DETERMINATION OF TRACE METALS AND COPPER COMPLEXATION IN FRESHWATER SYSTEMS OF THE BONAVISTA PENINSULA, NEWFOUNDLAND BY STRIPPING VOLTAMMETRY

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ABSTRACT

The speciation of trace metals in natural waters is important in determining their bioavailability and toxicity. For instance, inorganically bound (often referred to as labile) copper in natural waters is toxic to most phytoplankton species but complexation by natural organic ligands considerably reduces or eliminates this toxicity. In order to be able to understand the effects of trace metals, it is important to determine the amount of labile metals and degree of metal complexation (types and strengths of such complexation).

Labile zinc, cadmium and lead in freshwater samples from the Bonavista Peninsula area of Newfoundland were analyzed by differential pulse anodic stripping voltammetry (DPASV) and labile copper was determined by adsorptive-cathodic stripping voltammetry with 8-hydroxyquinoline (oxine) (ACSV or oxine-CSV). The oxine-CSV method used to determine labile copper in seawater has been modified for use in freshwater. Trace metal results reflected industrial and residential impacts on the watersheds even though concentrations of labile metals were within the typical range of the metals in freshwater.

Copper complexation was investigated by complexing capacity titrations using the oxine-CSV method to determine concentrations of natural copper complexing ligands and their conditional stability constants. The effect of the adsorption potential on the determination of copper complexation was also studied at three potentials of -0.15 V, -0.7V

and -1.1 V. It was found that the detected complexing ligand concentrations decreased 6.7 -69.4 % when the potential used was more negative than -0.15V. The decrease was more severe at more negative potential and lower detection windows. Detailed measurements of copper complexation in freshwater were carried out at three detection windows by varying the oxine concentrations from 7.3 to 36.7 μM and confirmed the presence of several complexing ligands. The detected complexing ligand concentrations were found to decrease with increasing detection window, whereas the conditional stability constants were found to increase.

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ABBREVIATIONS AND SYMBOLS

ACSV	adsorptive-cathodic stripping voltammetry
ASV	anodic stripping voltammetry
Beryllon III	4-[(4-diethylamino-2-hydroxyphenly)azo]-5-hydroxynaphthalene-2,7-disulphonic acid
CASS-2	coastal Atlantic seawater standard
CSV	cathodic stripping voltammetry
$[L_x]$	the concentration of total natural organic ligand
DASA	1,2-Dihydroxyanthraquinone-3-sulfonic acid
Dep. time	deposition time
D.L.	detection limit
DPASV	differential pulse anodic stripping voltammetry
dw	detection window
d.w.	distilled water
Fil. Acd. PE-bot.	filtered and acidified sample stored in low density polyethylene bottle
Fil. F-bot.	filtered freshwater sample stored in fluorinated-high density polyethylene bottle

Fil. F-bot. Fr.	filtered freshwater sample stored in fluorinated-high density polyethylene bottle in a deep freezer
Fil. PE-bot.	filtered freshwater sample stored in low density polyethylene bottle
FLPE	fluorinated-high density polyethylene
FW	freshwater
HEPA	high efficiency particle air
HEPES	4-(2-hydroxyethy)-1-piperazineethanesulfonic acid
ICP-AES	inductively coupled plasma-atomic emission spectrometry
ICP-MS	inductively coupled plasma-mass spectrometry
i_p	peak current
$K^{\bullet}_{a,i}$	stepwise stability constant for an acid
$K^{\bullet}_{i}$	stepwise stability constant for a complex
$K^{\bullet}$	stoichiometric concentration constant for monomeric complex
$\beta^{\bullet}$	stoichiometric concentration constant for dimeric complex
K'	conditional stability constants for monomeric complexand dimeric
β'	conditional stability constants for dimeric complex
LDPE	low density polyethylene
L_x	natural organic ligand
NASS-4	north Atlantic seawater standard

Nfil. PE-bot.	not filtered sample stored in low density polyethylene bottle
NF-G1 (May,1995)	Newfoundland Green Plan sample collected at site # 1 in May, 1995
oxine	8-hydroxyquinoline
PADPA	2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol
PAR	Princeton Applied Research
2,7-PADA	1-(2-pyridylazo)-2,7-dihydroxynaphthalene
Phen	1, 10-phenanthroline
Q-grade	double sub-boiling distilled in quartz
SA	salicylaldoxime
SATP	Salicylideneamino-2-thiophenol
S.D.	Standard deviation
SMDE	static mercury drop electrode
STD	standard
TAC	2-(2'-thiazolylazo)-p-cresol
TAN	1-(2-thiazolylazo)-2-naphthol
TAC	2-(2-thiazolylazo)-4-methylphenol
TAR	4-(2-thiazolylazo)-resorcinol
TAM	2-(2-thiazolylazo)-5-dimethylaminophenol
TSK-8HQ	Fractogel TSK-immobilized 8-hydroxyquinoline
UV-FW	UV-irradiated freshwater

XO	Xylenol orange
α	α-coefficient or side-reaction coefficient
[ ]	molar concentration

To Shuguang and Yixin

CHAPTER 1

INTRODUCTION

1.1 Trace Metals in Freshwater

1.1.1 Complexity of an Aquatic System

Natural aquatic systems are highly complex. Most of the elements of the periodic table may be present including a vast number of organic compounds with concentrations often at μg/L or lesser levels. In addition, the compounds in natural water have a continuum of sizes ranging from 1×10^{-8} cm to a few cm. Diameter classifications were based on the distinction between (**Figure 1.1**):

- particulates and dissolved components, or
- inorganic components, non-living organic components and living organisms.

The dissolved compounds are operationally defined as compounds which can pass through a 0.45 μm membrane filter. The filtration step arbitrarily separates dissolved components from particulates since the size distribution of some chemical species covers a broad range as shown in **Figure 1.1**. Similarly, there is no clear cutoff between organic and inorganic components: for example, particles such as hydrous Fe (III) oxides are usually considered to be inorganic, even though their surfaces are actually often covered with adsorbed organic components such as humic acid.

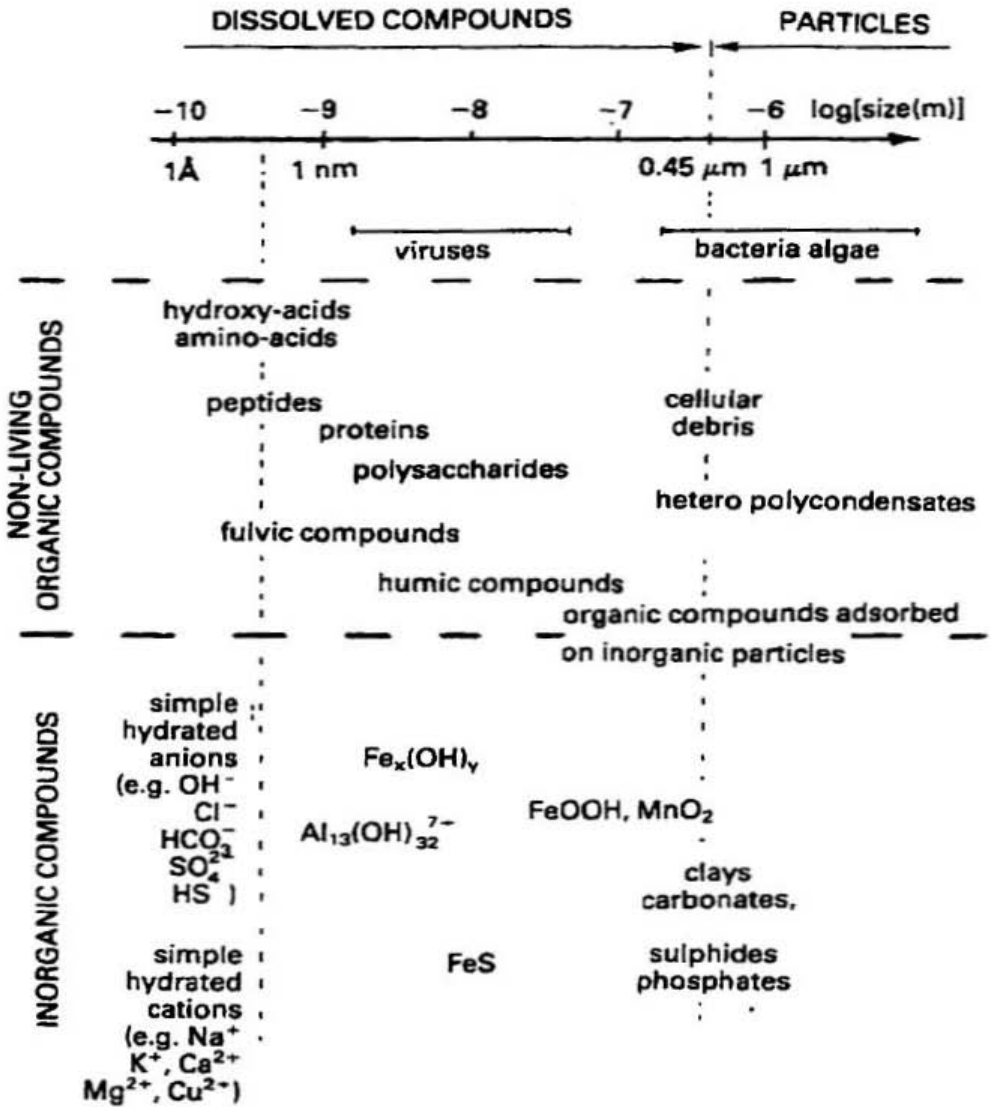

Figure 1.1 Schematic classification, by size, of important organic and inorganic water components (Buffle, 1988)

1.1.2 Trace Metals in Freshwater

It is known that trace metals in natural aquatic systems play important roles as either essential or toxic elements to aquatic organisms and humans. A trace metal, which is essential to a normal body function at lower concentrations, may be highly toxic when present at higher concentrations (Florence, 1982). Moore and Ramamoorthy (1984) discussed a range of trace metals in natural waters from an environmental point of view.

Zinc is an essential element to life because it mediates a variety of metalloenzymes and the biosynthesis of nucleic acids. The toxicity of zinc to aquatic organisms, under most conditions, is lower than mercury, copper, cadmium, nickel and arsenic. Zinc is always present in natural waters and most species can tolerate relatively high zinc levels.

Cadmium is acutely toxic to humans with as little as one gram being lethal as a single dose. At low concentration, humans are protected by complexation of Cd^{2+} with a protein which is subsequently eliminated in the urine. Higher levels are accumulated in the liver and kidneys with a lifetime of several decades (Hutchinson and Meema, 1987). However, cadmium is less toxic to aquatic life than to humans. This toxicity varies with species and environmental factors such as temperature and pH.

Lead is not very toxic to adults in low concentrations but is toxic to fetuses and children under age seven as it interferes with their brain development (Hutchinson and Meema, 1987). Accumulated lead in a mother's body can be passed to her newborn baby through her breast milk, while lead in tap water can also be harmful when the tap water is used to prepare formula for bottle-fed babies. The toxicity of lead to aquatic organisms is less

than that of mercury, but is similar to that of cadmium (Moore and Ramamoorthy, 1984).

Copper is not acutely toxic to humans but very toxic to most aquatic microorganisms even in very low concentrations (Moore and Ramamoorthy, 1984).

Four metals of prime environmental concern, Zinc, Cadmium, lead and Copper, are studied in this work because: (i) they are important to both aquatic organisms and humans; (ii) their labile fractions, which are usually considered to be the bioavailable[1] fractions, can be determined directly and simultaneously (section 1.2.2).

Figure 1.2 shows the typical range and average values of the total concentrations (labile and non-labile) of some metal ions in freshwater. The total concentrations of metals can vary significantly from one water system to another. Even though the total concentrations of a trace metal may be similar in two water systems, the physico-chemical forms of that metal (i.e. speciation) may be quite different. The different forms in which a metal ion (M) may be found in an aquatic system are summarized in **Figure 1.3** and some examples are given in **Table 1.1**. Variations in the chemical forms of trace metals can significantly change their bioavailability and toxicity. For environmental reasons, determination of the bioavailabile fraction of trace metals has gained more attention than total metal determination since the total metal determination provides little information about its bioavailability.

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[1] Bioavailable means that the metal can be taken in and used by organisms.

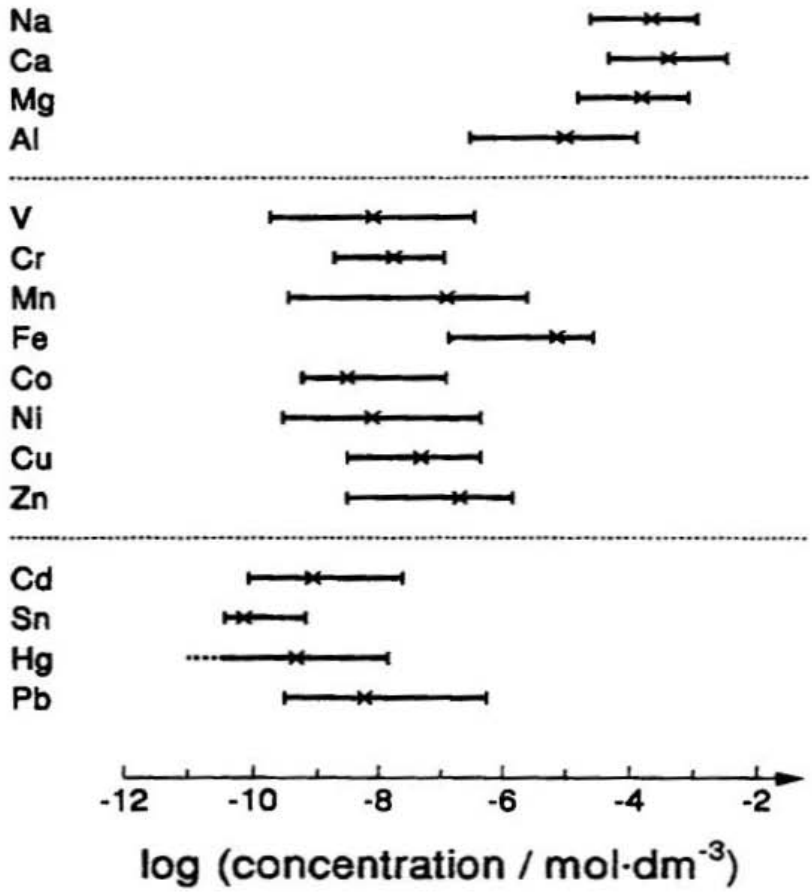


Figure 1.2 Range (—) and average values (×) of the total concentrations of some metal ions in freshwater (Filella *et al.*, 1995)

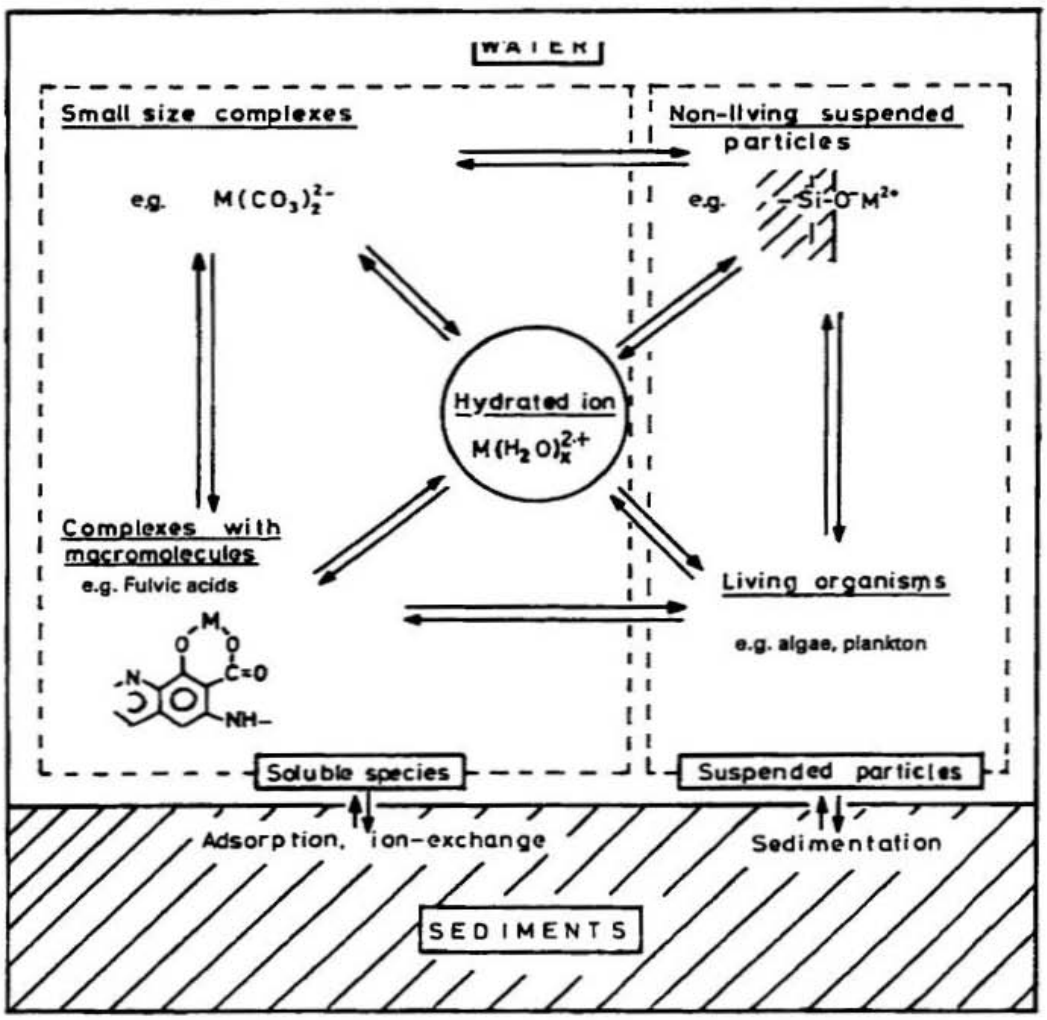


Figure1.3 The different forms in which metal ions (M) may be found in an aquatic system (Buffle, 1988)

Table 1.1 Possible physico-chemical forms of some metals in natural waters (Pickering, 1995)

Physico-chemical form	Example	Approximate diameter, nm
Particulate		>450
Precipitates	$PbCO_3$	
Mineral particles	PbS	
Metals absorbed by organisms	Metals in algae	
Colloidal		10-5000
Adsorbed on inorganic colloids	Cu^{2+}-Fe_2O_3	
Adsorbed on organic colloids	Pb^{2+}-humic acid	
Adsorbed on mixed colloids	Cu^{2+}-Fe_2O_3 /humic acid	
Soluble		<5
Simple hydrated metal ions[1]	$Cd(H_2O_6)_6^{2+}$	0.8
Simple inorganic complexes	$Pb(H_2O)_4Cl_2$	1
Simple organic complexes	Cu-glycinate	1-2
Stable inorganic compounds	PbS, $ZnCO_3$	1-2
Stable organic complexes	Cu-fulvate	1-2

[1] also called free metals.

Two distinctly different techniques: computer modelling and experimental measurement have been used for the study of trace metal speciation in natural waters.

The computer modelling method involves the use of known thermodynamic equilibrium stability constant data and concentrations of various components, in the water to compute the equilibrium concentrations of the different metal species (Jenne, 1979). Computer modelling is a powerful technique and makes a significant contribution towards

an understanding of speciation in natural waters. The limitations of the method are that the concentrations of natural organic ligands are usually not available and the interactions of metals with such natural ligands and with the colloidal particles present in natural waters are usually not clear (Buffle, 1988).

The experimental techniques for the study of trace metal speciation in natural waters include anodic stripping voltammetry (ASV) (Whitfield, 1975; Donat and Bruland, 1994), cathodic stripping voltammetry (CSV) (van den Berg *et al.*, 1990; Abolloi *et al.*, 1991), ion-exchange (Florence, 1977; Florence and Batley, 1980), ultrafiltration (Hart and Davies, 1978) and bioassay (Hoover, 1978).

ASV and CSV, two electrochemical approaches, determine the electrochemical labile fraction of the total metal. Labile metal is operationally defined as the fraction of the total metal which can be detected under experimental conditions. For instance, the ASV-labile metal is the fraction of the total metal that can be measured under a defined set of ASV and solution conditions (see section 1.2.2). The theory and applications of ASV and CSV to trace metal speciation measurements are discussed in section 1.2. Ion-exchange technique uses a chelating resin such as Chelex-100 to separate trace metals into different groups of "very labile", "moderately labile", "slowly labile" and "inert" due to the different rates of transfer to the resin. Ultrafiltration involves the use of ultrafiltration membranes to differentiate metal species by size, shape and charge characteristics rather than by molecular weight (Guy and Chakrabarti, 1975). The Chelex column technique and the Chelex batch technique are two commonly used ion-exchange approaches. The ultrafiltration technique,

when combined with characterization of the metal species in each size fraction by electrochemical and ion-exchange techniques, provides a more comprehensive picture of the metal speciation (Chakrabati *et al.*, 1993). Bioassay is a biological technique which involves the use of aquatic animals to determine the bioavailable fraction of trace metals based on the determination of trace metals taken up by the animals (Florence *et al.*, 1983; Morel *et al.*, 1991).

The speciation of some metals in freshwater by both computer modelling and anodic stripping voltammetry (ASV) are summarized in **Table 1.2**. The computer modelling results are reported as particular forms of the total inorganic metal, whereas experimental results are reported as the ASV-labile fraction of the total metal.

For Zn, computer modelling indicates that the dominant inorganic forms are free ion (50%) and zinc carbonate (38%). Experimental results indicate that 50 % of Zn is ASV-labile.

For Cd, computer modelling indicates that the main inorganic forms are free Cd ion and Cd carbonate. A high proportion (70%) of Cd is ASV labile.

For Pb, computer modelling suggests that carbonato species, such as Pb carbonate and Pb dihydroxy carbonate are the main inorganic Pb species, while little ASV-labile Pb is found in freshwater.

For Cu, computer modelling predicts that more than 90 % of inorganic Cu should be present as copper carbonate, although a small amount is likely to be associated with colloidal particles, such as hydrated iron oxide. Most freshwater streams also have little ASV-labile

Cu, and the percentage of organically bound Cu is usually high.

Table 1.2 Speciation for Zn, Cd, Pb and Cu in freshwater

Metal	Computer modelling, particular form / the total inorganic metal, %	Experimental measurement, ASV-labile fraction of the total metal, %	Reference
Zn	Zn^{2+} (50 %) $ZnCO_3$[1] (38 %)	50 %	Florence, 1980, 1982 Nordstrom, 1979
Cd	Cd^{2+}, $CdCO_3$[2]	70 %	Florence, 1977
Pb	$PbCO_3$ + $Pb_2(OH)_2CO_3$[1] (90 %)	Little ASV labile Pb	Florence, 1980, 1982
Cu	$CuCO_3$ + Cu^{2+} - Fe_2O_3 (colloidal particles)(90%)	-Little ASV labile Cu -Highly organically complexed	Florence, 1980, 1982, 1977

[1] may have low ASV lability.
[2] depending on pH.

1.2 Stripping Voltammetric Analysis

Among several analytical techniques available at present, stripping voltammetric analysis appears to be the most suitable for the study of trace metal speciation. The advantages of voltammetric analysis include:

- Determination of the labile fraction of metal which is believed, under certain conditions, to correlate well with the bioavailable fraction of metal (Florence, 1986).

• Simultaneous determination of four metals, Zn, Cd, Pb and Cu, with excellent sensitivity.

• Minimal sample preparation, less potential contamination and low cost of instrumentation.

1.2.1 Theory of Stripping Voltammetric Analysis

Stripping voltammetry is a two-step technique. During the first step, analyte is deposited (preconcentrated) into or onto the surface of an electrode (usually mercury) by controlled potential electrolysis. In the second step the deposited analyte is removed ("stripped") from the electrode by a potential scan and the resulting current peaks are used to determine the concentration of each analyte species in the sample.

There are two types of stripping voltammetry: anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV). Anodic stripping voltammetry (ASV) is used primarily for the determination of heavy metals. In this technique preconcentration is accomplished by the reduction of metal ions to the elemental state, and the stripping step is accomplished by a positive potential scan that gives an anodic current when the preconcentrated metals are oxidized (**Figure 1.4**). During deposition, an amalgam is formed by the elemental metal and the mercury on the electrode. Therefore, ASV can only be used to determine those metals that exhibit appreciable solubility in mercury. Examples of metal ions which have been determined by ASV at a mercury electrode are Bi^{3+}, Cd^{2+}, Cu^{2+}, Ga^{3+}, In^{3+}, Ni^{2+}, Pb^{2+}, Sb^{2+}, Sn^{2+}, Tl^{+}, and Zn^{2+}.

