DYNAMIC SEPARATION OF A SELECTION OF HEAVY METALS USING A DITHIZONE-IMPREGNATED POLYMER RESIN

by

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DECLARATION

I, Kim Thomas (214237168), declare that the contents of this dissertation represent my own unaided work and that the dissertation has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

This investigation focuses on optimising and separating metal ions using a dynamic (column) solid-phase extraction method. In this study, a polymer resin, Amberchrom CG-300, was successfully impregnated with dithizone. The investigation primarily focused on cadmium and nickel metal ions. Chelation was used as an adsorption method to optimise the separation of these metal ions. The study involved the selection and concentration of the eluent, flow rate, and column height of the resin to determine the optimal conditions for separating a copper-nickel solution. The recovery of these metal ions was determined using inductively coupled plasma and atomic absorption spectroscopy. This research was inspired by a recommendation from Dr Andre Spies in his 2020 doctoral dissertation.

All experimental work was carried out at a pH of 8. Hydrochloric acid was determined to be the most suitable eluent for both metal ion investigations, with a concentration of [HCl] = 0.1 M for cadmium and [HCl] = 0.4 M for nickel. This resulted in percentage recoveries of 41.82 % and 84.61 %, respectively.

Flow rate experiments were conducted within the range of 4 – 7 mL/min. It was observed that a flow rate of 7 mL/min resulted in the highest recovery rate during the cadmium study, reaching 24.15 %. In the nickel investigation, a flow rate of 6 mL/min yielded a recovery rate of 73.88 %. In the investigation of column height, it was found that the range studied did not significantly affect the elution of cadmium ions. The highest recovery was achieved with 0.4 g of resin for both studies, resulting in an 18.18 % recovery of cadmium ions and 95.30 % for nickel ions. This study was done within 0.2 g – 0.5 g range.

Copper and nickel metal ions in a binary solution were separated successfully at a pH of 8, resulting in a 94.40 % recovery of nickel ions and a mere 39.72 % recovery of copper ions. For this experiment 0.4 M hydrochloric acid was used as an eluent at a flow rate of 6 mL/min.

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ACRONYMS AND ABBREVIATIONS

DEHPA:	di-(2-ethyl) phosphoric acid
DETA:	diethylenetriamine
DMSPE:	dispersive magnetic SPE
EDMA:	ethylene dimethacrylate
EGDMA-MAH/Ni:	ethyleneglycoldimethacrylate-methacryloylhistidinedihydrate nickel(II)
FAAS:	flame atomic absorption spectroscopy
GMA:	glycidyl methacrylate
Mgo/SiO_2@coPPry-Th:	SiO_2-coated magnetic graphene oxide modified with polypyrrole-polythiophene
MSPE:	magnetic solid-phase extraction
MWCNT:	multi-walled carbon nanotubes
NR:	not reported
PMMA:	poly(methyl methacrylate) grafted agarose
SC:	sorption capacity
SPE:	solid-phase extraction

CHAPTER ONE

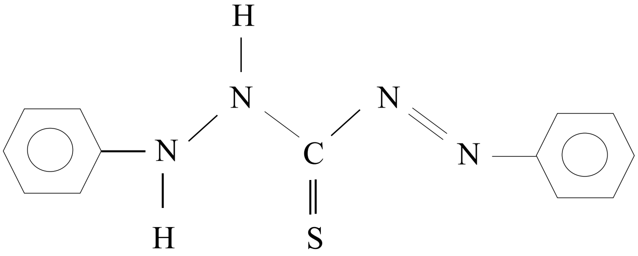
INTRODUCTION

1.1 Background

Water is a valuable resource that facilitates social and economic development. Literature broadly documents the pollution of both surface and groundwater owing to inadequately treated discharges originating from specific points, like industrial facilities, pharmaceutical plants, leachate sites, and sewage treatment plants. Effluents from industries have been reported to contain heavy metals such as zinc (Zn), mercury (Hg), cadmium (Cd), lead (Pb), and chromium (Cr), known for their stubborn behaviour that harms people, the environment, and animals (Ritter et al., 2002). Their release into the environment occurs readily through human activities such as metal plating operations, mining, and agricultural practices (Sultana, 2018). According to Liška (2000), many researchers are interested in finding ways to remove heavy metals from polluted water. These methods include chemical precipitation, membrane processes, solvent extraction, electrochemical treatment, ion exchange, and solid-phase extraction. Some of these methods are not economically feasible, and may be ineffective, especially at low metal ion concentrations (in the order of 1 to 100 $mg{\cdot}L^{-1}$).

