

DEVELOPMENT OF A NOVEL OFFLINE/ONLINE SEPARATION LC-ICP-MS
METHOD FOR THE ANALYSIS OF THE DISTRIBUTION OF METALS AND
METAL-COMPLEXES IN AQUEOUS SEDIMENT SLURRIES

by

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Abstract

DEVELOPMENT OF A NOVEL OFFLINE/ONLINE SEPARATION LC-ICP-MS METHOD FOR THE ANALYSIS OF THE DISTRIBUTION OF METAL AND METAL-COMPLEXES IN AQUEOUS SEDIMENT SLURRIES

By Duaa Abdu Hilbah

A new method for determining the distribution of trace metals in sediments containing complexing ligands such as EDTA and sediment was developed. This method required the coupling of an LC with an ICP-MS. The method uses an off-line or an on-line separation method prior to the injection of a sediment slurry containing both trace metals and EDTA. A certified estuarine sediment from NIST was identified and characterized as the test sediment to induce sorption of metals from water. The certified estuarine sediment was composed essentially of silica (SiO₂). The average concentration of C, H, and N in the sediment was 0.56 %, 0.20 % and 0.14 %, respectively. The LC separation was optimized using a weak anion exchange column containing a polystyrene divinylbenzene trimethylammonium stationary phase with a 5.4 mM sulfuric acid mobile phase. A sorption experiment was conducted, where target trace metals with EDTA are in contact with a sediment slurry. The preliminary results show that the metal sorption kinetics using time-dependent ICP-MS signals could be followed and measured.

June 13, 2016.

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LIST OF ABBREVIATIONS

CHN	Carbon, Hydrogen, and Nitrogen
EDS	Energy Dispersive Spectroscopy
EDTA	Ethylene-Diamine-Tetra-Acetic Acid
EPA	Environmental Protection Administration
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
HPLC	High-Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
NTA	Nitrilotriacetic Acid
PAHs	Polycyclic Aromatic Hydrocarbons
SEM	Scanning Electron Microscope
USEPA	U.S Environment Protection Agency
UV-vis	Ultraviolet-Visible spectrophotometry
XRD	X-ray Diffraction
TBABr	Tetrabutylammonium Bromide
WHO	World Health Organization
USGS	United States Geological Survey
DO	Dissolved Oxygen
PVDF	Poly Vinylidene Fluoride
TOC	Total Organic Carbon
PSDVB	PolyStyrene DiVinylBenzene

Chapter 1 INTRODUCTION

The study of heavy metals in environmental samples is important because of their potential negative impact on humans and the ecosystem. Heavy trace metals have become an increasingly serious environmental problem around the world due to heightened geologic and anthropogenic activities, and have become a focus of concern with regard to reducing global pollution.¹

Heavy metals refer to stable, high-density metals such as lead, cadmium, mercury, copper, nickel, and some metalloids such as arsenic and selenium. Most occur naturally in the crust of the Earth, and are present in air (as particulate matter), water (as dissolved and suspended matter), and soil. Because these metals can form complexes in the presence of ligands, it is difficult to predict or model the distribution of metals in the environmental system. Research indicates that most of the heavy metals, and their compounds, are toxic to humans.² The importance of studying heavy metals is illustrated from Table 1.1, which shows the route of entry of heavy metals into living organisms, as well as their toxic effects. As the table shows, these heavy metals have different toxicity levels depending on their nature, and in some cases can be carcinogenic. This area of study is all the more important because the contribution from heavy metals and their contents in air, soil, water and tissues of living organisms has increased greatly in recent years and continues to do so due to human activities.

Chen *et al.*³ reported that heavy metal contamination of the natural environment represents one of our most important problems in terms of human health and environmental quality. This danger starts at the very beginning of the food chain: uptake

of heavy metals by plants (including crops) can lead to high, accumulating levels of heavy metals along the food chain. About 100 ha of rural soils contaminated with Cd and Pb were identified by the Environmental Protection Administration of Taiwan (EPA-Taiwan) in 1988. This study mentioned that the concentration of Cd in brown rice growing at contaminated sites was higher than the critical health concentration of Cd (0.5 mg/kg dry weight) issued by the Department of Health of Taiwan.^{4,5}

Table 1.1: Toxicity of some trace heavy metals^{6,7}

Metal	Route of Exposure	Signs and symptoms of acute toxicity	Carcinogen
Cadmium	Inhalation and ingestion	Lung, liver and kidney damage; Irritation of respiratory system	Yes
Copper	Inhalation and ingestion	Brain, liver	No
Lead	Inhalation and ingestion	Headache, poor attention, loss of memory and appetite, nausea etc. Lung, and liver damage	Yes
Nickel	Inhalation, ingestion and skin absorption	Lung, prostate, liver and kidney damage	Yes
Zinc	Inhalation and ingestion	Brain, skin	No

Sediment contamination by heavy metals in rivers, lakes, and estuaries is also a major issue of environmental concern.¹ This transportation of heavy metals can result from anthropogenic activities, metal corrosion, atmospheric deposition, leaching in response to changes in environmental conditions, and distribution of water columns of rivers or lakes, as well as natural processes such as weathering and volcanic eruptions.⁶

Research indicates that the majority of the world's rivers and lakes are highly contaminated by the transportation of heavy metal pollution.¹

According to Waeles *et al.*,⁸ dissolved trace metals can be found in natural waters in the form of various chemical species, such as free hydrated cations, organic and inorganic complexes. These metals can be distributed as water soluble species, colloids, suspended forms and sedimentary phases within the aquatic environment. According to Salomons *et al.*,⁹ more than 99% of heavy metal entering into the aquatic environment are stored in various forms of sediments.

Importantly, heavy metals do not stay in sediments forever. They can re-enter the overlying water and become available to living organisms due to variation in water's physico-chemical characteristics.¹⁰ Thus, sediments act both as carriers and potential sources of metals in the aquatic environment.¹¹ The concentration of heavy metals can build up in sediments due to anthropogenic activities such as disposal of liquid effluent or via leachate-carrying chemicals originating from urban discharge and industrial wastewater.

The potential adverse effects metals might have on the biota and living organisms depends not only on concentration but also on their availability and mobility in soil or sediment. There are many factors that affect metal availability and mobility within soil or sediment, such as density, pH, the type of charge in soil/sediment colloids, and the degree of complexation with ligands.¹² Therefore, a thorough chemical and physical characterization of the test soil/sediment is required.

1.1. Metal species in the environment

The term “heavy metals” is most associated with transition metals, but also applies to some non-metals. The U.S. Environment Protection Agency (USEPA)¹³ classifies Cu, Pb, Cd, and Ni as priority pollutants in the environmental system. These metals are also called trace elements because of their presence in trace concentrations (ppb range to less than 10 ppm).¹⁴

Heavy metals are elements that have an atomic weight >20 and exhibit metallic properties. These properties include ductility, conductivity, cation stability and ligand specificity.¹²

The USEPA also identify these metals as important pollutants in the marine environment, due to their high toxicity, rapid accumulation in living organisms, and long persistence.¹⁵

The bioavailability of heavy metals is dependent on the nature of each metal ion present in the environment. Indeed, almost every metal can produce toxicity depending on their physico-chemical behavior. Some of them are important because they can generate toxicity at very low concentrations.¹³ A considerable number of articles have shown that the average Cd concentration in rice from some polluted areas of China was 0.59 mg/kg in 2006, which is 2.5 times higher than it was in 1987 and also higher than the Chinese Hygienic Standard for rice (0.20 mg/kg).¹⁶ Another study from Vietnamese Ministry of Health¹⁶ has shown that the Cd concentration in rice was 0.31 mg/kg, significantly higher than the maximum allowable concentration for Cd in rice (0.20 mg/kg) for Vietnam rice. According to Indian Journal of Pharmacology,¹⁷ the permissible limit for the heavy

metals and their effect on human health is: Cd, 0.06 mg/L; Pb, 0.1 mg/L; Cu, 0.1 mg/L; and Zn, 15 mg/L.

Sediment contamination by heavy metals in rivers and estuaries has become an issue of increasing environmental concern.¹³ Sediments have a high capacity for accumulating extremely low and undetectable concentrations of heavy metals from overlying waters. Therefore, the enrichment rate of heavy metals in river sediments is often a good indicator of the contamination level of rivers.¹⁸ Several researchers have demonstrated that heavy metals can be released to water bodies from sediments during dry seasons, thus increasing the potential ecological risk and toxicity to aquatic beings.¹⁹ El Bouraie, *et al.*²⁰ demonstrated that Rosetta Branch, the west part of the Nile Delta, suffered from various types of pollutants such as domestic, sewage, agricultural and industrial, and that their effects can become more pronounced during different seasons and years of low water flow in the river.

1.2. Heavy Metal Toxicology

Heavy metals are widely used as environmental monitoring factors because of their toxicity to humans, animals and plants.²¹ Although heavy metals are naturally found in the earth's crust, anthropogenic activities such as industrial production, mining, and smelting can lead to increased concentrations within the environment. Heavy metals, which are consequences of different processes such as chemical leaching of bedrock, water drainage and runoff from banks and discharge of urban industrial and rural

agricultural wastewaters, can serve as important indicators of water quality in the environment.²²

Some metals are essential to living organisms, whereas some are very toxic. Some of these heavy metals such as Co, Cu, Zn, Cr, Fe, Mg, Mn, Ni, Mo and Se are essential elements²³ with important biochemical and physiological functions, and an inadequate supply of these elements can lead to a variety of diseases or syndromes.²⁴

This work will focus on the distribution and interaction with organic ligand and sediments of five metals: Cu, Ni, Zn, Cd, and Pb. The rationale for selecting these five metals follows.

1.2.1. Copper

Copper (Cu, atomic mass 63.55 g/mol) is an essential trace element for all living organisms that can be found almost everywhere in the environment, including waters, sediments and soils. It is essential for normal healthy growth and reproduction in all higher plants and animals. Human blood cannot carry oxygen without Cu. The daily-recommended intakes of Cu for adults and children are 1.2 mg and 0.5-1 mg, respectively. According to the World Health Organization (WHO),²⁵ copper deficiency is more dangerous than copper toxicity, which can lead to health problems such as anemia, lungs, heart and circulation problems. Copper is also involved in the formation of collagen, the fibrous protein in bone, cartilage, tendons, and other connective tissue.²⁵ Agricultural production relies on proper levels of copper in soils. For example, with insufficient Cu levels, rice and wheat, the two most important crops in the world, grow in low yield and exhibit poor quality.²⁵

1.2.2. Nickel

Nickel (Ni, atomic mass 58.69 g/mol) is an abundant metal in the Earth's crust, and can be found in the environment at low levels. In the environment, the dissolution of soils, biological cycles, atmospheric fallout, industrial processes and waste disposal can lead to the accumulation of Ni in surface water.²⁶ Foodstuffs such as, butter nut (4.3 µg/g), cashew (5.0 µg/g), chocolate as cocoa powder (9.8 µg/g) and bittersweet chocolate (2.9 µg/g),²⁷ and fats contain high quantities of Ni. For some animals, Ni is essential in small amounts; however, it can be dangerous when concentrations exceed tolerable levels greater than 0.018 µg/m³ (by inhalation).^{28,29} Nickel can enter the human body via several different pathways: breathing air, drinking water, eating food and smoking cigarettes. Skin contact with nickel-contaminated soil or water may also result in nickel exposure.²⁹

1.2.3. Cadmium

Cadmium (Cd, atomic mass 112.41 g/mol), another naturally occurring metal, works as a cumulative poison in the environment. Cadmium is also known, in addition to lead, to bioaccumulate in the human body.³⁰ It is known to have a high degree of mobility and solubility compared to many other metals in soil, which allows it to easily bind with ligands.³¹ Cadmium and its compounds are highly toxic, and exposure to this metal is known to cause cancer.³² Each year, nearly 25,000 tons of cadmium are released into the environment. It is estimated that 10-30% of natural cadmium is transported by the wind and volcanic emissions.³² The main anthropogenic sources of cadmium are refining and

smelting, which lead to cadmium atmospheric loading that is mostly deposited into the bottom sediments.²⁶

1.2.4. Zinc

Zinc (Zn, atomic mass 65.39 g/mol) is common in the environment; in fact, it is the 23rd most abundant element in the Earth's crust.³³ Zinc can be found in different ores such as zinc blende (sphalerite), wurzite, smithsonite and hemimorphite. Zinc is also found in water, soil and air. Canada, USA, Peru and Russia and Australia are the world's largest zinc-producing countries. Drinking water becomes toxic when zinc concentrations become higher than the normal range. Due to the high prevalence of zinc, drinking water close to refineries, industry or sewage sludge can contain higher level of Zinc, which may cause health problems. Specifically, zinc at high concentrations can cause skin irritation, stomach cramps, vomiting, respiratory disorders, and may disturb protein metabolism. The recommended daily intakes of zinc for adults and infants are 8-11 mg and 2-3 mg, respectively.³³ Zinc also influences the activity of microorganism in soil, causing organic matter to break down slowly.

1.2.5. Lead

Lead (Pb, atomic mass 207.2 g/mol), also a naturally occurring element in the environment, is dangerous because it can accumulate in living organisms through entire food chains.³⁴ Human activities have resulted in an increase in lead concentrations in the environment partly because of leaded gasoline, which is responsible for an unnatural lead-cycle. Other human activities, such as fuel combustion, industrial processes and solid waste combustion, also contribute to an increase in lead concentration in the environment.

Lead can also be found in drinking water, mainly due to the corrosion of pipes. Very small concentrations of lead can result in lead poisoning in shellfish. Lead can also disturb phytoplankton, which is an important source of oxygen production. Because of the potential harmful impacts of lead, it is particularly important to study the ecological balances that are affected by lead pollution.³⁴

1.3. Transportation of heavy metals in the environment

The mobility and toxicological effects of heavy metals depend on the chemical properties of the metal species in surface interactions and exchange kinetics, which cannot be calculated from total concentrations of a metal.³⁵ The most important characteristic of heavy metals compared to other toxic pollutants is that they cannot easily be biodegraded.²⁶

For a given metal, the change in oxidation state is chiefly responsible for its unique physical and chemical properties. These oxidation states vary in their redox potential, complexation abilities, and hydration properties. The investigation of speciation of metals can distinguish the complexed and free forms of metal ions in order to identify their toxicity levels.³⁶ Sediments can add metals into a body of natural water through accumulation and incorporation, and can also release and remobilize metals into the water system when changes in physico-chemical conditions occur.²⁶ Accumulation of heavy metals in sediment may arise from discharge sources such as smelters, electroplating, paint and dye formulator industries, chemical manufacturing plants and petroleum refineries.²⁶

Copper and zinc, which are essential elements for life, can each form different oxidation states (e.g., Cu, Cu(I), Cu(II), and Zn, Zn(I) and Zn(II)).³⁶ The change of oxidation state of an element affects the degree of its bioavailability, as well as its toxicity. Cd and Pb may cause serious problems via accumulations within food chains.³⁷

Chen, *et al.*³⁸ indicated that most of the heavy metals are persistent in soil because of their immobile nature. Their geochemical forms can directly influence their phytoavailability, as well as the solubility of metals in soils.³¹

1.4. Sediment as environmental pollution indicator

Sediment is a naturally occurring material that is hard but loosely distributed on top of solid rock.³⁹ It is formed by processes such as weathering and erosion, and then transported by fluid flow, wind, glaciers and gravity.⁴⁰ There are various types of sediment, including detrital sediment, chemical sediment and biochemical sediment.³⁹ Sediment depends primarily on origin, with each of these groups formed by different natural phenomena. Detrital sediment is formed when weathering and erosion of rocks unearths at the surface. Chemical sediments form due to crystallization of minerals from waters containing different dissolved particles. Biochemical sediments originate from the hard parts of plants and animals and can be found on ocean floor.³⁹

Marine sediment differs from soil in a number of ways. In general, a marine sediment has a greater porosity than soil, with porosity ranging from 70-90% at the surface and from 50-60% at depths on the order of a meter or more. As well, pore water pHs vary between 7 and 8, which is a much narrower range than that shown by soils.

