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Studies on Chemical Speciation of Some Trace Metals in the Aquatic and the Terrestrial Environment

by

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

Doctor of Philosophy

Department of Chemistry

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acceptance of the thesis,

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in the Aquatic and the Terrestrial Environment”**

in partial fulfilment of the requirements
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ABSTRACT

New analytical techniques and methods for determining chemical speciation of some trace metals in the aquatic and the terrestrial environment have been investigated and developed. Rotating disk electrode voltammetry (RDEV) in conjunction with anodic stripping voltammetry (ASV) with a thin mercury film electrode (TMFE), or with a Nafion-coated thin mercury film electrode (NCTMFE) has been evaluated for direct determination of lead and cadmium speciation in aqueous solutions containing dissolved organic matter. The RDEV technique has been also employed to estimate dissociation rate constants and diffusion coefficients. Pseudopolarographic analysis of aqueous samples containing Cd or Pb and a well-characterized fulvic acid, and of samples of Rideau river surface water show that NCTMFEs provide interference-free determination, and give well-defined pseudopolarograms for determination of stability constants. For determination of dissociation rate constants of nickel complexes, Competitive Ligand Exchange / Adsorptive Cathodic Stripping Voltammetry (CLE/AdCSV) using high-frequency square-wave voltammetry has been developed as Anodic Stripping Voltammetry cannot take advantage of high-frequency square-wave voltammetry to achieve the extremely high sensitivity required for determining trace concentrations of nickel in unpolluted waters. Application of the CLE/AdCSV method to aqueous samples containing dissolved organic matter shows that complexes having dissociation rate constants from 10^{-3} s^{-1} to 10^{-6} s^{-1} can be resolved from very labile complexes, and also from inert complexes. For determination of chemical speciation of Zn and Cd in soil, High Performance Liquid Chromatography – Microfiltration Method was developed for determination of solution phase, labile-bound, and nonlabile Zn and Cd species. Extraction of metals from soil solids was accomplished by using online microfiltration which trapped soil solids from an injection of a soil slurry and allowed the mobile phase to extract metals and carry them to the chromatographic column for separation. The

results of this kinetic study of cadmium and zinc sorption/desorption on a well-characterized soil show that the metal sorption on the soil is a two-step process, involving an initial fast binding followed by a much slower sorption. Experimental differentiation of the Cd and Zn species on soil was found to be useful for understanding the mechanisms of sorption/desorption reactions of Zn(II) and Cd(II) on soil.

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1.1 Chemical Speciation

A large fraction of emitted chemical pollutants, including metals, pass through one or more of the many hydrological pathways in the natural environment. In natural waters, most vital and toxic elements are present at trace or even ultra-trace levels, typically 10^{-12} – 10^{-7} M [1,2]. The physico-chemical distribution of elements (chemical speciation) ranges from truly dissolved to particulate-bound chemical species and includes simple inorganic complexes, complexes with organic macromolecules, surface-bound metals (e.g. living cell walls, inorganic colloids and particles) and elements inside microorganisms. All these chemical species can co-exist and may or may not be in thermodynamic equilibrium [1]. Variations in the chemical speciation of an element will affect its bioavailability [3] as well as its mobility [4] in the water of interest.

For determination of chemical speciation of elements in the aquatic environment, difficulties lie less in the low concentrations of the components in the aquatic environment and more in the vast complexities of the aquatic environment. Physico-chemical studies of the numerous complexing components of aquatic systems are needed in order to understand their behaviour in the environmental medium to be studied, and inside the analytical system to be developed. Most of the existing methods of chemical speciation give information on “operationally-defined” species. For example, one such “operationally-defined” procedure that is very widely used is the sequential extraction for speciation of trace metals in soils [5], which has been severely criticized by Nirel and Morel [6] who described the “operationally-defined” results of this procedure as: “what is

measured is defined by what is measured.” What is needed is research and development of chemical speciation techniques and methods to characterize and quantify the bioavailable chemical species, by making *in situ* (at depth), real-time measurement at the concentrations (10^{-12} to 10^{-7} M) at which they may be present at the biological surface [1,2]. Because physical, chemical and biological processes continue in samples of natural waters after their collection and cannot be stopped without radically altering chemical speciation of the water samples, accurate determination of chemical speciation becomes extremely difficult from the moment water samples are collected from their natural environment [1]. Since sample collection, handling, and storage change the chemical speciation of samples, and since sediments and porewaters may have steep concentration gradients and fluxes of chemical species, *in situ* (at depth) submersible probes are needed for real-time, automatic measurement of bioavailable chemical species [1,2].

Three promising *in situ* techniques are:

- 1) Diffusive Gradients in Thin Films (DGT) [7,8,9,10,11].
- 2) Probes Based on Gel-Integrated Voltammetric Microelectrodes [1,2].
- 3) Supported Liquid Membrane Coupled to Voltammetry [1,2].

1) Diffusive Gradients in Thin Films (DGT) [7,8,9,10,11]

The technique of Diffusive Gradients in Thin Films (DGT) provides an *in situ* means of quantitatively measuring labile species in aqueous systems [11]. In its applications to trace metals, a layer of polyacrylamide hydrogel of known thickness is backed by a layer of ion-exchange resin (Chelex). The gel and resin layers are so arranged that

transport of metal ions is solely by molecular diffusion (Fick's First Law of Diffusion). DGT's major advance over previous *in situ* accumulation techniques, such as ion-exchange resins in dialysis bags [11,12] is that it constrains mass transport. By selection of an appropriate gel layer thickness (~ e.g. 1 mm), the mass of accumulated metal ions is independent of the hydrodynamics in solution above a threshold level of convection. Consequently, when DGT devices are deployed in uncontrolled convective regimes, such as rivers, effluents, and the well-mixed surface waters of lakes and seas, the measurements should be fully quantitative and independent of water flow. Moreover, because mass transport is so well-defined, there is a precise effective measurement time that can be calculated and used to define the measured species in terms of their lability. Deployment of DGT devices for 1 day results in a concentration factor of ~ 3000, allowing metals to be measured in extremely low levels (4 pmol L⁻¹). The technique has multielement capability [11].

The DGT is an *in situ* preconcentration technique which is specific for mobile metal species. DGT allows measurement of trace metals at unprecedented high spatial resolution, revealing geochemical processes occurring at sub-millimeter scale. The DGT results of geochemical samples have revealed that sources and sinks may be very localized, perhaps more than physiologically driven oxygen gradients which have been elegantly measured by microsensors [7]. Interpretation of metal fluxes and gradients and the role of microniches will be aided by the combined application of DGT and microsensors, particularly planar optrodes, which have provided fine-scale measurement of two-dimensional oxygen distributions [7]. In the DGT technique, a

plexiglass plate is first covered by a layer of a complexing agent such as Chelex resin (75-100 μm), followed by a relatively thick layer of polyacrylamide gel (500-1000 μm). This plexiglass plate assembly is placed direct in the water column or at the sediment-water interface. DGT measures the diffusible (mobile) metal species, both metal ions and metal complexes depending on their diffusibility. However, because the DGT is an integrating technique which integrates the analyte over a given period of time (typically, a few hours) and the plexiglass plates have to be removed and returned to the laboratory for analysis using conventional, equipment, it cannot give a real-time evolution of a water body or sediment. Nonetheless, DGT is presently the best technique available to obtain high resolution spatial concentration profiles of a large number of trace metals at the sediment-water interface.

2) Probes Based on Gel-Integrated Voltammetric Microelectrodes [1,2]

This technique employs voltammetric microelectrodes integrated in hydrophilic gels. A microdisk (or a microdisk array) electrode is covered with a gel layer much thicker than the radius of the disk, but thin enough to allow rapid (minutes) equilibration; voltammetric analysis is performed inside the gel. The gel is selected to allow rapid equilibration of metal cations and small complexes, while preventing the diffusion of potentially fouling colloids and macromolecules. The analytical device integrates fractionation by diffusion with voltammetric quantification. This enables the probes to be inserted direct inside sediments, in particular to measure concentration versus depth profiles and, therefore, fluxes of metals at the sediment-water interface. The

sensitivity limit obtained with single microelectrodes and microelectrode arrays is $10^{-10} - 10^{-11}$ M [1,2].

3) Supported Liquid Membrane Coupled to Voltammetry

This is a preconcentration technique based on the complexation of the metal ion of interest by a hydrophobic ligand dissolved in a non-miscible solvent which is immobilized in a porous inert membrane, followed by a simultaneous back extraction into an aqueous solutions (strip solution). This technique is selective (but not specific) for free metal ions, which is a key factor for environmental application [13]. In certain conditions, lipophilic complexes may also pass the membrane. However, since their determination is also important for the interpretation of environmental functioning, this is an advantage and not a limitation of this technique. Preconcentration factors of up to 3000 have been obtained, using small internal diameter (200-600 μm) hollow fibres as the porous membrane [14]. Simultaneous preconcentration of several metals is possible with preconcentration factors which depend on the stability of the metal complexes inside the membrane and in the strip solution. The coupling of this technique with highly sensitive quantification techniques such as fluorescence or voltammetry should allow determination of ultratrace free metal ion concentration below 10^{-12} M, i.e., at the levels encountered in environmental systems [13,14].

Among other chemical speciation techniques that are now being developed in this laboratory and which look promising are:

- 1) Rotating-disk electrode voltammetry with adsorptive cathodic stripping voltammetry or with anodic stripping voltammetry (RDEV/AdCSV) or (RDEV/ASV), which uses a thin mercury film electrode. RDEV/AdCSV is applicable to most elements of environmental relevance and has the highest sensitivity (limit of detection up to 10^{-12} M). The RDEV/AdCSV or RDEV/ASV measures the sum of metal aquo ions and labile metal complexes, and can characterize metal species by its speciation parameters: dissociation rate constant and diffusion coefficient, or by a pseudopolarographic approach can characterize the metal species by their half-wave potential, identify the metal complex that is undergoing electrode reactions, and measure the conditional stability constant of the metal complex (which is a measure of the availability of free metal ions **at equilibrium**).
- 2) Competing ligand exchange method (CLEM), which is a kinetic speciation method based on ligand exchange, and uses Chelex 100 cation-exchange resin, and characterizes metal complexes by their chemical speciation parameters: dissociation rate constants and diffusion coefficients, and provide kinetic spectrum of the metal complexes, yielding significant information on the identity and quantity of the kinetically distinguishable metal complexes – these advantages are unmatched by any other chemical speciation techniques.

RDEV/AdCSV or RDEV/ASV is not an *in situ*, real-time probe, but has the potential of being further developed into one; CLEM is a laboratory-based chemical speciation technique which, however, has the capability to characterize and quantify the metal complexes, and can provide quantitative values of speciation parameters: dissociation

rate constant and diffusion coefficient of metal species. For the purpose of bioavailability assessment, it is more important to be able to characterize and quantify the bioavailable metal complexes by their chemical reactivity (as measured by their relevant reaction kinetics), and by their mobility (as measured by their diffusion coefficients), and by their conditional stability constants (as measured by the pseudopolarographic technique) than to be able to identify and quantify them.

1.2 Speciation Parameters

Speciation parameters are quantitative measures of chemical characteristics of chemical species which are independent of the chemical methods used to measure them. It often requires a number of speciation parameters to characterize a system fully. Some important speciation parameters are given below.

- (1) Diffusion Coefficient – The mobility of a particular chemical species plays a very important role in the biological impact the element may have in the natural environment. Before an element may elicit a toxic response, it must come into contact with a biological membrane and eventually penetrate it [15, pp 210-211]. The diffusion coefficient is a direct measure of how quickly the chemical species can reach the biological membrane.
- (2) Stability Constant – The thermodynamic stability of a metal complex (which is a measure of the availability of free metal ions **at equilibrium**) is a very important property for classifying the metal species in the aquatic and the terrestrial environment [16,17,18]. The determination of stability constants relies on equilibrium

or pseudo-equilibrium condition to be established before the measurement can be made. While stability constants are useful for strong complexes, they are not very useful for slowly-dissociating complexes as equilibrium may not be established in a conveniently measurable time. Although measurement of this parameter is straightforward for homogeneous systems (such as inorganic complexes), it may be difficult to measure this parameter for heterogeneous samples such as those containing naturally occurring organic complexants. Owing to the different types of functional groups in humic substances, which are ubiquitous in the natural environment, humic substances are heterogeneous complexants. This chemical heterogeneity is a strong characteristic which determines the metal ion binding, and hence, the stability constants of metal complexes of organic complexants. In such cases, instead of discrete values for stability constants, a large spectrum of stability constants is often observed and the interpretation becomes uncertain and often an average conditional stability constant is estimated for a group of metal complexes having closely spaced values of stability constants.

- (3) Dissociation rate constants – The dissociation rate constant represents how rapidly a metal complex dissociates to form the metal-aquo complex and hence is a very important speciation parameter, especially in the case where free metal ion is the bioavailable species.

1.3 Organic Complexants such as Humic Substances in the Natural Environment, and their Effects on Metal Speciation in the Freshwater Environment

Organic substances are ubiquitous in the freshwater environment. The nature and amounts of these materials in natural waters arise from a number of complex processes including degradation of terrestrial and aquatic biomasses, leaching of soil by rainfall, chemical and biological activities and entrainment by aerosols [19]. The concentration of dissolved organic carbon (DOC) in typical streams and rivers ranges from 2 to 10 mg/L but can be as high as 50 mg/L in coloured waters [20].

The heterogeneous nature of DOC makes it necessary to fractionate it into a number of operationally defined chemical classes. The fractionation procedure on XAD resin and cation exchange resins is the most common and acceptable method of separating DOC constituents. The **humic** fraction is a general term used for the group of complex, high molecular weight organic compounds that are dissimilar to the biopolymers of microorganisms and higher plants (including lignin). The humic substances are fractionated on the basis of their solubility. The fraction that is not soluble in base (called humin) is separated from the dissolved fraction. This dissolved fraction is then acidified to give a precipitate referred to as **humic acid**. The fraction that stays in solution at pH 1 is named **fulvic acid**. These two fractions are the most important in metal ion binding as they contain the most number of functional groups. The fulvic acid fraction is especially important as it typically comprises ~50% of the total DOC [21].

Aquatic humic substances are frequently responsible for the regulation of trace metal speciation in freshwaters. Trace metal binding to these materials is complex due to the nature of these materials. A number of important properties for humic substances are listed below:

Polyfunctionality – each molecule can contain a number of different functional groups. Fulvic and humic acids primarily have carboxylic or phenolic groups, but moieties bearing sulphur and nitrogen are also known.

Polydispersity – humic substances as a class of compounds represent a wide variety of molecules with differing molecular masses.

Polyelectrolytical – each individual molecule carries a large number of electric charges due to its polyfunctionality.

These factors make metal binding with humic substances highly complex and variable depending on experimental conditions such as ionic strength and pH. These variables make the selection of an appropriate binding model difficult and sometimes uncertain.

1.4 Objectives and Goal

The objectives and goal of this research are:

- 1) to develop and study the applicability of electrochemical stripping techniques for measurement of speciation parameters in aqueous environmental samples.

This objective includes:

- i) development of thin mercury film electrodes for use with electrochemical stripping techniques for aqueous environmental samples
 - ii) development of techniques for the determination of kinetic speciation parameters
 - iii) development of techniques for the determination of thermodynamic speciation parameters
- 2) to develop a rapid extraction technique for determining the chemical speciation of some trace metals in soil.

This objective includes:

- i) development of the analytical instrumentation for rapid extraction
- ii) evaluation of the extraction efficiency of the technique
- iii) evaluation of the chemical species distinguishable by the technique

The goal of this research is to incorporate the above techniques into a comprehensive chemical speciation scheme. Parts of this comprehensive chemical speciation scheme have already been developed by Chakrabarti et al. [22,23,24,25,26,27,28,29,30,31]. Some aspects of filtration and ultrafiltration techniques will also be incorporated in this research, as they are essential to the comprehensive chemical speciation scheme.

**2. Pseudopolarography using Rotating Disk Electrode
/ Anodic Stripping Voltammetry with Thin
Mercury Film Electrodes for Determination of
Speciation Parameters**

2.1 Introduction

2.1.1 *The Advantages of Rotating Disk Electrode Voltammetry (RDEV)*

Rotating disk electrode (RDEV) voltammetry employs a disk shaped electrode rotated with an electric motor at a rate in the range of 50–4000 rpm. At a high rotation rate, there will be a thin, well defined diffusion layer close to the disk's surface (the Nernst diffusion layer) in which diffusion is the form of mass transport and a steady-state is rapidly established. RDEV provides a well-defined and reproducible mass transport regime. The current is entirely mass-transport controlled. In RDEV, there is no contribution from charging the electrical double layer current. Therefore, under the steady-state condition, the recorded current may be unequivocally equated with the faradaic current.

2.1.2 *Definition of Lability*

For many measurements involving ASV for studies in natural waters, the terms “free”, “labile” and “nonlabile” have been used loosely. It is useful to divide the total metal concentration as follows: $M_T = M_0 + M_L + M_I$, where M_T is the total metal, M_0 is the metal aquo complex, M_L is the labile metal complexes and M_I the inert metal complexes. The problem with dividing the metal into these classifications is how to differentiate between labile and inert species. The definition of labile used by inorganic chemist is “the ability of a particular complex ion to engage in reactions which result in replacing one or more ligands in its coordination sphere by others is called its lability. Those complexes for which reactions of this type are very rapid are called labile...and as a more explicit definition which says that complexes whose reactions may be studied by static methods

electrode fouling is possible because of interactions of the mercury surface with inorganic matrices, and with organic substances in freshwaters [34,36 p.104,37]. For example, TMFE deactivation can occur through the formation of Hg_2Cl_2 by chloride in water samples. However, the major limitation of TMFEs is the interference by organic surface-active substances present in freshwaters because of their adsorption on to the surface of the TMFE [34]. This adsorption can be prevented by using polymer-coated electrodes such as Nafion-coated electrodes; however, the mechanism of mass transport of the analyte to and from the TMFE surface in such electrodes is complex and depends on experimental conditions.

For anodic stripping voltammetry, the factors which affect fluxes towards the electrode surface must be known and controlled if the results are to be validly interpreted in terms of chemical speciation. For example, in the estimation of the diffusion coefficient D , the effective surface area of the electrode surface must be exactly known and held constant. However, TMFs on glassy carbon supports are not true films but a collection of fine droplets [34,38 p. 484]. If the droplets form a relatively homogeneous layer, and are of the same diameter, l , such that $l \ll \delta$ (where δ is the thickness of the Nernst diffusion layer), then these systems will behave as a true film [34,38 p. 484]. In a previous publication [22], δ has been found to be $4.5 \mu\text{m}$ at 4000 rpm rotation rate, thus satisfying the requirement that $l \ll \delta$ (the l is reported to be $< 1 \mu\text{m}$).

