THE DEVELOPMENT OF AN ANALYTICAL METHOD FOR THE

STUDY OF THE DISTRIBUTION OF METAL-COMPLEXES IN

AQUEOUS SLURRIES OF SEDIMENTS

By

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Abstract

A method has been developed to study the distribution of Cu-EDTA and Pb-EDTA complexes in aqueous slurries of sediment. This method involves separation of the metal-EDTA complexex by ion-exchange chromatography on a reversed-phase C_{18} column coated with Cetrimide (ion-pair reagent) followed by UV-detection measurement at 258 nm. The chromatographic method provided good separation of Cu-EDTA from Pb-EDTA within 15 min. The new method was tested with a sediment slurry spiked with Cu^{2+} and/or Pb^{2+}, and EDTA. For this purpose, a micro-extraction cell was added prior to the analytical column to allow the direct injection into the HPLC of whole slurries (suspended sediment in contact with metal ions and EDTA). Preliminary results showed that the time-dependent distribution of Cu-EDTA or Pb-EDTA in contact with sediment can be followed. The detection limits for the Cu-EDTA and Pb-EDTA sediment slurry were 1.32 nM and 2.03 nM, respectively.

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ACKNOWLEDGEMENT

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LIST OF ABBREVIATIONS

BSE	Backscattered Electron Detector
Cetrimide	Cetyltrimethylammonium bromide
DOC	Dissolved Organic Carbon
EDTA	Ethylenediaminetetraacetic acid
EDS or EDX	Energy Dispersive X-ray Spectroscopy
EPA	Environmental Protection Agency
GC	Gas Chromatography
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
g/L	gram/litre
HEPES	N-2-Hydroxyethyl Piperazine-N-2-Ethane or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	High Performance Liquid Chromatography
IC	Ion Chromatogram
ICP	Inductively Coupled Plasma
IPA	Ion Pair Agent
K_f	Formation/Stability Constant
mg/L	milligram/litre
μg/litre	microgram/litre
MS	Mass Spectrometer
NTA	Nitrilotriacetic Acid
PDA	Photo Diode Array Detector
RP	Reverse Phase
SEM	Scanning Electron Microscope
S/N	Signal to Noise Ratio
SRM	Solid Particulate Matter
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
UV	Ultraviolet

Dedicated to

Abba, Amma, Lamisa
&
Almighty Allah

CHAPTER ONE

1. INTRODUCTION

1.1. Outline of Environmental Pollution

The expression "heavy metals" in the environmental community stands for stable high-density metals (e.g., lead, cadmium, mercury, copper, nickel, etc.) and some metalloids (e.g. arsenic). Within the Earth's crust, these elements are natural constituents. Anthropogenic activities have increased the abundance heavy metals in the air, water, soil and tissues of living organisms. This constitutes a great problem as the heavy metals and their compounds possess pronounced toxic properties[1].

Toxicity of heavy metals is often increased by the formation of metal complexes with organic compounds (or ligands). The most common groups involved in complex formation include atoms such as oxygen, sulfur, and nitrogen. Metals bound to these groups can deactivate important enzyme systems or affect protein structure. As a result, these modified biological molecules lose their ability to function properly, and result in malfunction or death of the affected cells[2].

The most important heavy metals from the point of view of water pollution are Zn, Cu, Pb, Cd, Hg, Ni and Cr. Some of these metals (e.g. Cu, Ni, Cr and Zn) are essential to living organisms, but become toxic at higher concentrations. Others, such as Pb, Hg and Cd have no known biological function but are toxic elements[3]. Metals have many sources from which they can enter the water body, these sources are: natural sources, industrial sources, domestic wastewater, agricultural sources, mine runoff and

solid waste disposal areas and atmospheric deposition such as Solid Particulate Matter

(SRM), which can be deposited in a water reservoir.

From an analytical point of view, metal speciation (i.e. the chemical nature of the

metal compound) plays an increasing role in scientific research for furthering our

understanding of environmental processes and the effects metal species have on the

ecosystem and human health. Some of the possible interactions between this research

area and everyday life are shown in Figure 1.1.
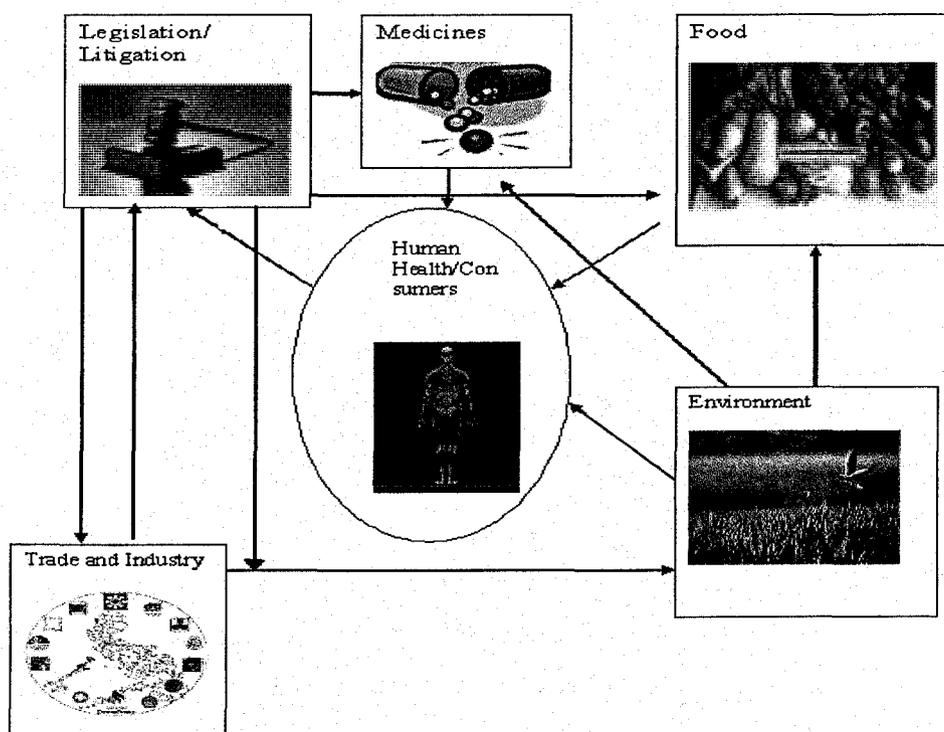
Figure 1.1. Diagram showing the relationships between some of the areas for which
the concept of organometallic speciation is important[4].

Although the diagram is a gross simplification, it attempts to highlight some of

the areas that are or will be affected by our knowledge about organometallic species.

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Metal speciation in the environment, of either anthropogenic or natural origin, has an impact on the environment itself, the food commodities that humans exploit and other products such as medicines, which are derived from natural resources. Usually these three areas are subject to legislation, which is aimed at protecting human health and the environment in general. Such legislation often has a direct impact on trade and industry, which in turn has a knock-on effect on the environment[4].

1.2. Heavy Metal Distribution

Heavy metals are ubiquitous in the environment and are introduced to riverine sediment naturally through the weathering of rocks, as well as from a variety of human activities. Sediment contamination by heavy metals in rivers and estuaries has become an issue of increasing environmental concern. Such contamination is often caused by human activities, including mining, smelting, electroplating and other industrial processes that have metal residues in their wastes and by non-point source surface runoff. In the past years, tremendous efforts have been made to characterize the fate, loading and distribution of heavy metals in rivers and estuaries[5,6,7,8,9,10,11,12,13,14,15].

The evaluation of the distribution, retention and release of heavy metals in soils is necessary for the application of the most appropriate technique and strategy for soil remediation. It is widely accepted that determining total content of heavy metals is insufficient to assess the environmental impact of contaminated soils[3,16].

The average concentrations of total metals in soils and coastal sediments in comparison with several reference values can be tabulated to show the ultimate degree of

contamination of the environment. Review of the extensive body[3,6,8,9,10] of work on the topic shows that in comparison with the Earth's crust[17], the high levels of copper in the studied soils and sediments are of major environmental concern. Copper is also related to the widespread mineralization of chalcopyrite ($CuFeS_2$) in the volcanic rocks of the area[18], and not necessarily related to anthropogenic contamination. Chromium (Cr) and iron (Fe) concentrations are found higher in sediments of Collins Harbour, KGI[19], though the different extraction techniques. In relation to local rocks[20,21] concentrations of most elements are similar, with the exception of Sr, Mg, Ca, and Ba, which are higher in rocks probably due to the dissolution processes operating in sub-aqueous sediments. The heavy metal contents indicate the influence of the geochemical weathering of terrigenous sources on Admiralty Bay rocks[17].

These studies have improved our understanding of heavy metal sediment contamination in river and estuary ecosystems. In the past, the river sediments were thought to protect the water column by removing pollutants. Now, this storage of heavy metals in sediment is viewed as harmful because of the resulting long-term exposure to the biota residing within or in close proximity to this metal-contaminated habitat. For example, several studies have been conducted to investigate heavy metal contamination in rivers and estuaries of Florida during the last decade. Schropp and Windom[13] evaluated the concentration of metals of concern to that of Al in sediments of coastal estuaries in Florida. These authors found that by using the metal to Al ratios, they could differentiate natural background sediment metal concentrations from anthropogenic ones. Their results showed that the concentration of Pb, Hg, Cu, Cd and Zn was high in Florida harbours. The historical profiles of metal enrichment in two Florida estuaries (i.e., the Lower St

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Jones River, LSJR and Hillsborough Bay) were examined by Alexander et al.[22] using core samples that represented approximately 50 years of sediment and metal accumulation. These historical profiles demonstrated that Cd, Pb and Zn in the sediments were enriched in these estuaries. In Hillsborough Bay, enrichment factors for some metals were relatively high and showed little change down core. Chromium (Cr), copper (Cu) and nickel (Ni) bordered on enrichment and lead (Pb), cadmium (Cd) and zinc (Zn) were enriched. This enrichment factor was calculated by metal:Al (Aluminium) ratios according to Schroop, et al.[23], where a normal probability plot correlation coefficient test was used to determine normally distributed elements.

1.2.1. Copper and Its Sources

Copper is required for the normal functioning of plants, animals and most microorganisms. It is incorporated into a variety of organics which perform specific metabolic functions. Because it is an essential metal, daily dietary requirements have been recommended by a number of agencies.

The chemical nature of copper is very important in determining its biological availability, both in the environment and in food. Although evidence of this continues to accumulate, the impact of excess copper is still far too frequently inferred from levels of "total copper" or even the "presence" of copper. Depression and schizophrenia also have links to high copper levels[24].

Copper is essential in the human diet. It helps in the process of making hemoglobin in the blood from iron-rich foods. In fact, it is essential for the normal

healthy growth and reproduction of all higher plants and animals. Copper is also involved in the formation of collagen (the fibrous protein in bone, cartilage, tendons, and other connective tissue) and protective coverings for nerves.

The American Medical Association has recommended 1.2 – 1.3 mg/day as the dietary requirements for copper[25]. Whereas the U.S. National Academy of Sciences' Food and Nutrition Board has issued a Recommended Daily Allowance (RDA) of 0.9 mg of copper per day for both men and women[24]. It is an especially important nutrient for expectant mothers, developing fetuses and newborns. The U.S. Department of Agriculture's Nutrition Center estimates that less than half of the U.S. population consumes the MDR for copper.

1.2.2. Lead and Its Sources

Depending on one's location on the planet, the food and water supply as well as the air we breathe expose us to lead. Areas of particular risk are places where the drinking water is obtained from geologic strata with significant lead content. Areas which have deposits of gold, zinc, and other economically useful metals also commonly have lead as an ore contaminant. As well, the "tailings" of the mining and purification of the ore often have a very high lead content. Before the systematic reduction of lead content in regular gasoline, the lead in the atmosphere in high automobile traffic areas was hazardous especially to small children. In old houses in which lead based paints were used there is a risk of toddlers consuming flaking paint chips with high lead content. This was a particular problem in the ghettos of large cities in the US until the ecologic consciousness was raised in the 60's and 70's. There are probably many old houses in

North America including Newfoundland with lead paint in their interiors, and there are known to be many communities with significant lead, zinc, and manganese concentrations in the ground water. In most individuals there is a "lead balance", i.e. the rate of intake matches the rate of excretion, and the tissue levels are below the concentrations which result in pathological changes. However an increase in the rate of intake will result in accumulation or a "positive lead balance". Lead and cadmium are known to bioaccumulate in human bone with half-lives of about 10 and 30 years, respectively[26].

1.2.2.1. Acute and Chronic Toxicity of Lead

Lead has no known biological role in the body. The toxicity comes from its ability to mimic other biologically important metals, the most notable of which are calcium, iron and zinc. Lead is able to bind to and interact with the same proteins and molecules as these metals, but after displacement, those molecules function differently and fail to carry out the same reactions, such as in producing enzymes necessary for certain biological processes. The biosynthesis of heme in general is deranged by the presence of lead. All actively dividing cells are especially susceptible; hence acute intoxication has major potential for gastrointestinal (GI) and renal mucosal damage. In addition there is a high risk of neurological damage. Acute lead poisoning is often treated with intravenous ethylenediaminetetraacetic acid (EDTA) because lead forms a very stable complex with EDTA ($K_f = 1.1 \times 10^{18}$). The lead-EDTA complex is then eliminated by the body through urine.

With a gradual build-up of a positive lead balance there is no sudden onset of symptoms as seen with acute poisoning. The initial symptoms include clumsiness, ataxia, vertigo, irritability and insomnia. In affected children, they are often considered "slow"; the real basis for the difficulty is not recognized sometimes. As the lead levels rise, hyper-excitability is seen. Confusion, delirium and convulsions may occur in some cases, while in others there is progressive lethargy leading to a comatose state[27].

1.2.3. Complex Equilibria Involving Metals

Practically every metal forms complexes of some kind in the presence of complexing ligands. Some metals form more numerous and more stable complexes than others, but in natural aquatic systems, the possibility of complex formation must always be considered. Metals ions in aqueous solution possess solvent molecules in their primary salvation shell. Attraction between a metal ion and water molecules is weak usually and the number of solvent molecules immediately surrounding each metal ion is variable. However, in the transition-metal ions and higher-valent metal ions, definite complexes such as $Cu(H_2O)_4^{2+}$ and $Al(H_2O)_6^{3+}$ exist in aqueous solutions. For this reason complex formation in aqueous solutions is often a ligand-exchange process in which solvent molecules in the coordination sheath surrounding a metal ion are replaced stepwise by other ligands.

Every anion is a potential electron donor and therefore can be considered as potential ligand. The stability of the complexes formed is related to the effective charge

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of the metal ion, the availability of atomic orbitals suitable for the formation of covalent bonds, and the electronegativity of the bonding atoms in the ligand group.

At least two sets of equilibria are involved in the process of complex formation. There is competition between solvent molecules and the ligand for the metal ion, and simultaneous competition between protons and the metal ion for the ligand. Reaction rates must also be considered.

1.2.4. EDTA as a Complexing Agent and Purpose of this Study

The synthetic complexing agent ethylenediaminetetraacetic acid (EDTA) is widely used in industrial, pharmaceutical, and agricultural applications. Due to its low biodegradability[28,29], it is present in sewage effluents[30,31], fresh water[32,33,34] and groundwater[35]. EDTA forms very stable complexes with heavy metals and has therefore often been suspected to remobilize adsorbed or precipitated heavy metals from river sediments or aquifers, which can be associated with the release of heavy metals in nature and their uptake by plants. Degradation of chelating agents is controversial, different studies having come to different conclusions[36,37], but it is known that EDTA is not likely to biodegrade easily[38].

In surface waters EDTA has been reported to be mainly associated with Fe(III) and Zn(II), but also with Ca, Mg, Mn, Cu and Pb to a minor extent[39]. The sensitive determination of these complexing agents requires the subsequent separation of these different metal complexes.

In this research we constrained ourselves to the determination of EDTA complexes of Cu^{2+} and Pb^{2+}. Because of its versatility we employed ion-interaction chromatography on C_{18} bonded silica with Cetrimide salts as ion-interaction reagents. This will be discussed further in Section 1.7.2. "Selection and Evaluation of Separation Methods".

1.2.4.1. Isocratic Characterisation of Free Chelators

The selected chelator EDTA is a synthetic agent, and first synthesized in an effort to create a substitute for citric acid by F. Munz in Germany in 1934-1935. In 1930 F. Bernsworth in 1930 developed a process for synthesizing EDTA and patented process in 1941[40].

The molecule is a substituted diamine (Figure 1.2) usually marketed as its sodium salt and it represents diverse aspects of the complexation chemistry as well as an environmental concern. It is a powerful complexing agent of metals and a highly stable molecule, offering considerable versatility in industrial and household uses (Table 1.1). The formal anionic charge of the species varies between -1 and -6. The metal complexes represent a broad range of equilibrium stability constants[41] and they play an important role in environmental metal speciation[42,43,44]. The selected metals react at different rates[45], for example Ni is representative of a slow, and Pb of a fast, reacting metal.

HOOC — CH$_2$ CH$_2$ —COOH
      N — CH$_2$ — CH$_2$ — N
HOOC — CH$_2$ CH$_2$ — COOH
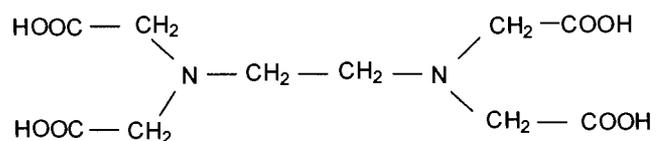
Figure 1.2. Structure of Ethylenediaminetetraacetic acid (EDTA).

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1.2.4.2. Correlation of EDTA with Waste Waters and Surface Waters

Following discussions on the effect of detergent phosphates on eutrophication of the aquatic environment, bans or limitations of detergent phosphate contents have been enforced in various industrialized countries. As a consequence, alternative detergent builders have been introduced or considered phosphate substitutes have been considered, namely citrate, aminopolycarboxylic acids, organic phosphonates, zeolites, or polycarboxylates [46,47]. All these compounds are ionic hydrophilic substances that serve the purpose of removing alkaline earth cations (water hardness) by complexation or ion exchange. Because of their hydrophilic nature, they are not expected to be adsorbed on sludges or sediments (with the exception of zeolites, which are minerals themselves): they are expected to stay in solution and to enter the aquatic environment if not biodegraded. In receiving waters they may be detrimental by remobilizing heavy metals from suspended particles or sediments owing to their complexing power[48,49].

Table 1.1.   Industrial and household uses of EDTA and its ligands (as percentages of the world market)[50]

Use	% of world market
Industrial Detergents	32
Household Detergents	16
Photo Industry	17
Paper products	7
Textiles	6
Agro Chemicals	5
Water Treatment	1
Other	16

Between 1950 and 1960, synthetic surface-active substances such as EDTA replaced "natural" soap products in many application areas in household and industry. These new surfactants offered extreme technical benefits but they had substantial disadvantages for water management. Because they showed only a very poor biodegradation profile, surfactants were soon found in increasing concentrations in sewage waters and rivers, but also in ground water for drinking water supplies as a result of soil infiltration. This occasionally led to extreme impairment of water quality management.

1.2.4.3. Protonation Constants of EDTA

EDTA is a polyprotic, multifunctional acid with several dissociation constants. In addition to the four carboxylic acid groups, deprotonation of the two amino groups must also be considered. Figure 1.3 shows the distribution of the different EDTA species and their dissociation constants pK_a[51].

From Figure 1.3, it can be deduced that at pH values commonly found in natural aquatic systems H_2EDTA^{2-} (H_2Y^{2-}) and $HEDTA^{3-}$ (HY^{3-}) are expected. For the least soluble form of EDTA, the range between pH 1.6 and 2.0 (H_4EDTA or H_4Y) is important.
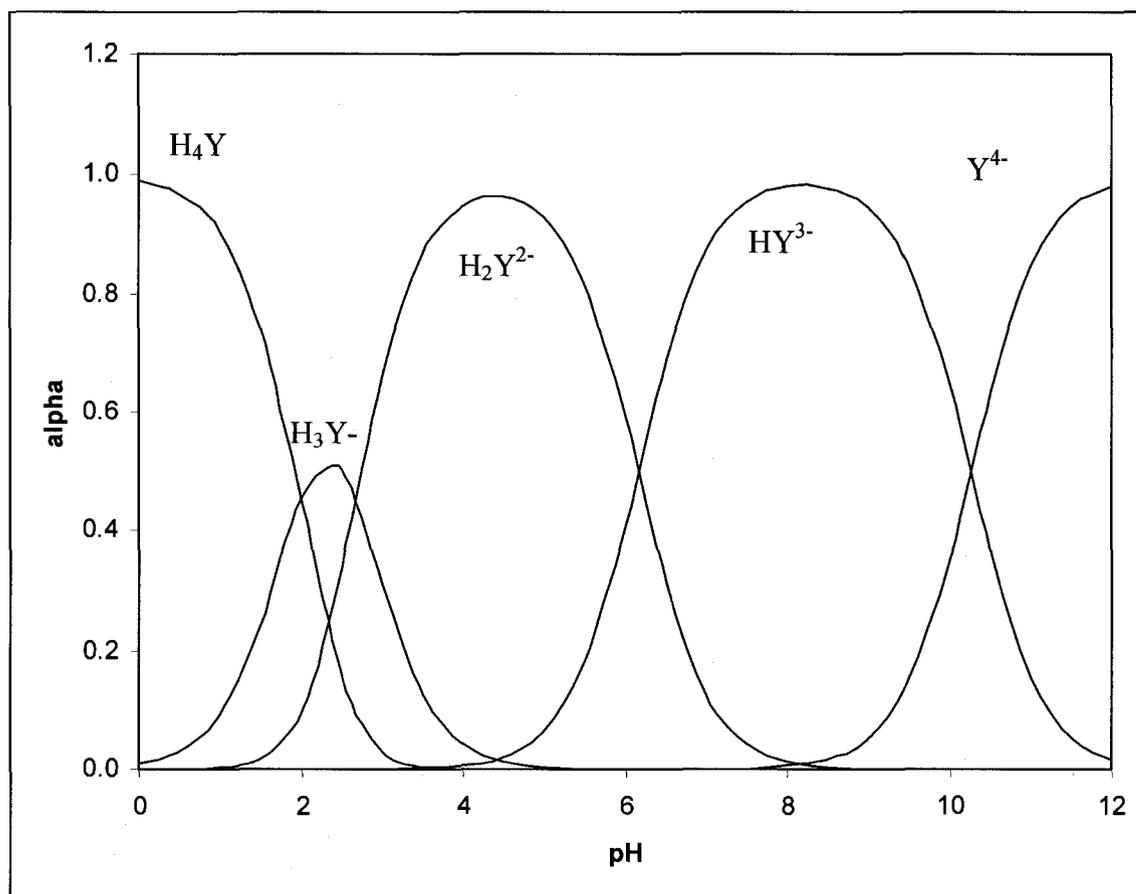
Figure 1.3.   Composition of EDTA (Ethylenediaminetetracetic acid) solutions as a function of pH.

1.3. THE PROBLEMS AND ITS SETTING

1.3.1. What is Sediment?

Sediments are unconsolidated particulate material that have been transported by fluid flow, wind, glaciers and gravity, and deposited as a layer of solid particles on the bed or bottom of a body of water or other fluids at or near the surface of the Earth. According to their size they are classified as gravel (>2.0 mm), sand (0.0625 to 2.0 mm), silt (0.0039 to 0.0625 mm) and clay (<0.0039 mm) using the Udden-Wentworth grainsize scale[52]. The sedimentation cycle originates primarily from natural processes, forestry, agriculture and other human disturbances. The impact of sediments on the ecosystem and urban life include: fisheries/ aquatic habitat, water supply, navigation, and energy production. They also act as a sink for pollutants. The major sediment contaminants are various nutrients (Phosphorous and Nitrogen), bulk organics including oil and grease, halogenated hydrocarbons or persistent organics (e.g., DDT), polycyclic aromatic hydrocarbons (PAHs) and metals, such as iron, manganese, lead, etc[53]. The origins of sediment contamination can be divided into point and non-point sources of pollution. Point source pollution comes from a specific, identifiable source such as a pipe. Point sources include municipal sewage treatment, overflows from combined sanitary and storm sewers, storm water discharges from municipal and industrial facilities and waste discharges from industry. Non-point sources can not be traced to a specific location. Non-point sources include storm water runoff from hazardous and solid-waste sites, runoff from croplands, livestock pens, mining and manufacturing operations, and storage sites. Atmospheric deposition is another source of non-point source of pollution[53].

1.3.2. Why is Contaminated Sediment an Important Issue?

In response to the need for national guidance on addressing contaminated sediments, the United States EPA (USEPA) released its Contaminated Sediment Management Strategy in 1998[54]. This document establishes four goals to manage the problem of contaminated sediment and it describes actions the USEPA intends to take to accomplish those goals. The goals are: (1) prevent the volume of contaminated sediment from increasing; (2) reduce the volume of existing contaminated sediments; (3) ensure that sediment dredging and dredged material disposal are managed in an environmentally sound manner; and (4) develop scientifically sound sediment management tools for use in pollution prevention, source control, remediation, and dredged material management[55].