Deposition: Applied potential more negative than $E_{1/2}$ of M^{n+}

$M^{n+} + ne^- \rightarrow M(Hg)$
sample solution

Stripping: Scan in the positive direction, peak current is proportional to the concentration of M

$M(Hg) \rightarrow M^{n+} + ne^-$
sample solution

Figure 1.4 Anodic stripping voltammetry

Cathodic stripping voltammetry (CSV) involves preconcentration by oxidation followed by stripping via a negative potential scan. Anions may be determined by deposition as an insoluble mercury salt on the electrode surface. The negative potential scan causes the reduction of the salt to Hg and X^-, giving a cathodic current peak (**Figure 1.5**). CSV has been applied to the determination of Cl^-, Br^-, I^-, S^{2-}, Se^{2-}, CrO_4^{2-}, WO_4^{2-}, MoO_4^{2-}, VO_3^-, and SO_4^{2-}. The determination of certain metals such as Ce^{3+}, Tl^+, Mn^{2+}, and Fe^{2+} is performed by cathodically stripping a film of insoluble $M(OH)_n$ deposited on a graphite electrode (Bond, 1980).

The remarkable sensitivity of stripping voltammetry is attributable to the deposition (preconcentration) step. In essence, a significant fraction of the analyte ions are electrochemically extracted from the sample solution as metal atoms into a mercury electrode (or as a salt onto the electrode surface). Since the volume of the mercury electrode is

considerably less than the volume of sample solution being analyzed, the resulting "solution" of metal atoms in the liquid mercury is much more concentrated than the solution of metal ions being determined.

Deposition: At a relatively positive potential where Hg_2^{2+} ions can be produced:

$$2Hg \rightarrow Hg_2^{2+} + 2e^-$$

then

$$Hg_2^{2+} + (2/n)\ X^{n-} \rightarrow Hg_2X_{(2/n)}$$

(insoluble film)

Stripping: Scan in the negative direction, peak current is proportional to the concentration of X^{n-}

$$Hg_2X_{(2/n)} + 2e^- \rightarrow 2Hg + (2/n)\ X^{n-}$$

Figure 1.5 Cathodic stripping voltammetry

There are several types of stripping techniques such as linear potential sweep stripping voltammetry, differential pulse stripping voltammetry and square wave stripping voltammetry. The differential pulse and square wave stripping techniques have higher signal to noise ratios than linear potential sweep stripping voltammetry and therefore, lower detection limits.

1.2.2 Differential Pulse Anodic Stripping Voltammetry (DPASV)

DPASV is the most widely applicable electrochemical technique for the study of trace

metal speciation in waters, because of its ability to determine the labile fraction of the total metal concentration which can be correlated to the bioavailable fraction. Florence (1986) compared the similarity of the mechanics of dissociation of metal complexes at an electrode and at a biomembrane (**Figure 1.6**).

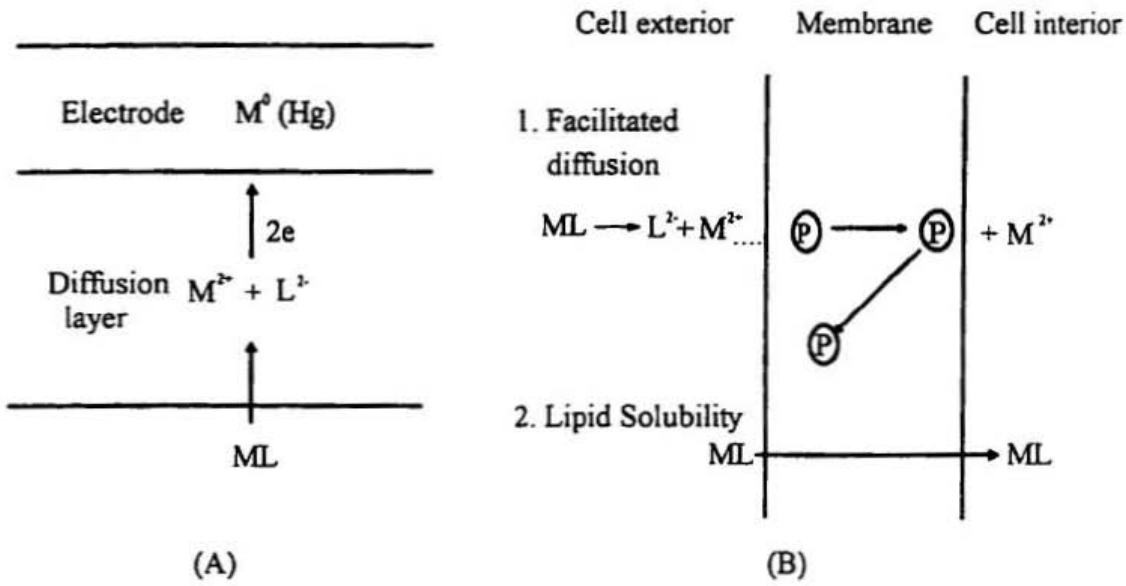


Figure 1.6 Comparison of mechanisms of dissociation of metal complexes at an electrode (A) and at a biomembrane (B); P: a protein

In **Figure 1.6A**, the metal deposited on the mercury electrode surface is due to the reduction of M^{2+} ions dissociated from the metal complex, ML. This fraction of metal is called the ASV-labile metal which consists of free metal ion and metal that can dissociate

in the double layer from complexes (usually inorganic complexes) or colloidal particles. In **Figure 1.6B**, one of the metal uptake mechanisms (shown as # 1 in **Figure 1.6B**) is a complexing agent (e. g. a protein) in the cell membrane binding the metal ion on the outer membrane surface, followed by the metal-protein complex diffusing to the interior of the membrane and releasing the metal ion into the cell interior. This metal uptake mechanism occurs when the metal complex is dissociated at a membrane surface. This fraction of metal is equivalent to the bioavailabile fraction which includes free metal ions and metal complexed with weak ligands. Since both the process of metal accumulation in an organism and metal deposition into mercury electrode involve the dissociation of metal complexes, the ASV-labile metal might therefore be used to monitor the toxic fraction of a metal.

Intensive studies (Florence, 1986) have been carried out to correlate the ASV-labile metal with its toxicity. Florence *et al.* (1983) found a good correlation between ASV-labile Cu and toxicity towards the marine diatom *Nitzschia closterium* (**Table 1.3**).

Table 1.3 Correlation between ASV-labile and toxic fractions of copper in seawater using the marine diatom *Nitzschia closterium* (Florence *et al.*, 1983).

Ligand	Conc.(M)	Copper (μM)	ASV-labile fraction[1], % - 0.6 V	-1.3 V	Toxic[2]fraction%
Fulvic acid	1×10^{-5}	32	1.5	2.9	7.5
Tannic acid	6×10^{-7}	32	5.5	10.5	12.5
Iron-humic acid colloid[3]	1.0 + 5.3 mg/L	32	70	74	60

[1] pH 8.2, with deposition potential of -0.6 V and -1.3 V.
[2] Fraction of added Cu appearing toxic compared with ligand-free solution.
[3] 1.0 mg/L of Fe + 5.3 mg/L of humic acid.

1.2.3 Adsorptive-Cathodic Stripping Voltammetry (ACSV)

As discussed in section 1.2.1, differential pulse cathodic stripping voltammetry (DPCSV) involves preconcentration by formation of an insoluble film on the mercury surface with subsequent stripping by a negative potential scan. This method has been applied to the determination of many anions and certain metals ($M(OH)_n$ as the insoluble film) (Vydra *et al.*, 1976). DPCSV has not been widely applied to trace metal speciation determination until recently with the development of adsorptive-CSV (ACSV). The adsorptive-CSV method involves the use of a surface-active reagent (complexing ligand) to complex the metal through which the metal-complex is selectively adsorbed onto the mercury electrode as a film and determined by cathodic stripping voltammetry as for example ACSV of Cu (**Figure 1.7**).

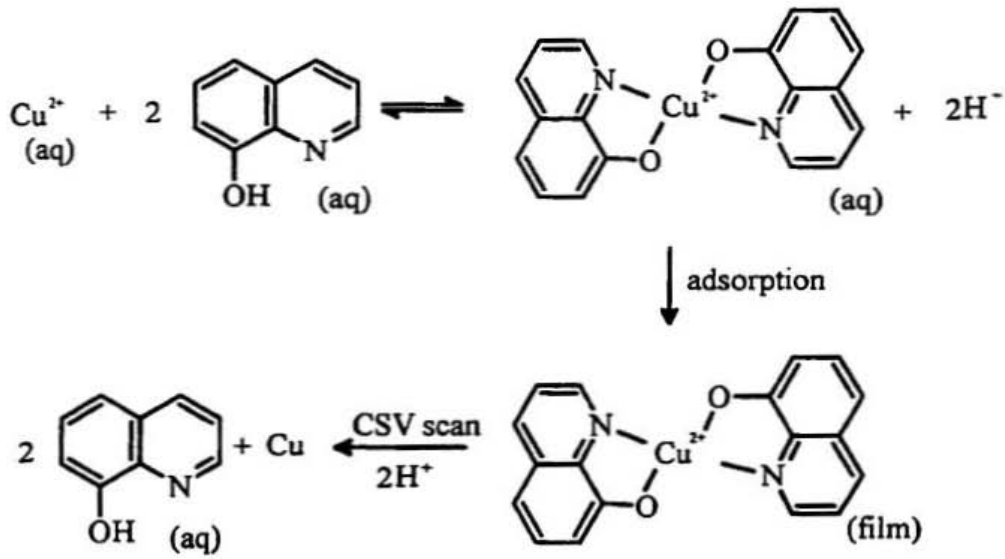


Figure 1.7 Adsorptive-cathodic stripping volammetry of Cu with oxine as the added ligand

The ACSV method has been applied to speciation studies of many elements in natural waters, such as copper speciation in estuarine waters (van den Berg *et al.*, 1990), selenium in seawater (van den Berg and Khan, 1990) and iron in lake water (Abolloi *et al.*, 1991). Different metals usually require different complexing ligands; therefore, recent applications have involved the use of adsorbed films of catechol for Cu, Fe, U and V (van den Berg, 1984 a and b; van den Berg *et al.*, 1984 a-c), ammonium tetra-methylene dithiocarbamate for Zn (van den Berg, 1984 c) and 8-hydroxyquinoline for Mo (van den Berg, 1985) and Cu (van den Berg, 1986). The discrimination of labile/inert species is based on their reactivity with the added organic ligand. Compared to DPASV, the adsorptive-CSV technique improves peak shape (sharper, flatter baseline) and provides lower detection limits.

Since the ASV peak for Cu is very close to the oxidation peak of mercury, there has been a need to further develop ACSV, resulting in the study of a great number of organic complexing ligands for Cu determination by ACSV (**Table 1.4**).

Oxine was chosen in this study for Cu determination in freshwater because of the lower detection limit for Cu.

Table 1.4 Summary of ligands for ACSV determination of Cu

Ligand	Sample	Detection limit	Buffer (pH)	Reference
XO[1]	Sea water	-	Acetate (5.0)	Wu *et al.*, 1995
SA[2]	Sea water	0.1 nM/1min	Borate (8.35)	Campos *et al.*, 1994
Thiocyanate ion	NASS-4[11], sea water	0.4 nM/1min	Hydrochloric Acid (2.5)	Yokoi *et al.*, 1994
Phen[3]	Sea water	0.5 nM/20min	HCl and NaOH (7.0)	Culjak *et al.*, 1994
TAC[4]	Sea water	0.8 nM/10min	Acetate (3.6)	Farias *et al.*, 1993
DASA[5]	Sea water	0.3 nM/1min	Borate (8.2)	Quentel *et al.*, 1994
2,7-PADA[6]	Tea, hair	8.0 nM/1min	Ammonia (9.8)	Zhang *et al.*, 1993
Beryllon III[7]	Hair, d.w.[12]	0.5 nM /-	Acetate	Zhao *et al.*, 1992
Tropolone	Sea water	0.6 nM/1min	Borate (8.35)	Donat *et al.*, 1992
TAN, TAC, TAR, TAM[8]	Freshwater	0.8 nM/5min	Acetate (3.7)	Farias *et al.*, 1992
Imidazole	-	2 nM/3min	Hydrogen carbonate(8.5)	Ertas *et al.*, 1991
SATP[9]	Rock	4 nM/1min	Nitric acid (2)	Tanaka *et al.*, 1990 a
1, 10-phenanthroline	-	0.9 nM/1min	Hydrochloric Acid (2.5)	Quentel *et al.*, 1990
PADPA[10]	-	50 nM/1min	Ammonia (9.0)	Tanaka *et al.*, 1990 b
Nioximate	River water	7 nM/1min	Ammonia (9.2)	Bobrowski, 1998
Oxine	Sea water	0.2 nM/1min	HEPES (7.7)	van den Berg, 1986
Catechol	Sea water	0.3 nM/1min	HEPES (7.8)	van den Berg, 1984 a
	River water			van den Berg *et al.*,1990
	Freshwater		Acetate (6.0)	Jones *et al.*, 1989

[1] Xylenol orange
[2] Salicylaldoxime
[3] 1, 10-phenanthroline
[4] 2-(2'-thiazolylazo)-p-cresol
[5] 1,2-Dihydroxyanthraquinone-3-sulfonic acid
[6] 1-(2-pyridylazo)-2,7-dihydroxynaphthalene
[7] 4-[(4-diethylamino-2-hydroxyphenly) azo]-5-hydroxynaphthalene-2,7-disulphonic acid
[8] 1-(2-thiazolylazo)-2-naphthol, TAN; 2-(2-thiazolylazo)-4-methylphenol, TAC; 4-(2-thiazolylazo)-resorcinol, TAR; and 2-(2-thiazolylazo)-5-dimethylaminophenol, TAM
[9] Salicylideneamino-2-thiophenol
[10] 2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol
[11] North Atlantic seawater standard
[12] Distilled water

1.2.4 Applications in Water Analysis

DPASV and ACSV have been used extensively for the determination of trace metals in natural waters. Representative applications are shown in **Table 1.5**.

Table 1.5 Typical applications of stripping voltammetry in natural water analysis

Place of Sampling	Metals determined	Stripping type	Reference
Seas			
Australian	Pb, Cd	DPASV	Florence, 1986
Barents	Cd, Pb, Cu, Ni	DPASV	Mart *et al.*, 1981
Japanese (near Japan)	Zn, Cu, Pb, Cd	DPASV	Miwa and Mizuike, 1977
Gulf of Mexico	Zn, Cd, Cu	DPASV	Florence, 1986
Antarctic	Cd, Cu, Fe, Ni, Zn	ACSV	Abollino *et al.*, 1995
Lake water			
Switzerland (Greifen)	Cu	ACSV	Xue and Sigg, 1993
Estuarine water			
Great Britain (Tamar)	Cu	ACSV	van den Berg *et al.*, 1990
Drinking water			
Germany (Ruhr)	Pb, Cu	DPASV	Arts *et al.*, 1984
Japan (Tokyo)	Sb	DPASV	Florence, 1986
(Kobe)	Cd	DPAVS	Florence, 1986
Rain water			
Germany (Ruhr)	Zn, Cd, Pb, Cu	DPASV	Nurnberg, 1984
Belgium (Brussels)	Zn, Cd, Pb, Cu, Mn, Co, Ni, Se	DPASV	Florence, 1986
Waste water			
Great Britain	Zn, Cd, Cr, Ni	DPASV	Clark *et al.*, 1988

1.3 Copper Complexation in Freshwater

Speciation studies of copper in natural waters have shown that > 90 % of dissolved Cu occurs complexed by natural organic material (Buckley *et al.*, 1986; Coale *et al.*, 1988). This complexation considerably reduces its toxicity (Gachter *et al.*, 1978; Swallow *et al.*, 1978; Sunda and Ferguson, 1983) to algae. Thus, the study of copper-organic interactions in freshwater is of interest. Among the several techniques for the determination of copper complexation (ASV, ion-exchange, liquid-liquid extraction and MnO_2-CSV), the DPCSV with an added organic reagent as a competitive ligand (discussed in section 1.2.3) is preferred due to the sensitivity, versatility and simplicity of this technique (van den Berg *et al.*, 1990; van den Berg, 1984 d). The oxine-CSV method was used in this study to determine the concentrations of copper complexing ligands and their conditional stability constants in freshwater.

1.3.1 Theory of Ligand Competition Between Oxine and Natural Organic Complexing Ligands

The theory of the determination of copper complexation by ACSV using oxine is directly analogous to that described for ACSV using catechol (van den Berg, 1984 d). In the presence of oxine, the total copper concentration in freshwater is distributed as follows:

$$[\,Cu\,]_{total} = [\,Cu\,]_{ino.} + [\,Cu\text{-}oxine\,] + [\,CuL_x] \qquad (1\text{-}1)$$

Where $[\,Cu\,]_{total}$ = the total copper concentration; $[\,Cu\,]_{ino.}$ = the concentration of inorganic copper; [Cu-oxine] = the concentration of copper complexed by oxine; and $[\,CuL_x]$ = the concentration of copper complexed by natural organic complexing ligands.

The CSV method determines the concentration of labile Cu which includes inorganic copper and copper complexed by oxine as shown in equation (1- 2):

$$[\,Cu\,]_{labile} = [\,Cu\,]_{ino.} + [\,Cu\text{-}oxine\,] \qquad (1-2)$$

therefore, the equation (1-1) can be rewritten as:

$$[\,Cu\,]_{total} = [Cu\,]_{labile} + [\,Cu\,L_x\,] \qquad (1-3)$$

In addition, there are the following relationships with the free metal ion concentration, $[\,Cu^{2+}\,]$:

$$[\,Cu\,]_{labile} = [\,Cu^{2+}\,]\,\alpha_{Cu} + [\,Cu^{2+}\,]\,\alpha_{Cuox}$$

$$= [\,Cu^{2+}\,]\,(\alpha_{Cu} + \alpha_{Cuox}) \qquad (1-4)$$

Where $[\,Cu^{2+}\,]$ = the concentration of free copper ion; α_{Cu} = the α-coefficient (Ringbom and Still, 1972) for copper, which corrects for inorganic side reactions of Cu^{2+} in freshwater; and α_{Cuox} = the α-coefficient of copper with oxine, which corrects for all occurring side reactions on the main reaction of copper with oxine.

α_{Cu} and α_{Cuox} were calculated in a similar manner described by van den Berg (1984 d):

$$\alpha_{Cu} = 1 + \sum (K^{\bullet}_{i}\;[L_j]^{i}) + \sum (K^{\bullet}_{a,i} / [H^{+}]^{i}) \qquad (1-5)$$

Where $K^{\bullet}_{i}$ (i=1, 2, 3, ...) is the stepwise stability constant for the complex of Cu^{2+} with ligand L_j (Cl^{-}, CO_3^{2-}, SO_4^{2-}); $[L_j]$ is the concentration of the ligand; and $K^{\bullet}_{a,i}$ (i=1 and 2) is the stepwise acidity constant of Cu^{2+} (for example, $Cu^{2+} + H_2O \rightleftharpoons Cu(OH)^{+} + H^{+}$ $K^{\bullet}_{a,1}$, $Cu(OH)^{+} + H_2O \rightleftharpoons Cu(OH)_2 + H^{+}$ $K^{\bullet}_{a,2}$). The α_{Cu} can be calculated at pH 7.6 and ionic strength 0.1 with the constants given in **Table 1.6** and values of average concentrations of the ligands present in freshwater (Turner *et al*, 1981).

Table 1.6 Stability constants used to calculate α_{Cu} in freshwater (values are valid at an ionic strength of 0.0 or 0.1M)

Complex	Log K^*	Reference
$CuCl^+$	0.4	Smith and Martell, 1976
$CuCO_3$	6.75	Smith and Martell, 1976
$Cu(CO_3)_2^{2-}$	9.92	Smith and Martell, 1976
$CuSO_4$	2.36	Turner *et al.*, 1981
$Cu(OH)^+$	-8.00	Turner *et al.*, 1981
$Cu(OH)_2$	-17.30	Turner *et al.*, 1981

According to the definition of α-coefficient (Ringbom and Still, 1972), the α-coefficient of copper with oxine can be expressed as follow:

$$\alpha_{Cuox} = K'_{Cuox}[ox'] + \beta'_{Cuox2}[ox']^2 \qquad (1-6)$$

The α-coefficient of oxine, α_{ox}, which corrects for side reactions of oxine, is computed from:

$$\alpha_{ox} = 1 + [H^+]^2 / (K^*_{a,1} K^*_{a,2}) + [H^+] / K^*_{a,2} \qquad (1-7)$$

Where [ox′] is the concentration of oxine not complexed by Cu; K'_{Cuox} and β'_{Cuox2} are the conditional stability constants for monomeric and dimeric complexes of Cu; the constants $K^*_{a,1}$, $K^*_{a,2}$ and K^*_1 are stoichiometric constants as indicated in **Table 1.7**. K'_{Cuox} and β'_{Cuox2} can be calculated for the present experimental conditions (ionic strength 0.1 M and pH 7.6) from the stoichiometric concentration constants of copper-oxine complex as follows:

$$K'_{Cuox} = K^*_{Cuox} / \alpha_{ox} \qquad (1-8)$$

$$\beta'_{Cuox2} = \beta^*_{Cuox2} / \alpha_{ox}^2 \qquad (1-9)$$

Values for the stoichiometric concentration constants listed in **Table 1.7** are valid at an ionic

strength of 0.1 M.

Table 1.7 Stability constants used to calculate α_{ox}, K'_{Cuox} and β'_{Cuox2} (values are valid at an ionic strength of 0.1M)(Smith and Martell, 1989)

Complex (L=oxine)	Log K^*	Type of constant
HL	- 9.65	$K^*_{a,2}$
H_2L^+	- 4.97	$K^*_{a,1}$
CuL^+	12.0	K^*_1
CuL_2	22.9	$\beta^*_{1,2}$

In the absence of oxine, the following relationship exists between [Cu^{2+}], [Cu L_x], [L_x] and K'_{CuLx} :

$$Cu^{2+} + L_x \rightleftharpoons CuL_x \qquad (1 - 10)$$

$$K'_{CuLx} = [\,CuL_x\,] / ([\,Cu^{2+}\,]\,[L'_x]) \qquad (1 - 11)$$

$$[\,L'_x\,] = [L_x] - [\,CuL_x] \qquad (1 - 12)$$

$$[\,Cu^{2+}] / [\,CuL_x] = [\,Cu^{2+}] / [L_x] + 1 / (K'_{CuLx}\,[L_x]) \qquad (1 - 13)$$

Where L_x is the organic complexing ligand; [L'_x] is the concentration of L_x not complexed by copper; [L_x] is the concentration of total organic complexing ligand; and K'_{CuLx} is the conditional stability constant of the copper-complex of the natural organic ligand.

Combined with equation (1 - 4), equation (1 - 13) can be rewritten as:

$$[\,Cu\,]_{labile} / [\,CuL_x] = [\,Cu\,]_{labile} / [L_x] + (\alpha_{Cu} + \alpha_{Cuox}) / (K'_{CuLx}\,[L_x]) \qquad (1 - 14)$$

Equation (1 - 14) indicates that a plot of [Cu]$_{labile}$ / [CuL_x] against [Cu]$_{labile}$ is a straight line with a slope which is 1/ [L_x] and an Y-intercept ($\alpha_{Cu} + \alpha_{Cuox}$) / ($K'_{CuLx}$ [L_x]).

The calculated values of α_{Cu}, α_{ox} and α_{Cuox} with experimental conditions of ionic strength 0.1 M, pH 7.6 and various concentrations of oxine are summarized in **Table 1.8**.

Table 1.8 The calculated values of α_{Cu}, α_{ox} and α_{Cuox} with experimental conditions of ionic strength 0.1 M, pH 7.6 and various concentrations of oxine in freshwater.

