**INVESTIGATION OF ANALYTICAL TECHNIQUES FOR DETERMINATION
OF METAL SPECIATION IN NATURAL WATERS**

by

Tahir Yapici

B.Sc. (Uludag University)

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Abstract

This thesis presents a critical appraisal of the following analytical techniques for quantitative determination of metal speciation: Diffusive Gradient in Thin Films (DGT) for *in situ* determination of DGT-labile metal species in aqueous systems; Ion Exchange Technique (IET) for quantitative determination of free metal ion concentrations in aqueous systems. Pseudopolarography employed for investigation of effect of heterogeneity and ligand loading in aqueous systems. It also presents a study of metal binding characteristics of a well-characterized Laurentian Fulvic Acid (LFA) as a function of the [metal]/[LFA] ratios and pH; Competing Ligand Exchange Method, coupled with Inductively-Coupled Plasma-Mass Spectrometry for determining dissociation rate constants of metal complexes in natural waters. Effects of various competing ligands: Chelex-100, Dowex MAC-3 and Dowex 50WX-8 in model solutions and in freshwater samples were also investigated.

Humic substances (HS) in natural waters are physically and chemically heterogeneous complexants. This work has investigated the efficacy of pseudopolarography for quantitative determination of effects of heterogeneity of humic substances on the release of metals from metal-HS complexes. The results clearly show that the heterogeneity parameter, Γ , strongly affects the release of metals from metal complexes of HS. The general trend observed for the heterogeneity parameter was $\Gamma_{Zn} \geq \Gamma_{Cd} > \Gamma_{Pb} > \Gamma_{Cu}$. It was found that the [metal]/[LFA] ratios and the heterogeneity of humic substances were main factors that affected the release of metals from their complexes. The environmental significance of this work is that it has thrown light into effects of heterogeneity of HS on the release of metals by metal-HS complexes in natural waters. An important contribution

made by this work to the knowledge of metal speciation in the aquatic environment is that this is first time a clear-cut differentiation between the concept of lability (an intrinsic property) and the metal release by labile complexes (an extensive property) of metal-HS complexes has been made.

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Dedicated to Gonul, Yusuf, and Nihal

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--Chapter 1--

Introduction

1.0. Introduction

1.1. Chemical Speciation

Chemical speciation refers to aspects of chemical and physical form of an element: oxidation state, stoichiometry, coordination (including the number and type of ligands), and physical state or association with other phases, which govern the chemical behavior of elements, whether in environmental settings or in human organs, and provide a link to ecotoxicity to biota [1].

The International Union of Pure and Applied Chemistry (IUPAC) defines ‘speciation’ as the distribution of an element among the defined chemical species in a system. A chemical species is a specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure [2]. However, ‘chemical speciation’ in this thesis refers to occurrence, distribution, and characterization of the various chemical species of an element, as well as other aquatic components with which the element may react [3].

1.1.1. Importance of Speciation

The chemical and physical aspects that define speciation of a metal control its reactivity, including its solubility and uptake behavior, and, in many circumstances, toxicity. Solubility and uptake behavior, in turn, influence mobility of the metal in the environment, and therefore constrain pathways of its exposure to organisms, including

humans. During exposure, the metal speciation directly influences absorption across a physiological membrane, which allows entry into systemic circulation. A transformation in speciation may occur in biological fluids (e.g., lung or gut fluids) prior to any absorption, which may affect absorption and subsequent toxicity. Within organ systems, detoxification processes may further alter speciation and toxicity and also influence transport, excretion, and storage. This over-simplified description illustrates the importance of metal speciation over the entire process, influencing the metal's fate and human impact.

It is well established that the forms in which metal ions exist, i.e., their speciation, determine their geochemical and biological cycling in the natural environment. In other words, chemical speciation of trace metals plays an important role in determining transport, fate, and bioavailability of metals in the aquatic environment [3-5]. Trace metals are important in the biogeochemistry of aquatic ecosystems, and their chemical speciation in natural waters has become a major concern to analytical and environmental chemists because of involvement of trace metals in a wide variety of chemical reactions in the freshwater environment. At elevated concentrations, certain trace metals may be toxic (Figure 1.1). Many metals are nutrients required by organisms, but they can have deleterious effects (become toxicants) at elevated concentrations (e.g., Cr, Cu, Ni, and Se). Furthermore, bioavailability of trace metals, and consequently, their ecotoxicity, is dependent on the form in which the metal ion is present in the solution (i.e., its chemical speciation) rather than on the total metal concentration. Metal ions in the aquatic environment can be found in a variety of chemical species in both particulate and solution phases.

1.1.2. *The Analytical Challenge*

Quantitative determination of low levels of trace metals is usually accomplished by atomic spectrometry methods, which are very appropriate for this analytical task (e.g., concentrations below 10 $\mu\text{g/g}$ in tissues, 10 $\mu\text{g/L}$ in biological fluids or less than 1 $\mu\text{g/L}$ in natural waters).

It is often not possible to determine the concentrations of the different chemical species that sum up to the total concentration of an element in a given matrix [7]. Often, chemical species present in a given sample are not stable enough to be determined as such. During the analysis, partitioning of the element among its various species may change. Also in many cases, the large number of individual species (e.g., in metal–humic acid complexes) makes it impossible to determine the exact metal speciation. Analytical approaches are needed for speciation measurements that can provide reliable criteria for determination of water quality and concentrations of potentially toxic metal species at ionic and molecular levels. This is a challenging task for modern analytical chemistry because of the following constraints [8]:

- a) It is very difficult to preserve the integrity of metal species during the whole analytical process i.e., sampling, storage, pre-treatment, filtration, and determination of metal speciation.
- b) Because of extremely low concentrations (down to $\mu\text{g/L}$ and below), it is difficult to fractionate them for determining their chemical speciation.
- c) The complex matrix (e.g., of a sediment), confounds interpretation of the results in terms of metal speciation.

1.2. Trace metals interactions in the freshwater environment

Understanding the fate of trace elements in their life cycle is of paramount importance to water quality. When metal ions enter the natural environment and living systems through natural or anthropogenic activities, only a small fraction of the metal remain as free metal ion. The major fraction of the metal is complexed with either inorganic or organic ligands. Natural methylation of metal ions under specific conditions is common. The new species can be much more toxic, as is the case with methyl mercury, or less toxic, as in the case of arsenic species.

Natural aquatic systems contain if not all, nearly all, of the elements of the Periodic Table as well as an almost infinite variety of organic compounds, which span a wide range of sizes. Interactions of those species with their surrounding environment are largely unknown, which makes the freshwater environment an extremely complex dynamic system, and as yet, an unexplored system. Natural Organic Matter (NOM) in natural waters plays a crucial role in freshwater environment by controlling metal speciation and, therefore, bioavailability and, hence, ecotoxicity to biota.

The nature of NOM found in aquatic systems depends on its provenance; primarily, it is aquagenic (i.e., derived from water), and/or pedogenic (i.e. derived from soil) [9], as shown in Figure 1.2 [11]. Humic substances (HS) are major components of NOM, and are naturally-occurring complexants, which are chemically and physically heterogeneous, and possess the following unique properties that make metal-HS complexation reactions

quite different from those that metals undergo with simple and well-defined homogeneous ligands such as citric acid [10].

Figure 1.2 Diagram of the occurrence and possible environmental flow paths of humic substances [11].

In recent years, Sutton and Sposito [12] have proposed a new description of humic substances, according to which humic substances has a supramolecular structure of relatively small bio-organic molecules (having molecular mass <1000 Da) that are self-assembled mainly by weak dispersive forces such as van der Waals, π - π , and CH- π bonds into apparently large molecular sizes. The resulting structures of humic substances are collections of diverse, relatively low molecular mass components forming dynamic associations, stabilized by hydrophobic interactions and hydrogen bonds. The above-mentioned collections are able to organize into micellar structures in suitable aqueous

environments. However, a large fraction of humic substances are represented by hydrophobic compounds such as long alkyl-chain alkanes, alkenes, fatty acids, sterols, terpenoids, and phenyl-alkyl residues of lignin degradation. These functional groups of HS allow self-association into supramolecular structures, which results in their long residence time in the natural environment.

One of the most important characteristics of humic substances is their possession of acidic functional groups, mostly carboxylic acids, which confer on these molecules the ability to form chelate complexes with multivalent cations such as Cu^{2+} , Ni^{2+} , and Ca^{2+} . Humic substances directly affect behaviour, bioavailability, and hence toxicity of trace metals in the freshwater environment. Understanding of chemical and physical structure of humic substances has long been a focus of environmental chemists; however, because of chemical and physical heterogeneity of HS, this challenge has until now eluded solution.

1.3. Chemical and Physical Heterogeneity of Humic Substances (HS) and its effects on release of metals from metal-HS complexes

The chemical heterogeneity of HS arises from their having different functional groups in the HS macromolecules, which makes them unique in terms of their metal-complexation characteristics. The geometric location of functional groups plays a significant role in their metal-binding properties. From the consideration of the geometric location, Zunion and Martin [13] have divided the functional groups, such as carboxylic acid and phenolic hydroxyl groups, into the following categories: (a) functional groups capable of forming chelate rings with high binding energy; (b) functional groups participating in open

complex formation with intermediate binding energy; and (c) functional groups, lying deeply buried within a macromolecule with low binding energy. The general consensus is that the metal-binding humic substances can be classified as having both minor (~1-10%) strong binding sites, and major (~99-90%) weak binding sites [14]. They are physically heterogeneous, polydisperse molecules, and are of various sizes. The reactivity of a functional group depends on its type and its environment. For many metals binding by the carboxylic and the phenolic OH groups are of importance. Chemically heterogeneous complexants such as humic substances are characterized by a unique property: the strength of their metal-binding is critically dependent on the metal loading, θ , which is the $[\text{metal}]/[\text{HS}]$ ratio. At very low $[\text{metal}]/[\text{HS}]$ ratios characteristic of unpolluted natural waters, only the strongest binding sites are used for metal binding, forming very strong metal complexes which are non-labile. Trace metals first bind strong binding sites (which are much fewer than weak binding sites) of HS; the remaining trace metals then bind to the weak binding sites of HS, forming weak complexes which are labile and yield free metal ions. Among the high affinity sites of humic substances are nitrogen-, sulphur-, or phosphorus-containing functional groups [15,16]. Heterogeneity, and hence, trace metal complexation is influenced by pH and ionic strength because of the dependence of ionization of acidic functional groups of humic substances on pH and ionic strength [8].

1.4. Approaches for studying chemical speciation: bulk and volume sample

Because of the low concentrations of trace elements and the variety of components present in natural waters, a combination of several analytical techniques is favoured for metal speciation purposes. Several analytical techniques needed to be used in combination.

The analytical methods having sufficiently low detection limits and high analytical sensitivity for the elements in question have been applied in this work. Some of the techniques are discussed below.

1.4.1. Anodic Stripping Voltammetry

Anodic Stripping Voltammetry (ASV) has been the most employed speciation technique used for environmentally important metals such as copper, lead, cadmium, and zinc a total of about fourteen metals which are soluble in mercury forming amalgam [17]. ASV can be used only for those metal species that are reducible at a mercury electrode at applied potential less negative than about -1.4 V vs. E_{SCE} . At more negative applied potentials, evolution of H_2 (g) and O_2 (g) occurs, till all water of the sample is exhausted by its electrolysis. The ASV-labile fractions of a metal species show some corrections with the bioavailable metal fraction of the metal species [18,19].

Measurements by ASV are controlled by both the kinetics of dissociation of metal complexes and by the amount of time the complex takes to diffuse through the diffusion layer at the mercury electrode surface. Among its limitations is the problem of adsorption of organics in contaminated samples, its unsuitability in low-resistance solutions, and the possible artifacts of dissolved oxygen, and the unsuitability (because of the presence of dissolved oxygen) that this imposes on *in situ* measurements in natural waters.

1.4.2. Ion Exchange Technique

Several equilibrium ion-exchange methods have been developed to measure free metal ion concentrations in natural waters. This approach involves the equilibration of a resin with the sample of interest. At equilibrium, the amount of metal adsorbed on the resin is proportional to the free metal ion concentration in solution. This fraction is measured spectrometrically following elution with a strong acid. Calibration is achieved by using matrix-matched solutions of known free metal ion concentration. The technique was first used by Cantwell and coworkers in the 1980's [20-22]. More recently, Fortin and Campbell [23] applied this technique to investigate cadmium and zinc speciation in freshwaters.

Figure 1.3. Concentrations of organic and inorganic compounds in three types of water [24].

Earlier, Figura and McDuffie used contact time with Chelex, including both column and batch techniques, to operationally discriminate between very labile, moderately labile, slowly labile, and inert fractions [25]. The technique assumes that free metal ion is the only species that binds to the resin. Results may be positively biased if there are other cationic metal species present which also interact with the Chelex resin [22].

1.4.3. *Diffusive Gradients in Thin Films Technique (DGT)*

The Diffusive Gradients in Thin Films Technique (DGT) was introduced by Davison and Zhang in 1994 [26]. It has been used in studies of natural waters, both in the laboratory and *in situ*, to measure labile trace metals in aquatic environments. Diffusion-limited separation of species can be achieved by using a diffusive gel of adequate thickness and pore size overlying a membrane impregnated with a chelating resin. It is an advance over the dialysis with receiving resins in that DGT functions by constraining mass transport so that it is independent of the hydrodynamics of a solution above a threshold level of convection, and is only controlled by molecular diffusion.

In theory, the DGT method (based on the **characteristic** measurement time) can discriminate between labile, inert, and partially labile metal complexes based on their ability to dissociate and exchange with Chelex during the measurement time. An advantage of DGT is its multielement capability and the fact that measurements are independent of pH, ionic strength, and flow over ranges practically encountered in natural water bodies. DGT is readily applicable for *in situ* deployment and can provide time-averaged information on metal speciation. Uncertainties are associated with the diffusion

coefficients and the thickness of the diffusive boundary layer. One of the disadvantages of DGT is biofilm formation in high humic-content freshwater, a possibility during long deployment periods, leading to an underestimation of the labile fraction [27].

1.5. Modelling Metal Bioavailability

Experimental results have indicated that the bioavailability of a metal is highly dependent on conditions such as pH and NOM concentrations. The purpose of the Free-Ion Activity Model (FIAM) or the Biotic Ligand Model (BLM) is to link simple and relatively inexpensive chemical determinations with the chemical reactivity and potential bioavailability of possibly toxic elements [17]. One suggested definition of bioavailability which we have adopted, is “the amount or concentration of a chemical that can be absorbed by an organism, thereby creating the potential for toxicity or the necessary concentration for survival” [28]. This definition reflects the duality of biological uptake, which integrates both the availability to satisfy physiological requirements and the possible excess uptake leading to toxicological effects.

1.5.1. The Free-Ion Activity Model (FIAM)

Although research in the mid-1970s had demonstrated that the uptake by and toxicity of copper and cadmium to algae, bacteria, and other organisms was proportional to the free metal ion activity in solution [29, 30], it was not until 1983 that Morel encapsulated this in the Free-Ion Activity Model (FIAM) of metal uptake [31]. In FIAM, the free metal ions [32] were initially assumed to interact with binding sites at the cell surface of an aquatic organism. The term 'Free-Ion Activity' is misleading since the free hydrated metal ion is not

the only reactive species; at chemical equilibrium, the free metal ion activity represents the reactivity of the metal.

There have been many experimental investigations of FIAM [33-37]. Campbell [32] critically reviewed the experimental data from experiments, examining the conformity of aquatic organisms to the FIAM. Out of the 59 cases examined in the laboratory, 52 confirmed the FIAM. Campbell [32] therefore concluded that exceptions to the FIAM are relatively rare, at least for aquatic organisms in laboratory experiments. However, as mentioned above, most of the experimental data supporting the FIAM have been generated from laboratory studies in media containing synthetic organic chelators to control inorganic metal speciation. As Campbell [32] pointed out, few studies have been carried out on environmental samples with natural Dissolved Organic Matter (DOM), which may affect metal uptake indirectly by binding metals in solution in competition with the cell or directly by binding at the cell surface, retarding metal diffusion [38]. The formation of biofilms and changes to cell membrane permeability occurring under real environmental conditions may lead to deviations from the FIAM. This is undoubtedly an area that requires further investigation.

1.5.2. The Biotic Ligand Model (BLM)

The BLM was initially developed as a model for fish toxicity. BLM proposed that the gill membrane contained specific active metal-binding sites, or so-called biotic ligands. The fixed amount of metal bound to the physiologically active gill sites caused toxicity. In freshwaters, metal-gill binding was dependent on competitive equilibria between the free metal ion, and other inorganic forms, organically bound metal, and other cations such as

calcium, magnesium, sodium, and protons, for the biotic ligand. Again, the basic assumption is that the system is at chemical equilibrium. Natural freshwaters are dynamic systems, often far removed from chemical equilibrium, which makes them unsuitable for application of models like the BLM that are based on chemical equilibrium. It should be noted that although the FIAM and BLM are often treated as separate models, they are mechanistically identical. The current BLM models place greater emphasis, however, on the link between surface-bound metal concentrations and predictable toxicological response. The application of the BLM requires a chemical speciation model that uses the known chemical composition and binding constants to compute the metal-BLM binding.

Figure 1.4. Schematic diagram of the role of complexing agents in the assimilation of metals (M) by aquatic organisms. Reprinted from Campbell et al. [39].

The BLM approach is appealing, in common with geochemical models of metal speciation, because of its predictive capability based on minimal chemical measurements. There are, however, important concerns. First the BLM addresses acute not chronic effects, and secondly, and more importantly, the critical applications of the BLM are at metal concentrations near the Water Quality Guideline values. The datasets showing the BLM-predicted versus measured metals are always plotted on a log-log scale [33], but, even so, in the case of copper as the most-studied metal, poor correlations are the norm for all data below $10\mu\text{g (Cu) L}^{-1}$ [33]. It is also noteworthy that complexation by natural dissolved organic matter only assumes importance at these low metal concentrations, so any model fits will usually be better at higher metal concentrations.

1.5.3. Geochemical Modelling of Metal Speciation and Bioavailability

Modelling the interactions between metals and natural ligands and colloids is still a challenge. The present models are limited in their approach to modelling metal complexation by Natural Organic Matter (NOM). Recent research endeavours in this area have been directed toward describing metal interactions with NOM. This is an immense challenge owing to the polydisperse, heterogeneous nature of NOM. The Windermere Humic Aqueous Model (WHAM) model V [40] and VI [41], is the most widely-used computer speciation code. It incorporates electrostatic interactions, multiple binding sites of varying strength and competition between various cations for binding sites, and represents some of the most significant advances over the last decade.

Equilibrium modelling necessarily assumes that the system is in thermodynamic equilibrium. In recent years, several examples of non-equilibrium effects in aquatic systems have been reported which make this fundamental assumption questionable. Metal speciation may also be influenced by thermodynamically unstable species resulting from slow reaction kinetics, e.g., the slow kinetics of nickel-HS complexation in natural freshwaters.

1.6. Weakness of the current knowledge

The dominant tool for the analysis of metal ion speciation has been the local equilibrium (or pseudo-equilibrium) approximation [42]; that is, complexation reactions are assumed to be fast relative to other processes, and hence, 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. All reactions that are considered to take place in the system are also considered to have reached equilibrium. In many cases, complexation of metals by ligands is indeed rapid relative to other processes of interest, and an equilibrium model then provides a good representation of complexation phenomena in natural waters [43]. However, natural waters are not chemical-equilibrium systems. They are dynamic and often far-removed from chemical equilibrium [29,42,44-46]; hence, equilibrium speciation methods may not apply [47]. Heterogeneous ligand systems such as humic substances, which are colloidal, macromolecular, polyelectrolyte ligands, can depart considerably in kinetic behaviour from simple ligands and can result in systems that are dynamic and far removed from chemical equilibrium [42]. In some instances, the rates of biogeochemical reactions may be influenced, or even controlled by the rates of metal complexation reactions [46].

Both the Free Ion Activity Model (FIAM) and the Biotic Ligand Model (BLM) [48] are based on the assumption of chemical equilibrium at the biological membrane surface and imply that diffusion of the bioactive free metal ion toward the organism is not rate-limiting. However, when the metal exists as metal complexes, sufficiently labile metal species might contribute to the biouptake via preceding dissociation.

The neglect of transport in the medium as an element of the overall metal uptake process has some important consequences [49]. First, the FIAM fails to describe the possible contributions from labile metal complexes. If transport of free ions to the uptake sites gives rise to a concentration gradient, a driving force for the net dissociation of sufficiently labile complexes is present. This would effectively lead to the uptake of metal originating from labile metal complexes, which is not taken into account by the FIAM. It is well-known that a variety of labile metal complexes can be present in aquatic environments [50]. Secondly, describing metal toxicity as solely determined by adsorption equilibria, as is done in the BLM model, completely neglects the nonequilibrium aspects of the processes involved. Also, uptake, accumulation, and effects of metals in living organisms are involved more than the mere binding at a collection of binding sites. Equilibrium distributions are not generally achieved; more common is some steady-state in which fluxes are constant but not zero. Between metal uptake and eventual effects there are several steps, such as the distribution over different tissues, and this requires a kinetic bicompartamental modeling approach. Besides these concerns, experimental data exist which do not correspond with the FIAM approach [32, 34, 37].

1.7. Hypothesis

Within the framework outlined above, the following hypothesis is made:

- 1- Metal-ion binding by heterogeneous, naturally-occurring complexants, viz. humic substances (HS), is determined by the metal loading, θ (the metal/binding site concentration) ratio.
- 2- Aggregation properties of HS are influenced by the pH and the metal loading, θ .
- 3- Diffusion Gradient in Thin Films (DGT), a relatively new technique for *in situ* deployment in natural waters including aqueous solutions of complex matrix, mining and municipal aqueous effluents and wastewaters, offers great promise of studying lability (an intrinsic property of the metal-HS complexes), and dependence of the amount of metal released by labile metal complexes on the metal loading, θ (an extensive property of the metal-HS complexes), and the pH of the sample solution.

1.8. Objectives

The objective of the present study is to test the above hypothesis using natural freshwaters by a comparative study of the above three experimental techniques and the theoretical predictions using Windermere Humic Aqueous Model (WHAM), which, however, is based on the assumption of chemical equilibrium. Natural freshwaters are however dynamic systems. Since the hypothesis covers several areas, its testing will be done by conducting the following experimental investigations.

1. To investigate speciation of Cd(II), Ni(II), Pb(II), Cu(II), and Zn(II) in model solutions of a well-characterized, naturally-occurring, organic complexant, a Laurentian Fulvic Acid (LFA), using Diffusive Gradients in Thin films, (DGT).
2. To investigate speciation of Cd(II), Ni(II), Pb(II), Cu(II), and Zn(II) in municipal wastewaters and mining aqueous effluents from Sudbury, ON, mining areas by DGT.
3. To investigate speciation of Cd(II), Ni(II), Pb(II), Cu(II), and Zn(II) by *in situ* deployment of DGT using various binding resins in the river water of St. Lawrence River, Morrisburg, ON.
4. To investigate capability of Ion Exchange Technique (IET) to determine free-metal-ion concentrations of Cd(II), Ni(II), Pb(II), Cu(II), and Zn(II) in a model solution of a well-characterized, naturally-occurring, organic complexant, Laurentian Fulvic Acid (LFA).
5. To investigate the effects of the heterogeneity of HS on the on the release of metals from the HS-complexes of Cd(II), Ni(II), Pb(II), Cu(II), and Zn(II) in model solutions of a well-characterized, naturally occurring, organic complexants (LFA), and to study the effects of various waveforms on the heterogeneity of LFA and on the release of metals from these metal complexes.
6. To investigate quantitatively the effects of HS heterogeneity on lability of HS-complexes of Cd(II), Ni(II), Pb(II), Cu(II), and Zn(II) in model

solutions of a well-characterized, naturally-occurring, organic complexant (LFA) by pseudopolarography

7. To investigate the effects of different chelating resins on the release of the metals from complexes of Cd(II), Ni(II), Pb(II), Cu(II), and Zn(II) in model solutions of a well-characterized, naturally-occurring, organic complexants (LFA), and also to study the effects of metal loading, θ , on the release of metals from their complexes with HS.

1.9. Outline of the Thesis

In this thesis, three strongly related topics: lability, heterogeneity, and free-metal-ion concentration are treated in an integrated way to determine metal speciation in natural waters. The thesis comprises 9 chapters. Chapter 1 provides an introduction and scientific background of this research. Chapters 2, 3, and 4 present a relatively new approach to metal speciation of Cd(II), Ni(II), Pb(II), Cu(II), and Zn(II) in model solutions of the well-characterized Laurentian Fulvic Acid (LFA), and in municipal wastewaters and mining aqueous effluents; investigation of binding by different binding resins for *in situ* deployment in the river water of Lawrence River (ON) using DGT. Chapter 5 presents study of the effects of metal-to- LFA ratio and pH on free metal ion concentration of Cd^{2+} , Ni^{2+} , Pb^{2+} , Cu^{2+} , and Zn^{2+} in model solutions of LFA. Chapter 7 provides a detailed study of the effect of metal loading, θ , on the release of metals from their complexes and also the effect of LFA (a heterogeneous complexant) on Cd(II), Pb(II), Cu(II), and Zn(II) complexes of LFA in model solutions. Chapter 7 also presents the effect of various waveforms in Anodic Stripping Voltammetry: Square Wave, Linear Scan, Differential

Pulse, on the release of metal from metal complexes. Chapter 8 presents the effects of various binding resins and metal-to-LFA ratio on the release of metal from complexes.

Chapter 9 presents conclusions.

2.0. References

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--Chapter 2--

**Simultaneous Determination of Cd(II), Cu(II), Ni(II), Zn(II)
and Pb(II) Speciation in Model Solution of a Well-
Characterized Fulvic Acid by Diffusive Gradients in Thin
Films (DGT)**

2.1. Introduction

Natural waters contain various complexants that can form complexes with trace metal ions [1,2]. Biogeochemical cycling of trace metals are profoundly influenced by their chemical speciation [3,4]. Complexation reactions of potentially toxic metals by natural organic complexants such as humic substances, which are ubiquitous in freshwaters, are increasingly being recognized as important factors in natural waters because these reactions determine, to a large extent, the metal speciation and its bioavailability [5]. Furthermore, metal speciation also determines the mobility and toxicity of trace metals in the natural environment; for example, complexation of metal ions as insoluble organic compounds greatly reduces their mobility, whereas formation of soluble metal complexes with dissolved organic compounds enhances their mobility in the natural environment [6-8].

Humic substances (HS) consist of typically 40–90% of Dissolved Organic Carbon (DOC) [9]. DOC may have terrestrial or aquatic origin, and may degrade or condense in the water column. The composition of DOC may vary spatially and temporally. Differences in their elemental composition and spectroscopic properties have been well-documented [8]. Humic substances comprise humic acid (HA), fulvic acid (FA) and humin. Fulvic acid represents a major fraction (~ 90 %) of DOC present in freshwaters. With molecular weights typically in the range of 500-2000 g mol⁻¹ for FA and 2000-5000 g mol⁻¹ for HA, FA and HA are considered representative humic substances in the aquatic environment. FA generally has a lower molecular weight, higher oxygen content, higher carboxylic acid content and higher acidity than humic acid [10], and is the more water-soluble fraction.

The complexing sites of heterogeneous, macromolecular, organic complexants can be classified into major and minor sites types [11]. The major sites are weak binding sites and are the ones present in large proportions [12]. There are only a few types of major sites, including carboxylate and phenolate functional groups. The range of the free energy of formation of complexes with the major sites does not exceed the equivalent of 1–2 log K units because the sites are chemically homogeneous [11]. The minor sites are strong binding sites and represent a small fraction of the total sites, but they consist of a very large number of sites types [11]. These sites form strong complexes with metals. Examples of minor sites include nitrogen- and sulphur-bearing functional groups. The distribution of formation constants for metal complexes with the minor sites is larger (at least several log K units) because of not only the physical factors mentioned earlier but also of the wide variety in the chemical nature of these sites [13].