Finally, the surface layers of sediment are oxic but become anoxic with increasing depth.⁴¹

Sedimentation cycles originate from the natural processes, mostly forestry and agriculture. Sediments impact many aspects of ecosystems and urban life, including fisheries/ aquatic habitats, water supply, navigation, energy production and sinks for pollutants.⁴⁰ Sediments are important indicators of the aquatic organisms present in an estuarine system. They also play a role in biological habitats because toxicants can enter into food chains via their presence in sediments. When contaminants are introduced into water bodies, they have affinities for sediment particles and can cause environmental disturbances for aquatic organisms such as benthic habitats, which are environmental reservoirs for different types of contaminants.⁴²

The equilibrium between water bodies and the metal pollutants present in bottom sediments can be disturbed by natural and anthropogenic activities. Therefore, the analysis of just concentration and sediment toxicity cannot provide a complete understanding of the influence that contaminated sediments have on the environment. To understand toxicity from metals in sediment, benthic organisms that are affected by toxicants in sediment mixtures need to be studied.⁴²

1.4.1. Sources of contaminants in sediments

According to the United States Geological Survey (USGS),⁴³ point source contaminants are defined as, “Any substance that degrades water quality and originates from discrete locations such as discharge pipes, drainage ditches, wells, concentrated livestock operations, or floating craft.”⁴³ Environmental pollution is very common in

point source areas for mining and smelting of metals, as well as other metal based industrial operations such as the production of batteries.¹

According to the USEPA,⁴⁴ there are hydrocarbons or persistent organics, nutrients including phosphorous and nitrogen and five other different types of major pollutants present in sediments: bulk organics, halogenated compounds (e.g. ammonia), polycyclic aromatic hydrocarbons (PAHs), and heavy metals such as iron (Fe), manganese (Mn), and lead (Pb). Sediment contamination may represent point or non-point sources of pollution, depending on origin. When pollution comes from a specific, identifiable source such as a pipe, it is considered a point source. Examples of point sources include municipal sewage treatment, storm water discharges from municipal and industrial facilities, overflows from combined sanitary and storm sewers, and waste discharges from industry. Non-point sources of contamination cannot be traced to one specific source. They can originate from hazardous and solid-waste sites of storm water runoff, croplands runoff, livestock pens, and atmospheric deposition.⁴⁵

1.5. Significance of speciation study with EDTA as chelating agents

Heavy metals can exhibit toxicity as a result of various electronic configuration behaviors. They can form different complex molecules with ligands and organic compounds, typically with those containing oxygen, sulphur and nitrogen. In some cases biological molecules, such as proteins and enzymes interact with these heavy metals, which prevent them from functioning properly.⁴⁶ Indeed, the resulting inactivity of such biological molecules can lead to the death of cells. Therefore, it is important to study metal speciation because it directly impacts the biochemistry of living organisms.

Nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA) are the most common ligands. Via interactions with these ligands, heavy metals can easily be remobilized from their sources. As a result, the metal release into water bodies increases which exhibits high concentration of heavy and transition metals into aquatic organisms.⁴⁷

Ethylenediaminetetraacetic acid (EDTA) is a cheap and suitable complexing agent for many industrial processes, and is thus used on a very large scale within industry.⁴⁸ Figure 1.1 shows the chemical composition of the chelating agents EDTA and NTA. The most common industries using EDTA and NTA are electroplating, pulp and paper, textile finishing, and leather manufacturing. These chelating agents are also known as substitutes for phosphate detergents.⁴⁷ EDTA can also be used to enrich micronutrients in alkaline soils. Due to its availability and widespread use, it is very difficult to remove EDTA from waste water. EDTA can complex strongly with almost every metal compared to humic substances.

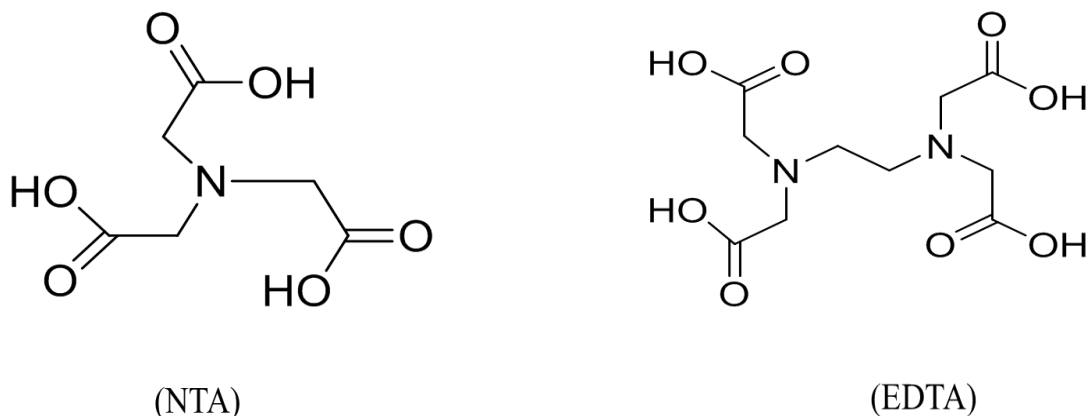


Figure 1.1: Chemical compositions of nitrilo-tri acetic acid (NTA) and ethylene-diamine-tetra acetic acid (EDTA)

1.5.1. Complexation of EDTA with metals

Due to their solubility, the distribution and transportation of heavy metals can be disrupted in the presence of EDTA. EDTA is a polyprotic which usually binds to a metal cation through its four carboxylic acid groups and deprotonation of the two amino groups.⁴⁹ When fully deprotonated (all acidic hydrogens removed), EDTA has the ability to complex or chelate with a metal ion in a one-to-one ratio.⁵⁰ Figure 1.2 shows the structure of EDTA- metal complex. The EDTA behaves as a hexadentate ligand where six donor atoms are bound with the metal cation. Table 1.2 lists the formation constants and logarithm values of the formation constant for EDTA with Cu, Ni, Pb, Zn and Cd at pH 4.

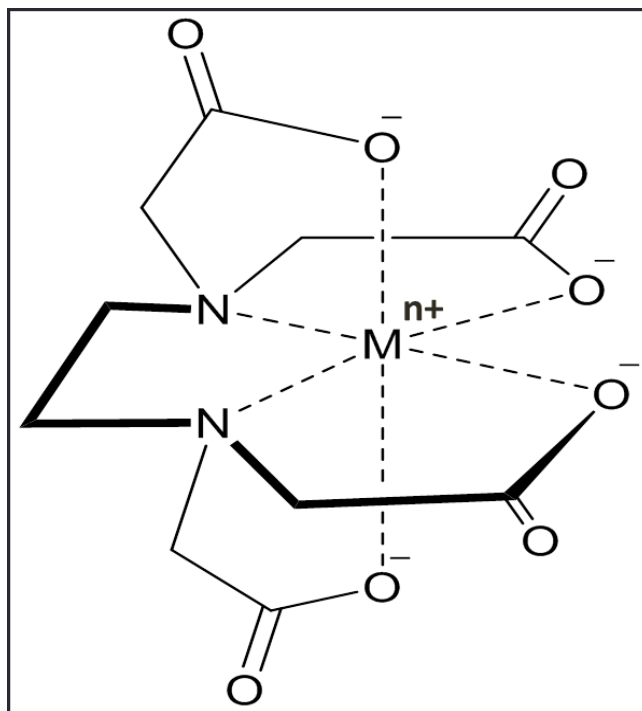


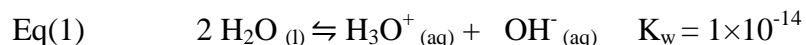
Figure 1.2: EDTA Metal Complex

Table 1.2: Formation constants for EDTA complexes⁵¹

Cation	K_{MY}	$\log K_{MY}$
Cu	6.3×10^{18}	18.80
Ni	4.2×10^{18}	18.62
Pb	1.1×10^{18}	18.04
Zn	3.2×10^{16}	16.50
Cd	2.9×10^{16}	16.46

1.6. Chemical equilibrium versus chemical kinetics

Chemical equilibrium occurs when the concentration of reactants and products remains constant as time progresses, which means that the forward and reverse reactions occur at equal rates.⁵² Initially, when metals arrive in the environment, they are not in chemical equilibrium with sediment. Various factors such as the type of sediment, ligands, temperature, and the metal will affect the equilibrium of a system. One example of the basic chemical equilibrium reaction is the ionization of water (H₂O) to form the hydronium ion (H₃O⁺) and the hydroxide ion (OH⁻)⁵³:



Chemical kinetics explores the rate at which chemical reactions take place. When a chemical reaction takes place, there are many factors that affect the reaction rate such as the nature of reactants, temperature, pressure, concentration, and physical state of reactants.⁵⁴

In environmental analysis, it is very important to understand the kinetics and mechanisms of environmental processes such as sorption and desorption because it affects directly the distribution of chemical species both physically and chemically. In natural systems, particularly in soils and sediments, the rates of reaction provide information about speciation, mobility, and bioavailability of contaminants in the environment.⁵⁵ Chemical kinetics can also be used to examine metal complexation with ligands and sorption onto sediment when looking at the effects of temperature, the nature of the metal ligand, and the type of sediment (sand, organic matter).

1.7. Objectives

It has been a challenge in analytical chemistry to accurately measure the distribution of metals in the environment, particularly low concentrations of metals in contact with sediment, because these distributions depend on metal concentrations, size, shape, composition of sediment/soil particles, the presence of ligands in the water, water T, and pH. A model that accurately accounts for such factors can provide information on the expected distribution of metals when environmental conditions are changing, thereby allowing appropriate remediation measures to be applied. There is no sensitive technique presently available that can provide quantitative determination of metal and metal organic complex in contact with sediment. This work attempts to investigate and determine the distribution of metals as dissolved, labile sorbed, and non-labile sorbed for the purpose of developing a predictive model at the molecular level in the context of changing environmental conditions.

The principle objective of this study is to develop a novel off-line and on-line separation method with High Performance Liquid Chromatography and Inductively Coupled Plasma Mass Spectrometry (LC-ICP-MS) to study the sorption dynamics of aqueous metal species and to assess the distributions of metals such as free metal ions, metal complexes, metal ions absorbed onto sediment, and metal complexes absorbed onto sediment. The novelty of the method is partly based on the utilization of an off-line and on-line separation process that segregates the sample solution from the sediment. This method will also examine the different parameters that control metal distribution.

Figure 1.3 depicts the sorption dynamics of aqueous metal species in contact with sediment particles. The sorption dynamics can be studied under different environmental conditions, such as varying mobile phase and pH at the ambient temperature.

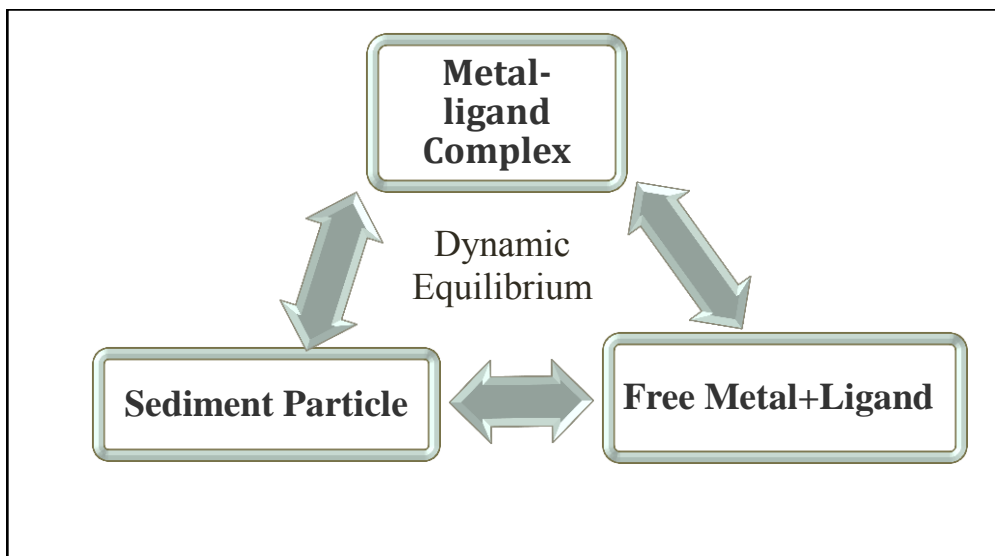


Figure 1.3: Schematic equilibrium process for aqueous metal species in contact with sediment particles under different environmental conditions.

This study can bring valuable information to our understanding of metals and their interactions with water-containing sediment particles. It can also enhance the knowledge about the chemistry of metal interactions in different areas, such as the environmental, geological, agricultural, and economic fields.⁵⁶ Further, this study may improve the design, selection, optimization, and monitoring of environmental remediation strategies applied to cleaning up contaminated sites.

In this study, the target metals are Cu, Cd, Ni, Pb and Zn and have been selected because of their important roles either as essential elements in the environment (Cu, Zn)

or their potential toxicity (Ni, Pb, Cd). In this work, metal complexation in the aqueous system is initiated with EDTA. The sorption dynamics of aqueous metal species in contact with sediment particles are studied using off-line and on-line separation LC-ICP-MS to quantify the dissolved, labile, and non-labile fractions of the target metal ions and metal complexes. Labile and non-labile sorbed refers to the ability of a chemical species to desorb (i.e. extracted) from a solid particle (e.g. soil particle) either easily (labile) or not at all (non-labile or also called bound residue).⁵⁷

1.8. Methodology

When a metal is released into an environment, containing an organic ligand such as EDTA, EDTA can increase its mobility in aquatic organisms. Due to the complex nature of metal-EDTA complexes during treatment and after discharge in the environment, a sensitive analytical technique is needed to study the fate of metal ions in the environment.⁵⁸

For separation of metal ions, ion exchange has become the most widely used technique in the presence of complexing agents such as EDTA.⁵⁹ Separation can be achieved via either cation or anion exchange resins. As EDTA forms very stable complexes with most transition metals, most ion exchange separation techniques use EDTA as a complexing agent for the separation of transition metals. There are other advantages to associating metal ions with their appropriate ligands, as these complexes can lead to selectivity of separation, as well as sensitivity of speciation.⁶⁰

Speciation analysis in analytical chemistry can be used to identify and/or measure the quantities of one or more individual chemical species within a sample. This research

concerns trace element analysis of copper, zinc, lead, cadmium and nickel with NTA and EDTA in well-defined sediment. For this purpose, inductively coupled plasma mass spectrometry (ICP-MS) coupled with liquid chromatography will be used. The technique is now widely used as a tool for a wide range of trace elements, due to its very low detection limits, accuracy and precision. It offers better sensitivity than graphite furnace atomic absorption spectrometry (GFAAS) with the rapid multi-element capability of inductively coupled plasma optical emission spectrometry (ICP-OES). In comparison to ICP-OES, the mass spectra are much simpler than the optical emission spectra⁶¹ and the ICP-MS ability to measure isotope ratios from different elements can provide new insight in the sorption/desorption mechanism of metals in contact with sediment. Therefore, ICP-MS has become the technique of choice in many analytical laboratories for accurate and precise measurements of environmental samples.

1.8.1. ICP-MS Operation

ICP-MS is a very versatile and rapid technique that can detect more than 70 elements of the periodic table, with detection limits below 1 µg/L for most elements. In a typical application, metals are brought into solution by acid digestion. The solution containing metals is introduced using pneumatic nebulization into the ICP-MS for mass spectrometric detection. The isotopes of the elements are identified by their mass-to-charge ratio (m/z), with the intensity of a specific peak in the mass spectrum being proportional to the amount of that isotope (element) in the original sample.⁶²

1.8.1.1. ICP-MS - Strengths and Weaknesses

Although ICP-MS is one of the best techniques for solution chemistry, the technique does have some drawbacks. For example, ICP-MS is a destructive technique, such that the sample cannot be recovered following analysis. In addition, it suffers from sample matrix, isobaric, poly-atomic and doubly charged ion interference. It also requires a minimum sample volume of 3-5 mL for a single run in the instrument when using pneumatic nebulization, which is the most common sample introduction device.

Table 1.3 shows the detection limits and the most abundant isotope of some elements that can be detected by ICP-MS.⁶³

Table 1.3: Detection of some elements with quadrupole ICP-MS

Element	LOD (ppt)	Most Abundant Isotope
Cu	1-10 ppt	63
Ni	1-10 ppt	58
Zn	1-10 ppt	64
Cd	1-10 ppt	114
Pb	<0.1-1 ppt	208

Note : ppt = part-per-trillion

1.8.2.High performance liquid chromatography

High Performance Liquid Chromatography (HPLC) is a powerful chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry to quantify or to purify the individual components of the mixture that have been dissolved into solution.⁶⁴ HPLC is generally used for the analysis

of non-volatile compounds that cannot be analyzed by gas chromatography. The liquid chromatography separation technique is based on the tendency of the dissolved species carried by eluent (the mobile phase) through a column which is packed with a solid support coated with a stationary phase, to partition between the mobile phase and the stationary phase. The separation is possible when there are some variations in their chemical and physical parameters. These parameters could be acidity or basicity, molecular structure, polarity, charge and polarizability.⁶⁵ Dissolved species will partition differently between the mobile phase and the stationary phase such that the migration rate of each dissolved chemical species through the column will be different and thus lead to the successive separation of each chemical species.

Liquid chromatography (LC) allows components of a complex sample to be separated based on differences in their affinity (or retention strength) for the stationary phase or mobile phase, then detects the separated components using UV, fluorescence, electrical conductivity or mass spectrometry.⁶⁶

1.8.2.1. Importance of using TBABr

Ion pair agents play a significant role in the reversed phase separation of metal ion complexes.⁶⁷ It helps to separate charged analytes, and improve peak shape and retention time when modifying mobile phase ratios or changing stationary phase.⁶⁸

In an ion pair reagent, there is an ionic terminal functional group at the end of a non-polar chain, which can interact with a positively charged chemical species that is dissolved in the mobile phase. The strength of the interaction holding the ion pair together depends on the chemical properties of the species interacting with the ion pair agent.