[ox], μM	α_{Cu}	Log α_{ox}	Log α_{Cuox}
1.5	6.25	2.15	6.95
7.3	6.25	2.15	8.33
36.7	6.25	2.15	9.73
73.4	6.25	2.15	10.33

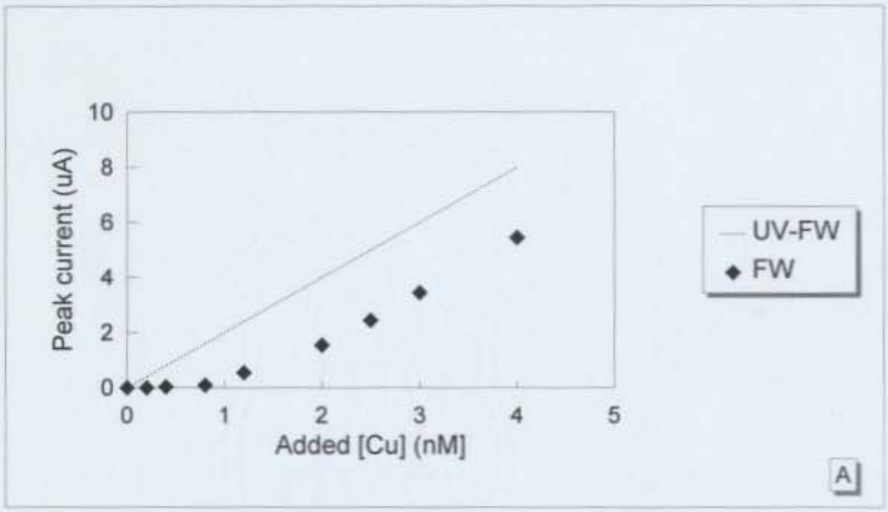
In practice, incrementally increasing amounts of Cu are added to aliquots of a freshwater sample to which oxine has been added as the competitive ligand. The added Cu is allowed to equilibrate with the natural ligands and the added oxine for 19-21 h. The Cu complexed by oxine (dominant) and by the major anions of the freshwater sample (minor) is measured by ACSV (equation (1-2)). A titration curve of the peak current (i_p) against the added Cu concentration is obtained (**Figure 1.8 A**). The peak current increases non-linearly at low added Cu concentration and linearly at high added Cu concentration when all the ligands are saturated with Cu. The slope of the upper portion of the titration curve is identical with the slope of the titration curve obtained in a UV-irradiated aliquot of the freshwater sample in which no natural ligand is present. A plot of equation (1-14) gives the transformed line of the titration data (**Figure 1.8 B**), from which the concentration of the natural ligand $[L_x]$ and conditional stability constant K'_{CuLx} are calculated.

1.3.2 Applications of ACSV in Copper Complexation Studies

The adsorptive-CSV method, based on competition between natural organic ligands and an added complexing ligand, has been applied to the study of copper complexation (**Table 1.9**).

Van den Berg (1984 d) first applied the ACSV method to study Cu complexation in seawater using catechol as the added ligand. He discussed the fundamental aspect of the method and compared two techniques for the treatment of the titration data. In 1990, Apte *et al.* (1990) employed the catechol-CSV method to assess the range of different types of natural complexing ligands occurring in seawater, estuarine water and freshwater. The great advantage of ACSV is that the concentration of the added catechol can be varied so as to compete at different degrees with natural ligands, allowing determination of ligands with various strengths. Arriving at the same result, van den Berg (1990) used two different ligands with different complexing strengths, oxine and catechol, to study Cu complexation in estuarine water.

The concept of "detection window" in relation to CSV was discussed in both papers by Apte *et al.* (1990) and van den Berg (1990). The detection window of ACSV was calculated from the complexing ability of the added ligand and expressed as $\log\alpha_{AL}$ (the α-coefficient for complexation of Cu by the added competing ligand). Van den Berg (1992) found that there is an excellent correlation between the detection window for a particular technique and the detected degree of complexation. This correlation was further confirmed by the work of Donat and van den Berg (1992) using catechol and tropolone as the competing ligands in seawater and the work of Campos *et al.* (1994) using salicylaldoxime, oxine and catechol in seawater.



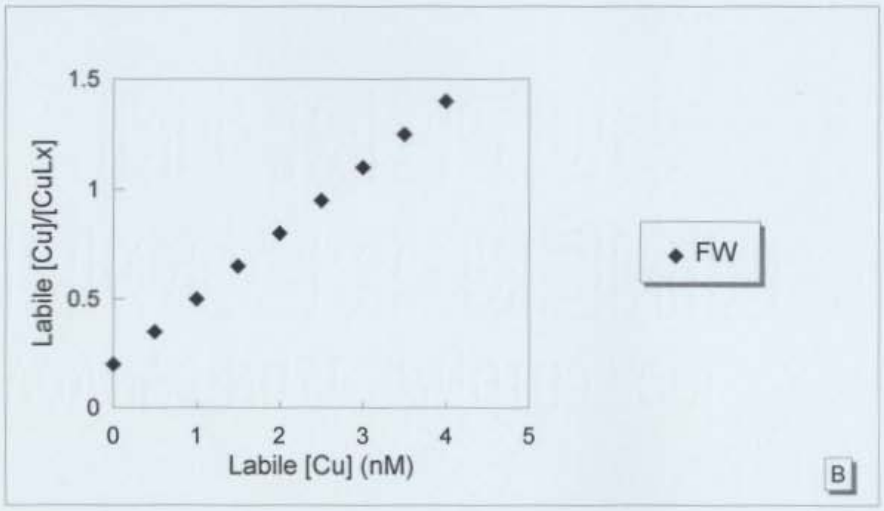


Figure 1.8 (A) Schematic representation of copper titration curve using oxine-CSV; (B) The transformed line of the titration data; FW: freshwater, UV-FW: UV-treated freshwater

Table 1.9 Applications of ACSV to copper complexation in natural waters.

Sample	Added ligand	[ligand]	Reference
Seawater	catechol	25 μM	van den Berg, 1984 d
Seawater and freshwater	catechol	1 μM - 2.5 mM	Apte *et al.*, 1990
Estuarine water	oxine catechol	0.01mM - 1 mM	van den Berg *et al.*, 1990
Seawater	catechol tropolone	0.158 mM -0.324 mM	Donat and van den Berg, 1992
Seawater	oxine catechol tropolone	0.83 μM - 0.702 mM	van den Berg and Donat , 1992
Lake water	catechol	10 μM - 80 μM	Xue and Sigg, 1993
Seawater	SA[1] oxine catechol	1μM - 100 μM	Campos *et al.*, 1994

[1] Salicylaldoxime

1. 4 Objectives

The Bonavista Peninsula of Newfoundland was chosen as the study area which includes three locations: Bonavista, Random Island and Come-By-Chance. Bonavista is a town on a major headland which had depended on the fishery for many generations. Random Island was selected

because information on freshwater was to be compared with data collected for the adjacent marine system. Come-By-Chance was examined to assess the impact of the local oil refinery on the water system.

This project is a sub-project of an interdisciplinary program called "the Tri-Council Eco-Research" program which was proposed during the collapse of the Newfoundland fisheries. The program entitled "Sustainability in a Changing Cold Ocean Coastal Environment", is funded by Environment Canada through the Green Plan and is admininstered at arms length by the three academic funding councils - the Medical Research Council (MRC), the Natural Sciences and Engineering Research Council (NSERC) and the Social Sciences and Humanities Research Council of Canada (SSHRCC). The principal objective of the Eco-Research was to "identify the central components required to achieve sustainability for cold coastal communities" (Ommer *et al.*, 1993). The objective of the sub-project was to "collect and analyze scientific data which will determine natural changes in diversity, biomass and food webs along the length of the watersheds" (Ommer *et al.*, 1993). The objective of this project was to measure trace metals and their complexation in the freshwater systems in the study area in order to determine the concentration of the bioavailable form and the ability of the water to complex added metals.

To accomplish the objective of this project, DPASV was employed to determine the ASV-labile concentrations of Zn, Cd and Pb and the oxine-CSV method using oxine as a competitive ligand to measure the CSV-labile concentration of Cu. Total concentrations of Zn, Cd, Pb and Cu in freshwater were determined by inductively coupled plasma-mass spectrometry (ICP-MS) and/or inductively coupled plasma-atomic emission spectrometry (ICP-AES) and/or

ASV. Determinations of both labile and total concentrations were used to estimate the labile fraction of total metal.

Copper complexation in freshwater was studied by complexing capacity titrations using oxine-CSV. The concentration of the natural organic complexing ligand and its conditional stability constant were then calculated. Different strengths of the natural organic complexing ligands were measured by varying the concentration of oxine (i.e. changing the detection window). These results were used to evaluate the ability of freshwater to complex copper.

CHAPTER 2

EXPERIMENTAL

2.1 Materials

2.1.1 Reagents

Potassium chloride (Suprapur) used as electrolyte was purchased from BDH Inc. Concentrated hydrochloric acid, nitric acid and ammonia used for the preparation of solutions and for final rinsings were Seastar double sub-boiling distilled in quartz (referred to as Q-grade). Fisher Scientific trace metal grade nitric acid was used for cleaning sample bottles. Standard solutions of zinc, cadmium, lead and copper were prepared by dilution of 1000 mg/L atomic absorption spectrometry standard solutions (Fisher Scientific). Water used (referred to as Nano-pure water) for dilution and rinsing was distilled and then deionised with a NANOpure II system (Barnstead). Other reagents, 8-hydroxyquinoline (commonly referred to as oxine, Fisher Scientific), glacial acetic acid (BDH), sodium acetate (trihydrate, BDH), 4-(2-hydroxyethy)-1-piperazineethanesulfonic acid (HEPES, 99%, Aldrich Chemical Company, Inc.) and sodium hydroxide (BDH) were ACS analytical grade. The 1 M acetic acid/sodium acetate (HAc/NaAc) buffer was purified by ion-exchange and the HEPES buffer (1 M HEPES in 0.5 M NaOH) by MnO_2 adsorption (van den Berg, 1986) before use (see section 2.1.2). In addition, 4-5 M ultra-pure hydrochloric acid for rinsing cells between measurements was prepared by isothermal distillation (EG & G PAR Model 303A Static Mercury Drop Electrode Instruction Manual, p17).

Reagents and standards used for DPASV and ACSV experiments are listed in **Table 2.1** and **Table 2.2.**

2.1.2 Purification of HAc/NaAc, HEPES and HCl

Purification of HAc/ NaAc Buffer

8-hydroxyquinoline (8HQ) is a well-characterized chelating agent which forms complexes with more than 60 metal ions (Sturgeon *et al.*, 1981). Immobilization of the chelating agent onto a solid support, such as Fractogel TSK, broadens its application in ion-exchange (Landing *et al.*, 1986). The Fractogel TSK-immobilized 8-hydroxyquinoline (TSK-8HQ) was synthesized[1] using Landing's procedure (1986) and was utilized to remove contaminating metal ions from the 1 M HAc/ NaAc buffer.

After acid and water cleaning, 2 mL of a TSK-8HQ gel slurry was pipetted onto a polyethylene column fitted with a porous polyethylene frit. Prior to use, the column loaded with 2 mL of TSK-8HQ, was rinsed by passing through five 1 mL portions of 2.0 M HCl/0.1 M HNO_3 (ultra-pure) acid mixture and 10-20 mL of water until the pH of the eluent from the column was about 6. Then an aliquot of 1M HAc/NaAc buffer solution (7 mL) was passed through the column. The concentrations of zinc, cadmium, lead and copper in the purified buffer were determined by ASV. The regeneration of the TSK-8HQ was performed by the passage of five 2 mL portions of the acid mixture followed by 20-30 mL of water.

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[1] We thank Mr. James Farrell for preparing this material.

Table 2.1 Reagents used for DPASV and ACSV experiments

Reagent	Grade	Used for	Procedure	Purification	Storage
1 M KCl	Suprapur	DPASV	4.47 g of KCl was dissolved in 59.95 mL of Nano-pure water	None	Clean bench
2 M KCl	Suprapur	ACSV	5.05 g of KCl was dissolved in 33.86 mL of Nano-pure water	None	Clean bench
1 M HAc/ 1M NaAc	Analytical	DPASV	13.61 g of $NaAc{\cdot}3H_2O$ and 5.72 mL HAc (glacial) were diluted to 100 mL, pH range 4.50 - 4.60	Ion-exchange	Clean bench
1 M HEPES buffer	Analytical	ACSV	9.54 g of HEPES was dissolved in 40 mL of 0.5 M NaOH solution, pH range 7.45 - 7.55	MnO_2 adsorption	Clean bench
0.1 M oxine	Analytical	ACSV	0.29 g of oxine was dissolved in 20 mL of 0.2 M HCl solution	None	Prepared monthly; Clean bench
1.6 mM oxine	Analytical	ACSV	250 μL of 0.1 M oxine was diluted with 15.37 mL of Nano-pure water	None	Prepared monthly; Clean bench
8.0 mM oxine	Analytical	ACSV	1.25 mL of 0.1 M oxine was diluted with 14.38 mL Nano-pure water	None	Prepared monthly, Clean bench
0.33 mM oxine	Analytical	ACSV	100 μL of 0.1 M oxine was diluted with 30.20 mL of Nano-pure water	None	Prepared monthly, Clean bench

Table 2.2 Standards used for DPASV and ACSV experiments

Standard (STD)	Used for	Procedure	Storage
10 mg/L metal STD (Zn, Cd, Pb and Cu)	Dilution	500 µL of 1000 mg/L STD was diluted with 49.50 mL of Nano-pure water	Clean bench
0.1 mg/L Zn, Cd, Pb and Cu STD	DPASV	100 µL of 10 mg/L Zn, Cd, Pb and Cu respectively was diluted with 9.60 mL of Nano-pure water	Prepared daily; Clean bench
0.4 mg/L Zn, 0.1 mg/L Cd, Pb and Cu STD	DPASV	400 µL of 10 mg/L Zn and 100 µl of 10 ppm Cd, Pb and Cu was diluted with 9.30 mL of water	Prepared daily; Clean bench
0.1 mg/L Cu STD	ACSV	100 µL of 10 mg/L Cu STD was diluted with 9.90 mL Nano-pure water	Prepared daily; Clean bench
0.2 mg/L Cu STD	ACSV	200 µL of 10 mg/L Cu STD was diluted with 9.80 mL Nano-pure water	Prepared daily; Clean bench
0.4 mg/L Cu STD	ACSV	400 µL of 10 mg/L Cu STD was diluted with 9.60 mL Nano-pure water	Prepared daily; Clean bench
1, 2, 3 and 4 mg/L Cu STD	ACSV	1, 2, 3 and 4 mL of 10 mg/L Cu STD was diluted to 10.00 mL with Nano-pure water respectively	Prepared daily; Clean bench

Purification of HEPES Buffer

The preparation of 40 mL of the HEPES buffer is listed in **Table 2.1**. About 0.1 g of manganese(IV) oxide was added to the 40 mL of buffer. The solution was stirred (overnight) using a magnetic stirrer and then filtered through a 0.45 μm Millipore filter. The trace copper in the purified buffer was determined by ACSV.

Purification of HCl

Ultra-pure HCl used for rinsing cells and electrodes was prepared by isothermal distillation. A 500 mL beaker filled with concentrated ACS grade HCl and a 500 mL wide mouth polyethylene bottle half-filled with water were placed in an acid cleaned desiccator. The desiccator was kept on a clean bench for 3 - 4 weeks. During this period, the volatile HCl transferred to the water in the polyethylene bottle. At the end of this time, the polyethylene bottle half-filled with purified acid was taken out from the desiccator. The concentration of the acid was determined by sodium hydroxide titration and the concentrations of the trace metals were determined by DPASV.

2.1.3 Filters

A 25 mm or 47 mm 0.45 μm Millipore filter membrane (HA type, Millipore Corporation) was used to remove particles from freshwater samples.

Filter holder A which held the 25 mm membrane was used with a 10 mL syringe to filter small amounts of sample before analysis. When a large amount of sample was required, for instance 500 mL of samples, filter holder B (Nalgene, Cat. No. 300-4000, 4050, 4100)

which held the 47 mm membrane was used with a receiver. This was operated under vacuum.

2.1.4 Cleaning

Trace metal analysis requires an extremely careful and lengthy cleaning process, because minute amounts of impurities from a variety of sources may contaminate samples. Only plastic-ware made of polyethylene, polypropylene or Teflon was used. All the plastic-ware was subjected to a thorough cleaning procedure, depending on the chemical resistance of the particular plastic and the individual container. There were three major steps in the cleaning process: pre-cleaning, reagent grade acid cleaning and trace metal grade acid cleaning. Nano-pure water was used for rinsing between each step.

Bottles for sample and reagent storage were cleaned in the following way. First, they were rinsed with ACS reagent grade acetone to remove organic impurities, then placed in a 5% micro detergent (Cole-Parmer Instrument Company) bath for 24 hours. Next, they were filled with 6 M reagent grade HCl and soaked in a 2 M reagent grade HCl bath for two weeks. Finally, they were filled with 0.1 M trace metal grade HNO_3, double-bagged in new Ziplock storage bags and stored in a plastic bucket for at least another two weeks. Bottles to be used with extremely low concentration samples were filled with 0.1 M Q-HCl instead of trace metal grade HNO_3. Before use, bottles were rinsed five times with Nano-pure water on the clean bench.

Small items, such as disposable tips, filter holders, syringes and teflon cells, were cleaned in a similar manner. After the detergent wash, items were placed in a hot (about

60 °C) 3 M reagent grade HCl bath for 48 hours, followed by soaking in a hot (60 °C) 7.5 M trace metal grade HNO_3 bath for 24 hours. Disposable tips were then rinsed with and soaked in Nano-pure water until use. Filter holders, syringes and Teflon cells were further cleaned in a 1 M Q-HCl bath and rinsed well with Nano-pure water and allowed to dry in the circulation of clean air on the clean bench.

The Millipore filter membranes were soaked in a 2 M Q-HCl bath for at least three weeks. Before use, they were rinsed well with a squirt bottle of Nano-pure water and placed in filter holders and then further rinsed by passing through large amounts of Nano-pure water. Containers which were to be used for different purposes, for instance the same voltammetric cell for different samples, were rinsed well with Nano-pure water, soaked in 0.1 M ultra-pure grade HCl, followed by final water rinsing.

2.2 Instruments

2.2.1 Mercury Electrode and Polarographic Analyzer

A EG&G Princeton Applied Research (PAR) Model 174A Polarographic Analyzer was interfaced with a PAR model 303A Static Mercury Drop Electrode (SMDE). The 303A SMDE was operated in the hanging mercury drop mode with the drop size on "large". The reference electrode was Ag/AgCl, saturated KCl and the counter electrode was a platinum wire. A mechanism for deaeration of the sample solution by purging with high purity nitrogen was incorporated in the electrode system. Prior to purging, the nitrogen was pre-saturated with Nano-pure water in order to prevent evaporative losses, by passing through

a scrubbing tower contained Nano-pure water. A Teflon voltammetric cell (Fisher Scientific) with a Teflon coated star-shaped stirring bar (Fisher Scientific) was used to contain the sample. A magnetic stirrer was employed to stir the sample solution during the deposition step and a BBC SE 780 x-y recorder to record the voltammogram. The 303A SMDE and the stirrer were operated on the clean bench.

2.2.2 The Clean Bench

As mentioned earlier, minute amounts of impurities from a variety of sources may contaminate samples. Thus, in order to prevent sample contamination from airborne dust, all sample filtration, handling and analysis were carried out on the clean bench. The clean bench consisted of a one side open wooden frame with a Lexan interior and a Class-100[1] high efficiency particle air (HEPA) filter (EACI, model # MAC 10) mounted on the top. The HEPA filter not only removed particles whose diameters were larger than 0.5 μm from air, but also provided laminar flow with a positive pressure to prevent dust from entering the clean area. The working surface of the clean bench was made of high density polyethylene. When the bench was not in use, the open side of the clean bench was covered by a plastic sheet, but the HEPA filter blower was never turned off.

All personnel working on the clean bench wore powder-free polyethylene gloves which were replaced daily, or more frequently if compromised by touching non-trace metal

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[1] Class 100 is defined by USA Federal Standard 209D as 100 or less particles greater than 0.5 μm permissible per cubic foot (0.0283 m^3) of air.

clean objects. The working area of the clean bench was rinsed with Nano-pure water every morning and wiped with Kimwipes. All the disposable tips were replaced daily.

2.2.3 UV-irradiation System

The home-built UV-irradiation apparatus[1] was a modified version of that described by Vega *et al.*(1994). A 125 W medium pressure mercury lamp was inserted into a double-layered tube with the lower part made of quartz. The double walled tube allowed cooling water running through to prevent a temperature increase during UV-irradiation. A 80 mL quartz sample tube (diameter 2 cm) with a Teflon stopper was placed 2 cm away from the lamp. To increase the radiation efficiency, the sample tube and the lamp were surrounded by aluminium foil. The system was operated in a regular fume hood with the aluminium covered window down.

2.2.4 pH Meters, Conductivity Meter and Pipettes

A Chemtrix pH meter (type 60A) with a glass electrode (Broadley James Corporation) was employed to measure the pH of all samples in laboratory. The pH meter was calibrated daily using two standard buffer solutions, pH = 8.68 and pH = 4.01. For sample collection, a portable pH meter (Model 290A, Orion) was used to measure pH on site. The pH meter was calibrated before each field trip using the same standard buffer solutions.

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[1] We thank Dr. Chet Jablonski for providing this apparatus.

A hand-held conductivity/temperature meter (model 122 and 123, Orion) was used to measure the conductivity and temperature on site when the sample was collected.

An Eppendorf Maxipettor pipette 4720 and Maxitip S Kit (barrel and piston) made of polypropylene were used to transfer sample aliquots on the clean bench. Three different volumes (50 μL, 100 μL, 250 μL) of Eppendorf Standard Pipettes 3130 were used to transfer small volumes of solution accurately. All the pipettes were purchased from Brinkman Instruments, Inc.

2.3 Methods

2.3.1 Sample Collection, Filtration and Storage

The freshwater samples were collected from the Bonavista Peninsula area of Newfoundland. Sample sites # 1 - 8 are located at Bonavista, sites # 9 - 13 on Random Island and sites # 14 - 17 at Come-By-Chance (see the map on the following page). Samples from each site (16 sites in total) were collected in field trips carried out in May and July 1995 and samples from selected sites were taken in April and July 1996 (**Table 2.3**).

Low density polyethylene (LDPE) bottles (500 and 1000 mL) were used for samples taken in 1995. LDPE and fluorinated-high density polyethylene (FLPE) bottles were used for samples taken in 1996.

Before field trips, bottles were rinsed with Nano-pure water and double-bagged in Ziploc storage bags. Polyethylene gloves were used on-site when handling sample bottles which were submerged, rinsed (3 times) and filled with the sample water. At least two

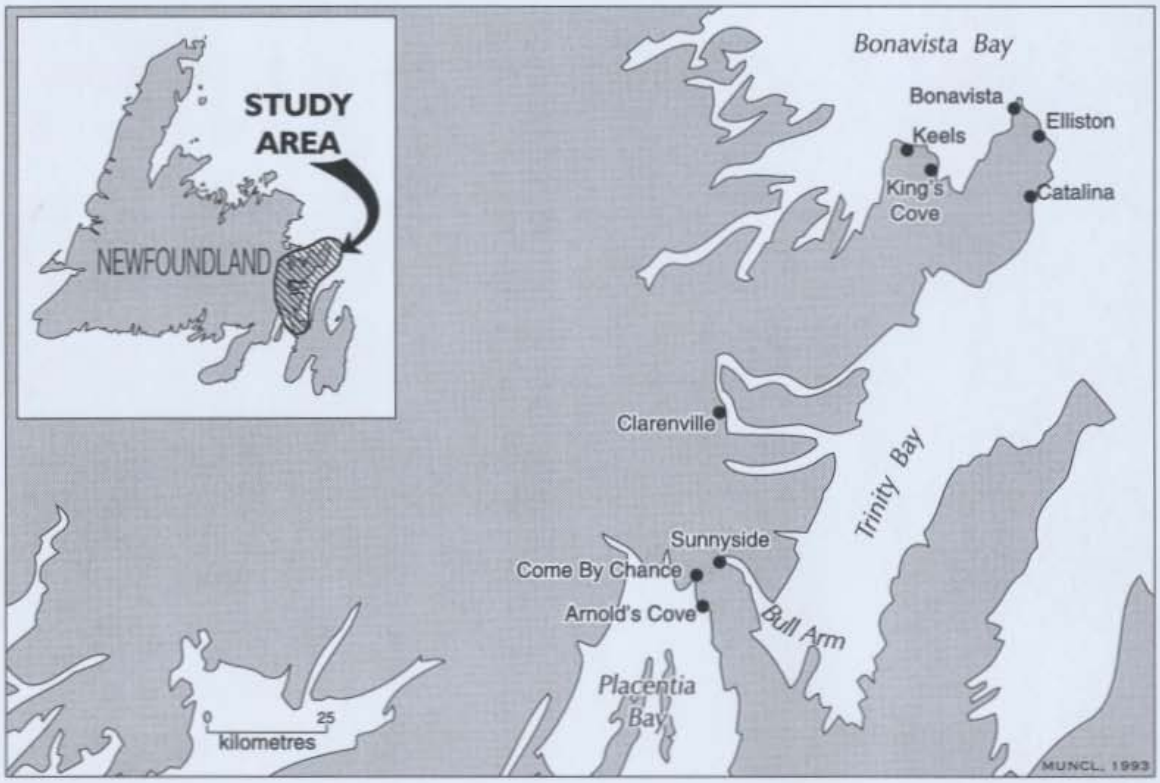


Figure 2.1 Map of the study area (detailed sample site locations are shown as an appendix)

samples, one in a 500 mL bottle and the other one in a 1000 mL bottle, were taken from each site. Temperature, pH and conductivity of the water were measured on site and general site observations were made.

Samples were stored and transported in coolers during the field trips and from the field to the laboratory. The 1000 mL samples were placed in a refrigerator at 4 °C, while the 500 mL samples were deep frozen to store for later studies.