Recent NMR studies of humic substances (HS) have led Sutton and Sposito [14] to propose that HS are collections of diverse, relatively low molecular mass components forming dynamic associations and stabilized by hydrophobic interactions and hydrogen bonds. These associations are capable of organizing into micellar structures in a suitable aqueous environment. As a result, conformational properties are one of the most important properties of humic substances that should be considered in interpretation of metal ion binding to humic substances. A number of factors such as metal and ligand concentrations, ionic strength and pH have been reported to affect conformational changes, degree of aggregation, coiling and uncoiling of the various molecules of humic

substances and consequently their metal ion binding [15]. This is probably due to changes in the accessibility of some ligand sites to metal ions because of conformational changes [16,17]. In particular, conformation depends on hydration-dehydration process, the formation of hydrogen bonds, electrostatic interactions among various functional groups on a single molecule or between neighbouring molecules, and formation of metallic bridges between two hydrodynamically separate molecules, which themselves depend on the degree of occupation of the complexing sites [18]. In all cases, the relative importance of these factors varies with metal concentrations, pH and the degree of occupation of the complexing sites. The literature shows that these aspects have not as yet been fully addressed.

Diffusive Gradient in Thin Films (DGT) when deployed *in situ* in natural waters represents a relatively new approach to determination of metal speciation with minimum or no sampling involved. It is a promising new approach to overcome some of the limitations outlined above and in the above-cited literature. In general, DGT samples labile (free metal ion plus weak metal complexes) metal species in aquatic systems, and *in situ* assays. Accordingly, the DGT approach clearly obviates contamination problems associated with the collection of water samples and filtration. To date, the DGT has been successfully used to determine concentrations of labile species of trace metals such as cadmium [19], and lead [20]. However, additional validation work is necessary to demonstrate that the DGT results are reliable and can be used routinely. This chapter aims: 1) to present the behaviour of Cd(II), Zn(II), Pb(II), Cu(II) and Ni(II) in the presence of a well-characterized Laurentian Fulvic Acid (LFA) in model (aqueous)

solutions containing low metal ion concentrations ($\sim 10^{-9}$ M), typical of pristine freshwaters; 2) to study the effect of various thicknesses of diffusion gel layers on DGT-labile fractions of trace metal species; 3) to investigate the effect of metal/LFA ratio on the metal binding properties of the LFA.

2.2. Theory

The theory on the DGT based on diffusional characteristics of metal species in a hydrogel, and of the ion exchange properties of a metal-binding resin has been established by Davison's research group [21,22]. Specifically, the DGT utilizes a hydrogel layer of precisely known thickness to constrain the diffusive transport of metal species to a cation-exchange resin, which binds the metal species of the sample solution. Chelex cation exchange resin is mostly used as a metal binding agent in the DGT. DGT provides an estimate of the DGT-labile fraction of metal, which is widely regarded as the bioavailable fraction of the metal species. Chelex resin is a strong metal complexing agent and rapidly binds free metal ion and /or labile metal species, at pH 5-8.3 [23,24]; the metal binding to Chelex at pH between 5 and 9, which makes it an excellent binding agent in natural freshwaters.

The DGT device consists of a three-layer system (Figure 2.1): (i) a resin-impregnated hydrogel layer; (ii) a hydrogel diffusion-layer, and (iii) a filter membrane. One component, polyacrylamide, is a hydrous polymer consisting of acrylamide-polymer chains, typically linked with bis or agarose cross-linkers [25]. Chelex is embedded in the backing layer of gel (referred to as the resin gel). Hence, the Chelex resin gel layer serves

as a sink for labile metal species which diffuse through the polyacrylamide diffusion layer [26]. The cellulose filter membrane (pore size= 0.45 μm) protects the fragile polyacrylamide surface from mechanical damage by rough particles.

Figure 2.1 Cross-section through the DGT device and the hydrogel layer, adapted from Dahlqvist et al. [27]

The DGT generally allows diffusion of both labile inorganic and organic species with different sensitivities depending on the mobility (diffusion coefficients) of each species [28-30]. The DGT has been used to investigate the speciation of trace metals [29,31-33, 38] and other elements [27,35] in natural waters. Detailed theory of DGT has been developed by Davison et al. (36,37). When the DGT is deployed in a sample solution, the flux of ions through the hydrogel is described by Fick's First Law of Diffusion [28,30] given below as Eq 2.1:

$$J = D \frac{dC}{dx} \quad 2.1$$

where J is the flux of ions (mass per m^2 per sec.), D the diffusion coefficient (cm^2s^{-1}), and dC/dx is the concentration gradient of the analyte metal in the hydrogel. Cations that pass

through the diffusive layer are rapidly immobilized in the Chelex resin. Hence, the concentration of cations in the chelex resin/diffusive layer interface can be expected to be effectively zero provided the chelex resin has not reached its point of saturation. Making the reasonable assumption that the chelex resin has not reached its point of saturation; dC/C can be taken to be equal to the dissolved concentration of the cation C in the bulk solution. In a sufficiently stirred sample solution where the diffusive boundary layer in the solution is negligibly small, the total thickness of the diffusive layer (dx) is the sum of the thickness of the hydrogel (Δg) and of the cellulose membrane filter. By simplifying and re-arranging Eq.2.1, one can write Eq. 2.2 as follows:

$$C = \frac{J\Delta g}{D} \quad 2.2$$

The flux of ions can be described as $J=M/At$, where M is the mass of cation accumulated by the chelex resin, A is the exposed surface area of the diffusive gel layer and t the time of exposure to the sample solution. One can write Eq 2.3.

$$C = M\Delta g / (DtA) \quad 2.3$$

where C is the concentration of the analyte metal ions which diffuse through a hydrogel layer of thickness, Δg , and are accumulated by the chelex resin phase embedded in the backing layer of the gel (referred to as the resin gel). The accumulated mass of metal, M , is measured after deployment for a known time, t , by eluting the metal with a strong acid and using Eq. 2.1 for calculating C in the deployment solution. D is the diffusion coefficient. As the DGT device can be configured as a simple disk in a plastic holder, it is readily deployable *in situ* [38] in natural waters. In this study, the bound metals on the

chelex resin were eluted in 1 mL of 1M HNO₃, and their concentrations in the eluted acid quantified by Inductively-Coupled Plasma Mass Spectrometry (ICP-MS).

The concentration (C) determined by DGT device was calculated from the amount of accumulated metal M , eluted from the chelex resin and quantified by ICP-MS. Equation 2.3 gives the metal mass (μmol) captured on the chelex resin and is calculated from the metal concentration in the eluted sample (C_e , $\mu\text{mol/L}$), the volume of the elution fluid ($V_{\text{HNO}_3} = 1.0 \times 10^{-3} \text{ L}$), and of the chelex resin ($V_{\text{gel}} = 1.6 \times 10^{-4} \text{ L}$), and an elution factor ($f_e = 0.8$) needed to compensate for incomplete metal removal from the chelex resin. From M , the free metal concentration ($\mu\text{mol cm}^{-3}$) is calculated by taking into account the thickness of the diffusion gel layer (Δg), the diffusion coefficient of the metals in the hydrogel (D , $\text{cm}^2 \text{ s}^{-1}$), the deployment time (t in s), and the area of exposure surface of the gel to the sample solution ($A = 3.14 \text{ cm}^2$). The diffusion coefficients in the APA hydrogel are similar to those in water, but may vary slightly depending on the cross-linker used in the preparation of the hydrogel [39].

2.3. Experimental Design

The experiment was so designed as to enable the hypothesis outlined above to be tested. Experiments were performed individually for each metal in same manner. As will be discussed later, the metal-to-LFA ratio was set as follows: a) keeping the metal concentration constant and increasing the LFA concentration b) keeping the LFA concentration constant while increasing the metal concentration. Diffusive gel layer was employed in three different thicknesses in order to study the effect of thickness on metal speciation parameters. Moreover, the DGT device was deployed in the metal-LFA

sample solutions for a selected time-period in order to determine any correlation between the deployment time and the accumulated metal mass.

2.3.1. The LFA Model Solutions

A stock solution of Laurentian Fulvic Acid (1.0000 ± 0.001 g/L) was prepared by dissolving 1.0000 g of freeze-dried Laurentian Fulvic Acid (Fredriks Research Products, The Netherlands) in ultrapure water. The concentration of carboxylic and phenolic groups in the Laurentian Fulvic Acid was 11.6 mmol/g [40,41]. All test solutions were prepared with ultrapure water, which was obtained direct from a Milli-Q UF Plus water purification system (Millipore), fitted with an organic purification column to remove organic matter.

2.3.2. Containers and their Cleaning Procedure

All containers used were made of Teflon, and they were cleaned as follows. After being cleaned with ultrapure water (resistivity 18.2 M Ω /cm), they were completely filled with ultrapure water containing 10% (v/v) nitric acid (AR grade) and allowed to stand at room temperature for 1 week. Then they were rinsed successively 7 times with ultrapure water, filled with ultrapure water, and allowed to stand until they were used; the filling water was renewed periodically to ensure continued contact with clean water.

2.3.3. Preparation of Model Solutions

Model solutions were prepared to contain the well-characterized Laurentian Fulvic Acid (LFA) and equimolar concentrations of Cd (II), Zn (II), Pb (II), Cu (II) and Ni (II), each metal being taken individually and separately. Each metal solution was prepared to contain a single metal solution (2L) of the above metals. The concentration of each metal

was 1.0×10^{-7} mol/L. The test solutions were left to equilibrate overnight. During this equilibration period, the test solutions were then stirred with a Teflon-coated magnetic stirring bar using stir rate of 1000 rpm, which was the highest possible stir rate that could be used to homogenize them.

2.3.4. *Preparation of the DGT probes*

Standard DGT units for metals were obtained from DGT Research Ltd., Lancaster, UK, and assembled according to the procedures described elsewhere [21]. The DGT devices were prepared following the procedure recommended by Zhang [42,43] and described in Odzak et al. [33]. To prepare the diffusive gel, 70 μ L of ammonium persulfate initiator 10 % (v/v) and 20 μ L of TEMED (N,N,N',N'-tetramethylethylenediamine) (99%) catalyst were added to a 40% (v/v) acrylamide solution (BIO-RAD), and agarose cross-linker (2 % v/v, DGT Research Ltd., Lancaster, UK) and ultrapure water. This solution was cast between two glass plates and allowed to set at 42-45 $^{\circ}$ C for 1 h. By using spacers of different thicknesses, diffusive gels of thickness 0.4, 0.8 and 1.2 mm were produced. All gels were hydrated in ultrapure water for at least 24 h to allow them to attain a new, stable dimension. During the above procedure, the water was changed several times to remove any impurities and unreacted reagents by diffusion. Gels were then conditioned and stored in 0.1 M NaNO₃ solution. The resin gel was prepared by adding 4 g of the ion-exchange resin (Chelex-100 mesh, sodium form) in 10 mL of the gel solution after washing with ultrapure water. Ammonium persulfate (50 μ L) and 15 μ L of TEMED (N,N,N',N'-tetramethylethylenediamine) (99 % v/v) catalyst were added, and the solution was cast in the same manner as the diffusive gel to produce a resin gel 0.4 mm thick. The

gel holder used was of a simple piston design (V) with a 2-cm-diameter window (DGT Research Ltd). The resin gel layer was placed on top of the piston, and then the diffusive gel layer was added, followed by the cellulose membrane filter. The front cap was then pressed down to form a seal between the cap and the gel surface.

DGT devices were deployed in acid-precleaned Teflon containers holding 2 L of continuously-stirred solutions. An assembled DGT unit was suspended in the sample solution by means of a synthetic wire. Each DGT measurement was done in triplicate, and the average values was obtained. The DGT units were deployed in sample solution for 24 h, after which they were removed and disassembled. During the deployment period, aliquots of the sample solution were tested to determine the DGT performance. For the determination of the accumulated metal, the Chelex-100 resin gel layer was removed from the DGT device and placed for 24 h in a Teflon bottle containing 1 ml of 1M HNO_3 (Startek, Canada) to extract the metal from the Chelex-100 resin. After dilution of the above extract with ultrapure water, the metals were quantified using Perkin Elmer SCIEX ELAN 6100 DRC ICP-MS in a CLASS 100 Clean Room Facility. The instrument operational parameters and the data acquisition protocol are presented in Table 2.1. The blank-corrected concentrations determined by DGT were obtained by following the well-documented procedures and equations reported elsewhere [21], and the diffusion coefficient for free metal ion (aqua complex) was obtained [44].

2.4. Discussion

2.4.1. *Effect of the deployment time on the metal mass accumulated by the DGT Chelex resin*

Figure 2.2 presents accumulated mass versus deployment time and shows that the relationship between the accumulated metal mass and the deployment time is linear, with a correlation coefficient of 0.958 to 0.995, as predicted by Equation 2.3. A negative intercept in the plot is common [45], and is due to the time required to establish a linear diffusion gradient across the gel layer.

Table 2.1 Instrumental operating conditions and data acquisition protocol for ICP-MS

ICP-MS	
RF power (kW)	1.1
cooling argon flow rate (L min ⁻¹)	16
carrier argon flow rate (L min ⁻¹)	0.8
auxiliary argon flow rate (L min ⁻¹)	1.0
Data acquisition parameters	
dwel time (ms)	50
scan mode	peak hop
signal measurement	counts s ⁻¹
points/spectral peak	1
resolution	Normal

Figure 2.2

Effect of deployment time of the DGT device on the accumulated mass of metal; $c_M = 1.0 \times 10^{-7}$ mol/L; $c_M/c_{LFA} = 3.6 \times 10^{-2}$, $T = 24 \pm 2$ °C, $I = 0.05$ mol/L,

Figure 2.2 confirms that DGT can be used successfully for a relatively long duration till the resin is saturated with the bound analyte metal. Study of the accumulated metal by DGT devices from aqueous solutions may provide useful information on the labile fractions of the M-LFA complexes, and also whether large uptake of M by the DGT device is required for the enhanced diffusion of trace metal ions over a long period of time (e.g. days).

Figure 2.2 clearly show that the uptake of the M is can be continued by prolonging the deployment time of the DGT device. However, the gentle slope of the curves of all these metals indicates that prolonged exposure does only a little or practically no enhancement in the amount of metal accumulated by the DGT device. Prolonged exposure of the DGT device, is hence unproductive in effect and wasteful of time and efforts, and is not recommended.

2.4.2. Assessment of the lability of M-LFA complexes by DGT

One of the promising advantages of the DGT technique is the possibility that its experimental results may be interpreted in terms of the metal-complex lability. To test this possibility, DGT samplers with different diffusion layer thickness were deployed in sample solutions containing the M-LFA complexes. Figure 2.3 shows the mass of metal accumulated in the Chelex-100 binding resin as a function of diffusive gel thickness: as the thickness of the diffusion layer was increased there was a decrease in the amount of metal accumulated for all of the metals studied. The mass of metal accumulated decreased with increase in the thickness of the diffusion layer, but the decrease was slight

Figure 2. 3

Effect of the diffusion layer thickness of the DGT device on the mass of metal accumulated from M(II)-LFA complexes. $c_M = 1.0 \times 10^{-7}$ mol/L; $c_M/c_{LFA} = 3.6 \times 10^{-2}$, $T = 24 \pm 2$ °C, $I = 0.05$ mol/L. The error bars represent three times the standard deviation of five replicate measurements. The trend lines of curves do not represent a fitted model.

for most of the metals; for Pb, there was hardly any decrease; only for Ni, the decrease was substantial. Unlike the curves for Pb, Cd, and Cu, the curve for Ni showed greater reactivity in the DGT, and its decrease in the accumulated metal mass as a function of the DGT thickness was much greater, especially at the early part of the curve, than those of the other three metals.

Additionally, when the thickness of diffusion layer was very small, there was insufficient time for M-LFA to dissociate, and hence there was essentially zero contribution from the M-LFA dissociation to the metal mass accumulated. In such circumstances, the DGT measured only the metal that resulted from diffusion of the labile fraction of the metal (metal aqua complex and other labile metal species).

Since in DGT, its total thickness defines its timescale of the measurement, as the thickness of the diffusion layer increased, the experimental timescale of measurement also increased [46]. Hence, more time was needed for M-LFA to dissociate, resulting in the observed increase in the mass of the accumulated metal. Additionally, Figure 2.3 shows that the individual metal behaved differently even under the same experimental conditions of $[M]_{\text{total}}$, $[LFA]$, diffusion layer thickness, pH, ionic strength and temperature.

In order to investigate the effect of the thickness of the DGT device on the accumulated metal mass, well-stirred, model solutions were used. The DGT devices with four different values of diffusive gel thickness, Δg (0.14 -for the filter only-, 0.54, 0.94, and 1.34 mm)

were deployed for 24 h in 2L synthetic solutions containing the following metals, studied one at a time: Co(II), Ni(II), Cu(II), Cd(II), and Pb(II), 1×10^{-7} mol/L, and $c_M/c_{LFA} = 7.1 \times 10^{-3}$. With the aim of improving the theoretically-predicted linear relationship between the mass of the accumulated metal and the inverse of the diffusive gel thickness ($1/\Delta g$), a longer deployment time was used in this initial experiment to increase the mass of metal accumulated by DGT. In order to have a Diffusive Boundary Layer (DBL) as thin as practicable, a small volume (2 L) and a high stir rate of 1000 rpm, which was the highest possible stir rate without decoupling the magnetic stir bar from the stir plate, were used.

Figure 2.4 presents a plot of the accumulated mass of Cu(II), Cd(II), Pb(II) and Ni(II) as a function of the inverse of the diffusive gel layer thickness. An inverse relationship between the thickness of the diffusive layer and the metal accumulated by the Chelex-100 binding resin was observed, which was in agreement with the predictions from equation 3. The slope of Ni(II)-LFA system was greater than those of the others, whereas that of Cu(II)-LFA complex was slightly greater than that of Cd(II)-LFA. The curve for Pb remained almost flat from the beginning to the end. From equation 2.3, the slopes of the curves in Figure 2.4 can be directly related to the diffusion coefficients of the metal-LFA species. The calculated mean diffusion coefficients for the Cu(II)-LFA, Cd(II)-LFA, Pb(II)-LFA and Ni(II)-LFA complexes are presented in Table 2.2.

The mean diffusion coefficient is an average diffusion coefficient of the free metal ion, D_M , and the diffusion coefficient of the metal complex, D_{ML} . When the metal is bound to colloidal or heterogeneous complexant such as humic substances, its diffusion coefficient

is primarily determined by the properties of the very much larger molecule of the complexing agent [8], such as humic substances. Hence, the observed mean diffusion coefficients can be used as an indirect indicator for degree of complexation of the system. More specifically, at high degree of complexation, more of the metal is present as ML, hence $\bar{D}_{ML}$ approaches D_{ML} .

Table 2.2 Mean diffusion coefficients $\bar{D}_M$ of the LFA complexes of Cu(II), Pb(II), Cd(II) and Ni(II) in model solutions of LFA. $c_M/c_{LFA} = 3.6 \times 10^{-2}$; $T = 24 \pm 2$ °C, $I = 0.051 \text{ mol/L}$

	$D_M / \text{cm}^2 \text{s}^{-1} \star$	$\bar{D}_M / \text{cm}^2 \text{s}^{-1}$
Cu(II)-LFA	6.06×10^{-6}	4.43×10^{-6}
Pb(II)-LFA	7.83×10^{-6}	9.07×10^{-7}
Cd(II)-LFA	5.93×10^{-6}	3.83×10^{-6}
Ni(II)-LFA	5.92×10^{-6}	4.82×10^{-6}

★ The diffusion coefficients for the aqua complexes of Cu^{+2} , Pb^{+2} , Cd^{+2} , and Zn^{+2} were obtained from Zhang (43)

Figure 2. 4

Effects of the inverse of diffusion layer thickness of DGT device on the amount of metal accumulated from M(II)-LFA complexes. $c_M = 1.0 \times 10^{-7}$ mol/L; $c_M/c_{LFA} = 3.6 \times 10^{-2}$, $T = 24 \pm 2$ °C, $I = 0.05$ mol/L. The error bars represent three times the standard deviation from five replicate measurements.

2.4.3. *Effect of the [metal] / [LFA] ratio on the lability of the LFA complexes of Ni(II), Pb(II), Cu(II) and Cd(II)*

Not much systematic data on the diffusion coefficients of humic substances in gels have been reported in the literature. Unquantified, slow diffusion of metal species through an agarose gel has been reported by Zhang et al. [28]. Tercier et al. [47] have reported that there is a good agreement between the diffusion coefficients of the metal ions in water reported in the literature with those obtained by the DGT experiments. However, there are no unique values for the diffusion coefficients for metal complexes of humic and fulvic substances in water. Humic substances (HS) are chemically and physically heterogeneous; their conformational and aggregation properties affect the measured diffusion coefficients.

Moreover, chemical heterogeneity, aggregation, and hence conformational properties of humic substances are strongly related to pH, ionic strength, concentration of the humic substances, concentrations of metals and of major anions and cations [48-50]. Therefore, clearly, diffusion coefficients of humic substances are not simply related to pore size only of the gel, but they also reflect the complexities of the gel and the molecular structures of the diffusing species.

Figure 2.5 presents the effect of the [metal(II)]/[LFA] ratio on the accumulated metal mass by the Chelex-100 cation exchange resin of the DGT device in a 24-hour deployment time. For Cu, Cd, and Pb, the accumulated metal mass decreased with

Figure 2.5 Effects of the ratio of M(II)/LFA on the mass of metal accumulated from M(II)-LFA complexes by the DGT Chelex resin in model solutions. $C_M = 1.0 \times 10^{-7}$ mol/L; C_{LFA} was varied to set the desired C_M/C_{LFA} ratios of the model solutions. Diffusion layer gel thickness = 0.4 mm; ■ Ni(II); ▲ Zn(II); ♦ Pb(II); ▼ Cd(II); ● Cu(II)., $T = 24 \pm 2$ °C, $I = 0.05$ mol/L. The error bars represent three times the standard deviation of five replicate measurements. The trend lines of curves do not represent a fitted model.

increasing [metal(II)]/[LFA] ratios, starting from the C_M/C_{LFA} ratio of 0.0018. For Cu, the decrease in the accumulated metal mass was drastic at the beginning of the curve, but the decrease became more slow and gradual with increasing C_M/C_{LFA} ratios, and the curve almost levelled off at long times. The LFA is a heterogeneous complexant, and the metal-LFA complexation is determined by the ratio of C_M/C_{LFA} ratio, as is the case with all metal complexes of heterogeneous complexants. However, nickel and zinc showed the reverse trend of what was predicted by the C_M/C_{LFA} ratio. The opposing trends displayed above by these two metals can be rationalized as follows. Ni and Zn follow the trend required by the C_M/C_{LFA} (i.e., [metal]/[ligand]) ratio: the larger this ratio, the greater is the amount of metal that becomes labile and available for accumulation by the DGT resin phase.

The curves for Ni and Zn, after the initial abrupt rise, soon levelled off into a plateau region at longer times, and this steady-state in the bulk solution was maintained probably by the balance of the rate of metal addition by the labile fraction of the DGT-labile complex, and that of the metal loss by metal accumulation by the resin phase of the DGT device. The curves for Cu, Cd, and Pb showed that with increasing C_M/C_{LFA} ratios, decreasing mass of the metal was bound as metal mass by the DGT, and soon this decrease ended in plateau (the trend for Cd and Pb was less than that of Cu). The observed trend ran counter to that predicted by the C_M/C_{LFA} ratio, which predicted that greater the ratio the greater the amount of metal that would be accumulated, not the decreased amount observed experimentally.

In Figure 2.5, the copper curve can be rationalized as follows. Copper is known [51] to preferentially occupy small number of binding sites of fulvic acid that have stability constants substantially stronger than the phenolic sites (which are stronger binding sites than the carboxylic sites of fulvic acid). The overall result is that for the copper curve, the predominant factor is the stronger (than those of the other four metals) intrinsic (covalent) binding to the LFA. Similar, but lesser covalent binding strength is operative for the other two metals, Pb^{2+} and Cd^{2+} . It is the intrinsic (covalent) binding strength of the metal ions: Cu^{2+} , Cd^{2+} and Pb^{2+} to the LFA that determines the stability of their LFA complexes, and also their DGT-lability (the rate of their dissociation), yielding metal for accumulation by the DGT resin phase. The observed trend runs counter to the trend expected from the C_M/C_{LFA} ratio. Comparison of the curves for the LFA complexes of Ni^{2+} and Zn^{2+} shows that the C_M/C_{LFA} ratio has more effect on the shapes of their curves, especially at, and following immediately the C_M/C_{LFA} ratio of 0.0018 i.e. up to 0.01, than at longer times throughout the entire measurement time of 2.5×10^5 s (Figure 2.2). The experimental results show that Cu^{2+} forms much stronger (covalent) complexes with the LFA (using the stronger phenolic sites of the LFA), and these strong Cu^{2+} -LFA complexes are DGT-nonlabile, and hence, do not contribute to the metal mass accumulation by the DGT resin phase. It seems that the covalent-bond strength of Cu^{2+} -LFA complexes has trumped the argument based on the C_M/C_{LFA} ratio. However, the following factors are also involved.

Both the stability (thermodynamics) and the lability (kinetics) of the metal complexes determine the amount of metal mass that is accumulated by the DGT resin phase [55].

For example, at 1 mm thickness of the DGT-gel-layer, the characteristic time available for metal-complex dissociation is ~ 5 min, and the DGT can be therefore be expected to measure those metal complexes that can readily dissociate in ~ 5 min and have a stability constant effectively smaller than that characterizing the binding of the metal to the Chelex resin [52]. For Ni, with the increasing $[\text{metal(II)}]/[\text{LFA}]$ ratios, the increase in the metal mass accumulated was drastic up to the $[\text{metal(II)}]/[\text{LFA}]$ ratio of about 0.01, probably because nickel after saturating all the strong binding sites ($\leq 1-10\%$) of the LFA was bound to the weak binding sites ($\geq 99-90\%$), forming weak complexes which were DGT-labile, and hence, increased the nickel mass accumulated. The curve for Ni, starting with a nearly exponential rise levelled off at about the C_M/C_{LFA} ratio of ~ 0.025 and this steady-state was probably maintained during the entire duration of the experimentation, by the exact balancing of the rate of gain of metals by the dissociation of metal-LFA complexes and the rate of loss of metals by capture of the metals by the DGT resin phase.

The curves in Figure 2.5 can be classified into two broad categories of metals: one category consists of Ni and Zn, and the other category of Pb, Cd, and Cu. These two categories of metals have displayed conflicting trends. The conflicting trends described above can be rationalized as follows. The metal group: Ni and Zn follow the trend unpredicted by the c_M/c_{LFA} ratio. Since the LFA is a chemically and physically heterogeneous complexant, like all other heterogeneous complexants, the stability and lability of its metal complexes are never fixed values of stability constants and lability, but they vary with the ratios of c_M/c_{LFA} . At extremely low ratios of $[\text{metal}]/[\text{concentration of binding sites}]$, the strong binding sites of the LFA are first filled, forming very strong

complexes, which are nonlabile. As the c_M/c_{LFA} ratio is increased, increasingly larger amounts of metals are bound to weaker binding sites (which are > 90% of the total binding sites), forming weak complexes which are labile and which add to the metal mass accumulated by the DGT. However, Figure 2.5 shows that as all the weak binding sites of LFA are filled up, a plateau region develops, presumably because the gain of metals from the dissociation of metal-LFA complex is exactly balanced by the loss of metals by being captured by the DGT resin binding phase, regardless of any variations in the c_M/c_{LFA} ratio. This trend is exacerbated for Ni by the slow kinetics of Ni-LFA complex-formation and dissociation. The plateau region of Zn ranges from c_M/c_{LFA} ratio ~ 0.005 to ~ 0.035 .

Contrary to the trend expected on the basis of c_M/c_{LFA} ratio, the curves formed by Pb, Cd, and Cu show a trend which conflicts with the trend shown by Ni and Zn. This opposite trend shown by Pb, Cd, and Cu can be rationalized by postulating that these metals, especially Cu have been reported to possess special binding affinity for heterogeneous complexants (e.g., the LFA), and may form supermolecules (using metal-binding groups to bridge hydrodynamically separate metal-LFA complexes) – such supermolecules have significantly lower diffusion coefficient and DGT-lability at increasing C_M/C_{LFA} ratios. For Cu, such drastic reduction in their diffusion coefficients occurs from c_M/c_{LFA} ratio 0.0018 to 0.01 probably because the Cu-LFA complex at the c_M/c_{LFA} ratio 0.0018 is small enough for the above accretion to the Cu-LFA complexes to produce a significant change in its diffusion coefficient and DGT-lability. However, it appears from the curves of this group (especially Cu), that further increase to the Cu-LFA complex on increasing the c_M/c_{LFA} ratio up to 0.035 (which is the range covered in Figure 2.5) decreases their DGT-

lability only slightly till the three curves of this group develop plateau at longer times. As stated above, this is a postulate, more a hypothesis which needs further experimental support before it can have predictive ability of an embryonic theory.