Various procedures for preparing TMFs have been reported in the literature. Frenzel [34] has reported that roughened GC supports produce mechanically stable mercury films with excellent electrochemical properties. However, the majority of work reported in the literature has been done using a smoothened electrode, typically, polished with 0.05 μm alumina or diamond paste. Such electrodes exhibit low background and reproducible currents [36, p.70]. To study the effect of deposition potential on mercury plating, other workers did microscopic examination of the mercury droplets and concluded that one quarter to a half of the geometric area of the electrode was covered by mercury droplets [39]. This finding is important as it means that the geometric surface area may not be the actual surface area used for reduction. Hence, calculations requiring the effective surface area of the electrode will be in error if this fact is not explicitly taken into account in making these calculations.

Formation of mercury films on smooth electrodes has been shown to be optimum under conditions of longer deposition times and lower deposition potentials to minimize H_2 gas bubble formation. Hydrogen gas bubbles produced at the bare GC surface have been shown to be disruptive to the mercury film [35]. It has also been suggested that TMFs be produced *in situ* to maintain a stable film [35,37]. Mercury films produced in this way have many desirable attributes; unfortunately, the large concentration of mercury required for *in situ* formation of a stable film is not practical as it may change the chemical speciation of the analyte species. Therefore, pre-formed, electrolytically-plated mercury film electrodes have been used in this work.

2.1.4 Pseudopolarography

The advantage of RDEV lies in its ability to differentiate between the equilibrium concentrations of the metal-aquo complex (produced by dissociation of ‘labile’ metal complexes) and the kinetic availability of dissolved metal complexes in aqueous environmental samples [33]. Studies on complex-formation analogous to those performed using a hanging mercury drop electrode (HMDE) can also be done using RDEV in which plots of stripping peak current vs. deposition potential are called pseudopolarograms. Pseudopolarograms (similar to D.C. polarograms) show the half-wave potential, $E_{1/2}$, which is a measure of the thermodynamic stability of the metal complex. When a metal-aquo complex forms a complex with a ligand other than H_2O , the $E_{1/2}$ for the metal complex is shifted to a potential more negative than the $E_{1/2}$ of the metal-aquo complex. Lewis et al. [40] developed equations based on work by DeFord and Hume [41] to relate stability constants to half-wave potentials. For the reduction of a metal complex, the position of the polarographic wave for a given metal-ligand species ($E'_{1/2}$) will be described by the following equation:

$$E'_{1/2} \sim E_{1/2} - (0.059/n) \log \frac{K_{ox}}{K_{red}} - (p - q) (0.059/n) \log [L] \quad (2.1)$$

where $E_{1/2}$ and $E'_{1/2}$ are the half-wave potentials for the reduction of the metal aquo ion and the metal-ligand complex, respectively, K_{ox} and K_{red} are the stability constants for the oxidized and reduced forms of the complex, and p and q are the number of ligand molecules L associated with each complex species. Brown et al. [42] and Vega et al. [43] have used the similar equations with a HMDE to study metal complexation with inorganic ligands. Under certain conditions, where the oxidized form of the complex is

reduced to the metal Hg-amalgam, with the destruction of the complex molecule, Equation 2 [44] simplifies to following equation at T=298K:

$$E_{1/2}' \sim E_{1/2} - (0.059/n) \log K_{ox} \quad (2.2)$$

Equation (2.2) can be used directly without knowledge of the exact activity of the ligand.

Pseudopolarograms also give information about the relative concentration and number of metal complexes that are in the sample whether they the metal-aquo complex or metal complexes with other ligands. Each wave in a pseudopolarogram is associated with a different reducible metal complex. The height of the polarographic wave corresponds to the limiting current in D.C. polarography. The concentration of the electroactive species is expressed by the following equation [42]:

$$[ML] = i_l/S \quad (2.3)$$

where i_l is the wave height and S is the analytical sensitivity (measured in $\mu A/\mu M$).

2.1.5 Nafion-Coated Thin Mercury Film Electrodes (NCTMFE)

Nafion, a non-cross-linked perfluorosulphonate cation-exchange resin has the following structure:

It has been used for electrode coating in a great variety of electrochemical studies, mostly in conjunction with immobilization of positively-charged redox couples within the

Nafion film coating [45,46]. The Nafion polymer is chemically inert, nonelectroactive, hydrophilic, and insoluble in water and thus possesses almost ideal properties for preparation of modified electrodes [47,48]. The sulphonate (SO_3^-) group of the polymer chain imparts a negatively-charged surface on the Nafion coating, thus giving an ionic size and charge dependent permselectivity which can be used in selective determination of metal species on the basis of size and charge [49,50,51,52,53,54]. Nafion coating also provides an effective shield against chemical and mechanical degradation of the TMFE [48,55,56]. Another important property of Nafion coating in electroanalysis of natural waters is that it provides an effective shield against electrode fouling by adsorption of surface-active substances [47,57,58] which are ubiquitous in natural waters [33,59]. Obviously, this protective mechanism can only be operative if the TMF is confined beneath or within the Nafion film; the thickness of the Nafion film satisfies this condition [48]. M.M. Correia dos Santos et al. [60] have studied the kinetics of dissociation and the effect of adsorption on a Nafion-coated electrode on the kinetics of the Cu(II)-proline complex by cyclic voltammetry. They concluded that: (a) adsorption of surface-active substances may influence the kinetics of a chemical reaction occurring before the electron transfer; (b) the use of NCTMFE seems to decrease or even to eliminate the interference due to adsorption of negatively charged organic compounds, and (c), the kinetics of the dissociation of Cu(II)-proline complexes at NCTMFE are almost unaffected by the adsorption.

Guy et al. [57] have reported that Nafion membrane contains two types of binding sites - a strong site that binds metal ions in a nonelectroactive form and cation exchange sites

that are useful for preconcentration prior to differential pulse voltammetry. The differential pulse currents are increased by a factor of 75 to 100 and a linear analytical calibration curve is obtained from about 25 nM to 1000 nM for Cu, Pb, Cd, and Zn. The ion exchange reaction (which is used for preconcentration of cations for electroanalysis) is very sensitive to ionic strength, and significant enhancement occur only in solutions of ionic strength below 0.10 M. They have concluded that Nafion-coated glassy carbon electrodes can be used for current enhancements in analysis under well-defined sample conditions of fixed ionic strength, pH, employing a standard addition method. It is clear from the literature [57,61,62] that Nafion coating adds an additional dimension of nonfaradaic preconcentration of some metals and ion-exchange preconcentration of other metals, making theoretical treatment of the electrode processes more complicated.

The majority of applications of Nafion coating in the literature report applications of 1-5 μL of a 0.2-1.0% Nafion solution onto a standard GC electrode 3 mm diameter [47,48,55,58,61,62,63]. Under these conditions, the Nafion film is considerably thicker than the TMF layer [48]. This sets up a second diffusional layer which the electroactive species must pass through before it is reduced at the TMF. Ugo et al. [64] have described the diffusion of an electroactive ion through such a Nafion film. They have defined an apparent diffusion coefficient D_{app} , which describes the overall diffusion-controlled mass-transport mechanisms through such a film. Although this D_{app} may be significantly different from D in the Nernstian diffusion layer, it has been reported that the Nafion film does not hinder mass-transport of metal ions since the thickness of the Nernstian diffusion layer is about one hundred times the thickness of the Nafion film [47].

prepared by dissolving an appropriate amount of NTA (99%, BDH) in ultrapure water containing 0.001M sodium hydroxide. A stock solution of Armadale fulvic acid (FA, see below) was prepared by dissolving 1.0000 grams of the freeze-dried FA into 1.00 L of ultrapure water. Nafion coating solutions were made from dilutions of a stock solution of 5% (w/w) Nafion solution obtained from Aldrich Chemicals. Dilutions of this Nafion solution were made by adding absolute ethanol (99%) to make a 0.5% (w/w) solution for direct application onto the electrode surface. A casting solvent of N,N-dimethylacetamide (DMAA) was used with the Nafion solution to form the Nafion film. Ultrapure water of resistivity 18.3 M Ω – cm was obtained direct from a Milli-Q UF Plus water purification system (Millipore Corporation), fitted with a purifying column to remove organic matter. All test solutions (unless otherwise stated) were prepared by serial dilution of the above stock solutions with ultrapure water immediately prior to determination.

2.2.2 Armadale Fulvic Acid

The fulvic acid (FA) used in this study was an Armadale BH horizon fulvic acid supplied by Dr. D.S. Gamble of Agriculture Canada [65]. The reported analysis of the functional groups gave the following results [65]: phenolic OH value of 3.0 mmol/g FA, total carboxyl groups by potentiometric titration of 7.71 mmol/g FA, and a bidentate chelating capacity (by potentiometric titration with Cu²⁺) of 5.43 mmol/g FA. FA is a heterogeneous, polyfunctional, polyelectrolyte, and polydisperse substance containing a mixture of different compounds having different molecular weights [38,59].

2.2.3 Samples

A 4-litre grab sample of Rideau River surface water was obtained using acid-washed Teflon containers. Immediately after collection, the sample was filtered through a 0.45 μm filter (Gelman) using a peristaltic pump, and the filtrate was retained. Cascade ultrafiltration was then carried out accord to Lu [66 p. 60-61] on the 0.45 μm membrane filtered sample using an Amicon (Lexington, MA, USA) ultrafiltration cell fitted with Amicon disc membrane filters (62 mm diameter). 50,000 molecular weight cutoff (MWCO), 10,000 MWCO and 1000 MWCO ultrafilter sizes were used.

The pH of the sample measured with an Accumet 925 pH/ion meter with a combination glass electrode containing an internal calomel reference electrode was 8.2.

2.2.4 Apparatus

Voltammetric measurements were done using an EG&G Princeton Applied Research Model 384B polarographic analyzer and a Rotel-2 rotating disk electrode. The working electrode, Rotel model R2/112/GC (EG&G PARC) of 6 mm diameter, was made of a glassy carbon substrate, surrounded with an epoxy resin insulating jacket. The reference electrode consisted of an Ag/AgCl electrode in a Teflon tube fitted with a porous Vycor tip filled with saturated KCl solution. The counter electrode was made of a coiled platinum wire. Data were acquired through the RS-232 interface of the polarograph to a model 486 personal computer and downloaded as an ASCII file for processing.

2.2.5 Formation of Thin Mercury Film Electrodes (TMFEs)

Prior to sample analysis, the GC electrode was prepared by polishing with 0.05 μm alumina powder, wetted with ethanol, and cleaned in an ultrasonic bath for 5 minutes. The TMFE was formed by immersing the electrodes into a 50 mL solution of deoxygenated, ultrapure water containing 0.04 M acetate buffer and 50 μL of 5000 $\mu\text{g/mL}$ Hg^{2+} solution. Deoxygenation was achieved by purging the samples for at least 10 min with oxygen-free nitrogen (BOC Gases, Extra Dry). With the rotation rate set at 2000 rpm, the potential was held constant at -0.3 V for 10 min as recommended in the literature [35]. After the 10 min deposition period, the solution was allowed to become quiescent for 5 s and the potential ramped from -0.3V to 0 V using a square-wave waveform (20 mV pulse height, 50 Hz frequency) to strip any impurities from the TMFE. The plating solution was then removed and the TMFE visually inspected. Non-uniform films or films containing bubbles were wiped off and the TMFE reformed. The plating solution was then replaced with a deoxygenated solution of the 0.04 M acetate buffer. Any excess mercury was removed from the electrode by dipping it in the above solution of acetate buffer and rotating the electrode at 2000 rpm for 1 minute.

2.2.6 Formation of Nafion-Coated Thin Mercury Film Electrodes

Nafion-coated thin mercury film electrodes (NCTMFE) were formed using a method developed by Hoyer et al. [63], who reported that the formation of NCTMFEs with n,n-dimethylacetamide (DMAA) as a casting solvent provided higher stability without significant loss in analytical sensitivity compared to a normal TMFE.

Coating of Nafion was done by applying 1 μL of a 0.5% (w/w) Nafion solution and 1 μL of DMAA with a 10 μL Hamilton Microlitre syringe onto the GCE rotated at 1000 rpm. A test tube was then put over the electrode and the solvents evaporated under low heat using a heat gun. After the evaporation was complete the Nafion was cured in a 100°C air stream from the heat gun. After cooling to room temperature the TMFE was formed on the Nafion-coated electrode in the usual manner.

2.2.7 Experimental Procedure

Pseudopolarograms were recorded for model solutions of metal-fulvic acid complexes (where the metal was one of the metals: Cu, Pb or Cd), and also for samples of Rideau River surface water. The procedure was started with the deposition of metal at -0.1 V at 2000 rpm rotation rate for 120 s for the model solutions, and for 500 s for the river surface water. After a 10 s quiescent period, the metal was stripped using linear sweep voltammetry (LSV), differential pulse polarography (DPP), or square-wave voltammetry (SWV) and the peak recorded. The deposition voltage was then increased -20 mV to -100 mV, and the deposition procedure repeated. Smaller increases in the deposition potential were used when approaching the expected half-wave potential of metal-complex. A limit for the deposition potential was set at -1.5 V vs Ag/AgCl due to the possible reduction of H_2O and data points for the pseudopolarogram were retrieved up to this voltage.

The data treatment described by Brown et al. [42], and more recently, by Vega et al. [43] was used to process the pseudopolarograms. First, the electrode reaction was checked for reversibility by plotting the deposition potential E_d vs. the $\log [(i_L - i) / i]$, where i_L was

the limiting current and i the peak current generated at the particular E_d . For a reversible electrode reaction, a straight line with a slope of $59.16/n \text{ mV}^{-1}$ is predicted, whereas curves having slopes different from the expected values were indicative of irreversible electrode reactions. $E_{1/2}$ values for irreversible systems were estimated by extrapolating the potential to that corresponding to one-half of the limiting current [42]. The estimated errors in these measurements were 1% for reversible systems and 3%-5% for irreversible systems [42].

2.3 Results and Discussion

2.3.1 The Effective Surface Area of the Electrode

The effective surface area of the TMFE can be found by using the Levich equation:

$$Q_0 = I_0 t = \frac{n F A D^{2/3} C^* \omega^{1/2}}{1.61 \nu^{1/6}} t \quad (2.4)$$

where n is the number of electrons involved in the electrode reaction, C^* is the bulk concentration, D is the diffusion coefficient of the electroactive species, F is the Faraday constant, A is the exposed surface area of the electrode, ω is the angular velocity of rotation (rad s^{-1}), and ν is the kinematic viscosity (viscosity/density). A more detailed explanation of the Levich equation can be found in section 3.2.1. The effective surface area of the TMFE was determined using a standard solution of lead. The charge Q_0 was measured as a function of the rotation rate of RDE, and the values of Q_0 vs. $\omega^{1/2}$ were plotted (Figure 1). Using the Levich equation (2.4) and the literature value of $7.50 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ [67] for the diffusion coefficient for Pb^{2+} ion, values for the effective surface area of the electrode

were determined under various plating conditions (Table 1). In all cases, the effective surface area was considerably smaller than the geometric surface area (which was 0.2826 cm²), typically about one quarter of the geometric area. A previous study using microscopic examination has shown that at a deposition potential of -1.2 V, an average of 29 Hg drops were formed with a diameter of 1.0×10^{-3} mm over an area of 2.07×10^{-4} mm² [39]. Assuming the Hg drops are of uniform radius and hemispheres [38] this works out to be approximately 23% of the geometric surface area, which agrees well with the results of this work.

The relative standard deviations over the duration of 4 days of this study was less than 3%, indicating that the Hg films were reproducibly formed when the experimental conditions were kept the same. Table 1 also shows that larger deposition potentials give smaller effective surface areas. This may be due to H₂ evolution which disrupts the formation of the Hg film [35]. Table 1 also shows that, the Nafion-coated electrode has a lower effective surface area.

2.3.2 Effects of Organic Matter

It is well known that organic matter interferes with voltammetry because adsorption of organics on the mercury film the covering electrode surface [34]. This fouling results in decreased peak currents, asymmetric peaks, shifts in peak position, and in some cases, complete electrode failure [36, p.106]. Furthermore, some organic substances in freshwaters are naturally-occurring complexants and may determine the trace metal speciation, which will also result in changes in peak current (i_p) and peak potential (E_p).

Table 1 - The effective surface area of thin mercury films. Experimental conditions: LSV stripping mode, scan rate: 100 mV/s, deposition time 600 s, The results are averages of at least 4 replicates.

Deposition Potential V vs. Ag/AgCl	Surface Area (cm ²)	Standard Deviation (cm ²)
-0.3	0.10	0.016
-0.7	0.094	0.022
-1.2	0.089	0.014
-0.7 (Nafion-coated)	0.075	0.0040

Figure 1 - Determination of the effective surface area of TMFs. Experimental conditions: LSV stripping mode, scan rate: 100 mV/s, deposition time 600 s. Sample contained 9.66×10^{-8} M Pb^{2+} in a model solution; ionic strength fixed at 0.12M using an acetate buffer (pH 4.7 ± 0.1). Solid line represents the result of linear regression.

As both of these effects are dependent on some of the same parameters such as pH and ionic strength, it is often difficult to distinguish between the two phenomena.

Figure 2 shows the effect of fulvic acid (an organic complexant) on the RDEV/ASV signal. Peak currents were measured in model solutions of Zn, Cd or Pb containing fulvic acid in aqueous solutions using various SWV frequencies. The change in peak current in ASV/SWV at a TMFE should be linear with respect to the frequency [68]:

$$\Delta i_p = (2.874 \times 10^4) n A C_o^* l f \quad (2.5)$$

where i_p is the peak current, l the thickness of the mercury film and f is the frequency. All other terms are as defined earlier.

Figure 2 shows that with increasing concentration of fulvic acid, the curves for all of the elements deviate from linearity. Although this deviation is severe for zinc, cadmium and lead seem to be less affected. Figure 2 shows that if a frequency of <50 Hz is used, the deviation from linearity in the curves for cadmium and lead in the presence of fulvic acid is relatively small. However, the interpretation of Figure 2 is complicated by the fact that FA is also an organic, polyfunctional, complexant besides being a surface-active substance. To investigate the effects of organic matter further, standard additions of lead were made to the model solutions of fulvic acid, i.e., the method of standard additions was adopted for titration. Figure 3 a-c shows some results of this study. At low concentration of fulvic acid, (Figure 3a), the analytical response was not significantly different from that of a sample containing no fulvic acid. In the plot for the titration of the solution containing 20 µg/mL of FA, there were two distinct areas in the curve. The first

region, near the beginning of the titration, showed a lower slope, indicating the expected contribution of complexation and or adsorption when the FA was below the saturation point of all fulvic acid sites (bulk solution FA sites + adsorbed FA sites). The second region, after the saturation point, showed a linear response to the added lead. These two regions of the titration curve were also noted by others to be due to the metal titration corresponding to the formation of two distinct metal complexes with the break point indicating where $[M] = [L]$ [69]. These two regions in the titration curve were also observed for the titration of a sample of a Rideau River surface water (Figure 3c).

