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Studies of Metal Speciation in Surface Waters from Grand River by Electrochemical Techniques

by

LIN SI

B.Sc. (Shandong Normal University, Jinan, China)

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

Department of Chemistry
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Ottawa, Ontario
July, 2003

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acceptance of the thesis,

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Chair, Department of Chemistry

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ABSTRACT

Chemical speciation is important for predicting bioavailability and toxicity of trace metals in freshwaters. The objectives of this research were to determine chemical speciation of the trace metals: Zn(II), Cd(II), Cu(II) and Pb(II) in Grand River surface waters and to study the effect of solar radiation on the chemical speciation of these metals.

Metal speciation was investigated by Anodic Stripping Voltammetry (ASV) at a Thin Mercury Film-Rotating Disk Electrode (TMF-RDE) and by Pseudopolarography at a Hanging Mercury Drop Electrode (HMDE) of surface water samples from Grand River which were exposed to sunlight for 0, 10 and 30 days.

This work involved investigation of a new way of studying metal complexes in freshwaters where the timescale of measurement is a key parameter to better understand metal complexation by heterogeneous complexants, Dissolved Organic Carbon (DOC). The results showed that when freshwaters are exposed to solar radiation the ability of DOC to protect aquatic organisms from exposure to toxic metal species was dramatically reduced. The results of this work suggest that the regulation of metal loading to freshwaters should take into consideration both concentrations of DOC and exposure of the waters to solar radiation.

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CHAPTER 1

INTRODUCTION

Freshwaters represents a small part of the aquatic environment, yet, it merits a much larger share of importance because it is indispensable for the life of humans and most other life forms. Freshwaters is a complex mixture of natural water and other substances, both of natural (geogenic) and anthropogenic origin, in addition to being the habitats of myriads of aquatic biota[1].

Trace elements play an important role in the functioning of life on our planet. Some elements can be highly toxic to various life forms; others are considered essential, but can become toxic at higher doses. In the freshwater systems, trace metals are present in various physicochemical forms (i.e. chemical speciation): free metal ions (properly called, metal-aqua complexes) and various metal complexes of naturally-occurring organic and inorganic complexants. In the study and control of toxic effects of trace metals, it was discovered at a relatively early stage that in many instances there is little sense in analysing only the total content of an element in environmental samples. This is because of the great differences in the toxic properties that can occur among the various chemical species of an element. Therefore, it is essential to understand the metal distribution among different chemical species in order to predict their potential beneficial or adverse environmental impacts [2].

In the freshwater systems, humic substances, the major constituents of Dissolved Organic Carbon (DOC), play an important role in regulating chemical speciation of elements. Because of binding of heavy metals by humic substances (humic and fulvic acids), the availability of the metals to organisms may decrease, whereas on the other hand, the mobility of the metals may increase because these natural complexing agents may act as solubilizers

in aquatic systems. Also, humic substances, because of their chemical heterogeneity, exert a buffering action on metal ions, resulting in chemostasis, preventing abrupt, major changes in free-ion activity of the metal when freshwaters receive large inputs of metal-containing effluents [3]. Thus, the study of interactions between trace metals and humic substances is important.

1.1 Trace Metals in the Freshwater Environment

Trace metals are natural constituents of every compartment of the natural environment. They take part in biogeochemical reactions and are transported between environmental compartments by natural processes. The rates of these processes are frequently altered by human activity.

Copper is an essential trace element for organisms, being present in many enzymes involved in metabolic processes that depend on redox reactions [4]. Nevertheless, at higher concentrations, copper is highly toxic to many organisms, especially in aquatic systems [5]. Copper is one of the most toxic metals, along with mercury and silver, to marine phytoplankton [6], bivalve larvae [7], echinoderm larvae [8] and crustacean larvae [9]. It is also a metal of environmental concern because its concentrations in some polluted areas reach toxic levels. Most pollution problems due to copper (II) are localized near the source of industrial wastes. It is probable that much of the copper (II) found in surface water is introduced by mining and milling wastes, metallurgical wastes and other industrial wastes. Runoff from agricultural lands and urban areas can also contribute to the input of copper into

surface waters. High amounts of copper may be introduced into freshwater systems through drinking water, or, one may be exposed to it because of swimming in lakes, or reservoirs recently treated with copper to control the growth of algae, or reservoirs receiving cooling water (from a power plant) that may have high amounts of dissolved copper. Vomiting, diarrhea, stomach cramps, nausea, liver and kidney damage and even death can be caused by exposure to higher levels of copper than normal.

Lead is an ancient protoplasmic poison that is cumulative, slow-acting and subtle. Lead can be found in all parts of our environment. Much of it comes from human activities including burning fossil fuels, mining, milling, and manufacturing. Lead has many different uses. It is used in the production of batteries, ammunition, metal products (solder and pipes), and devices to shield X-rays. Lead itself does not break down, but lead compounds are changed by sunlight, air, and water. When lead is released to the air, it may travel long distances before settling to the ground. Once lead falls onto soil, it usually sticks to soil particles. Movement of lead from soil into groundwater depends on the type of lead compound and the characteristics of the soil. When absorbed into the body by ingestion, inhalation and through the skin, it interacts with sulfhydryl, carboxyl and phosphoryl groups of the body and interferes with heme synthesis. As a consequence, a variety of symptoms may appear: central nervous problems, damages to kidneys and the reproductive system. At high lead levels, lead may decrease reaction time, cause weakness in fingers, wrists, or ankles, and possibly affect the memory. Lead may cause anemia, a disorder of the blood. It can also damage the male reproductive system.

Cadmium is used in numerous and varied industrial materials including batteries, pigments, metal coatings, and plastics, because of its great resistance to corrosion, its low melting-point and excellent electrical conduction. Cadmium may enter aquatic systems through weathering and erosion of soils and bedrocks, atmospheric deposition, direct discharge from industrial operations, leakage from landfills and contaminated sites, and the dispersive use of sludge and fertilisers in agriculture. Much of the cadmium entering freshwaters from industrial sources may be rapidly adsorbed by particulate matter, and thus sediment may be a significant sink for cadmium emitted to the aquatic environment. Rivers containing excess cadmium can contaminate surrounding land, either through irrigation for agricultural purposes, dumping of dredged sediments or flooding. It has also been demonstrated that rivers can transport cadmium for considerable distances, up to 50 km, from the source [10]. Cadmium compounds do not break down in the environment, but can change forms. Eating food or drinking water with very high levels of cadmium severely irritates the stomach, leading to vomiting and diarrhea. Long-term exposure to lower levels of cadmium in air, food, or water leads to a buildup of cadmium in the kidneys and possibly kidney disease. Other long-term effects are lung damage and fragile bones.

Zinc is an essential element for our bodies. It's found in air, soil, and water, and is present in all foods. Zinc has many commercial uses as coatings to prevent rust, in dry cell batteries, and mixed with other metals to make alloys like brass and bronze. Zinc combines with other elements to form zinc compounds. Common zinc compounds found at hazardous waste sites include zinc chloride, zinc oxide, zinc sulfate, and zinc sulfide. Zinc compounds are widely used in industry to make paint, rubber, dye, wood preservatives, and ointments. Some is

released into the environment by natural processes, but most comes from activities of people like mining, steel production, coal burning, and burning of wastes. Zinc compounds can directly move into the groundwater and into lakes, streams, and rivers. It can also attach to soil, sediments, and dust particles in the air and be transported to the aquatic environment by rain and snow. Exposure to high levels of zinc occurs mostly from eating food, drinking water, or breathing workplace air that is contaminated with zinc. Exposure to large amounts of zinc can cause stomach cramps, nausea, and vomiting. Long-term exposure to zinc can cause anemia, pancreas damage, and lower levels of high-density lipoprotein cholesterol.

Because of wide applications of copper, zinc, cadmium and lead and their toxicities of the metals, monitoring the contaminations of these metals is of great importance to public health.

When positively-charged, these metal ions have vacant orbitals to accept electrons from a ligand and thus can act as Lewis acids to form complexes with ligands. Cu(II), Zn(II), Cd(II) and Pb(II) have an incomplete set of electrons in either d, f or p orbitals, and are strongly influenced by their surroundings, particularly their coordinated ligands, such as those in Natural Organic Matter (NOM), especially humic substances (HS) because the unfilled orbitals project well out to the periphery of the ions. In the case of Cu(II), its d^9 complexes in octahedral substitution reactions are distorted octahedral (tetragonal) complexes because of Jahn-Teller effect (stabilization) [11]. It is well known that metal complexation with humic substances can modulate the exposure levels of trace metals and yet the complex reactions are not fully understood. It is therefore important to study the interaction between trace metals and humic substances in the freshwater environment.

1.2 Natural Organic Matter (NOM), Dissolved Organic Carbon (DOC) and Humic Substances (HS)

Natural Organic Matter, which is referred to as all the organic matter in a reservoir or natural ecosystem, other than living organisms and compounds of man-made origin is a key factor shaping aquatic ecosystems because it is the main complexing agent binding with trace metals. However, it is poorly characterized because of the complexity of the mixture.

Of the NOM, the most important part is Dissolved Organic Carbon (DOC), which represents between 80% and 95% of NOM in most freshwaters. DOC has an important role in the complexation and transport of trace metals such as Al, Fe and Hg. DOC is central to secondary production and microbial food webs in aquatic ecosystems. Typical DOC values are 0.4-2.5, 5-30 and 5-100 mg/L for seawaters, freshwaters, and sediment pore waters, respectively. Although it is still not well understood, DOC constitutes 50-90% humic substances, 1-6% proteins, 6-24% polysaccharides, and other compounds, i.e., free amino acids, glycolic acid, nucleic acids, combined uronic acids and certain phenolic compounds constitute ca. 1% of the DOC [12].

The major constituents of DOC, humic substances (HS), are important because of their ubiquitous presence in freshwaters. Humic substances are high-molecular-weight (usually from 1000 to 10^6), polyelectrolytic macromolecules and are refractory (i.e. degradation-resistant) materials. Elemental analysis shows humic substances consist of carbon, oxygen, hydrogen and sometimes small amounts of nitrogen and occasionally phosphorus and sulphur. Humic substances can be fractionated on the basis of their solubility characteristics:

(i) fulvic acid (FA), the low molecular weight fraction of humic substances is yellow-colored, biologically recalcitrant organic acids and is soluble in alkaline, acidic solutions and in water; (ii) humic acid (HA) is the fraction that is soluble in dilute alkaline solution, but is precipitated in acidic solution and in water (a tentative structure of HA is shown in Figure 1); and (iii) humin is the fraction that is not soluble in alkaline or acid solutions [13, 14].

Unlike simple homogeneous ligands, low-molecular-weight ligands with well-defined structures, humic substances not only are both chemically and physically heterogeneous [15-18], but also possess the following properties that make their metal-complexation reactions quite different from simple, well-defined ligands such as EDTA.

Lewis Bases. It is known that HA contains wide variety of chemical compounds such as carbohydrates, fatty acids, hydrocarbons, nitrogen-containing compounds, sulphur-containing compounds, phosphorus-containing compounds and aromatic compounds. These compounds, mostly, have lone pair of electrons (Lewis bases) which can be donated to the metal ions to form a chemical bond (covalent bond).

Polydispersity. Humic substances represent a variety of different molecules with a wide range of molecular masses and are therefore polydisperse.

Polyfunctional Properties. Each molecule contains a number of different functional groups. Fulvic and humic acids primarily have carboxylic or phenolic groups, but moieties bearing sulphur, nitrogen and phosphorus are also known; binding sites are present on humic

Figure 1. A tentative structure of Humic Acid [1]

substances: major sites and minor sites. Major sites are those present in large proportions in a given complexant system (~99-90 % of the total sites present). Major sites can be considered to be chemically homogeneous, including only one type of functional group, and are weak Lewis bases. Examples of major sites include the carboxylate and phenolate functional groups of humic and fulvic compounds, the carboxylate groups of polysaccharides and proteins, and the $\equiv \text{Fe-OH}$ surface groups of iron hydroxides. Minor sites are those present in trace amounts (~ 1-10 % of the total sites present) but whose chemical diversity may be very large. They can be considered to be chemically heterogeneous, and are strong Lewis bases. Examples of minor sites are the N and S complexing functional groups of fulvic/humic compounds, the -SH groups of proteins, and the $\equiv \text{Mn-OH}$ groups of iron hydroxides containing Mn impurities. Carboxyl groups play a prominent role in the binding of metal ions by humic substances.

Mixed COOH complexes are probably formed. The most prominent one involves both phenolic and carboxylic groups, i.e. a salicylic-acid-type [19,20].

Other possible combinations include two COOH e.g. a phthalic-acid-type site, two phenolic OH, quinone groups, NH_2 groups, sulfhydryl groups, and conjugated ketonic structures. As expected for any ligands, the degree of the ionization of the functional groups changes with

the pH. At low pH, the functional groups are protonated and thus less binding sites are available. At high pH, more functional groups are deprotonated, making more binding sites available to bind with metals.

Polyelectrolytic Properties. Each individual molecule of humic substances can acquire a large number of electric charges (negative charges) because of ionization of its functional groups. It is more accurate to call them oligoelectrolytic properties because of the smaller charge densities compared to true polyelectrolytes, such as proteins. Oligoelectrolyte properties depend on the pH, and are important at pH 5-8, typical of freshwaters, because of dissociation of the functional groups at this pH range [21]. As mentioned above, when pH increases, more negative charges will be generated, and thus more functional groups can bind with the metal. On the other hand, when pH decreases, less negative charges are available to bind with the metal. When a cation reacts with a functional group of polyanions such as a humic acid, the binding of metal ions by polyanion can be greatly strengthened by the coulombic attraction from the neighboring, nonreacting, negative sites. This is called polyelectrolyte effect (properly called oligoelectrolyte effect in the case of humic substances).