Figure 2.6 presents the effect of [nickel]/[LFA] ratio, and also of the equilibration time on the mass of nickel accumulated by the DGT device. The difference between the shape and the values of the two curves in Figure 2.6 can be rationalized by considering that the complexation of the LFA with nickel is slow, resulting in the curve for 96-hour equilibration representing nickel-LFA complex as at chemical equilibration, which allows its study as an equilibrium system. This equilibrium system presents a linear curve, with a slightly-decreasing slope with increasing C_M/C_{LFA} ratios. The overnight-equilibration (upper curve) represents a non-equilibrium system, of little use for the study of metal-LFA complexation reactions.

To investigate other possible contributory factors, further experiments were carried out: first, to validate the above results (Figure 2.5), experimental conditions identical to those of Figure 2.5 were maintained. However, no changes from the previously observed results were observed. Additional experiment was done for nickel by increasing equilibration time for Ni-LFA to 96 hours instead of overnight. The reason for this increase in the equilibration time was that nickel had slow rate for attaining complexation equilibrium [53,54]. Figure 2.6 shows the effect of 96-hour equilibration for Ni-LFA complexes. The amount of DGT-labile fraction of nickel decreased; however, this decrease was not as much as that for copper. Scally et al. [36], reported on the

Figure 2. 6

Effects of the [nickel]/[LFA] ratio and equilibration time on the mass of metal accumulated from Ni(II)-LFA complexes by the DGT Chelex resin. $c_M = 1.0 \times 10^{-7}$ mol/L. Diffusion layer gel thickness = 0.4 mm; ● = overnight equilibration, ▲ = 96-hour equilibration. $T = 24 \pm 2$ °C, $I = 0.05$ mol/L. The trend lines of curves do not represent a fitted model.

dissociation kinetics of Ni-NTA and Cu-NTA complexes by DGT in situ environment, using a simple ligand, NTA, as a complexing agent, and concluded that complexation of Ni-NTA had kinetic influence on the accumulated mass by the DGT resin phase while Cu-NTA complex had no kinetic influence. The findings of this work are in agreement with those of Scally et al.[37]. The results described above are the combined effect of the following factors [55]: The binding properties of the LFA, the combined effect of the covalent bonding due to Lewis-basicity (bonding by strong versus weak binding sites) and the coulombic (electrostatic) interactions (long-range) due to its oligoelectrolytical properties of the LFA are important determinants of the free energy of interactions between a metal (II) ion and the functional groups of the LFA. The electrostatic interactions are augmented by high pH (because of the greater ionization of the acidic functional groups) and low ionic strength, and are decreased by increased metal loading because of partial neutralization of the charge on the neighboring, non-reacting, negative sites of functional groups of fulvate anions by the increased metal loading, all of which contribute greatly to the long-range coulombic interactions.

2.5. Conclusion

Increasing DGT thickness decreases the mass of metals accumulated by the DGT resin phase (Chelex). Increasing deployment time of the DGT increases the metal mass accumulated by the DGT resin phase. If the deployment time of the DGT is longer than the characteristic measurement time (defined by the total thickness of DGT diffusive layer plus the cellulose filter protective layer overlying the DGT device) then the mass of metal accumulated by the DGT device is greater than the DGT-labile metal complex- the increase is probably due to quasi-labile DGT metal complex yielding more metal mass.

2.6. References

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--Chapter 3--

**Investigation of DGT as a Metal Speciation Technique for
Aqueous Municipal Wastes and Mine Effluents**

3.1. Introduction

Trace metals exist in a variety of inorganic and organic species ranging from simple hydrated molecules (e.g., aqua complexes) to complexes of naturally-occurring, large organic complexants, e.g., humic substances, which are ubiquitous in the aquatic environment. The bioavailability, and hence ecotoxicity of metals in aquatic systems is strongly dependent on the nature of the metal species present. Hence, determining the chemical form (i.e., chemical speciation) of metals in the natural environment is essential for acquiring predictive capability for their impact on the aquatic biota [1]. Pollutant metals may be present at relatively high concentrations in aqueous effluents from mines. When they are discharged into freshwaters, they present potential hazards in the freshwater environment. The bioavailability and toxicity of metal complexes of Dissolved Organic Carbon (DOC) depend on the chemical speciation of the metal; hence, chemical speciation of metals is important for determining water quality.

Metal speciation in aquatic systems has a large influence on the metal bioavailability [2]. Information on distribution of chemical species in natural waters helps understanding of geochemistry and impacts of the metals on aquatic biota. Substantial progress has been made in metal speciation during the last two decades, particularly through the application of the technique of Anodic Stripping Voltammetry (ASV) [3] and Competitive Ligand Exchange Method in conjunction with Adsorptive Cathodic Stripping Voltammetry (AdCSV) [4-6]. However, examination of the conventional analytical procedures reveals the operational nature of the determination of metal species by the operationally-defined metal speciation techniques, vis-à-vis the complexity of metal binding by DOC in natural

systems [7]. Moreover, the above analytical methods are mostly applicable in the laboratory, and they involve handling of the samples, which may consequently introduce errors and contamination, and risk of change in the metal speciation. Furthermore, they are often both time-consuming and not accurate at low metal concentrations [8].

The relatively new technique of Diffusive Gradient in Thin Films (DGT) represents a novel approach for *in situ* determination of labile metal species in aquatic systems. DGT is being increasingly used for determination of metal speciation in a wide variety of media such as, natural waters and soils (9-13). DGT can be readily deployed *in situ*, is a simple, robust device that accumulates metals after their transport through a well-defined diffusive layer. DGT has been used for determining labile metal species *in situ* in natural waters [14,15], and since it passively accumulates labile species from a sample solution while deployed *in situ*, contamination problems associated with conventional water collection and filtration procedures are eliminated. DGT provides an operationally-defined measure of the labile fraction; hence, inferences can be made with respect to metal toxicity. The mass of a particular metal, measured by DGT in a sample solution, is the sum of all complexes of the metal that are able to diffuse through the diffusive layer of the DGT. In other words, the measurable metal complexes are those which have adequate diffusion coefficients and dimensions small enough to diffuse through the diffusive layer, and which are labile enough to dissociate during the time involved in their passage through the total thickness of the diffusive gel layer of the DGT and its overlying protective cover of cellulose membrane filter paper.

The objective of this work was to investigate applicability of DGT to municipal waste waters and mine aqueous effluents for study of metal speciation of Cu(II), Zn(II), Pb(II), Cd(II), Co(II), and Ni(II); to make a detailed study of the various speciation parameters that DGT can determine and to validate the DGT technique by comparing the results of the DGT technique with the predictions of a well-established equilibrium model: a computer speciation code, Windermere Humic Aqueous Model, (WHAM) VI.

3.2. Theory

The theory for DGT has been presented earlier in Chapter 2.

3.3. Materials and Experimental Methods

3.3.1. Study sites:

Samples were collected in June 2006 from several sampling sites in the Sudbury area (Ontario, Canada); the Copper Cliff Waste Water Treatment Plant (CCWWTP), the Nolin Creek Waste Water Treatment Plant (NCWWTP), the Whistle Mine Effluent, the Crean Hill Mine Waste Water Treatment Plant, the Garson Mine Waste Water Treatment Plant; and the Sudbury Mine Aqueous Effluents. A 0.45- μm cellulose membrane filter (CN-6 Metricel, 74-mm diameter, 150- μm thickness, German Sciences, Inc.) was used to separate particulates from dissolved species in the water samples in this study, and only the filtered samples were studied.

The experiments consisted of testing the DGT device for 1) Municipal waste water samples; 2) Mine aqueous effluent samples; 3) comparing the DGT results with the

predictions of a computer speciation model: WHAM VI; and 4) comparing the results of speciation of trace metals: Cd, Cu, Ni, Pb, and Zn using various sizes of diffusive layers.

3.3.2. Preparation of the diffusive gels and the DGT Device

Preparation of the diffusive gels and the DGT Device has been described in Chapter 2.

3.3.3. Deployment of DGT probes

The DGT devices were deployed (in triplicate for each diffusive gel) in plastic containers holding 2 L of continuously stirred solution. The DGT units were deployed in solutions for 24 h. The DGT units, to be used as blanks, were not deployed. During the deployment, aliquots of the solution were sampled. After deployment, the gel layers were separated and the resin-gel layer was placed in a Teflon bottle. One milliliter of 1 M nitric acid (Seastar, Canada) was added, and the Teflon bottle was shaken and left standing overnight to extract the metal from the Chelex-100, the eluted metals were determined by ELAN 6100 DRC inductively coupled plasma mass spectrometry (ICP-MS) (Perkin-Elmer, SCIEX). Concentrations determined by the DGT (and blank-corrected) were calculated by the well-documented procedures and equations given elsewhere [14].

3.3.4 QA/QC

Quality control for metal analyses included repeated injections and periodic (two in every 2-h operation of the equipment) analysis of the Certified Reference Standard, NIST 1640. A prior set of samples was re-analyzed if the measured value differed from the certificate value by $> 10\%$. The relative standard deviation among the replicate determination was typically $\leq 5\%$.

3.4. Results

When using DGT, errors can arise from a number of different sources; some of the possible errors include the value of the elution factor (f_e) used to calculate the accumulated metal mass, temperature variations during the deployment that affect the value of the diffusion coefficients used, errors in the determination of the bulk solution concentration, variability in the thickness of the diffusive gels used, resin gel blank correction, errors associated with the measurement of the diffusion coefficients (D_{gel}), and the existence of a Diffusive Boundary Layer (DBL) of unknown thickness acting on the face of the device (16). Several of these sources of error can be greatly reduced or even eliminated altogether by 24-h deployments in the laboratory. When the diffusive gels are prepared, variations in thickness can be minimized by using spacers with a uniform thickness throughout their length (17). Thus, the primary sources of error still remaining are the values of the elution efficiency (18), diffusion coefficients used in the DGT calculations (19), and failure of the requirement that the thickness of the DBL is negligible compared to the thickness of Δg .

Quantitative determination of Co, Ni, Cu, Cd, Zn, and Pb in Municipal waste waters and mine aqueous effluents was investigated using three different gel thicknesses, 0.40, 0.80, 1.60 mm and a protective cellulose filter membrane (0.14 mm) under laboratory conditions. DGT devices were employed for 24 h, using 2 L volume and a stir rate of 1000 rpm, which was the highest rate possible without decoupling the magnetic stir bar from the stir plate. Diffusion coefficient values of the metals of interest were obtained from the literature [20]. For DGT determination, Δg is the sum of the thickness of the protective

cellulose filter membrane and of the polyacrylamide diffusive gel layers. The usual practice has been to use the same diffusion coefficient for the cellulose filter membrane and diffusive gel layers and to ignore the DBL for laboratory measurements in well-stirred solutions. Recent work by Scally et al. [21] has confirmed that the diffusion coefficient in the cellulose filter membrane is not statistically different from that in the diffusive gel layer.

Concentration of metals and DOC, and other chemical properties of the samples are presented in Table 3.1. Higher concentration of dissolved copper compared with the other metals were found in all sampling sites. Lead and cadmium had the lowest concentrations, while dissolved zinc and nickel concentrations were in the 10^{-7} mol/L levels. It should be noted that the dissolved concentrations of metals do not represent the bioavailable metal species because of DOC can complex metal, hence reducing the concentrations of M^{2+} (free metal ion). pH and concentrations of major cations of Ca^{2+} and Mg^{2+} are also major factors in determining M^{2+} concentrations. Necessity of studying DOC arises from M-DOC interactions which influence strongly behavior of toxic metal species, and hence, the toxicity and water quality.

3.4.1. Lability of metal complex ML assessed by DGT

The differentiation of labile species by DGT operates in a way similar to Anodic Stripping Voltammetry (ASV) (14). The Chelex resin is assumed to bind exclusively with the free metal ion plus all other labile metal species which are formed by dissociation of metal complexes within the experimental timescale of measurement. By analogy with

ASV, this measurement time is related to the residence time of species in the diffusion layer and is approximated by Eq 2.3 (22). With this scheme, inert complexes are not measured. Since each metal ion has its own characteristic rate of association with ligands, the rate of dissociation of a complex is related to the complex stability constant (2). Similar to ASV, knowledge of the diffusion coefficients of different species is essential for making accurate measurements of the concentrations of labile metal species. However, DGT has a number of potential advantages over ASV, such as being simple, easy to use *in situ*, robust, low in cost, multi-element capability, and independence of pH, ionic strength, matrix, and flow rate (above a threshold) (23).

One of the important advantages of the DGT technique is that the experimental results can be interpreted in terms of an operationally-defined lability, which is characterized by the detection window (the measurement time). The detection window is defined by the timescale of the measurement technique, which for the DGT is defined by the thickness of the diffusive gel layer. Figure 3.1 shows the mass of the metal accumulated by the binding phase as a function of diffusive gel layer thickness for Co, Ni, Cu, Cd, Zn and Pb in six different municipal and mine aqueous effluent samples. Figure 3.1, shows that as the thickness of the diffusion layer was increased a decrease in the mass of metal accumulated was observed for all samples of the metals studied in agreement of the theoretical principle of the DGT species determined by DGT were those which diffuse through the diffusive gel layer of the DGT and are dissociated during their passage through the DGT diffusive gel layer.

Table 3.1 Some properties of the mine effluent water samples, collected from Sudbury area, Ontario, Canada, in June 2006.

	CCWWTP	NCWWTP	Whistle	Crean	Sudbury	Garson
pH	8.8	8.7	9.0	8.8	6.9	8.4
Conductivity (mS/m)	20.1	359.0	255.0	26.9	76.3	157.1
DOC (mg/L) ^a	6.55	1.19	2.14	2.23	18.29	2.82
[M] ^b (mol/L)						
Pb	3.7×10^{-11}	9.4×10^{-10}	6.4×10^{-10}	6.4×10^{-10}	1.0×10^{-9}	5.9×10^{-10}
Cu	85.8×10^{-6}	3.2×10^{-6}	3.1×10^{-6}	2.7×10^{-6}	3.2×10^{-6}	1.5×10^{-6}
Cd	1.8×10^{-9}	1.1×10^{-9}	1.2×10^{-9}	1.1×10^{-9}	2.9×10^{-10}	1.5×10^{-9}
Ni	4.4×10^{-7}	2.9×10^{-7}	3.7×10^{-7}	8.4×10^{-7}	5.8×10^{-7}	1.1×10^{-7}
Zn	7.4×10^{-7}	3.7×10^{-7}	7.5×10^{-7}	7.7×10^{-7}	7.5×10^{-7}	8.4×10^{-7}
Co	8.1×10^{-9}	1.5×10^{-8}	6.3×10^{-9}	6.1×10^{-9}	1.1×10^{-8}	8.4×10^{-9}
Major Cations (M) ^c (mol/L)						
Mg	1.6×10^{-2}	1.6×10^{-3}	7.1×10^{-3}	2.4×10^{-3}	7.2×10^{-3}	1.4×10^{-2}
Ca	4.6×10^{-1}	7.5×10^{-2}	4.7×10^{-2}	3.5×10^{-2}	2.9×10^{-2}	2.5×10^{-1}
Na	1.4×10^{-1}	3.4×10^{-2}	2.1×10^{-3}	2.1×10^{-3}	9.3×10^{-2}	6.9×10^{-2}
K	3.9×10^{-2}	2.6×10^{-3}	5.3×10^{-3}	1.0×10^{-4}	5.9×10^{-3}	1.2×10^{-2}
Major Anions (M) ^d (mol/L)						
F	2.6×10^{-3}	8.1×10^{-5}	8.0×10^{-5}	1.2×10^{-5}	4.3×10^{-4}	1.1×10^{-4}
Cl	9.7×10^{-2}	9.1×10^{-2}	1.1×10^{-3}	4.5×10^{-3}	1.6×10^{-1}	1.5×10^{-1}
NO₃	5.2×10^{-3}	ND	5.8×10^{-3}	3.2×10^{-4}	1.4×10^{-3}	3.1×10^{-2}
SO₄	1.5×10^{-0}	2.2×10^{-1}	1.4×10^{-1}	6.8×10^{-2}	5.0×10^{-2}	7.4×10^{-1}

pH of the samples had an uncertainty of ± 0.1 .

^a Determined by Shimadzu Total Organic Carbon Analyzer, Model TOC-VCSH.

^b Concentration of total dissolved trace metals determined by ICP-MS.

^c Concentration of total dissolved major cations determined by Thermo Jarrel Ash IRIS Radial Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

^d Concentration determined by Dionex Series 4000i Ion Chromatograph.

ND: Not Detectable.

The key factors that determine the DGT-lability of the complex ML are the time for its dissociation and the thickness of diffusive layer (which provides the characteristic time). When the thickness of the diffusive layer is very small, the time for diffusion of the metal complex ML and of the free metal ion metal M^{2+} through the diffusive gel was very short, which meant that there was insufficient time for ML to dissociate; hence, the contribution from ML was essentially zero and the DGT measured effectively free metal ion M^{2+} .

When the thickness of the diffusive gel layer was increased, the experimental timescale of measurement was correspondingly increased as predicted by equation 2.3. Hence, more time was available for ML to dissociate, resulting in production of more M^{2+} , which reached a maximum, and then decreased exponentially as the mass became controlled by diffusion. The relevant diffusion equation predicted the observed exponential decay. Increase of the thickness of the diffusion layer enhanced the mass of metal uptake by DGT because of the corresponding increase in the characteristic measurement time. As reported by van Leeuwen et al. (24), even a small increase in the thickness of the diffusive layer greatly increases the time required to reach a steady-state.

The observed trend in Figure 3.1 for all samples with exceptions of CCWWTP sample is the accumulated mass of $Zn > Ni > Mn > Cu > Co > Cd$. This trend is in agreement with that of the total metal concentrations (Table 3.1); with the exception of copper, the concentration of which is much greater than those of the other metals, and hence, copper should have the greatest DGT-labile fraction and a maximum mass accumulation of by the DGT resin. However, this was not observed, and copper became an exception to the general rule.

Figure 3.1 Accumulated mass of metal versus thickness of the diffusive gel plus the cellulose filter (0.14mm) for the DGT sampler. $\blacktriangle$ = Zn(II); $\square$ = Ni(II); $\blacktriangledown$ = Mn(II); $\circ$ = Cu(II); Δ = Co(II); $\bullet$ = Pb(II); The uncertainties (error bars) represent three times the standard deviation of five replicate measurements. The trend lines of curves do not represent a fitted.

3.4.2. Mobility

Figure 3.2 presents plots of the accumulated mass of the individual metal as a function of the inverse of the diffusive gel thickness. An inverse relationship between the metal accumulated by Chelex-100 resin and the thickness of the diffusive layer was observed, which was in agreement with the results predicted from equation 2.3. The good fit of the experimental data to the theoretical line confirms the validity of the value of the diffusion coefficient of the metal-DOC complex. As can be seen in Figure 3.2, the mobility of metals changed from one field-site to another, probably as a result of the metals being complexed by humic substances of various properties, and hence, the metal mass accumulated by the DGT varied. However, the observed relationship between the metals and their mobility is as follows: $\text{Ni} > \text{Cu} > \text{Cd}$

3.4.3. Comparison of the experimental DGT results with the WHAM VI predictions

DGT should work particularly well as a speciation technique with peat-derived humic substances as their relatively large molecular weight ensures a good differentiation in the diffusion coefficients of inorganic species. Use of the restricted gel in a single measurement then should work well to give a correct differentiation between inorganic and organic species. With lower-molecular-weight species, greater reliance needs to be placed on parallel deployments with at least two gels. For the same concentration of Cu (e.g. 1.1 mM), the DGT showed increasing complexation with higher-molecular-weight humic substances, as had been reported previously [25].

Figure 3.2 Accumulated mass of the metals versus inverse of total thickness of the diffusive gel plus the filter (0.14mm) for the DGT samplers. $\blacktriangledown$ = Mn(II); $\blacktriangle$ = Zn(II); Δ = Co(II); $\square$ = Pb(II); $\bullet$ = Ni(II). The error bars represent three times the standard deviation of five replicate measurements. The trend lines of curves do not represent a fitted model.

Figure 3.3 presents a comparison of the experimentally measured results with the WHAM VI predictions for the metals Cu, Zn, Pb, Ni, and Cd in the Municipal Waste and Mine Aqueous Effluent samples collected from Sudbury, Canada, in June 2006.

It is important to note that for metal speciation, DGT [21, 33–38] gives an analytical signal which represents the total concentration of all labile complexes including free $[M^{2+}]$, i.e., the DGT measured value represents the sum of all labile metal complexes including the metal aqua complex. The WHAM computer modeling program gives all distinguishable species, however, inorganic species are not more than 1 %. In this work, WHAM shows all labile metal species, not only the free metal ion $[M^{2+}]$. Figure 3.3 shows that the WHAM VI predicted labile metal species, which include free metal ion and inorganic metal complexes concentrations, compare reasonably well with the sum of the concentrations of all the DGT-labile metal species.

The (- -) line represents 1:1 line. There was a very good agreement between the WHAM VI predictions and the DGT-measured values (Figure 3.3). The effective binding constants used in the WHAM VI model were derived by fitting the model to numerous reported data sets from laboratory experiments on the binding of metals to humic substances [24].

The concentration of humic substances was calculated from DOC concentration by assuming 60 % of the DOC was humic substances. The DOC values were determined by Total Organic Carbon Analyzer, TOC-V_{CPH} (Shimadzo, Japan) and are presented in Table 3.1. The concentrations of major cations were determined by graphite furnace atomic

Figure 3.3 Comparison of WHAM-VI-predicted pM ($-\log_{10}[M^{2+}]$) and the measured DGT-labile Zn(II), Ni(II), Mn(II), Cu(II), Co(II), and Pb(II)- species in the water samples collected in June 2006. (---) 1:1 line.

absorption spectrophotometry. Dionex ion exchange chromatography was used for determining anions. The concentrations of major anions and cations are presented in Table 3.1. Assuming that the mechanistic base of the WHAM VI adequately describes the effects of changes in the solution composition, such as ionic strength and pH, the model effectively acts as a conduit for comparing the past data on copper metal binding with the results and conditions of this work. Clearly, there is agreement between the copper measured by the DGT device and the predictions of WHAM VI for all effluent samples and for different diffusive gel layer thicknesses. This was not the case, however, for the other metals, which were determined by DGT, and the DGT results were compared with the WHAM VI predictions.

Before considering the WHAM VI calculations there are some issues that need to be clarified. It is not entirely reasonable to assume that FA is the only organic ligand in the effluent waters when ratios of FA/HA have been suggested to be 9:1.29 [52]. We assumed alternatively, therefore, that the organic ligands consist of 10% HA and 90% FA and recalculated the DGT results and the WHAM predictions. The concentrations of labile inorganic and organic Cu measured by DGT did not change significantly when 10% HA was introduced into the calculations compared with the no HA (100% FA) situation.

The concentration of humic substances is another important parameter for the WHAM VI prediction. It is not simple to obtain the individual concentrations of FA and HA in natural waters, and estimations made from DOC measurements are inherently inaccurate;

yet, they are the best way to represent binding properties of natural organic matters. Speciation models such as WHAM depend on an accurate set of input parameters, including ligand type and concentration, major cations and anions, and trace metals with a strong affinity for the ligands. Without performing non-routine measurements of input data, such as FA/HA ratios and total labile metal, they cannot be expected to reliably predict speciation, even if there are no problems with the mathematical description of the system and parameterization. Multiple DGT provides a direct measure of the total DGT-labile metal.

The WHAM predictions agreed well with the direct measurements of the DGT-labile metal species in the Municipal Waste and Aqueous Mine Effluent samples from the Sudbury (Ontario) area. Generally speaking, the WHAM predictions agreed well with the experimental results for all metals and samples. The DGT measurements were also made with various diffusive layer thicknesses: 0.4, 0.8, and 1.2 mm, and the results obtained were compared with the predictions of WHAM VI. Agreement between the results obtained from the increasing diffusive layer thicknesses (0.4, 0.8, and 1.2 mm) and the WHAM VI predictions were not as good for the diffusive layer thickness 0.4 mm (data not shown). WHAM VI slightly under-estimated the labile metal species in these metal-contaminated natural water samples.

3.5. Conclusions

The DGT is a simple, effective technique for studying chemical speciation of trace metals in natural water samples. However, as mentioned by van Leeuwen et al. (24), interpretation of the results and the overall performance of the DGT become complex as the results are controlled by three different types of detection windows: a) a size window related to the diffusive gel structure, b) a lability window defined by the thickness of the diffusive gel layer, and c) a detection window that depends on the strength of the metal binding phase of the DGT resin.

3.6. References

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--Chapter 4--

**Simultaneous Determination of Cd(II), Cu(II), Ni(II), Zn(II),
Co(II), and Pb(II) Speciation in St. Lawrence River water by
Diffusive Gradients in Thin Films Technique (DGT)**

4.1 Introduction

At present, determination of metal speciation in natural waters is usually done by collecting water samples and transporting them to the laboratory, where they are filtered and acidified to a $\text{pH} < 1.5$ in order to preserve the integrity of the samples, which are then tested by various methods of determining metal speciation in the preserved samples. This is a very complex and time-consuming process, involving serious risk of sample contamination and alteration of its chemical speciation [1]. Buffle et al.[2] have strongly argued that fully reliable information on the distribution of chemical species in natural waters can be achieved only if measurements are made *in situ* so that problems including delays involved in the sample collection and transport to the laboratory are avoided.

A relatively new technique, Diffusive Gradients in Thin Films (DGT), which can be readily deployed *in situ* in natural waters, and which uses a simple, robust device that accumulates metals after their transport through a well-defined diffusive layer, has recently emerged. DGT functions by constraining mass transport and measures only labile species, called the DGT-labile species. DGT has been used for measuring labile metal species *in situ* in natural waters [3,4] and has been tested in water samples containing various trace metals. DGT has been successfully developed as a promising *in situ* speciation technique for measurement of trace metals in freshwater, sea water and estuarine sediments [5,6]. DGT does not measure all the chemical species of a metal in solutions; it measures only those species called DGT-labile metal species that pass through the DGT gel layer and are accumulated by the metal-binding resin [7]. The sequestration of the free metal ion results in net dissociation of metal complexes; hence,

DGT measures free metal ion plus labile species, which are those complexes that can dissociate within the time determined by the diffusion layer thickness and the diffusion coefficient of the complex and are called 'DGT-Labile' complex [8]. The binding strength of the functional groups in the binding resin determines their competitive strength for ligand exchange with metal complexes in natural waters. Since different binding phases may have different binding strengths, it is expected that the amount of accumulated mass of metal depends not only on the thickness of the diffusive gel layer but also on the binding strength of the resin phase. The DGT device usually contains Chelex-100 resin embedded in a hydrogel as the binding resin phase. The Chelex resin layer is separated from the bulk solution by a well-defined diffusive layer of gel (the diffusive gel), the thickness of which determines the measurement time and is therefore the determining factor. The thickness of the diffusive gel layer can be varied. Chelex resin binds the metal ion that diffuses from the bulk solution through the diffusive layer and into the Chelex resin layer. Because the metal is continuously removed from the gel-entrained solution, a steady-state diffusive gradient is quickly established through the diffusive gel and is maintained by convective supply of metal complexes in the solution. The total amount of metal accumulated is determined by the amount of metal available to the resin during the deployment of the DGT device in the sample solution.

Some properties of the DGT need investigation. It is well known that the properties of the binding resin i.e., selectivity, and affinity for trace metals, affect the quantity of metal mass accumulated by the DGT device. However, more definitive investigation of the *in situ* deployment of DGT in the freshwater environment is needed. Diffusional properties

of DGT can be investigated by applying various thicknesses of the diffusive binding layer. In order to investigate the importance of the binding strength of different binding resins, it is necessary to compare DGT devices fitted with different binding phases containing various functional groups. The effect of various binding resins of different binding strengths and of various thickness of diffusive hydrogel layer on the performance of the DGT also needs further investigation [9].