Solid-phase extraction (SPE) is recognized as one of the most prevalent techniques in analytical chemistry due to its high efficiency and cost-effectiveness. This method is widely adopted for sample preparation because it simplifies the extraction process, reduces solvent consumption, and provides reliable results. SPE's versatility allows it to be applied across various fields, including environmental analysis, pharmaceuticals, and food safety testing, making it an essential tool for laboratories aiming for precise and efficient sample purification. It involves the distribution of solutes between two phases: a liquid phase (the substance to be separated) and a solid phase (the material used for separation). It is a sample processing method that allows the enrichment and washing of analytes from a solution through their adsorption onto the solid phase (Liška, 2000). Once the entire sample volume has passed through the solid phase, the retained analytes are recovered by elution using an appropriate solvent. Much work has been done to create new sorbent materials. The goal is to make them better at capturing specific substances and more stable physically and chemically. These improvements help in more accurate and reliable analysis of targeted analytes (Augusto et al., 2021). Chemical modification, especially with chelating agents, is one such improvement that has been researched extensively. Modification with a complexing reagent increases the capacity, stability, and selectivity of sorbents (Türker, 2012).

1,5-diphenylthiocarbazone (also known as dithizone) is an example of a powerful chelating agent which reacts with many metal ions (Nezhadali & Nazari, 2009), forming complexes with approximately 20 heavy metals (Lu et al., 2015). Below is the structural formula of dithizone:



In this study, the extraction and separation of Cd, Sn, Ni, and Cu from aqueous solutions onto a sorbent modified with dithizone, was investigated. Cadmium poses the most dangerous element to human life (even at low concentration levels). It can cause serious damage to the kidneys, liver, and lungs (Behbahani et al., 2013). Tin is a lethal heavy metal, which can be stored in the tissue of the human body and in animals (Esmaeilzadeh, 2019). Nickel is moderately toxic – inhalation of the metal and its compounds causes cancer to the respiratory system (Ferreira et al., 2001). Copper is an important element in biological systems and plays a role in lipid and carbohydrate metabolism. A level of 40 ng/mL of copper is needed for metabolism to occur; however, at higher levels, severe intoxication can affect the blood and kidneys (Tabrizi, 2007).

1.2 Research problem

Spies (2020) has studied the sorption mechanisms of Cd^{2+}, Ni^{2+}, Sn^{4+} and Cu^{2+} onto dithizone-impregnated Amberchrom-CG300m in batch experiments. However, the dynamic separation of metal ion pairs, and the parameters that influence their recovery have not been investigated. These parameters include sample flow rate, eluent type, eluent concentration and column height. The ability to recover metal ions after sorption is an important indicator of the reusability and efficiency of a sorbent. This study aims to optimise the separation of the metal ion pairs Cd/Sn and Cu/Ni, and the subsequent recovery of the sorbed metal ions by the column method.

1.3 Significance of the research

Chelating resins have been investigated for their action in various uses such as hydrometallurgy, chemical catalysis, nuclear waste, trace metal analysis, and wastewater treatment. It has also found use in nuclear chemistry to separate and refine byproducts during particle bombardment of the parent analogues called daughter isotopes. The latter application is essential in nuclear medicine where there is an ever-increasing demand for radionuclides used in theragnostic procedures in cancer treatment, which are both diagnostic and therapeutic; an example is that of a radionuclide that has been earmarked for “theragnostic” purposes. Radiopharmaceuticals have favourable applications in the comforting treatment of painful metastases in bone cancers (Spies, 2020).

1.4 Research aim and objectives

The primary aim of this study is to optimise the parameters for the dynamic separation of Cd from Sn, and Cu from Ni.

1.4.1 Specific objectives

- To impregnate Amberchrom CG-300m resin with dithizone.
- To evaluate the effects of eluent type, eluent concentration, flow rate, and column length on the separation of the metal ions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter offers a comprehensive overview of heavy metals, their environmental and human impacts, and the various techniques employed to remove them from sample analytes. The focus is on solid-phase extraction as a method, detailing its mechanisms, the traditional sorbents used, and its advantages and disadvantages. The use of dithizone as an extractant in SPE is explored in depth. The study also explains the parameters investigated and underscores the importance of optimization.

2.2 Heavy metals: Significance and separation techniques

2.2.1 Brief overview of heavy metals

Heavy metals are a group of metals and metalloids (semimetals) that are ecotoxic or potentially toxic. They are defined as natural metals with an elemental density larger than $5\,g\cdot cm^{-3}$, and an atomic number larger than 20 (Ali et al., 2019). Heavy metals such as zinc, nickel, lead, chromium, copper, mercury, and cadmium are also known as trace elements. They can be harmful if there are too many in the body, but they are also needed to keep the body's processes working properly (Sonone et al., 2020).

Effluents from industries have been reported to contain some heavy metals. These metals can cause genetic mutations and cancer. They are linked to various health problems in humans (Sultana, 2018) because they are hazardous inorganic pollutants (Laureano-Anzaldo et al., 2021). The number of heavy metals in soil and water has grown quickly in recent years. This is mainly due to waste disposal, use of fertilizers and pesticides, electronic waste, sewage, metal smelting, mining, and burning fossil fuels (Kim et al., 2019). These metals are also used in dyes and pigments, painting industries, metallurgy, electronics, electroplating, and agriculture (Laureano-Anzaldo et al., 2021). The United States Environmental Protection Agency (USEPA) has identified arsenic, copper, lead, cadmium, chromium, and mercury as priority control pollutants because of their persistent and toxic characteristics (Kim et al., 2019).