Depending on the strength of the interaction, the neutral ion pair will remain stable for a longer period of time if there is a greater degree of attraction, thus the variation in interactions between the species in the mobile phase and the stationary phase accounts for differences in retention time. Figure 1.4 demonstrates the mobile phase (TBABr) and the stationary phase (C18) used in this work

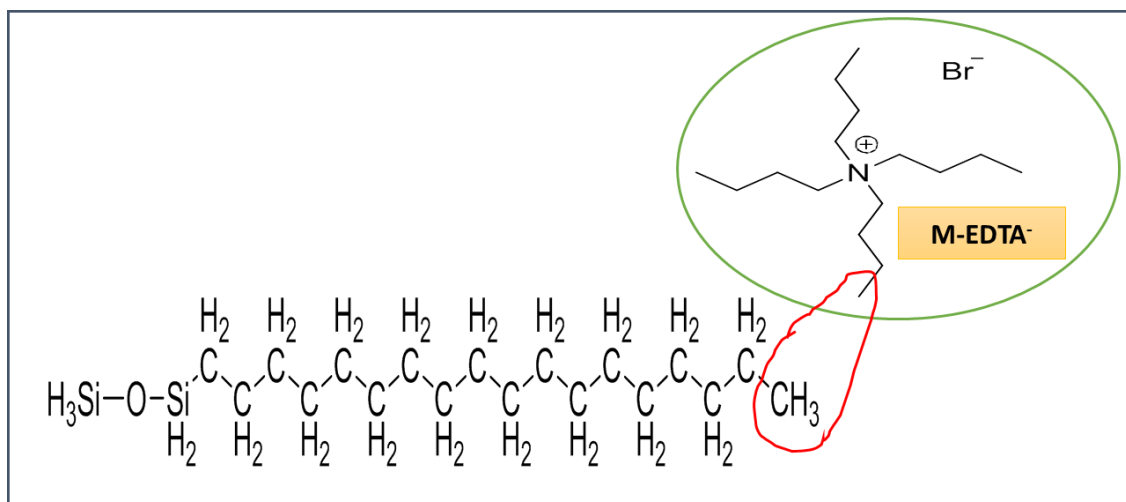


Figure 1.4: Chemical composition and interaction of stationary and mobile phase for LC

1.8.2.2. LC - Strengths and Weaknesses

The detectors used in LC primarily quantify substances based on their retention time, with quantitative measurements based on peak intensity and peak area. Although chromatography offers great resolution, accurately quantifying a substance can be difficult if multiple components elute at approximately the same time (co-elution problem), especially when simultaneous analysis is required for multi-analytes.

1.8.3. Hyphenated Techniques for Speciation

Research in the speciation analysis of metals and metal complexes in environmental samples strongly suggests using hyphenated techniques by coupling a powerful separation technique (e.g., chromatography) with a sensitive, element-specific detector (e.g., atomic spectrometry).

Heavy metal complexes are present in the environment in their anionic forms. This characteristic leads to direct ion-exchange process without any derivatisation preceding sample analysis. Ferrarello *et al.*⁶⁹ stated that speciation is not possible if the species are not separated properly before entering to the ICP–MS, which destroys the “compound” information. In the past, many different combinations (separation plus element-selective detection) of techniques have been applied in order to separate and quantify metal complexes in the environment. Of them, high-performance liquid chromatography (HPLC) coupled with inductively coupled plasma–mass spectrometry (ICP–MS) is known as one of the best combinations. HPLC can be applied to a wide range of environmental and biological samples because of its versatility with respect to separation mechanism. In addition, ICP–MS is the most widely used analytical technique because of its high sensitivity and robustness. Using HPLC and ICP-MS in conjunction is relatively easy and does not require any special arrangements.⁷⁰ Ferrarello *et al.*⁶⁹ claimed that ICP–MS is an almost ideal specific detector even for metal-biomolecules, because of its extremely low detection limits. Another advantage of ICP-MS is its multi-elemental detection for speciation analysis of bio-elements in biological material within a single run.

The type of chromatography (e.g. reversed-phase or ion exchange) should be chosen based on the physico-chemical characteristics of the sample, such as acid or base properties, solubility or polarity of the analyte species.⁷¹

Overall, the most sensitive technique is the anion exchange chromatography coupled with ICP-MS, primarily because this combination allows for accurate speciation analysis in several sample matrices and across a wide pH range.⁷²

1.8.4. Factors influencing HPLC coupled with ICP-MS

The distribution of metal ions in natural water containing sediment particles can be influenced by several factors, and even small variations in these factors can cause considerable changes in metal distributions. Therefore, it was necessary to prioritize certain parameters in order to get the best possible instrumental performance using HPLC coupled with ICP-MS. The influences of mobile phase pH and room temperature are of particular importance in this regard.^{1,73} The pH of the mobile phase can cause a displacement of time retention of the analyte. Large variations in room temperature can also affect the analyte retention time. An increase in temperature will decrease in retention time.⁷⁴

1.9. Factors affecting metal release/ uptake from sediment

1.9.1. pH effect

pH is a key factor in heavy metal transfer activities in sediments, as well as in the total environment. The distribution of metals and their toxicity in sediments are influenced by pH, especially when these metal ions are partitioned among sediment

particles and water bodies.^{75,76} When pH changes under different environmental conditions, metal ions and protons (H^+) compete to bind with ligands present in the sample.¹ As a result, metal ion adsorption on sediments decreases, reducing their bioavailability and increasing their mobility. It has also been reported that when pH changes from acidic to alkaline, phosphorus (P) release from sediment is increased.⁷³ At alkaline pH, phosphorus is mostly found as phosphate (PO_4^{3-}) or hydroxyl phosphate ($HOPO_2^{2-}$) which can combine with and solubilize Fe, Al, and/or Ca. The combination form of Fe-P and Al-P could exist in the sediment which can be uptaken easily by microorganisms due to the easy exchange between Fe-P and Al-P and OH^- under higher pH condition. Metal ions can also be released from sediments into water even under stable water conditions¹ if pH decreases to 1.2. For example, Table 1.4 shows that mobility of metals in sediment varies significantly with pH. The mobility of metal ions in sediment decreases as follows at pH 4.0: $Zn > Cd > Ni > As > Cu > Pb$.^{1, 77} This means that at pH 4, Zn is mobile in the sediment whereas Pb is fixed on the sediment (possibly as lead hydroxide).

Table 1.4: The limit pH values controlling heavy metals mobility in sediment¹

Metal species	pH limit
Zn	6.0-6.5
Cd	6.0
Ni	5.0-6.0
Cu	4.5
Pb	4.0

Research also indicates that soil pH is the major factor affecting metal availability in soil. When soil pH increases at 7.3, the uptake of some metals such as Cd and Zn to the roots of *Thlaspi caerulescens* (which is a flowering plant) decreases.¹²

Based on the above findings, this study uses a pH range of 4.0-9.0 in order to focus on the sorption capabilities of the target metals, Cu, Ni, Zn, Pb, and Cd, in sediment.

1.9.2. Influence of Temperature

With increasing temperature, dissolved oxygen (DO) concentrations in water decreases whereas dissolutions of carbonates and hydroxides increase in water. As a result, with increasing temperature, the rate of metal release of the water-soluble fraction, carbonate fraction, and exchangeable fraction from the sediment into the overlying water could be increased.⁷³

The effects of temperature (15-35°C) on metal ions sorption and desorption from sediments were studied by Haiyan, *et al.*⁷³ In their study, the release rates for different metals (Zn, Cu, Pb, Cr, and Cd) at low and high temperature were observed. It has been found that release rates were greater at high temperatures than low temperatures. It is well known that reaction rate increases with temperature.⁷⁸ This means that with increasing temperature, the desorption of metals from sediments will increase with time. Hence, it is important that the metal sorption and desorption from sediment be studied at constant temperature until equilibrium is attained. In light of the potential effects of temperature, the current research is conducted at room temperature conditions (between 20-23°C).

Chapter 2 EXPERIMENTAL SETUP AND PROCEDURE

2.1. Materials and Reagents

All reagent solutions were prepared by dissolving chemicals of the highest available purity in deionized water purified by a Nanopure II apparatus (Barnstead) and HPLC grade water.

2.1.1. HPLC

Copper (Cu), zinc (Zn) nickel (Ni), lead (Pb), and cadmium (Cd) were the test metals, and EDTA was the test complexing ligand. Standard stock solutions of Cu, Zn, Ni, Pb, and Cd (10^{-3} M) were prepared from commercial ICP-ES & ICP-MS standard (Plasma Cal, 1000 $\mu\text{g/mL}$, SCP Science). A stock solution of EDTA (20 mM) was prepared by dissolving disodium salt of EDTA (Reagent Grade, Caledon) in deionized water. Intermediate stock solutions of these reagents (1×10^{-3} M) were prepared by dilution in HPLC grade water, and working solutions were prepared by subsequent dilution of stock solution and adjusting the pH to 4.0 with nitric acid or ammonium hydroxide.

All chemicals used for preparation of mobile phases were of analytical-reagent grade.

The listed sources of the mobile phase used in this work are:

- Ammonium Acetate ($\text{C}_2\text{H}_7\text{NO}_2$) AnalaR Grade BDH INC.
- Sodium Acetate (CH_3COONa) AnalaR Grade BDH INC.
- Tetrabutylammonium Bromide (TBABr) Sigma-Aldrich.
- Ammonium Phosphate ($(\text{NH}_4)\text{H}_2\text{PO}_4$) Fisher Scientific Company.
- Sulfuric Acid (H_2SO_4) from J.T.Baker. Trace Metal grade.

The compositions of the mobile phases are listed in Table 2.1. The solutions were prepared by dissolving the listed compounds in water or in mixtures of methanol/water (methanol HPLC grade from Sigma-Aldrich and HPLC grade water from Caledon).

Table 2.1: Summary of the investigated chromatographic parameters for the HPLC separation of metals (Cu, Zn, Ni, Pb, and Cd) complexing with EDTA

Column	Mobile Phase
<p><i>Reversed phase</i></p> <p>Supelco C18 25 cm x 4.6 mm ID, 5 µm particle size.</p>	<p>Ammonium Acetate (C₂H₇NO₂) concentration; 50 mmol/L 30:70 Methanol: HPLC grade water buffer (pH adjusted to 4.0 with nitric acid).</p> <p>Sodium Acetate (CH₃COONa) concentration; 50 mmol/L 30:70 Methanol: HPLC grade water buffer (pH adjusted to 4.0 with nitric acid).</p> <p>Sodium Acetate (CH₃COONa) concentration; 10 mmol/L, Tetrabutylammonium Bromide (TBABr) concentration; 10 mmol/L 10:90 Methanol: HPLC grade water buffer (pH adjusted to 3.3 with nitric acid).</p> <p>Tetrabutylammonium Bromide (TBABr) 10 mM + EDTA 10 mM + Ammonium phosphate (NH₄)H₂PO₄ 50 mM (pH adjusted to 3.25 with either nitric acid or ammonium hydroxide).</p>
<p><i>Anion Exchange</i></p> <p>Hamilton PRP-X100 Anion, 150× 4.1 mm, 10 µm particle size.</p>	<p>4 different concentrations:</p> <ol style="list-style-type: none"> 1. 5.4 mM Sulfuric Acid prepared with HPLC grade water. pH 2.18 2. 3.6 mM Sulfuric Acid prepared with HPLC grade water. pH 2.25 3. 3.0 mM Sulfuric Acid prepared with HPLC grade water. pH 2.31 4. 2.0 mM Sulfuric Acid prepared with HPLC grade water. <p>(All these pHs adjusted with either nitric acid or ammonium hydroxide)</p>

The pH of the mobile phases were adjusted using 10% nitric acid or 10% ammonium hydroxide solution. The use of different mobile phases and their compositions are listed in Table 2.1, along with their respective pH values.

2.1.2. LC-ICP-MS

2.1.2.1. Internal standard

Internal standardization was used for all ICP-MS experiment. The following isotopes were selected for internal standardization: lithium (^7Li), scandium (^{45}Sc), yttrium (^{89}Y), indium (^{115}In), cerium (^{140}Ce), and thorium (^{232}Th). The mixed internal standard was prepared by combining all six internal standards into one solution. This was achieved by diluting of each isotope with deionized water from their 1000 ppm stock solution (Plasma Cal, 1000 $\mu\text{g/mL}$, SCP Science) into a 1.00 L volumetric flask. The internal standard solution was acidified to contain 1% of nitric acid.

2.1.2.2. Mobile phase

Concentrated sulfuric acid (H_2SO_4) was obtained from J.T.Baker with a purity of Trace Metal grade. The liquid chromatograph mobile phases were prepared by dilution of the concentrated acid with either deionized water (resistivity of 18.3 $\text{M}\Omega$) or HPLC grade water.

2.1.2.3. Preparation of working solutions

The following isotopes were selected for the ICP-MS analysis of the target metals: ^{63}Cu , ^{66}Zn , ^{60}Ni , ^{208}Pb , and ^{111}Cd . The mixed calibration standards were prepared by combining all five target metal into one solution. This was achieved by diluting of each

target metal with deionized water from their 1000 ppm stock solution (Plasma Cal, 1000 µg/mL, SCP Science) into a 1.00 L volumetric flask. The calibration standards were acidified to contain 1% of nitric acid.

The working test solutions were prepared in a similar fashion as the calibration standards except that their final concentration was different from the calibration standard.

A solution of 1000 µM of the disodium salt of EDTA (Reagent Grade, Caledon) was prepared by weighing 0.372 g of the salt in 1.0 L of deionized water.

2.2. Preparation of glassware

All glassware used in this work, such as volumetric flasks, beakers and funnels, were rinsed with tap water. Glassware was then soaked in 10% nitric acid for 48 hours to eliminate possible contamination from traces of residual metals. Finally, the glassware was washed three times with deionized water or HPLC grade water.

2.3. pH Meter

The pH measurements of all solution samples were performed following standard methodology with a pH meter (Corning pH meter 320). The pH meter was used to adjust the pH values in the Metal- EDTA complex and mobile phase solutions. Each time, the pH meter was calibrated using buffer solutions at pH 4.0 and pH 7.0.

2.4. Source of sediment

The sediment used in this study was a Standard Reference Material (SRM), obtained from the National Institute of Standards & Technology (NIST). The sediment was an estuarine sediment (SRM 1646a) which was collected by NIST by dredging the

Chesapeake Bay at a location of 37° 11.1' min N, 76° 17.11' min W. The certificate of analysis is provided in chapter three in table 3.2.⁷⁹

2.5. Scanning Electron Microscopy (SEM)

In this work, a Tescan Mira3 LM field Emission Scanning Electron Microscope equipped with an Oxford instruments X-max 80 mm² SDD EDS detector was used to characterize the SRM 1646a sediment. This instrument is located at the Electron Microscopy Laboratory (EMC) in the Science Building at Saint Mary's University.

2.6. CHN Analyser

The carbon, hydrogen, nitrogen concentrations in the estuarine sediment SRM 1646a was determined using a CHN Analyzer, Perkin Elmer 2400 Series II and performed by the Centre for Environmental Analysis and Remediation laboratory (CEAR) at Saint Mary's University.

2.7. X-ray Diffraction (XRD)

X-ray Powder Diffraction (Siemens D500 XRD) analysis of the sediment 1646a was done by the Department of Physics and Atmospheric Science at Dalhousie University.

2.8. Liquid Chromatography (LC)

Figure 2.1 shows the HPLC system used in this work, which is housed in the CEAR laboratory at SMU. This system consists of a Varian 9012 solvent delivery system with a Varian 9050 variable wavelength UV-VIS detector. The column used for this work was a C18, reversed phase column of dimension 250× 4.6 mm, with a 5µm coating of

stationary phase (Supelco, Octadecyl dimethylsiloxane phase), or a weak anion exchange column of dimension 150 ×4.1 mm and a 10 μm coating of stationary phase (Hamilton PRP-X100, PSDVB/ Trimethylammonium). The injection system was an Auto Sampler Varian Model Prostar 410 using an injection loop of 100 μL , and the effluent was monitored by the UV-VIS detector at 254 nm wavelength. A typical LC system is presented in Figure 2.2.



Figure 2.1: Photograph of HPLC system used for this study

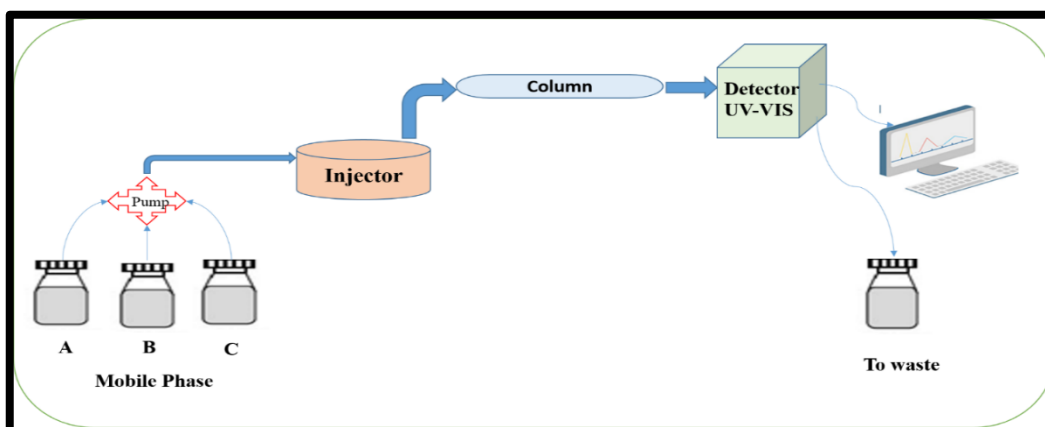


Figure 2.2: Block Diagram of a conventional HPLC system

2.9. UV-VIS Characterization of metal-EDTA complexes

Metal-EDTA complexes were studied using a Varian Cary 50 CONC UV Visible spectrophotometer. The UV-VIS spectra of metal-EDTA complexes (Cu-EDTA and Pb-EDTA) were collected from a wavelength of 200 to 800 nm.