4.2 Theory

The theory underlying the DGT, based on diffusion of metal species in a hydrogel and on the ion exchange properties of the metal-binding phase Chelex, has been established by Davison's research group [3,10]. A brief description of the theoretical background of DGT as proposed by Davison et al.[3,10] is given below. DGT utilizes a hydrogel diffusive layer to constrain diffusive transport of metals through the hydrogel to a cation-exchange resin, usually Chelex -100, which rapidly and strongly binds the DGT-labile fraction of the metal in the sample solution. This labile fraction is identified as free metal ion plus other DGT-labile metal species which are widely regarded as bioavailable.

DGT device consists of a three-layer system [11]: (i) a resin-impregnated hydrogel layer; (ii) a hydrogel diffusive layer, and (iii) a cellulose particulate filter membrane. One component, polyacrylamide, is a hydrous polymer consisting of acrylamide-polymer chains, typically linked with bis or agarose cross-linkers [12]. Chelex resin is embedded in the backing layer of the hydrogel, and will henceforth be referred to as the resin-gel. The resin-gel layer serves as a sink for labile metal species which diffuse through the

polyacrylamide gel diffusion layer [11]. The function of the cellulose filter membrane (pore size 0.45 μm) is to protect the fragile polyacrylamide surface gel layer from damage by adsorption of particles.

DGT generally allows diffusion of both labile inorganic and organic species with different sensitivities depending on the mobility (diffusion coefficients) of each species [1,13,14]. DGT has been used to investigate the speciation of trace metals [14,15-17,23] and other elements [18-20] in natural waters. Detailed theory, including kinetics of complex dissociation, has been developed [21,22]. When DGT is deployed in the sample solution, the flux of ions through the hydrogel is given by Fick's First Law of Diffusion [13,14], i.e., DGT is based on Fick's First Law of Diffusion as shown by equation 1:

$$J = -D \frac{dC}{dx} \quad (4.1)$$

where J is the flux of ions ($\text{mass per m}^2/\text{s}^{-1}$), D the diffusion coefficient (cm^2s^{-1}) (the negative sign indicates that the direction of diffusion is towards decreasing concentration), and dC/dx is the concentration gradient of the analyte metal species in the hydrogel. Cations that pass through the diffusive layer are rapidly immobilized in the resin. Hence, the concentration of cations in the resin/diffusive layer interface can be expected to be effectively zero provided the resin has not reached its point of saturation. Making this assumption of non-saturation, dC/C can be taken to be equal to the dissolved concentration, C , of the cation in the bulk solution. In a sufficiently stirred sample solution where the diffusive boundary layer in the solution is negligibly small, ions must

pass through the hydrogel layer of thickness Δg . By simplifying and re-arranging Eq.1, one can write Eq. 2 as follows:

$$C = \frac{J\Delta g}{D} \quad (4.2)$$

The flux (J) of ions can be described as $J=M/At$, where M is the mass of cation accumulated by the resin, A is the exposed surface area of the diffusive layer and t the time of exposure to the sample solution. One can write the following Eq.

$$C = M\Delta g / (DtA) \quad (4.3)$$

where C is the concentration of the analyte metal ions which diffuse through a hydrogel plus a particulate filter layer (used as a protective layer for the hydrogel against adsorption of particulates), and are accumulated by the Chelex resin bed embedded in the backing layer of the resin-gel. The accumulated mass of metal, $M(\mu\text{mol}/\text{dm}^3)$, is determined after deployment for a known time, t (s), by eluting the metal with a strong acid and using Eq. 4.1 for calculating the concentration, C , in the deployment solution. D ($\text{cm}^2 \text{ s}^{-1}$) is the diffusion coefficient, and A (3.14 cm^2), is the exposed surface area of the gel, and Δg is the thickness of the diffusion gel layer. As the DGT device can be configured as a simple disk in a plastic holder, it is readily deployable *in situ* in natural waters [23]. In this study, the bound metals on the resin were eluted in 1 mL of 1M HNO_3 , and their concentrations in the eluted solution were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The diffusion coefficients in the APA hydrogel are similar to those in water, but may vary slightly depending on the cross-linker used in synthesizing the hydrogel [24].

Within the framework outlined above, the overall objective of the present project is to test the hypothesis that in the natural waters the mass of metal accumulated by a DGT is dependent on the following parameters: diffusion coefficient of the metal species diffusing through the gel diffusive layer, the thickness of the gel diffusive layer and binding strength of the DGT binding resin-phase.

4.3 Experimental Section

In order to compare the performance characteristics of DGT devices containing each of the three different binding resin gels studied in this work were tested, one at a time: Chelex-100, Polymer 1.0 and Polymer 1.1 were individually (separately) deployed in natural waters. All DGT devices were so prepared as to contain a diffusive layer of 0.4 mm thickness. All field measurements were performed at the same time in St. Lawrence River, which is one of the world's largest rivers. With 1200 km total length, it drains an area of more than 1.3 million km², and connects the Great lakes with the Atlantic Ocean. The sampling location (44°45'40.48"N, 75°10'45.90"W) was upstream of Morrisburg (ON, Canada); the sampling was in early August 2006. Diffusion gel layers having three different thicknesses were employed in St. Lawrence River water in order to study the effect of the thickness of diffusion gel layers on metal accumulation.

4.3.1 Field deployment of DGT devices in natural waters

At a given test site, all DGT deployments were carried out for the same time periods: 45.3, 75, and 170 h. To prevent contamination, immediately after retrieving them, all

DGT devices were rinsed with deionized water (Milli-Q) thoroughly before disassembling them. At the deployment site, the flow of the water was reasonably fast without creating turbulence. The pH of the water was 8.5 ± 0.2 , the concentration of DOC was 12.81 mg/L, and the temperature was 23.5 ± 1.0 °C. Blanks were analyzed together with the analyte metals in the deployed DGT devices. The total metal concentrations of trace metals were determined using ICP-MS. Concentrations of major cations and anions were determined by graphite furnace atomic absorption spectrophotometry, using Perkin-Elmer equipment, and by ion exchange chromatography using Dionex equipment, respectively, (Table 4.1). The concentration of humic substances was taken to be same as the DOC concentration and was used for the WHAM predictions. Total Organic Carbon was determined by Total Organic Carbon Analyzer (TOC-VCPH, Shimadzu, Japan).

4.3.2. Preparation of the binding phase

The selected binding resins were synthesized and treated as reported in the literature [5,25-27]. Briefly, they were as follows:

Polymer 1.0 was synthesized according to the published procedures [25,26] by reacting 14.5 g (0.1mol) of 8-hydroxyquinoline and 17.2 mL (0.2 mol) of piperazine in 180 mL of dimethylformamide (DMF). To this solution, 19 mL (0.25 mol) of 37 % (v/v) aqueous formaldehyde was slowly added with stirring at room temperature. After 4 hours, the mixture was gradually heated on an oil bath to a temperature of 80 °C, and the heating was then continued for another period of 4 hours. An additional 10 mL of 37 % (v/v) formaldehyde was slowly added over a period of 4 hours to the reaction mixture in three

Table 4.1 Some properties of the St. Laurent River Water sample collected from Ontario in early August 2006.

St. Lawrence River Water Sample	Pb (µg/L)	Cu (µg/L)	Cd (µg/L)	Ni (µg/L)	Zn (µg/L)	Zn (µg/L)	Co (µg/L)	Mn (µg/L)
	3.4×10^{-1}	9.0×10^{-1}	5.8×10^{-3}	1.5×10^{-1}	2.0	4.7×10^{-2}	4.9	8.4
	Cl ⁻ (mg/L)	NO ₃ ⁻ (mg/L)	SO ₄ ²⁻ (mg/L)	Na ⁺ (mg/L)	K ⁺ (mg/L)	Mg ²⁺ (mg/L)	Ca ²⁺ (mg/L)	
	3.37	0.06	2.66	2.02	0.25	0.35	4.19	

Water sample was filtered through 0.45 µm AquaPrep™ 600 Capsules (Pall Corporation) and the filtrates were acidified to contain 1% (v/v) nitric acid, and the filtrates were analyzed.

The total dissolved metal concentrations of the sample were determined by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). All water samples were filtered through 0.45 µm AquaPrep™ 600 Capsules (Pall Corporation), and the filtrates were analyzed.

The major anion concentrations of the sample were determined by Ion Chromatography.

The major cation concentrations of the sample were determined by Flame Atomic Emission Spectrometry

DOC (mg/L) 12.81, pH 8.47

portions. After 12 hours of continuous heating, the reaction mixture was cooled and the precipitate was collected and washed with methanol. After soxhlet-extraction with methanol for 24 hours, the solid polymer was dried in vacuum, crushed, and sieved through mesh size 35– 60 (0.25–0.50 mm).

Polymer 1.1 was synthesized according to the published procedure [27] by dissolving 13.7 g (0.1 mol) of salicylaldehyde and 10.8 mL (0.1 mol) of N,N'-dimethylethylenediamine in 180 mL of DMF. To this solution, 19 mL (0.25 mol) of 37 % (v/v) aqueous formaldehyde was slowly added with stirring at room temperature. After 4 hours, the mixture was heated gradually on an oil bath to 90 °C, and the heating was then continued for 4 more hours. An additional 10 mL of 37 % (v/v) formaldehyde was slowly added to the reaction mixture in three portions. After 12 hours of continuous heating, the reaction mixture was cooled and the precipitate was collected and washed with methanol. After soxhlet-extraction with methanol for 24 hours, the polymer was dried in vacuum, and was then crushed, and sieved through mesh size 35 - 60 (0.25 - 0.50 mm).

Chelex-100 resin gel was prepared as reported by Davison et al.[5]; briefly, 4 g of the ion-exchange resin (Chelex-100 mesh, sodium form) was prepared for 10 mL of the gel solution by washing with ultrapure water. Ammonium persulfate (50 µL) and 15 µL of TEMED (N,N,N',N'-tetramethylethylenediamine) (99%) catalyst were added to the above solution, and the solution was cast in the same manner as the diffusive gel to produce a resin gel. Because of the presence of iminodiacetic acid group in Chelex, it induces

lability in metal complexes and competes effectively with natural ligands for metal ions in natural waters.

4.3.3 Preparation of the diffusive gels

Diffusive gels were prepared to a standard formula as described previously [13]; briefly, to prepare the diffusive gel, 70 μL of ammonium persulfate initiator (10 % (v/v)) and 25 μL of TEMED catalyst were added to 10 mL of the respective gels. After mixing it immediately the solution was injected between two glass plates and allowed to set for 1 h. Finally, the gel was conditioned and stored in a 0.1M NaNO_3 solution.

To investigate the effect of the thickness of diffusive gel layer on the performance of the DGT device including its use as a default diffusive layer, diffusive gel layers of thickness of 0.4, 0.8 and 1.2 mm were prepared, as reported in the recommended standard protocol [39]. They were then soaked for a minimum of 24 h in a 0.01 M NaNO_3 solution. The diffusive gel layer was overlaid with a protective 135 μm thick cellulose filter (Whatman). The diffusion coefficient of metal ions was assumed to be the same in the cellulose filter as in the diffusive gel [28].

4.4 Results and Discussion

4.4.1 Field deployment of the DGT device in natural waters

Concentrations of the labile species of cadmium, zinc, copper, lead, cobalt, and nickel in natural waters were determined by the DGT devices each, equipped with one of the three different binding resins: Chelex-100, Polymer 1.0 and Polymer 1.1, and which were then deployed in natural waters; this arrangement ensured that all the three resins were tested

in same way. The deployed DGT devices had a thickness of 0.4 mm; all field measurements were performed at the same time in St. Lawrence River water, at Morrisburg (ON, Canada), in early August 2006.

When metal ions accumulate in the binding layers, DGT is not able to differentiate the species that yielded the free metal ions. The uptake of metal by DGT depends on the metal speciation in the sample solutions, since the diffusion coefficients and the binding properties are different for different species. The meaning of metal uptake is that the metal is taken up from the solution by DGT, regardless of the metal species present. It is well accepted that the DGT device by design [8] does not measure all the chemical species of a metal in solutions; it only measures those species whose can pass through the cellulose filter plus DGT diffusive layer and are accumulated by the DGT binding phase [7], i.e., free metal ions (metal aqua complex), M^{n+} , plus labile fractions of the other complexes of the metal, which are collectively referred to as DGT-labile species [8]. Binding strength and properties of the binding phase which are due to its various functional groups, and thickness of the diffusive layer mainly determine the species that are DGT-labile, and hence, the metal mass accumulated by the binding phase.

4.4.2. Binding strength of the DGT resin phases used in this work

Polymer 1.0 is composed of 8-hydroxyquinoline and piperazine. The presence of piperazine moieties enhances the relative rigidity of the polymer backbone because of its cyclic structure. The binding capacity of polymer 1.0 was investigated in the pH range of 3.0-7.5; the best results were obtained at pH 7.0 [25,26]; this behaviour may be attributed

to the effect of pH on acid-base equilibria. Polymer 1.1 is oxime-containing polymer. Cross-linking is achieved by incorporating resorcinol. The binding strengths of Polymer 1.0 and 1.1 for binding a number of divalent metal ions in aqueous solutions are as follows [25]: $\text{Cu}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Cd}^{2+} > \text{Ca}^{2+} > \text{Mg}$. Chelex, the commonly used binding resin for DGT, owes its performance success as a binding agent to the presence of iminodiacetic acid functional group which competes effectively with natural ligands for metal ions in natural waters. The binding strengths of Chelex-100 for binding with a number of divalent metal ions in aqueous solutions are as follows [29]: $\text{Cu}^{2+} > \text{Pb}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Cd}^{2+}$

Li et al. [24] has reported that the amount and type of labile metal species accumulated by DGT depends only on the properties of the binding phase. However, previously in the studies reported on DGT, the binding phases were often considered to have no effect on the mass of metal accumulated [30]; they were treated merely as sinks for free metal ions. Recently, studies of various binding phases other than Chelex [8,31] on the DGT-labile metal fractions suggests that the labile metal fractions measured by different binding phases can be significantly different, implying that the proportion of metal complexes detected as DGT-labile is influenced by the binding strength of the binding phase used in DGT devices.

Figure 4.1 presents accumulated lead mass versus deployment time, and shows that under the same experimental conditions, i.e., identical duration of the deployment time, the same thickness of the diffusive gel layer, and the same location of the sample, the mass of lead accumulated varies, implying that the differences in the mass of lead

accumulated by various binding resins may be due to the differences in the binding strengths of the DGT binding resin phases. Chelex resin is most commonly used as the binding resin, and it can be successfully used as binding resin for all metals reported in this work. Polymer 1.0, has very low affinity for the studied metals; for some of them (copper and lead) low metal mass accumulation, and for some of them (cadmium, cobalt, nickel, and zinc) negative metal mass (after blank subtraction) has been observed in this work. Hence, Polymer 1.0 will not be discussed any further. However, it is worth mentioning that because of its low affinity for these metals Polymer 1.0 may be employed at sites heavily contaminated by these metals. Polymer 1.1 gave positive accumulation results for all these metals. Generally speaking, the accumulated masses of these metals were less than those of Chelex-100, with the exception of lead. However, Polymer 1.0 accumulated the metals more than Polymer 1.1. Amount of lead accumulated was higher with Polymer 1.1 than with Chelex-100. Less accumulation of lead by Chelex-100 binding resin has been attributed to the presence of the inert colloids mobilized from soils during the high flow conditions [32], which is consistent with the known bonding characteristics of lead [33]. The results of this work suggest that Polymer 1.1 might be suitable for determination of lead by DGT.

The results also suggest that replacing the functional groups of the binding resin may change the capability of DGT for metal uptake in aqueous samples. The binding strength of the DGT binding resin depends on the functional groups of the complexing agent (e.g., iminodiacetic acid in Chelex) in the binding resins. Only those metals are determined by DGT, the metal species of which can diffuse through diffusive boundary layer and are

Figure 4.1 Mass of lead accumulated as a function of different binding phases: Chelex-100, Polymer 1.0 and Polymer 1.1 binding phases. Accumulated mass of Pb by DGT in St. Lawrence River, ON. ■ Polymer 1.1 Resin, $r^2=0.982$; ● = Chelex-100 resin, $r^2=0.999$; ▲ = Polymer 1.0 Resin, $r^2=0.993$. DOC= 12.81mg/L, pH=8.5±0.2. The error bars represent three times the standard deviation of five replicate measurements.

Table 4.2

Concentration of DGT-labile metal species of Cd, Cu, Co, Ni, and Pb determined by in situ measurements in St. Lawrence River Water (ON), after deployment for 45, 75, and 170 h of the DGT device which is 0.4 mm thick and contains one of the following binding resins; Chelex-100, Polymer 1.0 and Polymer 1.1

Time DGT-labile Conc. of Metal, C_M ($\mu\text{g/L}$)	Binding Resins								
	Chelex-100			Polymer 1.0			Polymer 1.1		
	45 h	75 h	170 h	45 h	75 h	170 h	45 h	75 h	170 h
C_{Cd}	0.005	0.007	0.009	N/A	N/A	N/A	N/A	0.004	0.005
C_{Cu}	1.55	3.13	4.58	0.11	1.63	1.28	1.25	1.70	1.99
C_{Co}	0.045	0.065	0.081	N/A	N/A	N/A	0.006	0.007	0.010
C_{Ni}	0.45	0.63	0.81	N/A	N/A	N/A	0.099	0.134	0.203
C_{Zn}	1.29	2.58	3.64	N/A	N/A	N/A	0.37	1.87	3.23
C_{Pb}	0.03	0.05	0.09	0.002	0.008	0.02	0.08	0.15	0.26

The values shown above were the averages of two DGT devices; each with three replicate determinations.

N/A means: Not able to determine.

rapidly bound by the metal-binding resin within the measurement time determined by the combined thickness of the diffusive gel and the cellulose filter paper. Since different binding phases may have different binding strengths, it is expected that DGT-labile metal species will depend on the binding phase used [8]. The effects of different binding phases are clearly shown in Figure 4.2.

Table 4.2 presents the mass of accumulated metals by Chelex-100 resin, Polymer 1.0, and Polymer 1.1. The values presented in Table 4.2 are the resultant of several interacting factors: increasing duration of deployment results in increasing mass of metals accumulated by DGT, but this increase is different for different metals and for different binding resins, and for different deployment times. The metal exchange dynamics in solution determines the extent of net dissociation of metal complexes, penetration of complexes into the resin layer and consequently the proportion of metal that is measured. The metal ion competes with natural ligands in the solution and this competition is metal-specific; hence, the metal uptake by the binding phases is different for different metals, resulting in different concentration of DGT-labile species of Cd, Cu, Zn, Ni, Co, and Pb being determined under identical experimental conditions, viz., total deployment times, pH, and temperature.

4.4.3. Duration of the DGT deployment and biofouling of the DGT Device

One of the major drawbacks of DGT is microbial attack and biofouling, which needs to be taken into considerations in slow stream running water and/or in high-DOC-containing freshwaters. Fouling effect is a limitation for *in situ* deployment of DGT device,

particularly for relatively long deployment time. A superimposed layer of filter paper placed on the DGT device acts as a barrier for particles to pass, and hence, reduces the amount of metal species that pass through diffusive layer for possible capture by the resin layer. Zhang et al.[34], and Webb et al.[35] attempted to take the fouling effect into consideration by modifying the DGT uptake equation; however, their results show that the calculated values are under-estimations of the true environmental values, and also, the modified equations to be highly prone to errors [36]. Hence, a particular DGT device may not be applicable after certain time periods depending on sample solutions. It should also be possible to determine this effect directly by deploying DGT units with new membranes and DGT units with fouled membranes simultaneously. Alternatively, the problem can be avoided by using more DGT units and shorter deployment times as part of a monitoring program.

The literature reports that DGT has provided a satisfactory correlation between the metal mass accumulated by the binding resins and the deployment time with a slope of 0.99 [3]. However, in our study the slopes were less than the expected 0.99 level i.e., 0.85 to 0.97. All DGT devices were visibly fouled more or less by the end of the deployment periods. The lower correlations (slopes) between the accumulated mass versus the deployment time can be explained by the presence of a fouling layer over the filter paper [35], resulting in increased combined thickness Δg , (equation 2) of the diffusive boundary gel layer compared to the total thickness of the combined gel plus filter layer [3,35]. This observation should be kept in mind when interpreting the results. Even though DGT has been applied for relatively long deployment times successfully, DGT users should be

very careful about long time deployments in DOC-rich, or slow-stream environment because of the possible fouling effect.

Figure 4.2 presents effects of deployment-time and of the thickness of the diffusive gel layer of the DGT devices deployed in St. Lawrence River, ON, Canada. The DGT devices were of three different thicknesses of the diffusion layer 0.4, 0.8, and 1.2 mm (the results for 0.8, and 1.2 mm not shown). All the DGT devices were deployed for time-periods of 45.3, 75, and 170 h. The results show that the metal mass accumulated by the DGT binding phases decreased with increasing thickness of the diffusive gel layer (Figure 4.2) in agreement with the theoretical prediction. The results agreed with those of other workers [37]. Increased thickness of the diffusive gel layer meant more time was allowed for the diffusing species to pass through the increased path length through the diffusive gel layer. This allowed less time in contact with the binding phase for metal accumulation by the binding phase, and hence, the accumulated metal mass decreased with increasing thickness of the diffusive gel layer. Also, when the DGT device having the same thickness of diffusive gel layer was deployed for 45.3, 75, and 170 h, the accumulated mass increased with increasing deployment time, as expected from the theory.

Figure 4.3 presents plots of the accumulated mass of the metals with in the chelex resin as a function of the inverse of the diffusive gel thickness. An inverse relationship between the metal mass accumulated by the binding phase and the thickness of the diffusive layer was observed, which was in agreement with the predictions of equation 3.

Figure 4.2 Mass of zinc accumulated as a function of diffusion layer thickness. DGT-labile concentration of Zn in St. Lawrence River, ON. The thickness of the DGT diffusive gel layer 0.4 mm. ● = 45 h deployment; ▲ = 75 h deployment; ■ = 170 h deployment. DOC = 12.81 mg/L, pH = 8.5 ± 0.2. The error bars represent three times the standard deviation of five replicate measurements.

Figure 4.3 Mass of the metals accumulated as an inverse function of the diffusion layer thickness for the gel assemblies exposed to St. Lawrence River water, ON. ● = Cu, ■ = Pb, ▼ = Ni, ▲ = Zn, ◆ = Cd, and ★ = Co. DOC = 12.81 mg/L, pH = 8.5 ± 0.2 .

Additionally, equation 3 suggests that the slopes of the curves in Figure 4.3 can be directly related to the diffusion coefficients of the metal-DOC species. The experimental slopes are $\text{Cu(II)} > \text{Zn(II)} > \text{Ni(II)} > \text{Co(II)} > \text{Pb(II)} > \text{Cd(II)}$; this order suggests that Cu-DOC has highest mobility in the system while Cd-DOC has lowest mobility. The same order was also observed with the Laurentian Fulvic Acid in model solutions (not shown here). Interestingly, the observed slope changed with the deployment time periods. The reason for this change might probably be the fouling effect on the DGT device since there were no other changes in any of the experimental parameters. Figure 4.3 shows that the order of mobility of the studied metals for the duration of 45.3, 75, and 170 h is as follows $\text{Cu(II)} > \text{Zn(II)} > \text{Ni(II)} > \text{Co(II)} > \text{Pb(II)} > \text{Cd(II)}$ which was also the order of the mobility of the metal species noted earlier. Even though the slope of the curve of accumulated metal mass and the mobility changed with increasing deployment time, the above-mentioned order of mobility did not change.

4.4.4. Correlation with WHAM

The Windermere Humic Aqueous Model (WHAM) computer speciation code can be used to model and predict the aqueous trace metal speciation of a water system in chemical equilibrium [38]. The default database for metal binding to humic and fulvic acids for WHAM was obtained from a wide range of laboratory studies of metal ion binding. The model therefore proves a convenient way of comparing results from laboratory studies on model systems with the *in situ* measurements of natural water using DGT [39]. Interactions with inorganic ligands involve conventional equilibrium formulations and stability constants from the literature. The equilibrium speciation of

dissolved trace metals i.e., Ni, Cd, Cu, Zn, Pb in the St. Lawrence River was calculated using the WHAM VI [40,41]. Input data for major anions and cations of St. Lawrence River water samples were obtained for this work by their quantitative determination (Table 4.1). Speciation was computed using constant pH (8.47) and temperature (293 K), corresponding to the experimental conditions of the DGT deployment time in St. Lawrence River water.

Since the St. Lawrence River receives little anthropogenic organic matter from its catchments, it is reasonable to assume that humic substances (FS and HS) are the dominant general forms of Dissolved Organic Matter (DOM) that combine with trace metals in this system. Therefore, default- mode FA and HA, were used for the WHAM predictions of metal speciation. The experimental results by the DGT technique were compared with the computer-based predictions of WHAM VI (Figure 4.4). In Figure 4.4, WHAM VI values are only for free metal ion, whereas DGT values are for the DGT-labile metal species i.e. free metal ions plus other DGT-labile metal species. With the exception of copper, the agreement between the WHAM VI predictions and the DGT results was not good. The results of Pb and Cd were in poor agreement with the WHAM VI predictions.

A possible explanation of the above poor agreement was that the total binding capacity of DOM in the surface waters of St. Lawrence River was larger than the binding capacity used in the WHAM VI when 100% of the DOM was assumed to be fulvic acid [42].

Figure 4.4 Comparison of the WHAM-VI-predicted pM ($-\log_{10}[M^{2+}]$) and the measured concentrations in ($\mu\text{g/L}$) of DGT-labile species of Zn, Ni, Mn, Cu, Co, and Pb-complexes, measured *in situ* by DGT technique in St. Lawrence River, ON; on May 2006. (---) 1:1 line. Thickness of the diffusive gel layer was 0.4 mm; • Polymer 1.1; ▲ Chelex-100; DOC=12.81mg/L, pH= 8.5 $\pm$ 0.2

Another explanation was some of the properties of HS such as chemical heterogeneity and aggregation might not have been sufficiently accounted for in the default parameters of WHAM VI [42]. Any change in the properties of HS e.g., in its chemical heterogeneity would create a problem. Several studies reported in the literature tried to overcome this problem by manipulating the amount of HS using an empirical constant, 1.75 [42,43]; however, since each metal-DOC complexation was unique, and had many different variables, such empirical constant was not a good solution to the problem; also, this empirical constant affected all metals in same degree, and yet, it was well-known that each metal had unique interactions with DOM. Therefore, a better approach needs to be developed.

4.5. Conclusions

DGT has proved to be capable of determining DGT-labile species when deployed *in situ* in natural waters. The importance of different binding phases arises from the different functional groups that different binding phases are equipped with; these functional groups like iminodiacetic acid in Chelex bind metals from natural waters in competition with naturally-occurring ligands. Researchers should be careful about the selection of deployment time, diffusion layer thickness and location of deployment when the site is rich in HS, or located in a very slow stream. WHAM VI can be employed for comparison of the WHAM-predicted concentrations of the free metal ion with the experimentally-determined concentrations.

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--Chapter 5--

**Determination of free metal ion concentration in model
solutions of a well-characterized Laurentian Fulvic Acid by Ion
Exchange Technique (IET)**

5.1 Introduction

In natural systems, trace metals form complexes with a variety of inorganic and organic ligands, resulting in a heterogeneous distribution of complexes with a range of thermodynamic stabilities, kinetic labilities, and molecular sizes [1,2]. Because of complexity of natural systems, there is no single analytical method that can make quantitative determinations of all the species involved; each method can measure a certain proportion of the total complexes that lie within a given characteristic thermodynamic and kinetic window [1].

One of the goals of the metal speciation research is to determine free metal ion concentration of trace metals in natural freshwaters because of the crucial role of free metal ions in determining the reactivity, mobility, bioavailability, and toxicity [3,4] of trace metals. However, determination of metal speciation in natural waters is a challenging task because it requires very sensitive and selective analytical techniques for extremely low concentrations of metals. Several techniques have been employed to determine free metal ion concentrations; each technique has its own advantages and disadvantages. These advantages and disadvantages have been discussed somewhere else [1]. There is a need for analytical techniques capable of routinely measuring speciation parameters which are reliable predictors of bioavailability and bio-uptake and, thus, of potential ecotoxicological risks [5].