In amperometric titrations (as in the case in Figure 3), where the metal ion is added direct to the solution, both the bulk solution metal concentration ($[M_t]$) and the electrode surface metal concentration ($[M_0]$) are changed. To study the complexation of adsorbed complexes, Buffle has described the use of varying deposition time (t_d) for this investigation [38 p.555-562]. In these experiments, $[M]_t$ is held constant and t_d is varied, so that only $[M]_0$ is changed. This is because a longer deposition time will cause more metal to diffuse to the electrode surface. In this way, one can titrate the adsorbed FA sites and the “break-point” of the $i_p = f(t_d)$ curve will correspond to the saturation of the adsorbed sites. Figure 4, Figure 5 and Figure 6 show the results of the study, using Tl(I), Pb(II) and Cd(II) as titrants for a model solution containing 20 $\mu\text{g/mL}$ of Armadale FA and the filtrate of a Rideau River surface water sample that has been filtered through a

Figure 2 - Effect of SWV frequency changes on peak current in model solutions containing FA. ▲ - 1.53×10^{-8} M Zn, ■ - 8.90×10^{-8} M Cd, ● - 4.83×10^{-8} M Pb. A - no FA. B - 4 $\mu\text{g/mL}$ FA. C - 8 $\mu\text{g/mL}$ FA. D - 20 $\mu\text{g/mL}$ FA.

Figure 3 - Titration of FA Model Solutions and a 0.45 µm filtered Rideau River Surface Water with a Pb(II) Standard Solution. ■ - Additions of Pb(II) to acetate buffer solution only (Control). ● - Additions of Pb(II) to samples. A - sample contained 4 µg/mL FA. B sample contained 20 µg/mL FA. C - 0.45 µm filtered Rideau River Surface Water.

Figure 4 - Effect of varying deposition time on the peak current and potential for Tl complexes. Experimental conditions: $[Tl] = 2.82 \times 10^{-8} \text{ M}$, SWV for stripping, $f=50 \text{ Hz}$, pulse height = 25 mV, $\omega = 2000 \text{ rpm}$, ionic strength of the samples was fixed at $I=0.06 \text{ M}$ with acetate buffer pH 5.1. For all graphs, ● - Peak current. ■ - Peak potential. **A** - Tl in buffer solution only, **B** - Tl with 20 $\mu\text{g/mL}$ FA, **C** - Tl in 0.45 μm filtered Rideau River surface water.

Figure 5 - Effect of varying deposition time on the peak current and potential for Pb complexes. Experimental conditions: $[Pb] = 4.85 \times 10^{-8} \text{ M}$, SWV for stripping, $f=50 \text{ Hz}$, pulse height = 25 mV, $\omega = 2000 \text{ rpm}$, ionic strength of the samples was fixed at $I=0.06 \text{ M}$ with acetate buffer pH 5.1. For all graphs, ● - Peak current. ■ - Peak potential.

A - Pb in buffer solution only, B - Pb with 20 µg/mL FA, C - Pb in 0.45 µm filtered Rideau River surface water.

Figure 6 - Effect of varying deposition time on the peak current and potential for Cd complexes. Experimental conditions: $[Cd] = 3.55 \times 10^{-8} \text{ M}$, SWV for stripping, $f=50 \text{ Hz}$, pulse height = 25 mV, $\omega = 2000 \text{ rpm}$, ionic strength of the samples was fixed at $I=0.06 \text{ M}$ with acetate buffer pH 5.1. For all graphs, ● - Peak current. ■ - Peak potential. A - Cd in buffer solution only, B - Cd with 20 µg/mL FA, C - Cd in 0.45 µm filtered Rideau River surface water.

0.45 μm filter. Tl(I) was chosen initially as a titrant because it was expected to form very weak bonds with FA resulting in formation of weak complexes [79]. The $i_p = f(t_d)$ curves for the metals in all the matrices show no indication of break points. This is confirmed in Table 2 where r-squared values for the regression analysis of the $i_p = f(t_d)$ curves is <0.99 in all cases. This observation suggests that there is no formation of metal complexes with the adsorbed organic complexants on the electrode surface. This is probably because of the employment of fast-scan SWV. Oxidized metals close to the electrode surface are quickly re-reduced by the square-wave waveform and do not have time to become complexed by the adsorbed organic matter.

It is expected that titrations of polyfunctional ligands proceed with the binding of the strongest available sites first. This gives a $E_p = f(t_d)$ curve with a decay-type shape due to shifts in E_d to more positive potentials as t_d and hence $[M]_0$ is increased. Since these experiments were performed in matrices containing organic, polyfunctional complexants, the results obtained suggest that under these experimental conditions the metal ions do not form complexes with the adsorbed organic complexants as shown by the straight $E_p = f(t_d)$ plots. Since most freshwaters less than 20 $\mu\text{g/mL}$ of dissolved organic carbon [20], it can be concluded from the above results that these measurements are not affected by adsorbed organic matter in most freshwater samples. However, the lowered i_p values and E_p shifts encountered with the samples containing organic matter when compared to the controls (without organic matter) indicate that adsorption of organics still contribute to the voltammetric signal. Buffle et al. have suggested that in D.C. polarography with an HMDE, adsorbed FA onto the HMDE may influence charge transfer processes through

Table 2 - Effects of variation in deposition time of metal complexes on TMFE. See Figures 5-7 for detailed experimental conditions.

Metal	Sample Composition	Slope of i_p vs t_d ($\mu\text{A/s}$)	R-squared value
Tl(I)	Acetate buffer solution only	0.036	.9985
Tl(I)	Acetate buffer + FA	0.032	.9984
Tl(I)	Acetate buffered Rideau River surface water	0.030	.9995
Cd(II)	Acetate buffer solution only	0.040	.9983
Cd(II)	Acetate buffer + FA	0.032	.9962
Cd(II)	Acetate buffered Rideau River surface water	0.033	.9950
Pb(II)	Acetate buffer solution only	0.026	.9988
Pb(II)	Acetate buffer + FA	0.010	.9985
Pb(II)	Acetate buffered Rideau River surface water	0.0098	.9980

an electric effect [18]. Since FA is a neutral or negatively charged species, the adsorption of FA onto the electrode surface will result in a negative surface potential, (which Buffle has described as ψ) which is expected to increase the value of the experimentally measured stability constant.

2.3.3 Complexation of Cadmium by Fulvic Acid in Model Aqueous Solutions

The effect of complexation of Cd^{2+} by FA in an aqueous model solution was studied using the Armadale fulvic acid described earlier. Also, the effect of applying a Nafion-coating on the performance characteristics of the Nafion-coated TMFE was investigated. Figure 7 shows that Nafion coating has no effect on the pseudopolarograms of the Cd-aquo complex if LSV waveform is used. Figure 8, obtained with the uncoated TMFE and SWV shows that the pseudopolarogram for the sample containing Cd and FA had two polarographic waves. The first wave has an $E_{1/2}$ almost identical with the $E_{1/2}$ of the Cd-aquo complex, and hence, it is most probably a Cd-aquo complex. The second wave with a half-wave potential shifted to a more negative potential is probably due to a Cd-FA complex. The results as shown in section 2.3.2 shows that the magnitude of the wave shift is probably exaggerated because of the adsorption of organic matter onto the surface of the electrode. The wave-height of this pseudopolarogram is lower than that of the pseudopolarogram of Cd^{2+} alone. Since the wave-height represents the amount of Cd that is reduced at the electrode, the decreased wave-height is probably due to the fraction of the Cd that is bound to the strong sites of FA, forming strong complexes which are not dissociated nor are directly reduced at the applied potential. It is well known that FA consists of both strong sites (comprising ~ 3 -10% of the sites) and weak sites (comprising

the remaining ~90-97%) [70,71,72,73,74]. The complexation of Cd to the relatively large FA molecules will also cause a decrease in the diffusion coefficient, which will also decrease the current. Comparison of Figure 9, which was done using a Nafion-coated electrode with Figure 8 and does not show any significant difference in the pseudopolarograms for the Cd-aquo complex; however, there is a significant difference in those for the Cd-FA complex. Instead of the two polarographic waves observed in Figure 8, there is only one polarographic wave in Figure 9, with its $E_{1/2}$ about the same as the $E_{1/2}$ of the Cd-aquo complex. Nafion coating probably prevents the transport of the large, undissociated Cd-FA complex to the electrode, thereby eliminating the shift of $E_{1/2}$ to a more negative potential. The Nafion coating will also prevent adsorption of the organic matter which will eliminate the effect described in section 2.3.2. Table 3 shows the effect of the formation of Cd-FA complexes at a NCTMFE on the pseudopolarograms. For both the LSV and the SWV waveform, with the Cd^{2+} alone (i.e. without FA) there is no significant difference between the NCTMFE and the uncoated electrode. For the LSV waveform, the addition of 20 $\mu\text{g/mL}$ FA results in a pseudopolarogram with a single wave with a half-wave potential slightly shifted to a more negative potential relative to that of the pseudopolarogram of Cd^{2+} only. This Cd-FA complex with a log K of 1.92 is a very weak complex, which probably results from binding of Cd^{2+} by a weak site of FA (~90-97% of the total sites). For the LSV, the addition of FA to the Cd^{2+} solution decreased the peak current for both the NCTMFE and the uncoated electrode; the increasing amount of FA added to a constant amount of Cd^{2+} progressively decreased the peak current. This observation can be attributed to more and more Cd^{2+} being complexed by relatively large FA molecules causing a decrease in the diffusion coefficients and

Figure 7 - Pseudopolarograms of the Cd-aquo complex using LSV with NCTMFE and TMFE in model solutions containing 71.1×10^{-8} M Cd. ■ - using the NCTMFE ● - using the TMFE.

Figure 8 - Pseudopolarogram of Cd complexes containing 1.22×10^{-8} M Cd^{2+} in model solutions, using a TMFE and SWV. ●- Cd^{2+} with no FA, ■ - Cd^{2+} with 20 $\mu\text{g/mL}$ FA.

Figure 9 - Pseudopolarogram of Cd complexes containing 1.22×10^{-8} M Cd^{2+} in model solutions, using an NCTMFE and SWV. ●- Cd^{2+} with no FA, ■- Cd^{2+} with 20 $\mu\text{g/mL}$ FA.

Table 3- Results from the pseudopolarographic analysis of Cd-FA complexes. Experimental conditions- rotation rate:2000 rpm, scan rate: 100 mV/s. In LSV, deposition time was 240 sec. In SWV, deposition time 30 s, frequency 50 Hz, pulse height 25 mV. Ionic strength fixed at 0.12M using the acetate buffer (pH 4.7 $\pm$ 0.1).

Waveform	$[Cd^{2+}]$ $\times 10^8$ M	[FA] (μ g/mL)	Nafion Coated	Number of Waves	Relative Peak Current*		$\Delta E_{1/2}$	log K
					1st wave	2nd Wave		
LSV	71.1	0.0	Yes	Single wave	1.0	--	0.0	--
LSV	71.1	20	No	Single wave	0.60	--	-0.065	1.9
LSV	71.1	4.0	Yes	Single wave	0.88	--	+0.030	--
LSV	71.1	8.0	Yes	Single wave	0.58	--	+0.035	--
LSV	71.1	20	Yes	Single wave	0.48	--	+0.030	--
SWV	1.22	0.0	Yes	Single wave	1.1	--	0.0	--
SWV	1.22	20	Yes	Single wave	0.74	--	-0.011	--
SWV	1.22	20	No	Two waves	0.12	0.64	+0.012	3.5

* - These values measured relative to the values obtained using an uncoated electrode and the same stripping waveform and a sample containing the same concentration of Cd^{2+} only.

pH 8 and almost zero ionic strength. Starting with a low concentration of each metal ($\sim 10^{-8}$ M) simulating unpolluted freshwaters, and holding the concentration of the metal constant at the $\sim 10^{-8}$ M concentration, the concentration of the FA in three separate model solutions was increased to make the $[M] / [FA]$ ratio 0.0018, 0.018, and 0.18 in these solutions. The results of these studies have consistently shown that in the above solutions when the $[M] / [FA]$ ratio was 0.0018, 100% of all these four metals are bound to strong sites of FA, resulting in the formation of highly stable metal-FA complexes, which were non-labile [31,38,70,71,72,73,74,78 p.378]. When the $[M] / [FA]$ ratio was increased to 0.018 (holding the $[M]$ constant $\sim 10^{-8}$ M), a portion of the metal which were in excess of the strong binding sites of the FA became bound to weaker binding sites, forming weaker metal-FA complexes which were labile enough to be dissociated within the measurement time. The above observation can be explained by the fact that after all the strong sites of the FA are occupied by the metal, the remaining metal occupied some of the weak sites of the FA. With the increasing ratio of $[M] / [FA]$, progressively more of the FA weak sites are occupied by the metal, resulting in the formation of increasingly quantity of weak, labile metal-FA complexes, and hence, increasing dissociation of the metal-FA complex. The complexation will also cause a progressive decrease in the diffusion coefficient which will also decrease the wave-height.

2.3.4 Complexation of Lead by Fulvic Acid in Model Aqueous Solutions

Table 4 shows results of the Pb-FA system. There is no significant difference between the Nafion-coated and the uncoated electrode in the speciation of the Pb^{2+} without FA. However, for the uncoated electrode, the addition of increasing amounts of FA from 0 to

20 $\mu\text{g/mL}$ to a constant amount of $9.66 \times 10^{-8} \text{ M Pb}^{2+}$ caused a progressively smaller relative peak current. This effect has been explained earlier in discussing similar results with the binding of Cd^{2+} by FA. The pseudopolarogram for the NCTMFE (Figure 10) showed only a single polarographic wave corresponding to the Pb-aquo complex, whereas that for the uncoated electrode (Figure 11) showed two polarographic waves for both curves; the first wave was probably for the Pb-aquo complex, the second wave was probably for a strong Pb-FA complex, and was long-drawn-out (irreversible electrode reactions) for both curves. In Figure 10, the log K values for the Pb-FA complexes for the 8 $\mu\text{g/mL}$ [FA] and the 20 $\mu\text{g/mL}$ [FA] were 20 and 21, respectively. The difference between these two log K values may not be significant, considering the experimental uncertainty in the visual estimation of the $\Delta E_{1/2}$ values from which the log K values were determined. Probably, these two log K values correspond to the same Pb-FA complex, a strong complex formed by strong binding sites ($\sim 3\text{-}10\%$ of the total sites) of FA. It should be noted that the estimate of these stability constants should be used only for qualitative purposes. When dealing with heterogeneous, organic systems such as FA, many assumptions in the derivation of equation 2.2 may not be true. For example, the theory requires that the electrode reactions to be reversible and that the diffusion coefficient of the metal is approximately equal to the metal complex, i.e. $D_M \sim D_{ML}$. These conditions are almost never encountered in freshwater systems. This coupled with adsorption effects and the heterogeneous nature of FA makes the direct relationship between the stability constant and the $\Delta E_{1/2}$ values troublesome.

Comparison of Figure 10 with Figure 11 shows the advantage of the NCTMFE over the uncoated electrode for obtaining well-defined pseudopolarograms. In Figure 11, the smearing effect on the second waves, which makes the estimate of $E_{1/2}$ difficult, was due to both the heterogeneity of the FA ligand which provided a wide range of stability constants, resulting in the $E_{1/2}$ being an estimate of the weighted average of all the metal-FA complexes and not of a single complex and the adsorption of organic matter on the surface of the electrode.

2.3.5 Pseudopolarographic Analysis of a Rideau River Surface Water Sample

Figure 12 shows a pseudopolarogram of Cd^{2+} in a sample of Rideau River surface water. The total concentration of Cd was determined by Zeeman graphite furnace atomic absorption spectrometry to be 5 ng/mL ($\sim 4 \times 10^{-8}$ M). The pseudopolarogram obtained using the uncoated TMFE shows a single distinct polarographic wave with a half-wave potential similar to that of Cd-aquo complex. A second polarographic wave may exist between -1.1 V and -1.3 V, but the increase in the peak current is not sufficient enough to warrant an unambiguous conclusion. The pseudopolarogram for the NCTMFE was, as expected, noticeably different. Two distinct polarographic waves were evident; the first, with a half-wave potential similar to that of the Cd-aquo complex, and the second, with a half-wave potential 0.46 V more negative than that of the first. This, from equation (2.2), gives an estimate for log K of ~ 15 for the Cd complex. Since Nafion coating is expected to exclude relatively large organic complexes from the TMFE, the Cd complex producing the second wave is probably due to direct reduction of a relatively small Cd-organic complex and/or Cd^{2+} ions formed by dissociation of the Cd-complex. Anion-exchange

chromatography showed that Rideau River surface water contained $\sim 10 \mu\text{g/mL Cl}^-$ and $5\text{-}10 \mu\text{g/mL SO}_4^{2-}$. Since these inorganic ligands form only weak complexes with Cd^{2+} [79], the observed Cd-complex was probably formed by small organic ligands. This is confirmed in Figure 13 for Pb, where pseudopolarograms of Pb with $10 \mu\text{g/mL Cl}^-$ and Pb with $10 \mu\text{g/mL SO}_4^{2-}$ in model aqueous solutions show only single, well-defined waves with half-wave potentials similar to that of a pseudopolarogram of the model solution containing small, labile Pb complexes. The large difference between the limiting currents of the two curves in Figure 12 can then be attributed to large, Cd-organic complexes. Such Cd complexes will have smaller diffusion coefficients and can be reduced directly in the case of an uncoated TMFE, resulting in a larger peak current but are excluded by the Nafion coating, and hence, are not available for electrode reduction, whereas large Cd-organic complexes could not pass through the Nafion coating to reach the electrode surface. Figure 13 shows pseudopolarograms of the sample of Rideau River surface water (spiked with Pb) using both a Nafion-coated electrode and an uncoated electrode. Spiked samples were used because the total lead that was present in the Rideau River surface water was below the detection limit of lead by Zeeman graphite furnace atomic absorption spectrometry ($<10^{-8} \text{ M}$). The uncoated electrode gave a pseudopolarogram having two polarographic waves. The second wave (with the uncoated electrode) was very similar to the waves observed in Figure 11. In Figure 13, this wave was long-drawn-out and ill-defined, with a half-wave potential 0.42 V more negative than that of the Pb-aquo complex, giving a stability constant of $\log K \sim 14$ for the complex. This estimate can however be improved by using the half-wave potential from a Pb-aquo complex ($\bullet$ in Figure 13) as the $E_{1/2}$ for the first wave. The resulting estimate for the

Figure 10 - Pseudopolarogram of Pb complexes containing 9.66×10^{-8} M Pb^{2+} in model solutions, using an NCTMFE and SWV. ■ - Pb^{2+} with no FA, ● - Pb^{2+} with 4 $\mu\text{g/mL}$ FA, ▲ - Pb^{2+} with 20 $\mu\text{g/mL}$ FA.