Table 2.3 Sampling Locations in 1995 and 1996

Site #	Location	May 1995	July 1995	April 1996	July 1996
1		Y	Y	Y	Y
2		Y	Y	-	-
3		Y	Y	-	-
5	Bonavista	Y	-	-	-
6		Y	Y	Y	Y
7		Y	Y	Y	Y
8		Y	Y	-	-
9		Y	Y	-	-
10		Y	Y	Y	Y
11	Random Island	Y	Y	-	-
12		Y	-	-	-
13		Y	Y	Y	Y
14		Y	Y	Y	Y
15		Y	Y	-	-
16	Come-By-Chance	Y	Y	-	-
17		Y	Y	-	-

All samples, except samples collected in July 1996, were filtered through 0.45 μm

Millipore membranes using filter holder A (see Section 2.1.3) before sample analyses. Samples taken in July 1996 were filtered using filter holder B within 5 days after sample collection. For each site, one aliquot of the filtered sample was kept in the refrigerator, one in a deep freezer and another was acidified to a pH of about 2 by adding concentrated ultra pure HCl (Seastar) (2 mL/500 mL sample). The acidified samples were also stored in the refrigerator for total metal analyses.

2.3.2 Determination of Labile Zinc, Cadmium, Lead and Copper by DPASV

An aliquot (10 mL) of the filtered sample was pipetted into a Teflon cell. HAc/NaAc (250 μL, 1 M) was added to adjust the pH of the sample to 4.5 - 4.6 and KCl (250 μL, 1 M) to act as electrolyte. The solution was purged with nitrogen while stirred at a speed of

Table 2.4 Parameters of polarographic analyzer for DPASV and ACSV

Parameters	DPASV	ACSV
Potential scan rate	5 mV/s	5 mV/s
Scan direction	+	-
Potential scan range	1.5 V	1.5 V
Initial potential	-1.2 V	-1.1 V
Modulation Amplitude	25 mV	25 mV
Operating Mode	Differential pulse	Differential pulse
Current range dial	1 - 5	1 - 5
Current range switch	μA	μA
Drop time	0.5 s	0.5 s
Output offset	Off	Off
Display direction	-	+
Low pass filter	Off	Off

700 rev/min for 8 min. A deposition potential of -1.2 V was then applied for 10 min with stirring. The solution was left to rest for 30 s, then an anodic scan (in a positive direction) initiated at -1.2 V was started and a voltammogram recorded. The parameters for the analysis are listed in **Table 2.4**. The initial sample solution was scanned at least twice, while the standard additions were scanned once. In between scans, three mercury drops were discarded, whilst a fresh drop was used for each scan. Between two consecutive measurements, a deaeration time of 0.5 min was applied. A purge time of 2 min was used after the addition of standard solutions. The sensitivity was calibrated by three point standard addition to the sample and the initial metal concentrations were calculated by extrapolation.

2.3.3 Determination of Copper Concentration by ACSV

Determination of Copper in Seawater Reference Standard CASS-2

A method of cathodic stripping voltammetry with 8-hydroxyquinoline as a competitive ligand to determine labile copper in seawater has been reported by van den Berg (1986). Before the method was modified for freshwater analysis, it was first applied to determine the copper concentration in the seawater reference standard CASS-2 (National Research Council Canada). An aliquot (10 mL) of CASS-2 was neutralized with 0.2 mL of 1.2 M NH_3 in a Teflon cell, then 100 μL of 1 M HEPES and 50 μL of 4 mM oxine (final concentration 20 μM) were added. The adsorption potential was set to -1.1 V and the solution was stirred for 1 to 3 min. After a 10 s quiescent period, the potential was switched to -0.2 V within 10 s while the " hold " key on the polarographic analyzer was pressed down.

The DPCSV scan (in a negative direction) was initiated 20 s later. Other parameters were the same as described above for DPASV measurement (**Table 2.4**).

Optimization of the Oxine-ACSV Method and Determination of Labile Copper

The oxine-ACSV method for labile copper determination in seawater (van den Berg, 1986) was modified for freshwater. The optimization experiment was performed using 10 mL of Nano-pure water with 750 μL of 2 M KCl and 1 M HEPES buffer added. The experiment was designed by varying one parameter at a time. The parameters are pH, oxine concentration, deposition potential and deposition times.

The determination of labile copper in freshwater by oxine-ACSV was carried out in the following way. An aliquot (10 mL) of filtered sample was transferred into a cell. HEPES buffer (100 μL, 1 M) was added to the sample to adjust the pH to 7.5 - 7.6. KCl (750 μL, 2 M) and oxine (50 μL, 1.6 mM) (final concentration was 7.3 μM) were also added. After the solution was purged and stirred for 8 min, the deposition was carried out at potential -1.1 V for 3 - 10 min depending on copper concentrations of individual samples.

2. 3.4 Determination of Copper Complexing Ligand Concentrations

Copper complexing capacity titrations used to determine copper complexing ligands in sea water have been reported (van den Berg, 1984 d; Donat *et al.*, 1992; Campos *et al.*, 1994). Using such information, a modified procedure was designed for freshwater. Aliquots (10 mL) of filtered samples were pipetted into 10 Teflon cells along with 100 μL of the HEPES buffer, 750 μL of 2 M KCl and oxine (1.5 μM, 7.3 μM and 36.5 μM). Copper

stock solutions (0.1 ppm - 4 ppm) were pipetted into 9 of the 10 cells to final concentrations of between 0 and 20 nM in equal increments. The solutions, covered with acid cleaned Pyrex Petri dishes, were allowed to equilibrate for 19 to 21 h, whereafter the labile copper concentration in each cell was determined using the oxine-ACSV method. A deaeration time of 6 min and a deposition time of 1 min were used. The sensitivity was calculated from the linear portion of the titration curve where all ligands were saturated with copper.

2.3.5 Determination of Total Metals

A 70 mL aliquot of acidified (pH ~ 2) sample was transferred into an acid cleaned quartz tube. The sample was then UV-irradiated for 17 h using the UV-irradiation apparatus. An aliquot of 10 mL UV-irradiated sample was pipetted into the Teflon cell. Concentrated and 1:5 (V/V) ammonia were used to approximately neutralize the pH of the sample. The sample was then prepared for analysis by DPASV (see section 2.3.2) to determine the total dissolved zinc, cadmium, lead and copper. Another aliquot was prepared for ACSV analysis (see section 2.3.3) to determine total dissolved copper only if copper peaks were not observed for certain samples by the DPASV method. A deposition time of 1 min was sufficient for ACSV determination of total dissolved copper in the UV-irradiated sample.

Samples collected in May and July 1995 were analyzed by Inductively Coupled Plasma-Atomic Emission Spectrometry(ICP-AES) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) to determine a range of total metals in the samples, while ICP-MS and DPASV and ACSV methods were used for samples collected in April and July 1996.

CHAPTER 3

OPTIMIZATION OF THE ADSORPTIVE CATHODIC STRIPPING VOLTAMMETRY METHOD

3.1 Analysis of CASS-2 Standard

3.1.1 Cathodic Stripping Voltammetry in the Presence of Oxine

A near-shore seawater reference material, CASS-2, was analyzed by the oxine-CSV method to test accuracy and precision. A well-defined peak appeared at - 0.4 V after a DPCSV scan (**Figure 3.1**). The increase (the upper three scans) with increasing copper concentration, indicated that this peak was due to the reduction of Cu(II). Similar results (Cu peak at - 0.45 V, pH = 7.7) were observed by van den Berg (1986). No other peak was found when the scan was continued to potentials more negative than - 0.40 V.

Oxine complexes labile copper in solution. The copper(II)-oxine complex is adsorbed on the mercury drop surface at a potential of - 1.1 V to form a film. At this potential, the copper(II) in the adsorbed complex is reduced to metallic copper, which dissolves into the mercury drop as an amalgam. After the combined adsorption/reduction step, the potential was switched to - 0.2 or - 0.1 V for 20 s to allow the metallic copper in the mercury drop to migrate to the drop surface to be oxidized to copper(II), which complexes again with the oxine adsorbed in the mercury drop surface. When the potential was scanned in a more negative direction, the copper(II)-oxine complex underwent reduction at a potential of

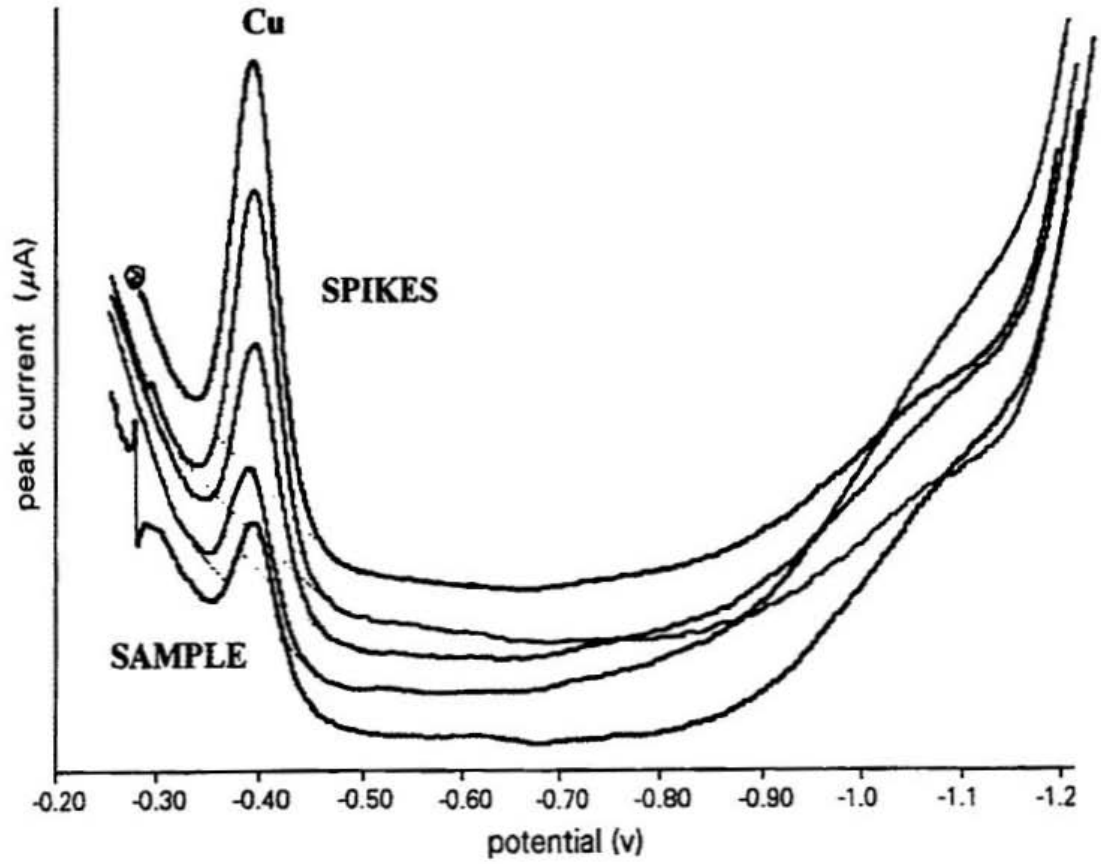


Figure 3.1 DPCSV voltammograms obtained for the seawater reference material CASS-2 in the presence of oxine. The lower two scans: 10 mL sample containing 0.024 M NH_3, 0.01 M HEPES and 20 μM oxine; the upper three scans: addition of Cu 0.48, 0.96, 1.43 μg/L respectively. The adsorption time was 1 min and adsorption potential was -1.1 V.

- 0.4 V. As discussed in Chapter 1, the peak height is proportional to the amount of copper (II) in the film which is proportional to the copper concentration in the sample, whereas the peak potential is related to the characteristic reduction of copper(II)-oxine complexes.

3.1.2 Results of Copper Determination of CASS-2 by the Oxine-CSV Method

The results of the copper determinations for CASS-2 are given in **Table 3.1**. The average copper concentration from eight determinations was 0.69 ± 0.06 μg/L, which is in agreement with the certified value of 0.675 ± 0.039 μg/L within experimental error.

Table 3.1 Copper determination in CASS-2 seawater reference material

# of measurement	1	2	3	4	5	6	7	8
[Cu] (μg/L)	0.63	0.73	0.69	0.73	0.65	0.77	0.75	0.59
Result (X ± SD)	0.69 ± 0.06							
RSD (%)	9.2							
Certified value	0.675 ± 0.039							

The reagent blank value of 0.05 μg/L is the average of four determinations obtained for 10 mL of Nano-pure water with 250 μL of 1 M KCl, 100 μL of 1 M HEPES and 50 μL of 4 mM oxine.

3.1.3 Test of the Linearity of the Differential Pulse Cathodic Stripping Voltammetry (DPCSV) Response with Adsorption Time

The DPCSV response (current i_p) will generally increase with increasing adsorption time until saturation is reached on the surface of the mercury drop. We tested this for a CASS-2 sample by varying the adsorption time from 1 - 12 min. The results are shown in **Table 3.2** and **Figure 3.2**.

At a copper concentration of 0.69 μg/L, the DPCSV response increased linearly with the adsorption time up to 6 min in CASS-2 seawater, between 7 and 10 min it increased linearly but at a slower rate and finally after 10 to 12 min it did not increase. The results indicate that the saturation process occurred gradually on the mercury drop surface with increasing adsorption time.

3.2 Optimization of the Oxine-CSV Method

3.2.1 Effect of pH

The DPCSV peak current was measured as a function of pH in Nano-pure water as shown in **Figure 3.3**. The experiment was performed in two ways. One was to prepare seven aliquots of sample in seven cells with different pH (range from 6.68 to 7.96). Sample pH was varied by additions of dilute HCl (1:6 V/V) or NH_3 (1:5 V/V) to the aqueous solution and measured after each CSV determination. The other way was to pipette 2 aliquots of sample into 2 cells. Sample pH was varied by additions of equal amount of dilute HCl or NH_3 (range from 6.78 to 8.23). At each addition, one sample was analyzed by CSV, while the other was

Table 3.2 Effect of adsorption time on the DPCSV peak height for CASS-2 seawater

Time (min)	Peak (μA)
1	0.19
2	0.33
3	0.47
4	0.58
5	0.68
6	0.76
7	0.83
8	0.91
9	1.00
10	1.07
12	1.10

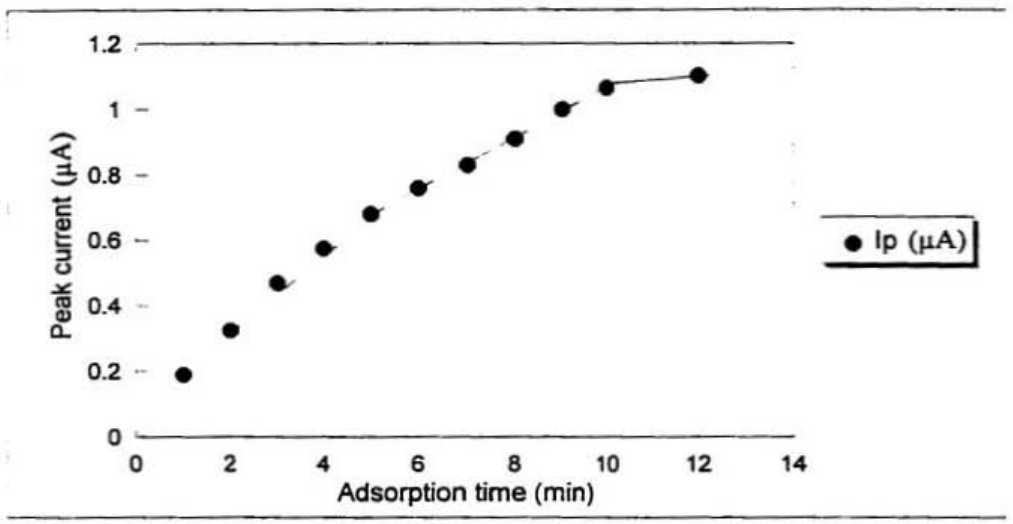


Figure 3.2 Effect of adsorption time on the DPCSV peak height obtained for CASS-2 containing 0.76 μg/L Cu, 20 uM oxine and 0.01 M HEPES at an adsorption potential of - 1.1 V

used to monitor sample pH.

The results obtained in the two ways revealed a similar trend but a slight difference in the maximum current. It was found that the peak current gradually increased with increasing pH up to 7.6 or 7.8, whereafter the peak current decreased with increasing pH. The maximum peak current obtained at pH 7.6 or 7.8 was approximately twice that at pH 6.7 or 6.8. The peak potential was shifted toward a more negative potential with increasing pH at approximately 0.1 V/pH unit at pH values between 6.7 and 8.2 (**Table 3.3**). The increase in the peak current as well as the negative shift in the peak potential with increasing pH may be the result of increased stability of the copper complexes with oxine, whereas the decrease in the peak current at pH value above 7.6 or 7.8 may be caused by copper hydrolysis which increasingly affected the formation of Cu-oxine complexes.

Table 3.3 Effect of pH on the DPCSV peak potential

Measured in same aliquot		Measured in separate aliquots	
pH	Peak Potential (-V)	pH	Peak Potential (-V)
6.78	0.28	6.78	0.27
7.05	0.32	7.05	0.32
7.20	0.36	7.37	0.36
7.57	0.39	7.57	0.38
7.75	0.40	7.75	0.39
7.90	0.42	7.76	0.41
8.23	0.43		

Even though the peak currents obtained by this method were sufficiently sensitive to allow the labile copper determination to be performed at any pH between 6.7 and 8.2, the pH

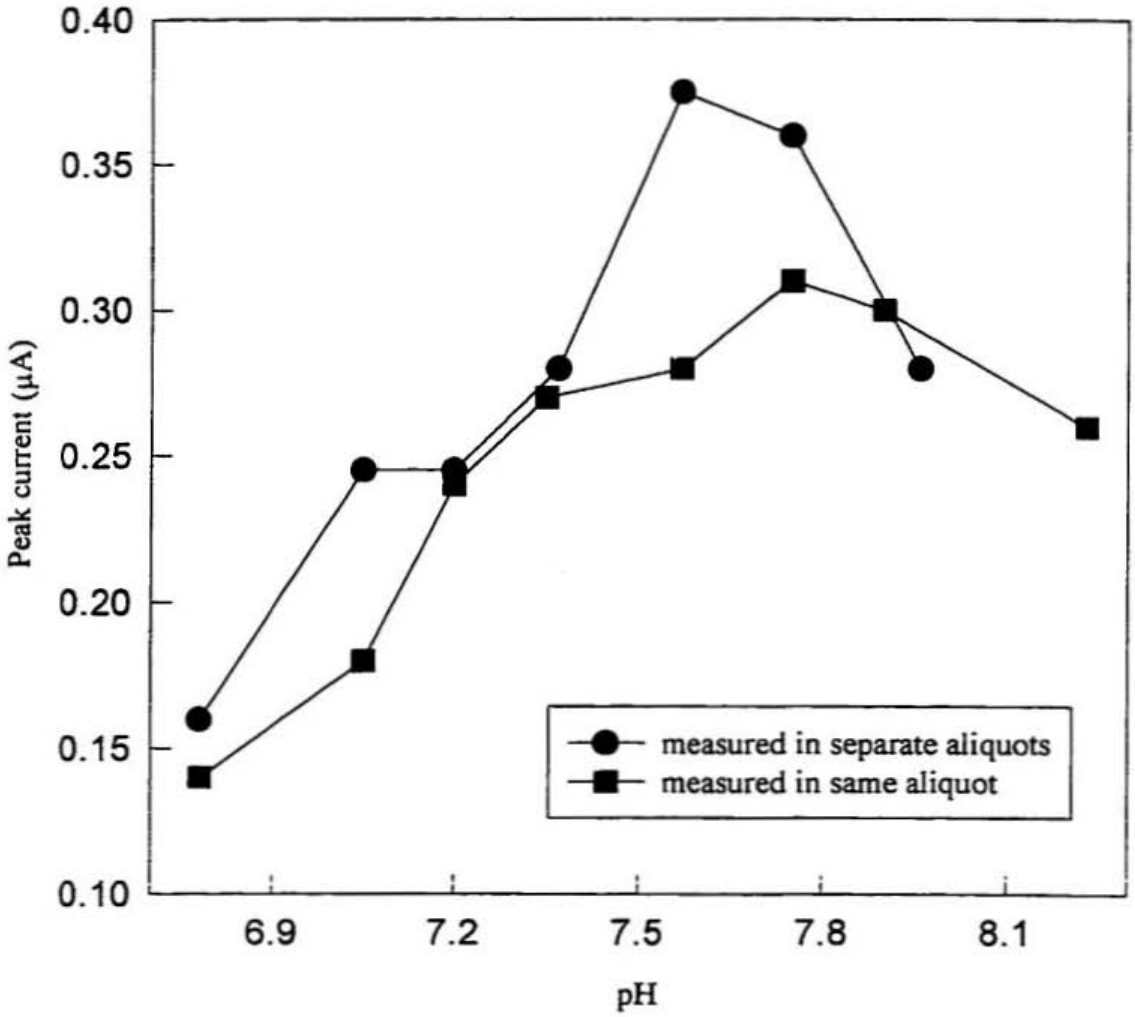


Figure 3.3 Effect of pH on the DPCSV peak height obtained for Nano-pure water containing 2×10^{-5} M oxine, 1 μg/L Cu and 0.15 M KCl. The adsorption potential was -1.1 V and the adsorption time was 1 min.

range 7.4 to 7.6 was chosen to be the optimal pH condition. Since this is close to the pH value of freshwater, it is possible to minimize the change of copper speciation with pH.

In order to understand the effect of pH on the DPCSV peak current, the distribution of three species in an oxine solution as a function of pH is shown in **Figure 3.4**. The distributions of these species were calculated using the thermodynamic equilibrium stability constants given by Smith and Martell (1989). The structures of the three species are presented in **Figure 3.5**.