Cation exchange techniques have some unique advantages over other techniques that determine labile metal species or free metal ion, such as ion selective electrode potentiometry, indicator dye titration methods, voltammetry, and polarography, when used

for ill-defined samples that have a high content of organic matter. Cation exchange techniques can be applied to determine speciation of many different metals; they are suitable for use at very low metal concentrations (e.g., 10^{-8} - 10^{-9} M) [6].

Ion Exchange Technique, (IET), is a relatively new technique with an excellent potential [7] to overcome many of the problems involved in determining trace metals at low concentration levels in natural waters. It has been first employed by Schubert in 1948, and then only a few research workers have used this technique [8]. Cantwell et al. [9] applied IET to their work on the investigation of free nickel ion concentration in sewage effluent waters. They used a variety of methods for adjusting ionic strength. After that the method was further developed for semiautomatic use in investigating copper in its complexation with EDTA [10].

Free metal ion concentration of copper was determined by Treit et al. [10] using miniaturized and calibrated version of IET. Sweileh et al [9], tested, further improved and compared the results of the improved version of IET with those of ion-selective electrode (ISE) potentiometry for measurement of free $[\text{Cu}^{2+}]$ with the calculated values for several Cu-ligand systems (phthalate, glycine, citrate, chloride, salicylate and fulvic acid). The results were identical for both the ISE and the IET in the presence of citrate and fulvic acid. However, concentrations of Cu^{2+} obtained with the IET were significantly higher than that determined potentiometrically when other ligands were used; these observations suggest interferences from neutral and/or cationic species. In the presence of chloride, the IET values were in agreement with those obtained from equilibrium speciation calculations. However, when the concentration of Cu^{2+} in any aqueous sample is below the detection limit of Cu^{2+}

for the ISE technique, i.e., when the concentration of total copper present in the sample is less than the concentration required to establish chemical equilibrium with the copper ISE, valid determination of free copper ion concentration by ISE potentiometry is not possible.

IET was employed to determine free Cd^{2+} and Zn^{2+} concentrations in model solutions: with Ca^{2+} and Mg^{2+} as counter-ions and NTA as a ligand [7], in the presence of chloride and sulphate as matrixes [11]. There was good agreement between the measured and the calculated concentrations of free-metal ions in the NTA experiment; also, good selectivity was observed for Cd^{2+} in the presence of chloride and sulphate as matrixes. IET was applied to urine samples for determination of Ca^{2+} and Mg^{2+} by Ren and Kratochvil et al. [12]. They calibrated the IET method for varying concentrations of major cations (Na, K, Ca and Mg).

IET is a multielement technique which can be employed to determine more than one free metal ion concentration at a time [9,11]. One requirement is that the concentration of the metal ions studied must be very low. Trace metals in natural waters easily satisfy this requirement.

The objectives of this work are to determine free-metal-ion concentrations of trace metals by employing IET, to study the factors that affect the quantitative determination of free metal ion concentrations of Cu, Cd, Zn, and Pb in model solutions of a well-characterized Laurentian Fulvic Acid by IET, and to study the effects of: i) metal-to-ligand ratio, and ii) pH on free metal ion concentrations of the above trace metals.

5.2 Theory

Complexation reaction may occur in the presence of an ion-exchange resin (in sodium form) between cation exchange resin and free metal ions (M^{z+}) when sample solution passes through the ion-exchange resin. This reaction can be described by the equilibrium constant K_{IE}^c ,

where R= resin, RNa = resin binding sites occupied by Na^+ , R_zM = resin binding sites occupied by free metal ion M^{z+} , and z is the number of binding sites involved in binding metal M. K_{IE}^c = ion-exchange equilibrium constant, defined as:

$$K_{IE}^c = \frac{[R_zM][Na^+]^z}{[M^{z+}][RNa]^z} \quad 5.2$$

As mentioned earlier [9], if the univalent metal ion concentration is kept constant and large relative to the concentration of M^{+n} , then the portion of resin in the R_nM form will be small, the activity coefficients and (RNa) in equations 5.2, become essentially constant, and (M^{n+}) becomes proportional to (R_nM).

The reaction can be considered at equilibrium when the following equality is established:

($[M]_{in}=[M]_{out}$) [13]. At that point, a steady-state condition is reached for the resin column.

At a fixed ionic strength, pH, and a for known amount of the resin and the elution volume, the distribution coefficient $\lambda_{0,i,pH}$ (expressed in $L \cdot g^{-1}$) of metal M can be determined by rearranging equation 5.2 to 5.3:

$$\lambda_{0,i,pH} = K_{IE}^c \frac{[RNa]^z}{[Na^+]^z} = \frac{[R_2M]}{[M^{z+}]} \quad 5.3$$

The metal bound to the resin (R_2M) can be measured experimentally by eluting the resin with a certain volume (V) of a strong acid. R_2M can be calculated by knowing the quantity of resin used (m_r), and the concentration of the metal in the eluate:

$$[R_2M] = \frac{[M_{(Eluate)}] * V}{m_r} \quad 5.4$$

By combining and rearranging equations 5.3 and 5.4 one gets equation 5.5 which gives the relationship between the concentration of the metal bound to the resin and the free-metal ion concentration in solution:

$$[M^{z+}] = \frac{[M_{Eluate}] * V}{\lambda_{0,i,pH} * m_r} \quad 5.5$$

The following experimental conditions must be maintained for the determination of the free metal ion in solution [13]:

- a) the sample and the standard are swamped with the same “electrolyte” (e.g. $NaNO_3$) to achieve the same bulk ionic composition;

- b) trace conditions are met (i.e. the number of resin binding sites are much greater than the M^{n+} in sample, which occupies less than 1% of the resin sites);
- c) M^{n+} species are the only species of the sample metal that will bind to the resin.

The first condition can be bypassed when dealing with samples of sufficiently high ionic strength that allows the method to be calibrated for a varying matrix of salts [14]. By analyzing eluent for the counter-ion (e.g. $[Na] \gg [M]$), second condition can be controlled easily any time. The third condition is also satisfied in principle by the evidence in the literature on the selectivity of sulphonic acid group resins for the free metal ion in the presence of positively charged metal-chloride complexes [14-16]

5.3 Experimental Section

5.3.1 Chemicals and Reagents

Stock solutions (1000 mg/L) of, copper, zinc, cadmium, and lead were purchased from SCP Science (ICP grade). Working standard solutions were prepared daily by dilution of the stock solutions with ultrapure water acidified to contain 1% (v/v) with ultrapure nitric acid just prior the analysis. A stock solution of Laurentian Fulvic Acid (1.0000 g/L) was prepared by dissolving 1.0000 g of freeze-dried Laurentian Fulvic Acid (Fredriks Research Products, The Netherlands) in 1 L of ultrapure water. The concentration of carboxylic and phenolic groups in the Laurentian Fulvic Acid is 11.6 mmol/g [17,18]. All standards and test solutions were prepared with ultrapure water, which was obtained direct from a Nanopure Diamond water purification system (Barnstead), fitted with an organic purification column to remove organic matter. The pH of solutions was adjusted

with a pH buffer of acetic acid and sodium acetate. A 2 M sodium acetate stock solution was prepared in a Class 100 Clean Room (Microzone, Ottawa, Canada) environment 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. ESCE for at least 48 h immediate prior to its use. A 2 M stock solution of acetic acid was prepared by diluting glacial acetic acid (ACS grade/Anachemia) with ultrapure water.

5.3.2. Apparatus

The analytical grade cationic resin (sulphonic acid functional group) was purchased from Sigma (Dowex 50W-X8, 50-100 mesh). Ion Exchange experiments were done with a Biologic LP System (Bio-Rad); pH was measured with a Fisher Accumet 20 pH/conductivity meter (Fisher). Concentrations of dissolved Cu, Cd, Zn, and Pb were determined using an ELAN 6100 DRC ICP-MS (PerkinElmer SCIEX). All tubing and connections on the system were made of Teflon except for the section going through the pump head (Technicon green-green Tygon tube). 1.5 M nitric acid was used for the elution step; this manual step ensured that the volume added was known with high accuracy [15], this being crucial for the calculation of the free metal ion concentration (eq. 5.5).

5.3.3. Cleaning Procedures for Containers

All experimental preparation and experiment was made in a Class 100 Clean Room Facility. All containers used were made of high density polyethylene (HDPE), or tetrafluoroethylene (TFE). These containers and other equipment used were acid-washed (10% v/v HNO₃) for at

least 72 hours, and successively rinsed thoroughly seven times with ultrapure water (18.2 M Ω – cm) and dried under the Class 100 Clean Room laminar flow hood. All manipulations were performed under the Clean Room laminar flow hood, wearing powder-free gloves, to minimize any contamination.

5.3.4. Resin Preparation

The protocol developed by Fortin and G.C. Campbell. [15] was adapted to convert the resin from a H^+ form to a Na^+ form. About 5 g of the resin was immersed into pure water until it settled. The supernatant water was poured out, to remove dust and undesirable particles. This procedure was repeated 10 times successively. Finally, the resin was transferred into a Teflon container; the container was sealed and stored. Amounts of resin the Dowex 50W-X8 (between 7.6 and 7.8 mg) was precisely weighed and then transferred to a small plastic Teflon column.

5.3.5. Procedure

The IET protocol was used as previously described [15]. Briefly, pre-treated resin (7.6 and 7.8 mg) was added into a small plastic column with purified water. First of all, the resin was rinsed with high-purity water; NaOH was then passed through the resin to convert the resin to Na^+ form. After that, the electrolyte solution (100 ± 10 ml) of the desired matrix (i.e., desired ions and ionic strength) was added to equilibrate with the resin until the desired pH was reached {the pH (outlet) was similar to the pH (inlet)}; and the sample or calibration standard was passed through the column for processing. The amount of sample to reach a steady state with the resin varied with the metal under test;

however, it was not more than 100 ± 10 ml for any metal. After that the solutions inside resin and pipes were drained by air. Finally, the metal captured on the resin was eluted using exactly 2.4 ml of 1.5 M HNO_3 into the column. This acidified solution was collected in a 4 ml HDPE container (Nalgene, Canada), and air was forced into the top of the column to collect all the interstitial liquid trapped between the resin beads. The samples were stored in a refrigerator until analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

5.3.6. Calibration

The distribution coefficient, λ , was determined by using Eq. (5.5) to obtain the ratio of the metal-resin to free metal ion concentration. In these experiments, the resin was exposed to different amount of known metal concentrations in a synthetic matrix. Since the NO_3^- ion is significantly complexing at this high NO_3^- ion concentration, the free-metal-ion concentrations of the metals in the standard solutions were not the same as the total metal concentration. The free metal ion concentration can be calculated using the computer speciation code WHAM VI [16]. Since metal nitrate complexes have been studied extensively, their thermodynamic values [19] with metals are believed to be reliable. By using Eq. (5), value of distribution coefficient was calculated for the specific metal, pH and ionic strength. The method was calibrated for pH 4.8 and 8.0, and three different metal concentrations (1×10^{-7} , 1×10^{-8} and 5×10^{-7} M). The metal solutions were prepared by spiking each of four different metals (Cu^{2+} , Cd^{2+} , Zn^{2+} , and Pb^{2+}), individually and separately.

5.3.7. QA/QC

To achieve quality control, the analysis schedule included a blank for every fifth sample, and a Certified Reference Standard (NIST 1640) was run at midway and near the end of the run. If a difference of >10% from certified value was observed, the selected set of samples were re-analyzed. All samples were analyzed in quadruplicate and the mean was used in the data analysis. The relative standard deviation of replicate determinations was typically $\leq 5\%$.

5.4. Results and Discussion

5.4.1. Calibration of the Column IET

The IET method was initially calibrated with the KNO_3 aqueous matrix to obtain the distribution coefficient values which would then enable $[\text{M}^{2+}]$ to be calculated by using WHAM VI. Table 5.1 presents the value of distribution coefficients of metals at two different pH's. The values obtained for distribution coefficient was 0.059 ± 0.02 , 0.310 ± 0.002 , 0.110 ± 0.04 , and $0.205 \pm 0.05 \text{ L g}^{-1}$ for Cu, Cd, Zn, and Pb, respectively, at pH 4.8; and 0.154 ± 0.02 , 0.410 ± 0.004 , 0.283 ± 0.08 , and $0.321 \pm 0.05 \text{ L g}^{-1}$ for Cu, Cd, Zn, and Pb, respectively, at pH 8.0. The order of the distribution coefficient was: $\text{Cd} > \text{Pb} > \text{Zn} > \text{Cu}$, indicating the affinity of Cd to the resin was the highest while that of Cu the lowest of the four metals.

The results also indicated that Pb had higher affinity than Zn. These values were lower than the literature values [15,16]. Since distribution coefficient was strongly dependent on pH, ionic strength and composition of the sample [7], the reason for low values was probably some differences in the pH and composition of the sample.

Table 5.1 Average distribution coefficient values for [M(II)] in model solutions of Laurentian Fulvic Acid (LFA); buffered at pH 4.8 ± 1.0 and pH 8.0 ± 1.0 , ionic strength 0.01 M, temperature = 23 ± 2 °C.

<i>M(II)</i>	$\lambda_o^M (Lg^{1-})$ (pH=4.0)	$\lambda_o^M (Lg^{1-})$ (pH=8.0)
Cd	0.030	0.041
Pb	0.205	0.321
Cu	0.059	0.154
Zn	0.110	0.283

Fortin and Campbell [20] had suggested that distribution coefficient might be affected by the properties of the solution flowing through the resin column. Table 5.1 suggests that the pH of the calibration solution may have an effect on the distribution coefficient factor. As the pH of the calibration solutions was increased the distribution coefficient values for all four metals increased. The same kind of behavior was observed for metals by Fortin et al. [16]. Fotovat and Naidu et al. [21] held that the increase in distribution coefficient values were due to increasing pH.

5.4.2. *Effect of metal-to-ligand ratio on the determination of free metal ion concentrations in model solutions of LFA*

The LFA, which has been characterized for the concentration of carboxylate and phenolate binding sites [6], allows for systematic tests of the chemical significance of the ion exchange technique over a wide range of metal-to-ligand ratios using its model solutions. The metal-to-ligand ratios were varied by keeping the metal concentration constant (1×10^{-7} M), and varying the LFA concentration. For the sake of simplicity of calculating metal-to-ligand ratio, the LFA will be considered to have $\sim 1\%$ strong binding sites, and $\sim 99\%$ weak binding sites. If FA has $\sim 1\%$ strong binding sites, then the ratio of metal to FA for occupation only of the strong binding sites of the FA was ~ 0.01 . To be on the safe side, the experiments were started with a metal-to-ligand ratio of 0.0037. The metal-to-ligand ratio was varied from 0.0037 to 0.12.

Figures 5.1 show the effect of metal-to-ligand ratio on the free metal ion concentrations of Cu(II), Cd (II), Zn (II) and Pb (II) in model solution of the LFA. In Figure 5.1, the top point of the curve represents the lowest metal-to-ligand ratio of 0.0037, whereas the bottom point in the curve represents the highest metal-to-ligand ratio of 0.12. As shown in Figure 5.1, the Cu^{2+} free metal ion concentration decreases with the increasing LFA concentration. This result can be interpreted as follows. At the metal-to-ligand ratio of 0.0037, the amount of fulvic acid was approximately 300 times higher than that of the metal (10^{-7} M), and the LFA had enough strong binding sites to bind all the metal ions, resulting in the formation of strong metal-LFA complexes, which were non-labile and did not dissociate to produce free Cu^{2+} ion. With further increase in the metal-to-ligand ratio

Figure 5.1 Effect of $[M(II)]/[FA]$ ratio on the free metal ion concentration of a) Pb(II), b) Zn(II), c) Cu(II), d) Cd(II), in model systems containing varying concentration of the Laurentian Fulvic Acid, measured IET. The concentration of Pb(II) was kept constant for all samples $[Pb(II)] = 1 \times 10^{-7}$ M; (□), pH 4.8 ± 0.1 , (●), pH 8.0 ± 0.1 ; ionic strength 1×10^{-2} M.

to 0.092 all the strong sites (~1%) of the LFA were completely filled, leaving the remaining metals to bind to the weak binding sites (~ 99 %) of the LFA, resulting in the formation of weak metal-FA complexes, which dissociated to yield free Cu^{2+} ion. Similar trends were also observed for Cd, Zn and Pb.

The concentrations of free metal ions varied in a reasonable way with the metal-to-ligand ratio, suggesting that the IET gave chemically significant metal species. The progressive decrease in the free metal ion concentration with decreasing metal-to-FA ratio can be attributed to the heterogeneous complex-formation characteristics of LFA, resulting in [metal]/[ligand] ratio determining the strength of the metal-ligand complexes. In other words, the metal-binding strength of heterogeneous complexants such as LFA are determined by the degree of metal loading of the system, θ [22].

The progressive decrease in the free metal ion concentrations with increasing metal-to-LFA ratio arises from the chemical and physical heterogeneity [22] of the LFA. These results confirm the expectation that the LFA has sites of closely spaced binding energies. The general trend observed in this study, in which the results were obtained using an equilibrium approach is in agreement with the results obtained by Fasfous et al. [23] by using a kinetic approach, based on a competing ligand exchange method to determine the dissociation rate constants of Mn(II), Zn(II), Cd(II) and Pb(II) complexes in model solutions of the LFA and in freshwater samples from Grand River. The above results agree well with those of Town and Filella [24] for the speciation of Cu (II), Zn (II), Cd (II), and Pb (II) complexes collected from a large data set of published literature on natural waters. However, the different slopes observed for the Zn(II)-, Cd(II)-, and

Cu(II)-LFA systems shown in Figures 5.1 suggest that the intrinsic binding properties of the LFA may differ considerably from one metal to another. The ratio at c_M/c_{FA} 0.0037 probably approached the geochemical limit [25], where the site occupation of the LFA was so small that only the strongest binding sites contributed significantly to metal complexation.

5.4.3. *Effect of pH*

Table 5.2 and Figure 5.2 show comparison of the free metal ion concentrations, determined by IET at pH 4.8 and 8.0. As expected, a change in the stability of the M(II)-FA complexes was observed as the pH was changed from 4.8 to 8.0. This was due to the fact that more functional groups of the LFA were ionized at higher pH and became available as binding sites. Increasing pH also had an effect on the size and/or aggregation on the polymeric ligand molecules because of their polyelectrolyte properties. Moreover, pH generally increases binding affinity of a metal to a complexant because of dissociation of protons on the acidic functional groups. This will increase the negative charge on the resin and hence increase the attraction of metal ions to the resin, and also, lower proton competition for the binding sites. Increasing pH is predicted to increase ionization of the functional groups of LFA, and the resulting negative charges on fulvate ion repelled other negative charges on the LFA macromolecule, resulting in changes in the conformation of the polymer chain, which make previously inaccessible binding sites accessible for binding. As a result, the stability of the M(II)-FA complexes increased with increasing pH; hence, $[M^{2+}]$ decreased with increasing pH.

Table 5.2 Effect of the $[M(II)]/[LFA]$ ratios on the free metal ion concentration $[M^{2+}]$ in model solutions of the Laurentian

Fulvic Acid. $c_{M(II)} = 1.0 \times 10^{-7}$ mol/L. Buffered at pH 4.8 ± 0.1 and 8.0 ± 0.1 ionic strength 0.01 M, $T = 23 \pm 2$ °C.

		$[Pb^{2+}]$		$[Cu^{2+}]$		$[Cd^{2+}]$		$[Zn^{2+}]$	
	$c_{Pb,T}/c_{LFA}$	IET	WHAM VI	IET	WHAM VI	IET	WHAM VI	IET	WHAM VI
pH 4.8	1.2×10^{-2}	1.4×10^{-7}	9.3×10^{-8}	1.6×10^{-7}	8.7×10^{-8}	1.9×10^{-7}	9.9×10^{-8}	9.3×10^{-8}	9.9×10^{-8}
	1.2×10^{-3}	7.4×10^{-8}	5.1×10^{-8}	7.7×10^{-8}	2.8×10^{-8}	1.3×10^{-7}	9.1×10^{-8}	8.0×10^{-8}	9.4×10^{-8}
	9.2×10^{-3}	2.4×10^{-8}	4.2×10^{-8}	1.0×10^{-8}	1.9×10^{-8}	5.9×10^{-8}	8.8×10^{-8}	7.6×10^{-8}	9.2×10^{-8}
	3.7×10^{-4}	1.7×10^{-8}	1.8×10^{-8}	8.1×10^{-9}	3.4×10^{-9}	4.8×10^{-8}	7.4×10^{-8}	4.9×10^{-8}	8.1×10^{-8}
pH 8.0	1.2×10^{-2}	5.1×10^{-8}	9.1×10^{-9}	1.6×10^{-8}	9.1×10^{-9}	7.9×10^{-7}	7.9×10^{-8}	2.8×10^{-8}	7.1×10^{-8}
	1.2×10^{-3}	1.9×10^{-8}	6.2×10^{-12}	2.4×10^{-8}	1.1×10^{-11}	7.7×10^{-7}	1.5×10^{-8}	6.3×10^{-8}	1.7×10^{-8}
	9.2×10^{-3}	1.3×10^{-8}	3.2×10^{-12}	2.5×10^{-8}	5.6×10^{-12}	3.6×10^{-8}	1.0×10^{-8}	2.8×10^{-8}	1.2×10^{-8}
	3.7×10^{-4}	1.1×10^{-8}	5.0×10^{-13}	5.1×10^{-9}	4.4×10^{-13}	2.9×10^{-8}	2.4×10^{-8}	1.4×10^{-8}	3.1×10^{-9}

Figure 5.2 Comparison of WHAM-VI-predicted pM ($-\text{Log}_{10}[\text{M}^{2+}]$) with the measured pM by Ion-Exchange Technique in model solutions of $[\text{M}(\text{II})]/[\text{LFA}]$; a) $[\text{Cu}(\text{II})]$, b) $[\text{Cd}(\text{II})]$, c) $[\text{Pb}(\text{II})]$, d) $[\text{Zn}(\text{II})]$, in model solutions of LFA. $c_M = 1.0 \times 10^{-7}$ mol/L. Ionic strength 0.01 M , temperature $= 23 \pm 2 \text{ }^\circ\text{C}$, o : pH 4.8 ± 0.1 ; Δ : pH 8.0 ± 0.1

5.4.4. Comparison of the WHAM predictions with the experimental results

The Windermere Humic Aqueous Model (WHAM), a chemical equilibrium model and a computer speciation code for waters, sediments, and soils, incorporating a discrete site/electrostatic model of ion-binding by humic substances [26], was used to predict the effects of different amounts of LFA on the binding of Cu(II), Cd(II), Zn(II) and Pb(II). The WHAM takes into account the interactions of metals with: (i) inorganic ligands (OH^- , HCO_3^- , CO_3^{2-} , SO_4^{2-} , Cl^-), using conventional equilibrium formulations and equilibrium constants from the literature; and (ii) humic substances using Humic Ion Binding Model VI [27]. Table 5.2 presents a comparison of WHAM VI predictions and the IET's experimental results obtained using different c_M/c_{FA} ratios at pH 4.8.

Figure 5.2 present a comparison of the free-metal-ion concentration determined by IET and those predicted by WHAM VI at pH 4.8 and 8.0. There were good agreement between the experimentally determined Cu^{2+} and the WHAM-predicted values at pH 4.8. Contrary to the above agreement, at pH 8.0, the WHAM-predicted values for free Cu^{2+} were much lower than the experimental values. However, these differences between the predicted and the experimentally measured values of Cu^{2+} may be due to the deficiencies of the WHAM database, which contains mainly data from soil humic and fulvic acids and does not contain aquatic humic substances [28]. The same kind of behavior was also reported by Fortin and Campbell [20], and Bernard [28].

Weak agreement was observed for cadmium complexation with LFA between the WHAM VI predicted values and the experimentally determined values. Better agreement

was found with higher pH at 8.0. As shown in Table 5.2 and Figure 5.2, the measured and the predicted concentration of Zn^{2+} in model solutions were in good agreement with each other for both the pH values. Even though, the experiments result at pH 8, were slightly higher than the measured values, overall, they were in good agreement with each other. The Pb-LFA complexes showed the same trend as the Cu-LFA complexes reported earlier. At pH 4.8, there was good agreement between the value of the free Pb^{2+} ion determined by IET and the WHAM prediction for other c_M/c_{FA} ratios. At pH 8.0, the WHAM over-estimated the Cu(II)-LFA complexation.

Overall, there were two different pictures of interest which emerged for metal complexation with the LFA at pH 4.8. Generally, there was reasonable agreement observed between the IET values and the WHAM predictions for the free metal ion concentrations. When the pH was increased to 8.0, this good agreement remained unchanged for Cd(II) and Zn(II)-LFA complexation even though there was a slight change with the WHAM predictions. At the pH 8.0, WHAM VI overestimated the complexations between the metal and the LFA. Good agreement between the IET experimental results and the WHAM VI predictions at pH 4.8 might have resulted from less complexation between the metal and the LFA at pH 4.8, because of lower concentrations of the ionized LFA.

The results show that the pH has strong effect on the metal-LFA complexation; for the same metal-to-LFA ratio the metal-LFA complexation was almost 5 times lower at pH

4.8 than at pH 8.0. As summarized in Table 5.2, WHAM VI predictions were in reasonably good agreement with the experimental results obtained by IET determinations, considering that the WHAM calculations did not take into account the particular characteristics of the LFA and that the WHAM was based on equilibrium calculations and did not take into account non-equilibrium speciation.

5.5. Conclusion

The main advantage of Ion Exchange Technique is that IET provides free metal ion concentrations even at very low concentrations. Moreover, it takes matrix effect into consideration. The results of this work provide a good agreement between the experimentally determined free metal ion concentrations by IET and those predicted by WHAM VI at pH 4.8; however, the experimental value of free metal ion concentration by IET was one order of magnitude lower than the value predicted by WHAM VI at pH 8.0. This discrepancy suggests that more work needs to be done to make the WHAM VI applicable at higher pH (e.g., pH 8.0), and also to take into account any conformational changes in the LFA macromolecule consequent on its conformational changes due to the repulsion between the negative charges that develop on its surface at high pH.

5.6. References

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--Chapter 6--

Determination of copper, lead, zinc and cadmium in model solutions of a well-characterized fulvic acid by Pseudopolarography, using a Hanging Mercury Drop Electrode. Effect of the various wave forms: SWASV, LSASV, and DPASV on the pseudopolarograms

6.1. Introduction

Distribution of divalent metal species e.g., M^{2+} and their properties i.e. mobility, bioavailability, in natural aquatic systems partly depends on the degree, and nature of their association with naturally-occurring ligands, mostly humic substances, which are perhaps best described as refractory, dark-colored, chemically and physically heterogeneous organic compounds produced as byproducts of microbial metabolism [1]. Humic substances are formed in both terrestrial and aquatic systems. They can be dissolved in ground or surface water. The soluble humics can also be bound to the other soil/sediment components. The humic matter composition of ecosystems is mainly controlled by a net balance of formation, degradation, and transfer [1-5]. Because of the widespread presence of humics, humic matter plays an important role in the aquatic environment, especially in binding of metals in natural environments.

Electrochemical techniques have been extensively applied to the study of metal speciation in aquatic systems [6]. They have with the advantage that the mathematical description of the fate of metal often is largely similar for an electrode and a natural interface such as a biological membrane. Among electrochemical techniques, the various voltammetric stripping techniques are favoured because of their low detection limits, and the potential to provide detailed information about the dynamic nature of the system [6-10].

Stripping voltammetry is a non-destructive technique for studying trace metal speciation in natural waters. It has the ability to distinguish between different metal species (e.g.,

labile vs. non-labile complexes) [11], and to detect very low metal concentrations, which are encountered in freshwater systems[11,12]. Instrumentation is inexpensive, reliable, versatile, and easy to operate and maintain [11]. The most common form of stripping analysis used for trace metal analysis is Anodic Stripping Voltammetry (ASV) using a Hanging Mercury Drop Electrode (HMDE). ASV involves automatic preconcentration of the metal ion by cathodic reduction onto a working Hg electrode, forming a metal amalgam (reduced metal dissolved in Hg), followed by an anodic potential scan that “strips” the metal from the mercury electrode by oxidizing it back into the solution [11] (Figure 6.1).