Figure 11 -Pseudopolarogram of Pb complexes containing 9.66×10^{-8} M Pb^{2+} in model solutions, using an uncoated TMF and SWV. ● - Pb^{2+} with no FA, ■ - Pb^{2+} with 4 $\mu\text{g/mL}$ FA, ▲ - Pb^{2+} with 20 $\mu\text{g/mL}$ FA.

Figure 12 - Pseudopolarogram of Cd species in a Rideau River surface water sample using SWV. Samples were buffered in 0.12M acetate buffer, pH 4.7 ± 0.1 . ■ - Using an uncoated TMFE, ● - Using an NCTMFE.

Figure 13 - Pseudopolarograms of Pb species in model solutions and in Rideau River surface water containing 9.66×10^{-8} M Pb^{2+} using NCTMFE and TMFE and SWV. Samples were buffered in 0.12M acetate buffer, pH 4.7 ± 0.1 . ● - Pb^{2+} only in a model solution using an uncoated TMFE, ■- Pb^{2+} and 10 $\mu\text{g/mL}$ Cl^- in a model solution using an uncoated TMFE, ◆ - Pb^{2+} and 10 $\mu\text{g/mL}$ SO_4^{2-} in a model solution using an uncoated TMFE, ▲ - Pb^{2+} in Rideau River surface water using an uncoated TMFE, ▼- Pb^{2+} in Rideau River surface water using an NCTMFE.

stability constant becomes 11 for the complex. The pseudopolarogram obtained using the Nafion-coated electrode was smaller than that from the pseudopolarogram obtained using the uncoated electrode. The first wave was well-defined and had a half-wave potential close to those found in Figure 11 and Figure 10 for the Pb-aquo complex. A small second wave was also evident, and was probably due to small-sized Pb-complexes which had crossed the Nafion coating barrier. Since sulphonate exchange sites, exchangeable counter-ions and sorbed water exist as ion clusters throughout Nafion coating with a spacing distance of about 50 Å [80] the Pb-complexes (which are probably Pb complexes of organic complexants) which cross the Nafion coating barrier are expected to be relatively small-sized. Also, the sulphonate groups on Nafion are negatively charged [62]; hence, these Pb-complexes must be neutral or positively-charged in order to cross the Nafion coating barrier. The wave for this Pb-complex was long-drawn-out, probably the result of a continuum of lead complexes (of similar stability) with heterogeneous, polyfunctional, polyelectrolytical organic complexants (e.g. humic compounds) which are ubiquitous in freshwaters [38,33,59]. Because of the close similarity of the stability constants of these lead complexes, they probably represent a continuum of lead complexes; hence, the long-drawn-out shape of the pseudopolarogram.

The Rideau River surface water was subjected to cascade ultrafiltration to investigate the metal complexes present in different size fractions in the river surface water. Each size fraction was spiked to contain 1.8×10^{-8} M Cd and the resulting pseudopolarograms were compared with a control sample (a model aqueous solution) containing the same amount of Cd in the acetate buffer solution, fixed at the same ionic strength, and the pH as those

of the fractions. Peak currents were then normalized to that of this control so that results for NCTMFE and TMFE could be compared. In Figure 14, using the TMFE, two polarographic waves were observed: one relatively sharp wave, corresponding to the Cd-aquo complex and / or very weak inorganic and/or organic complexes of Cd, and a long, drawn-out wave similar to the waves observed with model solutions of FA. The pseudopolarograms for the filtrate of the 0.45 μm filtered and <50,000 MWCO fractions were virtually identical and thus there is no significant difference between the two fractions. At the <10,000 MWCO fraction, the current for Cd increased for both the weak and strong Cd complexes. This seems reasonable, as a majority of the organic complexes >10,000 MW is expected to have been removed by the ultrafiltration procedure and would be unavailable for complexing with the cadmium. However, in the <1000 MWCO fraction, the wave-height decreases. There does not seem to be a reasonable explanation for this observation; however, the wave profile seems to be almost identical to that of the 10,000 MWCO fraction, indicating that the cadmium was complexed by the same type of ligands in the 1000 MWCO fraction.

The results for Cd complexes in Rideau River surface water fractions obtained by using the Nafion-coated electrode are presented in Figure 15. The results are markedly different when compared with those of the uncoated electrode. In the 0.45 μm membrane filtered fraction, only one long, drawn-out polarographic wave at high, negative potential is present. The sharp wave, normally present at potentials associated with weak Cd complexes is completely absent. The decrease in wave height for the NCTMFE relative to that of the TMFE may be due to the fact that the Nafion coating prevents large and

charged Cd complexes from reaching the surface of the electrode, thereby preventing them from being reduced. Also, unlike the results for the uncoated electrode, in which the pseudopolarograms for the 0.45 μm and <50,000 MWCO fractions were virtually identical, there is a large increase in wave-height for the <50,000 MWCO fraction relative to that of the 0.45 μm fraction. The 1000 MWCO fraction was not analyzed because of the lack of sufficient sample.

Although the Nafion-coated electrode is able to differentiate between the river surface water fractions more clearly than the uncoated electrode, the advantages of NCTMFES for metal speciation in real river water samples, are less certain than in model fulvic acid solutions. However, a clearer understanding of metal speciation in the river surface water samples can be obtained when results from both Nafion-coated electrodes and uncoated electrodes are compared. The similarity of the pseudopolarograms for the 0.45 μm and 50,000 MWCO fractions of the uncoated electrode would suggest that the similarity is due to the presence of similar Cd complexes. However, the results of the same ultrafiltered fractions obtained with the Nafion-coated electrode clearly demonstrate that they are quite different. The data from the Nafion-coated electrode suggest that the large molecular-weight species in the 0.45 μm fraction are being excluded by the Nafion coating while the smaller molecular-weight species in the 50,000 MWCO fraction are allowed to cross the Nafion coating barrier. This effect may be enhanced by preconcentration of metal ions in the case of the Nafion-coated electrode because of the presence of the sulphonic groups of Nafion coating in the 50,000 MWCO fractions. When taken together, the results suggest that although the composition of the 0.45 μm

and 50,000 MWCO fractions are quite different, they possess very similar Cd complexes. Conditional thermodynamic stability constants for Cd-river water complexes using both the Nafion-coated and the uncoated electrode are presented in Table 5 and Table 6. The Nafion-coated and uncoated electrodes identified two sets of Cd complexes: the Cd-aquo complex or very weak Cd complexes and a series of strong FA-bound forms very similar in thermodynamic stability. As for the FA model solutions, these stability constants are only rough estimates and should be used only for qualitative interpretation.

Table 5 - Conditional stability constants of Cd complexes in Rideau River surface water. Without Nafion coating. Experimental conditions: $[Cd]=1.8 \times 10^{-8}$ M, SWV for stripping, rotation rate: 2000 rpm, deposition time 120s, frequency 50Hz, pulse height 25mV, NaOAc/HOAc buffer (pH=5.3, I=0.06 M).

Fraction	1 st wave	2 nd wave
Size	log K_1	log K_2
0.45 μ m filtered	1.4	17
50,000 MWCO	1.0	19
10,000 MWCO	1.0	16
1000 MWCO	1.4	18

Table 6 - Conditional stability constants of Cd complexes in Rideau River surface water. With Nafion coating. Experimental conditions: $[Cd]=1.8 \times 10^{-8}$ M, SWV for stripping, rotation rate: 2000 rpm, deposition time 120s, frequency 50Hz, pulse height 25mV, NaOAc/HOAc buffer (pH=5.3, I=0.06 M).

Ultrafiltered	1 st wave	2 nd wave
Fraction	log K ₁	log K ₂
0.45 µm filtered	n/a	22
50,000 MWCO	2.0	22
10,000 MWCO	1.4	22

Figure 14 - Pseudopolarograms of Cd complexes in fractionated Rideau River surface water samples using a TMFE. Experimental conditions: $[\text{Cd}] = 1.8 \times 10^{-8} \text{ M}$, deposition time: 120 s, SWV for stripping, $f = 50 \text{ Hz}$, pulse height = 25 mV, ionic strength for the fractions were fixed at $I=0.06 \text{ M}$ with acetate buffer pH 7.7. ● - Cd^{2+} only. ▲ - 1000 MWCO. ▼ - 10,000 MWCO. ◆ - 50,000 MWCO. ■ - 0.45 μm filtered.

Figure 15 - Pseudopolarograms of Cd complexes in fractionated Rideau River surface water samples using a NCTMFE. Experimental conditions: $[\text{Cd}] = 1.8 \times 10^{-8} \text{ M}$, deposition time: 120 s, SWV for stripping, $f = 50 \text{ Hz}$, pulse height = 25 mV, ionic strength for the fractions were fixed at $I=0.06 \text{ M}$ with acetate buffer pH 7.7. ● - Cd^{2+} only. ▼ - 10,000 MWCO. ◆ - 50,000 MWCO. ■ - 0.45 μm filtered.

3. Determination of Kinetic Speciation Parameters Using Rotating Disk Electrode / Anodic Stripping Voltammetry

3.1 Introduction

Shuman and Michael reported the application of rotating disk electrode voltammetry (RDEV) with a thin mercury film electrode (TMFE) combined with anodic stripping voltammetry (ASV) for measuring dissociation kinetics of metal complexes in natural waters [81]. They applied RDEV/ASV to the measurement of rate constants for copper complex dissociation in marine coastal water samples by spiking copper ions into samples, and reported a first-order dissociation rate constant for the copper complex of the order of 2 s^{-1} [33].

A disk-shaped electrode rotating in a liquid has an important property: it is universally accessible to the sample solution. This makes a rotating disk electrode a unique tool for investigating heterogeneous kinetics of electrochemical reactions at solid electrode surfaces. The use of rotating disk electrode voltammetry (RDEV) as a kinetic tool has a distinct advantage: the current at the RDEV very rapidly assumes a steady-state value. RDEV is ideal for investigations in non-equilibrium situations (which are common in the aquatic environment), where kinetics of dissociation of metal complexes and their diffusion coefficients determine their availability at biological surfaces. Freshwaters are systems often far removed from chemical equilibrium. Rotating disk electrode voltammetry in conjunction with anodic stripping voltammetry (RDEV/ASV) has been used for trace metal speciation because of its high sensitivity and its ability to differentiate between operationally-defined labile and nonlabile metal species [33]. This distinction is important as the ASV-labile metal fraction is considered to be bioavailable and can be potentially toxic to organisms [36, p.116]. The power of this technique resides

in its ability to measure both equilibrium concentrations and kinetic availability of soluble metal in an aqueous environment and should be useful for studies of metal toxicity. Because of its extremely high analytical sensitivity, RDEV/ASV has the capability for direct (without pre-concentration) determination of trace elements in natural waters with minimal sample treatment so that chemical equilibrium is not appreciably disturbed; this capability for direct determination makes RDEV/ASV an obvious candidate for inclusion in a comprehensive scheme for trace metal speciation in natural waters [22,23]. It has been used for determination of rate constants of metal complexes and their diffusion coefficients in river surface water [23] and in model solutions and in snow [22].

3.2 Theory

3.2.1 Rotating Disk Electrode Voltammetry

Consider a metal ion M^{2+} and a ligand L^{2-} forming a metal complex, ML , in the bulk solution. Assume that at the applied potential, before ML can be reduced at the mercury film electrode, it must dissociate with a dissociation rate constant, k_d , and that the rate of equation (3.2) is much faster than that of equation (3.1).

For the electrode potentials at which the concentration of the electroactive species, M^{2+} , at the electrode surface is zero (i.e., when the rate of reduction is much faster than the rate of dissociation of the metal complex at the electrode surface), the quantitative relationship between the limiting current, I_0 , and the rotation rate may be expressed by the Levich equation [82,83]:

$$I_0 = \frac{n F A D^{2/3} C^* \omega^{1/2}}{1.61 \nu^{1/6}} \quad (3.3)$$

where n is the number of electrons involved in the electrode reaction, C^* is the bulk concentration, D is the diffusion coefficient of the electroactive species, F is the Faraday constant, A is the exposed surface area of the electrode, ω is the angular velocity of rotation (rad s^{-1}), and ν is the kinematic viscosity (viscosity/density). A typical value of ν for an aqueous solution is $10^{-2} \text{ cm}^2 \text{ s}^{-1}$, which has been adopted for this thesis [22]. The above derivation has been made on the assumption that $D = D_M = D_{ML}$. For anodic stripping voltammetry, it is more convenient to use the stripping peak area Q_0 in coulombs, which equals the integration of the limiting current over the electrodeposition time period t .

$$Q_0 = I_0 t = \frac{n F A D^{2/3} C^* \omega^{1/2}}{1.61 \nu^{1/6}} t \quad (3.4)$$

Kinetic information on the dissociation of metal complex ML can be obtained by using the equation given by Hale [84] (which is solved by numerical approximation), or by using a linearized approximation given by Koutecký and Levich [85]. The latter yields useful results only when the rates of metal complex dissociation are high, whereas the former is applicable for all conditions [22]. The limiting current observed at a RDEV is determined by the maximum rate of supply of the electroactive species to the electrode surface, and is characterized by a zero concentration, C , of the metal ion at the electrode surface, i.e.,

$$C = C^s = 0 \text{ at } x = 0 \text{ and } t > 0$$

where t is the time, x is the distance normal to the plane of the disk, and C^s is the concentration of the electroactive species at the electrode surface. There is also an initial condition

$$C = C^* \text{ at } t = 0, x \geq 0$$

and the usual condition for the bulk concentration:

$$C = C^* \text{ at } x \rightarrow \infty, t \geq 0$$

Because of the uniform accessibility of the RDEV surface and because both diffusion and convection are of similar importance, the convective-diffusion equation in one-dimensional form can be used [86]:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - V \frac{\partial C}{\partial x} \quad (3.5)$$

where V is the velocity of the stirred fluid in the direction normal to the plane of the disk.

For a 1:1 metal complex formed between a divalent metal ion, M , and a ligand, L , the dissociation of ML and the subsequent reduction of the metal ion during the deposition step (assuming that the complex itself is not reduced at the applied potential) are expressed as equations (3.1) and (3.2). The electrodeposited metal during the electrolysis step is contributed by the free metal ions already existing in the solution and/or are generated by the dissociation of the metal complex, ML . If the overall reaction is controlled by equation (3.1), i.e., when the dissociation reaction of ML is slow and the electron transfer reaction (3.2) is fast, the overall rate is that of the slow step, i.e., the dissociation of the ML complex.

From a theoretical treatment of hydrodynamic voltammetry, Koutecký and Levich [85] derived the following equation to be henceforth called the Koutecký-Levich equation:

$$\frac{I_o}{I_k} = 1 + \frac{D^{1/6} \omega^{1/2}}{1.61 [K(1 + K)]^{1/2} k_d^{1/2} \nu^{1/6}} = \frac{Q_o}{Q_k} \quad (3.6)$$

where:

K is the concentration ratio of the uncomplexed metal to the complexed metal ($K = [M]/[ML] = k_d/k_f[L]$)

Q_k is the stripping peak area (coulombs) representing the metal accumulated at the disk the reduction of the kinetically labile ML complex

Q_o is the stripping peak area in coulombs representing the metal accumulated by reduction of both $ML + M(II)$, i.e. for the reduction of total metal M_T

The Koutecký-Levich equation (3.6) [85] provides an approximate value of the rate constant k_d ; the error analysis made by Hale [84] shows that the reaction rate must be high for the k_d value to be correct. This restriction is removed by Hale [84] by using a numerical method. In the Hale method [84], a rate parameter λ is defined by:

$$\lambda = (1 + K) k_f \delta^2 / D \quad (3.7)$$

Where δ is the thickness of the Nernst diffusion layer given by:

$$\delta = 1.61 D_0^{1/3} \omega^{-1/2} \nu^{1/6} \quad (3.8)$$

A better approximation at small values of λ is:

$$\frac{I_d}{I_k} (1 + K) - K = \frac{1}{\sqrt{\lambda}} \tanh \sqrt{\lambda} \quad (3.9)$$

and

$$I_d = n F A D [M] / \delta. \quad (3.10)$$

the use of the ratio Q_k/Q_o for the calculation mostly cancels out the effect of the re-reduction of a part of metal ions on the estimation of K and k_d .

Analysis of the data is made by fitting the experimental data to either equation the Koutecký-Levich equation (3.6), or equation (3.13) (the Hale equation), in both of which the complex dissociation (equation 3.1) is assumed to be a first-order or pseudo-first-order reaction.

For the estimation of K and k_d , the simplest case is when there is only one metal complex present in the sample; the values of K and k_d can then be obtained by direct fitting of the experimental data to equation (3.6) or equation (3.13). However, in freshwaters, metals may exist as more than one complex. Theoretically, the contribution of each complex to the total stripping current is additive and a simple graphical procedure can be used to separate the contribution of the slower from the faster dissociating complex [87].

In general, Q_k increases as k_d and K increase because of increased availability of M to the electrode, and decreases as the rotation rate is increased because of less time is then available for dissociation. The data are plotted as the ratio Q_k/Q_o , or its inverse, as a function of the rotation rate. When Q_k/Q_o is plotted against $\omega^{1/2}$, $Q_k/Q_o \rightarrow 1.0$ as $\omega \rightarrow 0$ because at extremely slow rotation rates all complexes have time to dissociate. At high rotation rates, there is no time for dissociation, and only very labile metals complexes are reduced at the disk. Therefore, $Q_k/Q_o \rightarrow [M]/([M] + [ML]) = K/(K+1)$ as $\omega \rightarrow \infty$. Assuming only the metal-aquo complex value can dissociate $\omega \rightarrow \infty$, K is understood then as the ratio

of the amount of the metal-aquo ion to complexed metal and is related to the limiting value of Q_k/Q_o by $Q_k/Q_o = K/(K+1)$. As the rotation rate increases, less time is available for the metal complex to dissociate as it flows across the electrode surface, but the mass flux from the solution to the electrode surface increases with increasing rotation rates. At high rotation rates, there is no time for dissociation of the slowly-dissociating complex, and at the limit of high rotation rates, only the metal-aquo complex is reduced (it being assumed that the applied potential is not high enough for direct reduction of the metal complex, ML). According to equation (3.13), for a complex with a large dissociation rate constant, k_d , the plot of Q_o/Q_k versus $\omega^{1/2}$ should be a straight line with an intercept of 1.0. Hence, the slope of Q_o/Q_k versus $\omega^{1/2}$ at high rotation rates can be considered to be due to the fast-dissociating complex only. The value of Q_o/Q_k for the fast-dissociating complex is transformed by moving the linear portion in a parallel fashion to the position where its extrapolation to the ordinate equals 1.0. Considering that the composite Q_o/Q_k is a simple sum, minus 1.0, of the contributions of the two complexes, the value of Q_o/Q_k for the slowly-dissociating complex is obtained by subtracting the transformed values of Q_o/Q_k for the fast-dissociating complex from the composite value of Q_o/Q_k plus 1.0.