Conformational Properties. Humic substances in aqueous media are water-swollen gels—they are macromolecular and naturally-occurring colloidal substances. Factors such as pH and ionic strength affect conformational changes, degree of aggregation, coiling and uncoiling of the various molecules of humic substances and consequently metal ion binding. Conformational changes in humic substances may result in changes in the accessibility of

some ligand sites to metal ions [22]. In particular, conformation depends on hydration-dehydration process, formation of hydrogen bonds, formation of electrostatic interactions among the various functional groups on a single molecule, and formation of metallic bridges between two hydrodynamically separate molecules, which themselves depend on the degree of occupation of the complexing sites [23]. In all cases, the relative importance of these properties varies with pH, ionic strength and the degree of occupation of the complexing sites. Table 1 illustrates the effect of pH, electrolyte concentration, and NOM concentration on FA and HA conformation.

Table 1. The effect of pH, electrolyte concentration, and NOM concentration on conformation [12]

Concentration of FA or HA (g/l.)	Electrolyte (NaCl) Concentration M		pH	
	<0.005	>0.05	<2	>3.5
0.1 — 4
4 — 9

1.3 Metal Speciation and Bioavailability

The concept of bioavailability has different interpretations in biology and chemistry. Whereas biology is concerned with the assimilation properties of the organism, in chemistry bioavailability is defined as the fraction of total metal that is taken up by an organism and

subsequently transported to a site of action/receptor, or target organ [24]. In this thesis, the latter definition of bioavailability has been adopted. When the capacity of a cell to detoxify the accumulated metal is exceeded, damage to cytoplasmic constituents will occur. This is a toxic effect. Symptoms of intracellular toxicity include ultrastructural deformities, reductions in cell division rate, respiration, photosynthesis, motility, electron transport activity and ATP production.

Many of the either beneficial or adverse effects on living organisms depend strongly on the particular form in which the element is present in the system. For example, Cr(VI) ions are considered highly toxic, whereas Cr(III) is not [25]. On the other hand, while both methylmercury and inorganic mercury are toxic, they show different patterns of toxicity. Often these different chemical forms of a particular element or its compounds are referred to as “species”. There is no generally accepted definition of chemical speciation, and various meanings have been attributed to the term by various workers [26]. In this thesis, trace element speciation refers to our ability to define which forms of a given element or organometallic compound appear in a particular sample and at what precise quantitative levels such chemicals occur [27]. Speciation involves the analysis of chemical species leading to their identification in a defined sample; the measurement of the concentration of species of an element in a defined sample; the reactivity of a given species; and the transformation of one species into another. Speciation of trace metals, or distribution among aqueous complexes and particulate forms, varies both spatially and temporally. Metal speciation has been shown to govern important biotic uptake processes including the acquisition of essential metals and the regulation of growth in phytoplankton, the entry of

toxic metals into aquatic food chains, and the toxic effects of metals on higher organisms, such as fish.

The interaction of metals with intracellular compartments is highly dependent on chemical speciation of the metals. Only certain metal species can penetrate the biomembranes to cause a toxic effect. Hence, the total metal concentration is not enough for predicting metal toxicity to aquatic organisms. Furthermore, studies of toxicity suggest that metal toxicity is reduced in the oceans [28], rivers and lakes [29] by its complexation with organic substances. Metal speciation plays an important role in predicting bioavailability and toxicity of the metals in the environment.

1.4 DOC, Solar Radiation and Metal Speciation

Sunlight is important for living organisms in freshwaters. However, with the progressive thinning of the ozone layer, more UV radiation is reaching the earth's surface. Intense sunlight causes inhibition of phytoplankton photosynthesis and growth [30], and many studies have shown that ultraviolet radiation UV-B (280-320 nm) can negatively impact both bacteria and phytoplankton [31, 32]. For example, cellular DNA damage increases sharply with decreasing wavelength in laboratory experiments [33]; similarly, short-term photoinhibition decreases with increasing wavelength [34].

Dissolved Organic Carbon (DOC) has the ability to absorb and attenuate harmful UV radiation, thereby controlling the depth of UV penetration [35], and also to complex heavy metals, making them less bioavailable to phytoplankton [36].

However, solar radiation has been shown to degrade DOC photolytically; most of the DOC is of terrestrial origin and relatively resistant to other forces, and hence, called refractory [37]. Humic substances are fairly resistant to bacterial degradation, but after photolytic activity the products (e.g., formaldehyde, acetaldehyde, glyoxylate and pyruvate) are readily taken up by bacterioplankton [38]. Humic substances strongly absorb UV radiation. Thus, increased breakdown of DOC and subsequent consumption by bacteria increases the UV-B penetration into the water column.

Thus, knowledge of the ways in which DOC is changed with increased UV exposure is required to assess the potential consequences of greater UV to primary production in freshwaters.

1.5 Speciation Parameters

In the freshwaters and soils, it is often impossible to determine the identity and concentration of metal species, many of which are transient. It is therefore necessary to characterize these metal species by the speciation parameters of their metal complexes. Speciation parameters are quantitative measures of chemical characteristics of chemical species, which are

independent of the analytical methods that are used to measure them. It often requires a number of speciation parameters to characterize a system fully.

The following are some important speciation parameters.

(1) *Free Ion Activity*: As illustrated [39] in Figure 2, there are probably two mechanisms of assimilation of dissolved metal species by microorganisms been proposed. One is direct transfer of the hydrophobic and small species through the cellular membrane; the other is dissociation of hydrophilic or large complexes prior to their uptake by the cellular membrane. The latter mechanism is the usual case. Thus, Free Ion Activity Model (FIAM) is a good predictor of metal bioavailability [40]. It is emphasized that the FIAM does not mean that the hydrated metal ion is the species of a trace metal that binds to cell surface (transport) sites because the activity of the free metal ion is simply a measure of its chemical reactivity at the cell surface [41].

(2) *Diffusion Coefficient*: The mobility of a particular chemical species of an element plays a very important role in the transport and availability of the element in the environment. Before an element may elicit a toxic response, it must come into contact with a biological membrane and eventually penetrate it [42]. The diffusion coefficient is a direct measure of how quickly the chemical species can reach the biological membrane.

(3) *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 characterizing the metal species in the aquatic and the terrestrial environment [43, 44, 45].

Figure 2. A schematic diagram of the assimilation of metals (M) by aquatic organisms. L_1 is a natural ligand, L_2 is a transporter ligand, P is a membrane protein channel, and M_a is a metal ion absorbed on a site that will enable transport through the protein channel (Adapted from Wood [39]).

Determination of stability constants relies on equilibrium or pseudo-equilibrium condition to be established before the measurement can be made. Although the measurement of this parameter is straightforward for homogenous systems (such as inorganic complexes), it may be difficult to measure this parameter for heterogeneous systems such as those containing naturally-occurring organic complexants, such as humic substances. 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 humic substances. In such cases, instead of discrete values for stability constants, a large spectrum of stability constants is often observed and the interpretation becomes uncertain. Often an average stability constant is estimated for a group of metal complexes having closely spaced values of stability constants.

(4) *Dissociation Rate Coefficients*: The dissociation rate coefficients represents how rapidly a metal complex dissociates to form free metal ion (i.e. the metal-aqua complex), and hence, is a very important speciation parameter, especially in the case where free metal ion is the bioavailable species.

1.6 Organization of the Thesis

This thesis contains six chapters. Chapter 1 provides an introduction, explaining the driving force behind this work, and gives an outline of the Thesis. Chapter 2 presents theoretical background for studying the interactions between metal ions and humic substances. Chapter

3 contains the theories and their applications for metal speciation by two electrochemical techniques: Anodic Stripping Voltammetry at a Thin Mercury Film-Rotating Disk Electrode (TMF-RDE), and Pseudopolarography at a Hanging Mercury Drop Electrode (HMDE). Chapter 4 presents the results of determination of chemical speciation in freshwaters by Anodic Stripping Voltammetry at a Thin Mercury Film-Rotating Disk Electrode (TMF-RDE). Chapter 5 presents the experimental results of chemical speciation of Zn(II), Cu(II) and Pb(II) in freshwaters determined by Pseudopolarography at a Hanging Mercury Drop Electrode (HMDE). Chapter 6 presents the significance of this work in environmental chemistry.

CHAPTER 2

SCIENTIFIC BACKGROUND

2.1 Current State of Knowledge and Weaknesses

2.1.1 Free Ion Activity Model (FIAM) and Biotic Ligand Model (BLM)

2.1.1.1 Current State of Knowledge

The manifestations of the toxicities to an organism in the aquatic environment usually involve the interaction of trace metals with aquatic biota. It is well known that this interaction involves the following four steps:

- (1) diffusion of the metal from the bulk solution to the biological surface;
- (2) diffusion of the metal through the outer 'protective layer';
- (3) sorption/surface complexation of the metal at passive binding sites within the protective layer, or at sites on the outer surface of the plasma membrane;
- (4) uptake or 'internalization' of the metal (transport across the plasma membrane).

For a very long period, the emphasis of the research about metal-organism interactions was on the biomarkers of risks associated with the exposure to toxic metals such as life stage, nutrition, age. Seldom were the experiments performed on the exposure regime (e.g. metal speciation, pH, [Ca], alkalinity, ionic strength) [40].

Development of computing facilities and popularization of computer programs made it possible to perform bioassays in defined synthetic media, containing only known ligands. There is ample experimental evidence that bioavailability of metals in environmental systems is often related to the equilibrium activities of the bioactive species [46]. Two common

approaches applied in the interpretation of the biouptake of metals from complex media are the Free Ion Activity Model (FIAM) and the Biotic Ligand Model (BLM).

(a) Free Ion Activity Model (FIAM)

The biological response of the organism is directly related to M-X-cell surface complexes (X-cell is a cellular ligand present at the cell surface). These surface complexes can be formed either from the free-metal ion (M) ($M + X - cell \leftrightarrow M - X - cell$) or from a metal complex (ML) ($ML + X - cell \longleftrightarrow M - X - cell + L$). Under both conditions, provided the concentration of free-X-cell sites remains approximately constant, the biological response is determined by the activity of the free metal ion.

There are a number of either obvious or subtle key assumptions underlying the FIAM:

1. The metal interactions with living organisms is the surface complexation reaction that happens primarily on the plasma membrane and results in the formation of M-X-cell;
2. The concentration of free sites, {X-cell}, remains virtually constant in the range of metal concentrations of toxicological interest;
3. During exposure to the metal of interest, the nature of the biological surface i.e. the plasma membrane does not change; and
4. The biological response, whether it be metal uptake, nutrition, or toxicity, is strictly dependent on the concentration of the M-X-cell surface complex, {M-X-cell};
5. Diffusion of the (complexed) metal to near the cell surface, as well as adjunctive and/or disjunctive transfer of the metal from L to X-cell, occurs “rapidly” relative to both metal transport into the cell (if required) and the expression of the biological

response. As a result the cell surface is in equilibrium with the various metal species in the bulk solution. **This is an extremely important assumption.**

There is much experimental evidence in support of FIAM [47, 48, 49]. For example, S. D. Kim reported that when *Ceriodaphnia dubia* was exposed to the mixture of copper and various organic ligands the survival percentage of *C. dubia* was inversely proportional to the concentrations of the free metal ions in the presence of organic ligands [47].

However, apparent exception to the FIAM appears when assumption (4) fails, i.e. without forming a M-X-cell surface complex. This could either due to the presence of lipophilic metal species in solution that might traverse the plasma membrane or the formation of a mixed-ligand surface complex, such as L-M-X-cell, in which case the biological response is proportional to [ML] rather than to $[M^{Z+}]$ alone. However, the ligand concentrations at which enhanced metal bioavailability is observed are far greater than normal environmental levels (e.g., millimolar rather than submicromolar concentrations).

Assumption (5), the surface equilibrium assumption, implies:

First, the identity of the metal form(s) reacting with the cell surface is of no biological significance--no single species in solution can be considered more (or less) available than another. In other words, the dependence of toxicities on the concentration of the free metal ion does not mean that the free-metal ion is the 'toxic' or 'bioavailable' species [50], which has often been overlooked in the literature.

Secondly, other (cationic) species, especially the hardness cations, Ca^{2+} and Mg^{2+} , and the hydrogen ion, H^+ , may compete with the metal of interest, M, for binding at the surface complexation site. Such antagonistic interactions would tend to reduce the equilibrium concentration of the surface complex, M-X-cell, and thus diminish the biological response.

Finally, the FIAM will not apply if the rate-limiting step is metal transport to the cell surface or the reaction of M or ML at the cell surface rather than the surface equilibrium reaction. In this case, the biological response will be determined not by thermodynamic equilibrium considerations but by the kinetics of the complexation reaction or the rate of metal transport in the reaction layer outside the cell.

(b) Biotic Ligand Model (BLM)

Based on the cation competition concept suggested by Zitko and Carson [51], a recently resurrected surface-interaction model [52] of metal binding to gills, the Biotic Ligand Model (BLM), was first presented by Paderkop [53]. This model not only incorporates an equilibrium between M^{2+} and the concentration of metal accumulated at binding sites on the gill surface [54] but also incorporates competition of M^{2+} with other cations (e.g., Ca^{2+} , Mg^{2+} , H^+) for binding at the receptor sites.

BLM assumes that free-ion activity is a necessary but not sufficient condition to describe metal accumulation and, presumably, toxicity [55]. Thus, bioavailability of metals can be decreased in two ways: (1) by decreasing M^{2+} and, thus, decreasing the potential for M to bind to receptor sites (e.g. as increasing [DOC] does through equilibrium partitioning among

the dissolved ligands) or (2) by increasing the concentrations of competing cations and, thus decreasing the amount of M bound to receptor sites (e.g. as increasing Ca^{2+} does). A schematic diagram is presented in Figure 3 to summarize this model.

2.1.1.2 Weaknesses

Both the Free Ion Activity Model (FIAM) and the Biotic Ligand Model (BLM) [56] are based on the assumption of equilibrium at the biological membrane surface. However, this assumption may not be valid because the natural systems are dynamic and often far removed from equilibrium.