ASV has been applied widely to the study of metal ion complexation by humic substances because of its (i) high analytical sensitivity [13]; (ii) ability to determine both thermodynamic and kinetic parameters; and (iii) capability to allow metal-to-ligand ratios to be easily varied over a relatively wide range. The disadvantages of ASV is that the ASV data interpretation is compounded by (i) adsorption of organic matter on the working electrode surface [14-18]; (ii) the fact that it is the metal-to-ligand ratio at the electrode surface during the stripping step, not that in the bulk solution, which determines the resulting stripping peak characteristics (peak potential and peak half-width) and, consequently, a sufficient excess of the ligand must be present [19,20]; and (iii) for heterogeneous complexants (e.g. humic substances) instead of discrete values for stability constants, kinetic and diffusion coefficient values , only distributions are available [21]. Hence, extraction of valid binding parameters directly from stripping experiments is extremely difficult.

Figure 6.1 Steps in an ASV measurement of a metal ion: electrolytic reduction of the metal ion into the Hg electrode forming an amalgam, followed by oxidation of the metal to metal ion [11].

Pseudopolarography has been developed by Filella et al. [22] and Town et al.[23] to overcome some of the above-mentioned problems, such as adsorption of Natural Organic Matter (NOM) in natural waters. Pseudopolarograms are constructed by recording a series of ASV stripping currents as a function of deposition potentials. For reversible, readily dissociating complexes, the half-wave potential for the pseudo-polarographic curve is shifted to more negative potentials relative to that for the free metal ion, analogous to classical dc-polarography. Hence stability constants for complexes can be calculated from pseudopolarographic data using methods developed for dc-polarography. Pseudopolarography is simple, easily to use, and it provides a direct way of extracting binding parameters from experimental data. In short, pseudopolarography is successive running of ASV as a function of deposition potentials. The different ASV modes of pseudopolarography differ only in the nature of the potential ramp applied during the stripping step. In Linear-Scan (LS) mode, the

potential is changed linearly with time; Square-Wave (SW) and Differential Pulse (DP) make use of the different time dependencies of the faradaic and charging currents to discriminate against non-faradaic current to enhance the analytical signal, and hence, analytical sensitivity [24]. The signals obtained with DP and SW are more susceptible to interference from species adsorbed on the working electrode surface than LS because the adsorbed species can make multiple contributions to the stripping signal [25,26]. The different ASV modes utilized differ only in the nature of the potential ramp applied during the stripping step.

The potential-time relationship of the applied potential-excitation-signal at the working electrode is called the waveform [27,28]. Common waveforms used include Linear Scan, Differential Pulse, and Square Wave, as illustrated in Figure 6.2 [28].

Figure 6.2 Voltage profile for Linear Scan-, Differential Pulse-, and Square Wave waveform.

Linear Scan Voltammetry is a simple linear voltage ramp and offers no correction for background capacitive (charging) current. The Differential Pulse waveform is able to correct for the background capacitive (charging) current. The Differential Pulse waveform consists of small pulses superimposed on a linear voltage ramp. The current is measured twice – once just before the voltage pulse is superimposed and again during the last 17 milliseconds prior to the end of the voltage pulse (i.e., during the brief period when the nonfaradaic current has mostly decayed but the faradaic current is still large). The first measurement gives background current i.e., the blank signal (due to impurities in the sample solution) plus capacitive current (charging current) for subtraction from the background plus the analytical signal obtained in the second measurement. The difference between these two currents, i.e., the background-corrected, or the net current is plotted vs. potential, yielding a symmetrical voltammetric peak. This essentially compensates for the charging current background, because charging current decays much more rapidly than faradaic current after each voltage step. Hence, the current difference between the measurements taken prior to, and at the end of each pulse, is due mainly only to the faradic current (analytical signal), since charging current is essentially zero at both these sampling points. This significantly enhances the analytical sensitivity of this waveform [11,27,28].

This study aims to investigate 1) the effect of the ASV wave-form on the extracted parameters; 2) the effect of the metal to ligand ratio on the shape of pseudopolarograms; 3) the effect of increasing ligand loading on the extracted parameters, 4) the effect of heterogeneity on the shape of pseudopolarograms; and 5) the ability of waveforms to display this change in the pseudopolarograms.

6.2. Theory

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 voltammetry) [29 - 31]. As described earlier, pseudopolarography is based on repetitive anodic stripping voltammetric current as a function of deposition potentials. 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 pseudopolarographic 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 [32,33]. Hence, stability constants for complexes can be calculated from pseudopolarographic data using methods developed for conventional dc-polarography. The advantages of pseudopolarography over conventional polarography are its increased analytical sensitivity and much lower limits of detection, which enable electrochemical investigations at environmentally relevant (extremely low) concentrations of metals in natural freshwaters.

The polarographic wave for a solution containing 'simple', homogeneous ligands is described by the DeFord and Hume equation [34,35] shown as equation 6.1:

$$E = E^{\circ} + \frac{RT}{nF} \ln \left[\frac{1}{\alpha} \left(\frac{D_R}{D_{ML}} \right)^{1/2} \right] + \frac{RT}{nF} \ln \left(\frac{1 - i/i_{lim}}{i/i_{lim}} \right) \quad (6.1)$$

where E° is the normal redox potential (V) of the redox couple, R is the Universal Gas Constant (8.314 J K⁻¹ mol⁻¹), T is the absolute temperature (K), n is the number of

electrons exchanged in the balanced, full-cell reaction, F is Faraday Constant (96,485 C mol⁻¹), α is the degree of complexation of the metal, D_R is the diffusion coefficient of reduced M and, D_{ML} is the diffusion coefficient of ML , i_{lim} is the diffusion-limited limiting current and i is the measured current.

Like all other heterogeneous complexing agents, Laurentian Fulvic Acid (LFA) has a continuous distribution of complexing sites which leads to the formation of metal complexes having closely-spaced stability constants, resulting in a spread of the polarographic wave. In this case, stronger sites become active in controlling the free metal ions concentration as M is progressively depleted while maintaining the concentration of binding sites constant. 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 [36], as shown in Figure 6.3. 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. An equation to describe the shape of a polarographic wave in the presence of heterogeneous complexants was derived by Filella et al. [36] (shown in equation 6.2):

$$E = E_0 + \frac{RT}{nF} \ln \left[\frac{1}{A^{1/\Gamma} c_L} \left(\frac{c_M}{c_L} \right)^{(1-\Gamma)/\Gamma} \left(\frac{D_R}{D_{ML}} \right)^{1/2} \right] + \frac{RT}{nF} \ln \left[\frac{(1-i/i_{lim})^{1/\Gamma}}{i/i_{lim}} \right] \quad (6.2)$$

In the above equation, A is Freundlich constant; c_M and c_L are the concentrations of M and L , respectively, in the bulk solution. Γ is called Heterogeneity Parameter that reflects

the degree of heterogeneity of the system and is independent of experimental conditions. All other terms have been defined previously. For $[M]_b \gg [M]$, the heterogeneity parameter, Γ , may be used as a means of expressing the complexation buffer intensity of the system [37].

$$\Gamma = \frac{1}{[M]_b} \bullet \frac{d[M]_b}{d \log[M]} = \frac{\beta}{[M]_b} \quad (6.3)$$

where $[M]_b$ and $[M]$, respectively, stand for the concentrations of the bound and the free metal ion in the bulk solution, and β is the complexation buffer intensity of the system (which is indicative of the natural water's capacity to buffer the effect of sudden addition of metals [38]). The meaning of Γ (equation 6.3) and its constancy make it a very important parameter for describing heterogeneous complexant properties, especially as regards to interpretation of the voltammetric signals.

The advantage of equation (6.2) is that the heterogeneous property of the complexant (i.e. Γ) is explicitly considered. Comparison of equations (6.1) and (6.2.) reveals that the Deford and Hume equation (6.1) is simply a special case of equation (6.2) for the condition of a homogeneous, simple complexant (i.e. $\Gamma = 1$).

6.2.1. Diffusion coefficients

The mean diffusion coefficient ($\bar{D}$) of the metal complexes in the water samples can be estimated using the following equation [39]:

$$i_L = \frac{nFA\bar{D}^{1/2}c_{M,T}\omega^{1/2}}{g} \quad (6.4)$$

where i_L is the diffusion-limited limiting current, n is the number of electrons involved in

the balanced reaction at the electrode surface, A is the electrode surface area exposed to the analyte, $\bar{D}$ is the weighted average diffusion coefficient, ω is the angular velocity of rotation (rad s^{-1}), $C_{M,T}$ is the total metal concentration in the bulk solution, g is a constant that depends on the cell geometry.

Figure 6.3 Simulated polarographic curves for the reduction of M a) under non-complexing conditions, b) in the presence of a simple, homogeneous ligand (Equation 6.1), i.e. $\Gamma = 1$, and c) in the presence of a heterogeneous complexant (equation 6.2), with $\Gamma = 0.3$. [22].

6.3. Experimental Section

6.3.1. Apparatus

A computer-controlled Autolab PGSTAT30 potentiostat/galvanostat (Eco Chemie BV, The Netherlands) equipped with a Metrohm 663 VA stand (Metrohm, Switzerland) was employed for voltammetric measurements. The working electrode was a static mercury

drop electrode (Metrohm, Switzerland, ACS reagent mercury, purity 99.9995%), the auxiliary electrode was glassy carbon, and the reference electrode was Ag | AgCl | KCl(sat.) electrode in a glass tube filled with 4 M KCl and fitted with a porous Vycor tip (Bioanalytical Systems, Inc., USA). 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 were transferred to a Pentium computer and saved for processing.

6.3.2. *Electrochemical Parameters*

Differential Pulse Anodic Stripping Voltammetry (DPASV), Square Wave Anodic Stripping Voltammetry (SWASV), and Linear Scan Anodic Stripping Voltammetry (LSASV) were separately applied. For all ASV wave forms, the following conditions were used: the sample was purged with pure nitrogen gas for 10 min. immediately prior to analysis; the electrode-stirring rate was set at 2000 rpm, deposition time 120 s; equilibration time 15 s, at the initial scan potential: -0.4 V for Cu(II), -0.8 V for Pb(II), -0.2 V for Cd(II), and -1.2 V for Zn(II); step potential 0.0025 V, modulation amplitude 0.025 V, modulation time 0.5 s, interval time 0.5 s, frequency 50 Hz. The deposition time was chosen to avoid surface saturation effects during the stripping step.

6.3.3. *Materials and Reagents*

All solutions were prepared in ultrapure water of resistivity 18.2 M Ω .cm, obtained direct from a Milli-Q-Plus water purification system (Millipore Corporation). Standard solutions (1000 μ g/mL) of Cu(II), Cd(II), Zn(II) and Pb(II) were purchased from SCP Science. KNO₃ solutions were prepared from solid KNO₃ (BDH, AnalaR). 2 M sodium

acetate for the stock buffer solution (pH 5) was prepared with acetic acid and sodium acetate, by dissolving an appropriate quantity of sodium acetate trihydrate (ACS grade/BDH) in ultrapure water, which was then purified of metals by electrolysis at -1.5 V vs. E_{SCE} for at least 48 h. immediately prior to analysis. In order to make sure that the impurity metals removed by electrolysis did not go back into the sodium acetate aqueous solution on the termination of electrolysis, the electrolysis was continued while the clear, supernatant aqueous solution above the mercury cathode was siphoned off. A 2 M stock solution of acetic acid was prepared by diluting glacial acetic acid (ACS grade/Anachemia) with ultrapure water.

The Laurention Fulvic Acid (LFA) was obtained from Professor C.H. Langford, University of Calgary, Alberta, Canada. The elemental composition determined on the freeze-dried LFA was: C, 45.1; H, 4.1; N, 1.1; O, 49.7%. The determination of the number of sites in the chemically heterogeneous ligand (LFA) was always difficult, and never accurate, because of the large variety of groups present. However, the LFA was a well-characterized fulvic acid that had been stoichiometrically defined for the total concentration of binding sites, 11.63 mmol/g [40,41]

6.3.4. Model Solutions of Laurention Fulvic Acid

A series of model solutions (30 mL volume) were prepared by adding known amounts of the LFA stock solution to a 1.0×10^{-7} M metal solution to make the $[M(II)]/[LFA]$ ratios 0.01, 0.001, 0.0001 and 0.0002, which were chosen to model a wide range of natural freshwater samples. The model solutions were buffered to pH 5 (the pH buffer was prepared using acetic acid and sodium acetate), and were left to equilibrate overnight.

After the equilibration, the pH of the model solution was again determined immediately prior to analysis. KNO_3 was used to fix the ionic strength of all test solutions at 0.05 M.

6.3.5. Cleaning procedure

All containers used were made of Teflon. After cleaning with ultrapure water, the containers were completely filled with ultrapure water which was made to contain 10% (v/v) nitric acid (AR grade) and allowed to stand at room temperature for one week. They were then rinsed five times successively with ultrapure water. Finally, they were filled with ultrapure water, and left to stand until they were used. The filling water was renewed periodically to ensure continued contact with clean water.

6.3.6. Effect of the waveform on the speciation parameters

The effect of waveform on speciation parameters of the LFA complexes of Cu(II), Cd(II), Zn(II) and Pb(II) (for all the four metals $1 \times 10^{-7} \text{M}$), with varying concentrations of LFA, at pH 5, was investigated using pseudopolarography. Experiments on metal-LFA complexation in model solutions were performed by adding various concentrations of LFA in ultrapure water containing constant concentration of the M(II) (10^{-7}M). Aliquots of the model solution were weighed (30.00 g) and used as the test solutions. The effect of the metal to LFA ratios of these metals was investigated using all the wave forms: DPASV, SWASV and LSASV. The test solutions were purged with oxygen-free nitrogen prior to analysis (and also following the addition of any spikes), and a positive nitrogen pressure (a nitrogen blanket) was maintained over the solutions throughout the duration of all measurements. All measurements were performed in triplicate for each test solution, and the reproducibility was verified by repeat analysis of fresh solutions. The experimental data were fitted to equation 6.2 using a commercial software program, NLREG v5.2 (Phillip H. Sherrod, Brentwood, TN, USA).

6.4. Results and discussion

Typical pseudopolarograms obtained experimentally for the Cu(II)-LFA complexes are presented in Figure 6.4. X-axis shows deposition potential; y- axis shows normalized peak height of pseudopolarograms. From top of Figure 6.4, the 2nd, 3rd, and 4th pseudopolarograms were for a model solution containing 1×10^{-7} M Cu(II) and LFA and buffered with NaOAc/HOAc (pH 5); the top pseudopolarogram was for the same concentration of Cu(II) without any LFA. The pseudopolarograms were found to be well-defined with a flat plateau, indicating a fully reversible electrode process. The decrease in the limiting current for the Cu(II)-LFA model solution relative to that for the Cu(II)-aqua complex, was due to the lower mean diffusion coefficient of the Cu(II)-LFA complex, relative to that of Cu(II)-aqua complex. A further decrease in the limiting current was observed with the higher amounts of LFA in the Cu(II)-LFA model solution (Figure 6.4, the lowest pseudopolarogram). The reason for this further decrease in the limiting current was probably that the Cu(II)-LFA complex became so large as to make its diffusion coefficient almost equal to the diffusion coefficient of the LFA molecule. The pseudopolarogram was shifted to more negative potential with increasing metal-to-LFA ratio and the pseudopolarogram was broadened and flattened.

This broadening and flattening of the pseudopolarogram observed with increasing concentration of LFA can be explained by formation of increasingly stronger metal-LFA complexes with decreasing metal/LFA ratio, starting from second curve from top and continuing as one traverses Figure 6.4 from top to bottom. For a heterogeneous complexant (LFA), the metal loading, θ ($\equiv$ [metal]/[LFA] ratio), determines the complex stability and lability. At very low metal loading, the strongest binding sites are first

occupied by the metal, forming strong complexes which are nonlabile. In other words, the LFA being a heterogeneous complexant, 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[42]. The overall complexation energy therefore increases at the electrode surface relative to the bulk solution. This effect increases as the deposition potential becomes more and more negative.

Figure 6.4 Typical pseudopolarograms for the M(II)-LFA system in model solutions of Laurentian Fulvic Acid. $c_{Cu} = 1.00 \times 10^{-7}$ mol/L, (●), Cu(II) +nil LFA; (▲), $c_M/c_{LFA} = 1.0 \times 10^{-3}$; (■), $c_M/c_{LFA} = 1.0 \times 10^{-4}$; (▼), $c_M/c_{LFA} = 2.0 \times 10^{-4}$. HOAc-NaOAc buffer, pH 5, Linear Scan Anodic Stripping Voltammetry, deposition time 180s, equilibration time 30s, stirring rate 2000 rpm

6.4.1. Baseline correction

All pseudopolarograms were corrected for the dilution error, and normalized in relation to the reference solution which contains the metal aqua complex without any complexing agents. Similar trend was observed for all the three wave forms and for the reference solutions (Figure 6.5), whereas natural baseline varied from metal to metal and from waveform to waveform (Figure 6.6). Adsorptive accumulation of M(II)-LFA complexes on the surface of the mercury drop during the deposition time interfered with some of the baselines, and the adsorbed layer was directly reduced on switching the potential to more negative values prior to the stripping step. This phenomenon of baseline shifting in the pseudopolarogram was common in the pseudopolarograms for the solutions containing LFA [23]. Town et al. [23] tried to overcome this baseline-shifting problem using various experimental means. However, they were not able to overcome this problem; finally, they concluded that whatever caused the baseline to shift for the LFA-containing solutions must be a reasonably long time-scale event. Therefore, reliable correction could not be made to the data, and this aspect must be considered in application of pseudopolarogram to systems containing absorbable species e.g., the LFA

6.4.2. *Effects of various waveforms*

The various waveforms: LSASV, DPASV, SWASV, differ only in the nature of the potential ramp applied during the stripping step. For the same deposition time and bulk solution conditions, the same metal species will be accumulated and the same metal-to-ligand ratio will be generated in all cases at the electrode surface during stripping. LSASV gives improved analytical sensitivity by avoiding most of the charging current by sampling the total current as late as possible after the application of each potential pulse.

However, there still is some charging current left in the analytical signal. Another limitation of LSASV is that it employs a linear potential-ramp and gives poor resolution between neighboring waves because of drawn-out, sigmoidal I-E curves. DPASV is a polarographic technique that uses a series of discrete potential steps rather than a linear potential ramp to obtain the experimental polarogram. Some of the experimental parameters for Differential Pulse Polarography are the same as with Normal Pulse Polarography.

Figure 6.5 Typical pseudopolarogram for Cd(II)-LFA in model solutions of Laurentian Fulvic Acid. $c_M = 1.00 \times 10^{-7}$ mol/L, +nil LFA (●), Linear Scan Anodic Stripping Voltammetry (▲); Differential Pulse Anodic Stripping Voltammetry; (▼) Square Wave Anodic Stripping Voltammetry; buffered at pH 5, deposition time 180s, equilibration time 30s, stirring rate 2000 rpm

Unlike LSASV, in DPASV each potential step has the same amplitude and the return potential after each pulse is slightly negative of the potential prior to the step. DPASV has better ability to discriminate against capacitive current because it measures a difference in the current. SWASV has several advantages, among which it's excellent analytical sensitivity and its rejection of the background current; another is the speed, which coupled with computer control and signal averaging, allows for experiments to be performed repetitively and rapidly, and increases the signal to-noise ratio.

The signals obtained with DPASV and SWASV are more susceptible to interference from species adsorbed on the working electrode surface than is LSASV because adsorbed species can make multiple contributions to the stripping signal [43]. There are extensive reports on the adsorption of humic substances on mercury surfaces and accumulation of metal ions in this adsorbed layer [44,45 and 23 (for all other references cited in Ref. 23)].

For solutions containing only the free metal ion, the shape of the pseudopolarogram was independent of the ASV mode (Figure 6.5). In the presence of LFA, SWASV and DPASV gave higher limiting currents than did LSASV for the four metals studied (Figure 6.5), and for all the metal/ligand ratios. For cadmium at low metal/ligand ratios, SW and DP provided almost the same limiting current, whereas at higher metal/ligand ratios provided higher limiting current than the others waveforms. For the same metal/ligand ratio, LS provided the lowest limiting current. Another observation from the Figure 6.4 and 6.5 is that waveform had an effect on the shape of the pseudopolarogram. This effect can be seen most clearly in LS than DP and SW waveforms. The same observations apply to the

pseudopolarograms for Zn(II)-LFA. Zinc is more prone to adsorption interference than the other three metals; additionally, its pseudopolarograms are more spread-out than those of Cd(II)-LFA complexes. For Pb(II)-LFA complexes, DP provided higher limiting current than SW, and without any doubt, much higher than LS. The pseudopolarograms of Pb(II)-LFA complexes for all metal/ligand ratios were much more spread-out than those of the other three metals studied. The same trend was observed for the Cu(II)-LFA complexes, but its pseudopolarograms were less spread-out.

The pseudopolarogram obtained using the waveforms, LSASV, DPASV and SWASV, as a function of deposition potential for the Pb(II)-, Cu(II)-, Zn(II)-, and Cd(II)-LFA complexes, are shown in Figure 6.6. No difference in the signal between the waveforms in the absence of LFA was observed (Figure 6.5.). However, in the presence of LFA, the peak signal was broadened and flattened most probably because of the heterogeneity of LFA: in the solution containing LFA, a distribution of metal-LFA complexes having different stability constants is formed as a function of metal-to-ligand ratio at the surface of the electrode. As shown in Figures 6.7, with increasing metal-to-ligand ratios, a dramatic decrease in the limiting current was observed. Although the change in peak height differed from waveform to waveform and from metal to metal, dramatic decrease in the peak height was observed for all the metals and for all the waveforms. As expected, with increasing concentration of LFA, the deposition potential was shifted to more negative values (Figure 6.7), but remained unchanged as the applied potential was varied and shifted to negative values with increasing metal to the LFA ratios. It was the metal-to-ligand ratio at the electrode surface during the stripping step that determined the peak potential and peak half-width values of the resulting stripping signal [46].

Figure 6.6 Typical pseudopolarogram for M(II)-LFA in model solutions of Laurentian Fulvic Acid (LFA). $c_M = 1.00 \times 10^{-7}$ mol/L, $c_M/c_{LFA} = 1.0 \times 10^{-2}$ (●); Linear Scan Anodic Stripping Voltammetry (▲); Differential Pulse Anodic Stripping Voltammetry; (▼) Square Wave Anodic Stripping Voltammetry. HOAc-NaOAc buffer, pH 5, deposition time 180s, equilibration time 30s, stirring rate 2000 rpm

This limitation can be overcome by employing pseudopolarography since stripping peak is the only information taken from individual measurements. Hence, any variation in the peak potential and peak half-width and the metal-to-ligand ratio during the stripping step makes no contribution to the pseudopolarographic curves. This is a major advantage of the pseudopolarography technique.

The effects of Cu(II) -, Pb(II) -, Zn(II) -, and Cd(II) -LFA ratios were investigated by using LSASV, DPASV, and SWASV waveforms. Pb(II) was the only metal, among the studied metals which could be investigated using all three waveforms: LSASV, DPASV, and SWASV. DPASV also was the only waveform among the waveforms which could be used to investigate the above four metals successfully using various metal-LFA ratios. SWASV failed to reveal the effect of Cu(II) - and the Zn(II)-LFA ratios, whereas LSASV failed to reveal the effect of Cu(II)- and Cd(II)-LFA ratios, especially at relatively high M(II)-LFA ratios (i.e., 1×10^{-4} and 2×10^{-4}).

These experimental results suggest that Cd(II)-LFA and Zn(II)-LFA are more similar to each other in their electrochemical behaviour than are Pb(II)-LFA and Cu(II)-LFA. The results also show the importance of waveform to the study of the above complexes. Furthermore, the result emphasizes the importance of reporting the combination of the metal and the waveform used for a particular metal when reporting limiting currents for the metal.

Figure 6.7 Typical pseudopolarogram for M(II)-LFA in model solutions of Laurentian Fulvic Acid. $c_M = 1.00 \times 10^{-7}$ mol/L; (●), M(II) + nil LFA; (▲), $c_M/c_{LFA} = 1.0 \times 10^{-2}$; (▼), $c_M/c_{LFA} = 1.0 \times 10^{-3}$; (■), $c_M/c_{LFA} = 1.0 \times 10^{-4}$; (◇), $c_M/c_{LFA} = 2.0 \times 10^{-4}$. HOAc-NaOAc buffer, pH 5, Differential Pulse Anodic Stripping Voltammetry, deposition time 180s, equilibration time 30s, stirring rate 2000 rpm

6.4.3. Investigation of the effect of waveform and M(II)-LFA ratio on the electrochemical behaviour of M(II)-LFA complexes

It is the metal-to-ligand ratio at the surface of the electrode during stripping which determines the peak potential E_p and peak half-width $w_{1/2}$ values of the resulting stripping signal [52]. Therefore, this factor must be carefully considered when complexation parameters are extracted directly from individual stripping measurements. This limitation was eliminated by employing pseudopolarography because the magnitude of the ASV signal (stripping peak area or height) was the only information taken from each individual measurement. Hence, any variation in peak potential E_p or peak half-width $w_{1/2}$ due to variations in the metal-to-ligand ratio during stripping makes no contribution to the pseudopolarographic analysis. Other parameters (e.g. $E_{1/2}$) are extracted from the resulting plots of peak area (or height) as a function of the applied potential. This is a major advantage of the pseudopolarographic approach. The stripping step is extremely important for pseudopolarographic analysis since all needed data are obtained from this step. Also, special attention should be paid to the waveform to be used in pseudopolarographic analysis.

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--Chapter 7--

**Effect of Chemical Heterogeneity of Humic Substances (HS) on
the Lability of HS complexes of Pb(II), Zn(II), Cd(II), and
Cu(II)**

7.1 Introduction

Complexation of metal ions by humic substances (HS), including both humic and fulvic acids, is an important topic in environmental studies because heterogeneity of humic substances as organic complexants profoundly impacts on the thermodynamic stability and kinetic lability of the metal-HS complexes in the aquatic environment. [1-4]. HS originates from complex processes in which degradation and excretion products of living organisms produce disperse mixtures of natural organic polyelectrolytes that possess a great number of different functional groups which are responsible for their chemical and physical heterogeneity.

Humic substances are chemically heterogeneous ligands which are polydisperse and ubiquitous in environmental and biological media where they play a crucial role in buffering free metal ion concentrations and thus in the maintenance of life [5]. One of the characteristic features of HS is heterogeneity, both physical and chemical [6], which means that the ratio of metal/binding site concentration determines the thermodynamic stability of metal-HS complexes. Moreover its lability because the determining factor in metal-HS complexation is the dehydration of the metal-aqua ion, which results in a proportionality of the complexation stability constant of a metal ion with the dissociation rate constant of the metal complex. The thermodynamic stability of metal-HS complex is determined by metal/HS ratio and pH [5]. HS have a large number of binding sites, hence a variety of stability constants. Overall complex stability is no longer a singular characteristic parameter, but rather is given by some distribution that characterizes the stability as a function of the degree of binding site occupation, θ (called metal loading parameter). Because the lability of metal-HS complexes is also described by a

distribution, the diffusion coefficients of the metal complex species are characterized by a distribution. Unambiguous and quantitative characterization of heterogeneity parameters would facilitate prediction of metal ion binding by HS ligands and thus improve our understanding of metal-HS complexations. The heterogeneous properties of HS play a key role in metal speciation. Heterogeneity here refers to both physical and chemical properties. Heterogeneity of HS is due to the following properties: polyfunctionality: each macromolecule possesses many complexing sites of different functionalities and, further, in a particular group of complexants (e.g., fulvics) the constituent molecules are similar but not identical. Polyelectrolyte nature: since most complexing sites have acid-base properties, and each complexant molecule has a large number of them, all molecules bear a large number of electric charges, the total charge depending on the pH and the degree of site occupation, θ , by metals. Conformational properties: the electric charge and degree of hydration of these macromolecules and colloids affect the conformation, which in turn may affect the stability of metal complexes for steric reasons.