2.10. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

This work was performed at the CEAR laboratory using a Thermo Elemental / VG ICP/MS - PQ Plasma Quad Excell Mass Spectrometer for metal analysis. The isotope selected for ICP-MS analysis for each target metal were ^{63}Cu , ^{66}Zn , ^{60}Ni , ^{208}Pb , and ^{111}Cd . The ICP-MS signal for ^{208}Pb was actually the sum total of the signal collected from ^{206}Pb , ^{207}Pb and ^{208}Pb . This signal summation of these lead isotopes is possible because the natural abundance of these three lead isotopes is relatively stable and there are no isobaric interferences on any of these lead isotopes masses.

2.11. Coupling of LC with ICP-MS

Figure 2.3 shows the HPLC coupled with the ICP-MS. This coupling was achieved by connecting the outlet of the chromatographic column to the nebuliser of the VG PQ ExCell using T- mixing tube (to mix mobile phase with internal standard). Furthermore, the liquid HPLC sample was delivered to the ICP-MS nebuliser in a flow of the liquid mobile phase H_2SO_4 , which is pumped at 1.0 mL/min; exact element detection is provided by ICP-MS analyzer. The injection system was a HPLC Auto Sampler Varian Model Prostar 410 using 15 μL syringe. The volume of the injection loop was 100 μL and the inject volume of the sample was 20 μL . An anion exchange column (Hamilton PRP-X100, 150 mm \times 4.1 mm, 10 μm) was used for this experimental analysis. This analytical

approach has two pumps: one is a Varian 9012 solvent delivery system for HPLC which delivers the sample to the ICP-MS and the other is a peri-pump PERIMAX 12/ SPETEC for ICP-MS, which delivers the internal standard to the ICP-MS. The HPLC system promotes the separation of metal complexes via the differing affinities of the mobile phase and the stationary phase components. These separated compounds are pumped to the nebuliser of ICP-MS. Data was collected using acquisition of transient time.

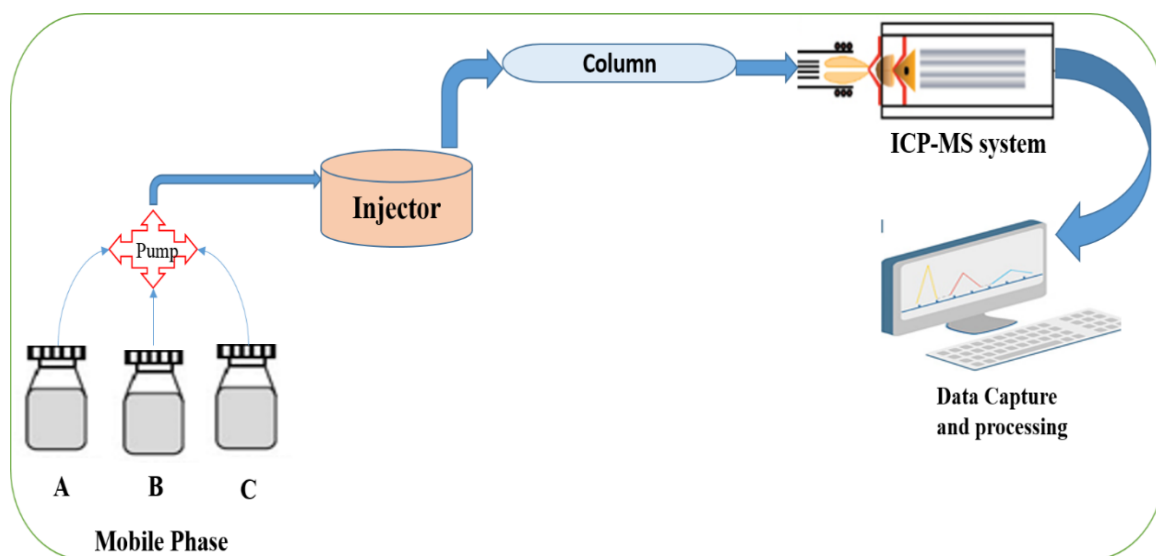


Figure 2.3: Schematic Diagram of HPLC coupled with an ICP-MS System

2.12. Preparation of sediment for LC-ICP-MS analysis- offline filtration

Intermediate mix standard solutions of Cu, Zn, Ni, Pb, and Cd were prepared from commercial ICP-ES & ICP-MS standard (Plasma Cal, 1000 $\mu\text{g/mL}$, SCP Science). Intermediate stock solution of these reagents (10 μM) was prepared in deionized water and 2 % of HNO_3 Disodium salt of EDTA (Reagent Grade, Caledon) 1000 μM was prepared with deionized water. All solutions were stored at room temperature in amber

glass bottles and sealed with Mininert® syringe valves 24-400 screw caps [Dynatech, VWR International] to avoid decomposition and evaporation. Slurries were prepared with 50 mg of estuarine sediment and kept in 30 mL amber glass vials capped with Mininert® syringe valves and kept stirring on stirrer (TALBOYS) during the sorption kinetic experiments. The deionized water used in this experiment was adjusted to pH 4 with nitric acid or ammonium hydroxide.

2.12.1 Offline separation analysis

Analyses of sediment slurries using an offline separation method were carried out using HPLC system. This system consisted of a Varian 9012 solvent delivery system. The column used for this work was a weak anion exchange column of dimension 150×4.1 mm and a 10 µM coating of stationary phase (Hamilton PRP-X100). The injection system was an Auto Sampler Varian Model Prostar 410 using an injection loop of 100 µL, and the effluent was monitored by the ICP-MS detector. In the offline system, whole slurries taken by 1 mL syringe from the amber glass vials were filtered with a 0.45 µm membrane filter (Millipore Millex-GV) made of polyvinylidene fluoride (PVDF).

Chapter 3 Characterization of sediment 1646a

3.1. SEM coupled to EDS Analysis for sediment particles

In this work, Scanning Electron Microscopy (SEM) coupled with Energy Dispersive Spectroscopy (EDS) was used to characterize the 1646a estuarine sediment sample. Figure 3.1 displays the various magnifications at which sediment particles were examined for their composition and distribution using SEM. This picture also shows the different areas of the sediment which were analyzed to provide information about the sediment homogeneity. Figure 3.1 shows that the sample is the chemically homogeneous because the composition in different elements investigated is the same in every area that was analysed.

Table 3.1 shows the elemental percent composition and results demonstrate the homogeneity of the estuarine sediment SRM 1646a. Table 3.2 shows the certified values for SRM 1646a⁷⁹. Table 3.3 shows the deviation (% error) of the SEM/EDS results from the certified values of SRM 1646a. When compared to the certificate of analysis of the SRM 1646a, the SEM/EDS results were significantly different except for silicon and titanium, where the %error are about 6% or less. The reason for this disagreement between SEM/EDS results and reported certified values for elements other than Si or Ti is probably due to the fact the SRM 1646a results were obtained with x-ray fluorescence spectrometry and/or inductively coupled plasma mass spectrometry, which are significantly more sensitive than SEM/EDS.⁸⁰ Therefore, the SEM/EDS results suggests

that the physical and chemical integrity of the sediment with respect to silicon and titanium has not changed significantly since its purchase.

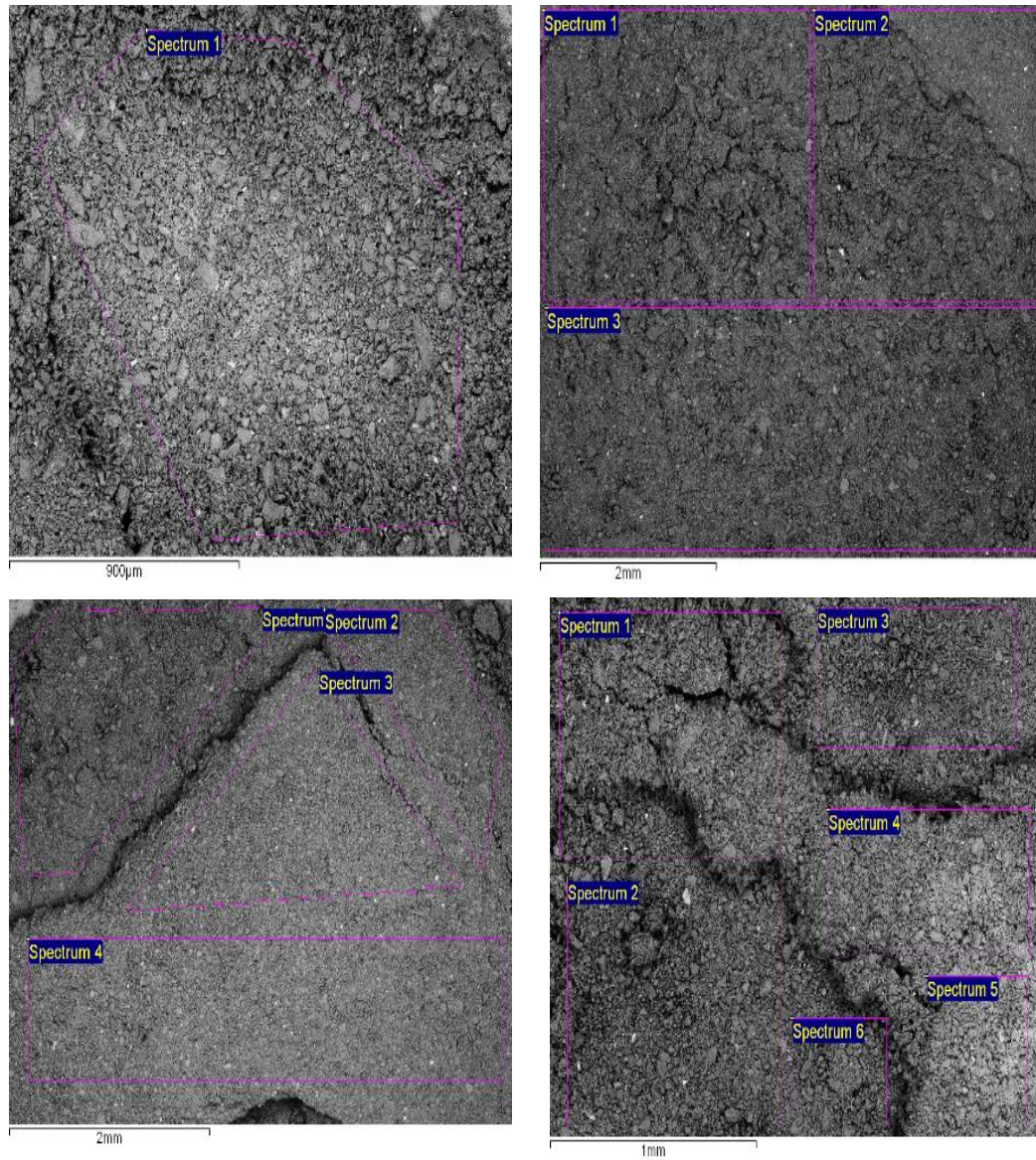


Figure 3.1: SEM/EDS/EDS/Analyzer of 1646a estuarine sediment sample at different magnifications

Table 3.1: The SEM/ EDS analysis of estuarine sediment 1646a. Elemental percent composition and of the estuarine sediment SRM 1646a.

Mineral	Concentration, %												
	Si	Ti	Al	Fe	Mn	Mg	Ca	Na	K	P	S	Cl	O
Area analysis	38.98	0.55	2.73	2.85		0.51	0.66	1	1.2		0.53	0.99	50
Area analysis	39.05	0.51	2.67	2.96		0.55	0.68	1.03	1.19		0.45	0.94	49.97
Area analysis	39.21	0.44	2.74	2.85		0.52	0.68	0.98	1.17		0.45	0.9	50.07
Area analysis	39.15	0.46	2.8	2.59		0.59	0.69	1.06	1.09		0.49	0.97	50.12
Area analysis	39.44	0.42	2.69	2.46	0.05	0.58	0.66	0.99	1.03		0.48	0.94	50.25
Area analysis	39.3	0.39	2.73	2.67		0.56	0.69	1	1.13		0.45	0.97	50.12
Area analysis	39.23	0.4	2.65	2.91		0.55	0.71	0.97	1.11		0.49	0.89	50.09
Area analysis	39.49	0.35	2.86	2.39		0.58	0.64	0.92	1.09		0.45	0.91	50.31
Area analysis	39.54	0.4	2.65	2.34		0.58	0.61	1.04	1.01	0.07	0.47	0.97	50.32
Area analysis	39.38	0.38	2.81	2.34		0.56	0.61	1.16	1.03		0.47	1.06	50.21
Area analysis	39.04	0.56	2.67	2.96	0.03	0.56	0.7	0.95	1.08		0.47	0.98	49.99
Area analysis	39.26	0.43	2.66	2.89		0.53	0.7	0.97	1.11		0.47	0.9	50.09
Area analysis	39.14	0.42	2.76	2.9		0.46	0.62	1	1.13		0.53	0.95	50.08
Area analysis	39.36	0.39	2.73	2.72		0.54	0.57	1.03	1.14		0.44	0.91	50.16
Area analysis	38.78	0.66	2.62	3.2		0.54	0.66	1.06	1.16	0.04	0.49	0.91	49.88
Area analysis	39.42	0.37	2.69	2.78		0.53	0.57	1	1.18		0.4	0.96	50.1
Area analysis	39.11	0.45	2.88	2.57		0.57	0.64	1.03	1.15	0	0.49	1	50.11
Area analysis	38.77	0.33	2.95	2.79		0.56	0.73	1.08	1.2	0.07	0.49	1.12	49.91
Area analysis	39.41	0.36	2.58	3.04		0.53	0.63	0.93	1.05		0.5	0.78	50.19
Area analysis	39.3	0.34	2.82	2.58		0.48	0.71	1.04	1.13	0.06	0.45	0.91	50.19
Average	39.218	0.4305	2.7345	2.7395	0.04	0.544	0.658	1.012	1.119	0.048	0.473	0.948	50.108
stdev	0.21739	0.08357	0.09473	0.24137	0.01414	0.03347	0.04595	0.0554	0.05794	0.0295	0.03063	0.06933	0.11954

Table 3.2: Certified values for the SRM 1646a sediment⁷⁹

Table 1. Certified Values			
Element	Mass Fraction, (%)	Element	Mass Fraction, (mg/kg)
Aluminum	2.297 ± 0.018	Arsenic	6.23 ± 0.21
Calcium	0.519 ± 0.020	Cadmium	0.148 ± 0.007
Iron	2.008 ± 0.039	Chromium	40.9 ± 1.9
Magnesium	0.388 ± 0.009	Copper	10.01 ± 0.34
Phosphorus	0.027 ± 0.001	Lead	11.7 ± 1.2
Potassium	0.864 ± 0.016	Manganese	234.5 ± 2.8
Silicon	40.00 ± 0.16	Selenium	0.193 ± 0.028
Sodium	0.741 ± 0.017	Vanadium	44.84 ± 0.76
Sulfur	0.352 ± 0.004	Zinc	48.9 ± 1.6
Titanium	0.456 ± 0.021		

Table 2. Noncertified Values			
Element	Mass Fraction, (mg/kg)	Element	Mass Fraction, (mg/kg)
Antimony	0.3	Neodymium	15
Barium	210	Nickel	23
Beryllium	<1	Rubidium	38
Cerium	34	Scandium	5
Cobalt	5	Silver	<0.3
Gallium	5	Strontium	68
Lanthanum	17	Thallium	<0.5
Lithium	18	Thorium	5.8
Mercury	0.04	Tin	1
Molybdenum	1.8	Uranium	2.0

Table 3.3: % error: Deviation from certified concentration for sediment SRM 1646a

Element	%Error	Element	%Error
Si	-2.00%	Mg	40.21%
Ti	-5.60%	Ca	26.78%
Al	19.04%	Na	36.57%
Fe	36.43%	K	29.51%
		S	34.37%

$$\text{Eq (2) \% Error} = \frac{(\text{SEM/ EDS measured concentration} - \text{certified concentration}) \times 100}{\text{certified concentration}}$$

3.2. CHN analysis

Sediment 1646a was analyzed for carbon, hydrogen, and nitrogen using a CHN analyzer; the results are summarized in Table 3.4. The average of a duplicate analysis showed that the % C, % H, and % N were 0.56 %, 0.20 % and 0.14 %, respectively.

Table 3.4: CHN analysis of sediment 1646a.

Sample results	weight mg	Carbon%	Hydrogen%	Nitrogen%
S1646a	6.63	0.56	0.20	0.16
S1646b	5.32	0.57	0.20	0.14
S1646c	6.14	0.56	0.20	0.11
Average	6.03	0.56	0.20	0.14
stdev	0.66	0.01	0.00	0.03

Table 3.5 shows typical concentration of organic carbon in different sediments. Hyland *et.al.*,⁸¹ indicated that organic carbon in sediment can be a useful indicator of

stress in marine benthos. Their study shows that the Total Organic Carbon (TOC) can vary from 8.0 mg/g (or 0.8%) for the Eastern Mediterranean Sea to a low of 4.0 mg/g (or 0.4 %) in the Northern Black Sea, and the Estuaries of South East USA averages 5.3 mg/g (0.53%) which compares well with the average 0.56% for the Estuarine Sediment 1646a.