Contaminated sediments may be directly toxic to aquatic life or can be a source of contaminants for bioaccumulation in the food chain. A wide range of physical, chemical, and biological factors have the potential to influence the bioavailability of sediment contaminants. The bioavailability of contaminants in sediment is a function of the type of chemical and the chemical speciation, as well as the behaviour and physiology of the organism. The two basic routes of exposure for organisms are (1) transport of dissolved contaminants in pore water across biological membranes and (2) ingestion of contaminated food or sediment particles with subsequent transport across the gut. For upper-trophic-level species, ingestion of contaminated prey is the predominant route of exposure, especially to hydrophobic chemicals. Uptake through ingestion of or direct exposure to water or sediment can also be important depending on the trophic level of the organism and the physical-chemical characteristics of the contaminant[56].

1.3.3. How Significant Is the Problem?

Studies conducted on sediment from the Great Lakes area have demonstrated that contaminated sediments are of great concern to humans and wildlife that live in the Great Lakes Basin. Years of industrial and municipal discharges, combined sewer overflows, and urban and agricultural non-point source runoff have contributed to the creation of vast amounts of highly polluted sediments that pose serious human and ecological health risks[57]. Sediments have been accumulating on the bottoms of the Great Lakes since they were formed by glacial scouring and melting. Even after cleanup efforts began in the late 1960s, little attention was paid to the toxic chemicals that accumulated in the bottom sediments. The first priority was to stop the discharge of new contaminants into waterways, and little concern was paid to pre-existing sediments[57]. It was not until the early 1980s that environmental problems caused by sediment contamination began to generate interest in this issue in the Great Lakes. The USEPA's Great Lakes National Program Office (GLNPO) has reported that polluted sediment is the largest major source of contaminants in Great Lakes rivers and harbours entering the food chain, including the current 42 Areas of Concern (AOC) designated by the United States and Canada, the parties to the Great Lakes Water Quality Agreement. Over the past several years, Great Lakes stakeholders have moved forward in the pursuit of sediment remediation. In the years 1997-2002, almost 2.3 million cubic yards of contaminated sediment has been removed from the U.S. Great Lakes Basin[57].

1.4. Why Should We Study Chemical Speciation

Why should speciation analyses be performed? The simple answer is that it is part of an overall approach to understand the complex chemistry and behaviour of contaminants in environmental and biological systems. However, there are other more practical reasons. One is that improved knowledge and understanding of the chemistry of metals and their interaction with environmental system chemistries would enable fundamental progress in a variety of environmental, geological, agricultural, and economic areas[58]. In the environmental field, an ultimate goal might be to use speciation analyses to accurately determine the human health or ecological risks posed by the metal species discovered and quantified at a site and redirect this understanding into the design, selection, optimization, and monitoring of remediation strategies applied to clean up the site, if necessary. Today, with the advent of in situ, submolecular research tools to probe the local environment of metals, that ultimate goal may be within our grasp.

1.4.1. Definitions of Speciation

The definition given by the International Union for Pure and Applied Chemistry (IUPAC)[59] for the terms "chemical species" and 'speciation analysis' are as follows:

Chemical species: specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.

Speciation analysis: analytical activities of identifying and/or measuring the

quantities of one or more individual chemical species in a

sample.

A major objective of metal speciation measurements is that they provide

quantitative information on metal bioavailability. Bioavailability as a concept has been

variously described, but is commonly assumed to mean the ability to be taken up by and

presumably cross a biological membrane. The recent US National Research Council

monograph on contaminant bioavailability recommends use of the term "bioavailability

processes" in discussing routes of metal uptake[60]. The term emphasizes the complexity of

this phenomenon.

The currently accepted model of metal bioavailability processes is illustrated in

Fig. 1.4. The extremes of this model are (a) where the rate of metal internalization across
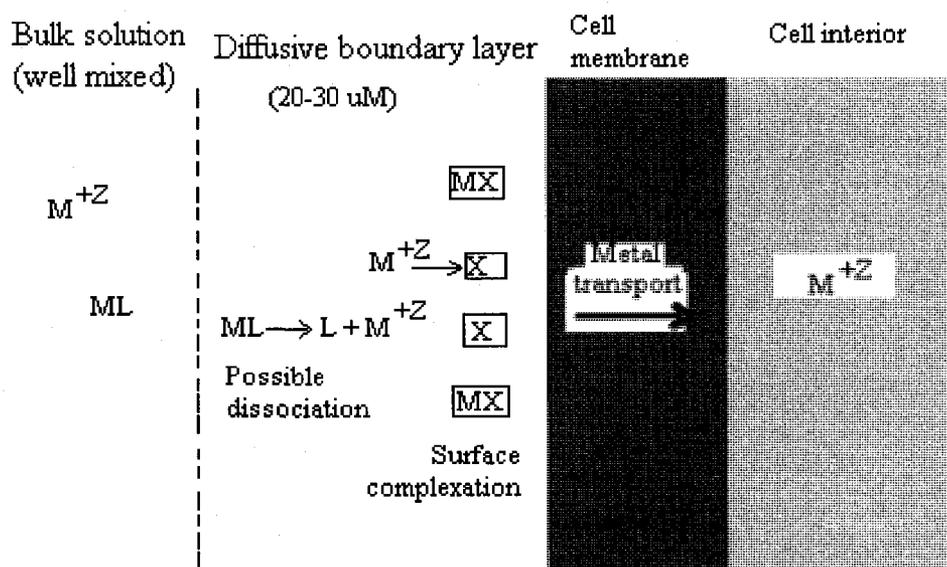
Figure 1.4.   Models of metal uptake by a biological cell.

a cell surface is relatively slow compared to the rate of diffusion to the cell surface, and the metal rapidly comes into pseudo-equilibrium with the metal in the bulk solution (metal bioavailability under thermodynamic control); and (b) where the rate of internalization is rapid compared to the rate of diffusion of simple metal species to the cell surface. Under these conditions there is a concentration gradient around the cell surface, and metal complexes may dissociate to counteract the local perturbation to equilibrium[61]. In this situation, metal bioavailability is controlled by kinetic factors. The rate of internalization of metals is therefore an important factor that distinguishes which of these models is a more appropriate descriptor, and attempts to determine this are the focus of current research activities in several laboratories.

1.4.2. Factors that Affect Metal Speciation and Toxicity

An aquatic organism is exposed to metals in both food (particulates) and solution (dissolved and potentially on colloids). Within each phase, the organism is exposed to a variety of physicochemical forms of each metal, and each form differs in its accessibility to the organism[62]. In general, it is thought that it is metals in the dissolved fraction, particularly the free metal ion, that are the most bioavailable species to aquatic organisms. There is much qualitative evidence to support this notion, especially in defined, synthetic media[63]. Short term (48 h) acute toxicity tests with the animal *Hyalella azteca* conducted in synthetic water demonstrated that the aqueous Ni concentrations required for lethality were greater than what could be significantly complexed by

environmentally relevant concentrations of dissolved organic carbon (DOC: 0.6–30.4 mg/L)[64].

However, the above-mentioned relationship appears to break down in natural waters, particularly in the presence of natural dissolved organic matter[63]. Metal uptake by aquatic organisms is controlled by environmental conditions at the biological membrane. Processes that control metal uptake include: (1) characteristics of the membrane itself, and (2) reactivity of each metal form with the biological membrane. Presence of other metals or major cations may antagonize or stimulate metal uptake factors, such as temperature, which affects uptake rates depending upon the ecological characteristics of the organism. Some of these tools may be of immediate use in monitoring metals in Canadian freshwater ecosystems, while others show promise with further development.

1.4.3. Influence of the Receiving Environment and Metal Speciation

The biological effect of dissolved metals involves metal speciation in effluents, and the potential transformations that may occur in moving from the chemical environment of effluent to the chemical environment of receiving water. For example, experimental studies have shown that Al toxicity in dynamic systems cannot be derived from the total concentration of the metal only[65].

Witters et al.[65] found that brown trout (*Salmo trutta* L.) experienced acute mortality (98% in 48 h) in neutral water mixed with acidic, Al-rich water, even though this mixture contained low Al concentrations (185 µg/L) and had a pH of 6.4. In contrast, mortality was lower when fish were exposed to higher Al levels (445 µg/L) and pH was lower (4.6). Thus, it is evident from this study that Al toxicity to fish is indirectly related

to the total Al concentration in the water. Based on these results, changes in chemical species and toxicity can occur when waters of different physicochemical quality (pH, temperature, hardness, alkalinity, DOC, suspended solids) meet at the confluence of rivers or when wastewater is discharged into receiving waters.

The exchange of metal ions between natural waters and sediments is an important process for maintaining environmental quality and for many technological processes, such as, the safe storage of radioactive wastes[66]. It is a very complex process and its general study presents a very demanding problem. The trace metal mobility, abundance and distribution in water and sediment depend on a number of physicochemical and environmental conditions. They include, for example, changes in adsorption-desorption equilibria due to pH changes, salinity, turbidity, the nature and amount of organic matter present, the formation of new particles, such as iron and manganese hydroxide, oxidation of organic particles containing trace metals or oxidation of metal sulfides, chlorinity, temperature, particle size and sedimentation rate[67,68,69,70,71,72,73,74,75,76,77,78] but the results of laboratory and field measurements have often been considerably different. Great attention has also been paid to the kinetics and mechanism of sorption of metal ions[67,74,79,80,81].

A number of attempts have been made to predict distribution constant values from theoretical and semi-empirical models[82,83,84,85,86]. These models always provide a specialized view that only holds for a certain system. It is desirable that the models be gradually unified and generalized. This is, of course, very difficult in view of the immense variability of natural systems and thus the solution can only be approached gradually.

1.5. Models to Predict Metal Speciation

Currently, there are a number of models that are used to predict metal speciation in freshwater environments. Some of these models, such as metal translators, are very simple empirical approaches that convert a total recoverable metal concentration to a dissolved fraction (i.e., water passed through a 0.45 μm filter). This approach is based on the assumption that the dissolved metal more closely approximates the bioavailable fraction of metal in the water column. The U.S. Environmental Protection Agency (U.S. EPA) has adopted these metal translators on a site-specific basis. An obvious implication of using dissolved metals in regulatory permits is that it would allow dischargers to monitor only this fraction as opposed to both total and dissolved. This would substantially lower analytical costs. Other potential monitoring tools include chemical equilibrium models, and an extension of these models, the fish gill model[87]. This project approaches to understanding chemical speciation of metals in the receiving environment for kinetic model of study.

1.5.1. Kinetic Model

Both metal speciation and biological availability (or toxicity) are functions of the tendency of the metal to react, as quantified by the free metal ion activity, under pseudo-equilibrium conditions[88,89,90]. For metals occurring as organic complexes, pseudo-equilibrium conditions among dissolved species may be maintained only if the rates of metal complexation reactions are fast compared with rates of metal uptake. If, however,

complex dissociation and ligand-exchange rates are slow compared to biological uptake, the rate of metal incorporation into the biota will be limited by abiotic chemical kinetics[90].

The apparent rate of other processes, such as precipitation, reduction, or adsorption, will be similarly influenced by the rates of metal complexation reactions if (i) the reacting metal occurs predominantly as an organic complex and (ii) the process of interest requires removal of the metal from the initial complex. Studies of the kinetics of ligand-exchange reactions of transition metals[91] have shown the reactions to proceed by direct attack of the incoming ligand (L') on the initial metal complex (ML) usually with rate-determining dissociation of the intermediate ternary complex (LML') thus releasing the initially bound ligand (L) and a new metal complex (ML'):

$$ML + L' \xrightleftharpoons{Fast} LML' \xrightarrow{Slow} L + ML'$$

The overall rate constants are influenced by steric and electrostatic factors and by protonation of the incoming ligand.

In contrast, Shuman and co-workers[92,93,94] have observed that ligand-exchange reactions of Cu-DOC complexes involve dissociation of the initial copper complexes and have reported rate constants for the dissociation of these complexes. Other studies of reactions of humate- or fulvate-metal complexes or of reactions of metals with humic or fulvic acids have focused on different metals-Fe[95,96,97], Al[98,99,100], Ni[101], and Th[102,103]. Two mechanistic pathways exist for the overall ligand-exchange reaction: an adjunctive pathway, which proceeds by direct attack of the incoming ligand on the initial complex, and a disjunctive pathway, which proceeds by dissociation of the initial complex. The overall (forward) reaction proposed by Shuman et al.[92] is as follows

$$CuL + D \rightarrow L + CuD$$
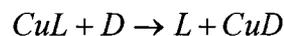
where the initial copper-ligand complex, CuL, undergoes dissociation in the presence of ligand D via a disjunctive pathways to form a new copper-ligand complex CuD.

1.6. OBJECTIVES

It is difficult to predict how analytical results will influence costs of aquatic effects programs; however, it is important to understand mechanisms of metal toxicity in natural waters. With this knowledge, it may be possible to develop treatment strategies that will minimize the effects of mine effluent on the receiving environment, which in the long run may decrease costs. Moreover, such studies would provide some insight into whether the total or dissolved fraction best explains toxicity on a site-specific basis and thus could be incorporated into a monitoring program.

The present work attempts to contribute to this task and is based on the following premises: (a) the basic interactions underlying the exchange of simple inorganic ions between water and a solid phase are adsorption, ion exchange and chemical complexation occurring both in the liquid and solid phase. Therefore, the initial experiments should be carried out with solid materials that can be found in natural sediments, are reasonably well defined and exhibit one predominating interaction. The composition of the aqueous phase should resemble that of natural water, but should be sufficiently simple for the description of the complexation equilibria. On the assumption of the additivity of these interactions, a more general model can then be formulated by solving the appropriate equilibrium equations for the individual interactions; (b) sufficiently sensitive and

reliable (i.e., good analytical precision) analytical methods must be available for trace determinations in the above system, and must be optimized for the study purpose and their analytical parameters must be verified[104]; (c) It is necessary to ensure that the system is in equilibrium, which not only ensures that the model is close to the real situation, but also guarantees reproducibility of the values measured and calculated. Considering these premises, lead and copper are selected as the model trace elements because they are of environmental interest, often occur in natural waters and sediments and are readily determined by two common analytical methods, ICP-MS and HPLC systems.

In this study, we have developed an analytical method that allows the time-dependent concentration of the test metal ions and metal complexes to be determined under environmentally relevant conditions of pH, temperature, and metal and ligand concentrations. The short term objectives are: (1) to adapt and optimize a chromatographic separation method based on ion-pairing agents for the separation of the test metal ions and metal-EDTA complex; (2) to adapt and optimize the chromatographic separation method in order to study the sorption/desorption kinetics of copper and lead in a EDTA-sediment slurry system under controlled environmental conditions (e.g. pH, temperature, ionic strength, etc.). The long term objective is to include the above experimentally determined parameters (sorption capacities, equilibrium constants, etc.) into an existing hydrology model to increase the level of predictability of Cu^{2+} and Pb^{2+} distribution in a dynamic system. This long term objective is important for future research but outside the scope of this project due to limited time frame.

Development of analytical chemical methodology in this project is described in Chapter Four and the application of this method to some preliminary sorption kinetics experiments are described in Chapter Five.

1.7. METHODOLOGY

1.7.1. Analytical Data Availability

In all analytical methods one is interested in measuring a signal that can be related to the concentration of a particular species in the original sample. An ideal analytical method would enable a species to be determined directly on diverse matrices and with a limited quantity of sample. The determination of trace elements in environmental solid samples and natural non-saline waters is an area of particular interest both for pollution control and drinking water quality monitoring[105]. Analytical data on the individual molecular species of an element present in a sample provides useful information in the study of e.g., its toxicity, essentiality, bioavailability, metabolism or transport[106]. For example, the toxicity of arsenic or mercury in biological systems greatly depends on the inorganic or organic forms present. The assessment of the human health risk associated with the ingestion of such elements via food should therefore be based on analytical data of the individual species.

1.7.2. Selection and Evaluation of Separation Methods

The basis of separation depends upon the existence of a difference in one physical-chemical property between two or more compounds. For two completely miscible substances to be separated, their molecules must differ in at least one physical-chemical property. The two major approaches employ either methods that create a second phase with a different concentration of the desired component or else methods that exploit differences within the single phase. The form of separation should be chosen so that a good separation from other components is assured. As is often the case in analytical chemistry, practice has consistently outstripped theory in many of the fields to be discussed. As a consequence, a certain amount of trial-and-error is inevitable, unless a procedure can be patterned after an analogous procedure found in the literature. Owing to distribution or solubility equilibria, separation is never theoretically complete. For the determination of major constituents, methods of practical value are those in which the separation is complete for both components within the limits of error of the subsequent determination. When interest centers on the purity of a desired component, the separation factor for an undesired constituent with respect to the desired component is of more concern than recovery or yield. On the other hand, the inverse situation pertains when the goal is the enrichment of some component that is present in a particular matrix. Partial separation also may be combined with the use of specific properties to facilitate analysis in cases where neither complete separation nor specificity can be obtained for the entire mixture. Sometimes the interfering elements or functional groups can be made "unreactive" through formation of masked species.

One significant fact about speciation analysis in analytical chemistry is that it could be used for analytical activities to identify and/or measure the quantities of one or more individual chemical species in a sample. This research study concerns trace element analysis of copper with EDTA in a well-defined sediment. For this purpose high performance liquid chromatography (HPLC) is used. The technique is now widely used as a tool for a range of trace elements because of its very low detection limits, good accuracy and precision.

The analysis of organometallic species in a particular sample requires a series of analytical steps. The most important aspects are highlighted as a flowchart in Figure 1.5 and comprise steps a) to e) below:

a) The sample of interest: The characteristics of the sample, such as organic carbon content, fat content, particle size, moisture content etc. have a significant influence on the performance of the analytical method. Therefore, the more that is known about the sample the better the analytical approach can be adapted to overcome potential difficulties caused by the physical, chemical and biological properties of the sample matrix. Depending on the physical characteristics of the sample, some pre-treatment such as fat removal, peeling, drying, grinding or sieving may be required to prepare the sample for the extraction step.

b) Extraction of the analyte(s) of interest from the sample: The extraction methodology is the first and often the most crucial step in the analytical process. In order to obtain accurate results, it is essential that the sample preparation is both quantitative (i.e. all of the analyte is extracted from the matrix) and representative (i.e. the chemical or physical form of the target analyte is not altered during the

extraction process). In addition, it should be reproducible in order to allow comparisons between different samples to be made.
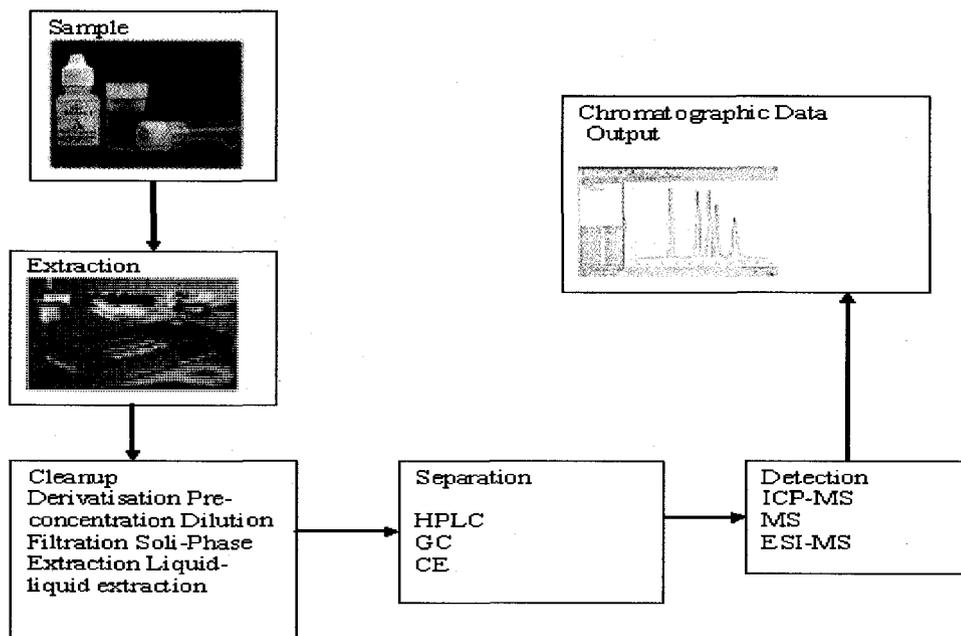
Figure 1.5.   Schematic Flow-chart of the analytical steps involved in organometallic speciation analysis.

c) Clean up/Derivatization/Pre-concentration Steps: At present no extraction methodologies exist that extract the target analyst exclusively while leaving interfering matrix components in the residue. Therefore, a clean-up step is sometimes necessary for interfering components that have been co-extracted and could interfere with the subsequent analytical steps. Derivatization is used to change non-volatile compounds into a volatile form and vice versa and this enables the use of either gas or liquid chromatography with compounds that can be treated in this way. If the device used for detection of the target analyte is not sensitive enough to detect the analyte at

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the normal concentration level in the sample, a pre-concentration step can facilitate the detection. Not all speciation methodologies require these steps (as indicated by the dashed arrows in Figure 1.5), but the performance of some can be significantly improved if additional sample pre-treatment is used.

d) Separation: The separation of the different species is an important step in the analytical procedure because, if successful, it allows the identification and quantification of individual compounds. It is usually achieved by means of chromatography and a variety of different separation mechanisms can be used according to the chemical and physical properties of the analytes. In addition, the separation of the analyte of interest from matrix components that have not been eliminated from the sample at this point by extraction and clean-up steps can be achieved in this way.

e) Detection: The instrument used for the detection of the compounds that have been separated in step d) has a significant impact on the performance of the method as a whole. The choice of instrument has an influence on the achievable detection limit, the analytes that can be minimized by the right detector choice for a given application. Due to the fact that some species can result in lethal or sub-lethal effects on organisms at concentration ranges of part per billion to part per million levels, the detector of choice must provide sufficient sensitivity to detect such species in the sample extracts.

1.7.3. Methods using Quantification Procedures

The ultimate goal in environmental analysis is the quantification of individual compounds, separately from all their isomers and/or homologs. This end cannot be reached by the application of nonspecific methods of analysis: on the contrary, sophisticated separation methods must be employed toward this end. Chromatographic procedures using High Performance Liquid Chromatography (HPLC) can be applied to achieve separations into classes of compounds in nearly all cases.

The main topic of this review is to cover the various analytical approaches for the analysis of aminopolycarboxylic acids, such as ethylenediaminetetraacetic acid (EDTA), which is expected to be present in surface waters in the low μg/Litre range.

1.7.3.1. Aminopolycarboxylic Acids

EDTA is an amphoteric compound containing a tertiary amine base and carboxylic acid residues; because of these properties it is soluble in water at all pH values. It cannot be extracted from water because its solubility in immiscible organic solvents is far less than in water. It has a large complex formation constant with some heavy metals, especially Bi, Fe, and Cu; therefore, the presence of significant amount of these cations interferes with some methods.

Colorimetric, polarographic, and HPLC determination methods have been proposed for EDTA and NTA (Nitrilotriacetic acid) analysis in water samples needing only a small amount of sample preparation apart from metal complex formation. GC

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determination methods have been developed that require more detailed sample preparation (derivatization) procedures. Far more sensitive detectors can be used for GC compared with the other methods; furthermore, specificity is also higher because of the superior separation power obtained on GC.

1.7.3.2. Polarography

In polarography, the different reduction potentials of metal complexes of NTA and EDTA are utilized for quantitative determination[48,107,108,109,110]. Hg^{107} and $Bi^{48,108,109,110}$ were employed as the complexed cation to be reduced. Generally, EDTA can be determined in the presence of NTA, and vice versa. As sample preparation, water samples are acidified to liberate complexed cations from the complexing agent; a cation exchange column then efficiently removes potentially interfering cations. After addition of an excess of Bi and pH adjustment, samples are usually ready for measurement[110]. Because suitable internal standards are not available, standard addition is employed in quantitative analysis[48,110].

The advantage of the polarographic method is that it tolerates fairly large concentrations of salt in the aqueous matrix. The detection limits, however, are higher than required for environmental analysis. Although lower detection limits have been claimed in the literature (to 15 µg/Litre[107]), the German standard procedure limits the method to concentrations higher than 100 µg/Litre[110].

1.7.3.3. HPLC and Ion Chromatography

High Performance Liquid Chromatography (HPLC) is well suited for the rapid and reliable determination of EDTA and metal-EDTA complexes. Relatively little sample pre-treatment is necessary when primary EDTA concentrations are relatively high. Also, a wider range of detection systems (e.g. amperometric detection)[111] and stationary phases can be used. Other authors have separated organic chelators like EDTA by ion chromatography[112] or applied ion pair chromatography to determine several aminopolycarboxylic acids[113].