With such a significant impact on humans and the environment, stricter legislation is being implemented worldwide to regulate and control these pollutants (Laureano-Anzaldo et al., 2021).

2.2.2 Impact of heavy metals on environment and human health

Humans are exposed to heavy metals in the environment through inhalation, ingestion, and absorption through the skin. Most people do not fully understand how heavy metals affect their health. They may be exposed to heavy metals in the workplace (occupational exposure) and the environment (nonoccupational/environmental exposure). Those who work in industrial operations and mining may breathe in particulate matter and particulates containing metals (dust). They are also exposed to Hg vapours through the amalgamation process while extracting gold. Welders have a drastically larger amount of Pb, Ni, Cr, and Cd in their blood due to prolonged occupational exposure to welding fumes (Ali et al., 2019).

The general population worldwide is largely exposed to heavy metals through the ingestion of food and drinking water, especially in areas where there is bioaccumulation of these metals in the media in which food is grown. The fast economic development, urbanisation and industrialization have led to an escalation in agricultural and industrial activities, which may be the cause of toxic heavy metal contamination of soil, water, and air (Ali et al., 2019).

The effects of Cd, Cu, Pb, and Ni on the environment and humans are discussed in the sections below.

2.2.2.1 Cadmium

Cadmium is one of the most harmful elements to human health. Even at low concentrations, it can cause damage to the kidneys, liver, and lungs. It also has harmful effects on metabolic processes in the human body (Behbahani et al., 2013). Human exposure to cadmium primarily occurs in two ways: inhalation (breathing) and ingestion (eating and drinking). However, dietary exposure, which can lead to kidney and bone damage, significantly impacts health. Occupational exposure and tobacco smoking can result in lung damage. For non-smokers, dietary intake accounts for approximately 90 % of all cadmium exposure, especially in diets rich in meat or products sourced from marine animals.

Because cadmium does not naturally break down into less toxic substances, it poses a significant threat to plants, animals, and various microorganisms. Exposure can also occur when contaminated soils are disturbed, releasing dust. Anthropological sources introduce cadmium into the environment through wastewater, industrial air emissions, and the widespread use of fertilisers in agriculture. Rice and tobacco grow in cadmium polluted soils – this increases the risk of human exposure (Mahurpawar, 2015).

2.2.2.2 Copper

Copper is an important element in biological systems and plays a role in lipid and carbohydrate metabolism. Copper levels of 100 – 250 μg/L are required for metabolism to occur. However, at higher levels, severe intoxication can affect the blood and kidneys (Tabrizi, 2007). In small amounts, copper is a necessary nutrient for plants, animals, and humans. In fact, copper deficiency may cause sicknesses (including osteoporosis in infants and children), low white blood cell levels, and skeletal problems because of its deficiency in connective tissue. Acute poisoning via ingestion results in temporary gastrointestinal distress, with symptoms such as abdominal pain, nausea, and vomiting. High levels of exposure to copper cause anaemia, a disease resulting from the destruction of red blood cells.

Mammals possess efficient physiological mechanisms to regulate copper levels within their bodies, primarily to prevent toxicity resulting from excessive ingestion. Under normal circumstances, these processes effectively guard against copper overload, maintaining homeostasis. However, certain genetic disorders, such as Wilson’s disease, disrupt this balance. Wilson’s disease is an inherited condition characterized by the abnormal accumulation of copper in the liver, which can lead to serious health complications if left untreated. Notably, symptoms of this disorder often remain dormant until puberty, when they may become more apparent. Research involving workers exposed to copper has indicated an increased risk of developing cancer, suggesting potential carcinogenic effects associated with occupational exposure. Conversely, studies conducted on animals have not demonstrated a similar increase in cancer risk, highlighting possible differences in susceptibility or exposure levels. Additionally, developmental studies in animals have revealed several adverse effects, including reduced litter sizes, delayed bone formation, decreased body weight, and slowed growth and development. Despite these findings in animal models, there have been no documented reports of developmental effects in humans exposed to copper, indicating a need for further research to understand the implications fully (Mahurpawar, 2015).

2.2.2.3 Tin

Compounds of inorganic tin generally have no harmful effects on humans and animals because they pass through the body rapidly after ingestion or inhalation. However, any potential harmful effects depend on the specific type of organotin compound involved. Cases resulting in death were typically due to the ingestion of large quantities of the compound.

Studies indicate that both animals and humans experience similar side effects when ingesting significant amounts of inorganic tin, including anaemia, liver and kidney problems, and stomach aches. No evidence suggests that inorganic tin causes cancer, birth defects, genetic mutations, or reproductive issues. Nevertheless, skin contact (dermal exposure), ingestion (oral), and inhalation (respiratory exposure) to some organotin compounds have been associated with adverse health effects in humans.

Some organotin compounds have demonstrated negative effects in humans following high-concentration exposure over a short period. These effects include gastrointestinal disturbances, respiratory issues, eye and skin irritation, and neurological problems. Notably, neurological symptoms resulting from such exposure have been reported to persist for years after the initial poisoning (Mahurpawar, 2015). Extreme tin mining activities cause acute environmental damage (Tiandho, 2019).