Table 3.5: Total Organic Carbon concentration in sediments⁸¹

Study region	TOC ranges (mg /g) ≤10
Eastern Mediterranean Sea	8.0
North Sea & coastal UK	7.8
Boston Harbor & Massachusetts Bay, USA	5.9
Estuaries of SE USA	5.3
Northern Black Sea	4.0

3.3. X-Ray Powder Diffraction analysis for sediment

X-ray powder diffraction is a technique used to study the crystal structure and atomic spacing of compounds. It is a non-destructive, fast analytical technique for phase identification of crystalline substances. It can also provide information about unit cell dimensions.⁸² Figure 3.2 shows the various diffraction lines collected from the estuarine Sediment 1646a. Analysis of these diffraction lines revealed that the sediment contained (in weight %) 89% silica (SiO_2), 6% anorthite ($\text{CaAl}_2\text{Si}_2\text{O}_8$), and 5% albite ($\text{NaAlSi}_3\text{O}_8$).

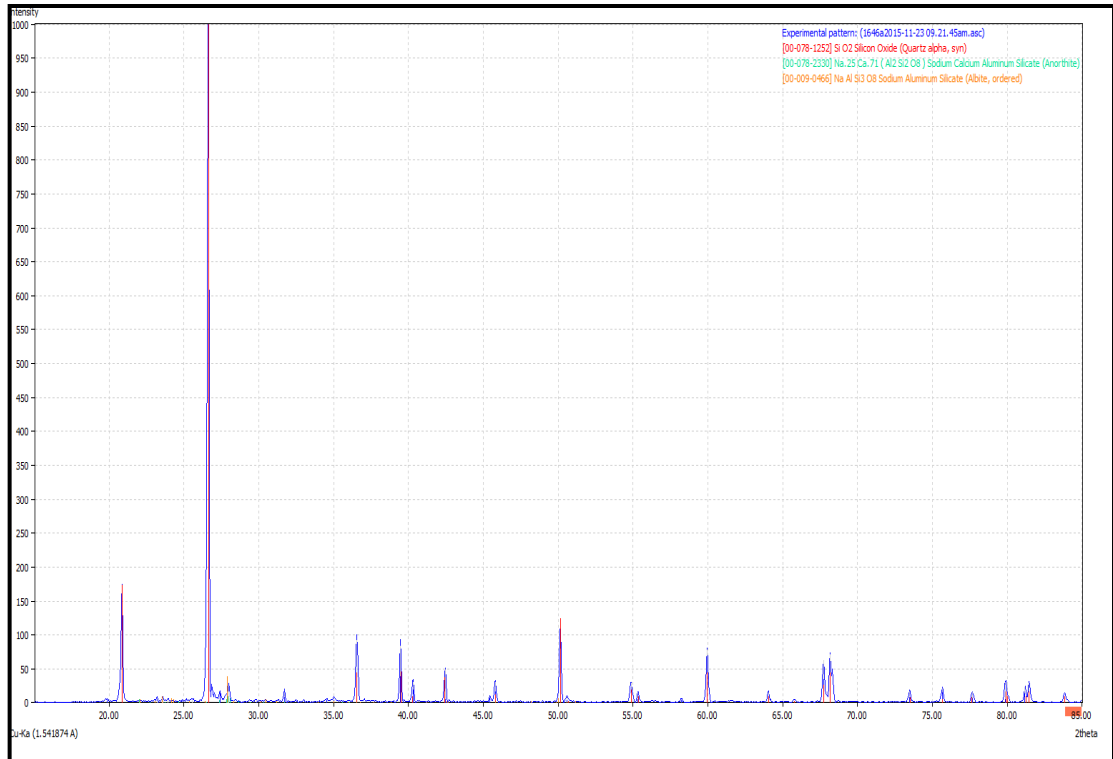


Figure 3.2: XRD Analysis for Estuarine Sediment

The X-ray diffraction results, combined with SEM/ EDS and CHN analyzer results, indicate that the estuarine sediment sample consists mostly of very fine density mineral, with a very small fraction of organic material. The small concentration of organic material in the sediment at this stage of the project is desirable because there will be less competition from the organic fraction to bind with the trace metals, thus providing a better control in this work for studying the interaction of EDTA with the target trace metals.

Chapter 4 RESULTS & DISCUSSION

4.1. UV-VIS study

UV-Vis spectroscopy was used to determine the ideal wavelength for studying metal-EDTA complexes using HPLC with UV-Vis detection. HPLC with UV-vis detection was used to determine the best separation conditions between metal-EDTA complexes. The optimum conditions would then be applied when LC is coupled to ICP-MS. This approach is relatively rapid and inexpensive (ICP-MS operation is significantly more costly than just the HPLC operation).

The purpose of this investigation was to use the absorption spectra of different metals at different concentrations and pHs to determine the optimum conditions for LC separation and detection. Figure 4.1 shows the wavelength and pH dependency of the UV-Vis light absorption spectrum for the Fe-EDTA complex at two different pHs, at 4 and 9, respectively. The range of pHs that natural water can experience is typically between 4 and 9,⁸³ thus the reason for choosing these two pHs for this study. The complex formation constant for [Fe-EDTA] is 1.7×10^{24} , which is one of the largest metal-EDTA complex formation constants.⁵¹ Therefore, when iron is in the presence of EDTA, it is expected to be found quantitatively as a metal-EDTA complex and little free iron ions will be left in solution. The Fe-EDTA complex has also a very high molar extinction coefficient⁵⁸ at 254 nm which translate into a great sensitivity towards UV detection at 254 nm. Figure 4.1 shows the optimum absorption wavelength for the Fe-EDTA complex is between 200 and 400 nm. For a 1.79 mM Fe-EDTA solution at pH 9, the absorbance intensity exceeds the measurable maximum intensity limit of the UV-Vis

detector (i.e., greater than absorbance value of 3) in the wavelength region of 200 to 400 nm, whereas at pH 4 for the same solution, the intensity is significantly reduced but yet remains at an acceptable absorbance reading of about 2.0 at 254 nm. Although the detection of the Fe-EDTA complex is more sensitive at pH 9, the possibility that Fe, or other metals from the list of target metals, forms insoluble hydroxides at pH above 4 is important and therefore pH 4 was chosen for the preparation of metal-EDTA complexes for all of the LC experiments with a detection wavelength of 254 nm.

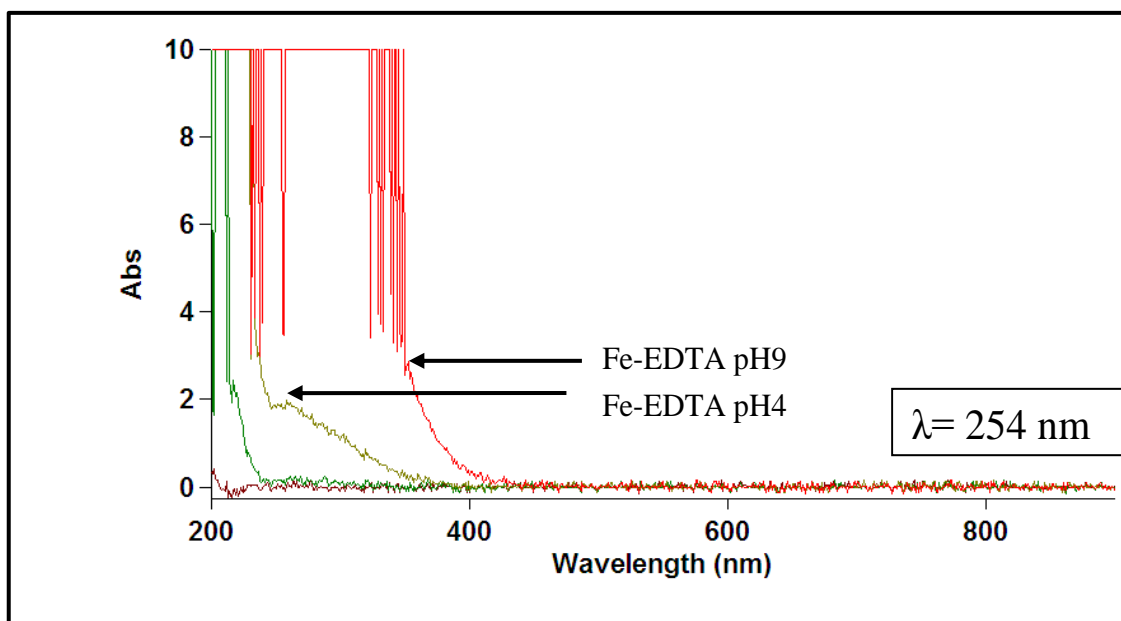


Figure 4.1: Wavelength and pH dependency of the Fe-EDTA complex. Fe-EDTA concentration is 1.79 mM at both pHs.

4.2. High Performance Liquid Chromatography Systems

High performance liquid chromatography (HPLC) was used to separate mixtures of metal-EDTA complexes. Target metals (Ni, Cu, Zn, Pb, Cd) were mixed with EDTA and put in contact with sediment particles. The following section reports the development

of the chromatographic separation of free metal ions from its EDTA complex and separation of metal-EDTA complexes from each other.

4.3. Mobile phase selection

In this work, different mobile phases were tested to determine the best mobile phase with ICP-MS for on-line and off-line separation technique.

4.3.1. Ammonium acetate and sodium acetate

This first mobile phase tested consisted of 50 mmol/L of ammonium acetate in 30:70 methanol:water at pH adjusted to 4.0. The use of ammonium acetate dissolved in methanol:water as an HPLC mobile phase for the separation of metal-EDTA has been reported before.⁸⁴ The mobile phase flow rate was maintained at 1.0 mL/min. This mobile phase was used with a reversed phase column C18 (Octadecyl dimethylsiloxane) phase, which is a non-polar stationary phase. Sodium acetate was also used instead ammonium acetate (all other conditions remaining same). Table 4.1 shows the conditions for the separation of metal-EDTA complexes with two different mobile phases; (a) ammonium acetate and (b) sodium acetate.

Figure 4.2 shows the chromatograms of the target metal ions (each at 1.0 mM) in contact with an excess of EDTA (20.0 mM) using 50 mM of (A) ammonium acetate in 30:70 methanol:water or (B) sodium acetate in 30:70 methanol:water as the mobile phase. The experimental results shows that the metal-EDTA complexes share more or less the same retention time (i.e. metal-EDTA complexes are not separated from each other) when using either ammonium acetate or sodium acetate with methanol:water as the mobile phase.

The likely reason why the metal-EDTA complexes are not separated from each other is because the difference in charge density (divalent metals forming doubly negatively charge metal-EDTA complex) and the difference in polarity between each complex is not large enough such that each complex experience more or less the same affinity for the stationary phase and mobile phase.

Table 4.1: HPLC conditions for separation of EDTA- metal complexes with Ammonium Acetate and Sodium Acetate

Condition	Description/Value
Columns	Analytical column: Supelco 25 cm x 4.6 mm ID, 5 μ M particle size, Guard column: Opti-Guard C18, 1.0 mm I.D. (USA)
Mobile Phase	A- 50 mmol/L ammonium acetate in 30:70 methanol:HPLC grade water at pH adjusted to 4.0 with nitric acid B- 50 mmol/L sodium scetate in 30:70 methanol: HPLC grade water at pH adjusted to 4.0 with nitric acid
Flow Rate	1.0 mL/min
Sample loop	100 μ L
Injection volume	20 μ L
Temperature	Room temperature
Detection	UV-VIS at 254 nm wavelength
Metals	Cu, Zn, Ni, Cd and Pb
Ligand	20 mM EDTA
pH	all metals adjusted to pH 4 or 9
Stationary phase	C18 (Octadecyl dimethylsiloxane) phase

4.3.2. Sodium acetate with tetrabutyleammonium bromide

The addition of an ion pairing agent to acetate was used to separate metal-EDTA complexes on a C18 column and this work has adapted and modified from work reported by Laine and co-workers.⁸⁵ The new mobile phase consisted of 10 mmol/L Sodium

acetate with 10 mmol/L tetrabutylammonium bromide (TBABr) as the ion pairing reagent and 10:90 methanol:water at a pH adjusted to 3.3. Table 4.2 shows the liquid chromatography conditions for the separation of metal-EDTA using sodium acetate with TBABr at pH 3.3.

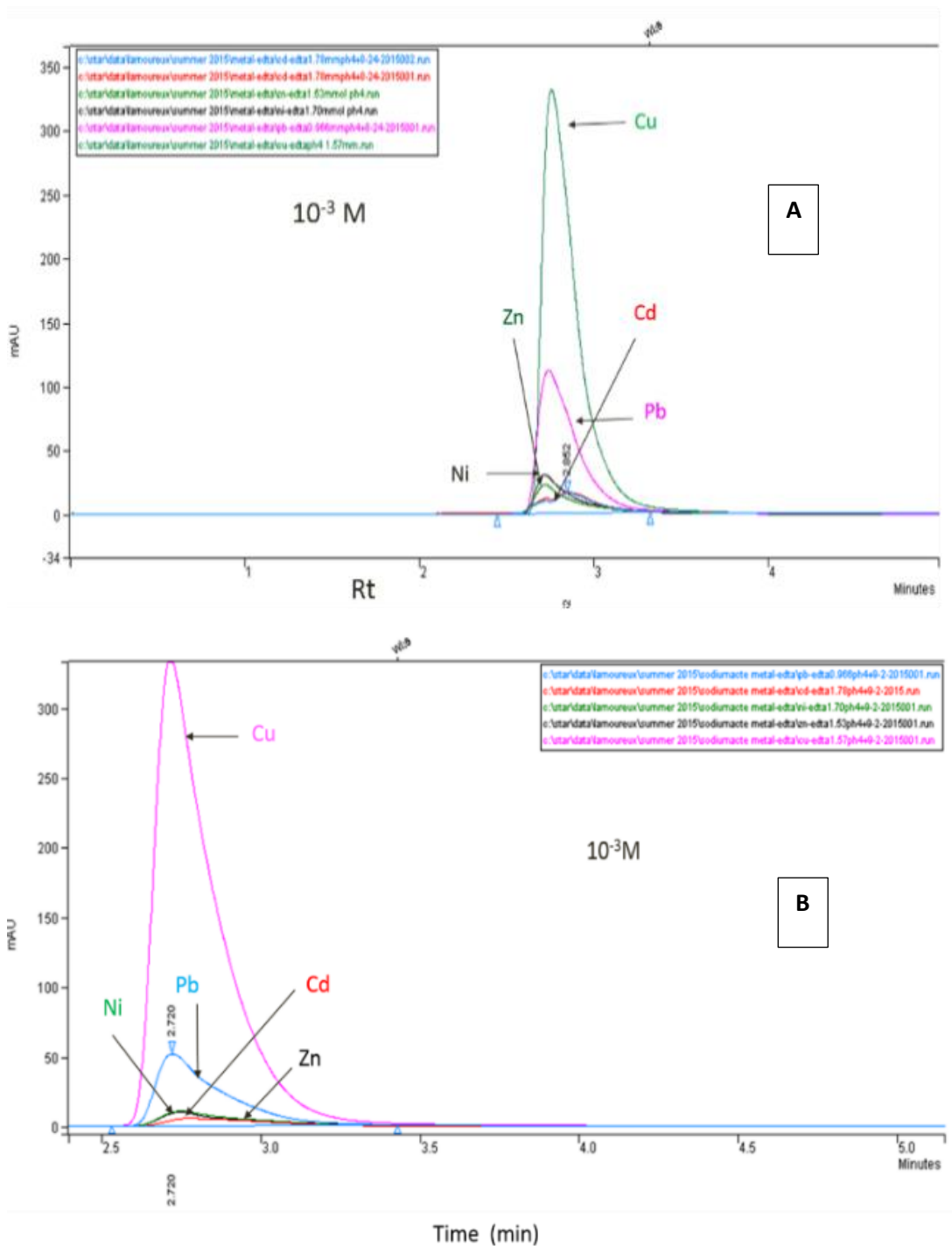


Figure 4.2: Chromatograms of metal ions in contact with EDTA using 50 mM of (A) Ammonium Acetate in 30:70 Methanol:Water; (B) Sodium Acetate in 30:70 Methanol:Water as the mobile phase.

Table 4.2: HPLC conditions for separations of EDTA metal complexes using Sodium Acetate with TBABr

Condition	Description/Value
Columns	Analytical column: Supelco 25 cm x 4.6 mm ID, 5 μ m particle size, Guard Column: Opti-Guard C18, 1.0 mm I.D. (USA)
Mobile Phase	10 mmol/L Sodium acetate , 10 mmol/ L tetrabutyleammonium bromide 10:90 methanol:HPLC grade water (pH adjusted to 3.3 with nitric acid)
Flow Rate	1.0 mL/min
Sample loop	100 μ L
Injection volume	20 μ L
Temperature	Room temperature
Detection	UV-VIS at 254 nm wavelength
metals	Cu, Zn, Ni, Cd and Pb
ligand	EDTA
pH	4 and 9
Stationary phase	Octadecyl dimethylsiloxane phase

Figure 4.3 shows the chromatograms of five target metal-EDTA complexes using 10 mM TBABr plus 10 mM Sodium Acetate with 90:10 HPLC grade water: MeOH at pH 3.3 has good separation for Pb and Cu using TBABr as the mobile phase. Due to Van der Waals forces the TBABr metal-complexes of Pb and Cu ion pair can be retained within the column to allow for separation. Peaks for Cd, Zn, and Ni were not reproducible and not well separated. Overall, the addition of TBABr is necessary to improve the separation on C18 column. Although the quality of separation was poor, it is anticipated that it can be improved with further method development.