Another weakness is that the transport of the metal species on aqueous systems in the overall metal uptake process was neglected in both models [57]. In terms of FIAM, this leads to the neglecting of the diffusion and kinetics aspects of metal species in the solution. In terms of BLM, this leads to the description of metal toxicity as solely determined by adsorption equilibria, which completely neglects the nonequilibrium aspects of the processes involved. However, uptake, accumulation, and effects of metals in living organisms are involved with more than the mere covering of a collection of binding sites. Equilibrium distributions are not generally achieved; more common is some steady state in which fluxes are constant but not zero.

Besides these shortcomings, experimental data exist which do not correspond with the FIAM or BLM approach [40, 58, 59, 60, 61, 62, 63].

Figure 3. A schematic representation of the Biotic Ligand Model (BLM) and the inter-relationships of chemistry, physiology, toxicology and the needs of regulatory agencies (adapted from [52]).

M represents a metal.

2.1.1.3. Speciation Dynamics in Aqueous Solutions

Study of speciation dynamics in aqueous solutions may provide a better understanding of interactions of metals with ligands in the freshwater environment, as shown below.

(a) Dynamic Nature of Volume Complexation Reactions

Many ionic reactions are quite fast in aqueous systems. For cationic metal species, the situation is that the complexation reaction of a x-valent, hydrated metal ion $M(H_2O)_m^{x+}$ with a ligand L^{y-} results in the formation of the complex species $M(H_2O)_mL^{(x-y)+}$. For the sake of simplicity we write this as:

where k_a and k_d denote the association rate constant (in $\text{mol}^{-1} \cdot \text{dm}^3 \cdot \text{s}^{-1}$) and the dissociation rate coefficient (in s^{-1}), respectively. Because of detailed balancing they are related to the equilibrium constant K through k_a/k_d .

The forward association reaction in Equation (1) involves the dehydration of the starting metal ion. According to the Eigen Mechanism [64, 65], the rate-limiting step is the removal of the first water molecule from the inner hydration sphere since this reaction is relatively slow compared to the preceding formation of an outer-sphere ML complex with an intact inner hydration sphere. So the overall association rate constant k_a is defined by: (1) the rate constant for water substitution k_w in the inner co-ordination sphere of the metal ion, and (2)

the stability constant for the intermediate outersphere complex K^* , which may be estimated on the basis of the electrostatics for the interaction [66], Thus we have:

$$k_a = k_{-w}K^* \quad (2)$$

where k_{-w} depends only on the nature of the metal, and its value can be taken from tables [65], K^* can be calculated or taken from a table [66] for a given set of charge and size data of M and L.

When the ligand is in large excess,

$$k'_a = k_a c_L \text{ and } K' = Kc_L = c_{ML}/c_M \quad (3)$$

Considering the characteristic life-times of the metal species that derive from the reaction rate coefficients in Reaction (1), i.e. for M it is $1/k'_a$ and for ML it is $1/k_d$, two limiting cases can be reached.

(i) *Dynamic Regime*: When the timescale of measurement is much larger than the life-times of M and ML, each individual metal ion frequently changes from M to ML and vice versa so that the equilibrium in the bulk solution is maintained. Such a situation is denoted as “dynamic” and obeys the condition:

$$k'_a t \text{ and } k_d t \gg 1 \quad (4)$$

(ii) *Static or Inert Regime*: This limiting case is reached when the life-times of M and ML are much larger than the measurement timescale. In this case, changes in species are not fast enough to attain significant (re)equilibration. The case is defined by:

$$k'_a t \text{ and } k_d t \ll 1 \quad (5)$$

Conditions (4) and (5) are concerned with the rate characteristics of a volume reaction. They provide information on both the capability of the system to attain equilibrium within a certain time and what is detected by consumptive bulk analytical methods like exchange or titration techniques. Even if nonconsumptive methods of analysis are applied to study equilibrium speciation, the above conditions are still useful for confirming whether or not equilibrium exists at all.

(b) Dynamic Nature of Interfacial Reactions

Now we shall consider what happens if M is taken up at an interface which is in contact with the bulk solution containing both M and ML. The interface may be the surface of some analytical sensor like an electrode, a species-selective membrane, or a size exclusion interface.

It should be noted that fulfillment of condition (4) do not necessarily imply that a dynamic system is capable of maintaining equilibrium between ML and M on any dimensional sub-scale. For systems that obey condition (4) there may be kinetic situations where inside the reaction layer equilibrium is not maintained, i.e., where the conversion of ML into M is not complete.

In dynamic regime, the general expression for the limiting metal flux J^* at the electrode surface is derived as [67, 68, 69]

$$J^*(t) = D_M^{1/2} k_d^{1/2} c_T^* \frac{(\varepsilon^{-1} + K')^{3/2}}{K'(1 + K')} \exp(\Lambda^2 t) \operatorname{erfc}(\Lambda t^{1/2}) \quad (6)$$

where D_M is the diffusion coefficient of the free metal ion, k_d is the dissociation rate coefficient of the metal complexes in the system, $c_T^* (= c_M^* + c_{ML}^*)$ is the total metal concentration in the bulk solution, $K' = Kc_L$, $\varepsilon = D_{ML}/D_M$ and

$$\Lambda = \frac{k_d^{1/2} \varepsilon^{-1/2} (\varepsilon^{-1} + K')}{K'(1 + K')^{1/2}} \quad (7)$$

The details of the derivation can be seen in the literature [68]. Three important situations can be reached based on extreme values of the argument $(\Lambda t^{1/2})$.

(i) *The case $\Lambda t^{1/2} \gg 1$:* For values of $(\Lambda t^{1/2})$ much larger than unity, $\exp(\Lambda^2 t) \operatorname{erfc}(\Lambda t^{1/2})$ comes to $\pi^{-1/2} \Lambda^{-1} t^{-1/2}$ and thus $J^*(t)$ is independent from t . This limiting case is known in electrochemistry as ‘labile’ [70]. Under this condition, the dynamic flux approaches

$$J^*(t) = \frac{D_M^{1/2} (1 + K' \varepsilon)^{1/2} c_T^*}{(\pi t)^{1/2} (1 + K')^{1/2}} \quad (8)$$

which is more usually written as

$$J^*(t) = \frac{\bar{D}^{1/2} c_T^*}{(\pi t)^{1/2}} \quad (9)$$

where $\bar{D}$ is the well-known mean diffusion coefficient of M and ML

$$\bar{D} = \frac{D_M c_M + D_{ML} c_{ML}}{c_T} (= D_M \frac{(1 + \epsilon K')}{(1 + K')}) \quad (10)$$

Under this condition, the flux is characterized by purely diffusion-controlled, coupled-transport of M and ML. As outlined by Kacena and Matousek [71], this means nothing but complete maintenance of the equilibrium between ML and M on the relevant dimensional scales and all the metal at the interface is available for consumption.

(ii) *The case $\Lambda t^{1/2} \approx 1$:* Under this condition, the system is denoted as ‘quasi-labile’ or ‘partially-labile’ [72]. In this regime, approaching the electrode from the bulk solution, there is a certain region with depletion of M, together with a metal flux low enough to be ‘followed’ by the complex so that equilibrium is maintained.

(iii) *The case $\Lambda t^{1/2} < 1$:* For very small values of $\Lambda t^{1/2}$, the $\exp(\Lambda^2 t) \operatorname{erfc}(\Lambda t^{1/2})$ is t-dependent according to a $(1-t^{1/2})$ function. In this regime, the system is denoted as ‘nonlabile’ [70]. In this case, the equation (6) reduces to

$$J^*(t) = D_M^{1/2} k_d^{1/2} c_T^* \frac{(\epsilon^{-1} + K')^{3/2}}{K'(1 + K')} \quad (11)$$

which represents a kinetically controlled flux.

The complete general classification of kinetic regimes of metal complexes is given in Figure 4.

Figure 4. A schematic representation of the characteristic kinetic regimes of metal complex systems as determined by the timescale of measurement (t) and the association/dissociation kinetics of the metal complex involved (adapted from van Leeuwen [73]).

$k'_a = k_a C_L$, k_a , k_d represent association rate constant and dissociation rate coefficient, respectively, for the reaction $M + L \xrightleftharpoons[k_d]{k_a} ML$, C_L is the concentration of the ligand in the complexing system, $\Delta t^{1/2}$ is the parameter related to the lability of metal complexes.

2.1.2 Solar Radiation

Solar radiation, as an important external factor affecting the aquatic ecosystems, is of highly interest due to recent evidence of increasing incident UV-B radiation as a result of stratospheric ozone depletion [74, 75].

The influence of UV on Antarctic ecosystems has been well documented [76, 77, 78]. The processes of the degradation of DOC was summarized in Figure 5 from a number of studies [79, 80, 81, 82] performed in marine systems. Experimental evidence, mostly obtained with artificial light sources, suggests that photochemical degradation of DOC often reduces DOC absorptivity in the UV range [83]. Because the concentration and absorptivity of DOC strongly influence water column transparency, it is suggested [84] that photodegradation of DOC may result in substantial temporal variability in the UV transparency in marine systems.

In contrast, UV effects on freshwaters are not well understood and may not be inferred from marine models [85]. Furthermore, previous studies concentrated on the changes in the optical properties of DOC [86]. However, it is indicated in the processes of photodegradation of DOC that the beneficial metal-binding properties of DOC may be altered when the freshwater systems are exposed to sunlight.

2.2 Objectives and Hypotheses

Within the framework outlined above, the overall objective of this work is to test the following hypothesis in the field (Grand River). The key hypothesis to be tested is that the

Figure 5. The processes of photodegradation of DOC, summarized from previous studies [79-82].

kinetic properties of the metal-DOC complexes i.e. inert or dynamic, labile or non-labile may be precisely defined by timescale of measurement, and are affected by exposure to solar radiation; the latter is an important external factor. The specific objectives of this work are: (i) to determine chemical speciation of the trace metals: Zn(II), Cd(II), Cu(II) and Pb(II) in the freshwater environment and to provide metal speciation data required for environmental impact assessment; (ii) to study the effect of solar radiation on chemical speciation of the trace metals: Zn(II), Cd(II), Cu(II) and Pb(II) in freshwater systems.

2.3 Testing of the Hypotheses

The first hypothesis is validated using two different metal speciation techniques, i.e. Anodic Stripping Voltammetry (ASV) at a Thin Mercury Film-Rotating Disk Electrode (TMF-RDE) (Chapter 4) and Pseudopolarography at a Hanging Mercury Drop Electrode (HMDE) (Chapter 5).

To test the second hypothesis, one needs to determine if the free metal ion availability (and hence bioavailability) is significantly changed after the exposure of the samples to sunlight (Chapter 4&5) and to the underlying reason of these changes, i.e. the variation of the metal binding properties of DOC as a function of the exposure to solar radiation (Chapter 5). The control is the freshwater samples collected on site.

2.4 Field-Study Area

Grand River is a Canadian heritage river and originates ($44^{\circ} 05.791' \text{ N}$, $80^{\circ} 22.431' \text{ W}$) in the highlands of Dufferin County, and flows through the heartland of southern Ontario. The watershed consists of all the land that drains into the Grand River through tributary creeks and rivers such as the Conestogo, Speed, Eramosa and Nith. Part of the Grand River's course flows through the Carolinian life zone, which contains a southern type of forest that is found only in this area of Canada. Many plants and animals that are rare in our country are found here. However, because of human activities, its water quality in the river deteriorated to the point where it was a public health concern. Thus, this area was chosen as the field-study area. The watershed map is shown in Figure 6.

Figure 6. Grand River Watershed

CHAPTER 3

ANODIC STRIPPING VOLTAMMETRY AT A THIN MERCURY FILM-ROTATING DISK ELECTRODE (TMF-RDE) AND PSEUDOPOLAROGRAPHY AT A HANGING MERCURY DROP ELECTRODE (HMDE)

3.1 Analytical Techniques

In many cases, the large number of individual species (e.g., in metal-humic acid complexes or metal complexes in biological fluids) will make it impossible to determine the metal speciation. The ideal method for metal speciation is one which is sensitive to the free metal ion activity, has a low detection limit and is selective for the metal in question [87].

The only analytical technique that is able to determine the activity of free metal ion species is ion selective electrode potentiometry [88]—all other analytical techniques measure concentration, not activity. However, this technique suffers from poor limit of detection -- typically 10^{-5} mol/L, which is not environmentally relevant under most circumstances.

Voltammetric methods are well suited for the investigation of speciation of trace elements in natural waters. It is not only because of their high sensitivity, the relative simplicity and compactness of the apparatus which make voltammetric methods easily applicable in the field but also because voltammetric signals are strongly linked to the physico-chemical characteristics of the electroactive species; in particular, these methods are sensitive to the stability, rates of association and dissociation (manifested in the measured lability, that is governed by the effective timescale of the technique), and mobility (diffusion coefficient) of metal complex species [89]. Thus, in principle, information on speciation of the element of interest can be obtained by voltammetric methods [90,91, 92, 93, 94].

Because of its unique properties (e.g. exceptional purity, fresh, reproducible electrode surface, and other properties stated below), mercury is the electrode material of choice for

electroanalytical stripping measurements. The mercury electrode makes convenient and sensitive determination of those metals that can form amalgam with mercury.

3.1.1 Anodic Stripping Voltammetry (ASV) combined with a Thin Mercury Film-Rotating Disk Electrode (TMF-RDE)

In addition to high analytical sensitivity of Anodic Stripping Voltammetry (ASV) which means that chemical equilibrium is not appreciably disturbed, ASV in conjunction with a TMF-RDE mainly has four advantages.

First, the timescale of measurement (t) can be precisely defined by the thickness of the Nernst diffusion layer. This is a unique advantage of this technique.

Secondly, it has the capability for DIRECT determination of trace metals in natural waters with minimal sample treatment, thereby eliminating the risk of contamination, loss of analytes or change in chemical speciation that may occur by the delay involved in preconcentration procedures.

Thirdly, TMF-RDE is ideal for investigations in nonequilibrium situations. This makes it particularly useful for the studies of chemical speciation in the aquatic systems, especially freshwater systems, which are often far removed from chemical equilibrium.

Finally, an important property of a disk-shaped electrode rotating in a liquid is that it is universally accessible to the sample solution. The conditions for transport of material to any

point on the disk surface are identical regardless of the distance from the axis of rotation. This makes a rotating disk electrode a suitable tool for investigating kinetics of electrochemical reactions at solid surfaces [141].