For systems exhibiting more than one polarographic wave, integration of the current of the first polarographic wave gives the charge passed for the stripping of the metal-aquo complex (Q_k) and the integrated stripping current (Q_o) of the second polarographic wave is assigned to the stripping of $M^{2+} + ML$. At very high rotation rates of the RDE, the metal complexes have no time for dissociation; hence, only the metal aquo complex is reduced at the electrode. The K ($= [M] / [ML]$) is evaluated as follows [33]:

$$\frac{Q_k}{Q_0} = \frac{[M]}{[M] + [ML]} = \frac{K}{K + 1} \quad (3.14)$$

The dissociation rate constant can be obtained by plotting Q_k/Q_0 vs. $\omega^{1/2}$ and fitting the data to the Hale equation (3.13). The diffusion coefficient, D , in the Hale equation can be determined by varying the rotation rate and measuring the Q_0 . The slope of the line can then be used in the Levich equation (3.4) to evaluate D .

3.3 Experimental

The apparatus, reagents, and sample solutions used for the experiments in this chapter were previously outlined in sections 2.2.1 to 2.2.5.

3.4 Results and Discussion

3.4.1 Determination of Diffusion Coefficients

Diffusion coefficients can be determined from the Levich equation (3.4) plots and the experimentally determined values of the electrode surface area (see section 2.3.1). It is often desirable in the determination of Q_0 to use SWV for stripping in order that higher analytical sensitivity and lower deposition times of SWV can be utilized. However, as Table 7 shows, for the Cu-NTA system, SWV gives values, similar to those of differential pulse polarography (DPP) obtained in earlier studies - the latter values are much lower than those given by LSV. The reason for these much lower D values from SWV (compared to LSV) is that in the pulsed voltammetric techniques (SWV and DPV), a portion of the oxidized metal cannot diffuse away from the electrode surface before the cycle is reversed and the reduction cycle restarts, resulting in re-reduction of a portion of

of the FA will be fully occupied first, forming strong complexes which will be inert [29, 59,70,71,72,73,74,78 p.378-400]. The dissociation rate constant of this work, $1.1 \times 10^{-1} \text{ s}^{-1}$, for the Cd-FA complex (Figure 18b) is however larger than the published value $1.7 \times 10^{-2} \text{ s}^{-1}$ [25]. It is also interesting to note that the Cd in the published study [25] comprised 98% of the fastest component, whereas the observed $K (= [M] / [ML])$ value of 0.196 would suggest otherwise. This difference may be due to the fact that the above publication [25] used a mixture of other metals in competition with the Cd^{2+} . At low [FA] to [M] ratios, we can expect the other metals having greater affinity for FA to fill up more binding sites of the FA, leaving Cd as the aquo complex.

3.4.3 Kinetic Study of Ultrafiltered Samples of Rideau River Surface Waters

Two samples of Rideau River surface water, collected on different days, were filtered through a $0.45 \mu\text{m}$ membrane filter, and the filtrates were then subjected to cascade (sequential) ultrafiltration, and the metal species in each ultrafiltered size fraction characterized by kinetic study of their dissociation parameters. Each ultrafiltered fraction was spiked with either $1.22 \times 10^{-8} \text{ M Cd}^{2+}$ or $4.88 \times 10^{-8} \text{ M Pb}^{2+}$ and allowed to equilibrate for 24 hours before testing.

Using the theory described earlier in Section 3.2.1 the speciation parameters (dissociation rate constants and diffusion coefficients) of Pb and Cd complexes in each ultrafiltered fraction described above was determined. The experiments were done in the same way as they were done for the Pb and Cd complexes of the fulvic acid as described earlier in Section 3.4.2. There was, however, one significant difference between the model

Table 7- Diffusion coefficients of Cu-NTA complexes in a model solution at pH 4.4, obtained by linear sweep voltammetry (LSV), and square wave voltammetry (SWV). In LSV, [Cu(II)] and NTA = 2.05×10^{-6} M and in SWV, [Cu(II)] and NTA = 4.72×10^{-6} M.

Waveform	Slope of the Levich Plot $\times 10^6 (\text{C s}^{-1/2})$	D $\times 10^6 (\text{cm}^2 \text{s}^{-1})$
LSV	0.364	2.9
SWV (f=5 Hz)	0.395	0.24
SWV (f=10 Hz)	0.465	0.30
SWV (f=50 Hz)	0.367	0.21
SWV (f=100 Hz)	0.424	0.26

Figure 16 – The Levich plots for the determination of D in a model solution of Cu-NTA pH 4.4. Q_0 measured at -1.35V (vs Ag/AgCl). ● - LSV, ○ - DPP, ◆ - SWV (f = 10 Hz), ◇ - SWV (f = 50 Hz), ▲ - SWV (f = 100 Hz).

Table 8 - Kinetic parameters of Pb and Cd fulvic acid complexes in model aqueous solutions. Experimental conditions - SWV stripping mode, scan rate: 100 mV/s, deposition time 30 s, frequency: 50 Hz, pulse height: 25 mV. [FA] = 20 $\mu\text{g/mL}$ (5.64×10^{-6} M of binding sites). Ionic strength fixed at 0.12M using the acetate buffer (pH 4.7 ± 0.1). Temperature = $21 \pm 2^\circ\text{C}$

Metal	$[\text{M}^{2+}] \times 10^8 \text{ (M)}$	K	$D \times 10^7 \text{ (cm}^2 \text{ s}^{-1}\text{)}$	$k_d \text{ (s}^{-1}\text{)}$
Cd ²⁺	1.22	0.196	8.95	0.11
Pb ²⁺	4.81	2.46	2.25	0.25×10^{-3}

Figure 17 -Determination of kinetic parameters for the Pb-FA complex in a model solution. Sample composition and experimental conditions as in Table 8. Q_0 and Q_k were measured at -1.3V and -0.7V vs. Ag/AgCl, respectively. **A** - The Levich Plot for determination of D for the Pb-FA complex. **B** - Q_0/Q_k vs $\omega^{1/2}$ plot for the Pb-FA complex. **C** - Q_k/Q_0 vs $\omega^{1/2}$ plot for the Pb-FA complex. ● - experimental data, — result of curve-fitting to the Hale equation.

Figure 18 - Determination of kinetic parameters for the Cd-FA complex in a model solution. Sample composition and experimental conditions as in Table 8. Q_0 and Q_k were measured at -1.3V and -0.8V vs Ag/AgCl, respectively. **A** - The Levich Plot for determination of D for the Cd-FA complex. **B** - Q_0/Q_k vs $\omega^{1/2}$ plot for the Cd-FA complex. ● - experimental data, — result of curve-fitting to the Hale equation. **C** - Q_k/Q_0 vs $\omega^{1/2}$ plot for the Cd-FA complex ● - experimental data, — result of curve fitting to the Hale equation.

solutions and the Rideau River surface water samples: In model solutions, the metal Pb or Cd was added to the aqueous solution of fulvic acid and the resulting solution was tested, whereas for the Rideau River surface water samples, which contained Pb or Cd below their limits of detection, the samples were first subjected to cascade (sequential) ultrafiltration, and each ultrafiltered fraction was in its turn spiked with a very small amount of Pb or Cd standard solution (of the stated concentration), simulating unpolluted freshwaters. Since the Pb and the Cd present in the above samples of Rideau River surface waters were not detectable, their native concentrations were ignored in the total counts. Hence, the effect of ultrafiltration was to fractionate the organic complexants according to their molecular weights. In freshwaters, humic substances are both dominant and ubiquitous as organic complexants and it is therefore reasonable to interpret the results of this study in terms of humic substances as the organic complexants for the Rideau River surface waters, to which (i.e. to each to its ultrafiltered fraction) the Pb and Cd spikes were added, as described. Therefore, the above complexants will henceforth be called simply 'organic complexants'.

Table 9, Figure 19, and Figure 20 show that the diffusion coefficient of both the Pb or Cd complexes increased with decreasing size fractions of the organic complexants. The observed increase is expected since with lower molecular weight fractions of the organic complexant, smaller Pb or Cd complexes should predominate; the smaller complexes should have larger diffusion coefficients. Table 9, and Figure 21 to Figure 24 show the faster and slower dissociation rate constants for both the Pb and Cd complexes. For the faster dissociation rate constant, both Pb and Cd complexes have increasing rate

constants with decreasing molecular weight fractions, while the slower dissociation rate constants decrease with decreasing molecular weight fractions.

Although the subject matter is undoubtedly more complicated for a simple explanation, some discussion of the observations are in order for understanding metal complexation in the Rideau River surface waters. The ultrafiltration process does two important things: 1) it progressively decreases the absolute amount of organic matter in each subsequent fraction and 2) it separates the types of binding sites available in each fraction. It is well known that the smaller molecular size fractions of humic substances have proportionally larger numbers of functional groups [88,89], resulting in proportionately more metals being bond to strong sites which means stronger complexes having low dissociation rate constants. This would explain the decrease in the slower dissociation rate constants in the smaller molecular weight fractions. This observation is supported by Ramamoorthy et al. who found that ~53% of the Pb binding capacity was found in the < 16,000 MWCO fraction, whereas ~54% of the Cd binding capacity was found in the < 1,400 MWCO fraction in an Ottawa River surface waters [90]. The smaller molecular weight fractions also contain less organic matter. Since these fractions have proportionally more strong sites, there will be proportionally less weak sites available for binding. Hence, this would cause the faster dissociating complexes (which consist of the metal aquo ion and other very labile metal complexes) to have larger dissociation rate constants.

Table 9 – Kinetic parameters for different size fractions of Rideau River Surface water samples. Ionic strength was fixed at 0.12M with the acetate buffer, pH 7.7±0.1, temperature = 21 ± 2°C.

		D				$k_{d,r}^*$ (s ⁻¹)		$k_{d,s}^{**}$ (s ⁻¹)	
		x 10 ⁶ (cm ² s ⁻¹)							
		06/09†	08/19‡	06/09†	08/19‡				
Metal									
<u>Fraction Size</u>									
45 µm filtered	Pb	0.93	2.0	2.7	2.5	1.9	1.3		
50k MWCO	Pb	0.91	2.5	5.1	4.7	1.8	1.7		
10k MWCO	Pb	2.1	3.2	6.5	5.2	0.34	0.080		
1k MWCO	Pb	2.3	4.5	7.9	6.9	0.43	0.12		

(Continued on following page)

Figure 19 - Diffusion coefficients of Pb species in Rideau River surface water samples containing 4.88×10^{-8} M Pb^{2+} using TMFE and SWV. Ionic strength was fixed at 0.12M with the acetate buffer, pH 4.7 ± 0.1 , temperature = $21 \pm 2^\circ\text{C}$.

Figure 20 - Diffusion coefficients of Cd species in Rideau River surface water samples containing 1.22×10^{-8} M Pb^{2+} using TMFE and SWV. Ionic strength was fixed at 0.12M with the acetate buffer, pH 4.7 ± 0.1 , temperature = $21 \pm 2^\circ\text{C}$.

Figure 21 - Faster rate constants for dissociation of Pb species in Rideau River surface water samples containing 4.88×10^{-8} M Pb^{2+} using TMFE and SWV. Ionic strength was fixed at 0.12M with the acetate buffer, pH 4.7 ± 0.1 , temperature = $21 \pm 2^\circ C$.

Figure 22 - Faster rate constants for dissociation of Cd species in Rideau River surface water samples containing 1.22×10^{-8} M Cd^{2+} using TMFE and SWV. Ionic strength was fixed at 0.12M with the acetate buffer, pH 4.7 ± 0.1 , temperature = $21 \pm 2^\circ C$.

Figure 23 - Slower rate constants for dissociation of Pb species in Rideau River surface water samples containing 4.88×10^{-8} M Pb^{2+} using TMFE and SWV. Ionic strength was fixed at 0.12M with the acetate buffer, pH 4.7 ± 0.1 , temperature = $21 \pm 2^\circ C$.

Figure 24 - Slower rate constants for dissociation of Cd species in Rideau River surface water samples containing 1.22×10^{-8} M Cd^{2+} using TMFE and SWV. Ionic strength was fixed at 0.12M with the acetate buffer, pH 4.7 ± 0.1 , temperature = $21 \pm 2^\circ C$.

**4. Determination of Chemical Speciation of Nickel in
Solutions containing Organic Complexants by
Competitive Ligand Exchange / Adsorptive
Cathodic Stripping Voltammetry**

4.1 Introduction

4.1.1 *Adsorptive Cathodic Stripping Voltammetry (AdCSV)*

Metals which can be determined by Anodic Stripping Voltammetry (ASV) using a mercury electrode must have a standard potential for reduction to the metallic state within the narrow analytical window of the analytical method, must be soluble in mercury (otherwise the reoxidation step is electrochemically irreversible), its reduction potential must be more negative than that of mercury and more positive than that of hydrogen ions or (in alkaline media) that of any major reducible ion in the electrolyte (otherwise the reoxidation current is masked by that of hydrogen or by that of the major reducible ion). The metal must also be present in natural waters in concentrations that do not require further preconcentration prior to its determination by ASV [91]. These requirements limit the elements that can be determined by ASV in natural waters, including seawater, to just four metals: Cu, Pb, Cd and Zn [91,92,93,94].

Many more metals and metalloids and non-metallic and organic compounds can be determined by voltammetry if a deposition step is used which does not utilize plating to the metallic state. The most promising of the alternative deposition techniques is Adsorptive Cathodic Stripping Voltammetry (AdCSV) which has achieved many successes with its low limits of detection and general applicability to almost any element.

There are two main advantages to AdCSV. First, any oxidation state can be collected rather than only the metallic state as in ASV. This aspect of AdCSV has opened up the

technique of electroanalysis to any element with a reduction potential (any reduction potential, not just that to the metallic state) falling within the stability range of mercury and hydrogen. C.M.G. van den Berg [91] has predicted "...it is fairly clear that there is much scope for the successful development of procedures to determine almost any element in the Periodic Table by AdCSV." The second advantage of AdCSV over ASV is that very high scan rates can be carried out in AdCSV by employing very high-frequency square-wave voltammetry (SWV). The most popular wave-form used for the potential scan in ASV is differential-pulse modulation which is adequate for ASV but is slow and gives low sensitivity. In AdCSV, the material is collected as a mononuclear layer on the electrode surface, so that all the material is instantaneously accessible to reduction (or oxidation); therefore, the reduction current is independent of diffusion of the reactive species and very fast potential scanning techniques (such as square-wave voltammetry) can be employed, producing larger currents which greatly enhance the sensitivity of the technique over that of ASV [91]. SWV has an additional important advantage over differential-pulse polarography: the high-frequency SWV is so fast that the oxygen-reduction can be eliminated as an interferent, making deaeration of water samples (which is time-consuming and inconvenient) unnecessary as dissolved oxygen does not interfere significantly.

Material that has been collected by AdCSV can be quantified by reduction (the usual method), or by oxidation (the exceptional method), of the metal or the ligand in the complex; occasionally, the catalytically accelerated reduction of dissolved reactants can be utilized to quantify the adsorbed material. Some elements (e.g., Al and Si) which have

reduction potentials too negative for reduction and therefore cannot be analyzed by ASV can be analyzed by AdCSV using suitable complexes of the metals for direct reduction of the ligand in the adsorbed complexes and quantification of the metals. For example, the Al-1,2-dihydroxyanthraquinone-3-sulphonic acid and Si-molybdate complexes are adsorbed complexes used for the quantification of Al and Si, respectively. The sensitivity of AdCSV for a large number of elements is very high, with typical limits of detection at the pM-nM level, allowing direct determination (without prior preconcentration of the sample), in freshwaters and saline solutions such as sea water.

Two additional advantages are as follows. First, in AdCSV, the reduction (or oxidation) current is independent of the diffusion of the reactive species, allowing use of very fast potential scanning techniques such as square-wave voltammetry with frequencies > 2 kHz; the scans can be carried out with less stringent deaeration as the reduction of dissolved oxygen contributes only very little to the faradaic current as its reduction is irreversible at high frequencies [95,96]. Second, the mercury film on a microelectrode would be very suitable for monitoring trace elements in the field and will be amenable for automatic monitoring of the environment.

4.1.2 Literature Survey of Electrochemical Determination of Ni(DMG)₂ Complexation

Adsorptive cathodic stripping voltammetry has been used for the determination of nickel and cobalt at ultratrace levels by the reduction of the adsorbed metal-dimethylglyoxime complex. This technique was first described by Pihlar [97] in 1981 and further investigated in 1986 by the same authors [98]. There has been considerable interest in

determining the mechanism of the electrochemical reaction because of the extraordinary sensitivity of the technique. Pihlar originally postulated that the stripping step of the adsorbed $\text{Ni}(\text{DMG})_2$ complex involved a 2 electron process [98]. Other studies have suggested however that the extraordinary sensitivity requires either that the mass transfer of the complex to the electrode is enhanced or that the reduction involves more than 2 electrons. Ma et al. [99] and Vukomanovic et al. [100] have independently found that the reduction process involves either 16 or 18 electrons. However, Baxter and coworkers [101], using spectroscopic and electrochemical techniques concluded that the stripping process was a 10 electron process. Since there is no consensus on the exact mechanism of the reduction, the work by Pihlar in 1986 will form the basis of the work in this chapter. In general, others have adapted this mechanism but differ only in the final reduction of the adsorbed $\text{Ni}(\text{DMG})_2$ complex.