2.2.2.4 Nickel

Nickel is found at very low concentrations in the environment. One of its primary applications is as a component in metal products and steel, particularly in the production of jewellery. Naturally, food contains small amounts of nickel; however, fats and chocolate are known to contain significantly higher quantities. Plants can accumulate nickel, which increases the importance of monitoring the levels of nickel in vegetables grown in contaminated soils, as humans and animals consume these vegetables. Human exposure to nickel occurs through various pathways, including ingestion of contaminated food and water, smoking cigarettes, and inhaling air containing nickel particles.

Exposure to nickel can also happen through skin contact with contaminated soil or water, as well as via contact with certain detergents. In small amounts, nickel is essential for human health; however, excessive exposure can be toxic. High levels of nickel exposure, whether through ingestion or inhalation, are associated with an increased risk of developing certain cancers, including prostate, nasal, laryngeal, and lung cancers.

Symptoms of acute nickel poisoning may include dizziness, nausea, and respiratory issues such as lung embolism, heart disorders, respiratory failure, asthma, and birth defects. Chronic exposure can lead to conditions like bronchitis, and allergic reactions such as skin rashes caused by jewellery (Mahurpawar, 2015).

2.2.3 Current techniques for heavy metals separation

Many removal technologies can be used for the separation of metal ions in sample analytes. A brief overview of chemical precipitation, adsorption onto activated carbon, electrochemical precipitation, membrane processes, ion-exchange chromatography, and solid-phase extraction is given below.

2.2.3.1 Chemical precipitation

A solution containing a mixture of metal ions can be separated using precipitation. Separation occurs when metal ions form insoluble salts with certain anions or cause them to settle out with specific metal ions (Chieh, 2019).

Precipitation processes distinguish between coagulation and flocculation:

- Coagulation involves the accumulation of finely divided, non-settleable solid particles into larger particles by interrupting the electric double layer around these particles.
- Flocculation refers to the formation of even larger particles by forming bridges between the coagulated particles. The bridging effect happens when large polymer molecules attach to particles, causing them to stick together. This process, called coagulation and flocculation, results in bigger particles that can be removed more easily through settling or filtering (Wang et al., 2005).

Compared to other methods such as solvent extraction or electrochemical separation, precipitation is often simpler and cheaper. For best results, it is important to carefully control factors like pH, temperature, and the amount of chemicals used, which helps increase the purity and amount of the metals recovered (Chieh, 2019). According to Wang et al (2005) the primary factors influencing the efficiency of precipitation processes include:

- Maintaining a basic medium ($pH > 7$)
- Pushing the precipitation reaction to completion by adding an excess of treatment ions
- Addition of a sufficient supply of sacrificial ions to guarantee precipitation and the removal of target ions.
- Successful removal of precipitated solids.

Advantages and disadvantages:

- One of the primary advantages of chemical precipitation is its cost-effectiveness, owing to its low capital investment and straightforward operational procedures. This technique has proven to be highly effective in removing pollutants from various industrial wastewaters.

- It functions efficiently under ambient conditions, making it suitable for continuous and automated control processes. Its simplicity and reliability have led to widespread adoption in wastewater treatment facilities, where it helps in achieving regulatory compliance and environmental protection.
- One main drawback of using chemical precipitation to separate substances is the high cost of chemicals and disposal of the sludge that forms. Additionally, this method can be limited because it does not target specific substances well. Problems can also occur when other chemicals, like chelating agents, interfere with the process during wastewater treatment (Wang et al., 2005).

2.2.3.2 Adsorption onto activated carbon

Activated carbon is the most applied adsorbent. Adsorption onto activated carbon involves processing carbon to increase its surface area by creating small, low-volume pores to allow adsorption or chemical reactions. 1 g of activated carbon has a surface area larger than 500 m^2, resulting in a high level of microporosity (Samer, 2021).

Same (2021) list the following factors that affect adsorption:

- Particle diameter: adsorption is directly proportional to the surface area but indirectly proportional to the particle size of the adsorbent.
- Adsorbate concentration is directly proportional to the adsorption.
- Temperature is directly proportional to the adsorption.
- Molecular weight: the adsorption is indirectly proportional to molecular weight depending on the compound weight and the structure of the diffusion control of the pore.
- pH: owing to surface charge, the adsorption is indirectly proportional to pH.
- Iodine number: the mass of iodine in grams that is consumed by 100 g of a substance.

Advantages and disadvantages:

- One of the major advantages of activated carbon is that it can be regenerated by steam oxidation/thermal oxidation and reused. The equipment is easy to use and is flexible for many treatments. This method has great capability to separate a wide range of pollutants. Also, when coupled with coagulation, it is highly efficient to lessen colour, chemical oxygen demand, and suspended solids.
- The regeneration of carbon is expensive and leads to a loss of the carbon material. The equipment for this method is costly, and the cost of materials is high. The performance of this method is dependent on the type of sample, and it is not as effective for removal of dyes and certain metals (Crini & Lichtfouse, 2018).