3.1.2 Pseudopolarography at a Hanging Mercury Drop Electrode (HMDE)

In order to obtain reliable data it is essential that the surface of the working electrode is well characterised and reproducible. The classical hanging mercury drop electrode (HMDE) clearly meets this criterion [89].

A direct calculation method for the extraction of metal ion binding parameters from reverse pulse polarograms for fulvic acids has been reported in the literature [95]. However, this technique lacks the sensitivity required for measurements relevant to environmental conditions. Pseudo-polarography allows measurements to be made at low (environmentally relevant) concentrations and overcomes problems associated with processes occurring during the stripping step.

Furthermore, when pseudopolarography is employed, the magnitude of the ASV signal, i.e. peak area or peak height, is the only information taken from each individual measurement. Since it is the metal-to-ligand ratio at the surface of the electrode during stripping that determines the E_p and $\omega^{1/2}$ values of the resulting stripping signal [96], this simply means any variation in E_p or $\omega^{1/2}$ due to variations in the metal-to-ligand ratio during stripping will not contribute to the pseudo-polarographic signal on the condition that surface saturation effects can be avoided [135]. Thus, the possible stripping effects could be avoided for the extraction

of complexation parameters. This feature is a major advantage of the pseudo-polarographic approach. The only other way is to perform medium exchange experiments [97, 98], but in practice it is very difficult to achieve rigorous well-defined experimental conditions.

Hence, Anodic Stripping Voltammetry (ASV) with a Thin Mercury Film Rotating Disk Electrode (TMF-RDE) and Pseudopolarography with a Hanging Mercury Drop Electrode (HMDE) were used for the metal speciation.

3.2 Theories

The speciation-sensitive step is the deposition (preconcentration) step, during which metal ions are reduced at a constant potential for a fixed time and the reduced metal is accumulated in the working electrode (usually mercury). The accumulated metal is quantified by a stripping (oxidation) step: in anodic stripping voltammetry (ASV) an anodically ramping voltage is applied and the resulting current is recorded as a function of potential.

However, without correct interpretation of the signals it is impossible to apply these techniques to real aquatic systems. This is currently a limiting factor. In particular, for metal complexation studies in aquatic media, most of the interpretations reported in the literature are based on theories developed for very simple systems, e.g., containing one metal and one (or a few) low-molecular-weight ligand with well defined structures. This situation seldom occurs in natural aquatic systems where particulate, colloidal or macromolecular complexants [92, 99, 100, 101], such as humics and fulvics, polysaccharides, clays and

hydrous metal oxides, play a major role. Recently, proper theories have been developed for ASV at a TMF-RDE and Pseudopolarography at a HMDE, which makes correct interpretations of the signals possible.

3.2.1 Anodic Stripping Voltammetry at a TMF-RDE

3.2.1.1 *A Thin Mercury Film Rotating Disk Electrode*

A schematic diagram of a Thin Mercury Film Rotating Disk Electrode is presented in Figure 7. When the electrode is rotated, it acts like a pump, transporting the bulk solution towards the electrode surface by laminar convection. Close to the electrode surface the solution is thrown radially outwards from the disk by the centrifugal force of rotation. And the result of this is that a well-defined and reproducible convective-diffusive mass transport regime is developed. At the electrode surface a thin layer of liquid, called the Nernstian diffusion layer, is formed. Inside the Nernstian Diffusion Layer, diffusion is the sole mode of mass transfer from the outer boundary of the diffusion layer to the electrode surface, whereas in the rest of the bulk solution, convective diffusion is the mode of mass transfer. Within the diffusion layer the metal complexes, ML may undergo a first-order dissociation, existing in equilibrium with the dissociation products, M and L as shown in Figure 7. When the rotation rate of the electrode is increased, the thickness of the Nernstian Diffusion Layer, δ , decreases according to the following equation [102]:

$$\delta = 1.62D^{1/3}\nu^{1/6}\omega^{-1/2} \quad (12)$$

where ω is the angular velocity (rad s^{-1}) and ν is the kinematical viscosity (viscosity/density). A typical value of ν for an aqueous solution, $10^{-6} \text{ m}^2 \text{ s}^{-1}$, was adopted in this work. With

Figure 7. A Schematic Diagram of a Thin Mercury Film Rotating Disk Electrode

increasing rates of rotation, the thickness of the diffusion layer, at first, decreases rapidly, at higher rates; the decrease slows down; finally, with further increase in the rotation rates, the diffusion layer tends to attain a constant value, as shown in Figure 8. The thickness of the diffusion layer can be precisely controlled by varying the rotation rate of the RDE [102], providing precise control over the analytical timescale of measurement – a great improvement over ill-defined diffusion layers provided by other types of electrodes.

3.2.1.2 Anodic Stripping Voltammetry (ASV)

Anodic Stripping Voltammetry (ASV) is of particular relevance for studies on metal speciation because adequate sensitivity can be obtained and the analytical signals contain direct speciation information on both the thermodynamic stability and kinetic lability of metal complex species. The application of ASV leads to the determination of “ASV-labile” and “ASV nonlabile” species. “ASV-labile” complexes are operationally defined as those metal complexes that have the ability to make and break bonds rapidly and to rapidly exchange a ligand within the metal coordination sphere for ligands outside this sphere and thus produce a current. A non-labile complex is one that dissociates very slowly and does not produce a current. Complexes that lie between the operational definitions of *labile* and *non-labile* complexes are quasi-labile or sometimes called as moderately labile [103]. These complexes contribute to the ASV current to an extent that depends on dissociation rate and concentration [104], as shown in Figure 9.

Although sometimes “ASV labile” and “ASV non-labile” species [104] have been correlated with metal bioavailability and toxicity [105], these terms are operationally-defined because

Figure 8. Thickness of diffusion layer (δ) as a function of the rotation rate. The Grand River surface water sample.

Figure 9. Concentration profile of labile, quasi-labile and non-labile metal complexes at electrode-solution interface.

δ is thickness of the Nernstain diffusion layer.

they are related to the observed stripping current. Van Leeuwen et al. [106] have presented a theoretical framework (Chapter 2) to replace these operationally-defined terms and to correctly interpret the electrochemical signals so as to be relevant to the environmental processes.

3.2.1.3 TMF-RDE combined with ASV

Van Leeuwen et al. [106] have reported that the labile and inert complexes can be differentiated according to the analytical timescale of electrochemical measurement. In Anodic Stripping Voltammetry, the analytical timescale of electrochemical measurement is defined by the thickness of the diffusion layer [106, 107]. Thus, by varying the rotation rate of a Rotating Disk Electrode, the analytical timescale of the measurement can be varied, and the kinetic characteristics of metal complexes, labile or inert, can be determined. With increasing rate of rotation, the thickness of the diffusion layer decreases; and at the higher rate of rotation the residence time of the metal complexes at the RDE surface becomes so small that only free metal ion and those labile complexes that can dissociate within that specific timescale of measurement contribute to the analytical signals. Different waveforms: linear scan, square-wave, differential-pulse, staircase, and reverse-pulse, can be applied when ASV is combined with TMF-RDE. It has been found that the staircase voltammetric results are similar to those obtained by linear scan voltammetry [108]. Cheng et al [122] have compared the performance of three waveforms, i.e. staircase voltammetry, differential-pulse voltammetry and square-wave voltammetry for determining Cu (II) and Pb (II) speciation in model solutions and snow samples, and have reported that staircase voltammetry gives the most satisfactory estimates of speciation parameters of the metal complexes, whereas square-

wave voltammetry and differential-pulse voltammetry have much higher analytical sensitivity than staircase voltammetry. Others have reported results of comparing the performance of linear scan, differential-pulse and square-wave ASV in aqueous solutions containing fulvic acid and have reported that the observed enhancement of the analytical signals in pulsed stripping techniques may be due to the adsorption of organic surface-active substances (present in model solutions of Pb(II)-FA complexes) to the surface of the TMFE [109]. The best evidence of this interference caused by the adsorption of organic surface-active substances was the absurd result that the diffusion coefficient of the Cd(II)-FA complexes was higher than that of the free metal ion [110].

3.2.1.4 The TMF-RDE Anodic Stripping Voltammetric Signals

The limiting current, i_L , is given by:

$$i_L = nFAD \frac{C^*}{\delta} \quad (13)$$

Combining Eqs. 12 and 13 yields the Levich Equation [102]:

$$i_L = \frac{nFAD^{2/3} c_{M,T} \omega^{1/2}}{1.62\nu^{1/6}} \quad (14)$$

The above equation describes the relationship between the limiting current i_L and the diffusion coefficient D at a rotating disk electrode.

The relationship between the timescale of measurement, t , and the thickness of the diffusion layer, δ can be expressed as follows:

$$t = \delta^2 / \bar{D} \quad (15)$$

Kinetic information about dissociation rate coefficient k_d and conditional stability constant K' can be obtained by combining Equation 6 (Chapter 2) and Equation 15:

$$J^*(t) = D_M^{1/2} k_d^{1/2} c_T^* \frac{(\varepsilon^{-1} + K')^{3/2}}{K'(1 + K')} \exp(\Lambda_{ss}^2 \delta^2) \operatorname{erfc}(\Lambda_{ss} \delta) \quad (16)$$

where δ is the thickness of the diffusion layer,

$$\Lambda_{ss} = \frac{k_d^{1/2} \varepsilon^{-1/2} (\varepsilon^{-1} + K')}{K'(1 + K')^{1/2} \bar{D}^{1/2}} \quad (17)$$

the other parameters are defined previously for Equation 6 (Chapter 2).

The limiting current i_L is related to the flux $J^*(t)$ by Equation 18:

$$i_L = nFAJ^*(t) \quad (18)$$

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

The combination of equations 17 and 18 gives the limiting current at a rotating disk electrode.

$$i_L = nFAD_M^{1/2} k_d^{1/2} c_{M,T} \frac{(\varepsilon^{-1} + K')^{3/2}}{K'(1 + K')} \exp(\Lambda_{ss}^2 \delta^2) \operatorname{erfc}(\Lambda_{ss} \delta) \quad (19)$$

3.2.2 Pseudopolarography at a Hanging Mercury Drop Electrode (HMDE)

3.2.2.1 A Hanging Mercury Drop Electrode (HMDE)

A typical Hanging Mercury Drop Electrode consists essentially of a capillary tubing connected to a levelling tube which contains a column of pure metallic mercury. A ‘hanging drop’ is dispensed at the end of the capillary by a valve and is used as a working electrode. This drop was then dislodged by a Drop Kicker. Because a fresh mercury drop is exposed to the analyte during each measurement, reproducible current-potential data are obtained.

As stated in Chapter 2, the crucial variable in testing lability is the timescale of the measurement, i.e. the stirring rate, when pseudopolarography at a HMDE is employed. However, since the thickness of the diffusion layer is ill-defined in HMDE, the timescale of the measurement cannot be defined precisely as that in TMF-RDE.

3.2.2.2 Pseudopolarograms

When pseudopolarography at a HMDE is employed, the currents are recorded at different deposition potentials. The graph of current versus potential is plotted as shown in Figure 10, which is called a pseudopolarogram.

The polarographic wave for a solution containing “simple” ligands is described by the DeFord and Hume equation [111,112].

$$E = E^{\circ} + \frac{RT}{nF} \ln \left[\frac{1}{\alpha} \left(\frac{D_R}{D_{ML}} \right)^{1/2} \right] + \frac{RT}{nF} \ln \left(\frac{1 - i/i_{lim}}{i/i_{lim}} \right) \quad (20)$$

Figure 10. Simulated polarographic curves for reduction of M^{n+} a) under non-complexing conditions, b) in the presence of a simple ligand, c) in the presence of a heterogeneous complexant [113]. I/I_L is the normalized peak height; the other parameters have the same meaning as stated in the text.

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

Since the metal complexes formed with heterogeneous complexants (such as humic substances) possess a nearly infinite number of different molecular-scale bonding characteristics, the pseudopolarographic curve (Figure 10 (c)) becomes asymmetrical, elongated and shifted towards negative potentials. This curve can be described by Equation 21 derived by Filella et al. [113]:

$$E = E_0 + \frac{RT}{nF} \ln \left[\frac{1}{A^{1/\Gamma} c_L} \left(\frac{c_M}{c_L} \right)^{(1-\Gamma)/\Gamma} \left(\frac{D_R}{D_{ML}} \right)^{1/2} \right] + \frac{RT}{nF} \ln \left[\frac{(1-i/i_{lim})^{1/\Gamma}}{i/i_{lim}} \right] \quad (21)$$

In the above equation, A is a Freundlich constant, Γ is a parameter that reflects the degree of heterogeneity of the system and is independent of experimental conditions. Γ has the following meaning [114]:

$$\Gamma = \frac{1}{[M]_b} \cdot \frac{d[M]_b}{d \log [M]} = \frac{\beta}{[M]_b} \quad (22)$$

where β is the complexation buffer intensity of the system (which is indicative of the natural-water's capacity to buffer the effect of sudden addition of metals [117], $[M]_b$ and $[M]$ represent the concentration of bound metal and metal in the bulk solution, respectively. The

meaning of Γ and its constancy make it a very important parameter for describing heterogeneous complexant properties.

3.2.2.3 The Pseudopolarographic Signals

Complexation constant (measure of the degree of metal-complexation) can be determined from [96, 115]

$$\ln \alpha = \frac{nF}{RT} (E_{1/2, \text{ref}} - E_{1/2, \text{sam}}) + \ln \left(\frac{I_{L, \text{ref}}}{I_{L, \text{sam}}} \right) \quad (23)$$

where $E_{1/2, \text{ref}}$, $E_{1/2, \text{sam}}$ are the half-wave potential for the reference solution and the spiked test solution, respectively. $I_{L, \text{ref}}$ and $I_{L, \text{sam}}$ are the limiting current for the metal aqua ion in the reference solution and for the complexes in the water sample, respectively. The heterogeneity of the complexants requires a continuous distribution function. This makes the extraction of such ideal system representations mathematically from experimental data impossible.