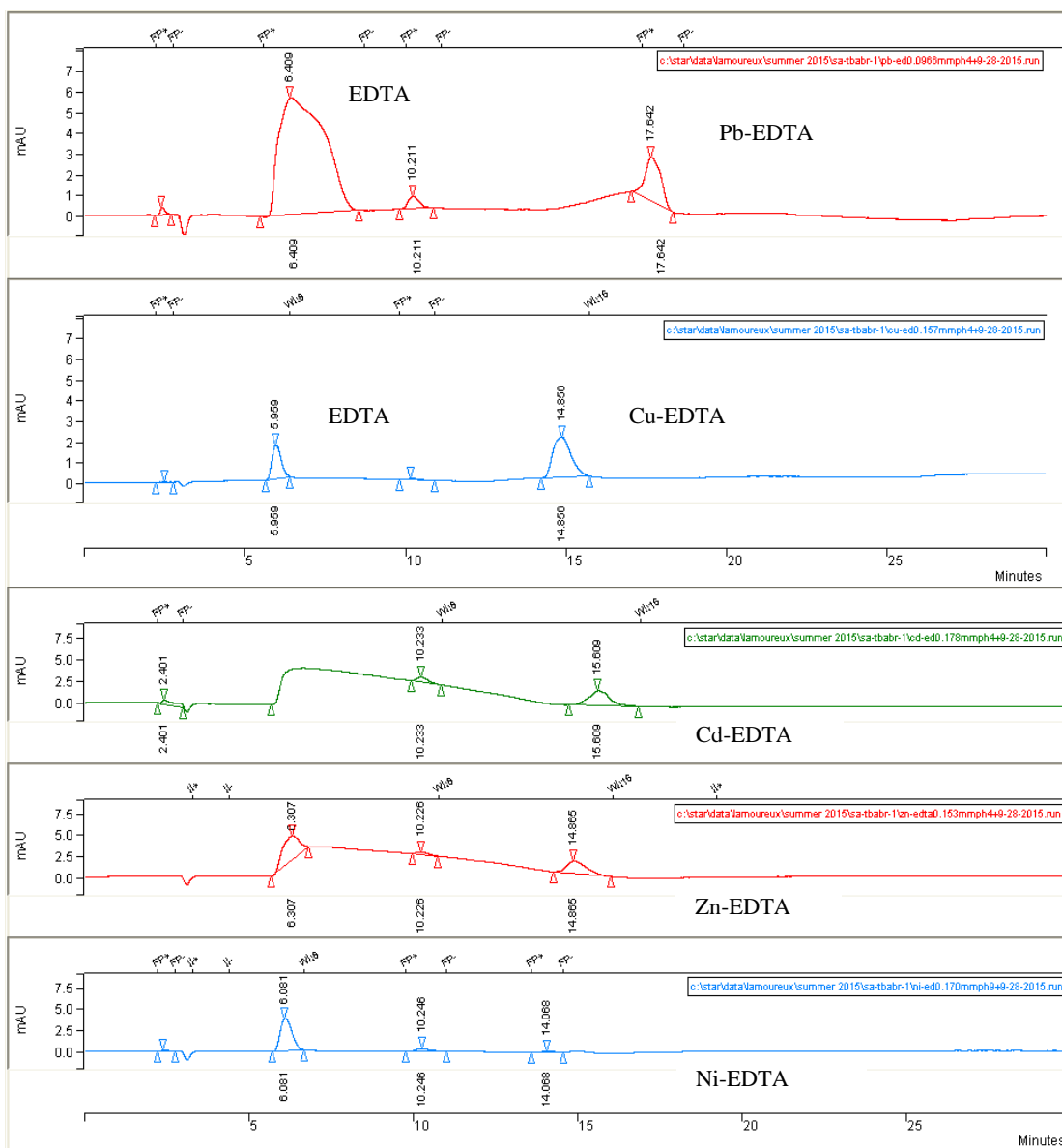


Figure 4.3: Chromatograms separation of EDTA- metal ions using TBABr 10 mM Sodium Acetate with 10 mM TBABr 90:10 HPLC grade water: MeOH at pH 3.3

4.3.3. Ammonium phosphate with tetrabutyleammonium bromide and EDTA

Marina and co-workers⁸⁶ used a phosphate buffer containing 0.02 M TBABr and 0.01 M EDTA for the mobile phase and a C18 column for the separation of some metal-EDTA complexes such as Ni-, Cu-, and Pb-EDTA complexes. Their separation method was adapted and the mobile phase that was used in this study consisted of 0.01 M TBABr, 0.01 M EDTA and 0.05 M (NH₄)H₂PO₄ at pH 3.25. The mobile phase also contained 20% methanol. Table 4.3 shows the HPLC conditions for separations of metal complexes using (NH₄) H₂PO₄ with TBABr and EDTA.

Table 4.3: HPLC conditions for separations of metal complexes using (NH₄) H₂PO₄ with TBABr and EDTA

Condition	Description/Value
Columns	Analytical column: Varian 15 cm x 4.6 mm ID, 5 μM particle size, Guard Column: Opti-Guard C18, 1.0 mm I.D. (USA)
Mobile Phase	10 mM Tetrabutyleammonium bromide (TBABr) + 10 mM EDTA + 50 mM(NH ₄)H ₂ PO ₄ , 10:90 methanol:HPLC grade water adjusted at pH 3.25
Flow Rate	1.0 mL/min
Sample loop	100 μL
Injection volume	20 μL
Temperature	Room temperature
Detection	UV-VIS at 254 nm wavelength
metals	Cu, Zn, Ni, Cd and Pb
ligand	EDTA
pH	4 and 9
Stationary phase	Octadecyl dimethylsiloxane phase

Figure 4.4 shows the chromatograms of the target metal-EDTA complexes using 10 mM TBABr + 10 mM EDTA + 50 mM (NH₄)₂PO₄ at pH 3.25 on a C18 column. Results from Figure 4.4 indicate that Cu and Pb are separated from each other and from

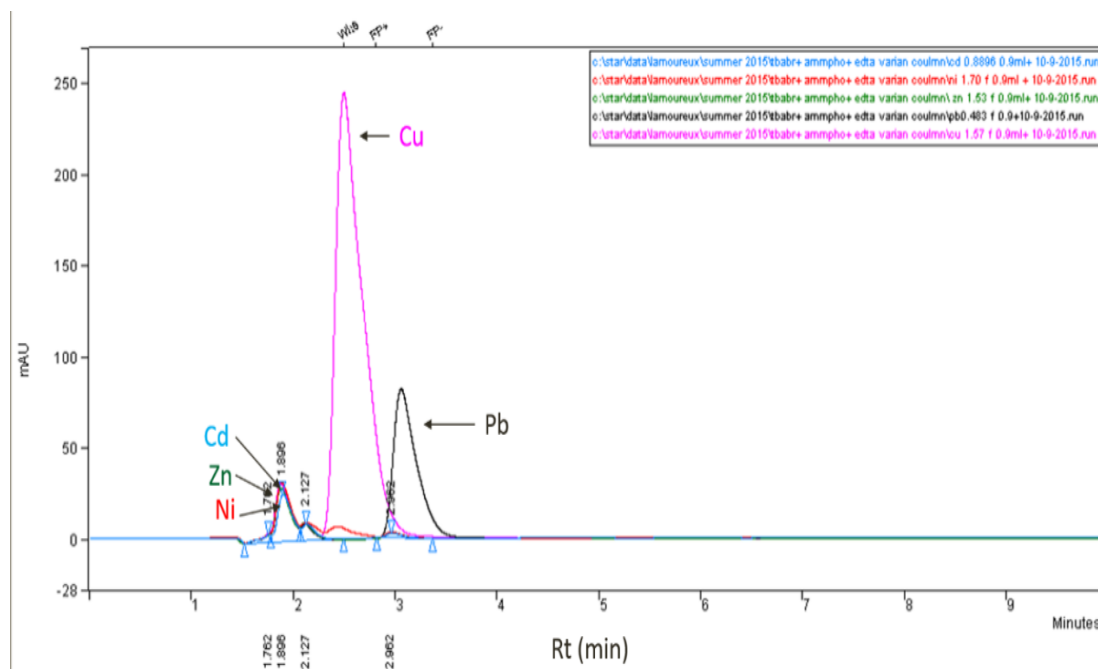


Figure 4.4: Chromatograms of target metal-EDTA complexes using 10 mM TBABr + 10 mM EDTA + 50 mM (NH₄)₂PO₄ at pH 3.25 on a C18 column.

Cd, Ni, and Zn, but that the separation of Cd, Ni, and Zn was not possible using the above-mentioned HPLC conditions.

Although the use of TBABr as an ion pairing agent in the mobile phase to separate metal-EDTA complexes with a C18 column showed some promise, it seems that the C18 stationary phase has limited power, regardless of the mobile phase used, in separating Ni-, Cd-, and Zn-EDTA complexes from each other. Consequently, a different type of stationary phase was used to separate the metal-EDTA complexes of this work.

4.3.4 Sulfuric acid

An application paper from Hamilton Company showed that a PRP-X100 anion exchange column was able to separate Cu-EDTA from other EDTA species when using a dilute sulfuric acid mobile phase.⁸⁷ Figure 4.5 shows that the PRP-X100 column consists of a stationary phase made of polystyrene-divinylbenzene (PSDVB) with a trimethyl ammonium head group. The PRP-X100 anion column with different concentrations of sulfuric acid as mobile phase was used to separate metal-EDTA complexes. Table 4.4 lists the HPLC conditions used for the separation of metal-EDTA complexes using four different concentrations of sulfuric acid.

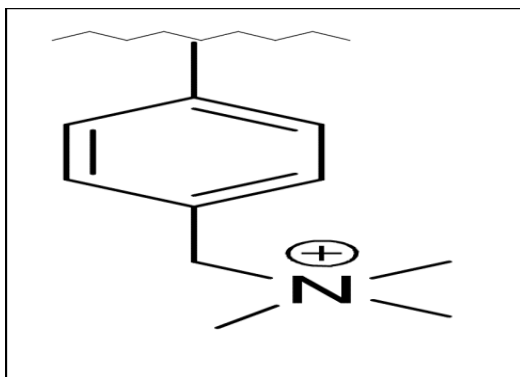


Figure 4.5: Packing Material type of PRP-X 100 column (PSDVB/trimethylammonium)

Different concentrations of sulfuric acid: 2.0 mM, 3.0 mM, 3.6 mM and 5.4 mM, were tested with the anion exchange PRP-X100 column for the separation of metal-EDTA complexes. Figure 4.6 shows the chromatograms for Cu-EDTA and Pb-EDTA at different concentrations of sulfuric acid. Table 4.5 shows the retention time for the five metal-EDTA complexes separated under the above-mentioned conditions. Figure 4.7

shows the LC chromatograms of the five target metals with a 5.4 mM H₂SO₄ mobile phase with the PRP-X100 column. For greater clarity and convenience, chromatograms of metal-EDTA complexes for other concentrations of sulfuric acid are not shown but only their retention time is shown in Table 4.5.. Figure 4.6 and Table 4.5 shows that for the five metal-EDTA complexes, the retention time decreases systematically with increasing concentration of sulfuric acid. However, the rate at which retention time decreases depends on the metal-EDTA complex.

Table 4.4: HPLC conditions used for the separation of Metal- EDTA complexes using four different concentrations of sulfuric acid

Condition	Description/Value
Columns	Analytical column: Hamilton PRP-X100 Anion , column dimension 150× 4.1 mm, Guard Column: Opti-Guard C18, 1.0 mm I.D. (USA)
Mobile Phase	4 different concentrations: 1. 5.4mM Sulfuric Acid 2. 3.6mM Sulfuric Acid 3. 3.0mM Sulfuric Acid 4. 2.0mM Sulfuric Acid
Flow Rate	1.0 mL/min
Sample loop	100 µL
Injection volume	20 µL
Temperature	Room temperature
Detection	UV-VIS at 254 nm wavelength
Metals	Cu, Zn, Ni, Cd and Pb
Ligand	20 mM EDTA
pH	Adjusted to pH 4 for all metals
Stationary phase	PSDVB/ Trimethylammonium

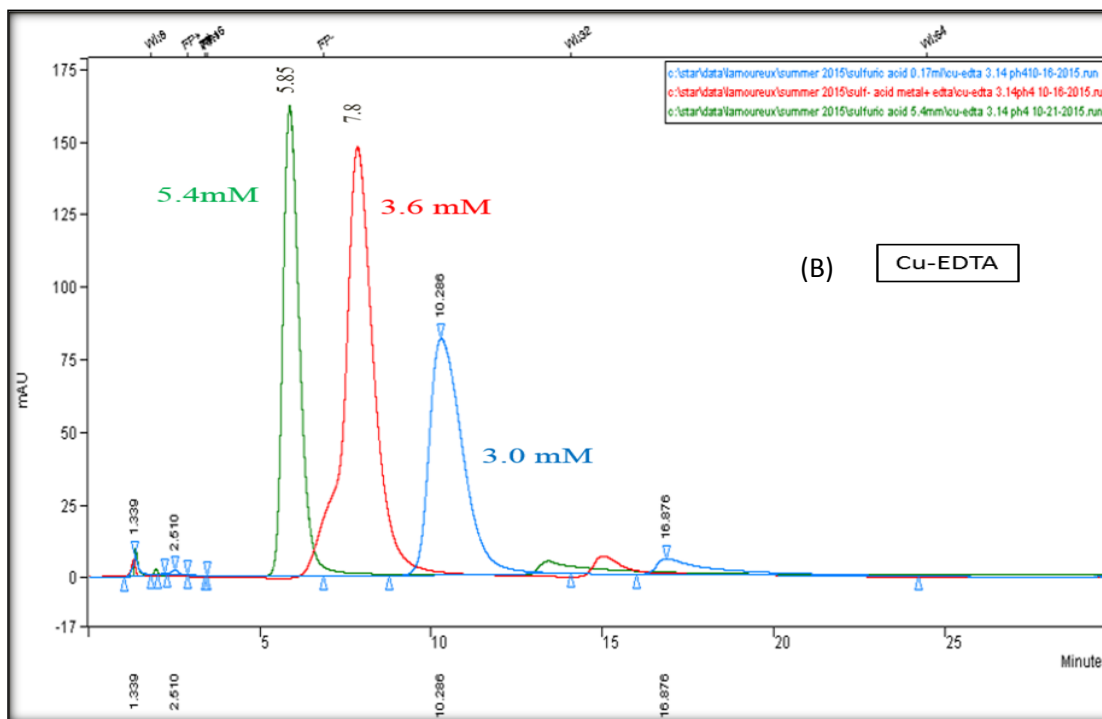
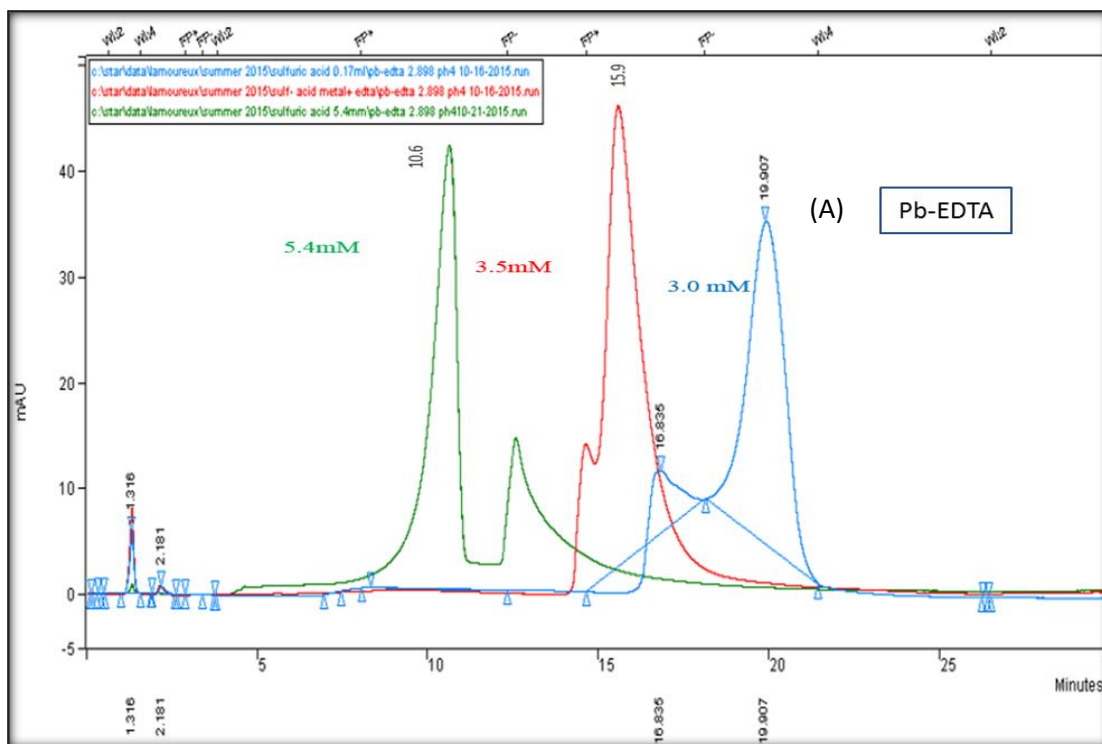


Figure 4.6: Chromatogram of (A) Pb-EDTA and (B) Cu-EDTA complexes with varying concentration of H₂SO₄ concentration.