2.2.3.3 Electrochemical treatment

Electrochemical treatment involves sophisticated oxidation and reduction techniques, which facilitate the production of reactive chemical species. These processes are highly effective in removing pollutants and contaminants from water sources, ensuring improved water quality. By leveraging electrochemical methods, it is possible to achieve efficient and environmentally friendly water purification, making this approach a valuable option in water treatment technologies (Feng et al., 2016). An electrochemical arrangement typically consists of an anode and a cathode (at least two electrodes) and a transitional space filled with electrolytes.

Advantages and disadvantages:

- Electrochemical treatment is useful because it can handle different types of pollutants. It creates chemicals right at the location and has a flexible design for the treatment cell (Chaplin, 2019).
- One problem with this method is that sludge can build up and the electrodes can become inactive, which can slow down the process. If the water has a lot of iron and aluminium, it needs extra cleaning afterward to remove these metals. Also, the equipment used is expensive to buy and maintain (Crini & Lichtfouse, 2018).

2.2.3.4 Membrane processes

Membrane processes entail a simple procedure in which a membrane is used as a specific filter that allows a solution to flow through while it catches substances and suspended solids. Different methods are employed to allow the passage of substances through the membrane, for example applying high pressures to keep the concentration gradient on both sides of the membrane the same, and to introduce an electric potential. A membrane filtration system can be used as an alternative for sediment purification techniques, flocculation, adsorption, distillation, and extraction.

Two factors that determine the effectivity of a membrane filtration process are productivity and selectivity. Productivity is measured by a parameter called flux, and selectivity by a parameter called retention or separation factor. Productivity and selectivity are membrane-dependent (Lenntech.com, n.d.).

Advantages and disadvantages:

- Membrane processes offer significant advantages due to the extensive variety of membranes available from numerous manufacturers. These processes are versatile, with a broad range of applications and various module configurations to suit different needs. They are known for their efficiency, simplicity, and speed, even when handling samples with high concentrations. Additionally, membrane systems require minimal space for installation, making them suitable for environments with limited space.
- Membrane methods produce high-quality effluent without using chemicals, which results in very little solid waste. These processes remove salts, minerals, dyes, inorganic matter, microorganisms, organic substances, particles, and suspended solids from water, making it cleaner and safer.
- Membrane processes have some disadvantages. They require a lot of energy and can be expensive to maintain and operate. When processing samples with high concentration, membranes can become clogged or fouled quickly. For small and medium industries, the main problem is that the initial investment costs are often too high (Crini & Lichtfouse, 2018).

2.2.3.5 Ion-exchange chromatography (IEC)

Ion-exchange chromatography is used for the separation of ionisable molecules according to their charge properties (whether they are negatively or positively charged). The charged molecules are separated by electrostatic forces of attraction, which are passed through an ionic resin at a certain temperature and pH. The separation occurs through a reversible interchange of ions found in both the solution and the ion-exchange resin. The success of this separation process relies greatly on the specific type of ionic resin used.

Types of ionic resins:

- Cation-exchange resins are acidic, negatively charged exchangers that have positively charged counter ions and are intended for preserving cations (positively charged ions) in the column.
- Anion-exchange resins are basic, positively charged exchangers that have negatively charged counter ions and are intended for preserving anions (negatively charged ions) in the column.

Advantages and disadvantages

- Ion exchange is a process used in various industries to remove or add ions in solutions. It is commonly used to soften hard water, making it suitable for household and industrial use. In research, ion exchange helps purify samples by removing unwanted ions. In biochemistry, it separates different organic compounds like proteins, nucleotides, and carbohydrates in mixtures. It also helps in isolating metabolites such as those found in urine, blood, and drugs. Additionally, ion exchange is used to purify enzymes from tissues of living organisms, ensuring they are free from contaminants. Overall, this process is valuable for cleaning and separating substances in many scientific and industrial applications (Masoodi et al., 2021).
- Ion exchangers present a limitation when employed for preconcentration purposes: although they effectively recover hydrated ions, they often lack the necessary selectivity and retention capabilities for major ions. This deficiency can impact the accuracy and efficiency of analytical procedures that rely on ion exchange methods, especially in complex sample matrices where selective separation is crucial.

2.2.3.6 Solid-phase extraction

Solid-phase extraction is a useful method for removing metals from very dilute samples. It helps prepare samples by separating certain compounds from a liquid mixture. This separation is based on the physical and chemical properties of the compounds involved (Nyamunda et al., 2019). SPE is widely used for concentrating and purifying samples for analytical purposes, offering the ability to isolate target analytes from a wide range of matrices.

A more in-depth review of SPE is given in Section 2.3.

2.3 Solid-phase extraction (SPE)

2.3.1 Basic principles of SPE

In recent years, the solid-phase extraction (SPE) technique has progressively become more favoured (Dave et al., 2010) because of its versatility, selectivity, low cost, high preconcentration factors, and simplicity (Shyam Sunder et al., 2020). It is a sample preparation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds according to their physical and chemical properties (Nyamunda et al., 2019). It involves the separating of solutes between two phases: a liquid phase that contains the sample matrix and a solid phase (sorbent). A variation of sorbent materials, and the capacity to use a minute or no amount of organic solvents make SPE an extremely ecologically friendly technique.