Currently, a variety of HPLC techniques can be used for the analysis of inorganic species. In fact, the use of ion exchangers as stationary phases in HPLC allows the achievement of multielemental separations with good sensitivity. On the other hand, chemically bonded phases in which polar and nonpolar organic moieties are permanently attached to a support of high surface area are the most commonly employed stationary phases in HPLC[114,115,116,117,118].

Generally, the use of this type of stationary phase gives better efficiencies than those obtained with ion exchangers. They also have the advantage that they can be used for the determination of both cations and anions. Recently, a new type of ion pair chromatography has been developed, whereby a mobile phase containing a hydrophobic counterion (ion-pairing agent) is used with a nonpolar chemically bonded stationary phase. The presence of the counterion increases the retention of the oppositely charged ionic solutes. The application of RP ion pair chromatography to the analysis of inorganic species has been reviewed. In the case of metal ions, the separation and determination of

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these species is achieved through the formation of an ion pair between an ion-pairing agent and the metal ion. This approach is, however, complicated by the fact that it is difficult to form a stable ion pair with a free metal ion and that detection of the metal ion-pair is weak due to the poor molar absorptivity of metal ion-pair species. Therefore, it is more common to form an ion pair with a metal complex, which allows, if the complexing agent is correctly chosen, the detection of the species by UV-vis spectrometry or fluorimetry. The complexes can be obtained in two ways. The first way is by the formation of a complex before the chromatographic system (pre-column complexation) in which case, the complexes must be stable enough to avoid decomposition during separation. The second procedure is based on the "*in situ*" formation of the complexes in the chromatographic column itself. The metal ions are introduced into the chromatographic system by dissolving their salts in water or in the mobile phase which must contain the complexing agent. *In situ* complexation saves analysis time by avoiding the prior complexation steps. However, it also has some drawbacks since the complexing agent must be noncorrosive and compatible with the detection system used.

The possibility of using ethylenediaminetetraacetic acid (EDTA) as a complexing agent with metal ions was investigated in this research. Polycarboxylic agents and their complexes are polyvalent anions. As such they are strongly retained by a polymeric anion exchanger. Review of papers[119,120,121,122,123,124] has showed that C_{18} bonded column worked for the separation of different metal complexes in environmental samples.

EDTA forms complexes of different stability with a great variety of metal ions[125] allowing the possibility of performing multi-elemental separations with this complexing ligand. Since EDTA forms negatively charged complexes with divalent and trivalent

metal ions, the possibility of the simultaneous separation of anions from metal ions is also possible as is the speciation of metal ions.

Among chromatographic methods for hydrophilic polar nonvolatile compounds such as NTA, EDTA, metal-NTA and metal-EDTA complexes, HPLC and ion chromatography (IC) are obvious choices for separation and quantification of these compounds. Detectability, however, is a problem with compounds containing only aliphatic residues. Direct detection methods usually use conductivity[126,127] or electrochemical detection[128,129], whereas indirect detection methods use metal complexation with a colorimetric (UV)[130,131,132,133,134] or atomic absorption measurements of the eluted fractions[135]. Some of the proposed methods have been applied only to commercial formulations[130] or standard solutions, but not to environmental samples. Thus, potential matrix interferences associated with these procedures cannot be assessed.

Water sample pretreatment before HPLC or IC separation is usually fairly simple: filtration of the sample to remove particulates, addition of the metal ions for complexation, and pH adjustment for indirect detection by UV[131,132,133,134]. For conductivity detection following IC of samples from biological matrices, a protein precipitation step was found to be sufficient sample pretreatment[127]. For electrochemical detection, either filtration[128] or filtration followed by removal of non-polar organic compounds on a C_{18} cartridge[129] produced solutions ready for chromatographic determination.

For analysis by indirect (UV) detection, separation of the Fe-EDTA and Fe-NTA complexes (which are anionic in charge) was effected by either chromatography on an anion exchange column[131] or by ion pair separation on C_{18} RP columns using

tetrabutylammonium as the counterion[132,133,134]. Separation for conductimetric detection was performed on a combination of column materials with an ion exclusion column as the step governing resolution. Thermostatting the detector system efficiently was mandatory for sensitive detection[128]. For electrochemical detection, chromatographic separation was effected on a C_{18} RP column or a styrene-divinylbenzene column with trichloroacetic acid or acetic acid as electrolytes/eluents[128] or on an ion chromatography (ion exchange) column using strongly acid (6.0 M HNO_3) conditions[129]. In all cases, NTA and EDTA were clearly separated from one another. Detection limits, were usually higher than 100 µg/Litre for each of these compounds, however, limiting the application of HPLC or IC to samples with a higher content of complexing agents, such as process waters or sewage influents.

1.7.3.3. GC

Capillary GC has an inherently higher resolution for organic compounds than HPLC. Compounds to be analyzed by GC, however, must be volatile under the conditions envisaged for chromatographic separation. For such hydrophilic polar ionic organic compounds as NTA or EDTA, chemical derivatization before GC separation is required to obtain sufficiently volatile derivatives. Consequently, sample preparation for GC analysis is a more complex and tedious procedure than for HPLC.

On the other hand, highly sensitive and specific detection systems like the nitrogen selective detector (NPD) or a mass spectrometer (MS) allow reliable

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quantification of EDTA and NTA at trace levels in the presence of organic matrix components.

The obvious choice for a derivatization method for carboxylic acids like EDTA or NTA is an esterification reaction. All published methods have used this approach, preparing methyl esters[136,137], propyl esters[137,138], trimethylsilyl esters[139], or butyl esters[48, 48,140,141,142,143,144,145]. Because derivatization reactions cannot be expected to have quantitative chemical yields, especially not for aminopolycarboxylic acids, the use of an internal standard is required to correct for non-quantitative yields (as well as for incomplete recovery during sample preparation). Aminopolycarboxylic acids seem to be the internal standard most similar in behavior to EDTA and NTA; compounds used for this purpose are cyclohexandiamine-(1,2)-tetraacetic acid[136], nitrilotripropionic acid (mainly for NTA)[48], and 1,2-diaminopropane-N,N,N',N'tetraacetic acid (DPTA). They are true internal standards because they cover the whole sample preparation procedure.

Two problems remain: in the presence of large concentrations of electrolytes[144], problems arise in the derivatization step. Second, the EDTA tetrabutyl ester, as well as the internal standard DPTA tetrabutyl ester are eluted from the GC column at very high oven temperatures; losses for unknown reasons among these two compounds compared with the control standards have irregularly been noticed.

Limits of detection of the proposed methods[48] are significantly lower than for polarography and HPLC: for the packed column GC/FID analytical method, a method detection limit of 25 µg/litre was reported, whereas for the capillary column GC/NPD combination, method detection limits as low as 0.2 µg/Litre were reported[143].

1.7.4. HPLC with Other Techniques

Hyphenation of HPLC with other techniques[106] offers many possibilities. In particular, the ability to couple an HPLC system with an inductively coupled plasma mass spectrometer (ICP-MS) allows a significant gain in analyte sensitivity, i.e., a lower limit of detection. Furthermore, the HPLC-ICP-MS hyphenation would provide an additional separation dimension, i.e., one that is time-dependent (due to the HPLC) and another dimension that is mass-dependent (due to the ICP-MS), thus minimizing significantly limitations due to co-elution of species from the analytical column.

The selection of the type of HPLC system (e.g. RP or ion exchange) should be based on physico-chemical characteristics such as acid or base properties, solubility or polarity of the analyte species. The following is a list of desirable properties and rationale for use for this work:

- Ion exchange HPLC techniques are well-suited for separation of ionic analyte species.

- Isocratic ion exchange HPLC systems with an aqueous mobile phase that employs organic buffer constituents operated at a pH that ensures that all analytes are partially or fully ionized provide good stability and robustness of the analytical system.

- Gradient elution with organic solvents due to risk of degradation of detector sensitivity and risk of memory effects in the sample introduction system.

• Crude extracts may be analyzed interference-free, but dilution of the sample extracts prior to injection may prevent chromatographic disturbances, e.g. double peaks or tailing.

• Two supplementary chromatographic systems may be of value if many analyte species need separation or if peaks of unknown identity occur in the sample.

• The risk of chromatographic peak broadening or memory problems may be minimized by low dead-volume sample transport and introduction systems.

• The S/N ratio of the ICP-MS detector used for elements of high first ionization energy may be improved by adding a few per cent of methanol to the mobile phase in combination with increased plasma RF power input.

• Spectroscopic interferences (e.g. from chlorine) may be prevented by separation by the same HPLC system or by bulk methods prior to injection (evaporation of chloroform).

• Establishing a mass balance of the analytes in all fractions of the procedure accounts for analyte extraction efficiency and helps evaluating the accuracy.

• Recovery experiments are useful in estimating the accuracy of analyses in liquid samples only.

• Comparison of results for the same analyte/sample combination using independent methods of analysis is useful for estimating accuracy for speciation in extracts of solid samples.

• CRMs with certified species contents are urgently needed for optimum evaluation of accuracy in speciation analysis.

• Verification of the identity of analyte species or elucidation of the identity of unknowns requires independent methods of analysis such as HPLC-ES-MS/MS, especially when standard substances are unavailable.

The technique of choice for this work (described in Chapter 2) was HPLC using C_{18} column and ion pair agents for the separation of metal-EDTA complexes. This was motivated by the availability of the instrument for the work, the ability to analyze real sediment slurry with the analytical system (which would not be possible with GC), and the future possibility of coupling the HPLC system with an ICP-MS to gain better limit of detection.

CHAPTER TWO

2. EXPERIMENTAL SETUP AND PROCEDURE

2.1. Introduction

Liquid chromatography has become one of the main powerful analytical tools for elemental analysis, and some reviews[146,147] with particular regard to ion chromatography[148] and complexation ion chromatography[149,150] summarize its potentialities.

A fundamental requirement for effective assessment of sorption/desorption mechanisms in natural waters is the ability to measure the distribution of complex species between solution and suspended sediment phases. Chemical analysis of the analyte in all free and sorbed species (or states) can give the complete analysis of the distribution of the analyte in the system. It has been usually impossible to monitor free and sorbed species using kinetic experiments. Gamble and collaborators[151,152,153,154,155] and Granados[156] have proposed analytical methods to assess quantitatively free, labile sorbed, and non-labile sorbed species (as free metal ions or as organic compounds). This thesis project proposes a new analytical approach that uses some aspects of the methods proposed by Gamble and Granados, i.e., the on-line micro-extraction cell for the direct analysis of sediment slurry samples.

2.1.1. Analytical Techniques for the Speciation of Metal Complexes

HPLC is a very flexible tool for separating different species from each other and for removing matrix interferences, and may be coupled with different detectors enabling determinations of specific or group of elements. Several devices including electrochemical detection (ED) systems and inductively coupled plasma (ICP) and graphite furnace atomic absorption (GFAA) spectrometers have been used as well as inductively coupled plasma mass spectrometry (ICP-MS) coupled with liquid chromatography[157,158,159].

Most batch methods do not distinguish between labile, presumably surface sorbed, species and bound residues (i.e., non-labile sorbed) which are either chemisorbed or result from intraparticle diffusion[160]. In contrast, a new on-line micro-filtration HPLC, MF-HPLC, technique developed by Gamble and collaborators[161,162] and further adapted by Granados[156] meets the requirements for kinetic speciation studies of complex uptake in heterogeneous systems.

This modified technique is unique among similar published methods in that it can quantitatively determine the sorbate distribution among dissolved, labile surface sorbed, and bound residue, as well as monitor their kinetics of mass transfer among the three states (dissolved, labile sorbed, and bound residue).

Generally, a variety of organic solvents of different polarity and mixtures of acids are used for extraction of organometallic species. The optimum extraction solvent is often specific to individual species, although simultaneous multi-species extractions can be performed for species with similar chemical characteristics.

The technique developed by Gamble and co-workers and further developed by Granados was modified and adapted in this study to use ion-pairing chromatography for the separation and analysis of aqueous Cu-EDTA and Pb-EDTA complexes. The parameters that were optimized included pH of mobile phase, mobile phase composition, and ion-pairing coating concentration. The optimized method was then used for a series of experiments to study the kinetics of sorption of some metal complexes in marine sediments. The experimental methodologies followed by the results of its evaluation are presented in details and data are discussed in the context of kinetic curves construction.

Specific instrumentation used for the different applications described in this work is given below.

2.2. Materials and Reagents

All experiments were done at the Centre for Environmental Analysis and Remediation Laboratory (CEAR), at Saint Mary's University.

All reagent solutions were prepared by dissolving chemicals of the highest available purity (see below) in deionized water purified by a Sybron Barnstead apparatus.

Copper and lead were the test metals (Cu and Pb) and EDTA was the test complexing ligand throughout this work. Standard solutions of copper, lead and EDTA were prepared from copper nitrate (Reagent Grade, Anachemia Chemicals Ltd.), lead nitrate (Baker Analyzed Reagent) and disodium salt of EDTA (Reagent Grade, J.T. Baker). Stock solutions of these reagents 1.000×10^{-3} M were prepared in distilled deionized water and working solutions were prepared by subsequent dilution of stock

solution. The solvents used in this experiments were methanol [Fisher and Caledon], acetonitrile [Caledon], iso-propanol [Fisher] were all HPLC grade. Reagent Grade cetyltrimethylammonium bromide (cetrimide) was obtained from BDH Chemicals. Ferric nitrate [BDH], sodium formate [Fisher], sodium sulphate [Caledon] and N-2-hydroxyethyl-piperazine-N-2-Ethane [HEPES] were obtained from BDH Chemicals.

The mobile phase consisted of 6 mM Na_2SO_4 and 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, adjusted to pH 7 with NH_4OH. The post-column reagent was prepared by adding 40 μM $Fe(NO_3)_3$ to a 200 mM sodium formate–formic acid buffer at pH 4.5.

The sediments used for this work, PACS-2 and HISS-1 were obtained from the Institute for National Measurement Standards (National Research Council of Canada). PACS-2 was collected in the harbour of Esquimalt, B.C., whereas HISS-1 was collected from the Hibernia Shelf, off the coast of Newfoundland[163]. The certificates of analysis are provided in Appendix I.

2.3. Preparation of Glassware

The glassware used in the experiments was first washed with a mild detergent followed by thorough rinsing with tap water. Glassware was then soaked in 10% nitric acid for 48 h to eliminate any trace of residual metals. The glassware was finally washed three times with distilled deionized water.

2.4. pH Meter

The pH measurements of all aqueous samples were performed following standard methods with pH meter (Model Accumet 910, Fisher Scientific). The meter was standardized using buffer solutions with the following pH values: pH 4.0, pH 7.0, and pH 10.0. The pH meter was used to adjust the pH values in the Metal-EDTA complex solutions, mobile phase solutions and to determine the alkalinity in aqueous slurries of sediments particles.

2.5. Scanning Electron Microscopy (SEM)

Scanning Electron Microscope is the best known and most widely used of the surface analytical techniques. SEM, accompanied by X-ray analysis, is considered a relatively rapid, inexpensive, and basically non-destructive approach to surface analysis[164] SEM with energy or wavelength dispersive X-ray detectors can provide qualitative chemical composition of rocks, minerals, and biological samples.

In this study, an environmental (i.e., variable pressure) SEM [SEM-LEO 1450 VP, UK] was used to characterize the surface and provide chemical composition at the percent and sub-percent concentration level of two different sediments, PACS-2 and HISS-1. The SEM is equipped with a Secondary Electron (SE) detector, a Backscattere Electron (BSE) detector, and an Energy Dispersive System (EDS). An accurate determination of sample particle size and shapes can be obtained from the three

dimensional images of these selected samples. The main objective of the SEM observation is to gather information on elemental composition of PACS-2 and HISS-1.

2.5.1. Relative Merits of BSE Images

In BSE images, thin section thickness does not influence image intensity, because BSE are essentially produced by electron impacts that do not penetrate more than a few microns into the sample. Contrast between grains and sedimentary matrix is clear. The possibility of superposing two features of interest with BSE images is greatly reduced because BSE images are produced at the surface of the sample sediment. BSE intensities are only proportional to the mean atomic number of the sample and crystallographic orientation does not affect BSE intensities. Some factors that influence of image intensity can be adjusted, including beam acceleration voltage, focus, tilt angle, working distance, and enlargement. BSE images have better qualities for image processing but sample preparation is time-consuming. When working with BSE for intrinsic characteristic of images it should be remember that there is potential for the introduction of uncertainties. For quantification of variations in the grain-size — along a sediment core for example — image-acquisition conditions must be similar and as closely controlled as possible. Therefore, BSE images are preferable, because observation conditions are easier to control.

2.5.2. Preparation of Samples for SEM

Small amount of sample (<0.01 mg) was placed on an SEM stub by pressing, which contained double sided carbon tape. Then compressed air was sprayed to blow off excess sample powder on the stub. The samples were coated with gold to improve image resolution. Coating was carried out using a SEM sputter coating unit PS3, BIO-RAD Microscience Division.

Ar was used to make an inert atmosphere and to work in the vacuum. The optimum coating time was determined to be 70 sec. The SEM was optimized and calibrated using a copper foil standard. A Backscattered Electron (BSE) detector Type 222 [UK] and an Energy Dispersive System (EDS), model INCA 200, were used to analyze the sediment composition. The SEM limit of detection (LOD) was 0.5%. The BSE detector provided images of sediments and the EDS provided (a) elemental mapping and (b) elemental X-ray spectrum. The optimum SEM working distance for the sediment samples was determined to be 18-20 mm.

2.6. High Performance Liquid Chromatography Systems

A diagram of a typical HPLC system[165] is shown in Figure 2.1. HPLC is generally applied to compounds that are nonvolatile and can therefore not be analyzed by gas chromatography.
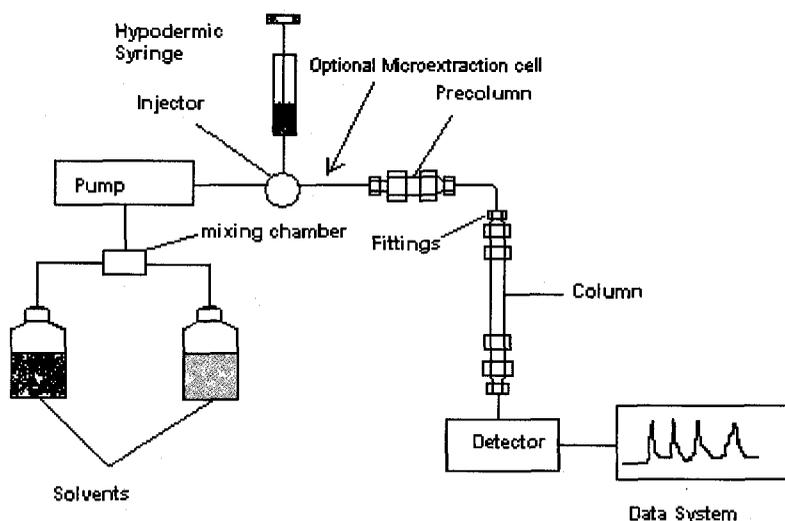
Figure 2.1. System diagram for liquid chromatography.

Figure 2.2 shows the HPLC system used for this work. It consisted of a Varian Prostar 230 solvent delivery system with a Varian 330 PDA detector for the isocratic run. A Varian Microsorb MV C_{18} column (reversed phase column, 250 × 4.6 mm I.D., 5 μM particle size) was used. The injection system was Rheodyne Model 7125 and the syringe used was a 1.0 mL plastic. The volume of the injection loop was 10 μL and 100 μL and the effluent was monitored at 258 nm. A typical LC system is presented in Figure 2.1.

A column inlet microfilter (extraction cell) set with a 0.5 μm stainless steel frit [Rheodyne 7335, Altech] and a two-position six port switching valve [Rheodyne 7000, Altech] were used for the extraction of sediment slurry from aqueous solutions.

Figure 2.2. Photograph of a HPLC System used for this work.

The UV–vis spectra of metal-EDTA complexes (Cu-EDTA and Pb-EDTA) were measured using a PDA detector and chromatograms were processed using the Varian Chemstation software. The Photo Diode Array detector is equipped with a standard flow cell of 10.0 mm path length and 17.0 μL cell volume. The cell has 9.0 bar pressure limit and can be operated up to a maximum flow rate of 10.0 mL/min. Chromatograms of Cu-EDTA and Pb-EDTA complexes were recorded at a wavelength 258 nm. The wavelength of 258 nm is one of the few wavelengths at which metal-EDTA complexes will absorb. It also allowed direct comparison of our results with the one reported by Bedsworth et al[166].

2.7. Column Coating

Prior to any analysis, the column was coated with cetrimide by pumping a 1 mM Cetrimide, methanol–water (30:70) solution through the column at 0.2 mL/min for 18 h. The column coating optimization is described in section 4. The column was then washed with water for 30–60 min before switching to the mobile phase. Analytical conditions are summarized in Table 2.1. To remove the cetrimide from the column after analysis, an acetonitrile–water gradient program was run from 50 to 95% acetonitrile at a constant rate over 3 h[166].

Table 2.1   Optimum Experimental conditions of HPLC for the analysis of Cu-EDTA and Pb-EDTA Samples.

Condition	Description/Value
Columns	Analytical Column: Varian Microsorb MV C_{18} 25 cm × 4.6 mm ID, 5 μM particle size, Guard Column: Opti-Guard C_{18}, 1.0 mm I.D. (USA)
Column Pretreatment	Coating for 18 h with 1 mM Cetrimide at 0.2 mL/min flow rate
Mobile Phase	10:90 v/v Methanol: 6 mM Na_2SO_4 with 1mM HEPES, pH 7.0 adjusted by NH_4OH
Flow Rate	1.0 mL/min
Sample Loop	10 μL and 100 μL
Other Parameters	All stainless steel tubing and needle assembly replaced with PEEK (polyether ether ketone)
Temperature	Room temperature
Detection	Photo Diode Array (PDA) at 258 nm wavelength

2.8. Mobile Phase Selection

Organic solvents or aqueous buffer solutions are used as the mobile phase and delivery to the analytical column is achieved by means of either a single pump for isocratic runs or dual or quaternary pumps where the composition of the mobile phase can be changed throughout the run resulting in a gradient elution. Varying amounts of methanol, acetonitrile or isopropanol (HPLC Grade, Fisher) were added to 6 mM Na_2SO_4 with 1 mM HEPES (pH 7.0) to determine the optimum mobile phase composition for the separation of Cu-EDTA and Pb-EDTA complex.

2.9. Post Column Reagent

Detection by post-column reaction (PCR) involves the chemical reaction of the solutes as they elute from the column on the fly, prior to their introduction to the detector. This can translate in enhanced analyte sensitivity of the HPLC system which can be critical when the analyte concentration is in the range of micromolar and/or in the present of large quantities of sample matrix interferences. For this work, the post-column reagent was prepared by adding 40 μM $Fe(NO_3)_3$ to a 200 mM sodium formate–formic acid buffer at pH 4.5, the HPLC flow-rate was 1.5 mL/min, whereas the post-column delivery pump was operated at 0.3 mL/min.

CHAPTER THREE

3. Sediment Characterization

In this study, the marine sediments PACS-2 and HISS-1 samples were obtained from National Research Council Canada (NRC-CNRC). HISS-1 sediment was collected from the Hibernia Shelf off the coast of Newfoundland, and PACS-2 sediment from the harbour of Esquimalt, B.C[163]. These sediments were used as received in powder form.

3.1. SEM Analysis for Sediment Particles

When an electron beam is scanned across a sample's surface in SEM the electrons strike the sample, a variety of signals are generated, and it is the detection of specific signals which produces an image or a sample's elemental composition. The three signals that provide the greatest amount of information in SEM are the secondary electrons, backscattered electrons, and X-rays.

Secondary electrons are emitted from the atoms occupying the top surface and produce a readily interpretable image of the surface. The contrast in the image is determined by the sample morphology. A high resolution image can be obtained because of the small diameter of the primary electron beam.

Backscattered electrons are primary beam electrons that are 'reflected' from atoms in the solid. The contrast in the image produced is determined by the atomic number of the elements in the sample. The image will therefore show the distribution of different

chemical phases in the sample. Because these electrons are emitted from a depth in the sample, the resolution in the image is not as good as for secondary electrons. Images and X-ray spectra of HISS-1 and PACS-2 sediments are presented in Figures (3.1-3.4). These images were obtained using Quadrant Backscattered Electron Detector (QBSD) at different distances from 2 μm to 100 μm.

Interaction of the primary beam with atoms in the sample causes shell transitions, which result in the emission of X-rays. The emitted X-rays have an energy characteristic of the parent element. Detection and measurement of the energy permits elemental analysis (Energy Dispersive X-ray Spectroscopy or EDS). EDS can provide rapid qualitative, or with adequate standards, quantitative analysis of elemental composition with a sampling depth of 1-2 microns. X-rays may also be used to form maps or line profiles, showing the elemental distribution in a sample surface. The system was calibrated with a Cu standard for SEM analysis.