There is a great difference between heterogeneous complexants (HS) and the classical "simple ligands". Some of the important features of heterogeneous complexants are the following [5]: (i) owing to the large number of site types, the complexant properties vis-à-vis a particular metal ion are not describable by a single complexation equilibrium constant, but must be described by an equilibrium complexation distribution function, which depends on the metal/complexing site ratio, i.e., metal-to-HS ratio [7,8]; (ii) similarly, the dissociation kinetics of metal ions from complexing sites, and the resulting so-called degree of lability of complexes, must be also described by a kinetic distribution function which depends on the metal-to-HS ratio; (iii) as the metal ion is bound to

macromolecules or colloids, the mobility of metal complexes is lower than that of the free metal ions because of the lower diffusion coefficient of heterogeneous complexants; this conflicts with the assumption often made in the case of complexes with "simple ligands", where the diffusion coefficients of the free metal ion and of the complex are considered to be equal [9]; (iv) because of the macromolecular structure of heterogeneous complexants, they may contain hydrophobic parts more often than the small "simple ligands" [10].

Recently, the subject of trace metals–HS interactions has received a great deal of attention in environmental and ecotoxicological sciences. Trace metal speciation in natural waters is complicated because complexing agents i.e., humic substances involved are often of a polyfunctional and/or polyelectrolytic nature, i.e., they are heterogeneous complexants so that their affinity to metal ions is a function of the actual metal-to-ligand ratio [11] and pH. Because of heterogeneity of the system, the apparent stability of the overall complex is then no longer a singular characteristic parameter, but must be given by some distribution which characterizes stability as a function of the degree of occupation of the sites, θ , under the given conditions (12). The effects of chemical heterogeneity of a metal complex system on the shape of the polarographic wave have been addressed in a earlier chapter.

The effects of chemical heterogeneity of humic substances on the lability of metal complexes have been studied recently by researchers from this laboratory [21]. By investigating the theoretical background, it was found that for infinite distributions of

complex stabilities, lability is always lost at some stage of the polarographic wave and, in principle; all limiting currents are to some extent kinetically controlled. The lability criteria were first developed by van Leeuwen [13] and were further investigated by Pinherio et al. [12] for chemically heterogeneous systems.

In this study, the effect of [metal]/[LFA] ratio on the lability of complexes of the chemically heterogeneous complexant LFA of the following metals Cd(II), Cu(II), Zn(II), and Pb(II), has been investigated.

7.2 Theory

The following theory is adapted from J.P.Pinherio et al. [12].

Complexation equilibrium of a metal ion M with a polyfunctional set of ligands (LFA), L, where k_a , and k_d are the association and dissociation rate constants, respectively, is described by the equilibrium constant, K, where $K=k_a/k_d$. The metal ion can be reduced to the metal M^0 on the mercury electrode:

Charges are omitted for the sake of simplicity.

Heterogeneity enters the set of conditions via the equilibrium relation between the c_M , c_L , and c_{ML} . One can express this in several ways. An obvious possibility is to use the average stability parameter, $\bar{K}$, defined as:

$$\bar{K} = \frac{\sum_i K_i c_{L_i}}{\sum_i c_{L_i}} \quad (7.2)$$

For simplicity, $c_{ML} = \sum_i c_{MLi}$ and $c_L = \sum_i c_{Li}$

Freundlich isotherm may also be used to describe the heterogeneity for natural systems, in the presence of a large excess of ligand in the system [14],

$$\theta = c_{ML} / c_L = A(c_M)^\Gamma \quad (7.3)$$

Where θ is the degree of metal loading, A, is a constant [for homogeneous systems, $A=K=c_{ML}/(c_M, c_L)$], Γ is the heterogeneity parameter, (introduced by Filella et al.[14]; for homogeneous systems, $\Gamma=1$, and for natural waters Γ varies from 0.3 to 0.6), and c_L , is the total ligand concentration.

The general lability criterion for a chemically heterogeneous system can be formulated as follows [12]:

$$\frac{\bar{k}_d^*}{(k_a c_L^*)^{1/2}} \varepsilon^{-3/2} \chi^{(1-\Gamma)/\Gamma} x \frac{[\chi^{(1-\Gamma)/\Gamma} + \varepsilon \bar{K}^* c_L^*]}{(\bar{K}^* c_L^*)^{1/2} [\chi^{(1-\Gamma)/\Gamma} + \bar{K}^* c_L^*]^{1/2}} t^{1/2} \gg 1 \quad (7.4)$$

This criterion is usually presented as: $\Lambda d t^{1/2} \gg 1$

The above criteria can be used to evaluate the lability of a complex if $\bar{K}^*$ (or $\bar{k}_d^*$), and

$\Gamma, \chi, c_{M,i}, D_M, D_{ML}$ values are known.

7.3 Experimental Section

Details of the experimental section have been given in Chapter 6.

7.4 Results and Discussion

It was observed in the experiments on pseudopolarography (Chapter 6) that the half-wave potential is shifted to more negative values in the presence of the ligand, LFA. This provides a first indication that the systems studied are labile; however, the system may become quasi-labile or inert with increasing ligand loading. This study aims to address this problem by studying the effect of effective timescale of the technique, the stirring rate and the metal to LFA ratio in Anodic Stripping Voltammetry (ASV). However, the range of the variation of the timescale of the techniques is rather small, which makes it difficult to obtain relevant results. This makes it more important to evaluate the systems studied using lability criteria as presented in Eq. (7.4).

The pseudopolarogram shifts to increasingly negative applied potentials with increasing ligand concentrations. Figures 6.6 and 6.7 provide a first indication about the studied systems whether they are inert, labile or quasi-labile. The reason for this shift is probably the metal concentration at the electrode surface. As the total metal concentration is decreased at the electrode surface, stronger sites become free and therefore active in controlling the free metal ion concentration. Hence, the overall complexation energy is increased at the electrode surface compared to the bulk solution. Evidently, during the recording of the wave, this effect increases as the potential becomes more and more negative and the total M concentration is more and more depleted at the electrode

surface. As a result, reduction of the remaining complexes requires more and more energy, and more negative potential is needed. Everything behaves as if, for each potential value, a new value of the constant in Equation 6.2 should be used; this value becomes increasingly larger as the potential becomes more negative.

Figure 7.1 clearly illustrates the effect of lability on the pseudopolarogram of Zn(II)-LFA complex. As the potential becomes more negative, the concentration of Zn(II) decreases at the electrode surface and the remaining complexes become increasingly more stable (K^* increases). This is reflected in the spreading of the pseudopolarographic wave compared to that of the fully labile case. However, in addition, the dissociation rate of the remaining complexes decrease (Fig. 7.1) since k_d is inversely proportional to K^* .

The studied metals: Cd(II), Cu(II), Zn(II), and Pb(II), with the c_M/c_{LFA} ratio ranging from 0.01 to 0.0002 makes it more important to evaluate the systems studied using lability criteria as presented in Eqs. 7.4. Figures 7.1 presents the effect of lability on pseudopolarograms; it can be seen that the M(II)-LFA complexes appear to be relatively labile under these experimental conditions, and they move in the direction of inert complexes with increasing ligand (LFA) loading at constant c_M values. The effect of metal loading and heterogeneity of the LFA on the lability needs to be investigated by the parameters obtained from pseudopolarograms such as Γ (called heterogeneity parameter) K , k_d (can be obtained from K). Heterogeneity parameter, Γ , is related to the complexation buffer intensity of the system and to the range of $\log K^*$ in which the heterogeneous complexant is effective. The smaller is Γ the larger is this range. This is

the reason why the spreading increases over the X axis (deposition potential) when heterogeneity Γ decreases.

There are two crucial elements for quantification of lability, heterogeneity, Γ , and metal-to-LFA ratio, χ . Lability criteria are affected greatly by the $\chi^{(1-\Gamma)/\Gamma}$. Let us assume that χ is 0.02 and two systems have heterogeneity parameter Γ 0.2 and 0.8. For relatively heterogeneous system Γ , 0.8, $\chi^{(1-\Gamma)/\Gamma}$ comes to $(0.02) (1-0.8) / 0.8 = 0.38$. For very heterogeneous systems with Γ , 0.2, lability works out as $(0.02) (1-0.2) / 0.2 = 1.6 \times 10^{-7}$, showing that an increasing degree of heterogeneity lowers the term $\chi^{(1-\Gamma)/\Gamma}$, thereby decreasing the lability dramatically. This shows clearly that the heterogeneity parameter, Γ , counts heavily in the lability features of chemically heterogeneous systems.

7.4.1 Effect of the ligand concentration

Increase in the ligand concentration causes the system to be less labile (quasi-labile) or inert, in both homogeneous and heterogeneous systems (12). This is so because c_L affects the thickness of the reaction layer, which decreases with increasing c_L concentration. Specifically in heterogeneous systems, when the value of χ decreases, as a result, the thickness of the reaction layer decreases; hence, the average local c becomes smaller. Moreover, when the concentration of the ligand is increased, stronger binding groups become more available for the metal; consequently, K^* increases and k^* decreases and this causes the system to be less labile, or inert.

Figure 7.1 Typical pseudopolarogram for Zn(II)-LFA in model solutions of the LFA.

$c_{\text{Zn}} = 1.00 \times 10^{-7}$ mol/L; ($\bullet$), Zn(II) +nil LFA; ($\blacktriangle$), $c_{\text{Zn}}/c_{\text{LFA}} = 1.0 \times 10^{-2}$; ($\blacktriangledown$), $c_{\text{Zn}}/c_{\text{LFA}} = 1.0 \times 10^{-3}$; ($\blacksquare$), $c_{\text{Zn}}/c_{\text{LFA}} = 1.0 \times 10^{-4}$; HOAc-NaOAc buffer, pH 5, Linear Scan Anodic Stripping Voltammetry, deposition time 180s, equilibration time 30s, stirring rate 2000 rpm

As mentioned before, the differences between the lability criteria for a homogeneous and heterogeneous system is governed by the heterogeneity parameter, Γ . The first key element is the estimation of $\chi = c_{\text{ML}}/c_{\text{ML}^*}$ since it directly affects $\chi^{(1-\Gamma)/\Gamma}$, heterogeneity, and hence, lability. It is important to note that if the system is heterogeneous then K increases near the electrode. Under limiting current conditions the ratio $c_{\text{LFA}}/c_{\text{M}}$ increases, tending to the value of $c_{\text{M,t}}/c_{\text{LFA}}$, while $c_{\text{M,t}}/c_{\text{LFA,t}}$ approaches zero [12]. Thus the average value of $c_{\text{M,t}}$ in the reaction layer can be used as an acceptable approximation of the value of c_{LFA} in χ .

Consequent to the above discussion, results are presented in Tables 7.1 - 7.4. It can be seen that the lability criterion is just fulfilled, confirming that for these experimental conditions, the M/LFA system is labile, or in some instances quasi-labile or inert. The values of $\log K^*$ for which the heterogeneous lability criterion would become unity are 6.0 to 6.5, implying that for ratios higher than these ratios, the system will be mostly inert. It should be noted that for describing a chemically heterogeneous system we need not only the value of K^* but also that of the ratio c_M/c_{LFA} .

Table 7.1 also shows the effect of increasing LFA concentration on the heterogeneity and lability of lead. Pb(II) behaves as homogeneous (i.e., $\Gamma=0.92$) in solutions containing low c_{Pb}/c_{LFA} ratios, and as the heterogeneity of Pb(II) increases to 0.34., the log stability constants of Pb(II)-LFA complexes increase from 6.1 to 9.5. As a result, the limiting current decreases dramatically (Table 7.1). Finally, the calculated lability decreases enormously from 9.8 to 1.1×10^{-12} . Another interesting point is that with increasing ligand concentrations, the heterogeneity of metal and, therefore, lability decreases.

Table 7.2 presents the effect of heterogeneity on Cd(II)-LFA complexes in the model solutions containing LFA. Heterogeneity of Cd(II)-LFA complexes is between that of the relatively homogeneous and the relatively heterogeneous systems (0.73 to 0.38).

Table 7.1 Effect of heterogeneity parameter (Γ) on the lability of lead (II) - LFA complexes

$c_{\text{Pb}}/c_{\text{LFA}}$	Γ	$\text{Log } K^*$	i_{lim} (normalized)	Lability
1.0×10^{-2}	0.92	6.1	0.95	9.8
1.0×10^{-3}	0.84	7.3	0.77	0.1
1.0×10^{-4}	0.63	8.3	0.38	2.9×10^{-5}
2.1×10^{-4}	0.34	9.5	0.19	1.1×10^{-12}

Table 7.2 Effect of heterogeneity parameter (Γ) on the lability of cadmium (II) -LFA complexes

$c_{\text{Cd}}/c_{\text{LFA}}$	Γ	$\text{Log } K^*$	i_{lim} (normalized)	Lability
1.0×10^{-2}	0.73	6.0	0.89	3.9
1.0×10^{-3}	0.69	6.2	0.78	0.4
1.0×10^{-4}	0.45	6.7	0.59	2.30×10^{-5}
2.1×10^{-4}	0.38	7.7	0.39	2.16×10^{-8}

The effect of heterogeneity is seen in the stability constants ($\text{Log } K^*$) and the normalized limiting currents (i_{lim}) of Cd(II)-LFA complexes changing from: 6.0 to 7.7, and 0.89 to 0.39, respectively. Lability of cadmium decreases from, 3.9 to 2.2×10^{-8} ; therefore, it can be stated that heterogeneity and lability of Cd(II)-LFA complexes are related to each other.

The effect of heterogeneity on the lability of Zn(II)-LFA complexes is presented in Table 7.3. Zn(II)-LFA complex behaves as an extreme example of both labile and inert species: Zn(II)-LFA complex is the most labile (Lability 14.0), and the most inert species (1.0×10^{-24}). The stability constant ($\text{Log } K^*$) of Zn(II)-LFA complex increases from 5.9 to 9.7, while heterogeneity parameter (Γ) decreases from 0.93 to 0.15.

Table 7.3 Effect of heterogeneity parameter (Γ) on the lability of zinc (II)-LFA complexes

$c_{\text{Zn}}/c_{\text{LFA}}$	Γ	$\text{Log } K^*$	i_{lim} (normalized)	Lability
1.0×10^{-2}	0.93	5.9	0.98	14.0
1.0×10^{-3}	0.84	6.5	0.88	0.8
1.0×10^{-4}	0.65	8.0	0.75	2.5×10^{-4}
2.1×10^{-4}	0.15	9.7	0.39	1.0×10^{-24}

Even at high metal loading, θ , i.e., high c_M/c_{LFA} ratios, Cu(II)-LFA complexes show quasi-labile to inert behaviour (Table 7.4). The stability constants and heterogeneity parameters show two clear trends: at relatively high metal loadings ($\theta=1.0 \times 10^{-2}$ and 1.0×10^{-3}), Cu(II)-LFA is quasi-labile (0.8 and 0.02), and its stability constant is enhanced ($\text{Log } K^*=7.3$ and 8.4, respectively); however, at low metal loadings ($\theta=1.0 \times 10^{-4}$ and 2.1×10^{-4}) drastic increase in the stability constants ($\text{Log } K^*=12.2$ and 11.5) and a decrease in the heterogeneity parameter (Γ) from 0.24 to 0.19 are observed.

Table 7.4 Effect of heterogeneity parameter (Γ) on the lability of Cu (II) - LFA complexes

c_{Cu}/c_{LFA}	Γ	$\text{Log } K^*$	i_{lim} (normalized)	Lability
1.0×10^{-2}	0.76	7.3	0.97	0.8
1.0×10^{-3}	0.74	8.4	0.81	0.021
1.0×10^{-4}	0.24	12.2	0.21	2.9×10^{-13}
2.1×10^{-4}	0.19	11.5	0.15	1.5×10^{-20}

When the spreading of dc-polarographic waves on the voltage axis is due only to the formation of labile heterogeneous complexants, it has been reported that Γ can be determined from the rising portion of the polarographic wave, even if the limiting current is not measurable [15]. This method has been successfully applied in this work to Pb(II)-LFA, Zn(II)-LFA, Cd(II)-LFA, and Cu(II)-LFA complexes. Although the experimental

results seemed to indicate that the pseudopolarograms tend to a partially kinetic limiting current, calculation of Γ values was attempted in the parts of the pseudopolarograms where complexes had a strong labile character. Results are shown for Pb(II)-LFA, Zn(II)-LFA, Cd(II)-LFA, and Cu(II)-LFA in Tables 7.1, 7.2, 7.3, and 7.4, respectively. These Tables include the values calculated from the pseudopolarograms plotted using the normalized peak height as a function of the deposition potential.

As suggested in earlier studies [15,16], the Γ values vary for each metal and for each metal to LFA ratio. However; the general trend observed for the metal heterogeneity is $\Gamma_{Zn} \geq \Gamma_{Cd} > \Gamma_{Pb} > \Gamma_{Cu}$; i.e., Zn(II)-LFA system is the most homogeneous, whereas Cu(II)-LFA is the most heterogeneous system. Cd(II)-LFA system also is a relatively homogeneous system, more than the Pb(II)-LFA and Cu-LFA systems, but less than the Zn(II)-LFA system. This is also seen in the degree of broadening of the ASV stripping peaks. Other studies have noted different degrees of heterogeneity for binding of various metal ions by humic substances [17-21]. However, the very flat shape of the Cu(II) pseudopolarograms (Figure 6.18) made fitting of these data extremely difficult. The Γ values obtained do not follow any consistent trend in the ASV mode, peak height versus peak area, or in concentration of the LFA (Table 4). The form of these curves suggests that there is a significant loss of lability in this system. This precludes collection of more definitive information i.e., the calculation of any binding parameter for Cu(II)-LFA.

Overall, all the studied metals showed two clear trends, which may be explained by the following considerations: heterogeneous complexants such as the LFA can be considered

consist of both major and minor sites types. Major site types of a complexant system are those present in large proportions, perhaps accounting up to 90 % of the total sites present, which are consequently few in number (22, 23). Major sites include the carboxylate and phenolate groups of humic substances. Minor sites comprise a small fraction of the total number of sites but a large number of site types, with a broad range of associated complexation constants (spanning several orders of magnitudes of $\log K$). Although minor as regards the mole fraction of total sites, the minor-site group may often be the most important as far as control of trace metals in aquatic systems is concerned because their K values are higher than those of the major sites (24). Examples of minor sites include the N and S complexing functional groups of fulvic/humic acids, the -SH groups of proteins.

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--Chapter 8--

**An evaluation of CLEM/ICP-MS Technique, using different
chelating resins in model solutions of a well-characterized
Laurentian Fulvic Acid (LFA)**

8.1. Introduction

Trace metals in natural waters are complexed with inorganic ligands: hydroxide, sulphate and fluoride, and with organic ligands, Natural Organic Matter (NOM). NOM plays an important role in trace metal speciation, transport, bioavailability, and hence, in ecotoxicity in the aquatic environment by regulating trace metal complexation and free metal ion concentration (1-3). NOM is ubiquitous in the aquatic and terrestrial environment. The NOM found in the aquatic environment possesses a large variety of properties, and is composed of an extremely complex mixture of compounds. NOM may have terrestrial or aquatic origin, and may degrade or condense in water column. Their composition varies spatially and temporally, and differences in their elemental composition and spectroscopic properties have been well-documented (4). Humic substances, such as fulvic acid (FA) and humic acid (HA), represent a major fraction of Dissolved Organic Carbon (DOC) present in freshwaters.

Humic substances (HS) in natural waters are formed by anionic heterogeneous ligands, having a large number of aromatic rings with many carboxylic and phenolic groups. The composition of HS depends on their provenance. HS are polydisperse systems with polyfunctional, polyelectrostatic and conformational properties because of the presence of different functional groups, to a high charge density and to stereochemistry (2). Each complexing site with specific chemical functional group and its surroundings acts differently in the complexation of the metal. The polyelectrolytic effects depend on the structure of HS, the pH and the macro constituents present in the solution. Owing to the

heterogeneity of HS, a continuous variation of the complexing parameters should be assumed in metal complexation studies of HS (5).

Chemical reactions and geochemical cycles of trace metals control their complexation, dissociation and biological uptake in natural waters (6). Even though great efforts have been made by earlier workers on investigation of metal complexation in the aquatic environment, yet it is very difficult to analyze systems for metal speciation and their reaction mechanisms. Complexations reactions of potentially toxic trace metals by naturally-occurring organic complexants such as humic substances are increasingly being recognized as important factors in many natural systems because these reactions determine, to a large extent, the metal speciation and bioavailability of the metal species(7). Complexation by humic substances has been of great interest to environmental studies, as interactions of HS with potentially toxic trace metals determines to a large extent their bioavailability, ecotoxicity and mobility of these trace metals in natural waters.

Chemical reactions (e.g., oxidation-reduction, complexation, or precipitation) which occur in the aquatic environment have often been modeled by assuming chemical equilibrium or steady-state conditions. Contrary to the usual chemical equilibrium assumption, complexation reactions with humic substances in natural waters, which are complex milieu of physically and chemically heterogeneous ligands, and are dynamic systems, often far removed from chemical equilibrium (11). Therefore, chemical equilibrium or steady-state conditions may not apply to natural waters (12). Additionally,

dissociation of the many trace metal complexes in natural waters are slow, and hence, chemical equilibrium conditions may not prevail.

Kinetic studies of metal speciation not only can differentiate chemical species according to their kinetic parameters, but also can give information on the distribution of the chemical species in the system at any time during the kinetic process (13). The kinetic approach has been most commonly used to study the dissociation kinetics for metal complexes of heterogeneous, organic complexants. van Leeuwen (14) and Jansen et al. (15), have reported the importance of considering dynamic processes for estimating metal bioavailability to aquatic organisms. Kinetic approaches for chemical speciation may more correctly reflect the realities of freshwater system, in which metal release is often kinetically controlled.

This study aims to investigate the effect of a chelating reagent and its mass on the kinetics of trace metal complexes of Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II) with a well-characterized Laurentian Fulvic Acid in model solutions. This study is expected to throw light on some of the complexities of natural freshwaters as dynamic systems.

8.2. Theory

8.2.1. The Kinetic Model

Competing Ligand Exchange Method (4,16)

Let us assume n represents the number of components in freshwater sample, in which each component, ML_i , exists in equilibrium with its dissociation products: the free metal

ion (i.e., metal aqua complex), M, and a naturally-occurring, heterogeneous complexant, L_i , such as humic substances which are ubiquitous in the aquatic environment (charges have been omitted for simplicity). Assume that both the rates of the reactions and the experimental conditions as specified below are present. The different binding sites on the naturally-occurring heterogeneous complexant are represented by subscript, i , .

where the formation and dissociation rate constants, $k_{f,i}$ and $k_{d,i}$, are coupled by the stability constant, $K = k_f/k_d$, through the principle of microreversibility (17). 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 (8.1) to the right.

Since the added competing ligand is in large excess, reaction (8.2) is pseudo-first-order.

Since $K_{M-\text{Chelex}} \gg K_{ML}$, M will be bound irreversibly to the Chelex and the net reaction represented by reaction (8.3) will lie far to the right.

If $k_{d,\text{Chelex}} \gg k_d$, then reaction (8.1) is the rate-determining step and the change in the concentration of ML_i can be described by a first-order rate law.

$$-\frac{dc_{ML_i}}{dt} = k_{d,i}c_{ML_i} \quad (8.4)$$

Integrating,

$$c_{ML_i}(t) = c_{ML_i}^{\circ} \cdot \exp(-k_{d,i} \cdot t) \quad (8.5)$$

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 (at a rate that depends on the nature of the functional group, its position on the macromolecule, and the residual charge), the total concentration of all complexes, c_{ML} , at any time, t , is given by a summation of exponentials (equation 8.6).

$$c_{\text{ML}}(t) = \sum_{i=1}^n c_{\text{ML},i}^{\circ} \cdot \exp(-k_{d,i} \cdot t) \quad (8.6)$$

In the case where the reactions can be described by equations (8.1) and (8.2), the rate coefficients measured for each component will be the dissociation rate coefficients of the corresponding complexing site.

8.3. Experimental Section

8.3.1. Materials and Reagents

Stock solutions (1000 mg/L) of Mn, Co, Ni, Cu, Zn, Cd, and Pb were purchased from SCP Science, Montreal, Canada. Ultrapure water of resistivity 18.2 MΩ-cm was obtained direct from a Milli-Q Plus water purification system (Millipore Corporation). Analytical grade (minimum 99% pure) Chelex 100 resin, sodium form, Bio-Rad), Dowex 50 WX 8, and Dowex Mac-3 (Bio-Rad) were prepared according to method of the Morel et al. (18). A stock solution (1.0000 g/L) of Laurentian Fulvic Acid (LFA) was prepared by dissolving 1.0000 g of freeze-dried LFA (Fedriks Research Products, The Netherlands) in ultrapure water. The determination of the number of sites in a chemically heterogeneous ligand is always difficult, and never accurate, because of the large variety of functional

groups present. However, the LFA is well-characterized fulvic acid that has been stoichiometrically defined for the total concentration of binding sites, 11.63 mmol/g [19,20]. All standards and test solutions were prepared with ultra pure water, which was obtained direct from a Milli-Q Plus water purification system (Millipore Corporation), fitted with an organic purification column to remove organic matter.

8.3.2. *Containers and their Cleaning Procedure*

All containers used were made of Teflon. After cleaning with ultrapure water, they were completely filled with 10 % nitric acid (AR grade) and allowed to stand at the room temperature for one week. Then, they were rinsed five times successively with ultra pure water (of resistivity 18.2 M Ω -cm), and then filled with ultrapure water and allowed to stand in the dark until they were used; the filling water was renewed periodically to ensure continued contact with clean water.

8.3.3. *Model Solutions*

Total volume of the model solutions for each metal was 300 mL, which was prepared in ultrapure water to contain various amount of Laurentian Fulvic Acid and the following metals: Mn, Co, Ni, Cu, Zn, Cd, and Pb; the concentration of each metal was 1.0×10^{-7} mol/L.

Kinetic experiments were performed in three parts as follows. Part 1) Dissociation kinetics of the metals complexes was studied using Chelex-100 as the competing resin. Part 2) Dissociation kinetics of the metals complexes was studied using three different

competing resins (Chelex 100 resin, Dowex 50 WX 8, and Dowex Mac-3) to investigate the effect of the competing ligands on the dissociation kinetics; and Part3) to study the effect of various amounts (0.5, 1, 1.5, 3 %) of Chelex-100 on the metal complexation reactions.

8.3.4. Kinetic experiments and data analysis

The dissociation kinetics of Mn, Co, Ni, Cu, Zn, Cd, and Pb complexes when undergoing simultaneous dissociation was studied using each of the above-mentioned three resins individually and separately, and an ELAN 6100 DRC ICP-MS (PerkinElmer SCIEX).

Part 1: Three grams (1% w/v) of Chelex 100 and a model solution containing separately every one of the above metals. Part 2: Three grams (1% w/v) of Chelex 100 resin, Dowex 50 WX 8, and Dowex Mac-3 were added separately to each of the model solution for all the above metals. Part 3: 0.5, 1.5, 3 and 4.5 grams (0.33, 0.5, 1 and 1.5 % w/v) of Chelex 100 were added separately to each of the model solution.

Certain amount of each competing resin along with the sample solution (300 mL) was taken in a cylindrical Teflon Reactor (500 mL volume) and the sample solution was stirred continuously with a Teflon-coated stirring bar. The data acquisition was initiated 15 s before the competing resin was added to the Teflon Reactor so that any contamination of the resin from its pre-treatment would be indicated by a sudden increase in the ICP-MS signal. In the case of any contamination, the experiment was terminated, and a fresh sample solution was studied. The sample solution was filtered with an online

0.40 μm polycarbonate membrane filter (Corning) to separate the competing resin from the filtrate before introducing the filtrate into the plasma torch of the ELAN ICP-MS through the solution nebulizer using a peristaltic pump at a flow rate of 1 mL/min. The time interval between the data points and the total time of the data acquisition was set for each experiment based on the dissociation kinetics of the metal complexes—a short time interval and short total data acquisition time for rapid kinetics, and a longer time interval and longer total data acquisition time for slow kinetics.

8.3.5. *QA/QC*

Quality control for metal analyses included repeated injections and periodic (two in every two-hour operation of the equipment) analysis of Certified Reference Standard, NIST 1643d. A prior set of samples was re-run if the analyzed value differed from the Reference Standard's certified value by $> 10\%$. The relative standard deviation among replicate determinations was typically $\leq 5\%$.