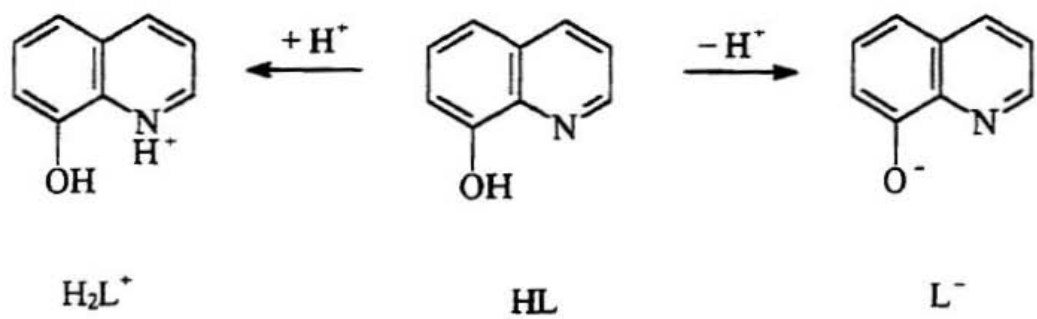


Figure 3.5 Structures of the three species in an oxine solution

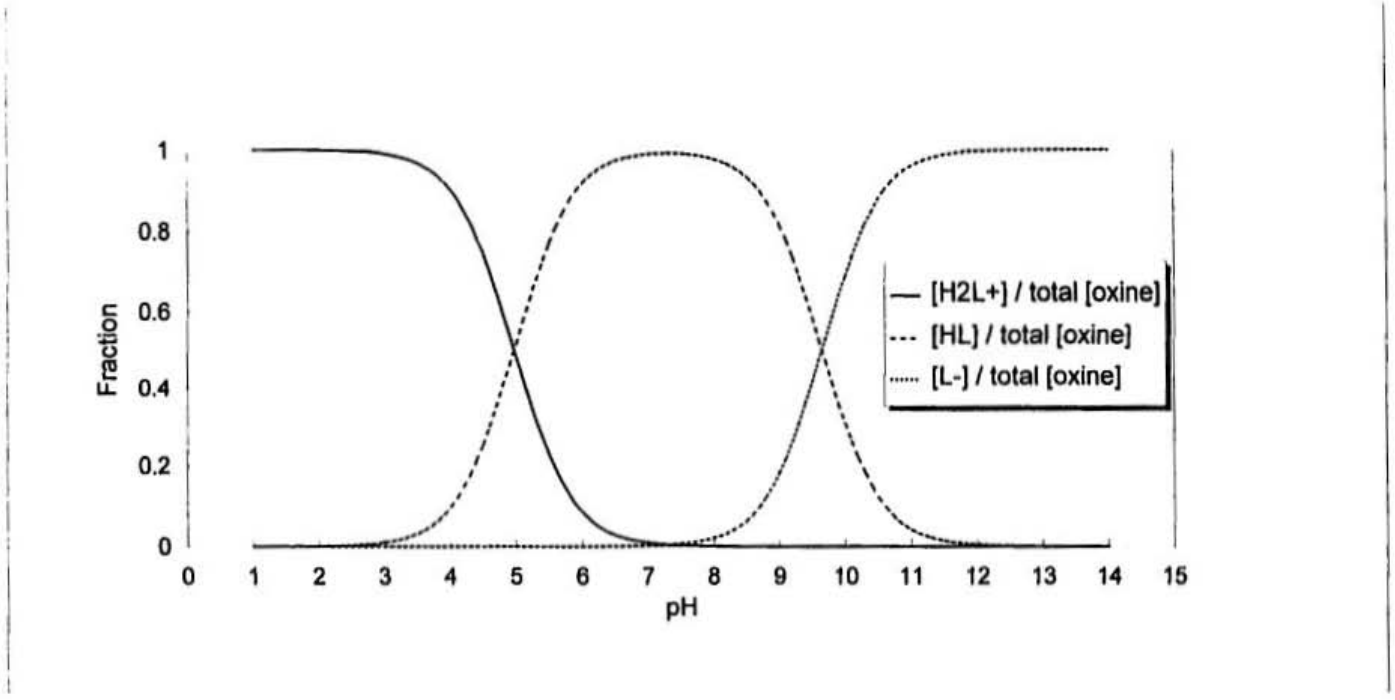


Figure 3.4 Distribution diagram for oxine calculated from the thermodynamic equilibrium constants of oxine

The thermodynamic stability constants for oxine at 25 °C and an ionic strength of 0.1 M are given below:

$$H^+ + HL \rightleftharpoons H_2L^+ \qquad \log K_{1'} = 4.97 \qquad (3-1)$$

$$H^+ + L^- \rightleftharpoons HL \qquad \log K_{2'} = 9.65 \qquad (3-2)$$

Therefore, the dissociation constants of H_2L^+ and HL are:

$$H_2L^+ \rightleftharpoons H^+ + HL \qquad K_1 = 1/K_{1'} = 1.072 \times 10^{-5} \qquad (3-3)$$

$$HL \rightleftharpoons H^+ + L^- \qquad K_2 = 1/K_{2'} = 2.239 \times 10^{-10} \qquad (3-4)$$

The distribution equations (3 - 5) and (3 - 7) of the three species are derived in a similar manner as described by Harris (1987).

$$[H_2L^+]/[\text{oxine}]_{total} = [H^+]^2/([H^+]^2 + [H^+]K_1 + K_1K_2) \qquad (3-5)$$

$$[HL]/[\text{oxine}]_{total} = K_1[H^+]/([H^+]^2 + [H^+]K_1 + K_1K_2) \qquad (3-6)$$

$$[L^-]/[\text{oxine}]_{total} = K_1K_2/([H^+]^2 + [H^+]K_1 + K_1K_2) \qquad (3-7)$$

The distribution diagram for oxine (**Figure 3.4**) is a graph of equations (3 - 5) and (3 - 7). The diagram shows that the dominant species (99%) is HL at the studied pH range 6.7 to 8.2, thus the formation equation of copper-oxine complex can be written as:

$$Cu^{2+} + 2\,HL \rightleftharpoons CuL_2 + 2\,H^+ \qquad (3-8)$$

Equation (3 - 8) indicates that the copper-oxine complex is more stable at low H^+ concentration (high pH). This increasing stability of copper-oxine complex results in increasing DPCSV peak height with increasing pH.

The negative shift of DPCSV peak potential with increasing pH can be explained by equation (3 - 9) which is the reaction that occurs during the scan step. The adsorbed copper-

oxine complexes (CuL_2) are more stable to reduction as the pH value increases, thus producing DPCSV peak at a more negative potential.

$$CuL_2 + 2\,e + 2\,H^+ \rightleftharpoons Cu + 2\,HL \qquad (3-9)$$

3.2.2 Effect of Oxine Concentration

The effect of oxine concentration on DPCSV peak height is shown in **Figure 3.6**. The two lines in **Figure 3.6** show the results obtained by varying the concentration of oxine in Nano-pure water and NF-G1(May, 1995) freshwater containing 0.01 M HEPES (pH = 7.6), 1 μg/L Cu and 0.15 M KCl. The experimental conditions were the same as in **Figure 3.3**.

A peak was observed in the Nano-pure water sample even when the oxine concentration was only 1.0×10^{-8} M. No peak was detected in the NF-G1 (May, 1995) freshwater sample until the oxine concentration was at least 6.3×10^{-7} M. The necessity for higher oxine concentration for the freshwater sample may be due to the natural organic ligands present in the freshwater. In both samples, the peak height increased with increasing oxine concentrations up to 7.3×10^{-6} M and decreased at oxine concentration higher than 7.3×10^{-6} M. Thus, an oxine concentration of 7.3×10^{-6}M was then used for the remaining optimization experiments and determinations of labile and total copper in freshwater.

It was also found that the peak potential shifted in a negative direction with increasing oxine concentration (**Table 3.3**). The increase of peak current and negative shift in peak potential with increasing oxine concentration were caused by increased stability of the adsorptive copper-oxine complex (see equations (3 - 8) and (3 - 9)). A similar discussion

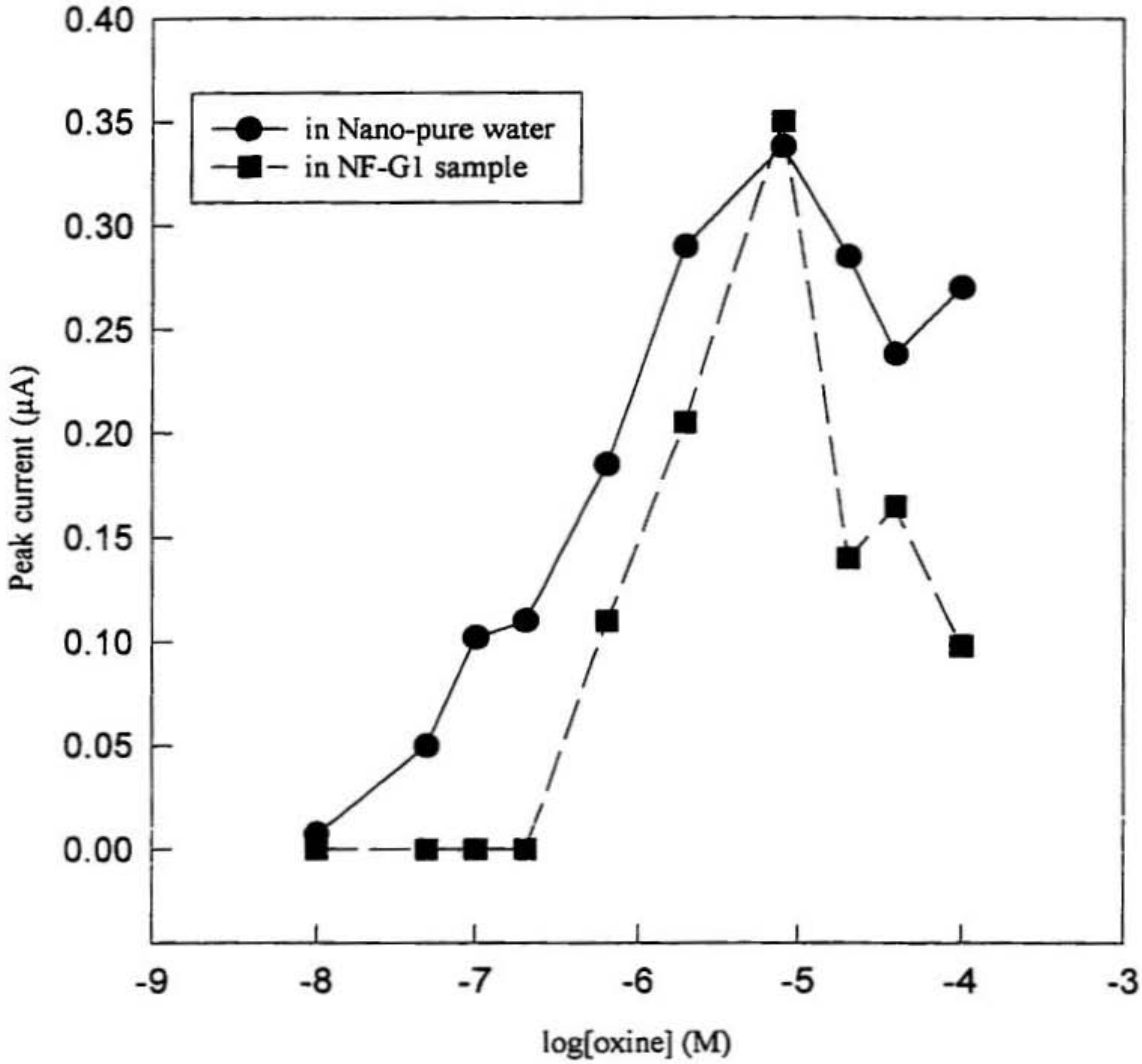


Figure 3.6 Effect of oxine concentrations on DPCSV peak height obtained in samples containing 0.01 M HEPES (pH = 7.6), 1 μg/L Cu and 0.15 M KCl. The experimental conditions were the same as in **Figure 3.3**

was given in section 3.2.1 for the effect of pH on the DPCSV peak. The decrease of peak current at oxine concentration higher than 7.3×10^{-6} M was presumably caused by competitive adsorption of oxine onto the mercury surface.

Table 3.4 Effect of oxine concentrations on DPCSV peak potential

-Log [oxine]	Nano-pure Water Peak Potential (-V)	NF-G1 Sample Peak Potential (-V)
8.0	0.320	-
7.3	0.330	-
7.0	0.340	-
6.7	0.360	-
6.2	0.380	0.370
5.7	0.420	0.415
5.1	0.435	0.435
4.7	0.445	0.435
4.4	0.450	0.440
4.0	0.460	0.450

In order to gain further information about the type of copper-oxine complexes adsorbed by the mercury drop, the distribution of the three species CuL_2, CuL^+, and Cu^{2+} in a copper-oxine solution at pH 7.5 as a function of the oxine concentration is presented in **Figure 3.7**. Hydrolysis of copper was ignored in the distribution diagram as the sample pH was controlled at 7.4 - 7.6. Values for the conditional stability constants, K_{CuL} and K_{CuL2} (at temperature 25 °C, ionic strength 0.1M), are from Smith and Martell (1989) and are listed in equation (3 - 10) and (3 - 11). In a similar manner used for the derivation of equation (3 -5) and (3 - 7), the fractional composition equations for the copper-oxine system are given

in equations (3 - 12) and (3 - 14).

$$Cu^{2+} + HL \rightleftharpoons CuL^{+} + H^{+} \qquad K_1 = 224 \qquad (3 - 10)$$

$$CuL^{+} + HL \rightleftharpoons CuL_2 + H^{+} \qquad K_2 = 178 \qquad (3 - 11)$$

If $[HL] / [H^{+}] = [F]$, then

$$[CuL_2] / [Cu]_{total} = K_1 K_2 [F]^2 / (1 + K_1 [F] + K_1 K_2 [F]^2) \qquad (3 - 12)$$

$$[CuL^{+}] / [Cu]_{total} = K_1 [F] / (1 + K_1 [F] + K_1 K_2 [F]^2) \qquad (3 - 13)$$

$$[Cu^{2+}] / [Cu]_{total} = 1 / (1 + K_1 [F] + K_1 K_2 [F]^2) \qquad (3 - 14)$$

Figure 3.7 shows that CuL_2 is the dominant species in copper-oxine system at an oxine concentration higher than 1.0×10^{-7} M. Therefore, the copper peak observed at −0.4 V for freshwater is caused by the reduction of CuL_2 complexes rather than CuL.

3.2.3 Effect of Adsorption Potential

In the DPCSV method, the potential applied on the HMDE affects the efficiency of the adsorption during the adsorption step as a result of coulombic effects and competitive adsorption (Donat and van den Berg, 1992; Campos and van den Berg, 1994). Thus, the applied potential range between − 0.1 V and − 1.3 V was tested (**Figure 3.8**).

The copper accumulation onto the mercury drop is most efficient at a potential of - 1.1 V. The increasing reduction current with more negative adsorption potentials may be due to both adsorption of copper-oxine complexes and reduction of copper to form an amalgam with the mercury electrode. In addition, competitive adsorption of complexes with metals such as uranium which do not form an amalgam is presumably prevented at a

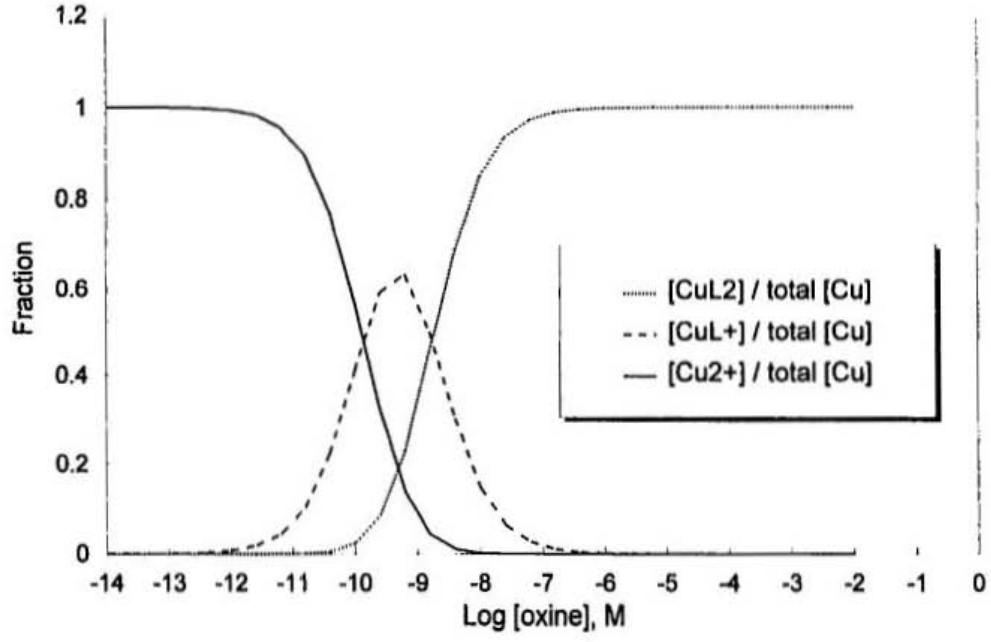


Figure 3.7 Distribution of copper-oxine system at pH 7.5

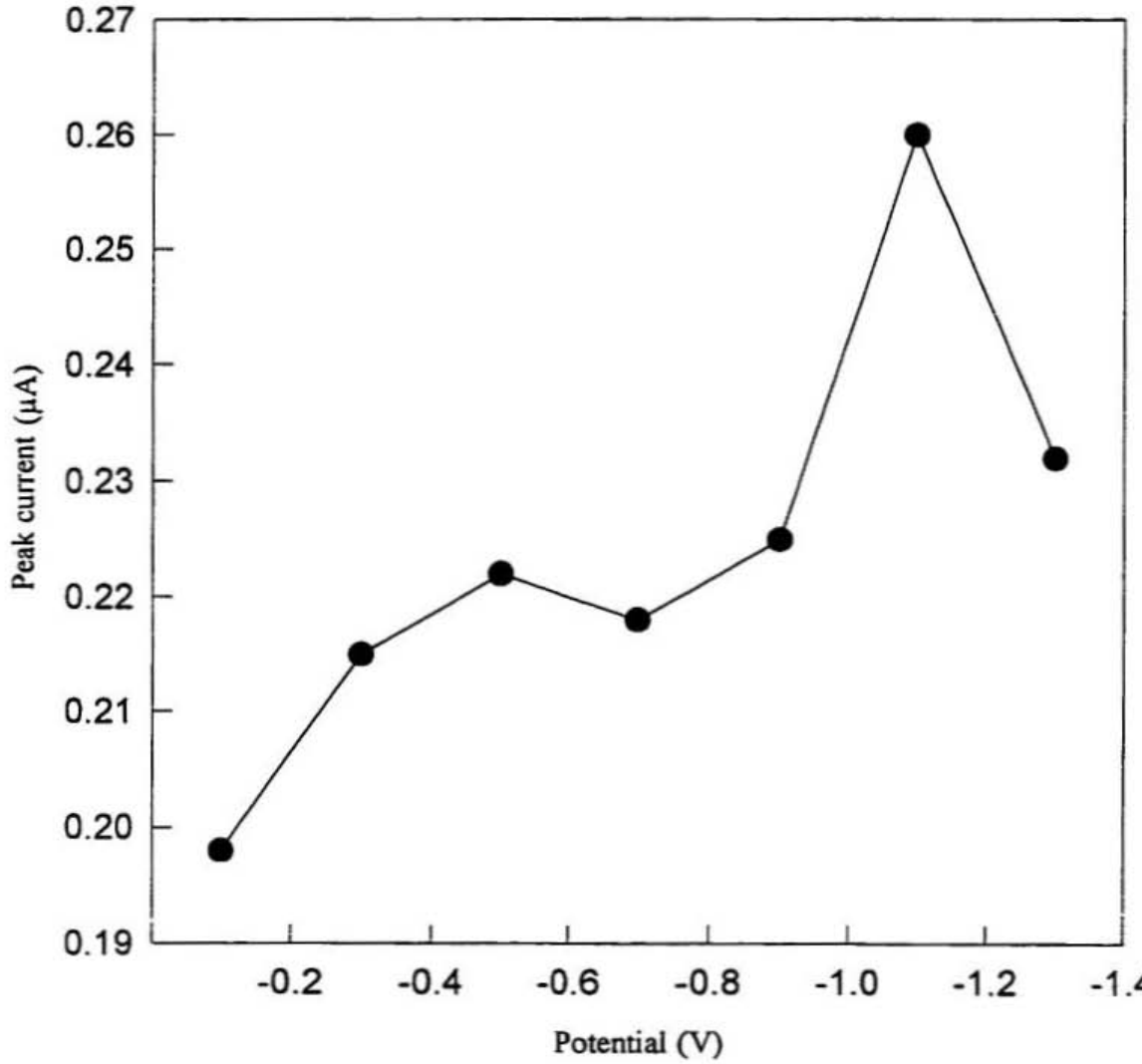


Figure 3.8 Effect of adsorption potential on DPCSV peak height obtained for Nano-pure water containing 0.01 M HEPES, 1 μg/L Cu, 0.15 M KCl and 7.3 x 10^{-6} M oxine.

negative potential of - 1.1 V (van den Berg, 1986). The decrease of the reduction current at potentials more negative than - 1.1 V is probably caused by hydrogen generation from water.

3.2.4 Effect of Adsorption Time

The effect on metal accumulation of increasing the adsorption time is given in **Figure 3.9**. It was found that the peak height increased linearly with a time of up to 5 min. A longer adsorption time will cause saturation of the drop surface or competitive adsorption of some other metal-oxine complexes, therefore, adsorption times of 1 to 5 min were used depending on the metal concentration in different water samples.

The optimal analytical conditions for Cu determination in freshwater (this study) and in seawater (van den Berg, 1986) are compared in **Table 3.5**.

Table 3.5 Comparison of the optimal conditions for Cu determination in freshwater and seawater

Sample	pH	[oxine] (M)	Time (min)	Adsorption potential (V)	Initial potential (V)	[KCl] (M)
Freshwater	7.5-7.7	8×10^{-6}	1-5	-1.1	-0.1	0.15
Seawater	7.7	2×10^{-5}	1-12.5	-1.1	-0.3	0

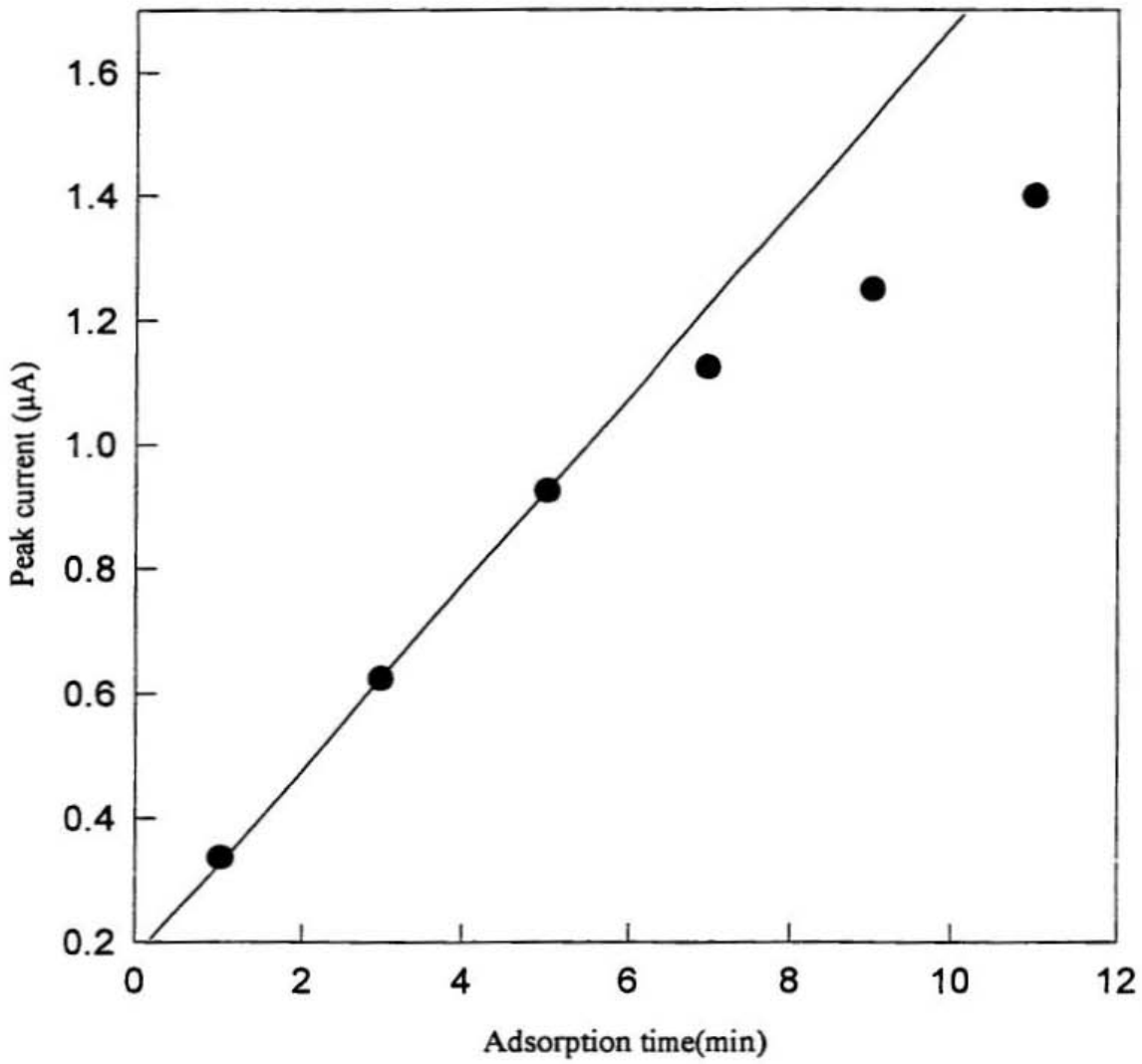


Figure 3.9 Effect of adsorption time on the DPCSV peak height obtained for Nano-pure water containing 1 μg/L of Cu at an adsorption potential of - 1.1 V. Other parameters were the same as previous optimal experiments.

CHAPTER 4

ANALYSIS OF FRESHWATER SAMPLES

4.1 Trace Metal Analysis

4.1.1 Determination of Labile Trace Metals by DPASV and ACSV

A typical DPASV voltammogram for a freshwater sample, NF-G1 (May,1996) is shown in **Figure 4.1**. The first two lines are sample scans and the top three are standard additions. The peaks of Zn, Cd and Pb are very well separated, but no Cu peak appeared at - 0.1 V even after standard additions. This may be due to Cu complexation with natural organic complexing ligands present in freshwater. The oxine-CSV method was, therefore, applied to determine the labile Cu concentration in freshwater samples, because the added strong oxine ligand can compete with natural organic ligands and results in more Cu available for CSV determination. Voltammograms obtained for freshwater samples were similar to that obtained for CASS-2 (see **Figure 3.1** in section 3.1.1).

Freshwater samples obtained in May and July, 1995 and April and July, 1996 were analyzed by ASV to determine labile Zn, Cd and Pb and by the oxine-CSV method to determine Cu. The results shown in **Table 4.1** and **Figure 4.2**, **Table 4.2** and **Figure 4.3** are the averages of three or two determinations.

The amounts of labile Cd in most of the freshwater samples collected in 1995 and 1996 were below the detection limit, the exceptions being sites 7, 8 and 14 in the July, 1995 samples (**Table 4.1**), site 7 in April, 1996 (**Table 4.2 A**) and site 14 in July, 1996 (**Table 4.2**

B). Similarly, the concentrations of labile Pb in most of the samples in the two years were undetectable except for sites 1 and 7 in July, 1995 (**Table 4.2 B**). Measurable amounts of labile Cu were found in all samples except for sites 3, 6, 7 and 14 in July, 1995 (**Table 4.1 B**) and sites 13 and 14 in July, 1996 (**Table 4.2 B**). Relatively high levels of labile Zn (compared to Cu) were observed in all samples (**Figures 4.2** and **4.3**).