Table 4.5: Retention time of metal-EDTA complexes with varying concentrations of H₂SO₄ as mobile phase with PRP- X100 column

Conc. Sulfuric acid	Cu t_R (min)	Ni t_R (min)	Pb t_R (min)	Cd t_R (min)	Zn t_R (min)
5.4 mM	5.9	8.5	10.6	14.0	14.9
3.6 mM	7.8	16.0	15.9	15.5	17.0
3.0 mM	10.3	17.0	20.0	16.8	17.0
2.0 mM	17.2	20.0	21.0	20.9	20.5

As shown in Figure 4.5, the anion exchange column consists of a polymer based stationary phase with trimethylammonium functional groups. Negatively charged species in the mobile phase will be attracted to the positively charged quaternary ammonium (NR₄⁺, R = methyl) head group of the stationary phase via electrostatic attraction (thus slowing elution from the column). The decrease in retention time with increasing sulfuric acid concentration can be explained by the increase in the amount of available sulfate counterion (SO₄²⁻) in the mobile phase which can compete for the available positively charged quaternary ammonium head group on the stationary phase, thus leaving fewer quaternary ammonium sites for the sorption of metal-EDTA complexes. The metal-EDTA complexes will spend less time on the stationary phase (because fewer sites are available for retention) which will lead to a reduction in retention time.

The best separation was obtained for a concentration of sulfuric acid of 5.4 mM (see Figure 4.7). At this sulfuric acid concentration, Table 4.5 and Figure 4.7 shows that the order of elution is Cu first, followed by Ni, Pb, Cd, and lastly Zn. The time separation

between the different metal-EDTA complexes is at least 0.9 min. The total separation time is less than 17 min, which is good owing to the fact that longer separation time translates into broader chromatographic peaks due mostly to non-equilibrium mass transfer of metal-EDTA complex to and from the stationary phase.

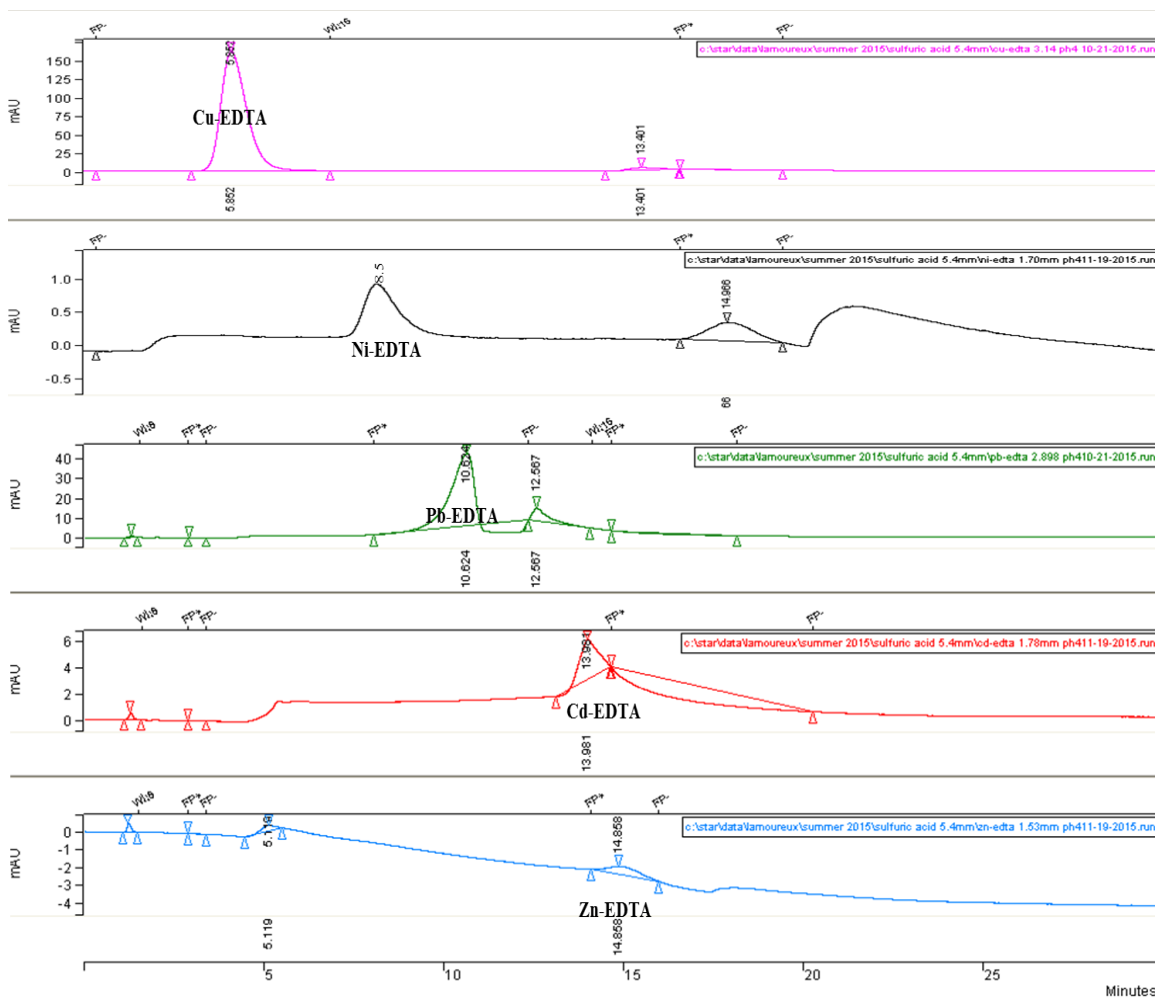


Figure 4.7: Chromatograms of EDTA-metal ions using H₂SO₄ mobile phase at 5.4mM and PRP-X100 column

The quality of this separation suggests that the coupling of LC with ICP-MS should provide a good separation of metal-EDTA complexes with good sensitivity due to the improved detection capability of ICP-MS.

4.4. Determining dissolved, labile sorbed, and non-labile sorbed (bound) fractions

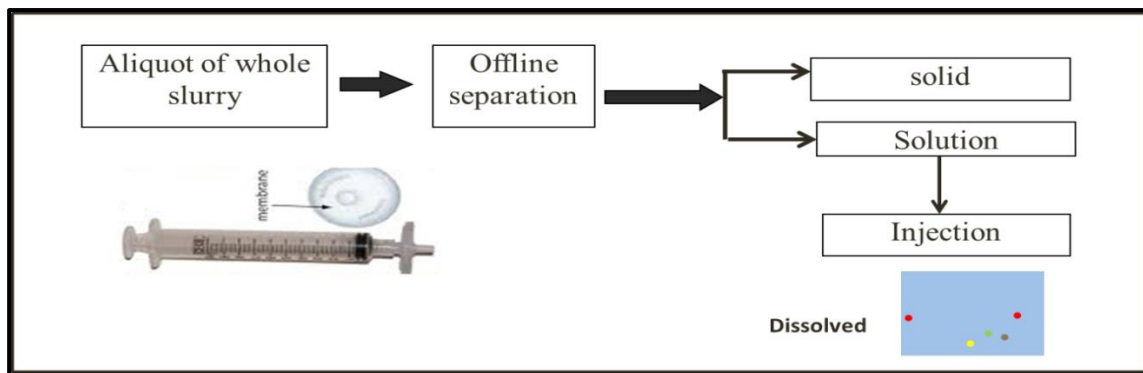
The process of off-line and on-line separation with LC-ICP-MS is used to determine the distribution of metals in contact with EDTA ligands and sediment particles. In this sediment slurry, one can assume that any metal in contact with sediment follows the simple relationship below:

$$\text{Eq (3) Total metal} = (\text{dissolved metal} + \text{labile sorbed metal} + \text{non-labile sorbed metal})$$

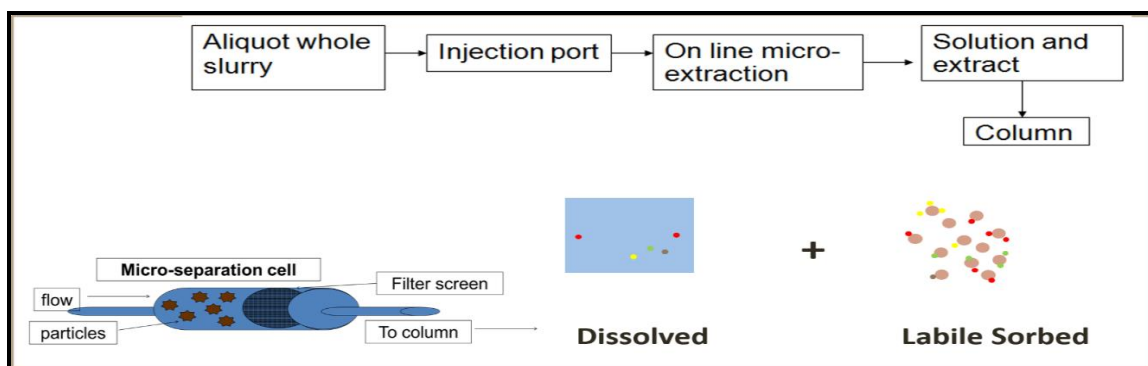
where labile sorbed metal is defined as metal sorbed onto sediment which is extractable with a mild solvent, and non-labile sorbed metal is defined as metal sorbed onto sediment which is not extractable with the same mild solvent.

Off-line and on-line separation via LC-ICP-MS will be used to quantify dissolved metals, labile sorbed metals (i.e. extractable by the mobile phase, the mild solvent), and non-labile sorbed or bound residue metals (non-extractable by the mobile phase).

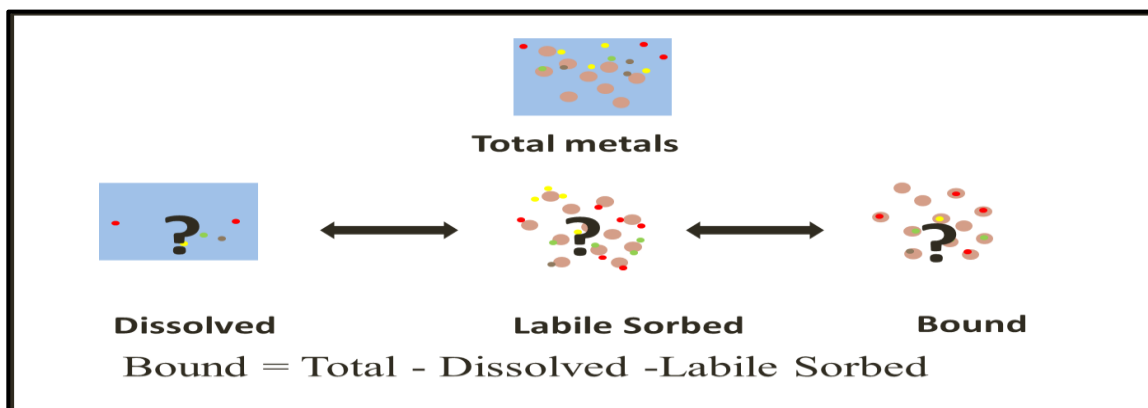
Figure 4.8(a) shows a schematic of the off-line extraction technique which allows recovery (or detection) of the dissolved fraction only from the sediment slurry. Figure 4.8(b) shows a schematic of the on-line extraction technique which allows recovery of both the labile and dissolved fractions. When the total metal concentration is known (after total metal analysis of the sediment slurry), the bound residue (or non-labile sorbed) metal fraction can easily be calculated by difference once the combined dissolved and labile sorbed metal fraction is determined using on-line separation. Figure 4.8(c) shows



(a) Off-Line Extraction



(b) On-Line Micro Extraction



(c) Total sediment

Figure 4.8: Analysis of metals in sediment using (a) off-line and (b) on-line separation; (c) shows the distribution of metals in sediment.

that the combined approach of off-line and on-line separation of whole sediment slurry will allow the quantitative partitioning of metals into three separate fractions: dissolved, labile sorbed, non-labile sorbed.

4.5. LC-ICP-MS method development

A liquid chromatograph (LC) was coupled to an ICP-MS to study the distribution of the target free metal ions and its EDTA complexes when in contact with sediment. The optimum LC separation conditions developed in Chapter 4.3.4 was applied to the LC-ICP-MS hyphenated technique. The combination of LC with ICP-MS will allow the possibility of a two-dimensional separation of the analytes, i.e. separation in time (because of the LC elution through the analytical column) and separation by mass (because of the mass spectrometer).

Table 4.6 shows the experimental conditions used for the LC-ICP-MS separation and detection of the free metal ions and metal-EDTA complexes. Table 4.7 shows the equation and correlation factor for the fit line of the calibration curve of each target metal using the LC-ICP-MS conditions of Table 4.6. Figure 4.9 shows the LC-ICP-MS calibration curve for zinc and lead. The calibration curve of Ni, Cu, and Cd is not shown for simplicity. The results from Table 4.7 and Figure 4.9 shows that the calibration curve of each metal has good linearity. Furthermore, the correlation coefficient, R^2 , is generally better than 0.99 indicating that the experimental data correlate well with the fit line.

Table 4.6: The operating parameters for HPLC and ICP-MS systems

HPLC parameters	
Columns	Analytical Column: Hamilton PRP-X100 Anion , column dimension 150 x 4.1 mm, Guard Column: Opti-Guard C18, 1.0 mm I.D. (USA)
Mobile Phase	5.4 mM Sulfuric Acid, pH= 2.18
HPLC pressure	67 atm
Flow Rate	1.0 mL/min
Sample loop	100 μ L
Injection volume	20 μ L
Column temperature	Room temperature, 20-23°C
Isotopes	^{63}Cu , ^{66}Zn , ^{60}Ni , ^{111}Cd and ^{208}Pb
Ligand	EDTA
pH	Adjust pH 4 for all metals
Stationary phase	PSDVB/ Trimethylammonium
ICP-MS parameters	
Nebulizer pressure	2.65 bar
Forward power	1348 W
Plasma reflected power	0.0 w
Analyzer pressure	5.0×10^{-8} mbar
Expansion pressure	1.4 mbar
Dwell time	100 s
Carrier gas	Ar
Internal standard	^7Li , ^{45}Sc , ^{89}Y , ^{115}In , ^{140}Ce , and ^{232}Th

Table 4.7: Equation and correlation factor for the fit line of the calibration curve

Isotope	Equation of the linear fit line	Correlation factor, R^2
Cu	$I_{\text{cps}} = 8425[\text{Cu}] + 3778$	0.9778
Ni	$I_{\text{cps}} = 6769[\text{Ni}] + 1314$	0.9911
Zn	$I_{\text{cps}} = 7290[\text{Zn}] + 324$	0.9998
Cd	$I_{\text{cps}} = 13718[\text{Cd}] + 597$	0.9894
Pb	$I_{\text{cps}} = 96039[\text{Pb}] + 2952$	0.9986

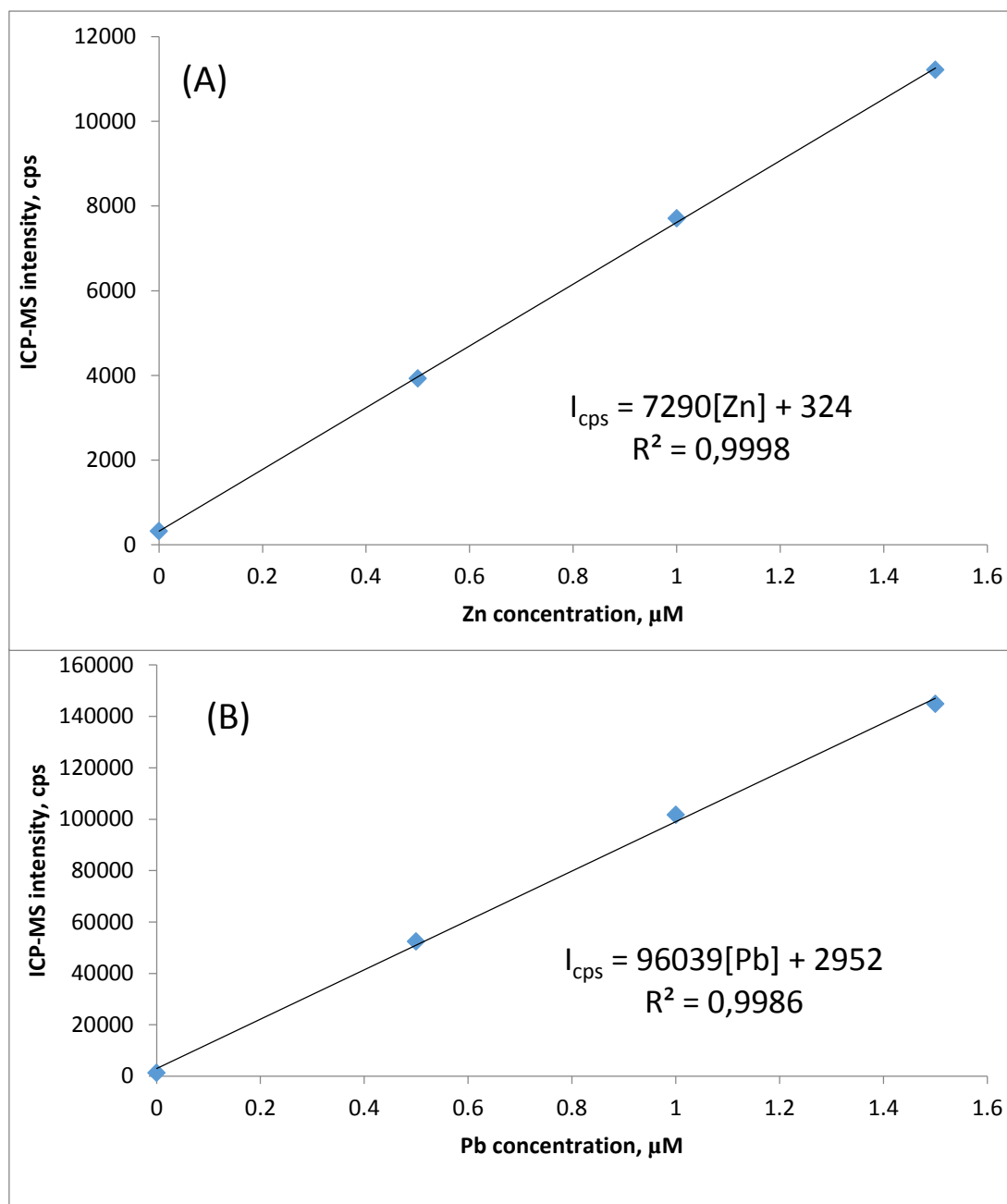


Figure 4.9: LC-ICP-MS calibration curve for (A) Zn and (B) Pb.