In practice, two ways are described to interpret the signals obtained by pseudopolarography at a HMDE so that information on the complexing properties of the system can be attained.

1. Average Equilibrium Function: The average equilibrium parameter, $\bar{K}$, corresponds to the average constant of an apparent equilibrium between M and the totality of sites L_i

$$\bar{K} = \frac{\sum_i c_{ML_i}}{c_M \sum_i c_{L_i}} \quad (24)$$

where c donates the concentration of the different species.

Its relation to the individual stability constants K_i of sites i is given by:

$$\bar{K} = \frac{\sum_i K_i c_{L_i}}{\sum_i c_{L_i}} \quad (25)$$

The value of $\bar{K}$ decreases as the degree of occupation ($\bar{\theta}$) of sites L by M increases since the averaging is carried out with an increasing number of weaker sites. Eq. (25) shows that $\bar{K}$ is an arithmetic mean of the true equilibrium constants weighted by the corresponding free site concentrations. Normally the knowledge of $\sum_i c_{L_i}$ is essential for the determination of $\bar{K}$; yet it is very difficult to determine experimentally. However, under conditions where there is a large excess of sites compared to metal complexes ($\bar{\theta} \ll 1$), the Freudlich constant, A, can be calculated using the following equation:

$$\alpha = A^{1/\Gamma} c_{L,T} \left(\frac{c_{L,T}}{c_{M,T}} \right)^{(1-\Gamma)/\Gamma} \quad (26)$$

where Γ is a parameter related to the heterogeneity of the complexant ($\Gamma = 1$ corresponds to the homogeneous case), $c_{L,T}$ and $c_{M,T}$ are the total concentrations of the ligands and the dissolved metals in the bulk solution, respectively, $\bar{K}$ can be determined by Equation 27:

$$\bar{K} = A^{1/\Gamma} \left(\frac{\sum_i c_{ML_i}}{\sum_i c_{L_i}} \right)^{(\Gamma-1)/\Gamma} \quad (27)$$

2. Differential Equilibrium Function (DEF).

A differential equilibrium parameter, K^* , can be defined as [113, 116, 117, 118, 119]

$$K^* = \frac{\sum_i K_i \theta_i (1 - \theta_i) c_{L_i,T}}{\sum_i \theta_i (1 - \theta_i) c_{L_i,T}} \quad (28)$$

where θ_i is the degree of occupation of site i ($= c_{ML_i} / c_{L_i,T}$) and $c_{L,T}$ = total concentration of site i ($= c_{ML_i} + c_{L_i}$).

The DEF is particularly useful in equilibrium studies because it considers the metal ion buffer intensity through the term $\theta_i (1 - \theta_i)$. Because the strongest sites are saturated first, K^* decreases with θ . It can be seen from Eq. (28) that K^* is a weighted arithmetic mean of the K_i values for all sites present in the system.

In principle, the DEF may take any shape. In practice, roughly straight log-log plots are always observed with chemically heterogeneous complexants:

$$\log \theta = \Gamma \log K_o^* - \Gamma \log K^* \quad (29)$$

where K_o^* is a constant with arbitrary physical meaning.

Since K_o^* can be determined by:

$$\alpha = K_o^* c_{L,T} \frac{\Gamma}{1-\Gamma} \left(\frac{c_{L,T}}{c_{M,T}} \right)^{(1-\Gamma)/\Gamma} \quad (30)$$

K^* can be readily calculated from experimental data using Equation (29) and it is independent of the complexation capacity.

These two sets of parameters, Freundlich-A and Γ , and DEF- K_o^* and Γ are independent of the experimental conditions and have predictive values, allowing $\bar{K}$ and K^* to be calculated for any metal-to-ligand ratio.

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

$$i_L = \frac{nFA\bar{D}^{1/2} c_{M,T} \omega^{1/2}}{g} \quad (31)$$

where i_L is the diffusion-limited limiting current, n is the number of electrons involved in the electrode reaction, A is the electrode surface area (exposed to the analyte), $\bar{D}$ is the weighted

average diffusion coefficient, ω is the angular velocity (rad s^{-1}), $c_{M,T}$ is the total metal concentration in the bulk solution, g is a constant that depends on the cell geometry [95].

3.3 Applications

The methodologies of Anodic Stripping Voltammetry at a TMF-RDE and Pseudopolarography at a HMDE have been used for the study of metal speciation because appropriate theories to correctly interpret the experimental results obtained by these techniques are available.

3.3.1 Anodic Stripping Voltammetry at a TMF-RDE

Rotating Disk Electrode (RDE) with a thin mercury film (TMF) combined with linear scan anodic stripping voltammetry (ASV) was first applied by Shuman and Michael [120] to measure the rate constants for copper complex dissociation in marine coastal water samples by spiking copper ions into samples, and a first-order dissociation rate constant for the copper complex of the order of 2 s^{-1} was reported.

Chakrabarti et al. [121] determined diffusion coefficients and dissociation rate coefficients of lead and cadmium species in a river surface water samples using square wave anodic stripping voltammetry with a thin mercury film electrode; their results agreed with a model of the metals forming complexes with humic substances (humic substances are ubiquitous in freshwaters), and showed that the thermodynamic stability and kinetic lability of the complexes were mainly determined by the polyfunctional and the oligoelectrolytic properties

of humic substances. Also, Chakrabarti et al. used this technique for studying kinetics of metal complex dissociation in model solutions and snow samples [122].

3.3.2 Pseudopolarography at a HMDE

The validity of the pseudopolarographic methodology has been demonstrated for simple reversible systems; e.g. the chloride complexes of Cd and Pb [123, 124, 125, 126, 127, 128, 129], labile Zn complexes with inorganic ligands [130] and in freshwaters [131], Pb-carbonate in seawater [132], Cd with L-aspartic acid [133]; and for the quasi-reversible Zn-chloride system [134]. For example, a labile system (Pb(II)-pyridine-2,6-dicarboxylic acid) and inert systems, (Pb(II)- and Cu(II)-EDTA), were studied by pseudopolarography, and the results were in good agreement with the known properties of those systems [135].

Pseudopolarography has been applied to the study of metal ion complexation in complex natural water systems, both seawater and estuarine samples (speciation dominated by inorganic complexation [136, 137, 138, 139, 140]) and to a lesser extent, in conditions relevant to freshwaters (higher organic matter content) [138, 141, 142, 143]. Several examples are given below.

The distribution of metal ions at a trace level of the Pb-nitrilotriacetic acid chelate system was analyzed and the stability constant $\log K = 11.9$ of the complex, the pseudopolarographic half-wave potential $E_{1/2} = 0.64$ V vs. Ag/AgCl, the charge transfer coefficient $\alpha 0.4$ and the standard rate constant $k = 2 \times 10^{-3}$ cm/s of its redox reaction were determined [144].

Dissolved copper (II) species in a seawater sample was determined using the pseudopolarographic approach [139]. It was found that in this natural seawater sample, copper (II) occurred mainly in the form of pseudopolarographically inert complexes (>95%).

Recently, Filella et al. [135] employed pseudopolarography to study the complexation of Pb(II) and Cu(II) by Suwannee River Fulvic Acids (SRFA) and information on the heterogeneity of Pb(II) and Cu(II) complexes was reported.

CHAPTER 4

EXPERIMENTAL DETERMINATION OF METAL SPECIATION IN FRESHWATERS BY ANODIC STRIPPING VOLTAMMETRY (ASV) AT A THIN MERCURY FILM- ROTATING DISK ELECTRODE (TMF-RDE)

4.1 Experimental

4.1.1 Reagents

A stock solution of 5000 $\mu\text{g/mL}$ $\text{Hg}(\text{NO}_3)_2$ for plating a Thin Mercury Film Electrode (TMFE) was prepared as follows: pure mercury was dissolved in ultrapure nitric acid and the solution was diluted with ultrapure water so that the final solution was made to contain 1% (v/v) nitric acid. Stock solutions of copper, lead, cadmium and zinc (1000 $\mu\text{g/mL}$) were prepared by dissolving chemically pure metal or metal oxide in ultrapure nitric acid (Ultrex II), followed by diluting the solution with ultrapure water, and finally adding proper amount of ultrapure nitric acid to the solution to make the final solutions contain 1% (v/v) nitric acid. In order to prepare a stock buffer solution, a stock solution of 2.0 M sodium hydroxide was prepared by dissolving an appropriate quantity of sodium hydroxide (ACS grade/BDH) in ultrapure water. This solution was purified by mercury-cathode electrolysis at -1.4 V vs. E_{SCE} for at least 72 h using a mercury-pool cathode to remove heavy metals. A stock solution of 0.9 M piperazine-N,N'-bis(2)-ethanesulfonic acid (PIPES) buffer was prepared by dissolving an appropriate quantity of sodium acetate trihydrate (ACS grade/BDH) in the least possible amount of 2M sodium hydroxide, followed by dilution with ultrapure water. Ultrapure water of resistivity 18.2 $\text{M}\Omega\cdot\text{cm}$ was obtained directly from a Nanopure Diamond water purification system (Barnstead Corporation), fitted with a purifying column to remove organic matter. The 2 mol/L potassium nitrate stock solution was prepared from solid KNO_3 (BDH, AnalaR) to fix the ionic strength and act as the supporting electrolyte. All test solutions (unless otherwise stated) were prepared by serial dilutions of the above stock solutions with ultrapure water immediately prior to use.

4.1.2 Apparatus

The Bioanalytical Systems (BAS) Electrochemical Analyzer, Model 100B/W analyzer, equipped with an RDE-1 Rotating Disk Electrode (BAS), was used to do voltammetric measurements. The working electrode was a 3 mm Glassy Carbon Electrode (GCE), surrounded by 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 (4mol/L) solution. The counter electrode was made of a coiled platinum wire. The data were obtained using the BAS 100W windows control software version 2.3 (BAS) and were then transferred to a computer and saved for processing.

4.1.3 Formation of the Thin Mercury Film Electrode (TMFE)

The glassy carbon electrode was prepared by polishing with 3 μm monocrystalline diamond paste (DP-Paste, M, Struers) prior to use. The mercury film was formed on the rotating disk electrode (RDE) by applying a potential of -1.0 V , rotating the RDE at 2000 rpm [145] in a plating solution containing 50 mL ultrapure water, 0.12 M nitric acid and 5000 $\mu\text{g/mL}$ Hg^{2+} that had been deoxygenated for 15 min using pure nitrogen gas immediately prior to this operation. A 15 min. deposition time was used, followed by a 5s quiescent period, after which the potential was ramped from -0.3 V to 0 V using a square-wave waveform (pulse height 20 mV, frequency 50 Hz) to strip any impurities from the TMFE. The plating solution was then removed and the TMFE visually inspected. Non-uniform films and films containing bubbles were wiped off and the TMFE reformed.

4.1.4 Electrochemical Parameters

Every sample was purged for 10 min. with ultra high-purity nitrogen gas (UHP, PRAXAIR) prior to use. Linear Scan Anodic Stripping Voltammetry (LSASV) was applied for the experiments. The experimental parameters were as follows: deposition time 180 s; equilibration time 15 s; step potential 2.0 mV/s; frequency 75 Hz, scan rate 10 mV/s, deposition potentials: Cd(II) -0.80 V, Cu(II) -0.10 V, Pb(II) -0.80 V, and Zn(II) -1.2 V, all versus E_{SCE} . The rotation rate of the RDE was from 25 to 7000 rpm.

4.1.5 Samples

The freshwater samples from the headwaters of Grand River were collected using acid-washed Teflon containers on June 23, 2002. 2.2-litre Teflon bottles were used for sample collection and storage. For pre-cleaning the Teflon containers, they were soaked in 10% HNO_3 (AR grade), and were left to stand for one week at room temperature in order to leach out trace metals and other impurities. The containers were then rinsed five times with ultrapure water, and left completely filled with the ultrapure water until they were used; the filling water was renewed periodically to ensure continued contact with ultrapure water.

Immediately after the water sample collection, they were filtered through 0.45 μm Gelman mixed cellulose ester filters using a peristaltic pump and the filtrate was used as the test sample and stored in the dark at 4 °C prior to use.

The water samples after filtration were then delivered to our collaborating laboratory at the University of Ottawa (Ottawa, Ontario) for solar irradiation. The samples were irradiated

under summer sunlight conditions in 2.2 L Teflon bottles (11.5 cm diameter) for ten and thirty days. The UV irradiation dose at the outside surface of the bottle (in air) was measured using OL754 spectroradiometer with dual monochromator (Optronics Ltd.), OL IS470-W submersible sphere, OL 730-7Q-WP waterproof fibre optic probe, and operated with OptoLab 754 software. The dose inside the bottle was measured by cutting a hole in the side of one of the bottles and sealing the probe's sensor inside the bottle with parafilm, then filling the bottle with deionized water. The average solar dose determined was 1.5 W/m^2 . Nonirradiated bottles were kept as controls. Irradiated water samples and non-irradiated bottles were stored in the dark at 4°C . The pH of the water samples were measured with an Accumet 925 pH/ion meter using a combination glass electrode containing an internal calomel reference electrode.

4.1.6 Determination of Metal Concentrations

Immediately after filtration the filtered water samples were acidified to pH 1.5 with ultrapure nitric acid (Seastar, Canada) in order to preserve the integrity (metals) of the samples, i.e. to prevent the loss of metal ions from the samples. Total dissolved metal concentrations were determined by inductively-coupled plasma mass spectrometry (ICP-MS) in our collaborating laboratory at Geological Survey of Canada. The Detection Limits for Cd (II), Cu (II), Zn (II) and Pb (II) are 0.6ppt, 3ppt, 1ppb and 15ppt, respectively.

QA/QC

Quality control for the determination of total metals by ICP-MS included replicate injections (typically $\leq 5\%$ relative standard deviation among metal concentrations determined in

replicate injections) and periodic (two in every 2h operation) analysis of NIST 1643d Certified Reference Material. A prior set of samples was reanalyzed if the analyzed value differed from the certified value by > 20%.