Additionally, the surface of the sorbent material can be engineered to be customized for specific applications, enhancing its effectiveness and efficiency in targeted uses. The transferal of analytes from the aqueous phase to the active sites of the solid phase is the most basic principle when using SPE for trace element ions.

SPE is commonly used to concentrate and purify samples for analysis and can be used to isolate analytes of significance from a wide variety of matrices. Sorption can take place via ion exchange, ion pairing, chelation, or adsorption processes. Typically, a liquid sample is passed through a column/cartridge/tube/disk containing an adsorbent that retains the analytes. The analytes are then retrieved upon elution with an appropriate solvent (Camel, 2003).

2.3.1.1 SPE operation steps

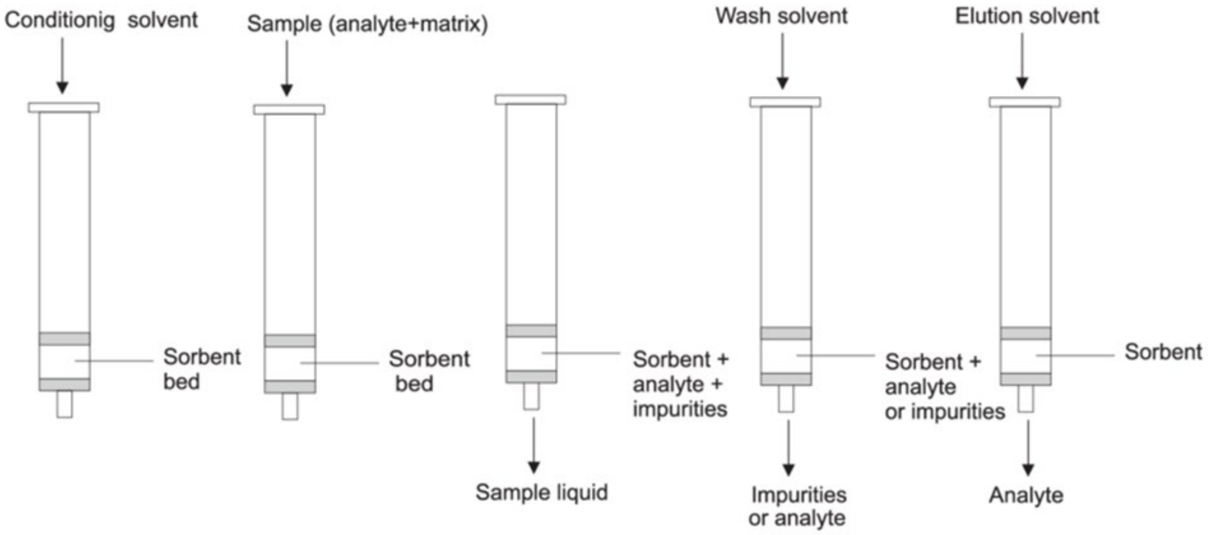


Figure 2.1: A schematic representation of the solid-phase extraction (SPE) process

1. Conditioning: the solid phase should be prepared using a suitable solvent, followed by the sample solvent. This is an important step, as it allows the wetting of the solid phase and the interaction between the solvent and the functional groups. This step may also remove any possible contamination initially held in the sorbent or the packaging. Any air present in the sorbent will be filled with the solvent during this step. Precautions should be taken to avoid the solid sorbent from drying between the conditioning and sample treatment steps. If this does occur, the sorbent must be reconditioned to avoid the inefficient retention of analytes and low recovery rates.

2. Percolation: the sample is introduced to the solid phase by an automated system, aspiration via a vacuum, gravity, or by using a pump. For this step, the flow rate of the sample passing through the sorbent must be high to avoid the duration of the method not being lengthy but low enough for the analytes to successfully retain onto the sorbent. During this step, there is a possibility that matrix components will also be retained in the solid phase.
3. Washing: this step is optional. The solid phase is washed with a suitable solvent to remove any matrix components without removing any analytes.
4. Elution: during the final step of the SPE operation, the analytes are removed from the solid phase using a suitable solvent while the matrix components are retained in the solid phase. The solvent volume must be altered to produce quantitative recoveries of the analyte without diluting the sample too much. A suitable flow rate must be used to ensure efficient elution (Camel, 2003).

2.3.1.2 Traditional sorbents used in SPE

Warshawsky and Grinstead developed solvent-impregnated resins (SIRs) as a new separation technique. SIRs are solid polymeric mediums (support resin) homogeneously mixed with a liquid complexing agent (liquid complexing agent) (Tang et al., 2017). This technique was developed on ion-exchange and solvent-extraction principles and is based on modifying a support material with an extractant.