Energy dispersive X-ray spectrometers (EDS) have the potential to enhance significantly the usefulness of SEM in sediments particle studies. EDS can give, in a matter of seconds, the qualitative chemical composition of any particle under observation in the SEM. The combination of the SEM and the EDS is usually called the analytical SEM (ASEM). With EDS analysis, mineral particles left in the residue (e.g. pyrite, black carbon particles in the light microscope) can be easily recognized on the basis of their elemental composition.

Figure 3.1.   SEM/EDS/Image Analyzer of HISS-1 marine sediment samples at different magnifications (clockwise, from upper left image: magnifications of 2110x, 1210x, 162x and 834x, respectively).

Figure 3.2.   EDS spectrum of marine sediment HISS-1 (Au is present due to coating).

Figure 3.3.   EDS spectrum of marine sediment PACS-2.
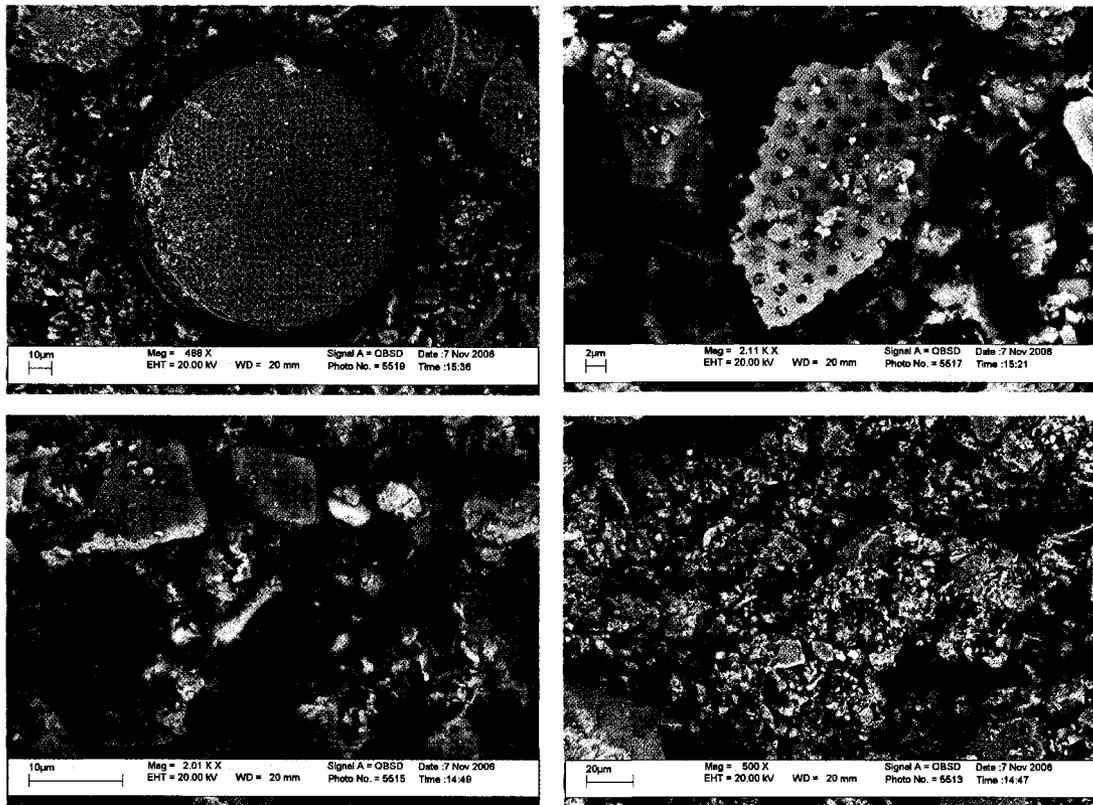
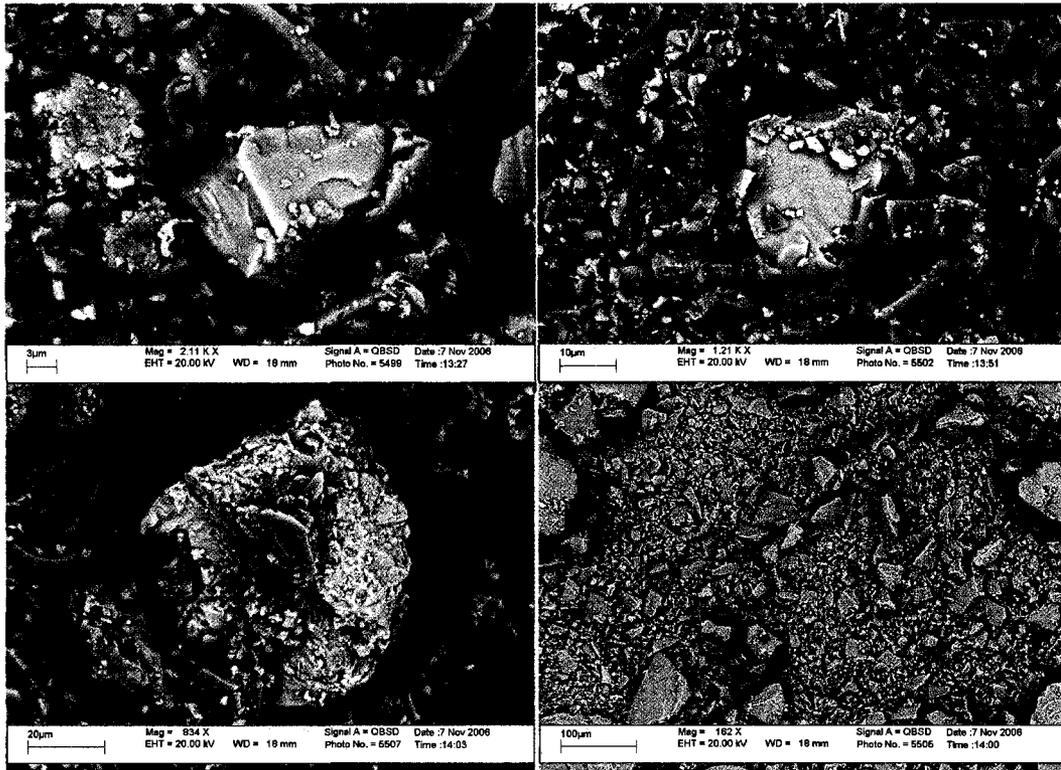

Figure 3.4.   SEM images of PACS-2 marine sediment samples at different magnifications (clockwise, from upper left image: magnifications of 2110x,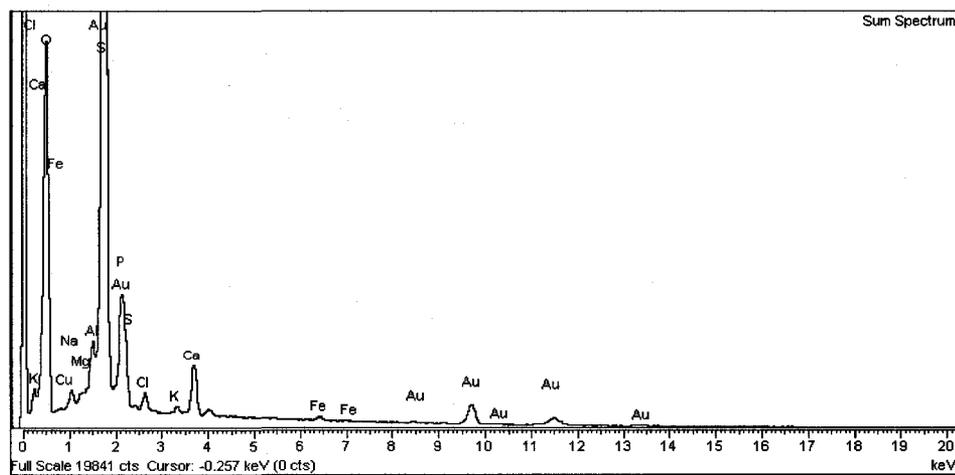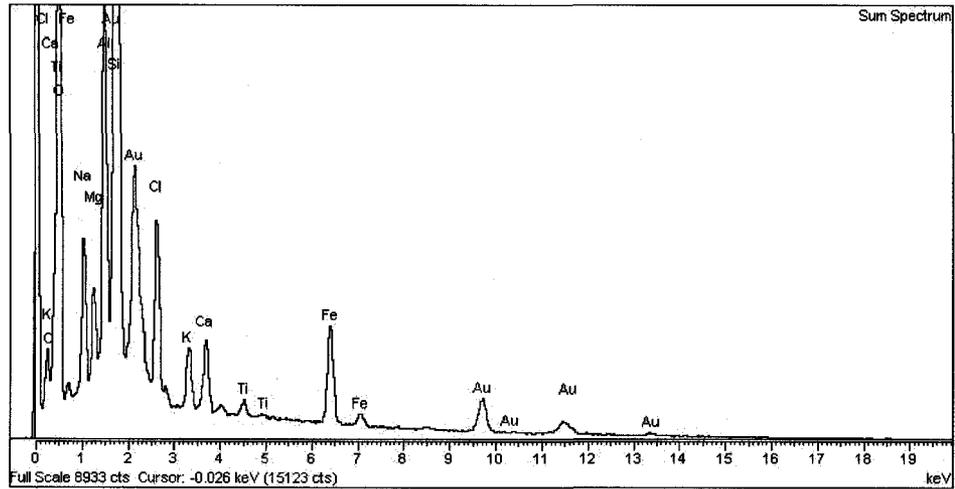 498x, 2010x, and 500x, respectively). The upper two images include diatoms (aquatic single-celled algae) as sediment particles.

In this study, EDS spectrometry coupled with SEM (Scanning Electron Microscope) was used to characterize HISS-1 and PACS-2 marine sediments samples. The shape of the sediment particles was investigated using SEM by generating images from measurements of the secondary electron intensities (see Figures 3.1 and 3.4). The SEM images show that both sediments are polymorphic with a heterogeneous distribution of material. In the case of PACS-2, the sediment particles include biogenic silica diatom frustules (Fig. 3.4). Table 3.1 shows a summary of the percent concentration (%weight) of the elements found in HISS-1 and PACS-2. This investigation shows that HISS-1 and PACS-2 are chemically different. The EDS analysis results focusing on small area show the strong presence of Si and O, which suggest the presence of quartz (SiO_2), and/or other silicate minerals (SiO_3^{2-}) and/or

Table 3.1.   Summary obtained from the EDS for HISS-1 (left Table) and PACS-2 (right Table).

Summary results: HISS-1

Element	Weight %	Atomic %
O	49.38 ± 0.26	63.54
Si	44.04 ± 0.23	32.28
Ca	2.47 ± 0.04	1.27
Na	1.04 ± 0.06	0.94
Al	1.13 ± 0.04	0.86
Cl	0.80 ± 0.04	0.46
P	0.31 ± 0.09	0.20
Mg	0.21 ± 0.04	0.18
Fe	0.41 ± 0.05	0.15
K	0.22 ± 0.03	0.12
Totals	100.00	100.00

Summary results: PACS-2

Element	Weight %	Atomic %
O	45.85 ± 0.33	61.78
Si	25.77 ± 0.18	19.78
Al	6.54 ± 0.08	5.23
Na	3.67 ± 0.09	3.44
Fe	7.08 ± 0.10	2.73
Cl	3.22 ± 0.06	1.96
Mg	1.83 ± 0.06	1.63
Ca	2.48 ± 0.05	1.33
S	1.25 ± 0.08	0.84
K	1.42 ± 0.04	0.78
P	0.39 ± 0.09	0.27
Ti	0.50 ± 0.04	0.23
Totals	100.00	100.00

amorphous biogenic silica (diatoms). In addition to the strong presence of Si, the EDS results show that both sediments contain significant amount of Ca, Na, and Al. The

higher Al in PACS-2 suggests the possible presence of more clay minerals (Al-Silicates) than HISS-1. In PACS-2, the additional strong presence of Fe and, to a lesser extent, Mg and K is noteworthy, indicating that PACS-2 is more diverse in terms of mineral content. The importance of the presence of Si, Mg, and Fe in these sediments is that these elements, when combined with oxygen to form oxides such as SiO_3^{2-} (silicate), Fe_2O_3 (hematite), and MgO_2, can either adsorb or absorb trace metal ions. These oxides generally have a negative charge density and thus would have an affinity for positively charged ions such as metal ions. The presence of oxides such as silicates, hematite or would have an immediate impact on the availability of aqueous trace metal ions in contact with the sediment and therefore would need to be taken into account in a model aimed at predicting the distribution of metals in a sediment slurry. Previous attempts to characterize the sediments by X-ray diffraction[156] (Appendix – II) did not reveal the presence of the above mentioned oxides. It is possible that the polydisperse, amorphous nature of the sediment prevented the detection of pure crystalline phases of the oxides[167]. The presence of gold in the EDS spectra of both sediments is attributable to the thin gold coating that each sample is subjected to during the sample preparation. Finally, no measurable Pb and Cu by EDS were found in either HISS-1 or PACS-2 sediment.

Figures 3.5 and 3.6 shows elemental maps of PACS-2 and HISS-1, respectively. The elemental maps were obtained by EDS X-ray measurements. The presence of an element is evidenced by bright, white colour whereas the black represents low concentration or zero. These maps can show correlation between different elements and sediment grains. For example, Figure 3.5 shows that Al, Mg, and Fe are concentrated around the same area on the map, which delimitates a particular sediment particle (see

"particle" on Fig 3.5). This suggests that the particle is likely made of Al-Mg-Fe, possibly associated with Si as a silicate since Si and O are quite abundant in most of the sediment grains, but it is not related with Cl since the Cl-map shows little Cl in the area of the particle, thus excluding the possibility that the particle be a chloride-containing mineral. It is expected that this particle could be the mineral Chinochlore based on the previous XRD analysis (Appendix - II) of this sediment samples.

Figure 3.6 shows that the elemental distribution in HISS-1 is more uniform, showing no or very few distinct mineral particles. From this analysis it is also relevant that HISS-1 is mostly composed of quartz. The elemental mapping analysis for Si is highly dense for HISS-1 in Figure 3.6. High Na and Cl detected in PACS-2 is, according to XRD, hosted Halite (NaCl), i.e. salt probably from seawater was still in this sample, whereas HISS-1 has only 0.6%.
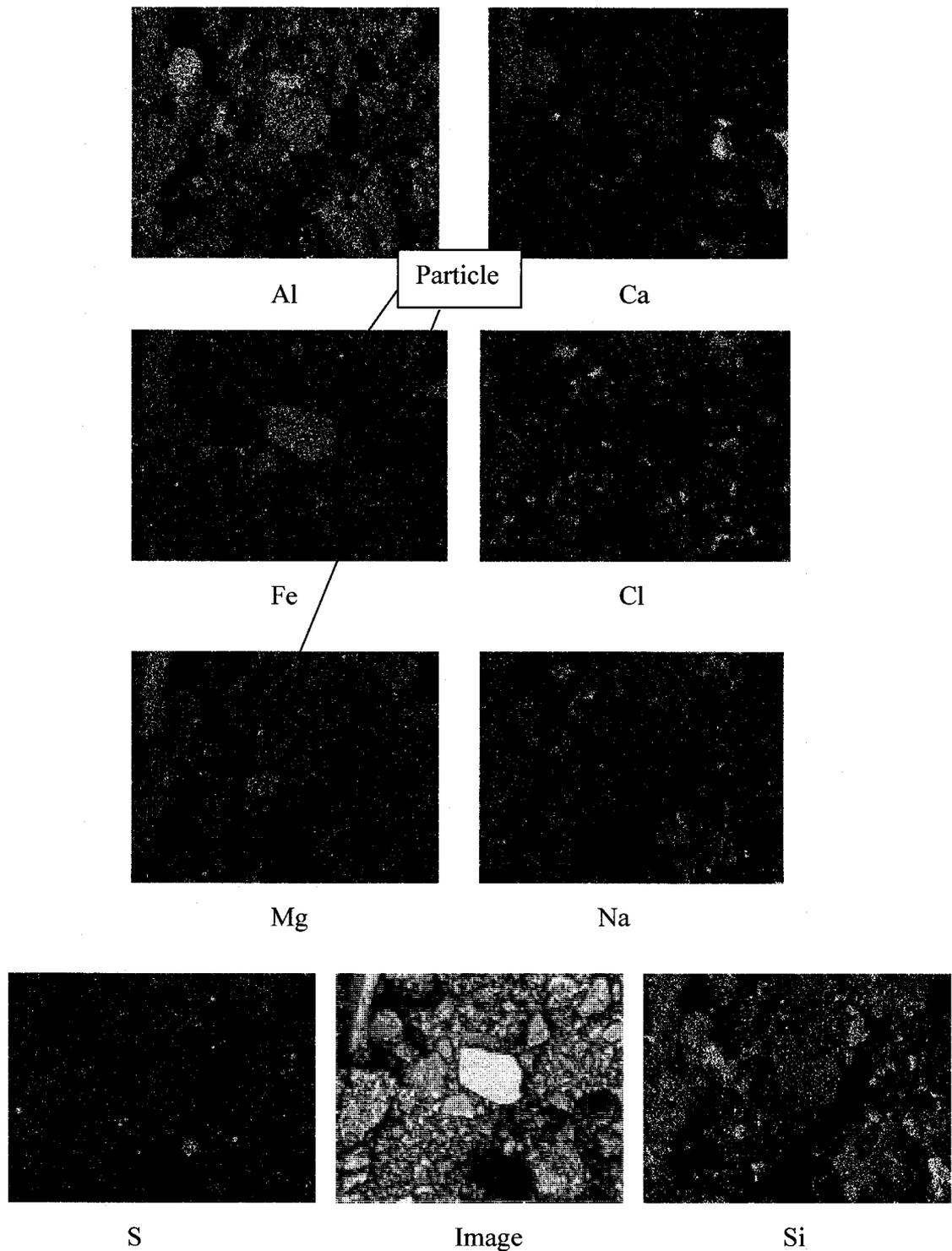
Figure 3.5.   Elemental mapping of PACS-2 from EDS analysis for different elements at 346x magnification.
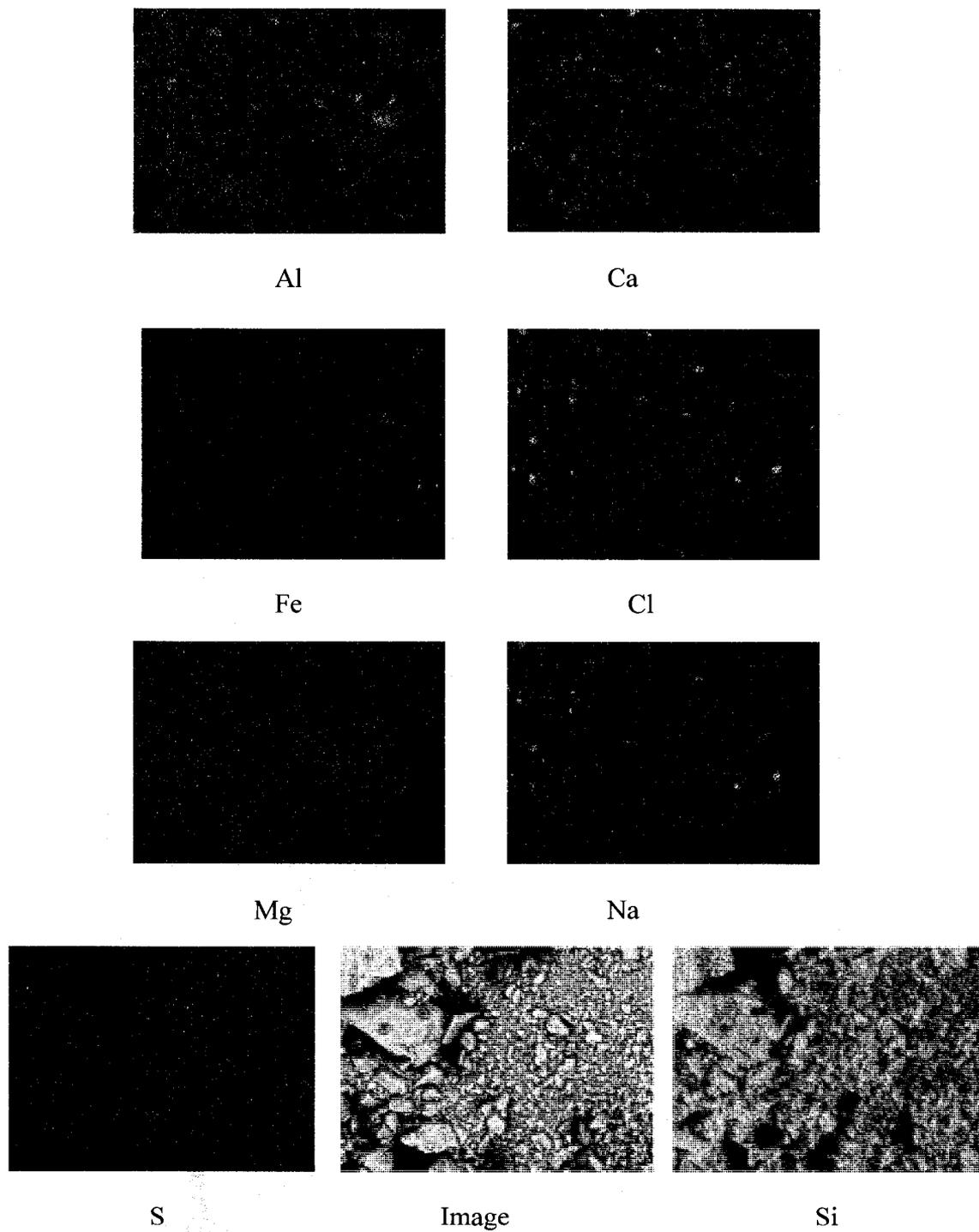
Figure 3.6. Elemental mapping of HISS-1 from EDS analysis for different elements at 297x magnification.

CHAPTER FOUR

4. RESULTS AND DISCUSSION FOR ANALYTICAL CHEMICAL METHODS

4.1. Introduction

The aim of this chapter is to develop a suitable quantitative separation method to study the distribution of EDTA, metal-EDTA complexes and metals in the aquatic environment.

4.2. Calculation of Capacity Factor of HPLC, k'

The capacity factor, k', of a sample component is a measure of the degree to which that component is retained by the column relative to an unretained component. The capacity factor is intimately dependent, among other factors, on the nature and composition of the mobile phase and stationary phase. Therefore, the optimization of a chromatographic method can be followed by monitoring k' as chromatographic parameters (e.g. mobile composition) are changed. The capacity factor can be calculated from measured retention times using the following equation:

$$k' = (t_r - t_o)/t_o$$

where t_r is the elution time of retained component, and t_o is the elution time of the unretained component.

Good resolution with reasonable retention time (i.e., not too long) is usually obtained for k' values between approximately 2 and 10[167].

4.3. Method Development with HPLC System

4.3.1. Optimum Concentration of Coating Reagent

Ion-pairing agents (IPA) are ionic compounds that contain a hydrocarbon chain that imparts a certain hydrophobicity such that the ion pair can be retained on a reversed-phase (RP) column.

In any RP-HPLC separation, ionic additives (like ion-pairing agents) in the mobile phase serve one or more of the following functions: pH control (buffering), suppression of adverse ionic interactions that can occur between silanols and basic analytes and between analyte molecules, or complexation with oppositely charged ionic groups to enhance RP retention[168]. The research in this study focused on using ion-pair chromatography with cetrimide (hexadecyltrimethylammonium bromide) for the separation of metal-EDTA complex.

The coated column acted as an anion-exchange column, without retaining metals in the system as might occur with a cation-exchange column. By preventing buildup of metals on surfaces, which could perturb metal–EDTA speciation during analysis[169], the speciation of metal–EDTA complexes are expected to be preserved. It has been shown that the larger the ion-interaction reagent, the greater the amount adsorbed on the stationary phase for a given concentration[170]. Therefore at equivalent concentrations, cetrimide will provide more ion-exchange sites.

The effect of IPA concentration on the separation of metal-EDTA complex was investigated by monitoring the change in the metal complex retention time versus the IPA concentration. The mobile phase (HEPES-Na_2SO_4) flow rate was maintained constant at 1.5 mL/min and contained either 0% or 10% acetonitrile (the influence or organic solvents is discussed in a later section). The pH of the mobile phase was adjusted at 7.0. Table 4.1 summarizes the experimental conditions tested. These conditions were chosen based on the work of Bedsworth et al.[166] which indicates that the eluent conditions do not lead to the formation of IPA micelles. Solutions having metal-EDTA complexes with Cu:Pb:EDTA mole ratio of 1:1:1 and 1:1:2 were used to investigate the effect of IPA concentration on the separation of the target metal-EDTA complex.

Figures 4.1A, 4.1B, and 4.1C show the chromatograms of Cu:Pb:EDTA (1:1:1) and Cu:Pb:EDTA (1:1:2) complexes using different concentrations of cetrimide coating of 0.0 mM, 1.0 mM and 5.0 mM, respectively.