The highest concentrations of labile Zn and Cu were obtained in the Bonavista area (sites # 1- 8) which is the most populated area among the three (Bonavista, Random Island and Come-By-Chance). The higher Zn level at site 1 (Hospital Pond) may be due to corrosion of storm pipes beneath the road and runoff from the nearby shopping center parking lot. The two occurrences of Cd in the site 14 samples (**Table 4.1 B** and **Table 4.2 B**) are interesting in view of its location which is close to the refinery in the Come-By-Chance watershed.

In general, concentrations of labile trace metals in the study area were at μg/L level in the range from undetectable up to 0.07 for Cd, 0.18 for Pb, 0.61 for Cu (by oxine-CSV) and range from 0.05 to 4.73 for Zn. These values were within the typical range of trace metals in freshwater systems reported in the literature (Florence, 1986).

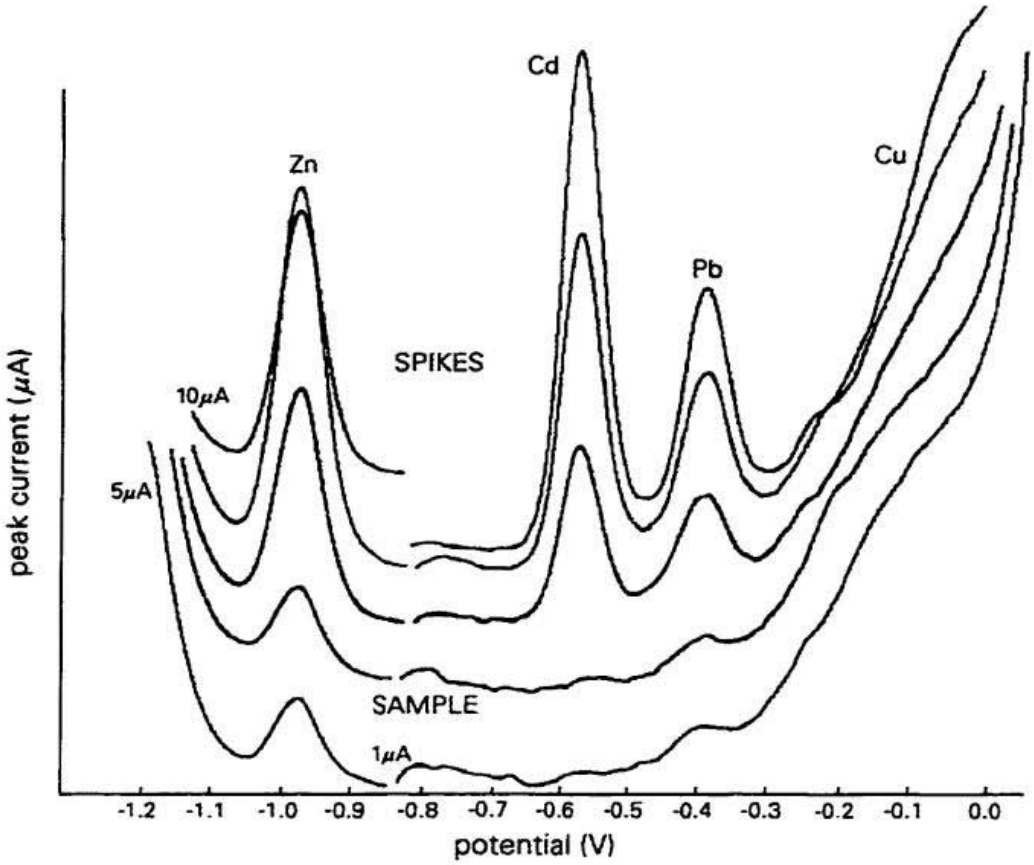


Figure 4.1 A voltammogram of ASV determination of a freshwater sample. The lower two scans are sample while the upper scans are for successive standand additioons.

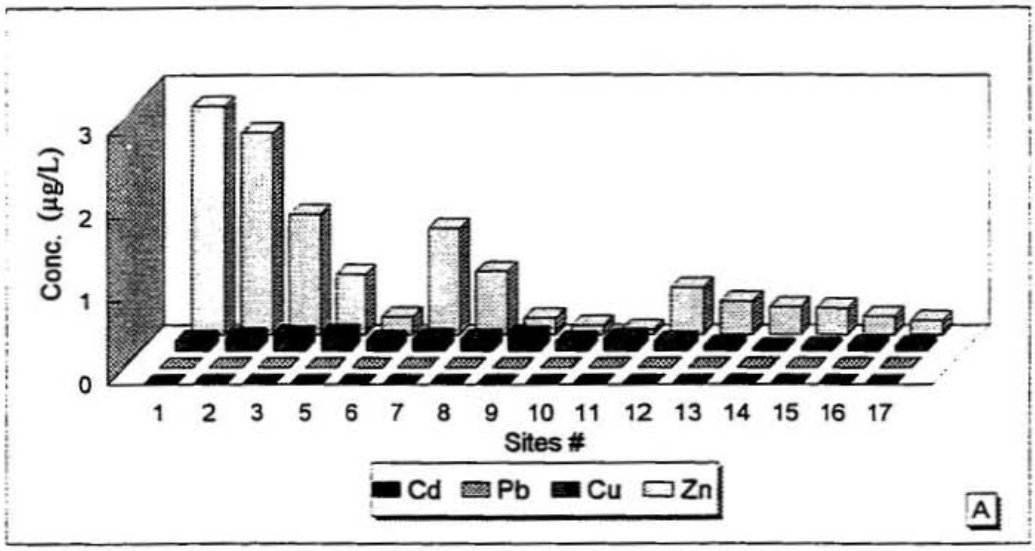
Table 4.1 Labile metal concentrations (μg/L, mean ± S.D., n = 3) in filtered freshwater samples collected in May (A) and July (B), 1995
(A)

Site #	Cd	Pb	Cu	Zn	Temp.(°C)	pH	Cond.(μS/cm²)
1	N.D.[1]	N.D.	0.13 ± 0.02	2.75 ± 0.23	12.0	6.35	68.5
2	N.D.	N.D.	0.16 ± 0.06	2.43 ± 0.43	8.9	6.13	52.2
3	N.D.	N.D.	0.18 ± 0.02	1.45 ± 0.30	10.0	6.01	44.2
5	N.D.	N.D.	0.20 ± 0.01	0.73 ± 0.09	11.2	5.55	39.7
6	N.D.	N.D.	0.13 ± 0.03	0.21 ± 0.07	13.1	7.35	62.2
7	N.D.	N.D.	0.15 ± 0.04	1.28 ± 0.13	10.1	5.80	46.4
8	N.D.	N.D.	0.14 ± 0.04	0.76 ± 0.11	11.3	5.35	41.9
9	N.D.	N.D.	0.18 ± 0.04	0.20 ± 0.04	12.5	7.20	91.5
10	N.D.	N.D.	0.12 ± 0.05	0.11 ± 0.03	11.8	6.86	106.0
11	N.D.	N.D.	0.15 ± 0.02	0.06 ± 0.02	11.1	7.08	84.2
12	N.D.	N.D.	0.11 ± 0.02	0.56 ± 0.04	N.A.[2]	N.A.	N.A.
13	N.D.	N.D.	0.06 ± 0.03	0.40 ± 0.11	9.9	6.97	34.2
14	N.D.	N.D.	0.03 ± 0.01	0.33 ± 0.08	11.9	6.82	28.2
15	N.D.	N.D.	0.05 ± 0.03	0.32 ± 0.08	11.6	6.82	49.1
16	N.D.	N.D.	0.07 ± 0.02	0.22 ± 0.03	11.3	6.7	32.7
17	N.D.	N.D.	0.06 ± 0.02	0.17 ± 0.03	11.7	6.76	39.4

[1] Not detectable
[2] Not Avalaible

Table 4.1 (continued)
(B)

Site #	Cd	Pb	Cu	Zn	Temp.(°C)	pH	Cond.(µs/cm²)
1	N.D.	0.18 ± 0.01	0.34 ± 0.01	3.33 ± 0.28	21.0	6.72	83.02
	N.D.	N.D.	0.36 ± 0.05	1.44 ± 0.22	20.0	5.60	55.9
3	N.D.	N.D.	N.D.	4.73 ± 0.63	19.0	5.42	49.0
5	N.A.						
6	N.D.	N.D.	N.D.	1.22 ± 0.07	22.0	5.87	49.7
7	0.03 ± 0.01	0.10 ± 0.01	N.D.	3.49 ± 0.13	19.0	5.71	52.7
8	0.03 ± 0.01	N.D.	0.24 ± 0.09	0.88 ± 0.06	24.0	5.43	46.3
9	N.D.	N.D.	0.30 ± 0.04	0.56 ± 0.06	19.0	7.33	89.6
10	N.D.	N.D.	0.32 ± 0.04	0.09 ± 0.02	12.0	6.56	87.0
11	N.D.	N.D.	0.31 ± 0.03	0.07 ± 0.02	19.0	7.14	80.5
12	N.A.						
13	N.D.	N.D.	0.14 ± 0.03	0.28 ± 0.02	19.0	6.70	39.7
14	0.07 ± 0.001	N.D.	N.D.	1.22 ± 0.16	16.0	5.33	35.5
15	N.D.	N.D.	0.08 ± 0.03	0.78 ± 0.03	17.0	6.10	43.7
16	N.D.	N.D.	0.08 ± 0.03	0.66 ± 0.08	18.0	6.04	38.0
17	N.D.	N.D.	0.13 ± 0.05	0.71 ± 0.24	17.0	6.06	45.4



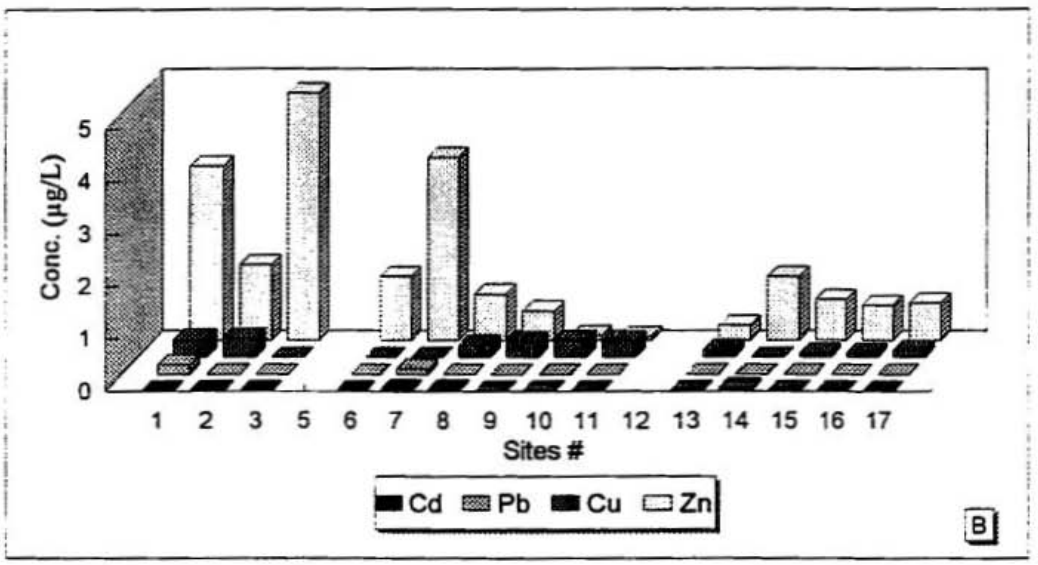


Figure 4.2 Labile metals in freshwater samples collected in May (A) and July (B), 1995

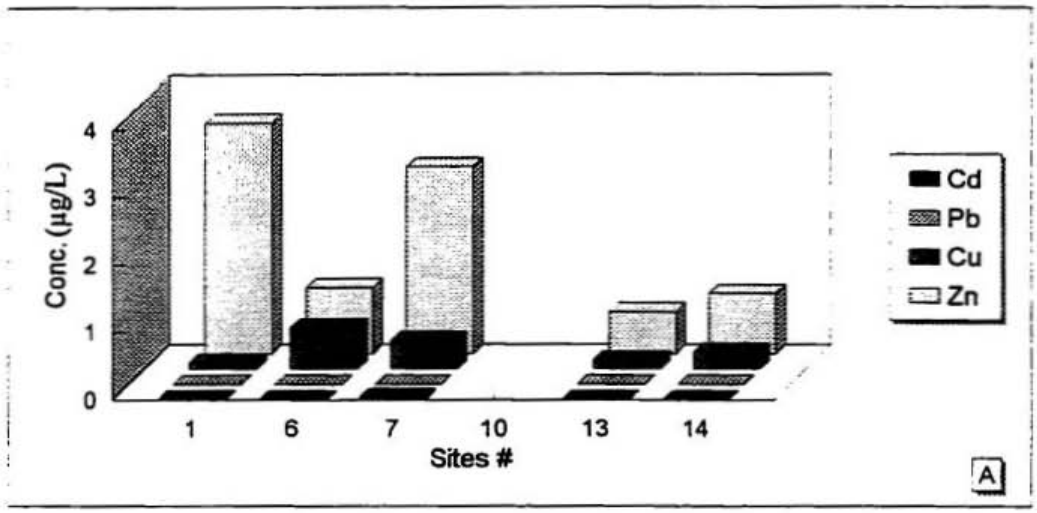
Table 4.2 Labile metal concentrations (μg/L, mean ± S.D., n = 2) in filtered freshwater samples collected in April (A) and July (B), 1996

(A)

Site #	Cd	Pb	Cu	Zn	Temp.(°C)	pH	Cond.(μs/cm²)
1	N.D.	N.D.	0.09 ± 0.04	3.42 ± 0.16	3.0	6.5	80.0
6	N.D.	N.D.	0.61 ± 0.13	0.98 ± 0.09	2.0	6.5	65.0
7	0.04 ± 0.00	N.D.	0.42 ± 0.05	2.77 ± 0.14	3.0	6.1	60.0
10	N.A						
13	N.D.	N.D.	0.13 ± 0.06	0.62 ± 0.12	6.0	7.8	48.0
14	N.D.	N.D.	0.25 ± 0.04	0.89 ± 0.20	8.0	6.8	45.0

(B)

Site #	Cd	Pb	Cu	Zn	Temp.(°C)	pH	Cond.(μs/cm²)
1	N.D.	N.D.	0.17 ± 0.04	0.62 ± 0.09	20.8	6.19	104.1
6	N.D.	N.D.	0.04 ± 0.03	0.24 ± 0.02	19.8	6.5	65.0
7	N.D.	N.D.	0.17 ± 0.08	0.66 ± 0.02	20.7	6.1	60.0
10	N.D.	N.D.	0.04 ± 0.06	0.05 ± 0.01	23.9	7.06	80.4
13	N.D.	N.D.	N.D.	0.25 ± 0.08	22.7	6.67	44.0
14	0.04 ± 0.01	N.D.	N.D.	0.91 ± 0.12	19.8	6.10	35.5



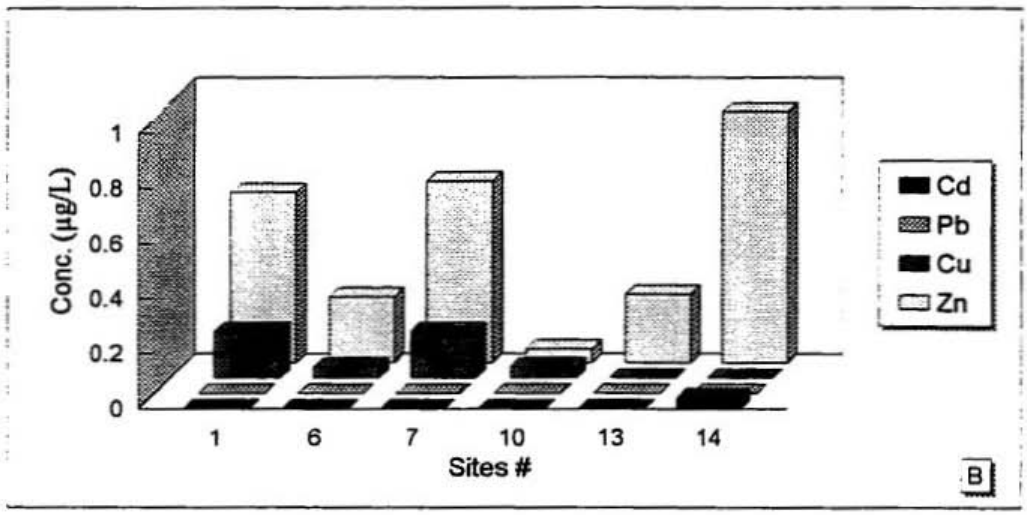


Figure 4.3 Labile metals in freshwater samples collected in April (A) and and July (B), 1996

Figure 4.4 shows comparisons of the May and July samples (1995) for labile Zn and Cu concentrations and **Figure 4.5** shows comparisons of the April and July samples (1996). Some seasonal variations were observed in the 1995 samples (**Figure 4.4**) with July concentrations being generally higher than May. This pattern seems reversed in the 1996 samples where April concentrations were generally higher than July (**Figure 4.5**). These observations are consistent with differences in water volume. For example, the volume of water was noticeably higher in July than it was in May, 1995, while the water level was higher in April than it was in July, 1996. If the higher water level was caused by rain, the higher concentrations with higher water volume might be explained as a result of acid rain. The average pH of rain samples collected between May and August, 1995 in Bonavista was 4.9 (Evans, 1996), which is lower than the average pH of freshwater which was 6.5. The lower pH of acid rain may cause the desorption and release of metal from lake sediment and surrounding soil and cause more of the metal ion to convert to the free form, such as hydrated metal ion, making it ASV labile (Florence, 1982).

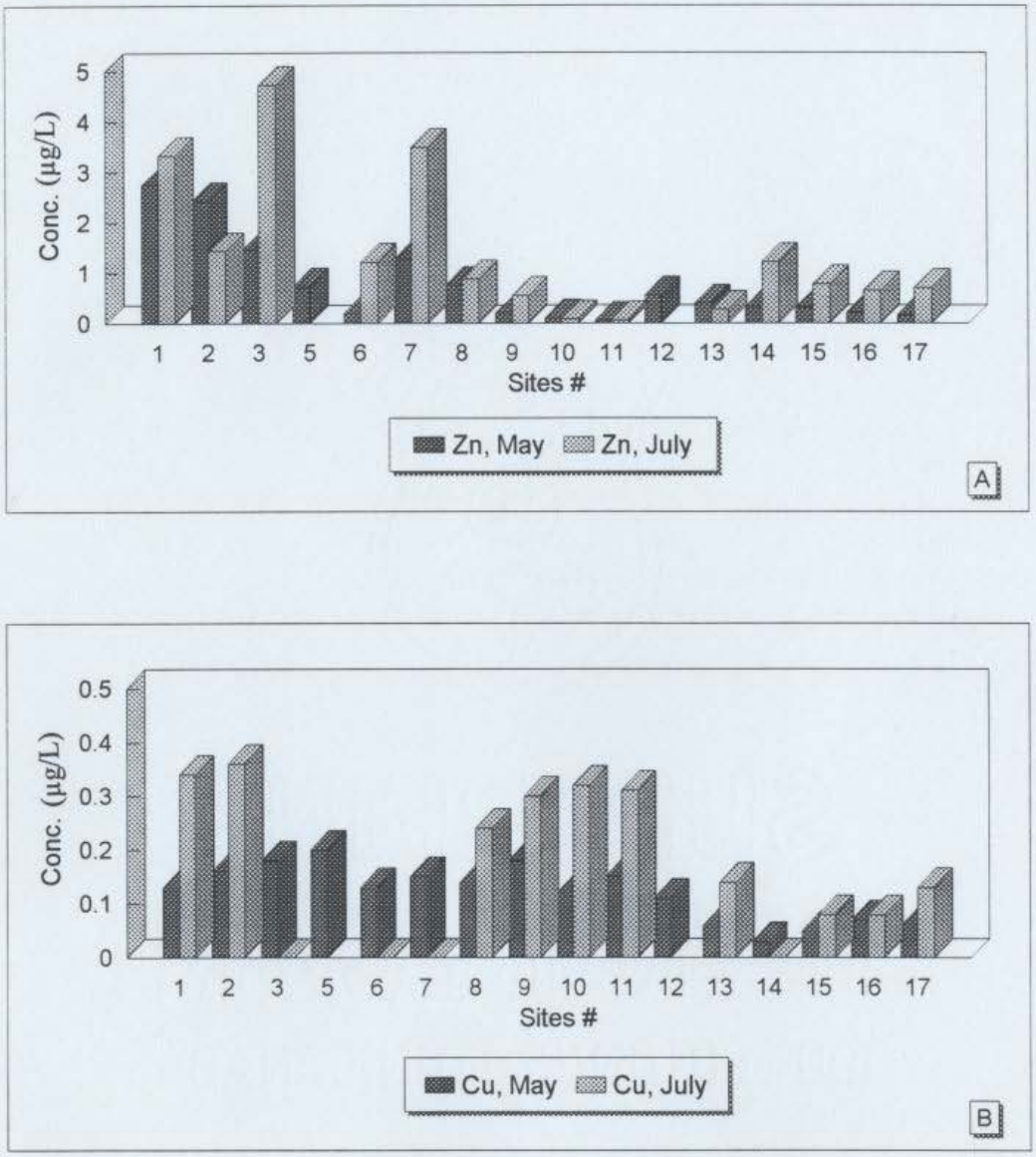


Figure 4.4 Comparisons of labile metal concentrations in samples collected in May and July, 1995: Zn (A) and Cu (B)

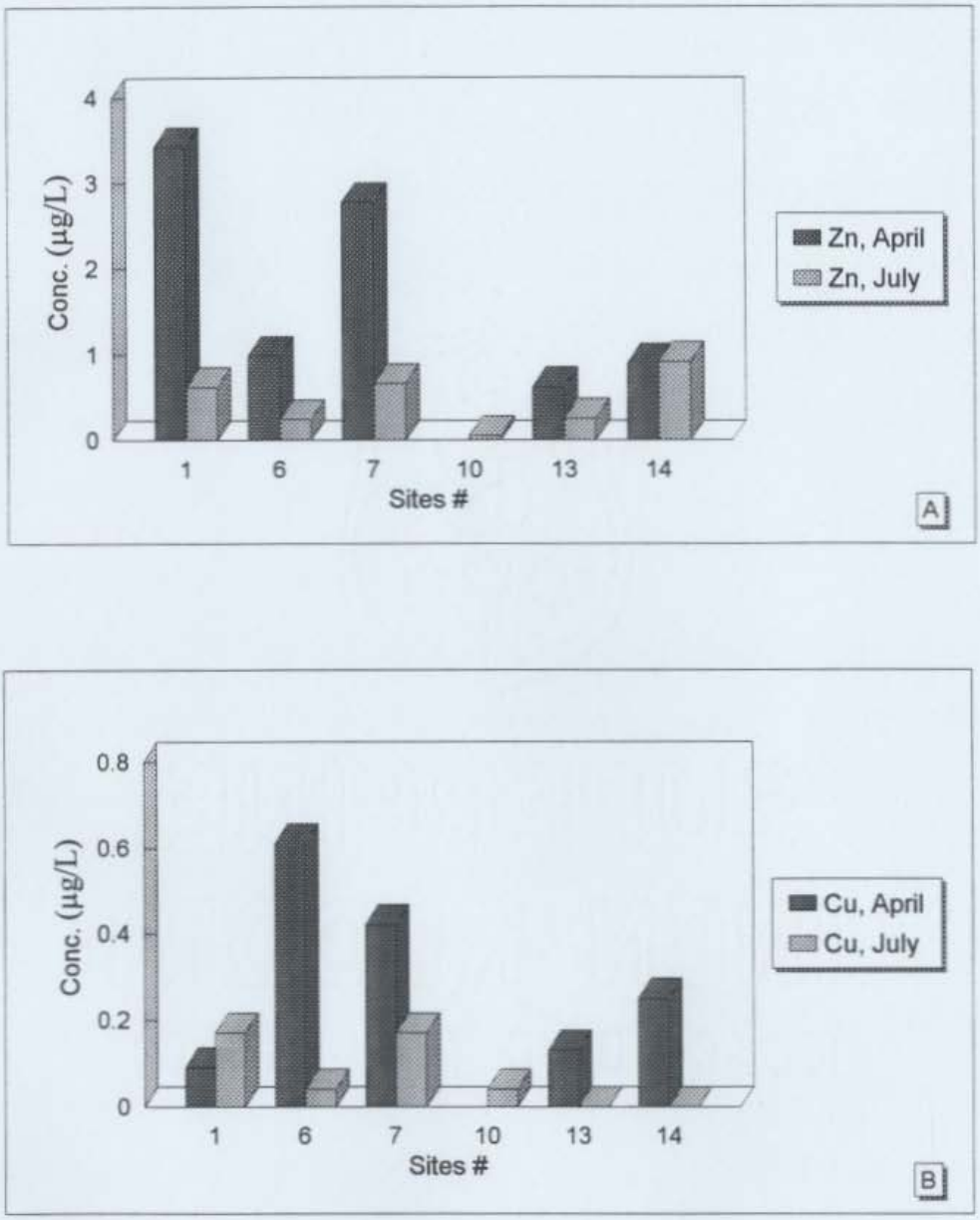


Figure 4.5 Comparisons of labile metal concentrations in samples collected in April and July, 1996: Zn (A) and Cu (B)

4.1.2 Total Metal Determination

Results of the total metal concentrations in the freshwater samples determined by ICP-AES and ICP-MS are listed in **Table 4.3**. Total concentrations of Cd and Pb in most of the freshwater samples collected in May, 1995 (**Table 4.3 A**) were below the detection limits, except at sites 1, 8 and 14 for Pb (1.4, 0.6 and 2.8 μg/L) determined by ICP-AES. Total amounts of Cu were in the range of 2 to 22 μg/L measured by ICP-AES, but undetectable by ICP-MS. For total Zn, ICP-AES results ranged from 1.50 to 19.30 μg/L, while ICP-MS results ranged from 0.91 to 3.15 μg/L. Overall, the ICP-AES results were generally higher than the ICP-MS results (**Table 4.3A** and **B**). They could be due to sample contaminations in ICP-AES analysis and/or metal loss to storage bottles in ICP-MS analysis. The ICP-AES analysis were carried out on samples taken in plastic bottles, which were not acid cleaned, and sample handling and analysis were not conducted in our laboratory.