4.1.3 Competitive Ligand Exchange

Most of the chemical speciation techniques for nickel and other metals reported in the literature comprise of competitive ligand equilibration procedures [16,102,103,104], anodic stripping voltammetry, and Chelex batch and column ion-exchange methods [16]. Cabaniss has reported that since many metal complexes in the natural environment may dissociate slowly, equilibrium conditions may not be established, and hence, equilibrium speciation models may not apply to these metal complexes [103]. Kinetic speciation methods have proven to be useful in investigating moderately to slowly dissociating metal complexes. Most of the kinetic speciation methods involve the addition of a competing ligand which must bind metals much faster and form thermodynamically

stronger complexes than the other complexants present in the aqueous samples. Removal of the metal from the system is then measured over time and rate constants for the dissociation of the metal complexes extracted from the $C = f(t)$ curve. For these methods to be useful for samples of natural waters, a very sensitive measurement technique must be used so that ambient concentrations of metals (which are very low) can be measured. Some workers have used 2,4'-pyridylazoresorcinol (PAR) as the competing ligand and measured the absorption by the metal-PAR complex at 500 nm [103]. This PAR method [103] provides a large quantity of data points yet suffers from poor sensitivity especially for Ni complexes in the μM level. Chelex batch techniques in combination with measurement by inductively-coupled plasma mass spectrometry (ICP-MS) are highly sensitive and can provide a large number of data points, but the ICP-MS measurement is limited to samples having low salt content. Competing Ligand Exchange (CLE) / Adsorptive Cathodic Stripping Voltammetry (AdCSV) is a promising method for highly sensitive and selective determination of nickel as nickel-dimethylglyoxime (DMG) complex. The high selectivity of dimethylglyoxime (DMG) for complexation and precipitation of Ni(II) does not result from extraordinarily great stability of the discrete $\text{Ni}(\text{DMG})_2$ complex, but from the unusual structure in the solid $\text{Ni}(\text{DMG})_2$ complex which leads to its low solubility in water. The square-planar bis(dimethylglyoximate)Ni(II) complex has two planar units stacked one on another so that the Ni "atoms" are bonded [105]. Previous studies on the quantitative determination of Ni as $\text{Ni}(\text{DMG})_2$ complex by AdCSV have reported a very low limit of detection for nickel, typically in nM range [97,106,107,108,109]. CLE/AdCSV has been used for determining the equilibrium speciation of copper and nickel in South San Francisco Bay

[16]. In the CLE/AdCSV method for Cu and Ni, the sample is titrated with copper or nickel and a competitive equilibrium is established for the metal cation between the naturally-occurring organic metal-complexant (such as humic substances). A well-characterized, competing complexant is then added to the sample at a set concentration. The amount of metal complexed by the added competing complexant, which is related to both the concentrations and strength of the added competing complexant, and the naturally-occurring complexant is then measured by AdCSV. For the nickel speciation, dimethylglyoxime (DMG), was added to a series of aliquots of a marine sample which were spiked with increasing concentrations of nickel and allowed to equilibrate overnight [16].

The objective of this research was to study the applicability of CLE/AdCSV to determine nickel speciation in model aqueous solutions containing a well-characterized fulvic acid, and in a sample of Rideau River Surface water, using DMG as the competing complexant, and a thin mercury film electrode (TMFE) on a glassy carbon substrate, and rotating disk electrode voltammetry (RDEV) / square wave voltammetry (SWV).

4.1.4 Theory

In previous competitive equilibrium methods with AdCSV the sample is titrated with nickel in the presence of a well-characterized complexant, dimethylglyoxime (DMG), which is added to establish a competing equilibrium with the naturally-occurring complexants (such as humic substances) for nickel. The nickel concentration complexed by the added competing ligand, DMG, is then determined by AdCSV at each

The most important reaction in the bulk solution in the presence of ammino and dimethylglyoximato ligands involve the equilibria:

In the absence of DMG, the nickel-ammino complex should be predominant at 0.1M concentration of NH_3 [98]; in the presence of a large excess of DMG, the Ni(DMG)_2 chelate should be formed almost exclusively:

DMG in equation (4.2) stands for the dissociated form of DMG (i.e., DMG^{-1}) which forms the Ni(DMG)_2 chelate. Under the conditions used throughout the experiments, the equilibrium represented by equation (4.2) lies far to the right, i.e. nickel is converted almost quantitatively into the Ni(DMG)_2 chelate.

Dimethylglyoxime (DMG) forms a red, square-planar, strong chelate, Ni(DMG)_2 with nickel; the chelate has the following structure [110].

In the AdCSV of nickel, the adsorption of $\text{Ni}(\text{DMG})_2$ on the TMFE proceeds in the above medium only if an external potential is applied to the cell. Pihlar et al. [98] have reported that the extent of the adsorption of $\text{Ni}(\text{DMG})_2$ is constant over a wide range of potentials. On the basis of AC measurements and the negligible influence of the deposition potential on the reaction rate, Pihlar et al. [98] have concluded that the adsorbed species at the polarized electrode is a neutral complex and that the desorption reaction proceeds by two consecutive steps:

followed by reduction:

Simultaneously with the reduction of the nickel (I) intermediate the desorption process proceeds, thus leading to a pseudo-faradaic discharge across the electrical double layer, and a corresponding increase in the total current passed through the cell is observed [98].

4.1.6 Measurement of the Kinetics of Nickel Complex Dissociation

If the nickel in the sample has been previously complexed with ligand L, it must dissociate first before being complexed with DMG. The dissociation of a nickel complex ML_i can be described as a first-order (or pseudo-first-order) dissociation of ML_i as shown below [31]:

where L_i is the i th ligand in the complexant L (a heterogeneous, macromolecular, polyfunctional, multifunctional, organic complexant, such as a humic substance which is ubiquitous in the aquatic and the terrestrial environment - the multifunctionality refers to its surface-active properties, such as adsorption on the electrode surface), and $k_{d,i}$ is the first-order (or pseudo-first-order) rate constant for the dissociation of the NiL_i complex. If equation (4.5) is slower than the reactions for the AdCSV, a series of consecutive reactions (equations 4.5 to 4.8) can be written:

Pihlar et al. [98] have reported that the rate of formation of $Ni(DMG)_2$ complex is fast:

where X represents a free “site” on the electrode surface, and $Ni(DMG)_2^{ads}$ represents the adsorbed bis(dimethylglyoximato) nickel (II) complex in the interface; k_{ads} and k_{des}

represent the rate constants (both dependent upon the electrical state of the system) for adsorption and desorption, respectively.

One can then use the following integrated rate equation (4.9) for the experimental determination of the rate of dissociation of the NiL_i complex.

$$[Ni(DMG)_2]_t^{ads} = \sum_{i=1}^n [NiL_i]_0 \{1 - e^{-k_i t}\} \quad (4.9)$$

Where $[Ni(DMG)_2]_t^{ads}$ is the concentration of the adsorbed $Ni(DMG)_2$ complex at time t , and $[NiL_i]_0$ is the concentration of the NiL_i complex at time zero. The relative amounts of $Ni(DMG)_2^{ads}$ can be related to the AdCSV signal and used in equation (4.9).

4.2 Experimental

4.2.1 Chemicals

A stock solution of 1000 $\mu\text{g/mL}$ $Ni(II)$ was prepared by dissolving pure nickel powder (SPEX, 99.999%) in ultrapure nitric acid (Baker, Inc, ULTREX II), diluting with ultrapure water acidified to contain 1% (v/v) nitric acid. A 0.1 M dimethylglyoxime (DMG) stock solution was prepared by dissolving an appropriate amount of solid DMG (Fisher Scientific, certified) in absolute ethanol. A stock solution of nitrilotriacetic acid (NTA) was prepared by dissolving an appropriate amount of solid NTA (BDH, 99%) in 0.0001 M sodium hydroxide (Fisher Scientific, ACS grade). The sodium hydroxide solution was purified by electrodeposition at -1.2V vs. SCE for at least 48 hours immediately prior to use. A 0.5% $Hg(II)$ solution for forming the thin mercury film electrode was prepared by dissolving doubly-distilled mercury in nitric acid and diluting the solution with acidified ultrapure water. A $NH_4Cl - NH_3$ buffer was prepared by

mixing appropriate amounts of aqueous NH_4Cl (which had been prepared by dissolving solid NH_4Cl (BDH, ACS grade) with ultrapure water) and NH_4OH (BDH, ACS grade). Ultrapure water of 18.3 $\text{M}\Omega\text{-cm}$ resistivity was obtained direct from a Mill-Q UF Plus water purification system (Millipore), fitted with a column to remove organic impurities.

4.2.2 *Reagents*

The fulvic acid (FA) used in this study was an Armadale BH horizon fulvic acid supplied by Dr. D.S. Gamble of Agriculture Canada and which had been fully characterized and described elsewhere [65]. The FA has the following properties: total phenolic -OH groups: 3.0 mmol/g FA, total carboxyl groups; 7.71 mmol/g FA, and a copper binding capacity of 5.43 mmol/g FA. A 1 g/L stock solution of this FA was prepared by weighing the freeze-dried FA and dissolving it in ultrapure water. The stock solution was stored in the dark at 4°C.

4.2.3 *Rideau River Surface Water Sample*

A sample of Rideau River Surface water (RRS) was collected from a sample collection location close to the Steacie Building at Carleton University in Ottawa, Ontario, using an acid-pre-cleaned Teflon container. Immediately after the collection, the sample was filtered through a 0.40 μm polycarbonate membrane (Millipore) using a peristaltic pump, and the pH and the conductivity of the sample were measured using an Accumet 20 pH/conductivity meter and found to be 8.1 and 322 μS , respectively.

4.2.4 Apparatus

Voltammetric measurements were done using a Bio-Analytical Systems (BAS) model 100B Electrochemical Analyzer, connected to a Pentium personal computer. The working electrode was a 3 mm glassy carbon electrode (BAS). The reference electrode was a Ag/AgCl electrode (BAS), and the counter electrode was a coiled Pt wire (BAS). Analysis of voltammetric peaks was done using the software provided with the BAS model 100B Electrochemical Analyzer.

4.2.5 Procedure

4.2.5.1 Preparation of the Electrode

The glassy carbon electrode was polished successively with 3 μm and 1 μm diamond paste and finally with a 0.05 μm alumina suspension until a mirror-like surface was achieved. The electrode was then rinsed with ultrapure water and ethanol and ultrasonicated for 5 minutes before the plating of the mercury film was started. The mercury film was formed by submerging the electrodes in a Teflon cell containing a de-oxygenated solution of 0.01 M Hg(II) and 0.12 M HNO₃ and applying a -1.0 V potential to the glassy carbon electrode and using a rotation rate of 2000 rpm for 10 minutes. After the plating, the electrodes were rinsed with ultrapure water and the mercury film visually inspected. Uneven or patchy films were wiped off and replated.

4.2.5.2 Determination of Nickel by AdCSV

A 100 mL solution buffered to pH 8.2 with 0.10 M $\text{NH}_3\text{Cl-NH}_4$ was de-oxygenated for 15 mins with pre-purified nitrogen (Extra-Dry, BOC Gases). To this blank, 500 μL of 0.1 M DMG was added and the adsorption of $\text{Ni}(\text{DMG})_2$ on the TMFE was done for 30 s using a potential of -0.7 V (vs. Ag-AgCl) and a rotation rate of 500 rpm. The solution was then allowed to become quiescent with a 10 s quiet period and the adsorbed $\text{Ni}(\text{DMG})_2$ complex stripped using a square-wave waveform. The stripping conditions were as follows: scan increment 10 mVs^{-1} , frequency 75 Hz, pulse height 25 mV. These conditions were previously found to be satisfactory for sensitive and reproducible determination of Ni(II) with a film electrode and yielded a detection limit of 14 ng /L for Ni with a 120 s preconcentration / adsorption time [106].

4.2.5.3 Measurement of Dissociation Kinetics of NiL_i Complex

To a 100 mL model solution or sample, which was prepared in the same manner mentioned above, 500 μL of 0.1 M DMG was added, and the experiment was started. After the first determination of Ni was completed and the voltammetric data recorded, the system was allowed to rest for 60 s and the determination was repeated. Further determinations of nickel were allowed to proceed until the peak heights did not increase appreciably or after 6000 s. Experiments over longer time were not attempted as the mercury film tended to deteriorate after this time. In the case of samples containing complexing ligands, a completely separate experiment was performed using the exactly same experimental conditions (including Ni(II) concentration) but without a complexant. This separate experiment was used as a control and also to find the maximum current in

the absence of ligand (i_0) so that the amount of complexed Ni(II) can be estimated from the sample containing ligands.

4.3 Results and Discussion

4.3.1 Adsorption Kinetics of Ni(DMG)₂ Complex onto the TMFE in the Absence of Other Complexants

Using a solution containing 3.44×10^{-8} M Ni(II) and DMG and without any other complexants the kinetics of preconcentration / adsorption of the Ni(DMG)₂ complex was measured. Figure 25 shows a series of square-wave voltammograms, each separated by 60 s, showing well-defined nickel peaks with half-wave potentials at -1010 mV and a broad hump-shaped wave centred around -1200 mV; the latter reported to be due to a surface-nature catalytic hydrogen wave [111]. Figure 26 shows peak currents given by the Ni(DMG)₂ complex plotted vs. time; the peak current increased with time and eventually plateaus at a maximum value (i_0). When this curve was fitted to a first-order rate equation, a rate constant (k_0) of $2.77 \times 10^{-3} \text{ s}^{-1}$ was found with a r^2 value 0.950. Similar experiments were also performed using various concentrations of Ni(II). The results presented in Table 10 show that the k_0 values are not very different and show no recognizable dependence on the nickel concentration, which is consistent with first-order adsorption kinetics of the Ni(DMG)₂ complex. To confirm that the observed rates are those of the Ni(DMG)₂ dissociation, two kinetic experiments were run consecutively with the same model solution containing 3.44×10^{-8} M Ni(II) to which

Table 10 - Rate constants for the preconcentration / adsorption of Ni(DMG)₂ onto the TMFE in the absence of any other complexant.

[Ni(II)] x 10 ⁸ (M)	k ₀ x 10 ³ (s ⁻¹)	r ² of curve fitting
3.44	2.77	0.950
6.88	6.95	0.979
1.70	4.61	0.967
10.3	7.57	0.983

Figure 25 - Square-wave voltammograms of Ni(II) in model solutions containing Ni(II) and DMG without any other complexants. Experimental conditions: scan increment =10 mVs⁻¹, frequency =75 Hz, pulse height =25 mV, deposition potential = -0.7 V, deposition time =30 s, rotation rate =500 rpm, voltammograms recorded at intervals of 60s. The sample was at pH 8.2, buffered in 0.12M NH₄Cl-NH₃ buffer. Temperature =21 ± 2°C, [Ni(II)] = 3.44 x 10⁻⁸ M.

Figure 26 - Kinetics of Ni(DMG)₂ preconcentration / adsorption in model solutions containing Ni(II). All other experimental conditions as in Figure 25.

$\sim 10^{-3}$ M DMG was added in the first experiment. After the first experiment was completed, the electrodes were removed from the model solution and were rinsed with ultrapure water. The model solution used for the first experiment was re-used for the second experiment without any further addition of DMG because it had been added at the beginning of the first experiment. The two experiments gave identical results, which indicates that the $\text{Ni}(\text{DMG})_2$ complex formation (equation 4.6) is not rate-determining.

The above experiments were performed using a wait period of 60 s between the determinations. This time was necessary as it was found that the peak currents tended to decay after the initial increase of the peak current. Figure 27, which was obtained using a Ni(II) concentration of 3.44×10^{-8} M Ni(II) and a wait period of 15 s, shows this phenomenon. It is not clear what is causing this effect; however, examination of equation (4.4) suggests that the desorbed DMG close to the electrode surface may not have enough time to diffuse away from the electrode surface and may interfere with the succeeding determination.

4.3.2 *Kinetics of $\text{Ni}(\text{DMG})_2$ Preconcentration / Adsorption in the presence of NTA*

Experiments using a series of solutions containing both Ni(II) and NTA was performed to investigate the kinetics of Ni-NTA dissociation when NTA was added in excess of Ni(II). NTA was chosen as it has been reported to form a homogeneous, well-characterized, moderately strong chelate complex with nickel ($K_f \sim 10^{14}$ [79]). It was found that when NTA was in large excess ($[\text{Ni}(\text{II})]/[\text{NTA}] = 0.033$ or 0.066), the Ni peak was too suppressed to provide accurate data for kinetic analysis. However, when $[\text{Ni}(\text{II})]/[\text{NTA}] =$

Figure 27 - Kinetics of Ni(DMG)₂ preconcentration / adsorption in model solutions containing Ni(II). Data points recorded at intervals of 15s. All other experimental conditions as Figure 25.

0.17 or 0.34, the voltammetric peaks were well-defined, and the resulting kinetics data were fitted to equation (4.9) using a two-component fitting. The results of the fitting shown in Figure 28 and Figure 29 for Ni(II) in the presence of NTA showed two kinetically distinguishable species. The first component (designated as component “A”) in both cases had $k_{d,a}$ values very similar to the k_0 values obtained in the absence of ligand (see Table 10). This suggests that this component was a Ni-complex which dissociates with a k_d value larger than 10^{-3} s^{-1} , which includes Ni-aquo complexes. The second component (designated as component “B”) involved a less labile species with a $k_{d,b}$ value which was much lower than that of the first component. Since the dissociation of this component is 100 times slower than the measured k_0 values (Table 10), the assumption that the rate of reaction of equation (4.7) is much faster than that of equation (4.5) for this system is justified. Table 11 presents the measured rate constants for the dissociation of NiL_i complex in model solutions in the presence of a number of ligands. As expected, when the $[\text{Ni(II)}]/[\text{NTA}]$ ratio was decreased, a larger proportion of the Ni(II) was released by the slower-dissociating component. This is evident from Table 11 from the increase in the % component B when the $[\text{Ni(II)}]/[\text{NTA}]$ ratio was decreased from 0.34 to 0.17. These values were then compared to values given by MINEQL, a chemical equilibrium program, giving the free nickel (consisting of the Ni(II) aquo ion, and nickel chloride ions) and the Ni-NTA complex. MINEQL predicts for the $[\text{Ni(II)}]/[\text{NTA}]$ ratio = 0.17, that 39% of the nickel will be free with 60% as the Ni-NTA complex. For the $[\text{Ni(II)}]/[\text{NTA}]$ ratio = 0.34, MINEQL predicts 70% free nickel and 30% Ni-NTA complex. Given the free nickel metal corresponds to component A and the

Table 11 - Kinetics of dissociation of NiL_i complex in the presence of a number of other complexants.

$[\text{Ni(II)}]$ $\times 10^8 (\text{M})$	$[\text{Complexant}]$ (μM)	$[\text{Ni(II)}]/$ $[\text{Complexant}]$	Component A (%)	$k_{d,a}$ $\times 10^3 (\text{s}^{-1})$	Component B (%)	$k_{d,b}$ $\times 10^5 (\text{s}^{-1})$	Component C %	$k_{d,c}$ (s^{-1})	r^2 of fitting
<u>NTA as a Complexant</u>									
3.44	1.04	0.033	**	**	**	**	**	**	**
3.44	0.520	0.066	**	**	**	**	**	**	**
3.44	0.208	0.17	22	2.9	78	6.9	--	--	0.98
6.88	0.208	0.34	47	2.8	53	9.0	--	--	0.98
<u>FA as a Complexant</u>									
3.44	1.06	0.032	61	6.1	--	--	38	inert	0.96
3.44	2.71	0.013	48	3.7	7.0	1.2	45	inert	0.98
3.44	5.43	0.0064	33	4.0	4.7	5.1	63	inert	0.97
<u>RRS water as a Complexant</u>									
6.88	**	**	9.9	4.7	88	0.42	--	--	0.95

** - Not Measurable

-- Not observed in curve fitting

Figure 28 - Kinetics of Ni(DMG)₂ preconcentration / adsorption in a model solution containing NTA. The sample was at pH 8.2, buffered in 0.12 M NH₄Cl-NH₃ buffer. temperature = 21 ± 2°C, [Ni(II)] = 6.88 × 10⁻⁸ M, [NTA] = 2.08 × 10⁻⁷ M, [Ni(II)] to [NTA] ratio = 0.352.