The absence of cetrimide coating leads to no separation of Cu-EDTA and Pb-EDTA as only one single peak can be observed (Fig. 4.1A). Figs. 4.1B and 4.1C show that good separation of Cu-EDTA and Pb-EDTA is achieved with 1 mM and 5 mM cetrimide. However, compared to the 1 mM cetrimide coating, the 5 mM cetrimide coating shows greater peak tailing and smaller peak height sensitivity for both Cu-EDTA and Pb-EDTA, which can affect the ability to determine accurately smaller concentration of Cu-EDTA and Pb-EDTA complexes. Therefore, the 1 mM concentration is chosen to be the optimum concentration for the cetrimide coating hereafter. It was also found that the Pb-EDTA:Cu-EDTA peak intensity ratio in the 1:1:1 Cu:Pb:EDTA complex was smaller than in 1:1:2 Cu:Pb:EDTA complex, indicating that EDTA complexes

preferentially with the Cu ion over the Pb ion. This is consistent with the fact that the K_f $_{Cu-EDTA}$ formation constant is about 6 times greater than K_f $_{Pb-EDTA}$, thus favoring the complexation of Cu over Pb.

Table 4.1.   HPLC conditions for complex separations with different coating conditions.

Columns	Analytical Column: Varian Microsorb MV C_{18} 25 cm × 4.6 mm ID, 5 μM particle size
	Guard Column: Opti-Guard C_{18}, 1.0 mm I.D. (USA)
Mobile Phase	Conditions A: 6mM Na_2SO_4 with 1 mM HEPES pH 7.0 adjusted by NH_4OH
	Conditions B: 10:90 v/v Acetonitrile: 6 mM Na_2SO_4 with 1mM HEPES pH 7.0 adjusted by NH_4OH
Column Coating Reagent	Conditions A: No Coating
	Conditions B: Coating for 18 h with 1 mM Cetrimide
	Conditions C: Coating for 18 h with 5 mM Cetrimide
Flow Rate	1.5 mL/min
Other Parameters	All stainless steel tubing and needle assembly replaced with PEEK (polyether ether ketone)
Reaction Temperature	Room temperature
Detection	Photo Diode Array (PDA) at 258 nm wavelength

Retention factors observed for the ion pairing agent Cetrimide with 0.0 mM (no coating), 1.0 mM and 5.0 mM concentration range are tabulated in Table 4.2. The retention times for the test anions $[Cu-EDTA]^{2-}$ and $[Pb-EDTA]^{2-}$ increased with increasing concentration of cetrimide coating on the column (Table 4.2). No separation of the metal-EDTA complexes was observed, regardless of the metal-EDTA mole ratio, when there was no coating, where a single peak at 3.05 to 3.13 min was observed (Fig. 4.1A).
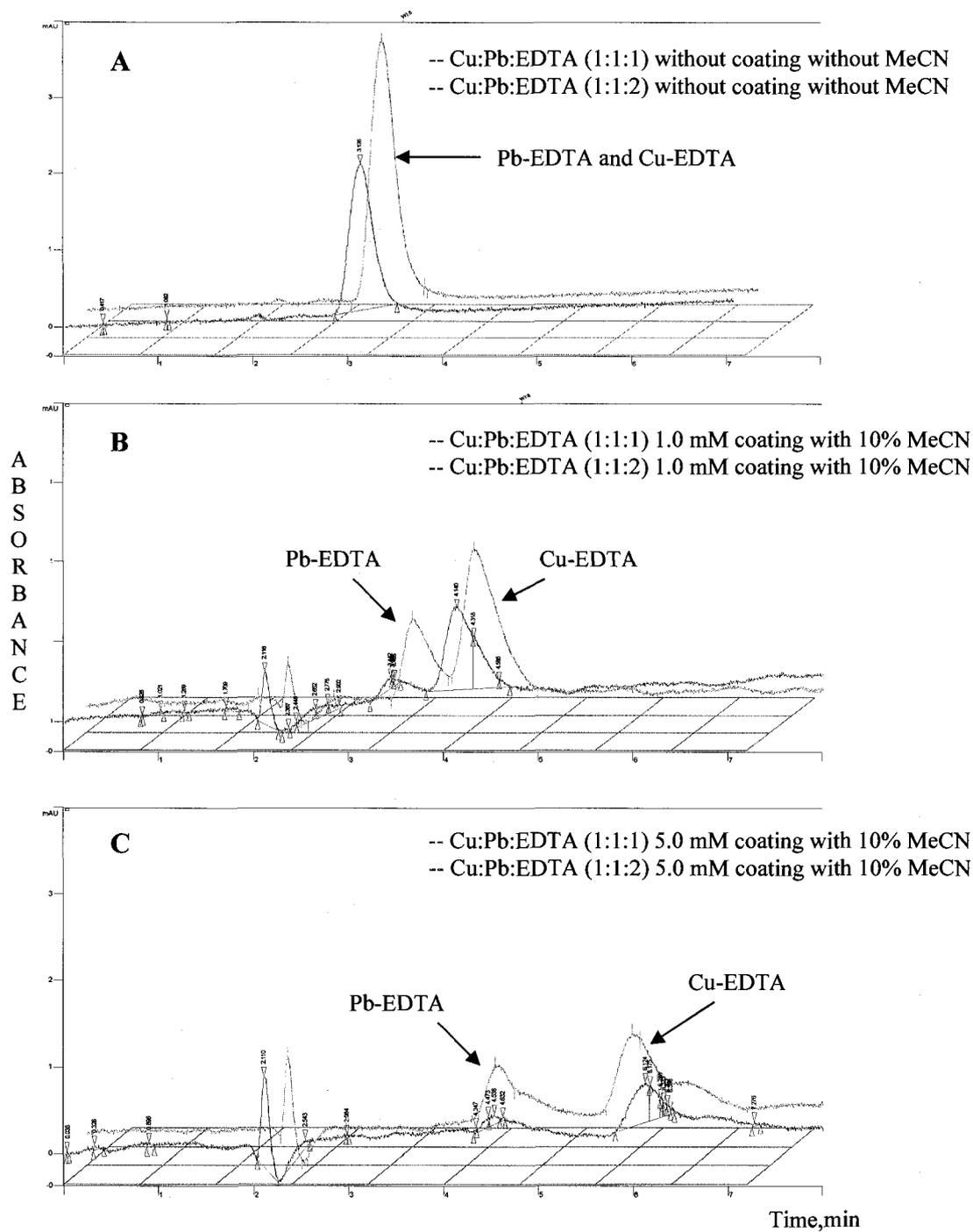
Figure 4.1.   Chromatograms of Cu:Pb:EDTA (1:1:1) and Cu:Pb:EDTA (1:1:2) complexes using different concentration of Cetrimide coating : A) 0.0 mM, B) 1.0 mM, and C) 5.0 mM.

Table 4.2. Effects of Concentration of ion pair agent on the retention behaviour of Cu-EDTA and Pb-EDTA complexes.

Metal-complex ratio Cu:Pb:EDTA	Cetrimide (mM)	Complex	Mobile Phase with or without modifier	Retention Time, RT (min)	Capacity Factor (k')
1:1:1	0.0 (no coating)	Cu-EDTA Pb-EDTA	No Organic	3.05	0.66
1:1:2	0.0 (no coating)	Cu-EDTA Pb-EDTA	No Organic	3.13	0.66
1:1:1	1.0	Cu-EDTA Pb-EDTA	10% MeCN	4.14 3.47	1.12 0.78
1:1:2	1.0	Cu-EDTA Pb-EDTA	10% MeCN	4.09 3.44	1.10 0.76
1:1:1	5.0	Cu-EDTA Pb-EDTA	10% MeCN	6.29 4.47	2.22 1.29
1:1:2	5.0	Cu-EDTA Pb-EDTA	10% MeCN	6.18 4.31	2.16 1.21

Retention time of metal-EDTA complexes increased with higher concentrated coating [Figure 4.1. (A-C)] regardless of the metal-EDTA mole ratio. Figure 4.1C shows that the base line drifted upward with the 5.0 mM cetrimide coating compared to 1.0 mM and 0.0 mM coating. This can affect the accuracy of peak integration which relies on the ability to define the baseline accurately. Fig. 4.1C also shows that both Cu-EDTA and Pb-EDTA complexes suffers significant peak tailing and decrease in peak height intensity with the 5.0 mM compared to the 1.0 mM coating, and this regardless of the metal-EDTA mole ratio. This can affect the ability to separate the two metal-EDTA complexes from each other or from metal-EDTA complexes that might be present in real environmental samples but absent in the test samples. Furthermore, the decrease in peak intensity with increasing cetrimide concentration will cause an increase of the limit of detection (the minimum amount of analyte that is statistically measurable above the noise), even though the peak area integration in conserved.

Table 4.2, and furthermore exemplifyied in Fig. 4.2, shows that the capacity factor, k',

increased for both Cu-EDTA and Pb-EDTA complexes with increasing cetrimide

concentration. The above results are consistent with the findings of Zou et al.[171], who

also found that capacity factors increased with increasing concentration of ion-pair agent

and that the sensitivity of metal complexes is reduced when the concentration of
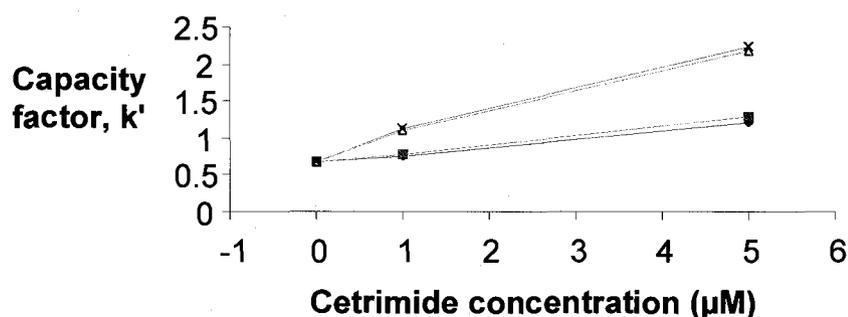
cetrimide

Figure 4.2.   Plots of the retention factor, k' of Pb-EDTA (♦ 1:1:1 and ■ 1:1:2), and
               Cu-EDTA (▲ 1:1:1 and × 1:1:2) versus cetrimide concentration.

coating is increased. Therefore, the 1.0 mM cetrimide coating was determined to be the

optimum concentration of cetrimide for this work which would allow sufficient

interaction ion-exchange sites. Storm and co-workers[172] also found that low concentration

of ion-pairing agent is desirable for the separation of metal-EDTA complexes.

4.3.2. Selection and Optimization of Mobile Phase Composition

The properties of the mobile phase are an important parameter in Ion-Pairing Chromatography. Changing ionic strength, pH or type of anions controls the elution strength. The mobile phases used in Ion-Pairing Chromatography are typically aqueous salt solutions, which depends on various factors including the compatibility with the detection mode, the nature of the competing ligand, the concentration of the competing ion, the pH and buffering capacity of the mobile phase, the ability to complex the ionic sample components, and the presence of organic modifiers (i.e., organic solvents).

4.3.2.1. Mobile Phase Considerations with Selection of Organic Modifiers

Determining the optimum organic modifier concentration is important because a variation in the content of the mobile phase in the organic modifier would also translate to a corresponding change in the concentration of the ion-pairing agent in the stationary phase, which can cause a change in the distribution of ion pairs to the hydrophobic stationary phase.

Organic solvents are added to elute analytes from a reversed phase column. The most commonly used solvents are aqueous buffers in combination with acetonitrile, methanol or tetrahydrofuran (THF). Other less commonly used solvents include ethanol, iso-propanol, dichlormethane or n-hexane. Elution can be performed isocratically (the solvent composition does not change as function of time during the separation process) or by using a concentration gradient (the solvent composition changes as a function of time).

This work tested the suitability of acetonitrile, methanol, and isopropanol as organic modifiers for the HPLC mobile phase. The k' values as well as selectivities of solutes separated by ion-pair chromatography with reversed-phase systems depend markedly on the mobile phase composition and in particular on the organic solvent selected[173].

Bedsworth et al.[166] and Buchberger et al.[178] carried out similar work and some of their investigation strategy was adopted here. Bedsworth and co-workers[166] used a HEPES buffer containing Na_2SO_4 for the mobile phase and found that variation in the sulphate concentration in the mobile phase would cause a significant change in the retention time of the EDTA complexes, but not in their relative retention times (i.e., the capacity factor remained unchanged). They also found that the stationary phase tended to clog in presence of high sulphate concentrations due to the formation of bubbles in the solvent tubing. Consequently, this work adopted the use of 1 mM HEPES buffer containing 6 mM Na_2SO_4 for the mobile phase. Methanol, acetonitrile and iso-propanol were studied in the presence of the ion-pair cetrimide (hexadecyltrimethylammonium bromide, 1 mM, pH 7.0).

Optimization experiments for the separation of Cu-EDTA and Pb-EDTA complexes at pH 7.0 revealed that sufficient selectivity of the mobile phase could be achieved only by using very hydrophobic ion-interaction reagents (such as cetrimide), together with certain amounts of organic modifier in the mobile phase.

The composition of the mobile phase was optimized by the addition of water miscible organic solvents. Three different solvents, methanol, acetonitrile and isopropanol, in varying % composition were added to 6 mM Na_2SO_4 with 1 mM HEPES

(pH 7.0) to determine the optimum mobile phase composition for the separation of copper-EDTA and lead-EDTA complex. The following solvent compositions were tested: (a) 10%, 15% and 40% acetonitrile added to 90%, 85%, and 60% of 6 mM Na_2SO_4 in 1mM HEPES (pH 7.0), respectively.

(b) 5%, 10% or 15% methanol added to 95%, 90%, and 85% of 6 mM Na_2SO_4 in 1mM HEPES (pH 7.0), respectively.

(c) 10% Isopropanol added to 90% of 6 mM Na_2SO_4 in 1mM HEPES (pH 7.0).

Varying % composition of added acetonitrile, methanol or isopropanol to the 1 mM HEPES in 6 mM Na_2SO_4 mobile phase was studied and the effect it had on the retention time and capacity factors of Cu-EDTA and Pb-EDTA was measured. Table 4.3 reports the different experimental conditions used to test the suitability of different solvents with the HEPES-Na_2SO_4 mobile phase.

Table 4.3.   HPLC conditions for complex separations with different solvent composition.

Columns	Analytical Column: Varian Microsorb MV C_{18} 25 cm × 4.6 mm ID, 5 µM particle size Guard Column: Opti-Guard C_{18}, 1.0 mm I.D. (USA)
Mobile Phase	Conditions A: 10:90 v/v Acetonitrile: 6 mM Na_2SO_4 with 1 mM HEPES pH 7.0 adjusted by NH_4OH Conditions B: 20:80 v/v Acetonitrile: 6 mM Na_2SO_4 with 1 mM HEPES pH 7.0 adjusted by NH_4OH Conditions C: 40:60 v/v Acetonitrile: 6 mM Na_2SO_4 with 1 mM HEPES pH 7.0 adjusted by NH_4OH Conditions D: 5:95 v/v Methanol: 6 mM Na_2SO_4 with 1 mM HEPES pH 7.0 adjusted by NH_4OH Conditions E: 10:90 v/v Methanol: 6 mM Na_2SO_4 with 1 mM HEPES pH 7.0 adjusted by NH_4OH Conditions F: 15:85 v/v Methanol: 6 mM Na_2SO_4 with 1 mM HEPES pH 7.0 adjusted by NH_4OH Conditions G: 10:90 v/v Isopronanol: 6 mM Na_2SO_4 with 1 mM HEPES pH 7.0 adjusted by NH_4OH
Flow Rate	0.5 ml/min, 1.0 mL/min and 1.5 mL/min
Column Coating Reagent	Coating for 18 h with 1 mM Cetrimide at 0.2 mL/min flow rate
Other Parameters	All stainless steel tubing and needle assembly replaced with PEEK (polyether ether ketone)
Temperature	Room temperature
Detection	Photo Diode Array (PDA) at 258 nm wavelength

Figure 4.3 and Figure 4.4 depict the chromatograms of Cu-EDTA and Pb-EDTA with acetonitrile, methanol and iso-propanol as organic modifiers using isocratic elution in ion-pair liquid chromatography. In Figure 4.3, it is apparent that the use of 10% acetonitrile with the mobile phase improves the separation of metal-EDTA complexes. For example, the red line in Fig. 4.3 shows that the valley between Pb-EDTA (first peak) and Cu-EDTA (second peak) is deeper compared to the blue line, indicating a better separation.
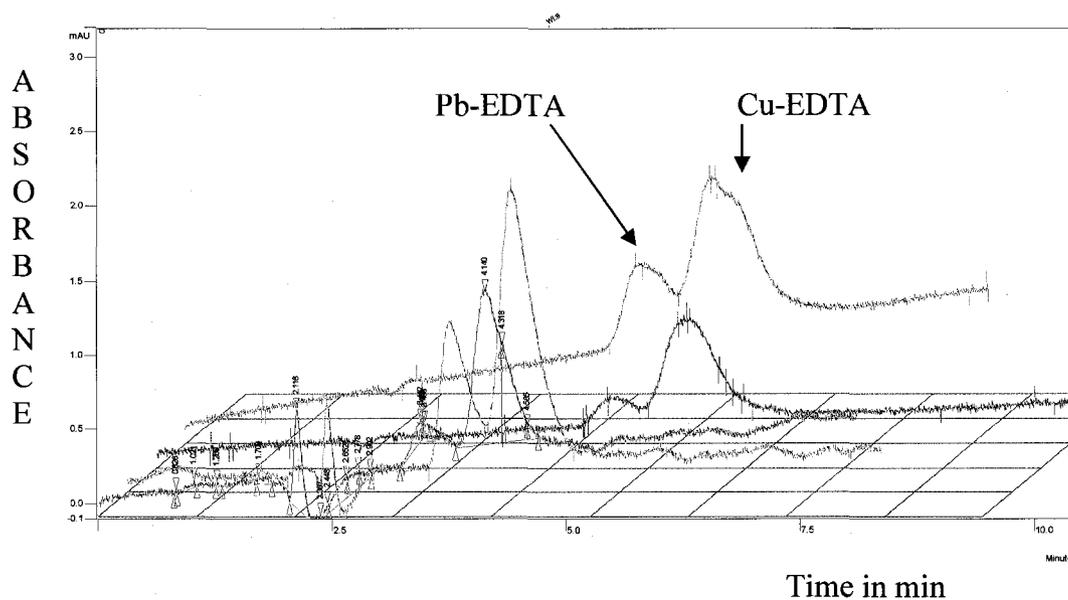
71

Figure 4.3. Chromatogram of 1:1:1 Cu:Pb:EDTA without MeCN (blue) and with 10% MeCN (red), and of 1:1:2 Cu:Pb:EDTA without MeCN (magenta) and with 10% MeCN (lime-green).

Acetonitrile was found to be a faster eluent than MeOH as 10% of the former was needed to elute the Cu-EDTA and Pb-EDTA while 10% of the latter was needed to elute the same peaks with the same ion-pairing concentration (Figure 4.3 and Figure 4.4 A). The eluting strength of MeCN in comparison to MeOH can be seen in Table 4.4 of the capacity retention factor (k) of complexes of organic modifier in the mobile phase added. The retention factor for 10% MeCN was lower, indicating that MeCN has higher elution strength. The difference in solvent behaviour between MeCN and MeOH can be partially attributed to their chemical nature. The dielectric constant (ϵ) is known to be an important parameter in defining the ability of a solvent to solvate ions[173]. Since the ability of a solvent to disperse electrostatic charges via ion dipole interactions is inversely related to the dielectric constant, MeOH (ϵ = 32.3) tends to exhibit more pronounced solvation effects for the hexa-decyl-trimethyl-ammonium ion than MeCN (ϵ= 38.8). In addition,

72
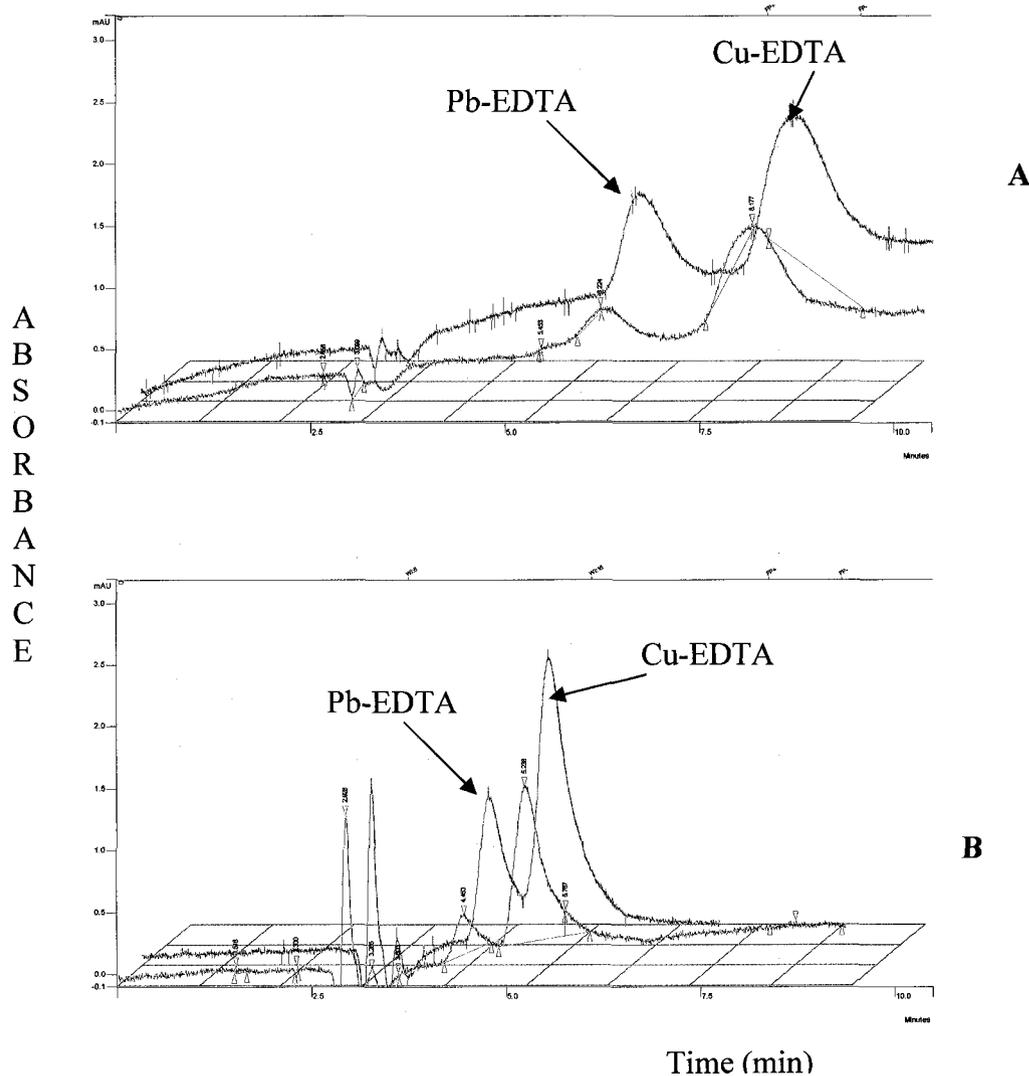
Figure 4.4. Chromatogram of 1:1:1 Cu:Pb:EDTA (red line) and 1:1:2 Cu:Pb:EDTA (blue line) complexes with (A) 10% MeOH or (B) 10% iso-propanol.

MeOH is a solvent, which can potentially solvate the ion-pair complex (Cu-EDTA and Pb-EDTA) via hydrogen bonding and result in a favourable extraction process whereas MeCN, due to lack of hydrogen bonding, could not show behaviour like MeOH. This explains the slower elution of Cu-EDTA and Pb-EDTA with MeOH.

Table 4.4. Effect of different organic modifier with retention time of different complexes.

Complexing ratio Cu (10 μM): Pb (10 μM):EDTA (10 μM)	Complex	Mobile Phase with and organic modifier	Retention Time, RT (min)	Capacity Factor (k')
1:1:1	Pb-EDTA	10% MeCN	3.44	0.76
	Cu-EDTA		4.09	1.10
1:1:2	Pb-EDTA	10% MeCN	3.47	0.78
	Cu-EDTA		4.14	1.12
1:1:1	Pb-EDTA	10% MeOH	6.28	1.32
	Cu-EDTA		8.18	2.01
1:1:2	Pb-EDTA	10% MeOH	6.30	1.32
	Cu-EDTA		8.22	2.03
1:1:1	Pb-EDTA	10% Iso-Propanol	4.45	0.64
	Cu-EDTA		5.24	0.93
1:1:2	Pb-EDTA	10% Iso-Propanol	4.46	0.64
	Cu-EDTA		5.24	0.93

This study also investigated different % amount of acetonitrile, 20% and 40%, in the mobile phase whereas only 10% MeCN showed some separation behaviour of metal complexes. There was no chromatographic peak with use of 20% and 40% acetonitrile. This could be explained by the IPA getting washed out i.e. the column lost its coating.