4.1.7 Determination of Dissolved Organic Carbon (DOC)

A Total Organic Carbon Analyzer (SHIMADZU, Model TOC-5000 or OI Analytical Model 1010 TOC Analyzer) was used to determine the concentrations of total carbon (TC) and inorganic carbon (IC) in the unfiltered freshwater samples. The concentrations of DOC in the samples were determined by subtracting the IC concentration from the TC concentration. For the TC determination, 25 μL of the freshwater sample was introduced into the analyzer and the TC components in the sample were combusted at 680°C using the catalyst (for regular sensitivity measurement P/N 017-42801-01) to form CO_2 , which was determined by a non-dispersive infrared gas analyzer (NDIR). For the IC measurement, 50 μL of the freshwater sample was injected into the analyzer, and the IC components in the sample were decomposed by the IC reagent (a solution of phosphoric acid) to form CO_2 , which was detected by NDIR.

4.1.8 Experimental Procedure

One of the following metals (M) was added (M = Pb, Zn or Cd) to the test solution to make the total metal concentration of the test metal (Pb, Zn or Cd) 8.52×10^{-7} mol/L. Since a higher concentration of Cu was required to get adequate sensitivity, the total Cu concentration was 1.57×10^{-6} M. The samples were then set aside overnight for equilibration. After the equilibration, a control solution containing 0.1 mol/L KNO_3 supporting electrolyte,

0.01 mol/L PIPES buffer, and the same concentration of the metal under test (copper, zinc, cadmium or lead) as that of the sample was prepared, and the samples were tested using the electrochemical conditions mentioned in Section 4.1.4. The sample under test was treated with the same amount of buffer as the control for the ASV measurement. The total volume of either control or sample was 50 mL. The quality of the thin mercury film was checked during the experimental runs to avoid the experimental errors caused by the damage of the film.

The data of both control and sample were fitted to the Levich equation i.e. Equation 14 (Chapter 3) [102] to evaluate the mean diffusion coefficient of M and ML complexes in the freshwater samples. Since theoretical values for diffusion coefficients of the aqua metal ions can be obtained in the literature [146], the mean diffusion coefficient of ML complexes can be determined. Then, by fitting the data to the Equation 19 (Chapter 3) using a commercial software program, NLREG v5.2 (Phillip H. Sherrod, Brentwood, TN, USA), the concentration of the free metal ion, the dissociation rate coefficients, the conditional stability constants, and the lability of ML complexes can be determined.

4.2 Results and Discussion

Figure 11 presents the fitting of the experimental data obtained by spiking Grand River water samples after 10 days UV irradiation with 8.52×10^{-7} mol/L Pb(II) aqua ion to the Equation 14 (Chapter 3) in order to determine the mean diffusion coefficient of the metal-DOC complexes. Since the metal species in the control solution was only free metal ion that is labile, in the experimental range (25 to 7000 rpm), the curve is linear. When the water

sample contains a variety of metals which formed complexes with Dissolved Organic Carbon (DOC), at low rates of electrode rotation, i.e. when the timescale of the measurement was long, all the metal complexes had enough time to dissociate to be reduced at the electrode surface; hence, the fitting displays a linear relationship. However, when the timescale of measurement was not long enough for some of the metal complexes to dissociate, the curves became nonlinear at high rotation rates of the RDE, as can be seen in Figures 11 and 12.

The speciation parameters determined by TMF-RDE in conjunction with ASV for Grand River surface water samples without solar irradiation are presented in Table 2. $D_{M^{2+}}$ and k_a are the literature values [146]; the rest are obtained from the above experimental results.

The mean diffusion coefficients of the above metal complexes are all significantly (at least 30%) lower than those of the corresponding metal-aqua complexes [147], probably because of the fact that most of the metals in the sample form complexes with macromolecular complexants, mainly DOC. The ratio of the mean diffusion coefficient of the copper-DOC complexes to the free Cu(II) aqua ion is about one-tenth, whereas those for the DOC complexes of Cd (II), Pb(II), and Zn(II), vary from about seven-tenths to four-tenths. These ratios reflect the relative strengths of M(II)-DOC binding — that of Cu(II)-DOC is much stronger than that of the DOC complexes of Cd(II), Pb(II), and Zn(II), resulting in a much smaller fraction of Cu(II) being present as free Cu(II) aqua ion and a much larger fraction as Cu(II)-DOC complexes compared with the DOC complexes of Cd(II), Pb(II), and Zn(II).

Figure 11. Modified Cottrell plot of Pb(II)-DOC complexes in Grand River surface water samples irradiated with sunlight for 10 days. pH = 6.2 ± 0.1 ; ionic strength = 0.1 mol/L, T = 295K. (♦), Reference solution $C_{\text{Pb(II)}, T} = 8.52 \times 10^{-7}$ M, $R^2 = 0.99$; (▲), the Grand River water samples irradiated with sunlight for 10 days, $C_{\text{Pb(II)}, T} = 8.52 \times 10^{-7}$ M, $C_{\text{DOC}} = 13.9 \pm 0.1$ mg/L, $R^2 = 0.98$.

Figure 12. Modified Cottrell plot of Pb(II)-DOC complexes in Grand River water samples irradiated for 30 days. pH = 6.2 ± 0.1 ; ionic strength = 0.1 M, T = 295 K. The solid line represents data that were fitted to Equation 19 (Chapter 3).

Table 2. Speciation Parameters in Grand River surface water samples without solar radiation. The samples were spiked with the following metals, one each for each of the following four experiments: 8.52×10^{-7} M Cd(II), Pb (II), Zn(II); 1.57×10^{-6} M Cu (II). The determination was done by TMF-RDE combined with ASV.

Metal	D_M (cm ² s ⁻¹)	$\overline{D}_{ML}$ (cm ² s ⁻¹)	k_a (M ⁻¹ s ⁻¹)	log K	$C_{M, Free}$ (mol/L)
Cd (II)	6.9×10^{-6}	4.8×10^{-6}	4.1×10^9	5.89	1.2×10^{-8}
Pb (II)	8.3×10^{-6}	3.4×10^{-6}	9.4×10^{10}	7.02	8.7×10^{-10}
Zn (II)	6.4×10^{-6}	4.3×10^{-6}	2.1×10^9	5.20	5.4×10^{-8}
Cu (II)	7.3×10^{-6}	6.8×10^{-7}	1.5×10^8	6.35	7.6×10^{-9}

D_M Mean diffusion coefficient for the metal aqua complex

$\overline{D}_{ML}$ Mean diffusion coefficient for metal-DOC complexes

k_a Rate constant for the formation of ML from M and L

K Conditional stability constant for the reaction $M + L \rightarrow ML$

$C_{M, Free}$ Concentration of free metal ion

The order of the availability of free metal ions at equilibrium reflected by the conditional stability constants was that: $\text{Pb (II)} < \text{Cd (II)} < \text{Zn(II)}$. The free metal ion concentrations has the same trend as shown in Table 2. This trend can be partially explained by the physicochemical properties of the metals, their charge-to-size ratio (Figure 13). The uppermost curve showed that the association rate constant (k_a) is linear with the charge-to-size ratio. This is because k_a can be estimated from the rate coefficient for water exchange, k_w , and the stability constant of the outer-sphere complex, k_{os} , and hence, is a characteristic of the metal itself. A less pronounced, similar trend was also found for the dissociation rate coefficient (k_d). The exception to the trend is Cu(II) , which has the shortened equatorial bonds and the lengthened axial bonds in octahedrally coordinated aqua complexes, $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$, because of the Ligand Field Stabilization Energy (LFSE) combined with Jahn-Teller effect [148]. This exception shown by copper was also observed by Sekaly et al. [149], who studied the complex dissociation kinetics using Chelex 100 as a competing ligand, and also by Achterberg et al. [150], who studied dissolved copper species in freshwaters employing adsorptive cathodic stripping voltammetry and radiotracers, and by Town and Filella [135], who studied the speciation of Cu (II) - and Pb(II) - fulvic acid complexes using pseudopolarography. However, the k_d of Cu (II) has shown only a minor deviation from the trend, probably because the amounts of Cu (II) added to the water samples were different from those of the other three metals, and also because of relatively large errors caused by the propagation of errors for estimation of dissociation rate coefficients of the metal complexes. Figure 14 shows that the changes in the concentration decreased with increasing duration of solar radiation; the DOC concentration decreased from an initial concentration of 14.9 mg/L

Figure 13. The relationship between charge-to-size ratio of the metal ions, and k_a , k_d , and K in Grand River surface water samples without solar irradiation, (●, ○), $\log k_a$; (■, □), $\log K$; (▼, ▽), $\log k_d$; from left to right the point is for Pb, Cd, Cu, Zn, respectively; (—), curve-fitting; pH, 6.2 ± 0.1 ; $T = 295$ K.

Figure 14. The changes in the concentration of DOC as the function of the duration of the exposure to sunlight for the Grand River surface water samples; $\text{pH} = 7.9 \pm 0.1$.

to 13.9 mg/L after exposure to sunlight for 10 days, and to 11.6 mg/L after 30 days of solar radiation, i.e. the concentration of DOC in Grand River surface water samples decreased by about 20% at the end of 30 days of solar irradiation.

The influence of solar radiation on the metal speciation in the surface water samples from the Grand River is presented in Table 3 and Figure 15. The last column of Table 3 presents the values of $\Lambda_{ss} \delta$; increasing values of $\Lambda_{ss} \delta$ with increasing duration of solar irradiation indicate increasing lability of the metal complexes. The speciation parameters: mean diffusion coefficients of the metal complexes, stability constant (Log K), and concentration of free metal ion, for Cu (II), Cd (II), Pb (II), increased dramatically with increasing duration of solar radiation. For example, the mean diffusion coefficients of the metal complexes increased ~50% for Cd (II), ~100% for Pb (II) and 5 times for Cu (II) after the surface water sample was exposed to sunlight for 30 days, whereas, the content of DOC only decreased ~20%. This suggests that the change of the chemical speciation in Grand River water samples may not be due only to the decrease in the concentration of DOC, but also to the change in the binding properties of DOC.

Table 3 also indicates that the metal speciation of Zn (II) changed only slightly on solar radiation, whereas that of other metals changed more noticeably. The values of $\Lambda_{ss} \delta$ indicate that, for Cd (II) and Pb (II), increasing duration of solar irradiation up to 30 days results in increasing lability of their metal complexes. The results of Zn (II) imply that Zn (II) is present mainly as free zinc metal ion or other complexes of inorganic ligands, such as carbonate, which are resistant to solar irradiation. Hence the Zn (II) speciation does not show

Table 3. The influence of solar radiation on the chemical speciation in the Grand River surface water samples. The samples were spiked with the following metals, one each for each of the following four experiments: 8.52×10^{-7} M Cd(II), Pb (II) or Zn(II); 1.57×10^{-6} M Cu (II). The determination was done by TMF-RDE combined with ASV. pH = 6.2 ± 0.1 , ionic strength = 0.1 M, T = 295 K.

Metal	Duration of Solar Radition	D_{ML} (cm ² /s)	Log K	$C_{M, Free}$ (mol/L)	$\Lambda_{ss} \delta$
Cd (II)	0 days	4.8×10^{-6}	5.89	1.2×10^{-8}	1
	10 days	6.1×10^{-6}	5.75	1.6×10^{-8}	2
	30 days	6.4×10^{-6}	5.72	1.7×10^{-8}	2
Pb (II)	0 days	3.4×10^{-6}	7.02	8.7×10^{-10}	0.6
	10 days	4.7×10^{-6}	6.72	1.7×10^{-9}	0.9
	30 days	7.3×10^{-6}	6.63	2.1×10^{-9}	0.9
Zn (II)	0 days	4.3×10^{-6}	5.20	5.4×10^{-8}	1
	10 days	4.9×10^{-6}	5.17	5.7×10^{-8}	1
	30 days	4.9×10^{-6}	5.17	5.7×10^{-8}	1
Cu (II)	0 days	6.8×10^{-7}	6.35	7.6×10^{-9}	0.4
	10 days	2.1×10^{-6}	6.12	1.3×10^{-8}	0.6
	30 days	3.4×10^{-6}	6.05	1.5×10^{-8}	0.6

Figure 15. The influence of solar radiation on the lability of metal-complexes in Grand River surface water samples, (●), Zn(II) 8.52×10^{-7} M; (○), Cd(II) 8.52×10^{-7} M; (▽), Cu (II) 1.57×10^{-6} M; (▼), Pb (II) 8.52×10^{-7} M; (—), curve-fitting; pH, 6.2 ± 0.1 ; ionic strength = 0.1 M; T = 295 K.

any observable change even though solar radiation reduces the complexing abilities of DOC, as can be seen from the results presented later on. However, although the complexation of Cu (II) with DOC is stabilized by its chemical structure making its complexes with DOC inert to the interface, when exposed to sunlight the complexes of copper with DOC changed to a more labile direction. This may adversely impact the environment and implies the importance of the external factor, solar radiation, on the chemical speciation.

As shown in Figure 15, the lability of metal complexes increases with increasing exposure to solar radiation, which suggests that solar radiation significantly reduces the ability of DOC to bind the metals. Furthermore, the highest increase in the free metal ion concentration occurs within the first 10 days. This implies that the beneficial metal-binding properties of DOC may be rather short-lived. The trend is consistent with that observed from bioassay toxicity tests [151].

The change of the chemical speciation may be due to the induction of a number of different photochemical processes, including breaking of bonds, change in functional groups and structures that may also change intramolecular interactions [152] when the samples are exposed to sunlight. However, this kind of information can not be extracted from the signals obtained by ASV combined with a RDE-TMF.

4.3. Conclusion

Dissolved Organic Carbon (DOC), an important metal complexing agent in freshwaters, can protect aquatic organisms by forming non-toxic metal complexes. However, DOC may be photooxidized upon exposure to UV radiation. The unique advantage of RDE, which is that the timescale of measurement (and hence, the metal species determined by ASV) can be precisely defined by controlling the thickness of the Nernstian diffusion layer, makes it extremely valuable for the study of chemical speciation in the aquatic environment. The results suggest that solar radiation significantly reduces the ability of DOC to bind the metals and the beneficial metal binding properties of DOC may be rather short-lived when DOC is exposed to solar radiation.