Several inorganic materials, such as organic polystyrene-divinylbenzene copolymers, silica gel, and polymethacrylate resins, are used as solid support (Tranter, 2011). Macroporous polymeric resins are the most suitable solid support for SIRs because of their high mechanical strength, high specific area, and minimal solvent swelling during the impregnation process, making integrating large amounts of extractant possible. Amberlite XAD resins are most used in SIR. However, for this study, Amberchrom CG-300 was used. It has smaller particle size, and is designed for enhanced purification performance, which is impossible with the larger particle size of the Amberlite resins.

The stationary phase is frequently an organic extractant. The selected organic extractant should meet certain basic requirements. It must not catch fire easily, be able to form complexes with metal ions, dissolve well in organic solvents, and react efficiently with metals in terms of speed and energy. Additionally, it should only slightly dissolve in water, not evaporate easily, and remain stable in acidic conditions (Adonis, 2023).

Seven parameters can be used to characterise the working of extraction chromatographic material: stability (physical, chemical, and radiolytic), reproducibility, selectivity, capacity, efficiency, and retention.

The effectiveness of isolating metal ions attached to the impregnated substrate relies on how quickly the complexes of metal ions are formed and broken apart (Spies, 2020). This efficiency depends on numerous factors, namely:

- the surface structure and configuration of the adsorbed ligand layer(s);
- the pH of the sorbate medium;
- the stability of the ligand layer(s);
- the nature of competing ligands and concentration within the bulk solution;
- the form of the metal ions in solution; and
- the nature of the functional group connected with the extractant.

SIRs have the following advantages and disadvantages over solvent-extraction and ion-exchange chromatography:

Advantages:

- high efficiency and selectivity;
- convenient preparation and ease of operation (Bao et al., 2016);
- short analysis time;
- minor use of organic solvents; and
- high reusability of resin, without any significant loss of metal extraction capacity.

Disadvantages:

- The preconcentration and stability capacities may be substandard compared to the supports to which the reagent has been covalently paired.
- SIRs tend to be unstable because the extractant they contain slowly leaks out into the water. This leakage happens even though the extractant is not very soluble in water and is very hydrophobic. As the extractant leaks out, the SIR's ability to absorb specific ions from water decreases. To fix this issue, two common methods are used: applying a protective coating or membrane on the surface of the SIR beads after they are made or removing any extractant that is loosely attached to the support. These solutions help keep the extractant inside the SIR, maintaining its effectiveness in water treatment processes (Spies, 2020).

2.3.1.3 Advantages and limitations of SPE

Most of the benefits of SPE methods are governed by the physical and chemical nature of the sorbent.

Solid-phase extraction is a useful method for separating substances. It offers many benefits, including being efficient, producing consistent results, using fewer organic solvents, and being safer for the environment. It also helps protect sensitive analytes from evaporation, reduces the amount of solvent needed, speeds up the extraction process, and lowers disposal costs (Türker, 2012). It is one of the best separation and pretreatment techniques due to its high concentration factor, simplicity, versatility, low cost, and selectivity (Tyagi & Jacob, 2020). Selective extraction is high because the trace element of interest is concentrated, and other ions are removed. This process allows for the identification of different forms of the metal by keeping the desired species and eliminating interfering ions (Castillo et al., 2001). Compared to liquid-liquid extraction, solid-phase extraction has a larger cost per sample (Płotka-Wasylka et al., 2015).

When analysing trace elements, it is important to prepare and transport samples carefully so that their chemical forms do not change before testing. Preparing samples helps keep metal species stable for several days. Using small amounts of solid sorbents saves space and avoids the need for large storage containers, which can be difficult to manage and require more effort (Yebra et al., 2001).

Due to the numerous parameters that can be adjusted, additional steps are often required to ensure a successful separation process. The complexity of optimizing these parameters can pose challenges, necessitating careful planning and execution to achieve optimal results in sample preparation and analysis. This could lead to confusion and difficulty in comprehending the method.

2.4 Dithizone and its role in heavy metal separation

2.4.1 Chemical structure and properties of dithizone

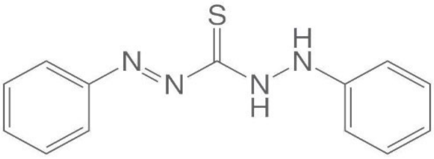


Figure 2.2: Structural formula of Dithizone

Dithizone ($C_{13}H_{12}N_4S$, MW = 256 g/mol) is a dark-green crystalline powder with a melting point of 168 ℃. It is soluble in alkaline solutions, chlorinated hydrocarbons, hydrocarbons, and alcohols, but insoluble in cold water. It is a weak acid that reacts with several metals to form colourful complexes. These colours range from distinct shades of red and orange to violet (Spies, 2020). Dithizone is highly sensitive and selective, which makes it an appropriate ligand for modification of sorbents for the preconcentration and removal of various metal ions.

Dithizone is a bidentate ligand and should therefore provide rapid and reversible sorption of metal ions. The elution of metal ions in stable complexes formed with the ligand requires highly concentrated acids through protonation of the functional groups.

As stated earlier, dithizone forms non-polar coloured complexes when binding to metal ions. Based on structural investigations, the metal ion binds to the sulphur atom and coordinately binds to the nitrogen atom.