For 1996 samples, the total metal concentrations were determined by DPASV or ACSV after samples were acidified and then UV-irradiated to destroy natural organic ligands present in freshwater. Results of those determinations and ICP-MS determinations are shown in **Table 4.4**. Total Cu concentrations determined by ACSV and DPASV were reasonably close with some exceptions, total Zn concentrations were different with most of ICP-MS results being higher. The discrepancies may be due to the incomplete dissociation of complexes of metals with natural organic ligands during the UV-irradiation prior to DPASV analysis.

Seasonal variations for total Zn and Cu concentrations (**Tables 4.3** and **4.4**) revealed

the same pattern as the labile metal concentrations (**Figure 4.4**): July concentrations were higher than May in 1995, while April concentrations were higher than July in 1996.

Figure 4.6 A and **B** show comparisons of labile and total Cu and Zn in July 1996 samples. It was found that the concentration of labile Cu ranged from 0 to 11.49 % of the total metal (with one exception at site 1) while labile Zn concentrations ranged from 14 to 69 %. Apparently Cu is highly complexed by natural organic ligands present in freshwater. Similar results have been reported by other researchers (Florence, 1986).

4.1.3 Reagent Blanks and Detection Limits

The reagent blank was determined by gradually increasing the amount of reagents added to 10 mL Nano-pure water and calculated by linear regression. The results are listed in **Table 4.5**.

The detection limits were calculated in three different ways: from 3 × the standard deviation of 9 repeated determinations of a spiked freshwater sample (van den Berg, 1986; Campos and van den Berg, 1994); from 3 × the standard deviation of 9 determinations of a blank sample (Wu and Batley, 1995) and from 2 × noise. Cu results presented in **Table 4.6** were obtained at an adsorption potential of - 1.1 V in the presence of 7.3×10^{-6} M oxine. Comparison of **Tables 4.6 A** and **B** shows that higher detection limits were obtained when higher initial concentrations were used for determinations. The detection limits estimated from noise (**Table 4.6 C**) were lowest for Zn, Cd and Cu.

4.1.4 Sample Storage

The adsorption of trace metals by storage bottles were tested by placing a freshwater sample in different types of bottles (Polyethylene or Fluorinated Polyethylene) with the sample stored after it was filtered or not filtered. Zn loss to storage bottles was observed for all cases with no obvious difference with different methods of storage (**Figure 4.7**).

Controversy over the adsorption of trace metals by storage bottles has been noted in the literature (Florence and Batley, 1980; Florence, 1982). Florence (1982) compared the disagreement and concluded that metal adsorption was observed when synthetic solutions were studied, while metal absorption was negligible when natural seawater and freshwater were tested. In this study, however, Zn adsorption by storage bottles was obvious in a natural freshwater sample even in an acidified condition.

Table4.3 Total metal concentrations (μg/L) in freshwater samples collected in May (A) and July (B), 1995

(A)

Site #	Cd ICP-AES	Cd ICP-MS	Pb ICP-AES	Pb ICP-MS	Cu ICP-AES	Cu ICP-MS	Zn ICP-AES	Zn ICP-MS
1	<0.1	<0.11	1.4	<0.07	2	<1.38	5.2	3.15
2	<0.1	<0.11	<0.2	<0.07	2	<1.38	5.0	5.20
3	<0.1	<0.11	<0.2	<0.07	3	<1.38	4.2	2.09
5	<0.1	<0.11	<0.2	<0.07	2	<1.38	2.6	1.34
6	<0.1	<0.11	<0.2	<0.07	2	<1.38	2.1	2.02
7	<0.1	<0.11	<0.2	<0.07	8	<1.38	4.3	<0.91
8	<0.1	<0.11	0.60	<0.07	5	<1.38	3.3	2.11
9	<0.1	<0.11	<0.2	<0.07	6	<1.38	2.6	<0.91
10	<0.1	<0.11	<0.2	<0.07	11	<1.38	2.2	<0.91
11	<0.1	<0.11	<0.2	<0.07	2	<1.38	1.5	<0.91
12	<0.1	<0.11	<0.2	<0.07	N.A.	<1.38	N.A.	<0.91
13	<0.1	N.A.	<0.2	N.A.	2	N.A.	1.5	<0.91
14	<0.1	N.A.	2.8	N.A.	22	N.A.	19.3	N.A.
15	<0.1	N.A.	<0.2	N.A.	2	N.A.	1.8	N.A.
16	<0.1	N.A.	<0.2	N.A.	2	N.A.	1.9	N.A.
17	<0.1	N.A.	<0.2	N.A.	3	N.A.	1.8	N.A.

Table 4.3 (continued)

(B)

Site #	Cd ICP-AES	Cd ICP-MS	Pb ICP-AES	Pb ICP-MS	Cu ICP-AES	Cu ICP-MS	Zn ICP-AES	Zn ICP-MS
1	<0.1	N.A.	<0.2	N.A.	12	N.A.	6.6	N.A.
2	<0.1	N.A.	<0.2	N.A.	11	N.A.	6.7	N.A.
3	<0.1	N.A.	<0.2	N.A.	27	N.A.	13.9	N.A.
5	<0.1	N.A.	<0.2	N.A.	N.A.	N.A.	N.A.	N.A.
6	<0.1	<0.02	<0.2	0.11	23	<0.27	11.5	1.71
7	<0.1	<0.02	<0.2	0.29	14	<0.27	6.6	4.59
8	<0.1	0.03	<0.2	0.13	11	<0.27	8.1	1.25
9	<0.1	0.05	<0.2	0.04	7	<0.27	3	1.20
10	<0.1	N.A.	<0.2	N.A.	8	N.A.	3.4	N.A.
11	<0.1	N.A.	<0.2	N.A.	5	N.A.	1.5	N.A.
12	<0.1	N.A.	<0.2	N.A.	N.A.	N.A.	N.A.	N.A.
13	<0.1	N.A.	<0.2	N.A.	5	N.A.	1	N.A.
14	<0.1	N.A.	<0.2	N.A.	N.A.	N.A.	N.A.	N.A.
15	<0.1	N.A.	<0.2	N.A.	7	N.A.	7.6	N.A.
16	<0.1	N.A.	<0.2	N.A.	6	N.A.	5.6	N.A.
17	<0.1	N.A.	<0.2	N.A.	9	N.A.	9.5	N.A.

Table 4.4 Total metal concentrations (µg/L) in filtered samples collected in April (A) and July (B), 1996

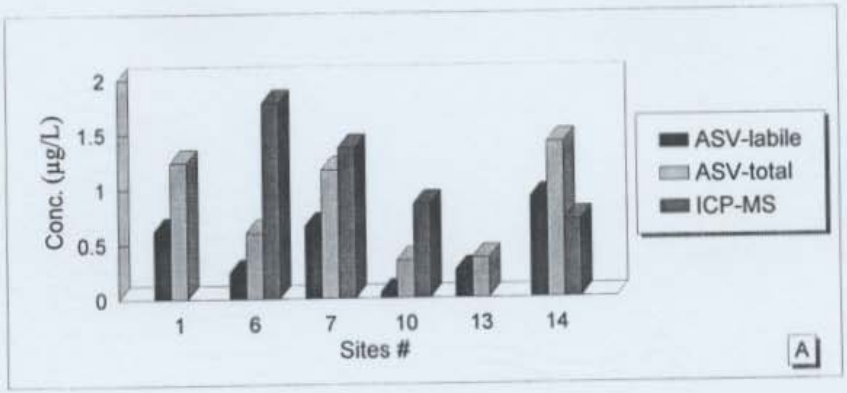
(A)

Site #	Cd ASV	Cd ICP-MS	Pb ASV	Pb ICP-MS	Cu CSV	Cu ICP-MS	Zn ASV	Zn ICP-MS
1	N.A.	<0.06	N.A.	<0.05	N.A.	<2.33	N.A.	3.87
6	N.A.	<0.06	N.A.	<0.05	N.A.	<2.33	N.A.	0.93
7	N.A.	<0.06	N.A.	<0.05	N.A.	<2.33	N.A.	2.86
10	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
13	N.A.	<0.06	N.A.	<0.05	N.A.	<2.33	N.A.	0.64
14	N.A.	<0.06	N.A.	<0.05	N.A.	<2.33	N.A.	0.64

(B)

Site #	Cd ASV	Cd ICP-MS	Pb ASV	Pb ICP-MS	Cu CSV	Cu ASV	Cu ICP-MS	Zn ASV	Zn ICP-MS
1	N.D.	0.28	N.D.	<0.05	0.45	0.79	<0.70	1.24	<2.75
6	N.D.	0.08	N.D.	<0.05	0.81	N.A.	<0.70	0.60	1.78
7	N.D.	0.54	N.D.	<0.05	1.48	1.47	1.10	1.17	1.37
10	N.D.	<0.06	N.D.	<0.05	0.42	N.A.	<0.70	0.34	0.85
13	N.D.	<0.06	N.D.	<0.05	0.48	0.72	<0.70	0.36	<0.64
14	0.01	<0.06	0.11	0.09	0.93	1.01	1.51	1.41	0.70

Site #	Labile Zn / ASV-total Zn (%)
1	50.00
6	40.00
7	56.41
10	14.71
13	69.44
14	64.54



Site #	Labile Cu / CSV-total Cu (%)
1	37.78
6	4.94
7	11.49
10	9.52
13	0.00
14	0.00

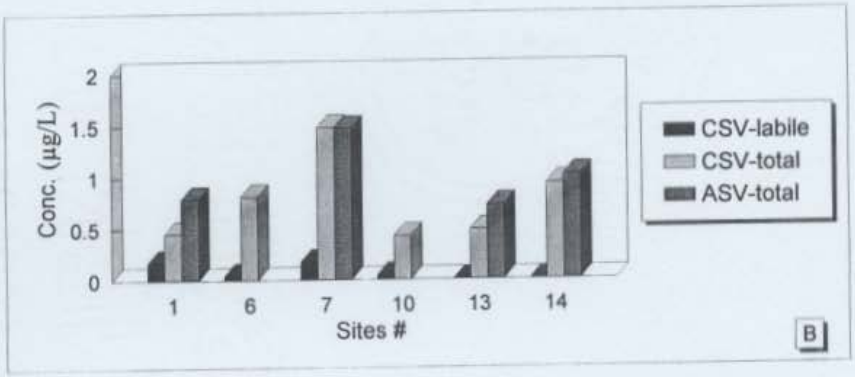


Figure 4.6 Labile and total metal concentrations in July 1996 samples: Zn (A) and Cu (B)

Table 4.5 Reagent blanks

	Conc.(μg/L)	S.D.
Zn(ASV)	0.021	0.015
Cd(ASV)	0.000	0.0005
Pb(ASV)	0.022	0.0000
Cu(CSV)	0.032	0.010

Table 4.6 Detection limits of Zn, Cd, Pb and Cu

(A)

	Conc.(μg/L)	S.D.	D.L.(μg/L)	Dep. time(min)
Zn	0.76	0.09	0.28	10
Cd	0.89	0.05	0.15	10
Pb	0.62	0.09	0.26	10
Cu (CSV)	1.93	0.12	0.37	1

(B)

Zn	0.15	0.05	0.14	10
Cd	0.00	0.01	0.03	10
Pb	0.01	0.03	0.09	10
Cu (CSV)	0.05	0.00	0.01	3

(C)

Zn	0.01
Cd	0.02
Pb	0.12
Cu (CSV)	0.007

(A) calculated from 3 x the S.D. of 9 determinations of a spiked freshwater sample;
(B) calculated from 3 x the S.D. of 9 determinations of a blank sample;
(C) estimated from noise.

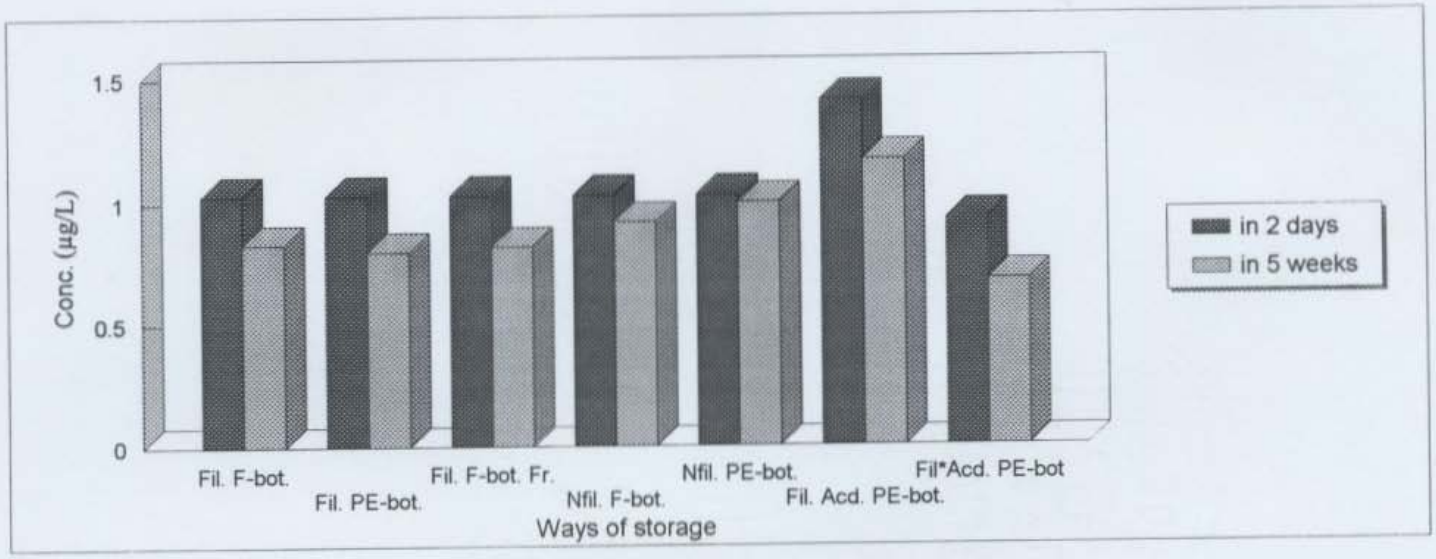


Figure 4.7 Effect of storage method on Zn concentrations in a freshwater sample collected in July 1996

Storage	Zn Conc.(µg/L) measured in 2 days	in 5 weeks	%
Fil. F-bot.	1.03	0.83	80.58
Fil. PE-bot.	1.03	0.80	77.67
Fil. F-bot. Fr.	1.03	0.82	79.61
Nfil. F-bot.	1.03	0.92	89.32
Nfil. PE-bot.	1.03	1.00	97.09
Fil. Acd. PE-bot.	1.41	1.17	82.98
Fil*Acd. PE-bot	0.93	0.68	73.12

4.2 Determination of Copper Complexation

4.2.1 Copper Complexation Titration Using ACSV

The results of the copper complexation titration of the NF-G6 (July, 1995) freshwater sample are given in **Table 4.7**. A titration curve which is a plot of peak current as a function of the total Cu concentrations is shown in **Figure 4.8 A**. In addition, a titration line obtained for an UV-irradiated aliquot of the freshwater sample is given in **Figure 4.8 B**. Compared to the UV-treated freshwater, all data points in the untreated freshwater show reduced concentrations of labile Cu as a result of competition by natural complexing ligands. The curved portion of the titration curve in **Figure 4.8 A** is due to excess natural organic complexing ligands present in the freshwater, while the straight portion is a result of saturation of the natural ligands with high concentrations of Cu. The slope of the straight line used to calculate the sensitivity is identical with the slope for the UV-sample. The linear transformation plot for the titration data is shown in **Figure 4.8 C**.

The technique used for the treatment of the titration data is based on the single-ligand model. This simple model assumes that over the range of copper concentrations studied, only one class of natural complexing ligands with similar stability constants form copper complexes having 1:1 stoichiometry. A straight line in the transformation plot indicates the validity of the model, while curvature would indicate the presence of two or more ligands with different stability constants (van den Berg, 1984 d; Apte *et al.*, 1988). Thus, a two-ligand model can be applied for the treatment of the titration data (van den Berg, 1984 d; Coale and Bruland, 1988,1990; Moffett *et al.* 1990; Sunda and Huntsman, 1991). However,

Table 4.7 Copper complexation titration of the NF-G6 (July, 1995) freshwater sample

Cell #	Total [Cu] (nM)	Peak current i_p (μA)
1	2.90[1]	0.000
2	3.81	0.000
3	4.72	0.010
4	5.62	0.029
5	7.47	0.180
6	9.23	0.248
7	12.03	0.401
8	14.61	0.476
9	17.44	0.530
10	21.16	0.639

[1] This value was the total concentration of copper initially present in the NF-G6 (July, 1995) sample and obtained for UV-treated sample by ACSV.

the two-ligand model is likely to be subject to greater uncertainty (Apte *et al.*, 1988; van den Berg *et al.*, 1990) because this involves calculation of two parameters per ligand (van den Berg, 1984 d). In addition, the titration data can usually be transformed to give a straight line since one class of ligand is predominant over the concentration range of titrated copper. Therefore, the single-ligand model has been used in recent studies (Donat and van den Berg, 1992; van den Berg and Donat, 1992; Campos and van den Berg, 1994).

The values for copper complexing ligand concentrations (C_{Lx}) and conditional stability constants ($\log K'_{CuLx}$) determined for five freshwater samples are listed in **Table 4.8**. Good reproducibility of the titration is demonstrated by duplicate titrations of two samples. The ligands detected in the five freshwater samples had concentrations between 0.75 and 3.22 nM and formed strong copper complexes with conditional stability constants of $10^{16.06}$ to $10^{18.30}$.

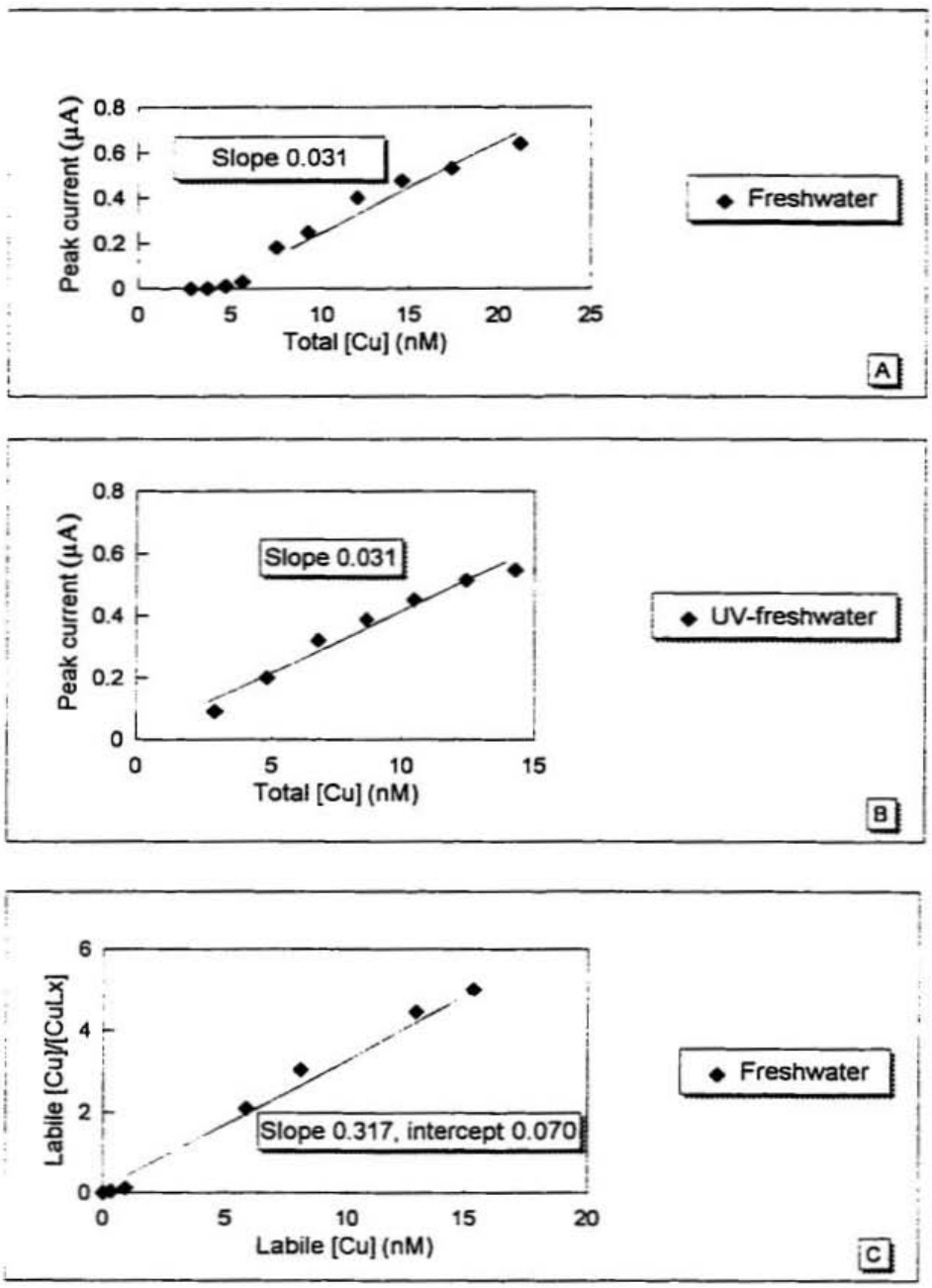


Figure 4.8 (A) Titration curve for filtered NF-G6 (July, 1995) sample containing 0.01 M HEPES, 0.15 M KCl and 7.3 uM oxine; (B) Titration line for UV-irradiated NF-G6 sample; (C) The transformed line of titration A

Table 4.8 Natural ligand concentrations and conditional stability constants for copper complexes in freshwater determined by ACSV at an adsorption potential of -1.1 V

Sample[1]	[oxine] (μM)	$Log\alpha_{Cuox}$	Total[Cu] (nM)		C_{lx} (nM)	$LogK'_{CuLx}$
NF-G6 (95M)	7.3	8.33	2.0[2]		3.12	17.97
NF-G6 (95J)	7.3	8.33	2.90[3]		3.15	17.99
NF-G15 (95J)	1.5	6.95	7[2]		2.37	16.00
	1.5	6.95	7		4.07	16.11
				Avg.	3.22	16.06
NF-G6 (96A)	7.3	8.33	0.79[4]		0.75	18.72
NF-G14 (96J)	7.3	8.33	1.01[4]		1.64	18.52
	7.3	8.33	1.01		1.14	18.08
				Avg.	1.39	18.30

[1] Samples are named NF-G-site # (time when collected);
The total Cu concentrations were obtained by: [2] ICP-AES; [3] ACSV; [4] DPASV

It has been realized that natural waters are likely to contain a broad range of complexing ligands with different stability constants. The values derived from the simplified model represent a summation of these different ligands or binding sites with similar stability constants. In addition, these ligand concentrations and stability constants are conditional and dependent on the methods used, the freshwater composition (pH, ionic strength etc.) and the treatment of data. In the case of the oxine-CSV method, the results obtained are also affected by factors such as the adsorption potential applied (section 4.2.2), the concentration of added oxine (section 4.2.3) and the total copper concentration determined. For instance, an overestimation of the total concentration of Cu leads to an overestimation of the ligand

concentrations. The values of the total Cu concentrations used for complexation calculations were determined by either ICP-AES or stripping voltammetry. As discussed in section 4.1.2, the total concentrations determined by ICP-AES were usually higher than the DPASV or ACSV results. In the case of ICP-AES, sample contamination may be one of the reasons for higher values of total Cu resulting in higher ligand concentrations. The low ligand concentrations from DPASV or ACSV may be due to an existence of strong organic ligands remaining complexed with Cu even after UV-irradiation.