Table 4.8 shows the retention time of the eluted target free metal ions and corresponding metal-EDTA complexes using LC-ICP-MS. The concentration of each target metal was 1.5 μM and the concentration of EDTA was 20 μM. For simplicity, the

chromatograms have been omitted. It should be noted that a variation of up to ± 0.2 min (12 sec) is normal. This offset arises from the fact that even though the injection of the sample into the LC is done automatically using the LC autosampler, the ICP-MS data acquisition had to be triggered manually. This created an offset in the order of about 6 to 12 s between the actual LC retention time and the retention recorded by the ICP-MS.

Table 4.8: Retention time of free metal ions and corresponding metal-EDTA complexes using LC-ICP-MS

Elements	Retention time, min		
	Standard only (1.5 μM)	Standard (1.5 μM) + EDTA (10 μM)	Standard (1.5 μM) + EDTA (10 μM) + sediment (50 mg) at $t = 0$ sec
Cu	3.0	6.1	2.4
Pb	2.7	2.9	2.0
Ni	2.4	9.7	1.8
Zn	2.4	2.4	1.8
Cd	2.5	2.4	1.8

The retention time of each free metal (column two of Table 4.8) corresponds to the retention time of unretained ions. The PRP-X100 is a weak anion exchange column and therefore has no affinity for the positively charged target metal cations. The retention time of free metal cations corresponds approximately to the dead time for an unretained peak to travel through the entire LC-ICP-MS system.

Table 4.8 shows that the retention time of free Cu and Ni ions (second column of the Table, standard only) is different from its corresponding EDTA complex (third column of the Table, standard + EDTA). On the other hand, the retention time for the Zn-, Cd-, and Pb-EDTA complex (third column of Table 4.8) is essentially the same as for

the free Zn, Cd, and Pb ions (second column of Table 4.8). Table 4.8 shows also results obtained at the very beginning of the sorption experiment, i.e. when sediment is present with the metal and EDTA containing solution (fourth column, standard + EDTA + sediment). For the sorption experiment, the concentration of each target metal and of EDTA in the slurry solution was 1.5 μM and 20 μM , respectively. It should be noted that the sediment slurry was subjected to a minimum of 24 hr wetting condition prior to being spiked with metals and EDTA at the begin of the sorption experiment. This is to ensure that the sediment behave as closely as possible to a natural sediment in water. It is understood, however, that the aqueous solution in which the sediment was introduced is not equivalent to the estuarine water from which the sediment was extracted from, i.e. Chesapeake Bay. The estuarine water composition of Chesapeake Bay at the time of collection is not provided with the certificate of analysis and therefore it remains unknown to this experimenter.

Column four of Table 4.8 shows that the observed retention time for all metals of interest corresponds essentially to that of free metal ions. This unexpected behaviour cannot be explained by shift in retention time due to a change in the pressure of the mobile phase after injection of a sample because the sample was injected using the off-line injection procedure, i.e. external filtration using a membrane filter was used prior to injection. It is possible, however, that the metal-EDTA complexes are strongly sorbed onto the sediment such that only the free metal ions remain in solution and are detected by LC-ICP-MS. The retention time of each metal ion with EDTA in contact with sediment (column four of Table 4.8) is shorter than those for the free metal ions (column

two of Table 4.8) and difference in retention time is approximately 0.6 min. No explanation can be offered at this time to explained this shift in retention time.

Figure 4.10 shows LC-ICP-MS signal intensity for the five target metals plus EDTA with and without sediment at the beginning of the sorption experiment. The

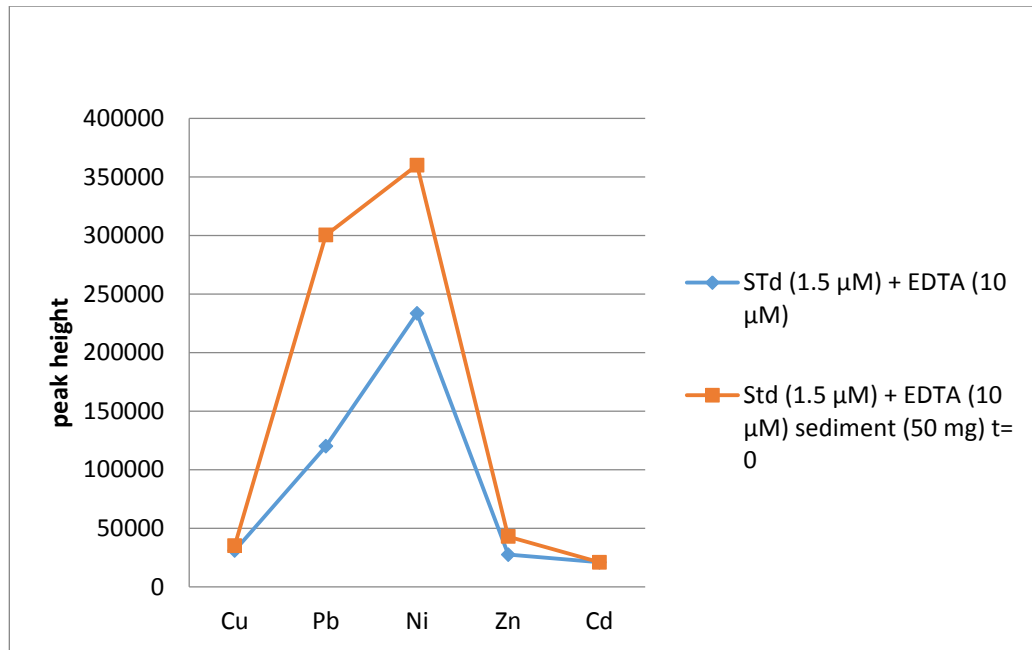


Figure 4.10: LC-ICP-MS signal intensity for the five target metals plus EDTA with and without sediment.

addition of sediment to the metal-EDTA solution causes an increase in the signal for all metals except for Cd, the increase being most important for Pb, Ni, and Zn. This signal increase is simply due to the fact that the sediment contains non-negligible amounts of trace metals. Table 3.2 shows that the concentration of Pb, Ni, Zn, Cd, and Cu is 11.7, 23, 48.9, 0.148, 10.01 mg/kg, respectively. One should not expect, however, to see the signal intensity increase due to the presence of the sediment to follow the increase in the

relative concentration of the sediment trace metals, i.e. a proportional relationship. The concentration of lead in the sediment is about half that of nickel and a quarter of zinc, and yet it produces a greater change in signal intensity than that of the Ni or Zn. The reason for this is, first, the ICP-MS intensity depends on the first ionization potential of the isotope being measured. The ICP-MS signal intensity depends on the ability of an element to ionize in the ICP plasma. The ionization efficiency is proportional to the logarithm of the first ionization potential of the element⁸⁰. The first ionization potentials for Pb, Ni, and Zn are 7.416 eV, 7.635 eV, and 9.394 eV, respectively.⁸⁰ One can see that the ionization efficiency of Zn is about one hundred time less than that of Pb or Ni and consequently, for equal concentrations and natural abundances, the ICP-MS signal of Zn would be about one hundred time less than that of Pb or Ni. The signal collected at mass-to-charge (m/z) ratio of 208 amu is the sum total of isotopes ²⁰⁶Pb, ²⁰⁷Pb, and ²⁰⁸Pb. The natural abundance of ²⁰⁶Pb, ²⁰⁷Pb, and ²⁰⁸Pb is 24.1%, 22.1%, 52.4%, respectively.⁸⁰ This amounts to a total natural abundance of Pb isotopes of 98.5%, all summed up as m/z 208 amu. The natural abundance of ⁶⁰Ni and ⁶⁶Zn is 26.2% and 27.9%, respectively.⁸⁰ Again, one can see that the natural abundance of the combined lead isotopes is about four times the natural abundance of either ⁶⁰Ni or ⁶⁶Zn and consequently, for equal concentrations and similar first ionization potential, the ICP-MS signal of Pb would be about four times greater than that of Zn or Ni. Therefore, the combined effect of low first ionization potential and the greater isotopic natural abundance will favour the greatest change in signal intensity for Pb compared to any of the other four target metals.

Figure 4.11 shows the time-dependent concentration of the sorption experiment for the five target metals in contact with EDTA and sediment. The results showed in Figure 4.11 were collected using the off-line separation procedure with LC-ICP-MS, i.e., the solution was separated from the sediment using a membrane filter prior to injection in the LC. Results were collected only for the first two days because the ICP-MS became out of order after the second day of the experiment. This situation is discussed below.

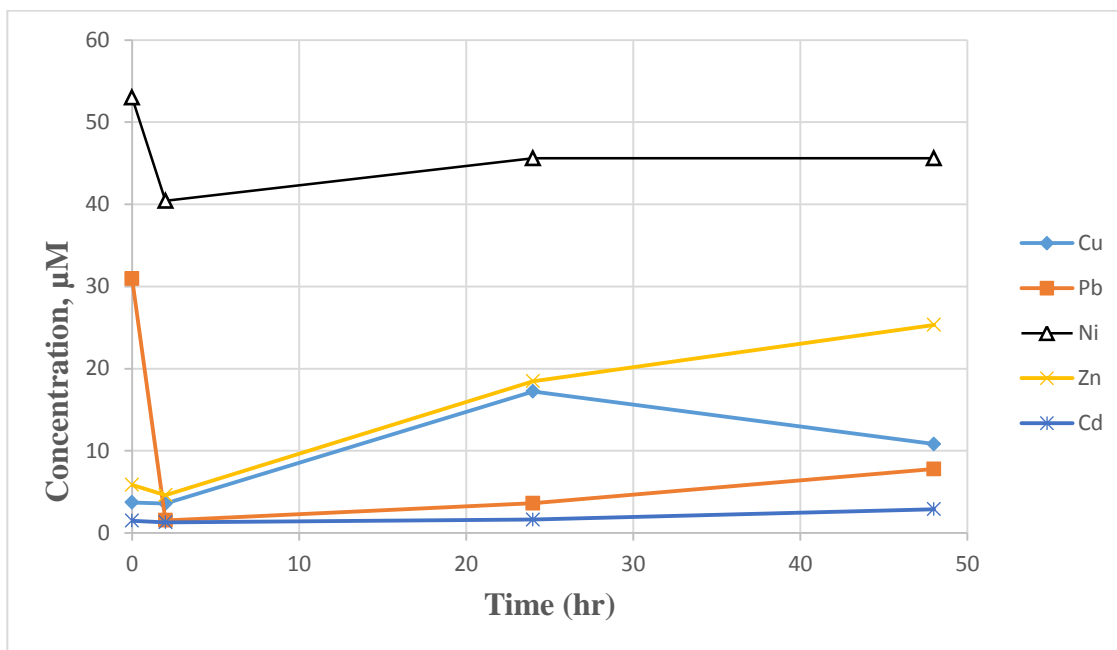


Figure 4.11: Time-dependent concentration for the five target metals (Cu, Pb, Ni, Zn, Cd) in contact with EDTA and sediment.

One can observed that in the first two hours of the sorption experiment, the concentration for all target metals initially decreased, i.e. that the concentration of dissolved target trace metals decreased. This is likely due to sorption onto the sediment particles. However, after the second hour of the sorption experiment, the concentration increased for all metals except Ni, which remained approximately the same after two hours. In fact, the increase in concentration is such that the signal after one day of

sorption experiment was greater than the initial concentration measured at the beginning of the experiment when the target metals had just been added to the sediment slurry. Results of Figure 4.11 suggest that the target metals already in the sediment (i.e part of the overall sediment composition) desorbed into the solution phase and thus causing an increase in the overall target metal concentration in the dissolved phase (or solution phase). This information together with results reported in Table 4.8, column four, suggests that the metal-EDTA complex dissociates in the presence of the sediment, leaving the free metal ions behind in solution. In order for the metal ions to remain in solution as free ions when EDTA is present, EDTA would have to be no longer available in solution to complex with metal ions in solution. Therefore, this suggests that EDTA could be sorbed onto the sediment, possibly complexed with the sediment metals such as Ca, Mg, and Al, which are at the percent concentration level in the sediment. There is no experimental evidence that suggest that the sorption of EDTA onto sediment is actually taking place. The fact remains, however, that the concentration of target trace metals in solution is increasing after one day of sorption experiment when the expectation is that it would decrease over time.

The sorption experiment had to be stopped due to the fact that the ICP-MS became out of order in March 2016, two days after the beginning of the sorption experiment, and has not been repaired since due to insufficient financial resource to effect the repair. No other ICP-MS or similar instruments with sensitivity and multi-element detection capability are available at SMU or locally. Consequently, the ability to continue the development and application of the off-line and on-line separation method with LC-ICP-MS was compromised.

Chapter 5 CONCLUSION

A new method for determining the distribution of trace metals in natural water containing complexing ligands such as EDTA and sediment was developed. This method required the coupling of an LC with an ICP-MS. The method uses an off-line or an on-line separation method prior to the injection of a sediment slurry containing both trace metals and EDTA. A certified estuarine sediment from NIST was identified and characterized as the test sediment to induce sorption of metals from water. The certified estuarine sediment was composed essentially of 89% silica (SiO_2), 6% anorthite ($\text{CaAl}_2\text{Si}_2\text{O}_8$), and 5% albite ($\text{NaAlSi}_3\text{O}_8$). The average % concentration of C, H, and N in the sediment was 0.56 %, 0.20 % and 0.14 %, respectively. The LC separation was optimized using a weak anion exchange column containing a polystyrene divinylbenzene trimethylammonium stationary phase with a 5.4 mM sulfuric acid mobile phase. A sorption experiment was conducted, where target trace metals with EDTA are in contact with a sediment slurry, and the preliminary results shows that the metal sorption kinetics using time-dependent ICP-MS signals could be followed and measured.

Chapter 6 FUTURE WORK

The sorption experiments of this thesis had to be stopped abruptly due to a breakdown of the ICP-MS, one of the main instrumentation required for this work. Financial resources are no longer available to repair the ICP-MS and it is presently targeted for decommissioning. Sorption experiments could be continued using other type

of instrument which have detection capability that are specific to metals. For example, an ICP-Atomic Emission Spectrometer (ICP-AES), which is currently now available at SMU or locally, could be used. ICP-AES has simultaneous multi-element detection capability similar to ICP-MS but the sensitivity would be about ten to one hundred time less than that of ICP-MS and thus the concentration of target trace metals would have to be raised by at least ten fold. A Flame Atomic Absorption Spectrometer (FAAS) could also be used instead of the ICP-MS. The Chemistry Department at SMU has an operating FAAS which could be used for this work. However, FAAS does not have simultaneous multi-element detection capability, i.e., it is a sequential, one element at a time, detection system, which means that for the five targeted metals of this work (Ni, Cu, Zn, Cd, and Pb), each sample would have to be analyzed five times and thus the length associated with sample analysis would be five time longer than with ICP-MS or ICP-AES. Furthermore, the sensitivity of FAAS is one hundred to one thousand times less than that of ICP-MS, and consequently, the concentration of target metals would have to be at least one hundred times greater than that used in this work. Understanding that trace metal concentration in natural water is typically at micromolar concentration or less, raising the concentration of target trace metals at nearly millimolar concentration could render the experiment irrelevant.

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