8.3.6. *Kinetic data analysis*

The experimental data were analyzed both for a distribution of rate constants and discrete values of the rate constants. Distributions of dissociation rate constants were obtained by fitting the kinetic data to equation (8.4); the overall reaction was treated as a pseudo-first-order and the dissociation rate coefficients and their associated kinetically distinguishable components (percentage compositions of the metal) were calculated by fitting the experimental data to a two-component exponential growth model by non-linear regression analysis using the Marquardt-Levenberg algorithm. The calculations were performed using SIGMAPLOT 10 computer program (SPSS Science). For the Kinetic

Model, the first condition which must be satisfied is that the regressions converge rapidly, and there should be a reasonable fit (as measured by correlation coefficient, r^2) with the kinetic data.

8.4. Results and Discussion

8.4.1 *Effect of the LFA ratios on the kinetic lability of the metal-LFA complexes*

Figure 8.1 shows the effect of [metal]/[LFA] ratios on the percentage composition of the complexes of Pb(II), Cu(II), Cd(II), and Zn(II) bound to the LFA in the model solutions as a function of time. The [metal]/[LFA] ratios were varied by keeping the metal concentration constant (1×10^{-7} M), and varying the LFA concentration. In Figure 8.1, the [metal]/[LFA] ratios were varied from 0.0001 to 0.01 (from top to bottom curve). For the sake of simplicity in calculating [metal]/[LFA] ratio, the LFA will be considered to have ~1- 10% strong binding sites, and ~ 90-99 % weak binding sites. If LFA has ~ 1- 10% strong binding sites, then the ratio of metal to LFA for occupation only of the strong binding sites of the LFA was ~ 0.01. To be on the safe side, the experiments were started with a [metal]/[LFA] ratio of 0.001.

In Figure 8.1, the top curves represent the [metal]/[LFA] ratio of 0.0001 for Pb(II)-, Cd(II)-, Cu(II)- and Zn(II)-LFA complexes. Regardless of the actual values of the [metal]/[LFA] ratios, the important factor to consider was that all these [metal]/[LFA] ratios were very small, resulting in formation of strong metal-LFA complexes which were not dissociated and represented very large percentages of ML remaining undissociated as a function of time. This case precisely as was expected since LFA was a heterogeneous complexant. At these [metal]/[LFA] ratios, the rates of dissociation of the

Figure 8.1 Effect of the increasing ligand concentration on the dissociation kinetics of metal complexes in model solutions of Laurentian Fulvic Acid, measured by ICP-MS. $c_M = 1 \times 10^{-7}$ M, pH 5.0 ± 0.1 , ionic strength ~ 0.056 M, and temperature 23 ± 2 °C. ($\bullet$), $c_M = 1 \times 10^{-7}$ M+ nil LFA; ($\circ$), $c_M / c_{LFA} = 0.01$; ($\blacktriangle$), $c_M / c_{LFA} = 0.001$; ($\triangle$), $c_M / c_{LFA} = 0.0001$.

metal-LFA complexes were very slow and hardly detectable over the entire period of the measurement since the concentration of metals was very low relative to the available number of strong binding sites of the LFA. When the [metal]/[LFA] ratios were increased by one order of magnitude to 0.001, the rate of dissociation increased initially, but became slow again at longer times. When the [metal]/[LFA] ratio was further increased by one order magnitude to 0.01, a rapid initial increase in the rate of dissociation with the increase levelling off to a slower rate of dissociation at longer times was observed. These results can be interpreted as follows. At the [metal]/[LFA] ratio of 0.0001, the metal concentration (1×10^{-7} M) was much lower than that of the LFA, and the LFA had enough strong binding sites which were all occupied by the metal ions, forming strong metal complexes which were inert ($k_d \leq 10^{-5} \text{ s}^{-1}$).

The strong binding sites on the LFA formed strong metal-LFA complexes which were non-labile, as expected. With further increase in the [metal]/[LFA] ratio to 0.001, and still further to 0.01, all the strong sites ($\sim 1\text{-}10\%$) were completely filled, leaving the remaining metal to bind to the weak binding sites ($\sim 90\text{-}99\%$) of the LFA, resulting in the formation of weak metal-LFA complexes, which were labile.

The experimental results presented in Figures 8.1 were fitted to the Kinetic Model (Eq.8.6), and analyzed using a non-linear regression method to obtain the numerical values of the kinetically distinguishable components and their associated dissociation rate coefficients. At the [metal]/[LFA] ratio of 0.0001, only one dissociation rate coefficient was obtained, whereas two kinetically distinguishable components with their associated rates of dissociation were obtained for the [M]/[LFA] ratios of 0.001 and 0.01.

The curves in Figure 8.1 are explained below: first, in general terms which are applicable to all the curves, secondly, in specific terms, applicable to specific curves. The interplay of three factors was the cause of stability of ML complexes, where M and L stand for metal and ligand (LFA), respectively. The three factors were: 1) The intrinsic strength of metal-LFA covalent bond. 2) The rate constant for water substitution in the inner coordination sphere of the metal ion, k_w . 3) Because LFA is a physically and chemically heterogeneous complexant like other humic substances (HS), the metal-LFA complex stability and lability are determined by a variable parameter called metal loading, θ , given by the ratio of [metal]/[concentration of the binding site denoted HS] i.e., by the ratio of $[M]/[HS]$. Of the above three factors, the rate constant for dehydration of metal ion k_w is often the controlling factor, being the slow, rate-determining step in the formation of the metal complex, ML. The greater the value of k_w , the more labile would be the metal complex, as is evidenced by the increasing trend of the ML dissociation: $Pb(II) > Cu(II) > Cd(II) > Zn(II)$. To a first approximation, the LFA complexes of $Pb(II)$, $Cu(II)$, $Cd(II)$, and $Zn(II)$ followed the trend expected on the basis of their water loss rate constant, k_w , of the metal ion. Increasing values of k_w confer greater lability on their metal-LFA complexes, as evidenced by the slope of the metal-LFA complex dissociation curves. Comparison of the curves of the four metals in Figure 8.1 shows that at the end of the set period of the complex dissociation, i.e., at 7000s, the ML complexes remaining undissociated followed the order of the stability of the ML complexes (the greater the affinity of the LFA for the metal ion the stronger and the more stable was the complex). The order of their rate constant for the dehydration of the metal ion k_w is: $Pb^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+}$. As a result, at the above point in time (at 7000 s), some of the

Pb(II)-LFA and Cu(II)-LFA complexes were still not dissociated, whereas Cd(II)-LFA and Zn(II)-LFA were almost completely dissociated; however, there are some variations among the curves probably based on the $[M]/[LFA]$ ratio.

As would be expected from the effect of $[metal]/[LFA]$ ratio, the fraction of the free plus other labile species and their associated dissociation rate coefficients increased with increasing $[metal]/[LFA]$ ratios and this observation applied to all the metals studied. The results are in agreement with the theory that as the $[metal]/[LFA]$ ratio is increased (by keeping the metal concentration constant and varying the LFA concentration), the strong binding sites of the LFA become saturated and the excess metal then binds to the weaker sites of the LFA, forming weak complexes which are labile, releasing free metal ions. Free metal ions are widely regarded as bioavailable and toxic.

The kinetic lability of the metal species varies in a reasonable way with the $[metal]/[LFA]$ ratio, suggesting that the kinetic speciation model gives chemically significant metal species. The change in the dissociation rate coefficient measured for each component of the metal-LFA complexes provides experimental evidence of the polyfunctionality of the LFA. The results show how kinetic data can detect the progressive occupation of strong and weak binding sites of a well-characterized LFA and the existence of strong and weak binding sites in the LFA.

The analysis showed that minimum of two components was required to describe the dissociation kinetics of the metal-LFA complexes at the $[metal]/[LFA]$ ratios of 0.0001 and 0.01 for all the studied metals. Again this is consistent with the presence of two

distinct classes of binding sites in the LFA. The existence of strong binding sites of humic substances, which was for the first time reported by Sunda and Hanson [21], using an initial metal concentration of 1×10^{-8} M. Earlier workers had failed to detect the existence of the strong site probably because of the higher concentration of metal (1×10^{-7} M) used in their titration experiments [17,21] and lower concentrations used by Sunda and Hanson [21].

At the [metal]/[LFA] ratio 0.0001 (the data not shown), only one rate coefficient was estimated about $k_d \sim 10^{-5} \text{ s}^{-1}$, for all the studied metals. With further increase in the ratio of [metal]/[LFA] by one order of magnitude from 0.0001 to 0.001, the second-faster kinetically distinguishable component appeared for all the metals. With additional increase in the ratio of [metal]/[LFA] by one order of magnitude from 0.001 to 0.01, while the percentage of the faster component increased, the percentage of the slower component decreased. Moreover, changes in the dissociation rate coefficients of the faster and the slower kinetically distinguishable components were also observed. All these observations are indicative of the dissociation rate coefficients of the metal-LFA complexes decreasing with the increasing [metal]/[LFA] ratio.

The observed change in the dissociation rate coefficients measured for each component of metal-LFA complexes presents experimental evidence of polyfunctionality of the LFA. Although carboxyl groups are reported to play a predominant role in the binding of metal ions, mixed complexes [22], and complexes with ligands belonging to two LFA molecules [23] probably also formed. Also, the nature of the complexing site may change

with varying metal-to-ligand ratio. When metal ions are added to the constant concentration of the LFA, metal-LFA complexes having different dissociation rate coefficients are formed. Slow dissociation rates may result from a chemical mechanism operating at the heterogeneous binding sites of LFA, and from secondary effects, such as slow diffusion of the metal ion into or out of the water-swollen cavernous gel colloidal macromolecules of the LFA. The results also show how kinetic data can detect progressive occupation of strong and weak binding sites of a well-characterized LFA, and the existence of strong and weak binding sites in the LFA.

8.4.2. Effect of different binding resins on the metal-LFA complexes

Dissociation curves of metal-LFA complexes allow interpretation of kinetic properties of metal-DOC complexes and their dissociation. Fitting of minimum two parameters are required for reasonable identification and interpretation of the system. There is no question that the type and amount of the employed binding resin and its properties is one of the key parameters. Chelex-100 is the most widely employed binding resin; however, the published literature lacks even very basic information on the other binding resins used in this work: Dowex-MAC3, Dowex -50XW8.

The most widely used group of binding resins for preconcentration of metals is chelating organic ion-exchange resins. Here, functional groups capable of forming chelates with metal ions are introduced into the binding resin. These functional groups which form chelate rings usually contain oxygen, nitrogen, or sulphur groups. Oxygen is usually present as a phenolic, carboxylic, carbonyl, hydroxyl, ether or phosphoryl group. Nitrogen can exist as a primary, secondary or tertiary amine, amide, azo, diazo, nitro,

nitroso, or nitrile group. Sulphur can occur as the thiocarbamate, thiol, thioether, disulphide or –SH group. Chelex-100 has proved to be most popular among the chelating resins, and it contains the iminodiacetic acid functional group, which is so powerful in its attraction for metal ions that it selectively binds metal ions in competition with the naturally-occurring other complexants in natural waters, resulting in special selectivity for multivalent ions. Dowex Mac-3, also competes with naturally-occurring ligands in natural waters and binds metal ions, and is a cation type complexing agent, and has polyacrylic macroporous matrix and contains carboxylic acid functional groups. The pH range for Dowex Mac-3 is 5-14. All these three binding resins have good chemical and physical stability.

Humic substances (HS) play an important role in the binding of trace metals in the aquatic environment. They have high complexation affinity toward metal cations, especially those in higher oxidation states ($Z \geq +2$). Various exchange resins have been used for chemical speciation and determination of metal humate complexation since they have comparable reactivity for trace metals. The external surface of the binding resin beds contributes little to the total surface area. In kinetic experiments, humic acid (HA) and the Ion Exchange Resin (IER) compete for the added metal ions as described below.

For this work, $[M]$ was kept constant (1×10^{-7} M) while the amount of the complexing agent (e.g. LFA) was successively increased. Dowex-50XW8, Chelex-100, and Dowex MAC-3 were employed as an ion exchange resin. All three resins were treated in same

way in order to obtain Na form of the binding resins. The detailed procedure, as given in Morel et al. [18], was followed.

Figure 8.2 presents the effect of three Ion Exchange Resins on M-IER, studied separately in model solutions containing the LFA. For all the studied metals excepting Cu, Chelex-100 and Dowex 50-WX8 behaved in same manner while Dowex MAC-3 had weaker affinity than the other two binding resins for the studied metals. The Cu-Chelex had the weakest affinity than the other two binding resins, and Dowex MAC-3 and Dowex 50-WX8 competed in the reaction at the some point even though they behaved differently along the way.

The plots in Figure 8.2 were obtained using all three binding resins separately, and the [metal]/[LFA] ratio 0.01. The behaviour of the studied metals with the binding resins, each studied separately, are as follows: cadmium, cobalt and zinc did not change their behaviour form resin to resin. Although lead and nickel followed the same trend in all three resins, Dowex-50WX8 resin showed its strong chelating properties. There was a clear difference between the effect of three binding resins, and the observed trend was: Dowex-50WX > Chelex-100 > Dowex MAC-3. Further increase in the ratio [M]/[LFA] to 0.001 and 0.0001 did not change the above trend. Cadmium, cobalt and zinc kept their same behaviour, whereas the other metals (lead and nickel) changed their behaviour from resin to resin. The above trend did not change with the change in the [M]/[LFA] ratio from 0.001 to 0.0001.

Figure 8.2 The effect of various chelating agents on the dissociation kinetics of metal complexes in model solutions, measured by ICP-MS. $c_M = 1 \times 10^{-7}$ M, pH 5.0 ± 0.1 , ionic strength ~ 0.056 M, temperature 23 ± 2 °C. $c_M / c_{LFA} = 0.01$. (●), Chelex-100; (○), Dowex 50WX 8; (▲), Dowex Mac-3

Overall, the results indicated that the competing resin type and the $[M]/[LFA]$ ratio had no significant effect on the following studied metals: cadmium, zinc and cobalt. However, the competing resin type had strong effect on lead, and nickel, depending on the ratio of $[M]/[LFA]$. The $[M]/[LFA]$ ratio and the competing resin type showed their greatest effect on Cu(II). Also, the results showed that the c_M/c_{LFA} ratios affected the dissociation of metal-LFA complex of the studied metals.

The results presented in Table 8.1 and 8.2 show that it is important to present all experiment parameters in publication of the results. The behaviour of the metals described above can be explained as follows. The $[M]/[LFA]$ ratio determines the labile fraction of the metal-LFA complexes. Increasing amounts of the LFA which provided lower $[M]/[LFA]$ ratios, resulted in the formation of strong complexes which were non-labile, making the dissociation of those metal-LFA complexes more and more difficult. Good agreement was observed between the results obtained by Dowex 50WX8 and Chelex-100. Complete dissociation of M-LFA was observed with Chelex-100 and Dowex 50WX8, while only around 30 % of M-LFA dissociation was observed with Dowex MAC 3.

8.4.3. *Effect of the amount of the binding resin on the metal(II)-LFA complexes*

The investigation of the effect of the concentrations of the exchange resin was performed for four Chelex concentrations (0.5, 1, 1.5, 3, and 4.5 g/L) with the ratio of metal to LFA 1×10^{-3} , using two hours of experimental time. Metal concentration was 1×10^{-7} M. Figure 8.3 shows that chelex concentrations had an effect on the studied metal-LFA dissociation rate. An increase in the Chelex concentration enhanced the dissociation rate.

Table 8.1 Kinetically distinguishable labile components of M(II) complexes in model solutions using Chelex 100 as the competing ligand and Inductively-Coupled Plasma - Mass Spectrometry (ICP-MS). $c_{M(II),T} / c_{LFA} = 1.0 \times 10^{-3}$. pH 5.0 ± 0.1 , T = 23°C. Ionic Strength ~ 0.056 M.

Metal	Amount of Chelex-100, g	<u>Kinetically distinguishable components</u>		<u>Dissociation rate coefficients</u>		R ²
		C ₁ (%)	C ₂ (%)	k ₁ (10 ⁻³ s ⁻¹)	k ₂ (10 ⁻⁴ s ⁻¹)	
Cd	0.5	7±1	93±0	0.1±0.1	3.0±0.0	0.999
Cd	1.5	33±6	67±6	0.4±0.0	7.5±0.2	0.999
Cd	3	93±1	7±1	0.8±0.0	2.0±0.2	0.999
Cd	4.5	99±1	1±0	2.1±0.0	0.5±0.2	0.997
Cu	0.5	7±1	93±0	1.1±0.1	0.3±0.0	0.962
Cu	1.5	3±1	97±0	25.6±13.1	0.2±0.0	0.938
Cu	3	10±0	90±1	3.5±0.3	0.6±0.0	0.959
Cu	4.5	14±0	86±1	3.0±0.1	0.4±0.0	0.984
Ni	0.5	7±1	93±1	1.1±0.1	0.5±0.0	0.986
Ni	1.5	8±1	92±0	2.2±0.6	0.7±0.0	0.992
Ni	3	14±0	86±1	1.6±0.1	1.2±0.0	0.997
Ni	4.5	22±1	78±0	1.8±0.0	1.4±0.0	0.996
Pb	0.5	2±1	98±0	9.5±5.9	0.5±0.0	0.992
Pb	1.5	1±1	99±0	-----	0.7±0.0	0.996
Pb	3	4±0	96±2	21.8±11.4	0.9±0.0	0.980
Pb	4.5	10±1	90±1	0.9±0.0	1.4±0.0	0.998
Zn	0.5	49±1	51±0	1.6±0.1	1.5±0.1	0.999
Zn	1.5	32±0	68±1	2.3±0.0	3.4±0.0	0.999
Zn	3	28±2	72±1	0.3±0.9	9.4±1.5	0.999
Zn	4.5	94±0	6±1	2.1±0.1	2.5±0.1	0.998

The experimental results agreed with other studies [24,25]; the mass of the Ion-Exchange resin increased the dissociation of metal-ligand complexes (Figures 8.2, 8.3, Table 8.2). Moreover, these Figures clearly shows two different pictures: the mass of Chelex, except the lowest amount 0.5 g, has almost no effect on the dissociation of Cd, Co, and Zn; Cd-, Co-, and Zn-LFA complexes dissociate completely with any mass of the Chelex (e.g., 1, 3, and 4.5 g) used. However, for the other studied metal-LFA complexes (Cu, Ni, and Pb), the mass of binding resin does not affect the metal-LFA complexes' dissociation rates. Sixty percent of copper-LFA complexes remain undissociated, whereas forty percent of lead-LFA complexes and thirty percent of nickel-LFA complexes remain undissociated even with the higher mass, 4.5 g, of the Chelex resin employed. However, the above result was obtained with the ratio of $[M]/[LFA] = 1 \times 10^{-3}$ and $[M] = 1 \times 10^{-7} \text{ M}$, which shows that the amount of the binding resin employed is extremely important, especially for the complexes of copper, nickel, and lead.

The results of this work (Figures 8.2, 8.3, and Table 8.2) agree with those reported by earlier workers [15, 26], and clearly show two different pictures: the amounts of Chelex with the exception of 0.5 g, had almost no effect of the dissociation of Cu(II), Co(II), and Zn(II) complexes of the LFA. The results included metal complex dissociation in all the range of the mass of Chelex used: 1, 3, and 4.5g. Sixty percent of Cu(II)-LFA complexes remained undissociated, whereas forty percent of Pb(II)-LFA complexes and thirty percent of Ni(II)-LFA complexes remained undissociated even with the higher amounts (4.5 g) of Chelex resin. The above results conclusively show the importance of the amount Chelex (competing ligand) employed for the study of the complex-dissociation kinetics of the metal(II)-LFA complexes of the above metals.

Figure 8.3 Dissociation kinetics of M (II)-LFA complexes in model solutions using Chelex 100 as the competing ligand and Inductively-Coupled Plasma - Mass Spectrometry (ICP-MS) to measure the dissociation kinetics. $C_{M(II),T} = 1.0 \times 10^{-7}$ mol/L, pH 5.0, $T = 23^{\circ}\text{C}$. Ionic Strength ~ 0.056 M; $\bullet$ 0.5 g (0.17 %) Chelex; $\circ$ 1.5 g (0.33 %) Chelex; $\blacktriangle$ 3.0 g (1.0 %) Chelex; $\triangle$ 4.5 g (1.5 %) Chelex

Table 8.2 Kinetically distinguishable labile components M (II) complexes in model solutions using the three different binding resins as the competing ligand and Inductively-Coupled Plasma - Mass Spectrometry (ICP-MS). pH 5.0 ± 0.1 , ionic strength ~ 0.056 M, temperature 23 ± 2 °C. $c_M / c_{LFA} = 0.01$.

Metal	Resin	<u>Kinetically distinguishable components</u>		<u>Dissociation rate coefficients</u>		R ²
		C ₁ (%)	C ₂ (%)	k ₁ (10 ⁻³ s ⁻¹)	k ₂ (10 ⁻⁴ s ⁻¹)	
Cd	Chelex-100	93±1	7±1	1.0±0.0	2.0±0.2	0.999
Cd	Dowex 50wx-8	91±1	9±0	2.2±0.0	3.1±0.0	0.995
Cd	Dowex Mac-3	99±1	----	0.2±0.0	---	0.972
Cu	Chelex-100	92±0	-		6.6±0.0	0.954
Cu	Dowex 50wx-8	53±1	47±1	1.0±0.0	1.0±0.0	0.987
Cu	Dowex Mac-3	81±2	19±1	4.7±0.3	0.4±0.0	0.932
Pb	Chelex-100	4±2	96±0	5.4±1.1	0.1±0.0	0.999
Pb	Dowex 50wx-8	65±1	35±1	1.1±0.0	2.0±0.1	0.997
Pb	Dowex Mac-3	97±1	----	0.3±0.0	----	0.655
Zn	Chelex-100	28±2	72±1	1.1±0.9	9.4±1.5	0.999
Zn	Dowex 50wx-8	96±1	4±0	1.8±0.0	0.2±0.2	0.981
Zn	Dowex Mac-3	25±2	75±2	22±2.2	1.1±0.2	0.971
Ni	Chelex-100	8±2	92±0	4.8±0.1	1.4±0.0	0.997
Ni	Dowex 50wx-8	62±1	38±1	1.0±0.1	1.7±0.0	0.996
Ni	Dowex Mac-3	94±2	---	0.1±0.0	----	0.921

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--Chapter 9--

Conclusions

9.1. Conclusions

This work has been a comparative study of several analytical techniques which has revealed their strengths and weakness. This thesis has examined the complexation of Ni(II), Cd(II), Cu(II), Zn(II), and Pb(II) in model systems containing a well-characterized Laurention Fulvic Acid (LFA), and in natural freshwaters and municipal wastes and mine effluents, using various analytical techniques, so that relationship between metal complexation and metal speciation may be better understood. IET, DGT, and Pseudopolarography, which are based on different physico-chemical principles, have yielded estimates of free-metal ions (aqua complex) and other labile species in aqueous solutions, which are regarded as indicators of metal's bioavailability and ecotoxicity. The comparative study of the IET, DGT techniques along with the predictions capabilities of a computer speciation code, WHAM VI, has yielded a bounteous harvest of speciation data for metals in natural waters and in mining, industrial, and municipal wastewaters, which are unprecedented in the annals of metal speciation research in the aquatic environment.

The free metal ion $[M^{n+}]$, has been reported to be usually a good indicator of toxicity in freshwaters; however, it is an extremely challenging task to measure the $[M^{n+}]$ at environmentally relevant conditions. This work has explored the capability of comparative study of several speciation techniques to provide insight into the process of complexation, transport, fate, and bioavailability of metal species in natural freshwaters.

Diffusive Gradient in Thin Film Technique (DGT) applied to model solutions of the LFA, and municipal and mining aqueous effluents, and *in situ* deployment in natural freshwaters has provided a new insight into DGT-lability of metal-LFA complexes as follows: DGT is capable of determining labile species in the above-mentioned matrices, including municipal and mining aqueous effluents which present formidable challenge to existing speciation techniques. For long deployment times at high $[M]/[LFA]$ ratios, fouling of DGT apparatus was observed. The conclusion is that extra precaution against fouling of DGT should be taken in DOC-rich and/or slow-stream environments. The effect of Diffusive Boundary Layer (DBL) on the amount and type of accumulated metal strictly depends on the size of DBL. The effect of the binding resin deployed *in situ* in natural freshwaters is as follows: contrary to prevailing assumption that only DBL has effect on the type and the amount of captured metal species by DGT, the type of binding resin strongly influences the type and mass of the metals accumulated by the DGT resin phase. For example, Polymer 1.1 has higher affinity for lead while Chelex-100 has higher affinity for the other studied metals, and Polymer 1.0 has very little affinity for the studied metals. These findings can be helpful for employment of DGT in different matrices.

Ion Exchange Technique (IET) has been found to be the only analytical technique capable of determining free-metal-ion concentration in environmentally relevant conditions. IET is able to determine $[M^{n+}]$ in the aqueous environment. The main limitations of IET are the problem of contamination, and its assumption that only free metal ion is captured by the binding resin. Since IET is based on preconcentration, even

trace amount of contamination may end up causing dramatic change in the free metal ion concentration. Moreover, matrix effect is another challenge, especially in natural freshwater samples, which has as yet defied solution.

Heterogeneity of metal-NOM complexes is reflected experimentally in their apparent lability under given conditions. Lability of metal-NOM complexes, under given experimental conditions are strongly influenced by heterogeneity of NOM. Under the same experimental conditions e.g., pH, ionic strength, ligand and metal concentrations, different metals show different heterogeneity because of the different complexing sites of the complexants might be available to the metal ion, and therefore stability of complexes formed by the strongest and weakest sites of the complexant may vary from metal to metal. The metal loading, θ , and heterogeneity parameter, Γ , are the main factors that affect stability, and hence, lability of metal-NOM complexes. Heterogeneity of DOC influences M-DOC complexations and hence, the amount and the type of labile species, especially free metal ions which are widely reported to be bioavailable and toxic. Yet, because of the inherent physicochemical complexity of DOC it is extremely difficult to determine the direct effect of heterogeneity on lability. This thesis advances the existing knowledge a step forward by using model solutions to provide understanding of the effect of heterogeneity on lability. The observed trend for the metal heterogeneity ($\Gamma_{Zn} > \Gamma_{Cd} > \Gamma_{Pb} > \Gamma_{Cu}$) did not change on changing $[M]/[LFA]$ ratio.

Chelex-100 is the most commonly employed chelating agent to investigate M-DOC complexation. Dowex 50WX8, Dowex MAC-3, and Chelex -100 chelating agents when they are employed individually (separately) do not depend for their performance on the

amounts and types of the chelating agent employed. However, the speciation parameters (i.e., dissociation rate coefficients, and kinetically distinguishable components) and the amounts of the labile fraction is strongly influenced by the type and amounts of the chelating agents employed.

Natural freshwaters and aqueous effluent samples are difficult to study because of the numerous reactions and interactions that are occurring simultaneously. WHAM, a computer-based speciation code, has been modeled by other workers using natural freshwaters. However, WHAM is based on chemical equilibrium, whereas natural freshwater are dynamic systems, often far removed from chemical equilibrium. WHAM is able to model all natural freshwaters of different chemical compositions, based on its data base and the input parameters. The experimental results of this work, compared with the predictions of WHAM VI, have yielded the conclusions that the WHAM VI predictions over-estimates the experimental results by about two orders of magnitude, depending on the metal; only four metals, Cu, Zn, Ni, and Pb have been studied.

9.2. *Environmental Significance*

The environmental significance of this work for the natural freshwater is that it has provided the knowledge of complex interactions that occur between metals and naturally-occurring organic ligands, which has heretofore been limited because of the enormous complexities of metal speciation in natural freshwaters. The findings of this work are that comparative study of the rival analytical techniques and methods holds promise of unlocking one of the well-guarded secrets of nature: the relationship between metal

complexation and metal speciation in natural freshwaters, with the possibility of finding the link between the lability of complexes (an intrinsic property) and metal released by the metal complexes (an extensive property): the ever elusive HOLY GRAIL of environmental research, which has long been hotly pursued by modern knights of the Arthurian Legend--the environmental researchers.