4.2.2 Effect of Adsorption Potential

The optimal adsorption potential -1.1 V was used for determination of both the labile and total Cu concentrations in freshwater samples. The same potential was employed for the determination of organic complexation of Cu (**Table 4.8**). However, there has been discussion focusing on the possible effect of such a negative potential on dissociation of natural complexes of copper (Donat and van den Berg, 1992; van den Berg, 1992). In order to study the effect of adsorption potential on natural complexing ligand determination by oxine-CSV, adsorption potentials of -0.15 V, -0.7 V and -1.1V were used in five titrations of three freshwater samples. A potential of -0.15 V was selected to avoid the oxidation of mercury, -1.1 V as the optimal potential and -0.7 V as a value in between. The determined ligand concentrations and the conditional stability constants of the samples are listed in **Table 4.9**.

It was found that the detected ligand concentrations in the samples decreased with

increasingly negative potential, while the values for the conditional stability constants, at a fixed $\log\alpha_{Cuox}$ value (i.e. a fixed detection window), showed no obvious difference at each potential, indicating that the same class of ligands was detected.

Table 4.9 Effect of adsorption potential on ligand concentrations, C_{Lx} (nM), and conditional stability constants, $\log K'_{CuLx}$, for copper complexes in freshwater determined by ACSV

Sample[1]	$\log\alpha_{Cuox}$	-0.15 V		-0.7 V		-1.1 V	
		C_{Lx}	$\log K'_{CuLx}$	C_{Lx}	$\log K'_{CuLx}$	C_{Lx}	$\log K'_{CuLx}$
NF-G	6.95	10.78	16.87	4.69	16.34	2.37	16.00
-15(95J)	6.95	10.30	16.50	5.02	16.94	4.07	16.11
	8.33	9.23	17.28	8.84	18.20	7.97	19.53
NF-G -6 (96A)	8.33	1.44	18.73	1.19	18.72	0.75	18.72
NF-G -14 (96J)	8.33	1.41	18.78	1.26	18.48	1.39	18.30

The results from **Table 4.9** are rearranged in **Table 4.10** which shows an average ligand concentration of one class of ligands with an average conditional stability constant at two different detection windows. At a detection window of 6.95, ligands with an average conditional stability constant, $10^{16.46}$, were detected and an average ligand concentration of 10.54 nM was obtained at a potential of -0.15 V, which changed to 4.86 nM at -0.7 V and 3.22 nM at -1.1 V. The ligand concentrations decreased by 53.80 % at -0.7 V and by 69.45 % at -1.1 V compared with the concentration at -0.15 V. The decrease in concentrations can be explained as a result of dissociation of the natural complexes, CuL_x, at the more negative

potential since only ligands remaining complexed with Cu are determined by the titration. When the detection window was increased to 8.33, the same trend was observed (**Figure 4.9**) but the ligand concentrations decreased by only 6.70 % at -0.7 V and 16.38 % at -1.1V. The slower rate of decrease in concentrations at a higher detection window might be because a negative potential has less effect on the dissociation of the more stable complexes, CuL_x, with a higher conditional stability constant of $10^{18.53}$. The results are consistent with the values reported by van den Berg (1992). At detection windows of 2.5 and 3.0, an underestimation of the ligand concentration by an average of 61 % was observed at potential -0.7 V compared with -0.15 V for seawater using tropolone-CSV. The value, 61 %, is higher than the value obtained in this study (53.80 %) because of the lower detection window used by van den Berg.

Table 4.10 Effect of adsorption potential on average ligand concentrations and conditional stability constants for copper complexes in freshwater determined by ACSV

$Log\alpha_{Cuox}$	Average $LogK'_{CuLx}$	Average ligand concentration -0.15 V	-0.7 V	-1.1 V	
6.95	16.46	10.54	4.87	3.22	
			53.80	69.45	Decrease, %
8.33	18.53	4.03	3.76	3.37	
			6.70	16.38	Decrease, %

Since a more negative potential caused the dissociation of natural complexes thereby underestimating the concentrations of natural ligands, the more positive potential of -0.15

V was then used for the determination of copper complexation (**Table 4.11**). The determined ligand concentrations ranged from 1.41 to 10.78 nM and values of conditional stability constants ranged from $10^{15.68}$ to $10^{18.87}$.

Table 4.11 Natural ligand concentrations and conditional stability constants for copper complexes in freshwater determined by ACSV at an adsorption potential of -0.15 V

Sample[1]	[oxine] (μM)	$Log\alpha_{Cuox}$	Total[Cu] (nM)	C_{Lx} (nM)	$LogK'_{CuLx}$
NF-G6 (95M)	1.5	6.95	2.0[2]	4.66	15.68
	7.3	8.33	2.0	3.34	17.57
	36.7	9.73	2.0	2.04	18.30
NF-G15 (95M)	1.5	6.95	2[2]	6.25	17.26
	7.3	8.33	2	3.34	17.97
	36.7	9.73	2	2.06	18.28
NF-G15 (95J)	1.5	6.95	7[2]	10.78	16.87
	1.5	6.95	7	10.30	16.50
	7.3	8.33	7	9.23	17.28
	36.7	9.73	7	3.12	18.25
NF-G6 (96A)	7.3	8.33	0.79[4]	1.44	18.73
NF-G14 (96J)	7.3	8.33	1.01[4]	1.41	18.78

[1], [2] and [4] are the same as in **Table 4.8**.

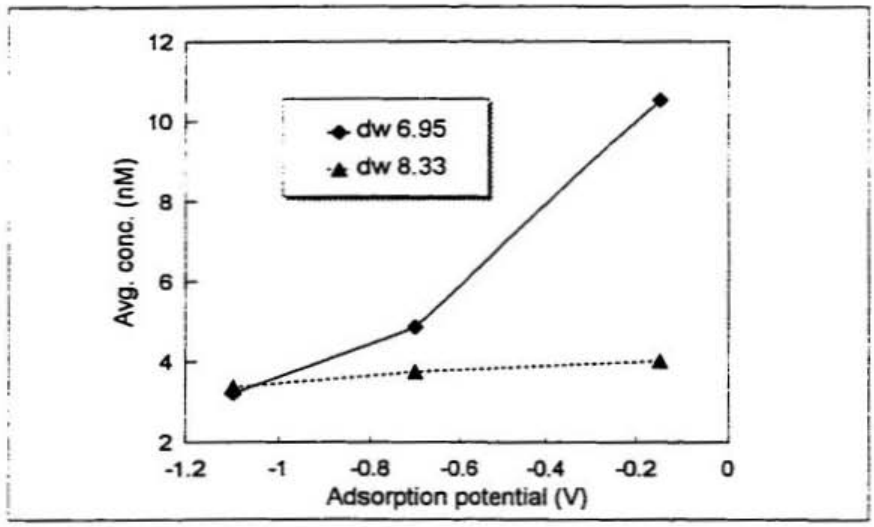


Figure 4.9 Effect of adsorption potential on average ligand concentrations determined by ACSV at different detection windows (dw)

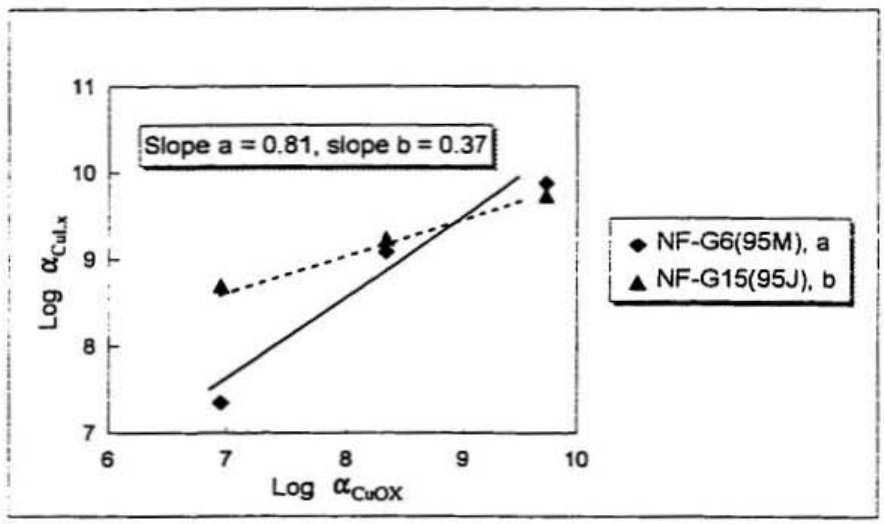


Figure 4.10 Copper complexation, log α_{CuLx} , determined at different detection windows for two freshwater samples

4.2.3 Detection Windows

The ligand concentrations obtained in this study were low and the conditional stability constants were high compared with values reported for seawater in the literature (**Table 4.12**). The differences are probably because a great variety of ligands exist in natural water and because different analytical techniques, having different detection windows, determine organic complexing ligands of different strengths. Hence, any technique at any single detection window may detect only some of the complexing ligands in natural water.

As discussed in section 1.3.2, the detection window in ACSV is calculated from α_{CuAL}, which is the centre of the detection window. It has been reported that natural organic ligands with α_{CuLx} values, (where α_{CuLx} is the α-coefficient of copper with the natural ligands), approximately 1-2 orders higher or lower than α_{CuAL} can be accurately measured using the detection window of α_{CuAL} (Buckley and van den Berg, 1986; van den Berg and Donat, 1992).

To obtain a more meaningful picture of the complexation of copper in natural water, it is important to select analytical techniques that cover a range of detection windows. The ACSV method meets this requirement because the center of the detection window, α_{CuAL}, can be varied over several orders of magnitude by changing the concentrations of the added ligands and by selecting different ligands with different stability constants.

In this study, three values of α_{CuAL} (6.95, 8.33, 9.73) were used to determine copper complexation by using various levels of added oxine. It can be seen (**Table 4.13**) that the

Table 4.12 Literature values of natural ligand concentrations and conditional stability constants for copper complexes in seawater determined by ACSV at different detection windows

Sample	added ligand	$Log\alpha_{CuAL}$	C_{Lx} (nM)	$LogK'_{CuLx}$	Ref.
Mediterranean sea	1 μM SA	3.61	14.4	13.08	1
	2 μM SA	3.99	10.5	13.16	1
	1 μM oxine	5.02	3.2	14.26	1
Atlantic Ocean	0.3 μM SA	3.03	12.8	12.2	1
	0.5 μM SA	3.27	8.1	12.7	1
	2 μM SA	3.99	4.9	13.1	1
North sea	0.324 mM troplolone	3.12	16.2	12.4	2
	0.83 μM oxine	4.85	4.0	14.2	3
	0.193 mM catechol	6.24	7.82	15.6	2
Indian ocean	0.158 mM troplolone	-	4.13	12.6	2
Channel sea	0.01mM oxine	6.95	6.0	15.10	4
Sargasso sea	0.70 mM tropolone	3.75	3.5	12.4	3
	4.71 μM oxine	6.36	2.0	15.9	3

1. Campos and van den Berg, 1994.
2. Donat and van den Berg, 1992.
3. Van den Berg and Donat, 1992.
4. Van den Berg *et al.*, 1990.

determined ligand concentrations decreased with increasing α_{Cuox} , whereas the values of conditional stability constants increased. This result indicates that several natural ligands were present in the samples and stronger ligands or complexing sites detected at higher detection windows (greater α_{Cuox}) were present at lower concentrations. Similar results were obtained previously for seawater and estuarine waters (van den Berg *et al.*, 1990; van den Berg and Donat, 1992; Campos and van den Berg, 1994).

Table 4.13 Natural ligand concentrations and conditional stability constants determined by ACSV at different detection windows using an adsorption potential of -0.15 V

Sample	[oxine] (μM)	$Log\alpha_{Cuox}$	C_{Lx} (nM)	$LogK'_{CuLx}$	$Log\ \alpha_{CuLx}$
NF-G6 (95M)	1.5	6.95	4.66	15.68	7.35
	7.3	8.33	3.34	17.57	9.09
	36.7	9.73	2.04	18.30	9.61
NF-G15 (95M)	1.5	6.95	6.25	17.26	9.06
	7.3	8.33	3.34	17.97	9.49
	36.7	9.73	2.06	18.28	9.59
NF-G15 (95J)	1.5	6.95	10.54	16.87	8.70
	7.3	8.33	9.23	17.28	9.24
	36.7	9.73	3.12	18.25	9.74

Linear regressions: $log\alpha_{CuLx} = A\ log\alpha_{Cuox} + B$

Sample	A	B	R^2
NF-G6 (95M)	0.81	1.91	0.91
NF-G15 (95M)	0.19	7.79	0.88
NF-G15 (95J)	0.37	6.23	0.85

The overall stability (as expressed by α_{CuLx}, being calculated from C_{Lx} K'_{CuLx}) of the complexes of copper with natural ligands, L_x, increased with increasing α_{Cuox} (**Table 4.13**). A good linear relation between $\log\alpha_{CuLx}$ and $\log\alpha_{Cuox}$ was demonstrated in **Table 4.7** and **Figure 4.10)**. The various slopes of the regression lines for different samples suggests that variation of the detection window has different effects on the observed organic copper complexation in different samples. All three slopes were not equal to unity, indicating that the observed complexing ligands present in the samples did not follow a continuous range of complex stabilities.

CHAPTER 5

CONCLUSION

It is known that concentrations of labile metal and not the total metal provide information about the biological availability of trace metals in terms of nutrient limitation and toxicity. Therefore, the concentrations of labile Zn, Cd and Pb in the freshwater samples collected from the study area were determined using differential pulse anodic stripping voltammetry and the labile concentrations of Cu were determined by the adsorption-CSV method using oxine as the competing ligand.

Before the oxine-CSV method was applied to the determination of labile Cu in freshwater, analytical conditions were optimized by varying such parameters as pH, oxine concentration, adsorption potential and adsorption time. The optimal conditions were at pH 7.4-7.6, oxine concentration 7.3 μM, adsorption potential -1.1V and adsorption time 1-7min.

Concentrations of labile metals in the freshwater of the study area were comparable with the reported literature values. Metals of interest in order of increasing concentrations in freshwater were Cd, Pb, Cu and Zn. The highest concentrations of labile Zn and Cu obtained in the Bonavista area indicated a residential impact on the water system, while the two occurrences of Cd in the refinery watershed suggested the possible impact of industry, although the concentration did not exceed that allowable by Canada Drinking Water Standards (Henry and Heinke, 1996). Seasonal variations observed in the 1995 and 1996 samples were consistent with the effect of acid rain on metal speciation in freshwater.

Total metal concentrations were measured by ICP-AES and/or ICP-MS and/or DPASV and/or ACSV. Comparisons of the labile and total metal concentrations in the July 1996 samples revealed that labile Cu ranged from 0 to 11.49% of the total metal while Zn ranged from 14 to 69%.

Copper complexation in freshwater was studied by competitive complexing titration using ACSV to determine the concentrations of natural organic ligands and conditional stability constants. Very strong complexing ligands in the samples were detected under the conditions used in this study. It was also found that more negative potential (-0.7 V, -1.1 V) caused the detected ligand concentrations to be underestimated due to the dissociation of natural complexes of copper. Furthermore, the underestimation was more severe at more negative potential and lower detection windows. Thus a relative positive potential of -0.15V is suitable for copper complexation titration to avoid the dissociation of natural complexes of copper.

The effects of the detection window on copper complexation determination were also discussed. It was found that the detected ligand concentrations decreased with increasingly higher detection windows, whereas the values of the conditional stability constants increased. These findings confirmed that a spectrum of natural complexing ligands exist in freshwater with weaker ligands being determined at lower detection windows and stronger ligands being determined at higher detection windows. A good linear relation was found between $\log\alpha_{CuLx}$ and $\log\alpha_{Cuox}$.

As mentioned in section 1.4, this study is part of an interdisciplinary research program. The results obtained in this study will be linked to the other natural sciences results and then considered with the health and social sciences. In order to obtain an overall picture of the water chemistry, the labile metal results will be compared with the full range of total metals determined by ICP-MS and ICP-AES, then compared with the results on atmospheric inputs of both trace metals and acid and finally compared with the trace metal analysis of sediments and lichens. The picture will be merged with results from Biology and Nursing. Combining all scientific results with those of the social sciences may provide a better picture of the sustainability of the communities.

More studies on trace metals and metal complexation in the watersheds would be useful. For example, the determination of trace metal concentrations in coastal water could be performed in order to obtain information on the fate and speciation changes of the metals when they enter the marine environment. Water samples taken throughout the year should be determined for trace metal speciation to gain a better understanding on seasonal variations. In addition, determination of copper complexation at different detection windows by using different competing ligands other than oxine could be carried out to measure a whole range of strengths of natural ligands.

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Figure A.1 Sample sites # 1 - 8 in Bonavista area

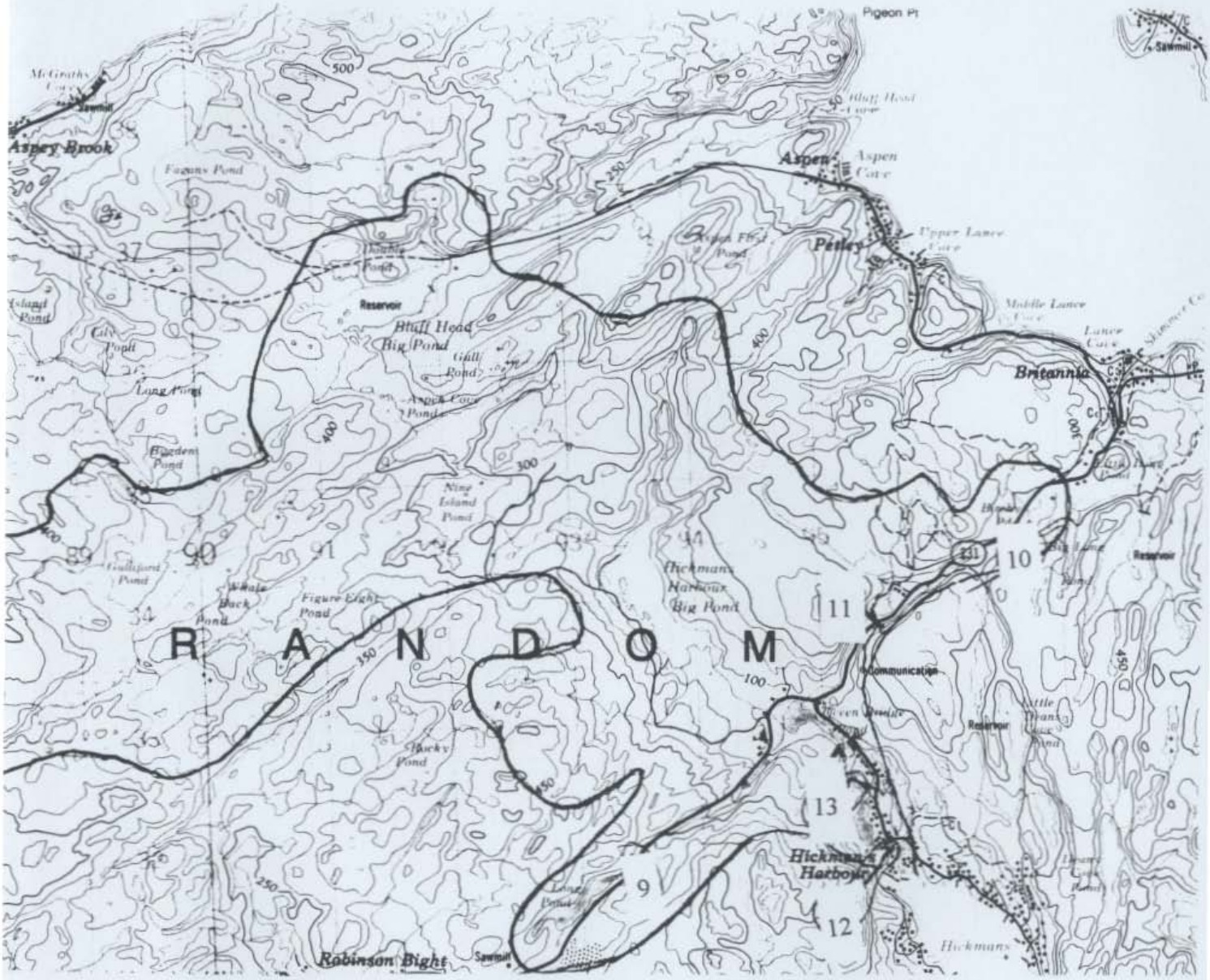

Figure A.2 Sample sites # 9 - 13 in Random Island

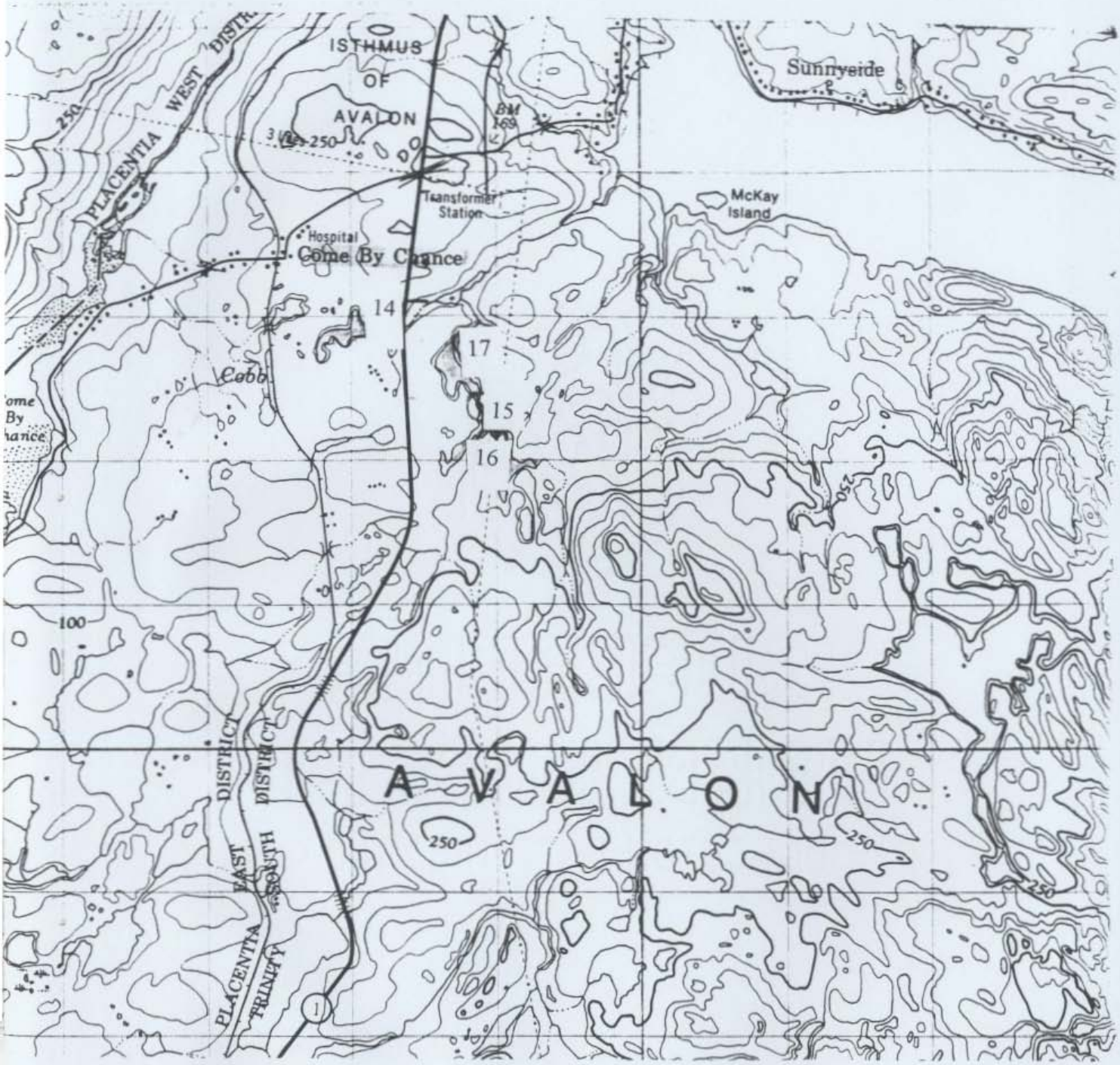

Figure A. 3 Sample sites # 14 - 17 in Come By Chance