CHAPTER 5

DETERMINATION OF CHEMICAL SPECIATION OF Zn(II), Cu(II) AND Pb(II) IN FRESHWATERS BY PSEUDO- POLAROGRAPHY AT A HANGING MERCURY DROP ELECTRODE (HMDE)

5.1 Experimental

5.1.1 Apparatus

A computer-controlled Autolab PGSTAT30 potentiostat/galvanostat (Eco Chemie BV, The Netherlands) equipped with a Metrohm 663 VA stand (Metrohm, Switzerland) was used for voltammetric measurements. The working electrode was a Static Mercury Drop Electrode (Metrohm, Switzerland). The reference electrode was an Ag/AgCl electrode in a glass tube filled with saturated KCl (4 M) and fitted with a porous Vycor tip (Bioanalytical Systems, Inc., USA). An electrode made of platinum rod (Metrohm, Switzerland) was used as the counter electrode. Analysis of voltammetric peaks was done using the General Purpose Electrochemical Software v4.8 (Eco Chemie BV, The Netherlands). The data were transferred to a computer and saved for processing.

5.1.2 Reagents and Materials

Ultrapure water of resistivity 18.2 M Ω -cm was obtained directly from a Nanopure Diamond water purification system (Barnstead Corporation) fitted with a purification column to remove organic matter. All the solutions were diluted with ultrapure water. A stock solution of 2.0 M HEPES buffer at pH 7.5 was prepared by dissolving an appropriate quantity of HEPES (N-2-Hydroxyethylpiperazine-N'-3-ethanesulfonic acid, BDH 99%) in 2.0 M sodium hydroxide (ANACHEMIA) solution. The 2.0 M sodium hydroxide solution was prepared by dissolving an appropriate quantity of sodium hydroxide (ACS grade/BDH) in ultrapure water followed by purification to remove heavy metals by mercury-cathode electrolysis at -1.4 V vs. E_{SCE} for at least 72 h using a mercury-pool cathode. To fix the ionic strength, the 2 mol/L

potassium nitrate stock solution was prepared from solid KNO_3 (BDH, AnalR). Stock solutions of copper, lead and zinc ($1000 \mu\text{g/mL}$) were prepared, each metal separately, by dissolving chemically pure metal or metal oxide in ultrapure nitric acid (Ultrex II), diluting the solution with ultrapure water, and acidifying the solution with ultrapure nitric acid to make the final solutions contain 1% (v/v) ultrapure nitric acid.

5.1.3 Electrochemical Parameters

Every sample was purged with nitrogen gas for 10 min. prior to the determination. The electrode stirring rate was set at 2000 rpm. For LSASV: deposition time 180 s, equilibration time 15 s, initial potential -1.2 V, end potential 0 V, step potential 2.5 mV. For studies on the chemical speciation of Cu (II), Pb(II), Zn (II), deposition potential was varied from 0.1V to -1.2V, -0.1V to 1.3V, -0.1V to 1.3V, respectively.

5.1.4 Water Sample Collection

The freshwater samples from the headwaters of Grand River were collected on June 23, 2002, using acid-washed Teflon containers. Immediately after the collection, the water samples were filtered through $0.45 \mu\text{m}$ Gelman mixed cellulose ester filters using a peristaltic pump and the filtrate was used as the test sample and stored in dark at 4°C prior to use. The water samples collected from Grand River after filtration were treated to UV radiation according to the procedures outlined in Section 4.1.5 (Chapter 4).

5.1.5 Determinations of Total Dissolved Metal Concentrations and Dissolved Organic Carbon (DOC)

Total dissolved metal concentrations and the DOC content in the filtered water samples were determined by inductively-coupled plasma mass spectrometry (ICP-MS), and with an IO Analytical 1010 Total Organic Carbon Analyzer (persulfate oxidation method), respectively, according to the procedures outlined in Section 4.1.6 and Section 4.1.7 (Chapter 4).

5.1.6 Sample Treatment and Methodology for Pseudopolarography

The water samples to be analyzed by pseudopolarography were prepared to be 0.1 mol/L in KNO_3 and 0.02 mol/L in HEPES buffer. To every test solution one of the following metals (M) was added (M = Cu, Pb or Zn) to make the total M (II) concentration 8.52×10^{-7} mol/L. Eppendorf digital micropipettes with disposable tips were used to add small aliquots of the reagents to the measurement cell; bulb pipettes were used to dispense larger volumes. A cell volume of approximately 15ml was used for all experiments. All solutions were purged with oxygen-free nitrogen for 10 min. prior to the determination.

All spiked test solutions were subjected to overnight equilibration. Following the measurement of the pseudopolarogram for the reference solution which was a model solution containing the test metal only as the metal-aqua complex, the equilibrated spiked test solutions were analyzed following the experimental procedure described earlier. Data were transferred to, and saved on a computer for subsequent analysis. A commercial software program, NLREG v5.2 (Phillip H. Sherrod, Brentwood, TN, USA), was used for the curve fitting purpose.

5.2 Results and Discussion

An example of LSASV curves recorded as a function of deposition potential for the reference solution containing 8.52×10^{-7} mol/L Pb (II) aqua ion only, and the water samples containing Pb-DOC complexes is shown in Figure 16, a and b, respectively. As can be seen from the curves, compared to the homogenous system (curve a), the stripping peaks were broadened and flattened in the presence of DOC. This was due to the heterogeneity of the complexant (DOC), i.e. a range of complexes of different stability were formed at the electrode surface [153].

The pseudopolarograms for determination of metal speciation in Grand River surface water samples (without solar radiation) are presented in Figure 17. The top curve, the pseudopolarogram for the Pb^{2+} aqua complex, is a sharp, well-defined wave with a flat plateau. This indicates that it represents a fully reversible electrode process. The lower curve is for the water sample spiked with the same total concentration of metal as that in the reference solution and equilibrated overnight. This curve is not steep as that for the metal aqua complex, but is elongated reflecting heterogeneity of the complexant (DOC). Also, the limiting current of Pb (II)- DOC complex has a lower value than that of lead aqua ion alone. This was probably due to the mean diffusion coefficient of the Pb(II)-DOC complexes being lower than that of the Pb(II) aqua complex.

5.2.1 Some Important Properties of Heterogeneous Complexants

Table 4 shows the difference in the half-wave potential between metal aqua complexes and metal-DOC complexes of Grand River UV irradiated sample of surface water, and the

Figure 16. Variation of LSASV peaks as a function of deposition potential for (a) Pb (II) aqua ion; and (b) Pb (II)-DOC complexes, $c_{M,T} = 8.52 \times 10^{-7}$ M, $c_{DOC} = 14.9$ mg/L.

Figure 17. Pseudopolarograms for Grand River surface water samples without solar radiation, (●), 8.52×10^{-7} mol/L $\text{Pb}^{2+}(\text{aq})$; (○) Pb (II)-DOC complex.

heterogeneity parameter, Γ . The order of decrease in the value of Γ is $\Gamma_{\text{Zn}} > \Gamma_{\text{Pb}} > \Gamma_{\text{Cu}}$. Since for a homogeneous complexant, $\Gamma = 1$, the above order suggests that for Zn (II), the DOC behaves as a relatively homogeneous complexant, whereas for Pb (II) it behaves as a relatively heterogeneous complexant, and for Cu (II) it behaves as a very heterogeneous complexant. Although the heterogeneous parameter, Γ , is not affected by the ligand concentration [95], it is metal-dependent. Other workers have observed the difference in the heterogeneity of humic substances for the complexation with metal ions. For example, Pinheiro et al. [154] have reported that a peat-derived humic acid is more heterogeneous for Pb (II) complexation than for Cd (II) complexation. It has been reported by Town and Filella [135] that Suwannee River fulvic acid (SRFA) is less heterogeneous for Pb (II) complexation than for Cu(II) complexation.

Table 4. Experimental parameters for metal-DOC complexes in Grand River surface water samples without solar radiation, $c_{\text{M,T}} = 8.52 \times 10^{-7}$ mol/L, $c_{\text{DOC}} = 14.9$ mg/L, LSASV, HEPES buffer, pH = 7.5, I = 0.1M, T = 295K.

Metal	$\Delta E_{1/2}$, V	Γ	i_{lim} (normalized)
Zn(II)	0.002	0.82	0.73
Pb(II)	0.006	0.65	0.48
Cu(II)	0.055	0.017	0.69

Two sets of parameters which reflect the equilibrium properties of the heterogeneous complexants, A , Γ , $\bar{K}$, and K_0^* , Γ , K^* can be determined as shown in Table 5 and Table 6, respectively. Since the stronger sites of the heterogeneous ligands are occupied first when they bind to one kind of metal, as the degree of occupation of all site types, $\bar{\theta}$, increases, the averaging of the apparent equilibrium between M and the site L_i is carried out with an increasing number of weaker sites and thus both $\bar{K}$ and K^* decreases. As shown in Table 5 and Table 6, the order of $\bar{\theta}$ is $\bar{\theta}_{Zn} < \bar{\theta}_{Pb} < \bar{\theta}_{Cu}$, whereas $\bar{K}$ and K^* have the same trend, ruling out the possibility that the change in $\bar{K}$ and K^* are due to that of $\bar{\theta}$. This implies that Cu (II)-DOC complexes is the most stable of these three metals, whereas the second most-stable is Pb(II), and the least stable is Zn(II)-DOC complex. These results are in agreement with those obtained by Thin Mercury Film at a Rotating Disk Electrode combined with Anodic Stripping Voltammetry (Chapter 4).

Table 5. Calculated Freundlich constant A and average equilibrium function $\bar{K}$ for M (II)-DOC complexes in Grand River surface water samples without solar radiation, $c_{M,T} = 8.52 \times 10^{-7}$ mol/L, $c_{DOC} = 14.9$ mg/L, $M = Zn(II)$, $Pb(II)$ or $Cu(II)$, LSASV, HEPES buffer, pH = 7.5, $I = 0.1M$, $T = 295K$.

Metal	$\bar{\theta}$	$\log A$	$\log \bar{K}$
Zn(II)	2.2×10^{-3}	3.03	4.16
Pb(II)	3.4×10^{-3}	3.67	4.32
Cu(II)	9.0×10^{-3}	-1.90	6.08

Table 6. DEF parameters, K_o^* , Γ and K^* , for M-DOC complexes (M = Zn(II), Pb(II) or Cu(II)) in Grand River surface water samples without solar radiation, $c_{M,T} = 8.52 \times 10^{-7}$ mol/L, $c_{DOC} = 14.9$ mg/L, LSASV, HEPES buffer, pH = 7.5, I = 0.1M, T = 295K.

Metal	$\bar{\theta}$	$\ln K_o^*$	$\log K^*$
Zn(II)	2.2×10^{-3}	7.01	6.38
Pb(II)	3.4×10^{-3}	7.34	7.38
Cu(II)	9.0×10^{-3}	-248.00	10.1

Note: K^* Differential Equilibrium Parameter

K_o^* Constant (= value of K^* for $\theta = 1$)

$\bar{\theta}$ Degree of occupation of all site types

There is lack of data on diffusion coefficients for heterogeneous complexants in natural waters [155, 156]. Diffusion coefficients reflect the mobility of the metal species, and hence, have important impacts on the transport and bioavailability of the metals in the aquatic environment. Table 7 represents average diffusion coefficients of metal-DOC complexes in the Grand River surface water samples. D_{ML} has much lower values than the corresponding D_M , indicating that the metal complexes with the natural complexant molecules are significantly larger than those with water molecules (metal-aqua complexes). Table 7 also indicates that the complexes formed by Zn(II) with DOC are the smallest, whereas those of Cu(II) are much larger. The same trend was found in the results obtained by using Thin Mercury Film at a Rotating Disk Electrode combined with Anodic Stripping Voltammetry

(Chapter 4). The extremely low mobility of Cu-DOC complexes may be related to its inert behaviour in the natural systems [139].

Table 7 Diffusion coefficients for metal-DOC complexes in Grand River surface water samples without solar radiation, determined by LSASV, $c_{M,T} = 8.52 \times 10^{-7}$ mol/L, $c_{DOC} = 14.9$ mg/L, HEPES buffer, pH = 7.5, I = 0.1M, T = 295K.

Metal	D_M (cm ² /s)	$\overline{D}_{ML}$ (cm ² /s)
Zn(II)	6.4×10^{-6}	3.4×10^{-6}
Pb(II)	8.3×10^{-6}	1.9×10^{-6}
Cu(II)	7.3×10^{-6}	7.9×10^{-7}

5.2.2 Influence of Solar Radiation on the Complexing Properties of DOC

Table 8 presents effects on the degree of heterogeneity of the complexants when the freshwater samples were exposed to sunlight. The heterogeneity parameter, Γ , increases for all the three metals with increasing duration of solar irradiation, implying that the complexing systems became less heterogeneous upon exposure to sunlight.

Since Γ is a constant for a given metal under different ligand concentrations [95], the change in Γ indicates different binding characteristics of DOC after it is exposed to solar radiation.

Table 8. Effect of solar radiation on the heterogeneity of the complexants in Grand River surface water samples after exposure to solar radiation, $c_{M,T} = 8.52 \times 10^{-7}$ mol/L, HEPES buffer, pH = 7.5, I = 0.1M, T = 295K, determined by LSASV.

Metal	Duration of Solar Radiation	$\Delta E_{1/2}$, V	Γ
Cu (II)	0 days	0.055	0.017
	10 days	0.015	0.080
	30 days	0.001	0.19
Pb (II)	0 days	0.006	0.65
	10 days	0.003	0.65
	30 days	0.001	0.89
Zn (II)	0 days	0.002	0.82
	10 days	0.001	0.92
	30 days	0.001	0.97

However, the exposure to sunlight did not change the order of the heterogeneity of the complexant among different metals, probably because the chemical properties of the metals such as charge-to-size ratio (constant), and metal-binding properties of DOC may be primary factor that affect the heterogeneity of the DOC.