The selectivity of mobile phases containing 10% isopropanol was practically the same because no good separations of chromatographic peaks of Cu-EDTA and Pb-EDTA resulted (Figure 4.9.B). Moreover the complexes were eluted faster than methanol as in the polarity index acetonitrile > methanol > isopropanol (5.8>5.1>3.9). From this polarity index it is found that iso-propanol is highly non-polar. It was also shown that in presence of highly hydrophobic cetrimide, iso-propanol could not be retained in the column for a longer time. The complexes were eluted faster with iso-propanol than methanol. As a result only methanol was used as organic modifiers for further experiments.

In isocratic mode and at a constant concentration of ion-pairing agent, the elution of the most charged complexes required a high concentration of organic modifier. But in this case, Cu-EDTA and Pb-EDTA co-eluted with overlapping peaks. Considering the fore mentioned discussion, this research study selected an isocratic mode of MeOH, while the ion-pairing agent was also kept constant. Work was also performed to achieve the best separation with 5%, 10% and 15% MeOH as organic modifier. Figure 4.5 and Table 4.5 shows how retention varies with change of methanol composition. The retention behavior decreased with higher concentration of methanol. All of these experiments were carried out at a 1.0 ml/min flow rate. The separation factor for use of 5% methanol and 10% methanol were close enough to each other. It was also found that with 5% MeOH, the retention time changed with every run. This observation could be explained by the equilibrium shift of the complexes by the organic modifier. Use of 15% MeOH resulted in a lower capacity factor, summarized in Table 4.5 and Figure 4.5. From these investigations the optimum separation of Pb-EDTA and Cu-EDTA complexes from each other was obtained with a mobile phase composition of 10% methanol with 90% of 6 mM Na_2SO_4 in 1mM HEPES (pH 7.0).

Table 4.5.   Effect of different percentages of methanol with retention time of different complexes.

Complexing ratio Cu (10 μM): Pb (10 μM):EDTA (10 μM)	Complex	Mobile Phase with and organic modifier	Retention Time, RT (min)	Capacity Factor (k')
1:1:1	Pb-EDTA	5% MeOH	6.92	1.38
	Cu-EDTA		8.78	2.01
1:1:2	Pb-EDTA	5% MeOH	7.25	1.49
	Cu-EDTA		9.07	2.12
1:1:1	Pb-EDTA	10% MeOH	6.28	1.32
	Cu-EDTA		8.18	2.01
1:1:2	Pb-EDTA	10% MeOH	6.30	1.32
	Cu-EDTA		8.22	2.03
1:1:1	Pb-EDTA	15% MeOH	6.21	1.18
	Cu-EDTA		8.30	1.92
1:1:2	Pb-EDTA	15% MeOH	6.34	1.23
			8.53	1.99
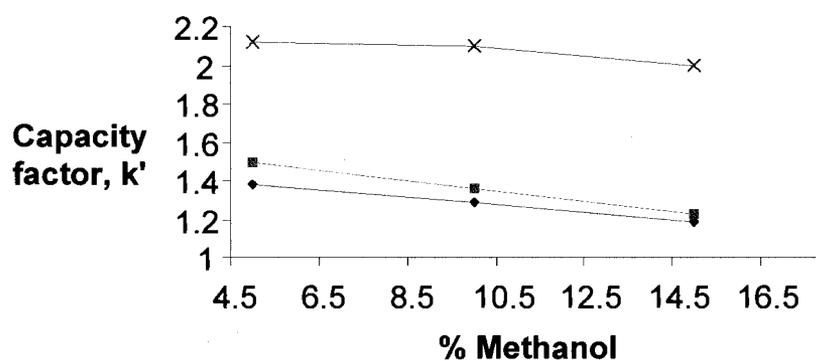
Figure 4.5.   Plots of the capacity factors, k', of Pb-EDTA (♦ 1:1:1 and ■ 1:1:2 Cu:Pb:EDTA mole ratio), and Cu-EDTA (1:1:1 and × 1:1:2 Cu:Pb:EDTA mole ratio) versus % methanol in the mobile phase.

4.3.3. Influence of pH

The pH of the mobile phase can have an important role on the retention of an analyte. Charged molecules elute earlier than their neutral counterpart. The pH of the mobile phase should be at least 1.5 pH units away from the pK_a of the analyte to avoid the presence of charged and neutral analyte components leading to broad elution peaks. When analysing charged analytes the addition of ion-pairing reagents into the mobile phase can increase the retention time of the analyte.

The variation of the retention time as a function of the pH of the solution is shown in Figure 4.6. It can be seen that pH affects the retention of ions. The retention decrease of the anions with increasing pH shows the effect of this parameter on the ion pairing equilibrium. Since the increase of the negative charge of uncomplexed EDTA with pH can give rise to a competing effect in regards to the solutes for the formation of the ion pair with Cetrimide this means a decrease in retention.
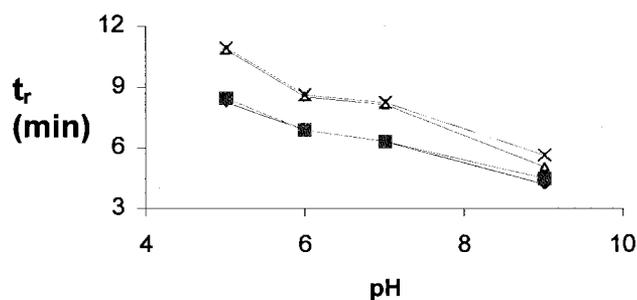
Figure 4.6.   Plots of the retention time, t_r, of Pb-EDTA (♦ 1:1:1 and ■ 1:1:2), and Cu-EDTA (▲ 1:1:1 and × 1:1:2) versus pH of the mobile phase.

The study made to optimize the separation conditions by applying different applications of the method.

As the pH of the solution appeared to be the main variable responsible for the LC separation of different EDTA complexes, screening for Cu-EDTA and Pb-EDTA were carried out in the pH range 5.0–9.0. The pH of the eluent can be adjusted to a broad range down to acidic pH, if necessary, without compromising eluent strength. Most metal complexes, however, are destabilized under non-neutral pH conditions ($5.0 > pH < 9.0$). An increasing amount of NH_3 at higher values ($pH > 8.0$) may help to destabilize some complexes. At acidic pH, the loss of buffering capacity of the eluent requires the sample and eluent pH to be identical.

The RT of the EDTA complexes decreased as the pH became higher. As can be seen in Figure 4.7 the best separation of these complexes was achieved under acidic conditions. However, the uncharged metal ions may cause disturbance to the equilibrium of complexes.

The retention time pH profiles resemble the plots of the equilibrium fractions of the respective uncharged species as functions of pH (Figure 4.6), suggesting that the retention is due to the uncharged species. Working with different pH at 5.0, 6.0, 7.0 and 9.0, different retention times for the Cu-EDTA and Pb-EDTA complexes (Figure 4.7) were found. Working with pH 5.0 showed best separation but the retention time was not reproducible. Moreover, to get the reproducible retention time in every run the complexes were found to move towards longer RT. For pH 9.0 the retention times were moving to the shortest intervals. The separation was also not good enough to be reliable. At pH 9.0 hydroxide ions may also react with Cu^{2+} and Pb^{2+}, causing disturbances in the

equilibrium. It was also evident that mobile phases of low or high pH could lead to severe disturbances of the equilibria between metal ions and EDTA in the samples during the chromatographic separation[178].

Working with pH 6.0 and 7.0 found good separation but the pH 7.0 runs demonstrated better separation than pH 6.0 due to base line drift. At pH 6.0 the complexes were highly sensitive to the LC systems. The retention time at pH 6.0 did not show reproducibility, because the peaks were distorting to shorter and longer retention times. It was evident that the mobile phases in the neutral pH range were considered to be advantageous for this purpose.

Table 4.6.   HPLC conditions for complex separations at different pH.

Columns	Analytical Column: Varian Microsorb MV C_{18} 25 cm × 4.6 mm ID, 5 μM particle size Guard Column: Opti-Guard C_{18}, 1.0 mm I.D. (USA)
Mobile Phase	Conditions A: 10:90 v/v Methanol: 6 mM Na_2SO_4 with 1 mM HEPES pH 5.0 adjusted by HNO_3 Conditions B: 10:90 v/v Methanol: 6 mM Na_2SO_4 with 1 mM HEPES pH 6.0 adjusted by NH_4OH Conditions C: 10:90 v/v Methanol: 6 mM Na_2SO_4 with 1 mM HEPES pH 7.0 adjusted by NH_4OH Conditions D: 10:90 v/v Methanol: 6 mM Na_2SO_4 with 1 mM HEPES pH 9.0 adjusted by NH_4OH
Flow Rate	1.0 mL/min
Column Coating Reagent	Coating for 18 h with 1 mM Cetrimide at 0.2 ml/min flow rate
Other Parameters	All stainless steel tubing and needle assembly replaced with PEEK (polyether ether ketone)
Temperature	Room temperature
Detection	Photo Diode Array (PDA) at 258 nm wavelength

An increase in pH from 5.0 to 9.0 could decrease the capacity factors. Thus the effect of pH was relatively large for the mobile phases. The increase in the capacity factor for pH 5.0 was expected due to increased formation of the uncharged form at the lower

pH. The uncharged form of the analytes is probably the most retained form due to the hydrophobic nature in the stationary phases. EDTA was expected to act in a similar way as it possesses four carboxylic and two amino groups. At a higher pH EDTA will be less retained due to a net charge of Y^{4-} instead of HY^{3-}. However, the experimental ranges of the x-variables were not large enough to induce any notable variance in the capacity factors for EDTA with different metal ions. To increase the retention the concentration of uncharged complexes in the mobile phase must be decreased below the lowest settings in this study. Working below pH 7 would probably increase the retention time as EDTA changes from a total charge of HY^{3-} to H^2Y^{2-}. The addition of organic modifiers as 5% or 10% methanol to the mobile phase did not have any large average effect on the capacity factors according to the results in Figure 4.6. However, a small average effect cannot exclude any counter ion effect. This small effect from adding organic modifiers to the mobile phase was expected. A reason for the low ion-pairing effect might be low ion-pair formation constants due to the high content of uncharged modifiers in the mobile phases or that the distribution constants for the analytes as uncharged analytes and as ion-pairs were quite similar in size. Figure 4.7C shows the separation of Cu(II)- Pb(II) at pH 7 in a mobile phase containing 10 % methanol.

It was also found that metal ions with toxicological features such as Pb(II) can be separated from other divalent metal ions by using Cetrimide as counterion and also in a characteristic sequence. Therefore, the presence of several metal complexes from the same chelator can provide a separation pattern that can identify the chelator. This is the unique advantage of element-specific detection: the use of such a metal species separation pattern for ligand identification.
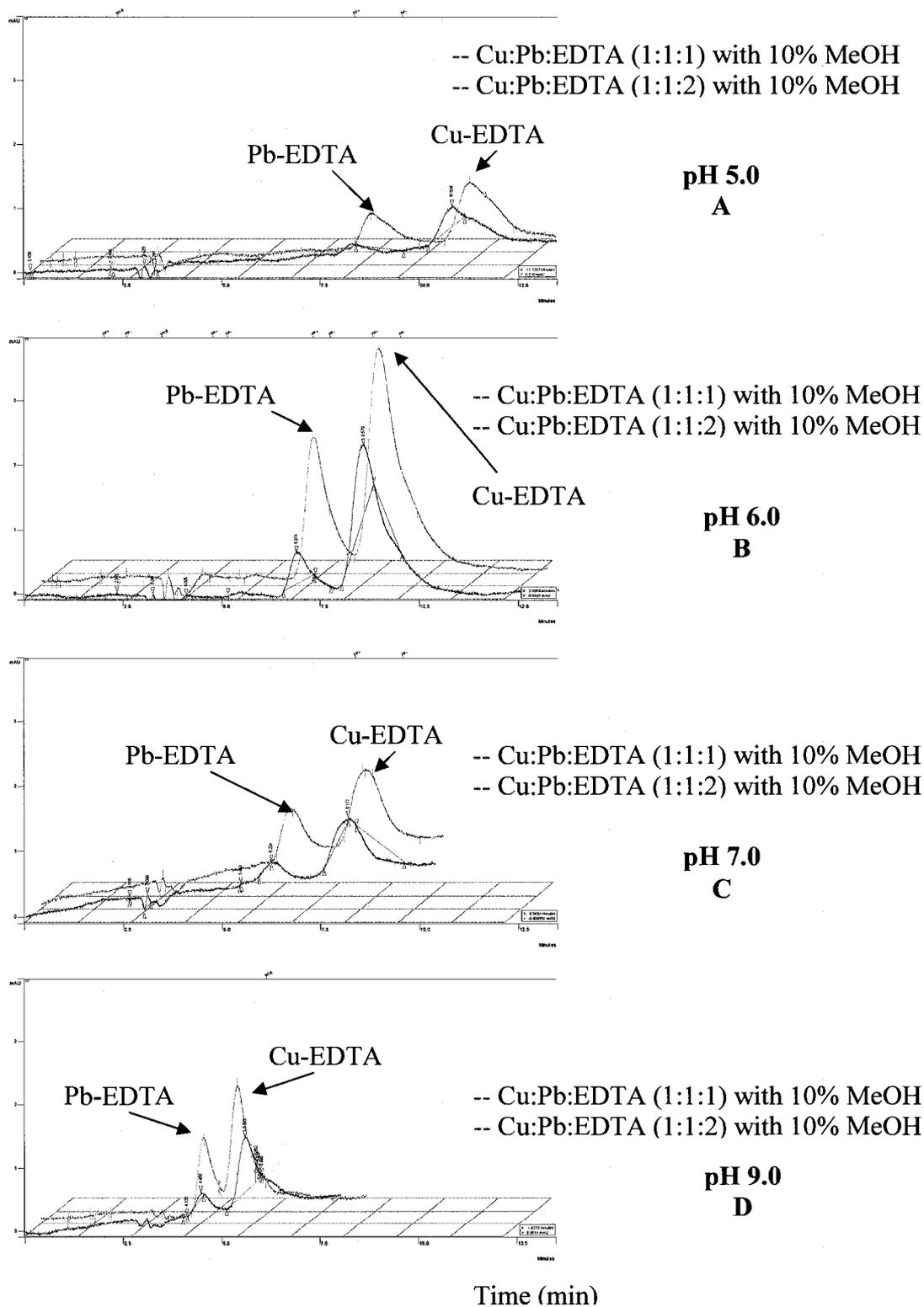
Figure 4.7.   Chromatogram of Cu:Pb:EDTA (1:1:1) and Cu:Pb:EDTA (1:1:2)
complexes at different pH (5.0, 6.0, 7.0 and 9.0)

Since both mobile phase and solutes ions can be affected by the pH, the buffering capacity of the mobile phase is very important, and should be maintained high. HEPES buffer (pH 7.0) at concentration of 1 mM and 5 mM were tested. The HEPES buffer with concentration of 1 mM was found to be best, providing enough buffering capacity (the pH of the mobile phase was verified before and after chromatographic separation) while providing good baseline stability.

The detection mode to be used is the major factor that determines the types of mobile phases suitable for the desired separation. The detector signal obtained by the background, i.e., the mobile phase itself, must not be too high; otherwise it would be difficult to obtain linearity, wide dynamic range and stability of the baseline. Initially, the mobile phase chosen was 6 mM Na_2SO_4 with HEPES buffer. The optimum pH was determined to be 7.0 (optimization described below). The Na_2SO_4 with HEPES buffer was compatible with Varian ProStar 330 Photo Diode Array Detector (PDA).

4.3.4. Selection of Flow Rate

The flow rate is a very important parameter for LC detection in the flow cell for PDA detector having 10.0 mm path length and 17.0 µL cell volume. It is not difficult to imagine that system pressure is affected by flow rate. The system pressure is important because it can tell the status of the instrument. It reflects the status of column and the system. A very low pressure (e.g. <6 atm) indicates leak in the system. A very high pressure indicates some blockage in the column or tubing. If the pressure fluctuates widely (e.g. change from 6 atm to above 400 atm), the pump inlet may have been

blocked, some bubble could be in the system, or the inlet valve is malfunctioning. It is a good habit to monitor the pressure while the instrument is running.

Flow rate impacts HPLC system pressure, chromatographic quality, and analysis time. One must choose a flow rate that is appropriate for the HPLC system and column. A higher than usual flow rate may adversely affect the quality of the chromatography by not giving the analyte sufficient time to interact with the stationary phase (i.e., faster is not always better). On the other hand, a lower than usual flow rate may leave the analyst waiting for a long time for the peak to appear at the detector. Long retention time is generally associated with greater peak broadening mostly due to the longitudinal diffusion broadening effect throughout the chromatographic system.

Separations with narrow bore, micro bore and even nanobore LC operating at flow rates from a few hundred µL/min down to a few hundred nL/min[174] are becoming more and more common due to lower solvent and stationary phase consumption, less dilution of the analyte, and good compatibility with the LC devices. Flow rates of 0.5, 1.0, and 1.5 mL/min were tested with the optimum mobile phase of 10% methanol with 90% of 6 mM Na_2SO_4 in 1mM HEPES (pH 7.0).

Maintaining mobile phase linear velocity is also important when attempting to reproduce chromatography obtained on columns of differing flow rates. For example to maintain mobile phase linear velocity from the standard column, 4.6 X 250 mm, the standard flow rate is 1 mL/min.

The effect of the flow rate was investigated using different flow rate for complex separations at 0.5 mL/min, 1.0 mL/min and 1.5 mL/min. Conditions are presented in Table 4.7. It was expected that with low flow rate the retention should be higher, which

the result was found (Figure 4.8). We found that with 0.5 mL/min the retention time was higher compared to 1.0 mL/min and 1.5 mL/min flow rate. In terms of capacity factor (Table 4.8) for 0.5 mL/min and 1.0 mL/min, they were very close. But for 1.5 mL/min flow rate, the capacity factor was less as well as the separation of complexes. In order to ensure efficiency in the chromatogram for separation of Cu-EDTA and Pb-EDTA complexes, 1.0 mL/min was chosen as the optimum flow rate for the complex solutions, with the mobile phase containing 10% methanol as an organic modifier. The capacity factor from 1.32 to 2.03, is also in agreement to achieve the highest separation of complexes with good resolutions[167].

Table 4.7.   HPLC conditions for complex separations at different flow rate composition.

Columns	Analytical Column: Varian Microsorb MV C_{18} 25 cm × 4.6 mm ID, 5 µM particle size Guard Column: Opti-Guard C_{18}, 1.0 mm I.D. (USA)
Mobile Phase	10:90 v/v Methanol: 6 mM Na_2SO_4 with 1 mM HEPES pH 7.0 adjusted by NH_4OH
Flow Rate	0.5 mL/min, 1.0 mL/min and 1.5 mL/min
Column Coating Reagent	Coating for 18 h with 1 mM Cetrimide at 0.2 mL/min flow rate
Other Parameters	All stainless steel tubing and needle assembly replaced with PEEK (polyether ether ketone)
Temperature	Room temperature (25 ± 1 °C)
Detection	Photo Diode Array (PDA) at 258 nm wavelength

Table 4.8.   Effect of different flow rates with retention time of different complexes.

Complexing ratio Cu (10 µM): Pb (10 µM):EDTA (10 µM)	Complex	Flow rate of mobile phase (ml/min)	Retention Time, RT (min)	Capacity Factor (k')
1:1:1	Pb-EDTA	0.5	12.85	1.30
	Cu-EDTA		16.85	2.01
1:1:2	Pb-EDTA	0.5	12.96	1.32
	Cu-EDTA		16.93	2.03
1:1:1	Pb-EDTA	1.0	6.28	1.32
	Cu-EDTA		8.18	2.01
1:1:2	Pb-EDTA	1.0	6.30	1.32
	Cu-EDTA		8.22	2.03
1:1:1	Pb-EDTA	1.5	4.57	1.24
	Cu-EDTA		5.12	1.49
1:1:2	Pb-EDTA	1.5	4.60	1.26
	Cu-EDTA		5.15	1.51
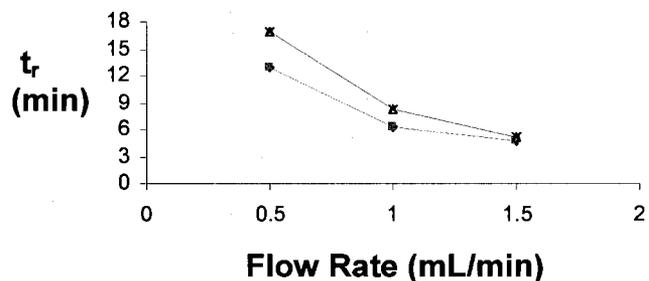
Figure 4.8.   Plots of the retention time, t_r, of Pb-EDTA (♦ 1:1:1 and ■ 1:1:2), and Cu-EDTA (▲ 1:1:1 and × 1:1:2) versus flow rate of the mobile phase.

4.3.5. Influence of Temperature

An increase in the column temperature shifted the equilibrium towards desorption which also led to a decrease of the capacity factors[175]. The effect of the column

temperature on the capacity factors was quite small in this range (10-35 $^\circ$C)[176]. However, an increase in the column temperature resulted in a decrease of the capacity factors.

System pressure is also affected by temperature change. The viscosity of the mobile phase will decrease with increasing temperature. For example if the HPLC system pressure is too high for a given solvent system the chromatographer may choose to raise the temperature of the column compartment to 40°C or even 60°C. The retention decreased with increasing temperature for all solutes and there was a decrease in the reduced plate height. But the net result for the actual solutes was lower resolution with increasing temperature[177]. This study strictly followed the laboratory temperature at 25±1° C, there was therefore to effort this study.

4.3.6. Post Column Reagent

Previous work[166] on varying this project to work with post-column reagent $Fe(NO_3)_3$ to produce the best separation of Cu-EDTA and Pb-EDTA complexes did not find any significant improvement. Table 4.5 shows the set up of experiments in this study for the post column use.

The suitability of this method also depends on the two chemical factors: (i) high thermodynamic stability of the complexes formed in reactions in the coil and (ii) rapid kinetics of ligand substitution in both reactions.

The stability constant of the Fe(III)-EDTA complex (log k_1 = 25.1) is higher than that of the Cu(II)-EDTA (log k_1 = 18.8) and Pb(II)-EDTA complex (log k_1 = 18.0). To work with Fe(III), it was necessary to use a mobile phase of pH 4.5 in order to obtain

satisfactory peak shape[178]. The post column reagent iron (III) nitrate for this study was prepared in sodium formate – formic acid buffered at pH 4.5. In this study the mobile phase followed the pH at 7.0, therefore when the two solutions were mixed together (mobile phase and post column reagent) in the coil their combined pH may be shifted to between 4.5 and 7.0.

In the case of Fe(III)-EDTA, the free Fe^{3+} concentration at neutral pH is very low due to the insolubility of iron hydroxides. Hence the conversion of Cu-EDTA and Pb-EDTA into Fe(III)-EDTA could be affected by use of high pH.

It was also evident that the Fe(III) complex is thermodynamically favoured over all other metal complexes, but some of the other complexes are also kinetically slow. Therefore, it seemed advisable to heat the samples after adding Fe(III) and acid to accelerate the equilibration[179] because some metal-EDTA species react very slowly with Fe(III).

Table 4.9.   HPLC conditions for complex separations with Post Column reagent.