Figure 29 - Kinetics of $\text{Ni}(\text{DMG})_2$ pre-concentration / adsorption in a model solution containing NTA. Sample was at pH 8.2 buffered in 0.12 M $\text{NH}_4\text{Cl-NH}_3$ buffer. Temperature $\approx 21 \pm 2^\circ\text{C}$, $[\text{Ni}(\text{II})] = 3.44 \times 10^{-8}$ M, $[\text{NTA}] = 2.08 \times 10^{-7}$ M, $[\text{Ni}(\text{II})]$ to $[\text{NTA}]$ ratio = 0.176.

Ni-NTA to component B, the values show give reasonable agreement with MINEQL values.

4.3.3 Kinetics of Ni(DMG)₂ Preconcentration / Adsorption in the presence of FA

To study the kinetics of Ni(DMG)₂ preconcentration /adsorption on the electrode in the presence of naturally-occurring organic substances such as humic substances which are ubiquitous in freshwaters, model solutions containing aqueous solutions of a well characterized FA [65] were tested. It is well known that the presence of surface-active substances in the sample solution can seriously affect the measurement because of inhibition of adsorption of Ni(DMG)₂ at the electrode surface [98,112]. In freshwaters, naturally-occurring organic substances can act as surfactants and these include humic and fulvic acids [59 p.579-586]. These substances also act as naturally-occurring complexants and it is often difficult to separate the effects of the adsorption and complexation [36]. In the Ni(DMG)₂ system, adsorption of charged surface-active organics can be distinguished from complexation as the adsorption of the charged surface-active organics is highly sensitive to the deposition potential [98]. In systems with no interference due to surface-active agents, peak currents due to nickel remain constant between the deposition potentials of -0.2 V to -0.85 V [106,98]. To test the effect of adsorption of FA to the electrode surface, determinations of Ni(II) were done at -0.2V, -0.6 V and -0.85 V in a model solution containing 10 mg / L FA. At all potentials, the peak current due to nickel remained the same which was surprising since FA was known to be a strong surface-active agent [36 p. 105]. Adsorption of the FA may have been minimized because of the combined effect of the extremely high speed of square-wave voltammetry (the same

reason why oxygen reduction cannot interfere with SWV [113]), and the high rotation rate of the electrode. It should be noted that this observation may not be true for all natural waters and this test should be performed for each type of natural waters.

The results of the studies of kinetics of $\text{Ni}(\text{DMG})_2$ preconcentration / adsorption in model solutions containing the three FA concentrations are shown in Figure 30. In all of the model solutions, an inert component (designated component “C”) representing a certain amount of $\text{Ni}(\text{II})$ that did not dissociate during the time of the experiment was observed. The amount of this inert fraction increased with increasing concentrations of FA. This result confirms what other workers [74,72,73,114] have reported: that polyfunctional fulvic acid has two types of binding sites; minor sites (~ 3 to 10%), which are **strong** binding sites (strong Lewis bases) which bind metals forming strong complexes which are **inert**, and major sites (~ 90 to 97%), which are weak binding sites (weak Lewis bases) which bind metals forming weak complexes which are **labile**. The metals first occupy all the strong sites; after all the strong sites are fully occupied, the metals start occupying the weak sites. The competition among different metals for any of these sites is determined by their water-loss rate constants - the greater the value of water-loss rate constant of a certain metal, the greater is the competitive advantage of that metal over other metals in binding to a given binding site. Assuming that at a constant pH and ionic strength used in this series of experiments, the bonding due to coulombic interactions between the metal cations and the FA polyanions has fixed values, the observed changes in the bonding can be attributed to chemical (covalent) interactions [78 p. 381] Hence, the observed rate constant for the dissociation of metal-FA complexes can be considered as the combined

Figure 30 - Kinetics of $\text{Ni}(\text{DMG})_2$ preconcentration / adsorption in model solutions containing the FA. Sample was at pH 8.2 buffered in 0.12 M $\text{NH}_4\text{Cl}-\text{NH}_3$ buffer. Temperature $\approx 21 \pm 2^\circ\text{C}$, $[\text{Ni}(\text{II})] = 3.44 \times 10^{-8}$ M, $[\text{FA}] = \bullet - 1.09 \times 10^{-6}$ M, $\circ - 2.71 \times 10^{-6}$, $\blacktriangledown - 5.43 \times 10^{-6}$, $[\text{Ni}(\text{II})]$ to $[\text{FA}]$ Ratios $\bullet - 0.032$, $\circ - 0.013$, $\blacktriangledown - 0.0064$.

result of the above polyfunctional ligand effect and the water-loss rate from the metal-aquo complex in the process of the metal forming inner-sphere coordination complexes with other (than aquo) ligands. The loss of a water molecule from the primary hydration sphere of a metal ion is the rate-determining step in metal-ligand (other than aquo) complexation reactions. However, the rates of formation of the metal-FA complexes and of dissociation of these complexes are determined more by the above-mentioned polyfunctional ligand effect and less by the water-loss rate even though the effect of the water-loss rate is very strong. The water-loss rate constant encompasses an enormous range spanning 16 orders of magnitude with metals like Pb having a second-order water-loss rate constant $\sim 10^9 \text{ M}^{-1} \text{ s}^{-1}$ and Cr $\sim 10^{-7} \text{ M}^{-1} \text{ s}^{-1}$ [59 p.301,78 p.400]. The dissociation of the FA-complexes of nickel which has a second-order water-loss rate constant $\sim 10^4 \text{ M}^{-1} \text{ s}^{-1}$ is determined primarily by the above-mentioned polyfunctional ligand effect. This has been experimentally confirmed by careful, exhaustive, experimental investigation done by Chakrabarti et al. employing a Competitive Ligand Exchange Method, using Chelex-100, a strong cation-exchange resin, as the competitive ligand and inductively-coupled plasma mass spectrometry and graphite furnace atomic absorption spectrometry for monitoring the kinetics of dissociation of complexes of Ni(II), Co(II), Pb(II), Cu(II) and Al(III) with well-characterized samples of fulvic acid [75,76,77]. Since the added Ni(II) will bind to the strong sites of the FA till all the strong sites are fully occupied by Ni(II), an increase in $[\text{FA}] / [\text{Ni(II)}]$ mole ratio should cause an increase in the inert fraction of the Ni-FA complex. The solutions with $[\text{Ni(II)}]$ to $[\text{FA}]$ ratios of 0.013 and 0.0064 have a small component with $k_{d,b}$ values of $\sim 10^{-5} \text{ s}^{-1}$. This would suggest that the Ni complexes are still dissociating and are experimentally

observable. However, visual examination of Figure 30 show that this is not the case (from the zero slope) and so component B is likely due to an artifact from the curve fitting. The remaining fraction of the Ni-FA complex (the Component A in Table 11) shows that it exists as very labile component with k_0 values similar to those found for the kinetics of Ni(DMG)₂ preconcentration / adsorption in the absence of any other complexant. This labile component represents the nickel species in the model solution with k_d values greater than 10^{-3} s^{-1} , which includes the labile nickel complexes and the Ni-aquo complex.

4.3.4 Kinetics of Dissociation of Nickel Complexes in a Sample of Rideau River Surface Water (RRS)

Some properties of the RRS water sample are presented in Table 12, which shows that the concentration of nickel was very low. Study of kinetics of this sample at the natural concentration proved to be unsuccessful, as the nickel was undetectable. To simulate a slightly polluted water sample, the RRS water was spiked with a total concentration of $6.88 \times 10^{-8} \text{ M Ni(II)}$ (added as the nickel nitrate), and the water sample was left standing overnight for equilibration. A test for interference by surface-active substances in the water sample was then performed as in the experiments with the FA. Again, no appreciable difference was detected in the peak currents was detected using deposition potentials of -0.2V, -0.6V and -0.85V.

The kinetics of dissociation of the nickel-complex in the polluted sample of the RRS water is shown in Figure 31. The best fitting was obtained using a two-component system

Table 12 - Some properties of samples of Rideau River surface waters

PH	8.1*, 8.0-8.2**
Conductivity	325 μS * 300-400 μS ***
[Ni(II)]	1.6 $\mu\text{g/L}$ *, 2.5 $\mu\text{g/L}$ **
Major Inorganic Ions	10 mg/L Cl^{-1} *, 12.56 mg/L Cl^{-1} ** 5-10 mg/mL SO_4^{2-} *
Dissolved Organic Carbon	6.6 mg/L**

* - Sample used in this work

** - Reference 115

***- Reference 66

Figure 31 - Kinetics of $\text{Ni}(\text{DMG})_2$ preconcentration / adsorption in a Rideau River surface water sample spiked with $\text{Ni}(\text{II})$. The sample was at pH 8.2, buffered in 0.12M NH_4Cl - NH_3 buffer, temperature $=21\pm 2^\circ\text{C}$, $[\text{Ni}(\text{II})] = 6.88 \times 10^{-8} \text{ M}$.

**5. Kinetics of Metal Sorption / Desorption Reactions
on Soil using High Performance Liquid
Chromatography – Microfiltration**

5.1 Introduction

Addition of municipal sewage sludge onto farmlands has been an inexpensive but environmentally hazardous way to fertilize fields and dispose of unwanted waste materials [116,117,118,119,120]. In some municipalities, high quantities and varieties of toxic metals are found in these sludges [116,117]. This, coupled with the release of heavy metals by dissolution and leaching of the earth's crust, atmospheric deposition, mining operations and industrial effluents, has made these metals a significant environmental hazard [121, p.227]. Although regulations have been established to limit the exposure of these metals to the natural environment, uptake of metals by plants and leaching of these metals from soils has been observed to contribute to contamination of ground and surface waters [122,123,124,125]

The toxicity of heavy metals in the dissolved phase has been linked to the free metal ion concentration [78, p.400]. When considering this link, however, one must not only consider the equilibrium concentration of the free metal ion but also the kinetic availability of metal ions due to lability of metal complexes, as they may dissociate to form additional aquo ions [25]. When metal ions are bound by solid soil particles, there is also a possibility of metal complexes having different labilities. Perturbation of such a system can cause the release of these bound metal ions as free metal ions, thus increasing the free metal ion concentration. This perturbation can be caused by a number of environmental conditions, including atmospheric precipitation, percolating through the soil column [121 p. 227], biological activities of organisms in the soil [119], or changes in the ambient temperature [126]. Since soil is a heterogeneous mixture of many different

types of organic and inorganic substances, kinetics of metal release may also differ depending on the type of material to which the metal ion is bound.

Soil is a storage/controlled release system for metals that plants take up only from the soil solution phase. Sposito [127] has reported that many positive correlations have been published, describing the plant uptake of metal ions with their thermodynamic activities in the soil solution. Streit et al. [128] regard metal ion transfer from soil to plant as poorly understood, with predictions by computer model being impossible. Instead of regarding such predictions as totally impossible, Eich et al. [129,130] and Sposito [131] have suggested that predictive computer models must account for the chemical processes involved. An important review by Sposito [131] reveals a large literature on the physical chemistry of metal ion reactions with soils, sediments, and their component materials. At least five types of samples have been investigated, including humic materials, metal oxides of Fe(II) and Al(III), clays, and such carbonates as limestone and chalk, and a number of whole soils. [132,133,134,135,136,137,138,139,140,141,142]. The chemical reactions fall into the four broad categories described in Table 13 [129,130,132,133,134,135,136,137,138,139,140,141,142]. In addition to these direct reactions with soil components, precipitation of hydroxides or carbonates onto surfaces can take place in higher pH ranges [143,144]. The half-lives reported for these categories range cover a range spanning as many as fifteen orders of magnitude [130,131]. One group of reactions are fast compared to the rates of plant uptake and transport by flowing water. The second group includes those that are slow enough compared with plant uptake rate and the hydrological transport rates to be rate-determining under field conditions.

Table 13 - Categories of Metal Ion Reactions With Soil Components

I Cation exchange reactions.

literature reports on humic materials and clays [130,132]

kinetics mostly observable by relaxation methods

some cases with vermiculites slow enough to be monitored by methods other than relaxation kinetics [133].

II Proton displacement reactions.

literature reports for humic materials, $\text{-Al}_2\text{O}_3$, goethite, limestone, chalk, and 38 Danish soils

cases with 1 mechanism step [141,148]

cases with 2 mechanism steps [134,135,136,137,138,139,140]

III Hydrolysis with subsequent chemisorption.

literature reports for goethite & synthetic zeolites

2 mechanism steps

cases with 1 or 2 displaced proton [119].

IV Intraparticle diffusion.

literature reports for goethite, $\text{Al}_2\text{O}_3 \cdot x\text{H}_2\text{O}$, & synthetic zeolites

2 mechanism steps proposed [134,140,141]

Several previous studies have investigated the binding of transition metals to soil surfaces [6,126,145,146,147,148]. The distribution of metal ions between solids and solutions has been correlated to such factors as soil composition, soil and solution pH and the cation exchange capacity of the soil. Earlier studies were done using experimental methods which typically consist of single or sequential extraction procedures using many different types of extracting agents such as strong electrolytes, acids, bases, and chelating agents [145]. Since the concentration of the extracted metal ions is dependent on the chemical properties of the extractant and the extraction procedures used, the resulting fraction is operationally defined [6]. The most commonly used Sequential Extraction Procedure for bottom sediments of freshwaters is that of Tessier et al. [5], which has been criticized by Nirel et al. [6] for its inability to determine chemical entities definable by more than the analytical method itself. Sequential extraction procedures are generally not very selective, and suffer from co-extraction of other metals, and from re-distribution of metals between phases and between solid phases, and are sometimes not quantitative [6]. The operationally-defined nature of such extraction procedures is seen in examples such as metal ions extractable using a combination of nitric acid and hydrogen peroxide are classified as "oxidizable", whereas metal ions extracted using ammonium acetate are considered "leachable" [126]. These classifications lead to some important limitations for chemical speciation studies in that they do not clearly distinguish between the solution phase, labile-bound, and nonlabile forms of the metal. Also, conventional extraction procedures are often time-consuming, laborious, and labour intensive, making them difficult or impractical for use in kinetic studies in which a large number of data points are required.

5.1.1 Kinetics of Metal Sorption/Desorption Reactions on Soil

Models of metal sorption based on macroscopic measurements of metal sorption reactions on soils are ubiquitous in the soil and environmental sciences literature. In some cases, these models equally well describe sorption data and are often useful for describing metal sorption by soil over a range of pH and ionic strength values. However, many of these models have numerous adjustable parameters, and, thus it is not surprising that sorption data can be well described using them. In effect, most sorption models that employ macroscopic data are often curve-fitting exercises. Despite this, many investigators have used them to make mechanistic interpretations about metal sorption on soil surfaces. However, some of these models can describe several different sorption mechanisms. Thus, conformity of material balance data to a particular model does not prove that a particular mechanism is operation. Only by combining kinetic and *in situ* spectroscopic studies can one make definitive determination of mechanisms for metal retention/release from soils [149,150,151,152,153].

Most of the studies of metal sorption/desorption by soils have been equilibrium-based. Equilibrium-based modeling of metal sorption on clay minerals, oxides, sediments, and soils have been productive [129,133,134,141,148]. Much useful information has been obtained from such macroscopic studies. However, one cannot obtain mechanistic information from such approaches [149]. Moreover, equilibrium conditions are often not appropriate to simulate field conditions since soils are seldom, if ever, at equilibrium with respect to ion and molecular transformations and interactions [149,150,151]

To understand the fate of metals in soils properly, and to comprehend their mobility with time, kinetic investigations are necessary. Such time-dependent data can be used to derive mechanisms for metal sorption and desorption on soils. Of course, to ascertain sorption mechanisms definitively, one should employ surface spectroscopic or microscopic techniques [149,150,151,152,153].

Only within the last 50 years, and particularly in the past decade, have kinetic investigations of metal sorption and desorption on soils appeared in the literature. Soils are indeed complex, heterogeneous systems, and the application of kinetics to such solid surface is arduous and fraught with many pitfalls. While many advances have been made in describing the kinetics of metal reactions in soils, it is widely believed that we are only in the infancy of this important area of soil and environmental chemistry [150].

Metal sorption reactions can involve physical sorption, outer-sphere complexation (electrostatic bonding) and inner-sphere complexation (ligand exchange, covalent bonding). Surface complexation can occur on time scales of microseconds to months [130,131]. Metal sorption reactions are often rapid on clay materials such as kaolinite and smectite and much slower on mica and vermiculite, particularly with metals such as NH_4^+ , Cs^+ , and K^+ with small hydrated radii [133,153]. The kinetics of metal sorption are greatly affected by the structural properties of the clay minerals. With kaolinite, only easily accessible planar external sites are available for metal ion exchange [153].

Although considerable progress has been recently made in understanding the rates of metal retention on soils, much is left to be done. There is particular need to obtain more experimental kinetic parameters that can be used in transport models to predict the fate of metals from contaminated soil, particularly if we are going to develop effective strategies for decontaminating soils [149].

The objective of this research was to do a kinetic study of metal sorption/desorption reactions on soil by developing a simple and rapid analytical method for the differentiation of dissolved, labile-bound and nonlabile Zn(II) and Cd(II) ions in selected, contaminated soil using High Performance Liquid Chromatography (HPLC) - Microfiltration technique.

5.2 Experimental Section

5.2.1 HPLC System

The instrumentation was largely a conventional arrangement for metal ion determination by HPLC shown in Figure 32 [154]. It consisted of a Varian Star 9010 solvent delivery system, a Dionex postcolumn reactor (PCR) and a Beckman Model 165 variable wavelength UV–VIS detector. The injection system (detailed in Figure 33), modified for on-line extraction [155,156] and removal of the extracted solids by backflushing, consisted of a Rheodyne 7723 injection valve equipped with a 20 mL sample loop, a Rheodyne 7010 switching valve, an Alltech on-line microfilter equipped with 2.0 and 0.5 μm frits and a Alltex 100 HPLC pump. Separation was achieved using a Supelcosil LC–18 (75 x 4.6 mm) reverse phase column and an Alltech refillable guard column filled with pellicular 10 μm C₁₈ packing.

Figure 32 – HPLC set-up used in this study. Taken from reference [154].

Figure 33 - Injection - micro extraction system. A: injection valve in the "inject" position and the switching valve in the "run" position. B: injection valve as above, and the switching valve in the "bypass" position.