The effect of solar radiation on the equilibrium properties of metal complexation with DOC are presented in Table 9 and Table 10. When the Grand River water samples were exposed to sunlight from 0, to 10 and 30 days, more binding sites in the DOC became free as reflected in the decrease of $\bar{\theta}$. It is also found that both the average equilibrium function and the differential equilibrium function of the complexants in freshwater samples significantly decrease with increasing duration of solar radiation. This is due to less binding sites being occupied by the metal (lower $\bar{\theta}$) and the changes both in the functional group content of DOC and in molecular size [152]. Investigations performed by J.M. Monsallier show that the complexation strength decreases with decreasing size fractions of humic acid [157].

Table 11 presents the effects of solar radiation on the speciation parameters, the diffusion coefficient of the metal-DOC complexes and the concentration of the free metal ions in Grand River surface water samples. The results indicate that with increasing duration of the exposure to solar radiation, the average diffusion coefficient, D_{ML} , increases probably because the new average now consists of proportionally greater number of small metal complexes (e.g. metal complexes with inorganic ligands) which has greater diffusion coefficient than the diffusion coefficient of the metal-DOC complex, ML .

Table 9. Influence of solar radiation on average equilibrium function for metal-DOC complexes in Grand River surface water samples exposed to solar radiation, $c_{M,T} = 8.52 \times 10^{-7}$ mol/L, HEPES buffer, pH = 7.5, I = 0.1M, T = 295K, determined by LSASV.

Metal	Duration of Solar	$\bar{\theta}$	log A	log $\bar{K}$
	Radiation			
Cu (II)	0 day	9.0×10^{-3}	-1.90	6.08
	10 days	8.2×10^{-3}	-1.48	5.03
	30 days	3.4×10^{-3}	-0.87	4.24
Pb (II)	0 day	3.4×10^{-3}	3.67	4.36
	10 days	4.8×10^{-3}	3.67	4.24
	30 days	9.3×10^{-4}	1.92	4.08
Zn (II)	0 day	2.2×10^{-3}	4.27	4.16
	10 days	1.2×10^{-3}	3.22	4.15
	30 days	1.5×10^{-4}	3.03	3.71

Table 10. Influence of solar radiation on differential equilibrium function for metal-DOC complexes in Grand River surface water samples exposed to solar radiation, $c_{M,T} = 8.52 \times 10^{-7}$ mol/L, HEPES buffer, pH = 7.5, I = 0.1M, T = 295K, determined by LSASV.

Metal	Duration of Solar	$\bar{\theta}$	$\ln K_o^*$	$\log K^*$
	Radiation			
Cu (II)	0 day	9.0×10^{-3}	-2.48×10^{-2}	10.10
	10 days	8.2×10^{-3}	-4.02×10	8.71
	30 days	3.4×10^{-3}	-9.35	6.92
Pb (II)	0 day	3.4×10^{-3}	7.34	7.38
	10 days	4.8×10^{-3}	6.57	6.67
	30 days	9.4×10^{-4}	6.22	5.79
Zn (II)	0 day	2.2×10^{-3}	7.01	6.38
	10 days	1.2×10^{-3}	6.58	6.28
	30 days	1.5×10^{-4}	5.62	6.02

Table 11. Change in the diffusion coefficient of metal-DOC complexes and in the concentration of free metal ions in Grand River surface water samples exposed to solar radiation, $c_{M,T} = 8.52 \times 10^{-7}$ mol/L, HEPES buffer, pH = 7.5, I = 0.1M, T = 295K, determined by LSASV.

Metal	Duration of Solar Radiation	$\overline{D}_{ML}$ (cm ² s ⁻¹)	C _{M, Free} (mol/L)
Cu (II)	0 day	7.9×10^{-7}	1.6×10^{-9}
	10 days	3.3×10^{-6}	1.9×10^{-8}
	30 days	3.5×10^{-6}	1.3×10^{-7}
Pb (II)	0 day	1.9×10^{-6}	4.3×10^{-8}
	10 days	2.4×10^{-6}	6.0×10^{-8}
	30 days	3.7×10^{-6}	9.9×10^{-8}
Zn (II)	0 day	3.4×10^{-6}	3.5×10^{-8}
	10 days	5.1×10^{-6}	7.3×10^{-8}
	30 days	5.3×10^{-6}	2.0×10^{-7}

5.2.3 The Kinetic Behaviour of the Metal Complexes

In pseudopolarography at a HMDE, the important variable to define the kinetic properties of the metal complexes, the timescale of measurement, is related to the stirring rate of this technique. Figure 18 presents pseudopolarographic waves under varied timescales of the electrochemical measurement, i.e. the varying stirring rate of the experiment. In the reference solution, the only metal species is the metal aqua ion; therefore, its pseudopolarogram (the upper most curve in Figure 18) remains the same whatever the stirring rate is. However, for metal-complexes with polyfunctional, heterogeneous DOC, the higher the stirring rate is (and hence, the shorter the timescale of measurement), the lower is the limiting current of the metal complexes. Since the limiting current is determined by the lability of the metal complexes in the system [158], the decreased limiting current at the higher stirring rate suggests transition of the metal-DOC complex from the labile to the non-labile state as the analytical timescale of measurement is decreased.

The information on the lability of the metal-DOC complexes can be obtained by comparing the calculated values of $\Delta t^{1/2}$ with 1, as mentioned earlier in Chapter 2 of this Thesis. Although with the Hanging Mercury Drop Electrode (HMDE), the thickness of the diffusion layer (and hence, the timescale of measurement) is not precisely defined, it is estimated that under our experimental conditions, t is approximately 0.3s [153], which is used in preparing Table 12. The lability of the Cu (II)-DOC complexes is significantly lower than those of Pb (II) and Zn (II). Upon the exposure to sunlight, the Cu (II)-DOC complexes change from the non-labile to the quasi-labile state, suggesting that solar radiation has the ability to change the kinetic behaviours of the complex systems and thus may affect the bioavailability of

Figure 18. Influence of the timescale of measurement on the dynamic nature of the Cu (II)-DOC complexes in Grand River surface water samples without solar radiation, • the reference solution, ○ 500 rpm, ■ 1000 rpm, △ 2000 rpm, $c_{M,T} = 8.52 \times 10^{-7}$ mol/L, HEPES buffer, pH = 7.5, I = 0.1M, T = 295K, determined by LSASV.

the metals in freshwaters. Under our experimental conditions, the Pb (II)-DOC and Zn (II)-DOC complexes are labile.

Table 12. The calculated $\Delta t^{1/2}$ values of metal-DOC complexing systems in Grand River surface water samples exposed to solar radiation, $c_{M,T} = 8.52 \times 10^{-7}$ mol/L, LSASV, HEPES buffer, pH = 7.5, I = 0.1M, T = 295K, determined by LSASV.

$\Delta t^{1/2}$ Values				
Metal	Sunlight	0 day	10 days	30 days
Cu (II)		0.3	2	2
Pb (II)		376	466	593
Zn (II)		26	45	150

5.2.4 Comparison of Speciation Parameters determined by TMF-RDE and with those determined by HMDE

Speciation parameters provide important information on chemical speciation in the freshwaters and soils and are independent of the analytical methods that are used to measure them; they quantitatively measure the chemical characteristics of chemical species. To fully characterize a system, usually, a number of speciation parameters are required.

Speciation Parameters determined by ASV at a TMF-RDE and by pseudopolarography for Pb (II)-DOC and Zn (II)-DOC complexes at a HMDE in Grand River UV-irradiated surface

water samples are compared in Table 13. A consistent trend in important Speciation Parameters was found in the results obtained by employing these two different techniques.

The difference between the average equilibrium constants and the concentration of free metal ions determined by TMF-RDE using ASV and by pseudopolarography at a HMDE is at least one order of magnitude. This difference may arise from the very small shifts observed in the pseudopolarographic half-wave potentials [95]. Since $\bar{K}$ increases with the increasing A, the lower values of A may be the cause for the underestimation of the average equilibrium functions of the metal-DOC systems, and so also, for the free metal ion concentrations.

The diffusion coefficients of the metal-DOC complexes in the water samples determined by ASV at a TMF-RDE are higher than those determined by pseudopolarography at a HMDE. This is may be due to the different chemical treatment of the water samples for these two electrochemical experiments. When TMF-RDE in conjunction with ASV was used, the samples were buffered to the pH of 6.2 using PIPES buffer, whereas when performing the experiments using HMDE, HEPES buffer was used to adjust pH to 7.5.

5.3 Conclusion

Pseudopolarography has two main advantages for the extraction of metal-complexation parameters of heterogeneous freshwater systems; one is the avoidance of the problems associated with changes in the individual peak potentials and peak widths caused by the metal-to-ligand ratio at the electrode surface; the other is that by doing one experiment, the

Table 13. Speciation Parameters determined by Anodic Stripping Voltammetry at a TMF-RDE and by Pseudopolarography at a Hanging Mercury Drop Electrode (HMDE) in Grand River surface water samples exposed to solar radiation, $c_{M,T} = 8.52 \times 10^{-7}$ mol/L, HEPES buffer, pH = 7.5, I = 0.1M, T = 295K, determined by LSASV.

Metal	Sunlight Duration	$\overline{D}_{ML,1}$ (cm ² s ⁻¹)	$\overline{D}_{ML,2}$ (cm ² s ⁻¹)	Log K ₁	Log K ₂	C _{M, Free,1} (mol/L)	C _{M, Free,2} (mol/L)
Pb (II)	0 day	3.4 x 10 ⁻⁶	1.9 x 10 ⁻⁶	7.02	4.36	8.7 x 10 ⁻¹⁰	4.3 x 10 ⁻⁸
	10 days	4.7 x 10 ⁻⁶	2.4 x 10 ⁻⁶	6.72	4.24	1.7 x 10 ⁻⁹	6.0 x 10 ⁻⁸
	30 days	7.3 x 10 ⁻⁶	3.7 x 10 ⁻⁶	6.63	4.08	2.1 x 10 ⁻⁹	9.9 x 10 ⁻⁸
Zn (II)	0 day	4.3 x 10 ⁻⁶	3.4 x 10 ⁻⁶	5.20	4.45	5.4 x 10 ⁻⁸	3.5 x 10 ⁻⁸
	10 days	4.9 x 10 ⁻⁶	5.1 x 10 ⁻⁶	5.17	4.15	5.7 x 10 ⁻⁸	7.3 x 10 ⁻⁸
	30 days	4.9 x 10 ⁻⁶	5.3 x 10 ⁻⁶	5.17	3.71	5.7 x 10 ⁻⁸	2.0 x 10 ⁻⁷

$\overline{D}_{ML,1}$, K₁ and C_{M, Free,1} are diffusion coefficients, average equilibrium constants, and the concentrations of free metal ions, determined by TMF-RDE in conjunction with ASV, respectively; $\overline{D}_{ML,2}$, K₂ and C_{M, Free,2} are diffusion coefficients, average equilibrium constants, and the concentrations of free metal ions, determined by pseudopolarography at a HMDE; M = Pb (II) or Zn (II).

polarographic wave can be used to completely define two sets of equilibrium functions: Average Equilibrium Function and Differential Equilibrium Function. Two sets of parameters for these two functions, Freundlich-A and Γ , and DEF- K_0^* and Γ are not affected by experimental conditions, and thus have predictive values, allowing $\bar{K}$ and K^* to be calculated for any metal-to-ligand ratio. It is found that the metal complexes with DOC become less labile when the stirring rate (and hence, the timescale of measurement) decreases. Also, the results show that exposure to sunlight significantly change the binding properties of DOC in Grand River surface water samples. These results agree with those obtained using TMF-RDE in conjunction with ASV. Furthermore, these changes may be due to both the release of free metal ion and variation in the functional groups of DOC and in molecular size.

CHAPTER 6

SIGNIFICANCE OF THIS WORK IN ENVIRONMENTAL

CHEMISTRY

The dynamic nature of metal speciation in natural waters involves not only the equilibrium distribution of the different metal species but also the kinetic characteristics of their interconversion. In these dynamic systems, the assumption of equilibrium at the surface of the biological membrane, which requires association and dissociation kinetics and transport of the metal species towards the biological membrane to be more rapid than the uptake of metals by the organism, may not be valid, and this may result in Free Ion Activity Model (FIAM) and Biotic Ligand Model (BLM) being not applicable to these dynamic systems. More rigorous criteria with the timescale of measurement as a key parameter were developed for investigating metal complexes in heterogeneous systems, resulting in better understanding of the dynamics of metal complexation in freshwater.

Anodic Stripping Voltammetry at a Rotating Disk Electrode with a Thin Mercury Film provides high sensitivity and has the unique advantage of defining precisely the timescale of measurement by its control of the thickness of the Nernstian diffusion layer. In freshwater samples without exposure to sunlight, DOC complexes of Cd (II), Zn (II) and Pb (II) are quasi-labile, whereas those of Cu (II) are non-labile. The lability of metal complexes increases with the duration of solar radiation, which suggests that solar radiation significantly reduces the ability of DOC to bind metals. Although DOC can prevent the toxic effects of aquatic organisms caused by changes in metal loading in aquatic systems, these beneficial metal binding properties of DOC may be rather short-lived when DOC is exposed to solar radiation.

Pseudopolarography at a Hanging Mercury Drop Electrode (HMDE) provides an insight into the heterogeneity of organic, macromolecular complexant (DOC) in freshwaters. In Grand River surface water samples without solar radiation, in the presence of Zn (II), the complexants behaved as homogeneous, whereas in the case of Cu (II), these complexants behaved as very heterogeneous. Solar radiation changes the properties of DOC to make it more homogeneous and reduces the stability of the complexes formed between the metal and DOC. The increase in the lability of these complexing systems under irradiation by sunlight may increase their bioavailability and ecotoxicity.

The significance of this work is that the timescale of measurement was identified as a crucial parameter for defining the metal species in freshwater systems and that this work has brought out the need for regulating metal loading to freshwaters on the basis of not only the concentration of DOC but also solar radiation.

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