Columns	Analytical Column: Varian Microsorb MV C_{18} 25 cm × 4.6 mm ID, 5 µM particle size Guard Column: Opti-Guard C_{18}, 1.0 mm I.D. (USA)
Mobile Phase	6 mM Na_2SO_4 with 1 mM HEPES pH 7.0 adjusted by NH_4OH
Column Coating Reagent	Coating for 18 h with 1 mM Cetrimide at 0.2 mL/min flow rate
Post Column Reagent	Condition A: use of only 6.0 m coil Condition B: 50 mM sodium formate-150 mM formic Acid with 40 µM $Fe(NO_3)_3$ without coil Condition C: 50 mM sodium formate-150 mM formic Acid with 400 µM $Fe(NO_3)_3$ with 6.0 m coil
Flow Rate	1.5 mL/min
Other Parameters	All stainless steel tubing and needle assembly replaced with PEEK (polyether ether ketone)
Reaction Temperature	Room temperature (25 ± 1 °C)
Detection	Photo Diode Array (PDA) at 258 nm wavelength

Based on this expectation it would be advisable to heat the solution at 90 °C for 0.5 h. But all other stages of the experiment in this study were performed at room temperature i.e. 25° C so the exchange of Cu-EDTA and Pb-EDTA into Fe(III)-EDTA was not suitable in this parameter as there is no important change of the peak heights and also peak retention times of these complexes (Figure 4.9). Calculation of the capacity factor was found to yield little increase for (1:1:1) complex (1:1:2) in Figure 4.10. From this analysis this study found no value to using a post column reagent of Fe(III) salt.
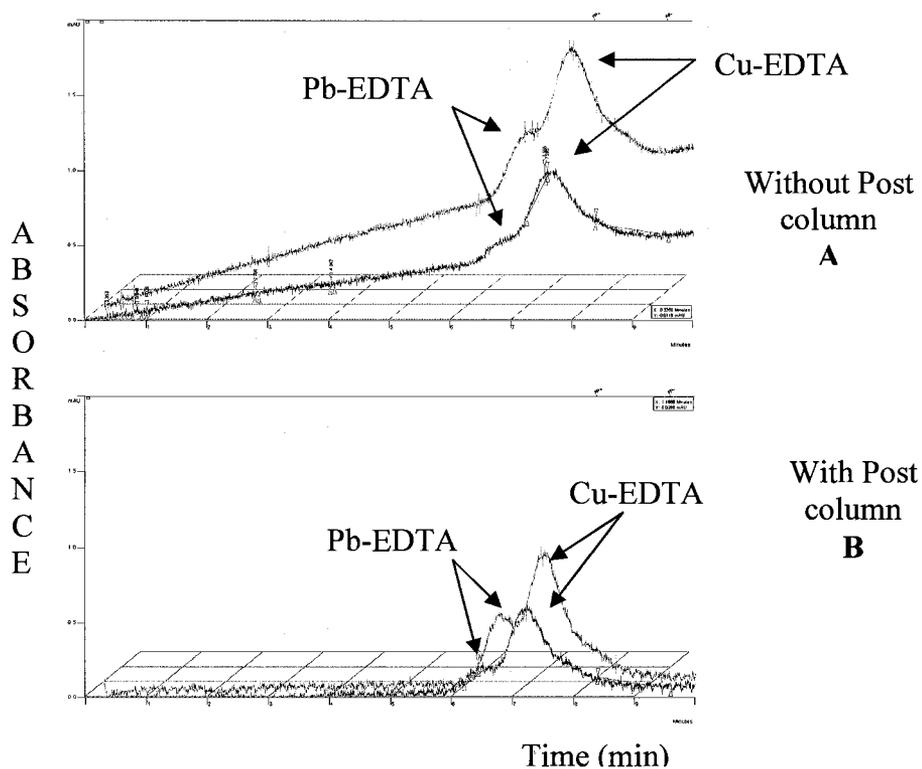
Figure 4.9. Chromatogram of Cu:Pb:EDTA (1:1:1) and Cu:Pb:EDTA (1:1:2) complexes using the mixing coil.
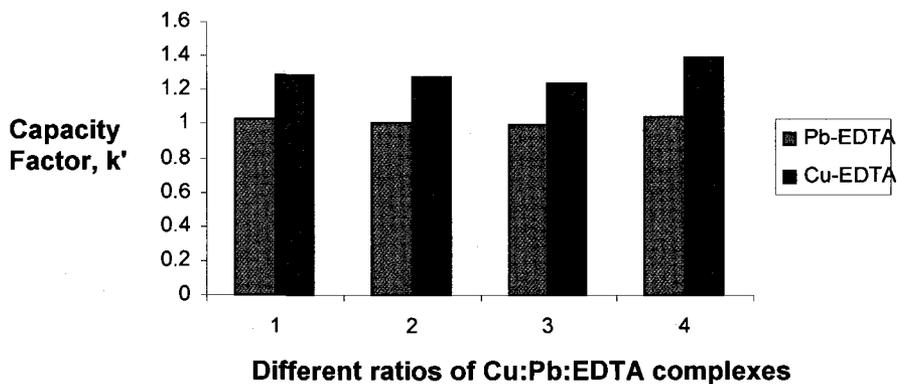
Figure 4.10.   Chromatogram of Cu:Pb:EDTA (1:1:1) and Cu:Pb:EDTA (1:1:2) complexes using the mixing coil (1 & 2 without post column) and with post column (3 & 4).

4.3.7. Nature of the Competing Ligand

The affinity of the competing ligand ions (EDTA mostly as HY^{3-}) to the stationary phase is governed by the same factors that affect the affinity of the solute ions, i.e., charge density, degree of hydration, polarizability, etc. Ligand ions of higher affinity to the stationary phase are stronger, and will result in lower interactions of the sample ions with the stationary phase; hence, lower retention times. In this experiment, Pb-EDTA eluted before Cu-EDTA. The concentration of the ligand ion in the mobile phase affects the retention of the sample ions. As well, higher concentrations result in stronger competition, and displacement of the sample ions from the stationary phase; hence, lower retention. The Cu-EDTA stability constant ($K_f = 6.3 \times 10^{18}$) is greater than the Pb-EDTA ($K_f = 1.1 \times 10^{18}$), therefore the expected retention time of Pb-EDTA should be shorter than that for the Cu-EDTA complex.

To test the stability of metal-EDTA complexes with the ion-pairing anion-exchange procedure, varying concentration of Cu and Pb and EDTA were tested using isocratic elution (flow rate at 1.0 mL/min) with optimum mobile phase (1 mM HEPES with 6 mM Na_2SO_4 at pH 7.0). The chelator (EDTA) and the cetrimide ion-pairing agent that is coating the column both compete for metal ions. Therefore, the stability of MeL (i.e. metal-ligand complex) is an important prerequisite that can determine whether the complex remains intact or is decomposed during chromatography, whereby the metal can possibly interact with the ion-pair coating on the column. The variation of capacity factors with EDTA concentration is shown in Table 4.10 ranging from 1.0×10^{-5} to 2.0×10^{-5} M. There is a slow decrease of the retention for Pb-EDTA and Cu-EDTA complexes alone with different ratios of Cu:Pb:EDTA (1:1:1) and Cu:Pb:EDTA (1:1:2) complexes. This behaviour is observed not only for metal ions but also for the EDTA anion. The conclusion is that this retention variation with EDTA concentration is due to a modification of the ion pairing equilibrium and the formation constant of those metal ions with EDTA [Pb-EDTA - 1.1×10^{18} and Cu-EDTA – 6.3×10^{18}, respectively].

Table 4.10.   Effect of different EDTA concentration with different metal ions.

Complex Ratio		EDTA Conc. (μM)	Metal ion Conc. (μM)	Retention time, t_r (min)	Capacity Factor (k')
Pb-EDTA (1:1)		10	10	4.60	1.26
Cu-EDTA (1:1)		10	10	5.14	1.50
Cu-Pb-EDTA (1:1:1)	Pb-EDTA	10	10	4.82	1.39
	Cu-EDTA	10	10	5.68	1.82
Cu-Pb-EDTA (1:1:2)	Pb-EDTA	20	10	4.82	1.39
	Cu-EDTA	20	10	5.61	1.83

This sequence can again be explained by the lower charge density of [M-EDTA] formed by larger metal ions. In fact, it is possible to explain the retention increase for high concentrations of EDTA because once the complex is formed an increase of the EDTA concentration causes an increase in the concentration of uncomplexed, negatively charged EDTA giving rise to a competing effect with respect to the metal complexes for the formation of the ion pair (shown in Figure 4.11 and 4.12). The influence of the EDTA concentration on the complexation equilibrium is observed when a broadening in the chromatographic peak and a loss in sensitivity is obtained for low values of EDTA concentration. In fact, an increase in the EDTA concentration displaces the equilibrium towards the formation of the complex, implying an increase in the amount of complex formed for the same amount of metal ion injected; that is, an increase in sensitivity. Likewise, high EDTA concentrations improve the peak shape matching the results obtained by other authors[180]. For the above-mentioned reasons, this study chose a concentration of 10^{-6} M EDTA in order to obtain good retention and selectivity.
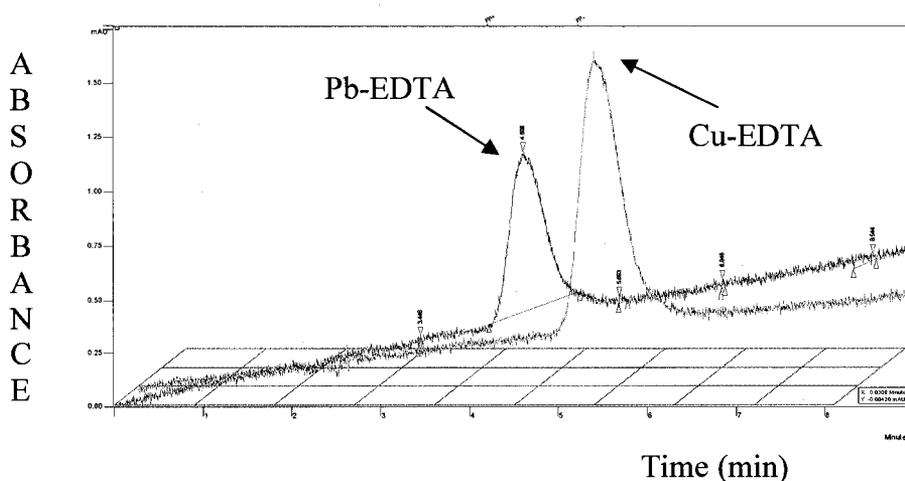
Figure 4.11.   Chromatogram of Pb-EDTA (1:1) and Cu-EDTA (1:1) complexes with 1.0 mM coating and without Organics.
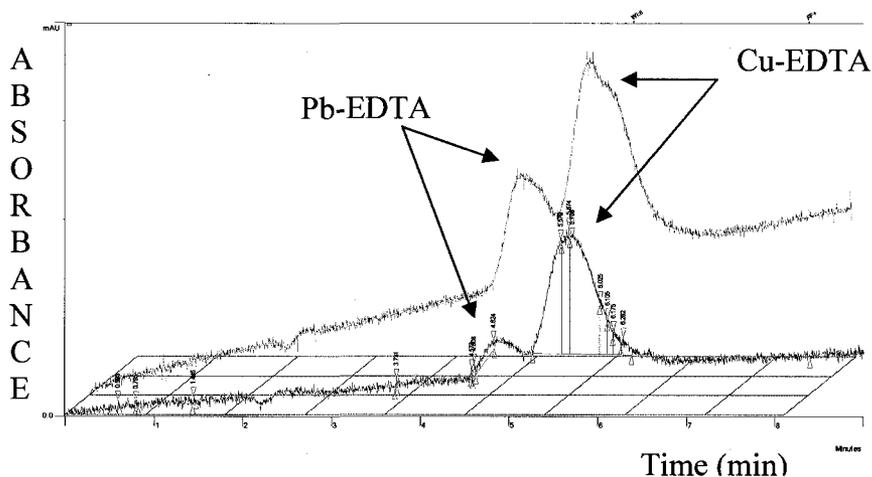
Figure 4.12. Chromatogram of Red - Cu:Pb:EDTA (1:1:1) and Lime Green-Cu:Pb:EDTA (1:1:2) complexes at different EDTA concentration (10.0 μM and 20.0 μM respectively).

4.3.8. Effect of Metal Ions

Due to their strong complexing properties DTPA, EDDS, EDTA and NTA do not occur as free acids in environmental samples but are complexed with different metal ions. Therefore, the presence of cations in the samples could alter the separation and LC detection of these compounds. Two cations, Cu(II) and Pb(II) were selected for this study.

Metal ions are useful to characterize organic ligands dissolved in seawater[181]. Copper is a candidate due to its strong complexation ability. The study of Cu-complexing capacity of dissolved organic matter in natural water has been developed since about 1970[182,183]. Measurements of the metal complexing capacity of natural water have been widely adopted as a methodology for chemical speciation. The complexing capacity of a

sample, defined as moles of added metal (usually Cu^{2+}), which are complexed per litre of sample, is a measure of the metal-buffering capacity, and is of fundamental importance for a quantitative assessment of the fate of polluting metals in natural water[182]. The complexing capacity is also a measure of the abundance of the organic ligand in natural water. However, Cu is insufficient to specify organic ligands because Cu^{2+}, belonging to boundary metal ions in Pearson's classification[184], forms complexes with ligands having oxygen, nitrogen and sulfur as a donor atom. In order to characterize organic ligands in natural water, the use of typical hard or soft metals is effective and organic speciation studies based on this concept have been carried out[184].

Samples containing 1.0 µM of the chelating agent with variable amounts of each of the cations were prepared and the chromatograms were obtained for the calibration curve. The variation of EDTA (1.0×10^{-5} to 2.0×10^{-5} M) with 1.0×10^{-5} M of both metal ions was also studied.

If metal ions are present at concentrations higher than their tolerable limits (>10.0 µM), a sample that contained both free and complexed metal ions need to be percolated. After that free cations were retained in the column, whereas the complexes pass unhindered. The resultant solution only contains the cations in the form of the 1:1 complexes of these ligands, and therefore is appropriate for the analysis. This project only tested copper, lead and EDTA as 1:1:1 complex and 1:1:2 complex, to study the complexation behaviour of EDTA towards copper and lead for these two ratios (1:1:1) and (1:1:2).

4.4. Measurement of the Analytical Responses

4.4.1. Calibration Curves

To obtain a calibration curve for a series of experiments with concentration ranging from 0.0 to 1.0×10^{-6} M standard solutions of Copper with constant 1.0×10^{-6} M EDTA and Lead from 0.0 to 1.0×10^{-6} M standard solutions with constant 1.0×10^{-6} M of EDTA were prepared and analyzed. The calibration curves were prepared using the method of least squares regression. Correlation coefficients (r^2) were 0.9988 and 0.9971 for Cu and Pb, respectively. Figure 4.13 and Figure 4.14 show the examples of a typical calibration curve obtained for a series of Cu-EDTA and Pb-EDTA solutions.
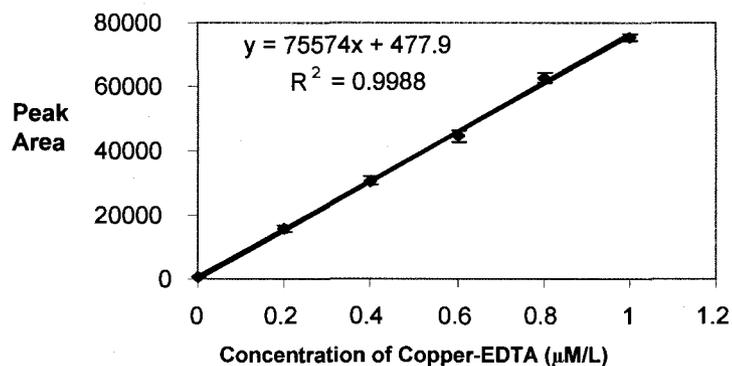
Figure 4.13.   Calibration curve for Cu-EDTA standard solutions.
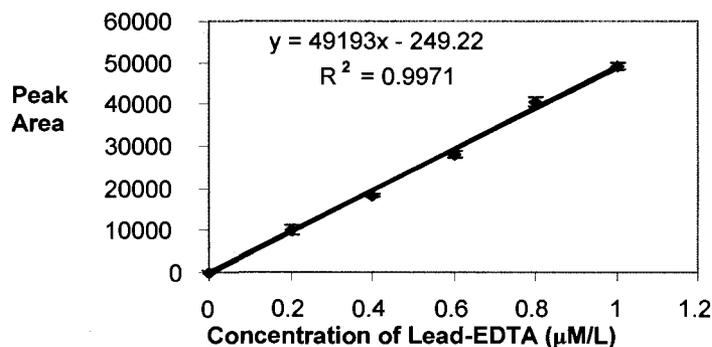
Figure 4.14. Calibration curve for Pb-EDTA standard solutions.

4.4.2. Limit of Detection

In analytical chemistry, the detection limit, lower limit of detection, or LOD (limit of detection), is the lowest quantity of a substance that can be distinguished from the absence of that substance (a *blank value*) within a stated confidence limit (generally 1%)[185,186].

Marine sediment blank sample PACS-2 was analyzed by online HPLC microextration separation methodologies to determine the limits of detection (LD) of the analytical instruments. The detection limit is estimated from the mean of the blank, the standard deviation of the blank and some confidence factor, i.e. three times standard deviation of the blank.

The limit of detection for the calibration curve typically falls in the range of 0.08×10^{-8} to 7.35×10^{-8} M for Cu-EDTA and 0.11×10^{-8} to 7.13×10^{-8} M for Pb-EDTA,

with the average LOD being 4.4×10^{-8} M. This LOD is at least one order of magnitude lower than the concentration of the smallest copper and lead standard solutions used.

4.5. Statistical Analysis of Chromatographic Data

Under the experimental conditions described above, good correlation was observed between the peak area ratio of EDTA and the corresponding concentration as shown in Table 4.11. The correlation coefficient (r^2) and the standard error of estimate (S_r) of the calibration curves are also given, along with the standard deviation (S.D.) of the slopes and intercepts.

The limit of detection attained, as defined by IUPAC[185],[187] $LOD_{(k=3)}=kSa/b$, where b is the slope of the calibration graph and Sa is the S.D. of the blank signal, estimated on a signal to noise ratio of 3, were found to be 0.05 μg/L and 0.23 μg/L for Cu-EDTA and Pb-EDTA without pre-concentration, respectively. The limit of quantitation (LOQ) was also attained according to the IUPAC definition $LOQ_{(k=10)}=kSb/b$, and were found to be 0.16 μg/L and 0.77 μg/L for for Cu-EDTA and Pb-EDTA, respectively.

For the precision and accuracy of the method, values in Table 4.11 indicate for Cu-EDTA R.S.D.%=1.5–7.3 and $Er\%$=0.21-2.78. Moreover, the Pb-EDTA R.S.D.% and $Er\%$ values (Table 4.12) were ranged from 1.8 to 10.3 and 0.11 to 5.57, respectively. The accuracy of the method was also assessed by analyzing model solutions spiked with known amounts of (1 μM/L) EDTA corresponding to 0.2-1.0 μM/L of Cu and Pb solution variables. These experiments were performed in triplicate. The statistical evaluation of EDTA reveals its good linearity and reproducibility and led us to the

conclusion that it could be applied for determination of metal-EDTA complexes in environmental samples.

Table 4.11.   Accuracy and precision of the proposed HPLC method for the determination of different EDTA complexes.

EDTA Complex	Nominal Concentration (μM/L)	Mean ± S.D.	R.S.D. (%)	Error of Estimation (Er %)
Cu-EDTA	0.2	15807±1148	7.3	1.4
	0.4	30644±1281	4.2	0.2
	0.6	44639±1851	4.2	2.6
	0.8	62616±1370	2.2	2.8
	1.0	75400±1141	1.5	0.9
Pb-EDTA	0.2	10147±1045	10.3	5.6
	0.4	18398±347	1.9	5.2
	0.6	27960±723	2.6	4.4
	0.8	40393±1169	2.9	3.3
	1.0	48997±864	1.8	0.1

CHAPTER FIVE

5. APPLICATIONS OF THE METAL COMPLEX METODS TO THE STUDY OF A SEDIMENT FROM NATIONAL RESEARCH COUNCIL, CANADA

The methodologies described in the previous chapters for the separation of Cu-EDTA and Pb-EDTA were applied to samples from SRMs sediments PACS-2.

5.1. History of PACS-2

In this study, the marine sediment PACS-2 sample was obtained from National Research Council Canada (NRC-CNRC). This sediment was collected from Esquimalt Harbour, B.C[163].

5.2. SAMPLE PREPARATION AND ANALYSIS

In order to apply the separation method PACS-2 was chosen for further experiments. Only PACS-2 was chosen due to fixed time frame for this project. It was assumed to survey the sorption/desorption of metal-complexes in aqueous slurries of sediments as presented in Figure 5.1. This material was used as received without wetting for this study. It is established that these type of sediments should be wetted before being used because wetting of the sediment makes a homogeneous mixture and thus improves its original chemical and physical characteristics in nature. Slurry composition was prepared by analyzing the strategy used by other researchers[120,153,156].

The sediment composition was chosen in such a way so that the pressure of the HPLC system is minimized. Higher slurry concentrations could clog the microfilter by build up of pressure.
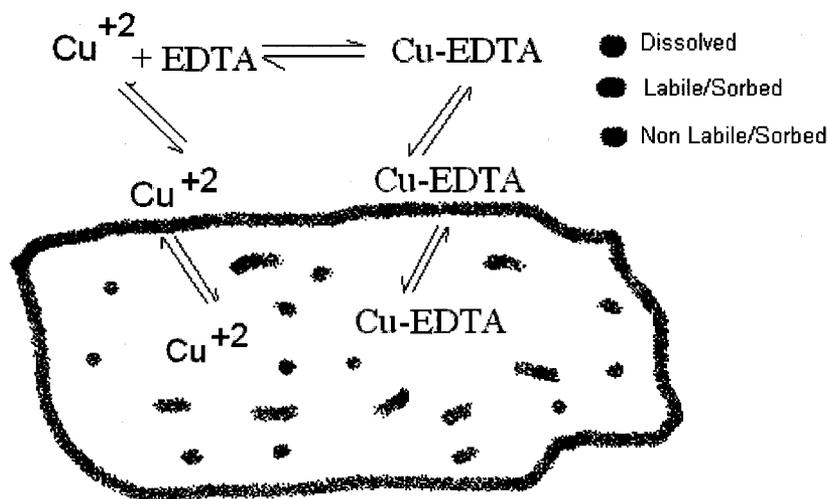

Figure 5.1.   Model of Cu with EDTA in aqueous slurries of sediments.

The final slurry composition was 50.0 ± 0.2 mg of sediment in 100.0 ± 0.5 mL of solution. A flowsheet for this sample preparation is shown in the Figure 5.2.

```
┌─────────────────────┐              ┌─────────────────────┐
│ 10 ml of 1mM        │              │  Marine Sediment    │
│ NH₄NO₃ buffer and   │              │  PACS-2 (0.5 g)     │
│ 90 ml of 1 µM EDTA  │              │                     │
└─────────────────────┘              └─────────────────────┘
           │                                    │
           │                                    │
           └──────────────┐      ┌──────────────┘
                          ▼      ▼
              ┌──────────────────────────┐
              │  Mixing together in a    │
              │  closed vessel with      │
              │  constant stirring       │
              └──────────────────────────┘
                          │
                          ▼
   Discard by       ┌──────────────────────┐
   backflushing  ◄──│  C₁₈ Reverse         │
                    │  Phase Column        │
                    └──────────────────────┘
                          │
                          ▼
                    PDA Detection
```
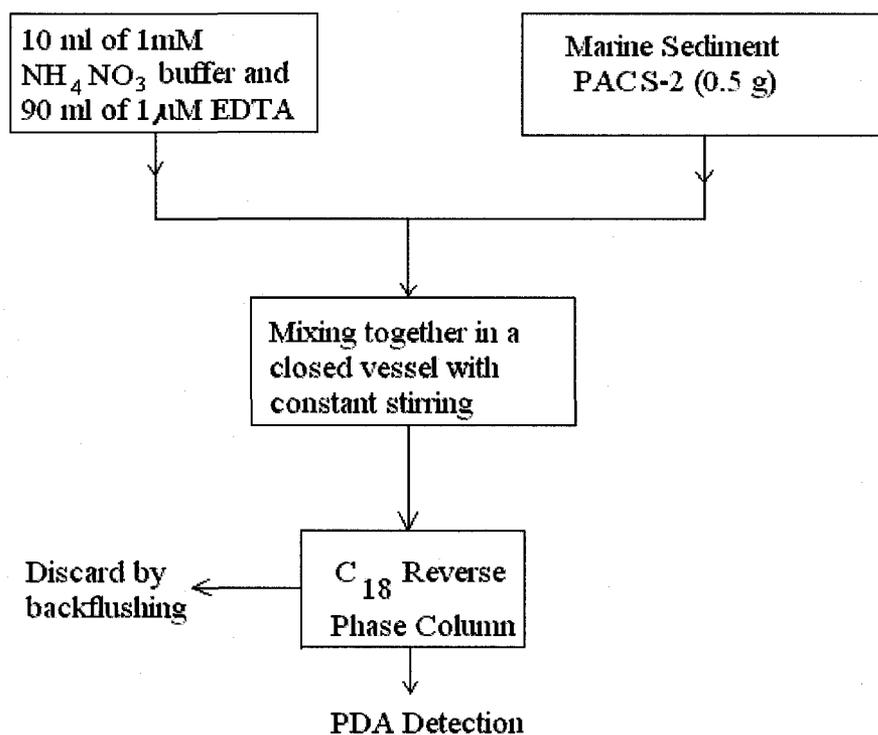
Figure 5.2. Flow sheet for sample preparation for the HPLC analysis of marine
            sediment PACS-2.