5.2.2 Detection and Quantification by HPLC

Figure 32 shows details of the membrane reactor, which is the heart of the detection system. The eluate that is pumped out of the column proceeds into a porous membrane coil. The 4-(2-pyridylazo)resorcinol (PAR) reagent surrounds the coil on the outside and can penetrate the coil when it is under pressure. The PAR reagent and the eluted metal ions react irreversibly inside the coil to form an intensely red metal complex. This complex is detected using a UV-Vis detector at 520 nm. Quantification of the metal ions was achieved by using analytical calibration curves. Prior to the commencement of the experiment, an analytical calibration curve was prepared using standard solutions. Linear analytical calibration curves for the concentration range of 1.00×10^{-6} M to 2.00×10^{-5} M for both Zn(II) and Cd(II) was established.

5.2.3 Reagents and Soil Samples

5.2.3.1 Mobile Phase

The mobile phase was a tartaric acid eluent composed of 50 mM tartaric acid - 2 mM octane sulfonate which was prepared by dissolving 0.94 g of sodium octane sulfonate (Fluka brand, HPLC grade), 15.0 g of tartaric acid (BDH, ACS grade) into 100 mL of methanol (BDH, HPLC grade) and 1800 mL of ultrapure water. The pH of the resulting solution was adjusted to 3.5 ± 0.1 using NaOH and HCl, and the solution diluted to a volume of 2L. Before use, the eluent was passed through a 0.22 μ m filter and degassed using helium gas (99.995% purity).

5.2.3.2 Post-Column Reaction Reagent

The Post-Column Reaction Reagent was prepared by dissolving 0.05 g of PAR (Aldrich, 98+%) into 400 mL of ultrapure water and 200 mL of 30% ammonium hydroxide (BDH, ACS grade). After the PAR was dissolved, 60 mL of glacial acetic acid (BDH, ACS grade) was added carefully with mixing. The resulting solution was diluted to 1L with ultrapure water, passed through a 0.22 µm filter, and degassed with helium. The solution was used within 1 week after which it was discarded because degradation of the reagent resulted in an unacceptable loss in instrumental sensitivity.

5.2.3.3 Soil

The soil used was obtained from the surface (<15 cm depth) of a cultivated field in the Raisin River basin, 30 km from Cornwall, Ontario. The soil was left open to the air for 3 days to dry and then gently ground to separate aggregates. The resulting grind was then passed through a 150 mesh sieve. The soil pH, cation-exchange capacity, organic carbon content and particle size distribution were determined using standard analytical methods [157] and the results are listed in Table 14. The pH was determined by the CaCl₂ method [157, p. 143], organic carbon by dry combustion [157, p. 189], particle size distribution by the pipette method [157, p. 499] and cation exchange capacity by the ammonium acetate method [157, p.173]. The mineral composition was semi-quantitatively determined by the method described by Kodama et al. [158] and the results are shown in Table 15.

Table 14 - Physical and chemical characteristics of the soil sample used for this research.

<u>Property</u>	<u>Value</u>	<u>Notes</u>
pH	6.6 ± 0.1	Slurry prepared by adding the soil (in g) to the solvent (in mL) in the ratio of 1:2
Cation Exchange Capacity	3.1 mmol / 100g	
Organic Carbon	$10.94 \pm 0.18 \mu\text{g/mg}$	

Particle Size Distribution

%Clay	2%	particles < 2 μm
% Silt	3%	particles between 2 μm and 20 μm
% Sand	95%	particles > 20 μm

Table 15 – Semi-quantitative determination of mineral materials in the soil by X-Ray diffraction analysis

Mineral	Fraction Size		
	150 μm	20 μm	2 μm
Vermiculite	Trace	Trace	Trace
Chlorite	--	Trace	Trace
Mica	Trace	Trace	Trace
Amphiboles	Minor	Trace	Trace
Quartz	Major	Major	Major
Microcline	Minor	Trace	Trace
Plagioclase	Minor	Trace	Trace
Smectite	Minor	Minor	Minor
X-Ray Amorphous	Trace	Trace	Trace

Major – Mineral is present at >20% of fraction

Minor – Mineral is present at 1% to 20% of fraction

Trace – Mineral is present at <1% of fraction

5.2.3.4 A Model, Contaminated Soil

A model, contaminated soil was prepared as follows. An aliquot (4.00 g) of the above mentioned soil was weighed and slurried in an aqueous solution of 50.00 mL of a 1.0×10^{-3} M Zn(II) and 1.0×10^{-3} M Cd(II) and stirred overnight with a magnetic stirrer. The resulting slurry was then allowed to settle and the supernatant solution was decanted. This process was repeated three times over the course of a week. The soil was then washed with ultrapure water until no Zn(II) or Cd(II) in the wash solution was detected by HPLC. The soil was then left open to the air for 3 days to remove excess moisture and was gently ground to separate aggregates.

5.2.3.5 Standards Solutions

Two standard solutions, one of Cd(II) and the other of Zn(II), each 1.00×10^{-5} M, were prepared by dissolving solid sulphate salts (Aldrich, 99.999% purity) in ultrapure water. These solutions were then standardized against certified atomic absorption reference standards (Fisher Scientific) by flame atomic absorption spectrometry (AAS). The concentrations were also checked biweekly by flame AAS against the certified atomic absorption reference standards to ensure calibration stability. Spike solutions and analysis standards were made by serial dilutions of the working stock standards with ultrapure water.

5.2.4 Procedure

5.2.4.1 HPLC-Microfiltration Method

The online extraction of Zn(II) and Cd(II) from the soil required the injection of the soil slurry without filtration into the HPLC. To avoid fouling of the guard column by the soil particles, the HPLC was equipped with a microfiltration system (Figure 33). After injection of a soil slurry into the sample loop, the injection valve was switched, causing the mobile phase to carry the slurry to the microfilter. Solid particles were trapped at the microfilter and extracted by the mobile phase. The resulting extract is then carried to the column for the chromatographic separation. To avoid filter blockage, the microfilter was mounted on a switching valve. This allowed the microfilter to be bypassed and flushed with water without interruption of the flow of the mobile phase through the column.

The separation of Zn(II) and Cd(II) was done using a C₁₈ reversed-phase column. To permit retention of these metal ions, the column required preconditioning. This was accomplished by running 100 mL of the mobile phase through the column at 1.0 mL/min. Separation of the metal ions was achieved by using an eluent flow of 1.0 mL/min. The Post-Column Reaction Reagent (PAR), pumped at 0.5 mL/min at a pressure of 50 psi of He, was mixed at the membrane reactor with the column eluate.

5.2.4.2 Metal Binding by the Soil

A 0.0800 g aliquot of the uncontaminated soil was slurried with 30.00 mL of water in a 7.3 x 3.0 cm screwcap Pyrex vial and stirred for 2 days using a Teflon stirring bar. During the 2 day period, the pH was measured periodically to monitor any desorption of protons from the soil. The vial was thermostated at 25.0°C using a Pyrex jacket connected to a circulating water bath. The soil suspension was then spiked with 10.00 mL

of the standard solution containing 7.0×10^{-5} M Zn(II) and 7.0×10^{-5} M Cd(II) to start the metal binding experiment. To maintain uniform distribution of solids in the soil slurry, it was stirred with a magnetic stirrer throughout the experiment. The concentration of metal ions in the solution phase was determined by taking up 300 μ L aliquots of the slurry from the vial using 1 mL disposable syringes (B-D, Tuberculin) and passing the slurry through 0.22 μ m Nylon 66 filters (MSI cameo). Forty microlitres of the filtrate was injected into the HPLC using 100 μ L HPLC syringes (Hamilton, model 710). The analysis of the filtrate was preceded and followed by an analysis of a standard solution to monitor and to correct for any instrument drift. Metal ions bound to the soil were then determined by injecting 40 μ L of the soil slurry direct into the HPLC using modified HPLC syringes equipped with a 350 μ m bore needle (Hamilton, model 710SNR). Slurry injections were also bracketed by injection of reference standards. After analysis of the slurry, the switching valve was used to bypass the on-line microfilter so that the eluent was pumped direct to the column. Water was then pumped in the opposite direction of the eluent flow through the microfilter to remove trapped soil particles, thus preparing the system for subsequent injections (Figure 33). The pH of the soil slurry was measured continuously during the first hour of the experiment and daily afterwards.

Concurrent with the metal binding experiment, a blank (contained in another Pyrex vial fitted with a Teflon stirring bar) was prepared as follows using the standard solution of metal ions. The blank consisted of the filtrate of a 0.0800g aliquot of the slurry of the soil with 30.00 mL of water filtered through a 0.22 μ m filter. This blank represented the metal ions binding by colloidal particles which escaped the filtration of the solution phase fraction plus any metal ions binding to the Pyrex vial and the Teflon stirring bar. Since no significant decrease in the Zn(II) and Cd(II) concentration were observed, no correction for the blank was made.

The concentrations of the Zn(II) and Cd(II) in the filtrate and slurry were plotted as a function of time. The resulting curves were fitted by a least-squares method using an appropriate polynomial equation. The labile-bound fraction was determined by subtraction of the filtrate concentration from the slurry concentration. Concentrations of the nonlabile fraction was determined by subtracting the analyte concentration in the slurry from the initial spike concentration.

5.2.4.3 Comparison of the Batch Extraction Method and the HPLC–Microfiltration Extraction Method

To evaluate the usefulness of the HPLC-microfiltration technique, extractions of the metal ions from the model, contaminated soil were performed by the Batch technique and by the HPLC-Microfiltration Method. The concentrations of metal ions extracted at the same time intervals for both the methods were then compared.

The batch extraction of the model, contaminated soil was started by mixing 0.0800 g of the soil in 40.00 mL of the tartaric acid octane sulphonate eluent in a Pyrex vial. The suspension was stirred with a magnetic stirrer throughout the experiment to facilitate extraction. To determine the metal ions extracted by the eluent, 300 μ L of the soil suspension was taken up and filtered in the same manner as in the analysis of the solution phase fraction in the metal binding experiment. Forty microliters of the filtrate was then injected into the HPLC for determination of the extracted metal ions. This analysis was done after 1, 24 and 72 hours of stirring of the soil suspension.

For comparison, the HPLC-microfiltration technique was also used to extract Zn(II) and Cd(II) from the model, contaminated soil. A 0.0800 g aliquot of the model, contaminated soil was mixed with 40.00 mL of ultrapure water in a Pyrex vial and stirred with a

magnetic stirrer. The Zn(II) and Cd(II) of the soil suspension were then extracted by injecting 40 μ L of the soil slurry into the HPLC as in the metal binding experiment. Extraction of the Zn(II) and Cd(II) from the slurry of the model, contaminated soil at 1,24 and 72 hours was studied.

5.3 Results and Discussion

5.3.1 Evaluation of the HPLC Separation

Figure 34 shows typical chromatograms of metal ion standards, unfiltered aliquots and filtered aliquots of the spiked soil slurry samples. Zn(II) had a typical retention time of about 3 minutes, and Cd(II) had a typical retention time of just over 6 minutes. An unknown peak appearing after Cd(II) at about 6.5 minutes was present in all samples, including the metal ion standards. By comparing the peak with that of a Fe (II) standard, this peak was identified as Fe(II). A peak at 4.94 minutes for the filtered and unfiltered aliquots was also observed. This unidentified peak was due to a component in the soil matrix and was adequately resolved from neighbouring peaks. The chromatograms and the Figures of Merit (Table 16) show that good resolution was achieved with k' values of 3.08 to 5.16 and resolution between 1.26 and 5.13, where k' is defined as the capacity factor. The HETP (height equivalent of theoretical plates) for the two analytes ranged from 0.02 to 0.31 mm which is typical for this type of separation system [159]. Band broadening was observed with the metal ion standard and unfiltered aliquots when compared with the filtered aliquot, and since this was common to the metal ion standards and unfiltered aliquots, this was attributed to a larger analyte concentration and not to a time lag between transport of the metal ions in the solution and metal ions released from the solids in unfiltered aliquots. The limits of detection (152 ng/mL for Cd(II) and 27 ng/mL for Zn(II)) and the analytical sensitivities (8 mg/mL for Cd(II) and 60 mg/mL for

Figure 34 - HPLC chromatographic peaks for Zn(II) and Cd(II). Injection 1: off-line filtrate, for solution phase analysis. Injection 2: whole slurry with on-line micro extraction, for total extractable metal from solution + solids. Injection 3: analytical standard.

Table 16 - Figures of Merit for the HPLC detection system

Chromatographic Parameters

Analyte	Sample Type	k'	H	HETP(mm)
Cd(II)	Filtered	5.16	3795	.020
Cd(II)	Unfiltered	5.15	1681	.044
Cd(II)	Standard **	5.14	1681	.044
Zn(II)	Filtered	3.11	430	0.17
Zn(II)	Unfiltered	3.12	243	0.31
Zn(II)	Standard **	3.08	237	0.32

where: k' is the capacity factor

H is the plate height

HETP is the height equivalent to theoretical plate.

Resolution

Between Zn(II) and Cd(II)

Sample type	Resolution
Filtered	5.13
Unfiltered	4.33
Standard **	4.37

(Continued on the following page)

Table 16 (continued from the previous page)

Between Cd(II) and Fe(II)

Sample type	Resolution
Filtered	1.53
Unfiltered	1.24
Standard **	1.26

Limits of Detection and Analytical Sensitivity

Analyte	Limit of Detection [†] (ng/mL)	Analytical Sensitivity [‡] (ng/mL)
Cd(II)	152	8
Zn(II)	27	60

** Standard used was an equimolar mixture of 1.75×10^{-5} M Zn(II) and Cd(II) in 1% nitric acid. Fe(II) was present in trace amounts as a contaminant.

[†] - Limit of Detection is defined as the concentration giving a signal-to-noise ratio of 2.

[‡] - Sensitivity defined as the concentration giving an absorbance of 0.0044.

Figure 35- Zn(II) extraction from the model, contaminated soil. A comparison of the Batch Extraction Method with the On-line Microfiltration Method. Error bars represent standard deviations of three replicate measurements. ■- On-line Microfiltration, ▲ - Batch extraction. Temperature: $25 \pm 0.1^\circ\text{C}$.

Figure 36- Cd(II) extraction from the model contaminated soil. A comparison of Batch Extraction Method with the On-line Microfiltration Method. Error bars represent standard deviations of three replicate measurements. ■- On-line microfiltration, ▲ - Batch extraction. Temperature: $25 \pm 0.1^{\circ}\text{C}$.

Method. Equilibrium is also more rapidly established in the HPLC-Microfiltration Method probably because the continuous flow of the mobile phase progressively washes the soil with fresh extractant.

5.3.3 *Metal Binding by the Soil*

Plots of the experimental data for Zn(II) and Cd(II) for the HPLC-Microfiltration Method are shown in Figure 37 and Figure 38. The Zn(II) filtrate curve was fitted using a second degree polynomial while the Cd(II) curve was adequately fitted using a linear equation. The standard deviation for the Zn(II) and Cd(II) curves were 4.8×10^{-7} and 7.6×10^{-7} M/day, respectively. The curves for both Zn(II) and Cd(II) show an initial fast decrease in concentration which correspond to a binding of 46% and 69% of the respective metal ions. This decrease occurred within the first 30 seconds of spiking, during which time the process of spiking, sampling, and injection precluded earlier measurement of the metal concentration. This initial fast binding of metal ions was also accompanied by a drop in pH from 6.3 ± 0.1 (measured before spiking) to 5.1 ± 0.1 , (measured immediately following the spike), which was the minimum pH measured over the course of the experiment. Following the fast initial binding (decay) of the metal ions, both curves thereafter showed a much slower decay. The concentrations of Zn(II) and Cd(II) decreased by an additional 41% and 23%, respectively, from their initial concentrations during a 3 week period, after which no significant decay was observed. During this time, the pH increased slowly and exceeded the initial pH of 6.3 ± 0.1 and reached 6.5 ± 0.1 . This slow increase in the pH probably resulted from some of the H^+ ions being lost by the adsorption on the surface of the reaction vessel on standing for 3 weeks. Figure 37 shows

of large pores. The non-labile Zn(II) is that which has diffused into the particle interior. If the interior represents an “infinite sink” and the surface concentration is nearly constant, as the almost flat curve (the dotted curve) for the labile sorption suggests, the zero-order kinetics is understandable. The model suggested below by equation (5.1) can account for the above observation.

The fitted plots showing the solution phase, the labile and the nonlabile fractions provide insight into the behaviour of the metal ions. The initial fast binding of metal ions to soil has also been observed previously in other soils [160,161,162]. It has been suggested that this initial binding is due to ion-exchange reactions [160]. The observed pH drop and the fast binding of Zn(II) and Cd(II) in the metal binding experiment support this. The difference between the labile and the nonlabile-bound fraction provides information with which to investigate the slower processes. It is known that binding of metal ions can occur within the top layers or the interiors of soil minerals. Previous studies have shown that diffusion of metal ions into goethite structures, illites, smectites, manganese oxides, and other minerals is possible [162]. Table 15 shows that the soil sample has a significant amount of smectite in the clay fraction, which could potentially bind the metals in this manner. To release these occluded metal ions a treatment with a reducing agent (usually $\text{NH}_2\text{OH}\cdot\text{HCl}$) is necessary [145,147]. Since no reducing agent was used in the HPLC-Microfiltration method, it is likely that metal ions bound in this manner are nonlabile by this method.

The results presented in this chapter show the promise of the HPLC-Microfiltration Method to be a simple, rapid and reliable for the determination of the labile-bound, the nonlabile-bound and the solution phase Zn(II) and Cd(II) in soils. Experimental differentiation of these fractions is necessary for understanding the mechanisms of sorption/desorption reactions of Zn(II) and Cd(II) on soil.

Figure 37 - Plot of Zn(II) in the metal binding experiment of the soil, spiked with Zn(II) and Cd(II). Temperature = $25.0 \pm 0.1^\circ \text{C}$. Initial conditions: Zn(II) and Cd(II) each $1.75 \times 10^{-5} \text{ M}$, pH = 6.3 ± 0.1 . ■ - slurry sample ● - filtrate sample.

Figure 38 - Plot of Cd(II) for the metal binding experiment in the soil spiked with Zn(II) and Cd(II). Temperature = $25.0 \pm 0.1^\circ \text{C}$. Initial conditions: Zn(II) and Cd(II) each $1.75 \times 10^{-5} \text{ M}$, pH = 6.3 ± 0.1 . ■ - slurry sample ● - filtrate sample.

Figure 39 - Plots of Zn(II) obtained by curve-fitting of the experimental data from the HPLC-Microfiltration Method. Solid line - Zn(II) in solution, dotted line — labile-bound Zn(II), dashed line — nonlabile Zn(II).

Figure 40 - Plots of Cd(II) obtained by curve-fitting of the experimental data from the HPLC-Microfiltration Method. Solid line - Cd(II) in solution, dotted line – labile-bound Cd(II), dashed line – nonlabile Cd(II).

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