5.3. High Performance Liquid Chromatography Systems

5.3.1. Online Microfiltration Analysis Setup

For online microfiltration system, the conventional HPLC system [Varian,

Canada] was used to work with the solid particles. The instrumental set up consisted of a

ProStar 230 solvent delivery system, a ProStar 330 photo-diode array (PDA) and an

injection system, all in series. The injection system was modified to carry out the

subsequent removal of the sorbent particles by forcing a solvent to flow at countercurrent

(i.e., backflush) through the extraction cell. This injection system is comprised of two valves: 1) an injection valve [Rheodyne 7125, Altech] equipped with a 100 μL sample loop and, a column inlet microfilter (i.e., the extraction cell) set with a 0.5 μm stainless steel frit [Rheodyne 7335, Altech] and; 2) a two-position, six-port switching valve [Rheodyne 7000, Altech]. An HPLC ternary pump [Rose Scientific] connected to one of the ports of the injection valve was used to backflush of the microfilter. This injection system is presented schematically in Figure 5.3.

5.3.2. Working with Online Microfiltration

The LC conditions for the HPLC system are presented in Table 5.1. For the online microfiltration system, cleaning of the microfilters was performed after each injection of slurries by forcing the mobile phase to flow through the microfilter in the reverse direction or countercurrent of sample injection, thus removing the particulate matter from the microfilter cartridge. This process is hence forth referred to as backflushing the microfilter. This process was checked with blank slurry composition before using the metal-EDTA spiked sediment mixture.

Table 5.1.   HPLC conditions for complex separations with sediment particles.

Columns	Analytical Column: Varian Microsorb MV C_{18} 25 cm × 4.6 mm ID, 5 μM particle size
	Guard Column: Opti-Guard C_{18}, 1.0 mm I.D. (USA)
Mobile Phase	Conditions: 10:90 v/v Methanol: 6 mM Na_2SO_4 with 1 mM HEPES pH 7.0 adjusted by NH_4OH
Flow Rate	1.0 mL/min
Column Coating Reagent	Coating for 18 h with 1 mM Cetrimide at 0.2 mL/min flow rate
Other Parameters	All stainless steel tubing and needle assembly replaced with PEEK (polyether ether ketone)
Temperature	Room temperature (25 ± 1 $^{\circ}C$)
Detection	Photo Diode Array (PDA) at 258 nm wavelength

CONFIGURATION A

CONFIGURATION B

CONFIGURATION C

CONFIGURATION D

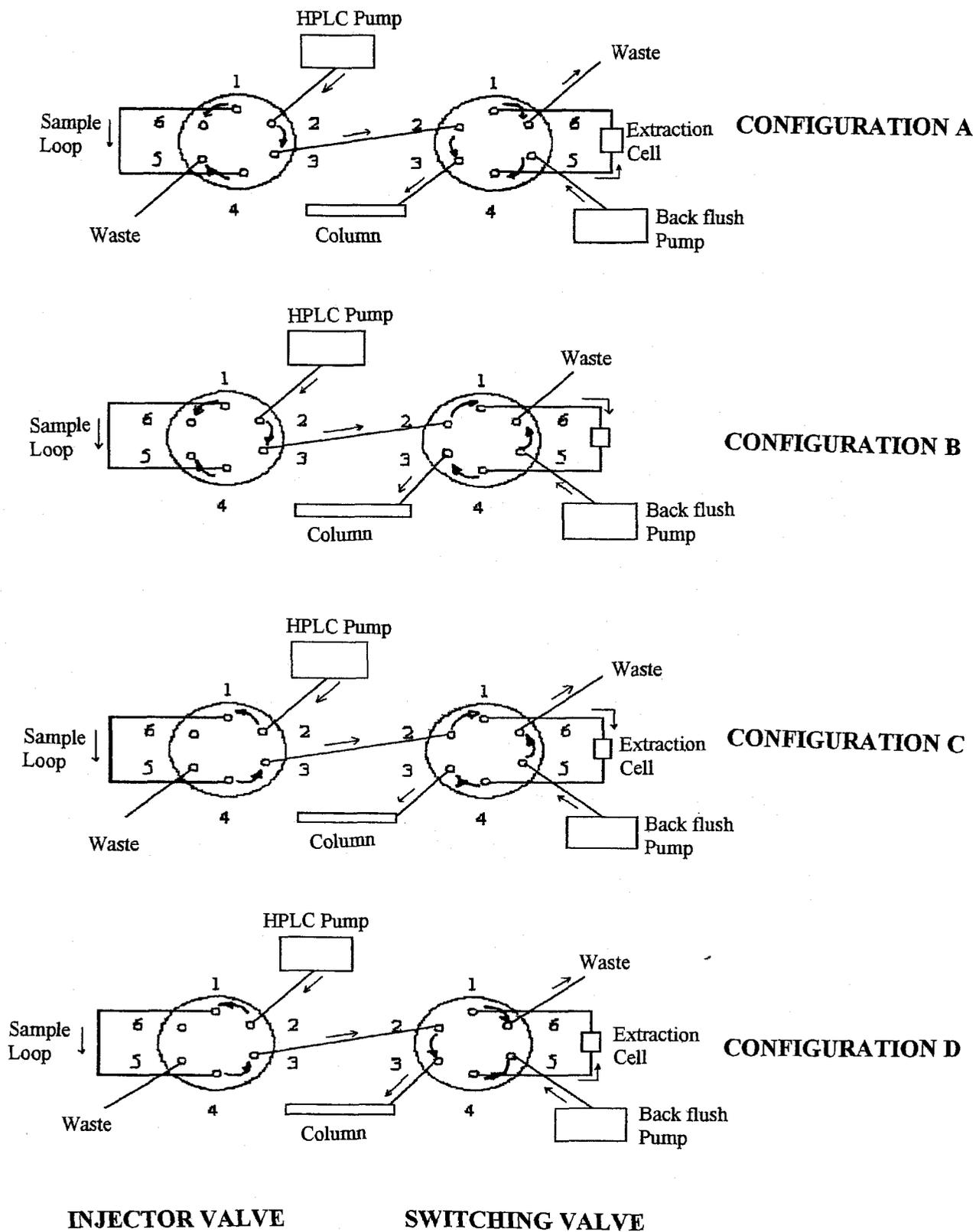**INJECTOR VALVE**   **SWITCHING VALVE**

Figure 5.3.   Graphic representation of injection mode of online micro extraction system. Adapted from Ref. 156.

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The stepwise operation of the injector and switching valves during an online microextraction HPLC experiment is demonstrated in Figure 5.3. At the time of sample loading the valves position are presented in Configuration A of Fig. 5.3. Sample is injected to the 100 μL sample loop and excess amount of sample is removed to waste by port No. 5 of the injector valve. The switching valve is then rotated (Configuration B) allowing the mobile phase from the HPLC pump to flow through the extraction cell. The sample slurry is then loaded in the extracted cell by positioning the injector valve in the inject position (Configuration C). Solid particles are filtered out by the microfilter of the extraction cell to prevent clogging of the HPLC flow system. Dissolved and extractable species from sample and the mobile phase continue to go through the guard column, analytical column and finally the PDA detector. The slurry was extracted for 30 seconds, after which the extraction cell was isolated from the rest of the HPLC system by rotating the switching valve back to its initial position. A rinse solution (90% HEPES & Na_2SO_4 – 10% methanol) was forced through the extraction cell at countercurrent via port No. 5 of the switching valve at a flow rate of 10 mL/min for 5 min using a separate HPLC pump.

This rising step or backflushing help to clean out the extraction system. If the solid particles are presented in the system they will cause increase of pressure and then the system can be shut down. So, monitoring of HPLC pressure requires inspection for the possibility of sample clogging.

Samples were taken by injecting 0.100 mL aliquots of metal-EDTA complex spiked slurries (50 mg sediment/mL solvent) in the online microfiltration HPLC system. The samples were left in the online extraction cell for different extraction times and the resultant peak area was used for further calculation.

It was not possible to do the offline separation technique due to time constraints arising from the construction of Science Building Complex of Saint Mary's University.

5.4. Results and Discussions

Table 5.2 and Table 5.3 below shows the results for Pb-EDTA and Cu-EDTA spiked in the SRM PACS-2. The complexes were quantified using the methodology developed in this research. The blank value was quantified with three times standard deviation and was found 1.32 nM (0.08 µg/L) and 2.03 nM (0.42 µg/L) for Cu-EDTA and Pb-EDTA using calibration curve (Figure 4.13 and Figure 4.14). The method yields good results that show it is fully effective for sediment matrices. It is also shown that for sediment with 1:1:2 complex, the peak areas for both metal complexes (Cu-EDTA and Pb-EDTA) decreased due to adsorption (Figure 5.4).

Table 5.2.   Complexation behaviour of different complexes in the PACS-2 sediments.

Complexing ratio		Complex	Area after 2 min of mixing	Area after 1 h of mixing
Cu (10 µM): Pb (10 µM):EDTA (10 µM)	1:1:2	Pb-EDTA	38630	12472
		Cu-EDTA	51166	24605
Cu (10 µM): EDTA (10 µM)	1:1	Cu-EDTA	52729	81473
Pb (10 µM) :EDTA (10 µM)	1:1	Pb-EDTA	55278	64918

Table 5.3.   Retention behaviour of different complexes in the PACS-2 sediments.

Complexing ratio		Complex	Capacity Factor, k', 2 min later	Capacity Factor, k', 1 h later
Cu (10 μM): Pb (10 μM):EDTA (10 μM)	1:1:2	Pb-EDTA	1.06	1.69
	1:1:2	Cu-EDTA	1.71	2.13
Cu (10 μM): EDTA (10 μM)	1:1	Cu-EDTA	1.80	1.46
Pb (10 μM):EDTA (10 μM)	1:1	Pb-EDTA	1.22	0.91

It was also found that for both single (Pb or Cu with EDTA) complexation the peak area increased (Fig. 5.5 and 5.6). It was not consistent with Cu:Pb:EDTA of 1:1:2 complex. It could be explained by many different ways, like as: 1) for this analysis only 50 mg of sediment sample was taken, which was not representative of the whole sample according to the certificate value (Appendix-I), 2) this sediment sample was not wetted before use, as wetting could improve the chemical and physical nature of sediment, and 3) there may be some kinetic factors that could influenced during this analysis. Another interesting observation was that the capacity factor decreased with time for both Cu-EDTA and Pb-EDTA complexes, while for the Cu:Pb:EDTA (1:1:2) complex the capacity factor increased with time. Figures 5.4 to 5.6 show different chromatograms for this sediment with the different complexes. It shows that the peaks are well separated and quantified so this method is an excellent method to study the distribution of metals with respect to the ligands in sediment particles.
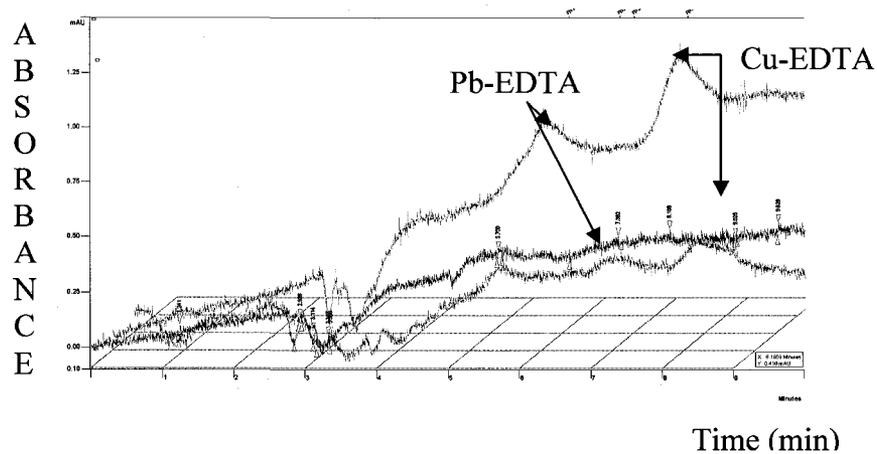
Figure 5.4.   Chromatogram of Cu:Pb:EDTA (1:1:2) complexes with Sediment PACS-2. [Red-sediment only no spiking, Green-Cu:Pb:EDTA (1:1:2) complexes with sediment, Purple-Cu:Pb:EDTA (1:1:2) complexes 1 h later].
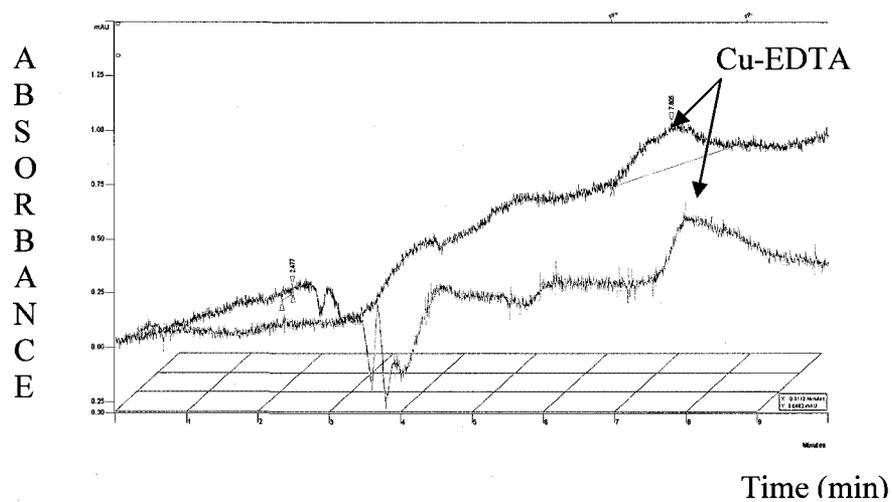

Figure 5.5.   Chromatogram of Cu:EDTA (1:1) complexes with PACS-2 Sediment. [Red-sediment with spiking 2 min later, Green-Cu:EDTA (1:1) complexes with sediment 1 h later].
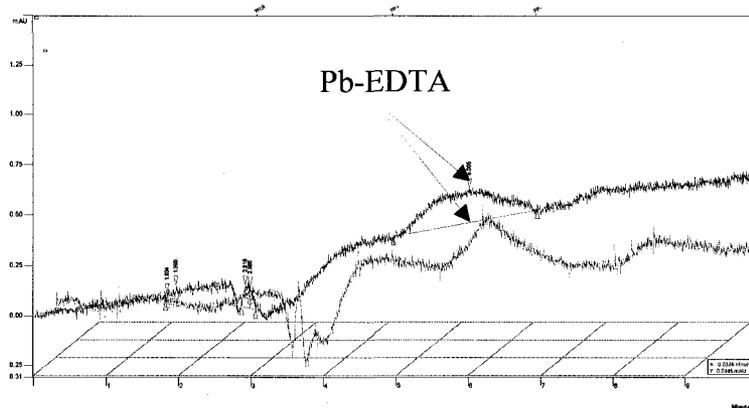
Figure 5.6.   Chromatogram of Pb:EDTA (1:1) complexes with Sediment. [Red-sediment with spiking 2 min later, Green-Pb:EDTA (1:1) complexes with sediment 1 h later].

CONCLUSIONS

A number of chromatographic separation methodologies for Cu-EDTA and Pb-EDTA complexes were developed by reverse phase anion exchange liquid chromatography with PDA detection system. Based on the experimental results and image analysis performed in this research, the following conclusions can be drawn:

1. Advantages over previously developed complex separations method include improvements in analyte resolution, reductions in analysis time, reduction of use of chemicals, increased analyte capabilities and/or reduced limits of detection.

2. This highlighted the benefits of RP-LC such as superior analyte capabilities and measurement precision, which were linked to the greater resolution, higher sensitivity, and more reproducible peak integration by this technique. Reverse phase anion exchange chromatographies were developed and considered to be a more time-efficient option for certain analytes of interest.

3. An existing RP-LC method for Cu-EDTA and Pb-EDTA was improved to enable longer retention times of previously found with post column reagent for such complexes. The addition of organic modifiers significantly increased the sensitivity for those complexes.

4. The developed methodologies formed the basis for evaluating a range of extraction techniques (ion-pair agent, temperature, mobile phase pH, addition of organic modifier, concentration of ligand and use of post column reagent) for the extraction of Cu-EDTA and Pb-EDTA complexes from environmental matrices, including sediments.

5. The SEM micrographs showed the elemental composition of the marine sediments PACS-2 and HISS-1, and are compatible with XRF analysis from previous work. The X-ray analysis revealed the presence of different metal atoms bonding crystal structure of minerals.

6. Ion exchange can be considered as an important mechanism for the adsorption of analytes in stationary and mobile phase.

7. This study showed that the Pb-EDTA complex elutes earlier than Cu-EDTA due to the high charge of lead.

8. It was observed that the IPA concentration strongly affects the retention times of the two complexes.

9. The efficiency of complex separation was influenced by the addition of organic modifiers. Use of a different modifier resulted in different retention times.

10. Best separation of complexes was achieved by use of 10% methanol at a flow rate of 1.0 mL/min.

11. The pH of the mobile phase was found to affect the degree of separation of the metal-EDTA complexes. It was found that a lower and higher pH may affect the equilibrium of complexes. The best results were at neutral pH (7.0).

12. For our purposes the effect of post column treatment with Fe(III) on the exchange process was not found to be significant for metal-complex separations.

Although several of the techniques examined delivered promising results for the extraction of Cu and Pb complexes in a range available SRMs, Reverse Phase Anion Exchange was selected for further method development in an attempt to develop a novel simultaneous extraction approach for EDTA complexes for different elements (Cu and

Pb). This method was also chosen because the technique provided the greatest scope for accurate data for separate extractions of complexes from sediments as well as Cu-EDTA and Pb-EDTA from a range of marine SRMs.

RECOMMENDATIONS FOR FUTURE WORK

The separation methodologies for Cu-EDTA and Pb-EDTA that were employed in this study are required to fully characterize the marine sediments for SRMs and also for the real world samples.

As we determining the metal quantitatively so that our target will be as much as contamination we can avoid to get accurate results. So in future all LC system should be metal free such as column, needle, syringe and fittings.

The separation and quantitation of metal complexes in a wide range of environmental samples would also warrant further investigation at different pH ranges, such as 6.5 and pH 7.5.

At the beginning of my research I planned to work with a coupled system like as LC-ICP-MS, but it was not possible for break down of the instrument. But for very low-level investigation this would be a highly sensitive technique to study further.

The kinetics of Cu-EDTA and Pb-EDTA were not explored in this study but is also an area that is important for future research.

The pre-treatment of sediment slurries should be considered to improve their physical and chemical reliability. Characterization of the wetting process of sediments for their homogenous behavior is necessary.

SEM analysis would not show significant changes that would matter (Cu and Pb concentrations too low). There would be differences, but almost entirely due to wetting and drying rather than any metal complex interactions.

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HISS-1, MESS-2, PACS-2

Marine Sediment Reference Materials for Trace Metals and other Constituents

The following tables show those constituents for which certified and information values have been established. Certified values are based on the results of determinations by at least two independent methods of analysis. The uncertainties represent 95% confidence limits for an individual sub-sample of 250 mg or greater.

Trace Metals
(milligrams per kilogram)

	HISS-1			MESS-2			PACS-2		
Antimony	(0.13)*			1.09	±	0.13	11.3	±	2.6
Arsenic	0.801	±	0.099	20.7	±	0.8	26.2	±	1.5
Beryllium	0.129	±	0.023	2.32	±	0.12	1.0	±	0.2
Cadmium	0.024	±	0.009	0.24	±	0.01	2.11	±	0.15
Chromium	30.0	±	6.8[†]	106	±	8	90.7	±	4.6
Cobalt	(0.65)*			13.8	±	1.4	11.5	±	0.3
Copper	2.29	±	0.37	39.3	±	2.0	310	±	12
Lead	3.13	±	0.40	21.9	±	1.2	183	±	8
Lithium	2.83	±	0.54	73.9	±	0.7	32.2	±	2.0
Manganese	66.1	±	4.2	365	±	21	440	±	19
Mercury	(0.01)*			0.092	±	0.009	3.04	±	0.20
Molybdenum	(0.13)*			2.85	±	0.12	5.43	±	0.28
Nickel	2.16	±	0.29	49.3	±	1.8	39.5	±	2.3
Selenium	0.050	±	0.007	0.72	±	0.09	0.92	±	0.22
Silver	0.016	±	0.002	0.18	±	0.02	1.22	±	0.14
Strontium	96.9	±	11.2	125	±	10	276	±	30
Thallium	(0.06)*			(0.98)*			(0.6)*		
Tin	(0.11)*			2.27	±	0.42	19.8	±	2.5
Uranium	(0.26)*			---			(3.)*		
Vanadium	6.80	±	0.78	252	±	10	133	±	5
Zinc	4.94	±	0.79	172	±	16	364	±	23
Tributyltin (as Sn)	---			---			0.98	±	0.13
Dibutyltin "	---			—			1.09	±	0.15
Monobutyltin "	---			---			(0.3)*		

*information value only
† see page 3

NRC · CNRC

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Matrix and Minor Constituents - Percent

	HISS-1			MESS-2			PACS-2		
Al_2O_3 (f,i,n,x)	1.37	±	0.09	16.2	±	0.49	12.5	±	0.6
C (e,r)		---		2.14	±	0.03		---	
CaO (f,i,n,p,x)	1.60	±	0.14		---		2.75	±	0.25
Cl (n,v,x)	(0.35)*				---		(3.)*		
Fe_2O_3 (f,i,n,p,x)	0.352	±	0.013	6.22	±	0.31	5.85	±	0.08
K_2O (f,n,x)	0.400	±	0.016		---		1.49	±	0.06
MgO (f,i,p)	0.124	±	0.027		---		2.44	±	0.22
Na_2O (f,i,n,p)	0.503	±	0.035		---		4.65	±	0.23
P_2O_5 (i,x)		---		0.28	±	0.03	0.22	±	0.01
S (i,x)		---		0.18	±	0.04	1.29	±	0.13
SiO_2 (f,x)	(93.)*			59.4	±	2.3	(59.)*		
TiO_2 (f,i,n,p,x)	0.127	±	0.006		---		0.739	±	0.053

Methods of Determination for Trace Metals

Antimony (b,g,h,i,n,q)
Arsenic (b,g,h,i,n,x)
Beryllium (b,g,i)
Cadmium (b,g,i,q,x)
Chromium (b,g,i,n,q,x)
Cobalt (b,g,i,n,x)
Copper (f,g,i,n,q,x)
Lead (g,i,q,x)
Lithium (g,i,q)
Manganese (b,g,i,n,x)
Mercury (a,c,q)

Molybdenum (g,i,q)
Nickel (g,i,q,x)
Selenium (b,g,h)
Silver (g,i,q)
Strontium (f,i,n,q,x)
Thallium (b,q)
Tin (b,g,i,q)
Uranium (q)
Vanadium (b,g,i,n,x)
Zinc (f,g,i,n,q,x)

Coding

a - Atomic fluorescence spectrometry
b - Inductively coupled plasma mass spectrometry
c - Cold vapour atomic absorption spectrometry
e - Coulometry
f - Flame atomic absorption spectrometry
g - Graphite furnace atomic absorption spectrometry

h - Hydride generation atomic absorption spectrometry
i - Inductively coupled plasma atomic emission spectrometry
n - Instrumental neutron activation analysis
q - Isotope dilution inductively coupled plasma mass spectrometry
r - Infrared spectrometry
x - X-ray fluorescence spectrometry

Not all the methods listed above were applied to all three certified reference materials.

Chemical Characterization of Marine Sediments from Reference[156].

Property	Units	PACS-2	HISS-1	Notes
pH		6.4	8.7	Measured as a 1:2 (mass:volume) ratio
Electrical Conductivity	mS/cm	21.09	4.39	Measured as a 1:2 (mass:volume) ratio
Carbon	% (w)	3.21 ± 0.15	0.39 ± 0.02	Soils results are expressed on an air dried basis
Sulfur	% (w)	1.55 ± 0.01	0.03 ± 0.01	
Nitrogen	% (w)	0.38 ± 0.03	nd	
Hydrogen*	% (w)	1.2	nd	*Estimated Quantitation Limit (EQL) 0.5. nd: not detected above standard EQL

Major Constituents				
SiO_2	%	56.24	95.00	X-ray fluorescence
TiO_2	%	0.698	0.146	
Al_2O_3	%	12.09	1.28	
Fe_2O_3	%	5.69	0.36	
MnO	%	0.051	0.005	
MgO	%	2.35	0.07	
CaO	%	2.73	1.57	
Na_2O	%	4.54	0.44	
K_2O	%	1.45	0.40	
P_2O_5	%	0.222	0.027	
V	ppm	116	26	
Cr	ppm	75	14	
Zr	ppm	135	163	
Ba	ppm	1031	389	
Ni	ppm	24	<3	
Zn	ppm	352	9	
Ga	ppm	13	<5	
Sr	ppm	272	109	
Nb	ppm	9	<1	
Total	%	93.32	100.03	
Loss of ignition	%	13.47	1.60	

Mineralogical composition				
Quartz (SiO_2)	%	62.6	99.4	X-ray powder diffraction analysis
Halite (NaCl)	%	14.3	0.6	
Albite, ordered ($NaAlSi_3O_8$)	%	18.8		
Clinochlore, ferron $(Mg,Fe,Al)_6(Si,Al)_4O_{10}(OH)_8$	%	2.8		
Calcite ($CaCO_3$)	%	1.5		

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