When dithizone coordinates as a monobasic acid anion (HDz-), primary dithizonates are formed, $M(HDz)^{n-1}$, where M is the metal ion of charge n+. Secondary dithizonate complexes are formed with certain metals (Pd, Cu, Hg, Au, Pt, Ag). The definite form of secondary complexes is elusive, with researchers having different opinions on their nature. Some argue that it has the form M_2Dz, while others claim it has the form $M\text{-}DzH_2$, which is formed at either a high pH or ligand-to-metal concentration (Spies, 2020).

Dithizone exists in two tautomeric forms – keto and enol. Keto forms are formed mainly in neutral or acidic solutions, whereas enol forms are formed in alkaline solutions or when there is little dithizone. When dithizone bonds with bivalent metal ions, it replaces the hydrogen atom in either the sulfhydryl (-SH) or imide (-NH) group. In alkaline conditions, the metal ion takes the place of the hydrogen in the sulfhydryl group.

In nearly neutral or acidic conditions, the hydrogen in the imide group is replaced. So, at low pH or low metal ion concentration, the keto form is preferred. At high pH or high metal ion concentration, the enol form is preferred (Spies, 2020).

2.4.2 Dithizone as a chelating agent in heavy metal separation

Dithizone is a powerful chelating agent that reacts with many metal ions, forming coloured chelating complexes (Nezhadali & Nazari, 2009). When dithizone acts as a ligand, it reacts with metals to form its neutral complex species (Lu et al., 2015). At different acidities, dithizone can form complexes with approximately 20 heavy metals. In its natural state, the complexity of the dithizone-metal complex can be established when the extraction process uses the ligand to interact with the extraction material. However, interfering ions may prevent the extraction of metal species. A disadvantage of dithizone is that it decomposes in the presence of light (Urasa et al., 1997). In addition, metal-dithizone complexes are non-polar, making them soluble in organic solvents but slightly soluble in water.

2.4.3 Dithizone impregnation on polymer resins

Below are summarised investigations where dithizone was used as a chelating agent in SPE.

The sorption behaviour of heavy metals Ag^+, Bi^{3+}, Cd^{2+}, Co^{2+}, Cu^{2+}, In^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} onto Amberchrom CG-300m resin impregnated with dithizone in was investigated in batch and column experiments (Spies, 2020). The loading capacity of the impregnated resin was found to be 3.2 mg dithizone per gram of sorbent.

The resin was characterised using FTIR and SEM before and after impregnation. SEM revealed that pore size decreased after impregnation. The parameters of interest were pH, initial metal ion concentration, temperature, V/m ratio, and time. The sorption capacity of the metal onto the resin lowered in the order: $Ag^+ > Bi^{3+} > Pb^{2+} > Co^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+} > Cd^{2+} > Mn^{2+}$, with the sorption increasing with initial concentration and pH. Most of the metals were quantitatively sorbed at modest initial concentrations. However, Ag^+ sorbed at 4.104 mg/L, and Bi^{3+} sorbed at 3.30 mg/L (which is considered a high concentration). Hydrolysis was found to occur at higher pH levels even when tartrate ion was used for most metals; however, Ag+ (pH < 3), Bi^{3+} (pH < 2), and Cu^{2+} (pH < 5.5) were sorbed quantitatively at lower pH levels. Mn(II) was slightly sorbed in the pH range of 4.57 – 8.54.

Ag^+ and Bi^{3+} completely sorbed in the temperature range 283 K – 298 K. Positive ΔH_0 values indicated that Pb, Cd, Cu, Co, and Ni sorption was endothermic, whereas Zn sorption was exothermic. Mn was the only metal that remained unabsorbed in this temperature range. The consistency and productivity of the dynamic method were unquestionable and were quantitatively confirmed by kinetic studies of Pb. Sorption kinetics for all column studies were adequately fast, with steady-state approximation being recognised within three minutes. Temkin and Dubinin-Rudeshkivich, Freundlich, and Langmuir sorption isotherms were studied at 294 K. Langmuir isotherms provided suitable sorption data, except for Cu – Temkin, Langmuir, and Freundlich isotherms best modelled sorption of Cu. Except for Cd, the Freundlich isotherm proved a reliable model for the sorption of all metals. All metal ions followed pseudo-second-order reaction kinetics. However, the sorption of Cu onto the dry-impregnated resin followed pseudo-first-order kinetics.

The Weber-Morris and Homogeneous Particle Diffusion models successfully modelled diffusion kinetics. Based on Weber-Morris plots, sorption of all metal ions was controlled by both pore and film diffusion mechanisms. Resin pore size did not affect diffusion of Co(II) and Cu(II) ions. Qualitative separation of Cd^{2+}-Sn^{4+}, Mn^{2+}-Co^{2+}-Ni^{2+} and Bi^{3+}-Pb^{2+} ion mixtures was successfully applied to the dithizone-impregnated resin. In all cases, separation was achieved quantitatively within five minutes.

The following are the SEM and FTIR results presented in the study of Spies (2020):

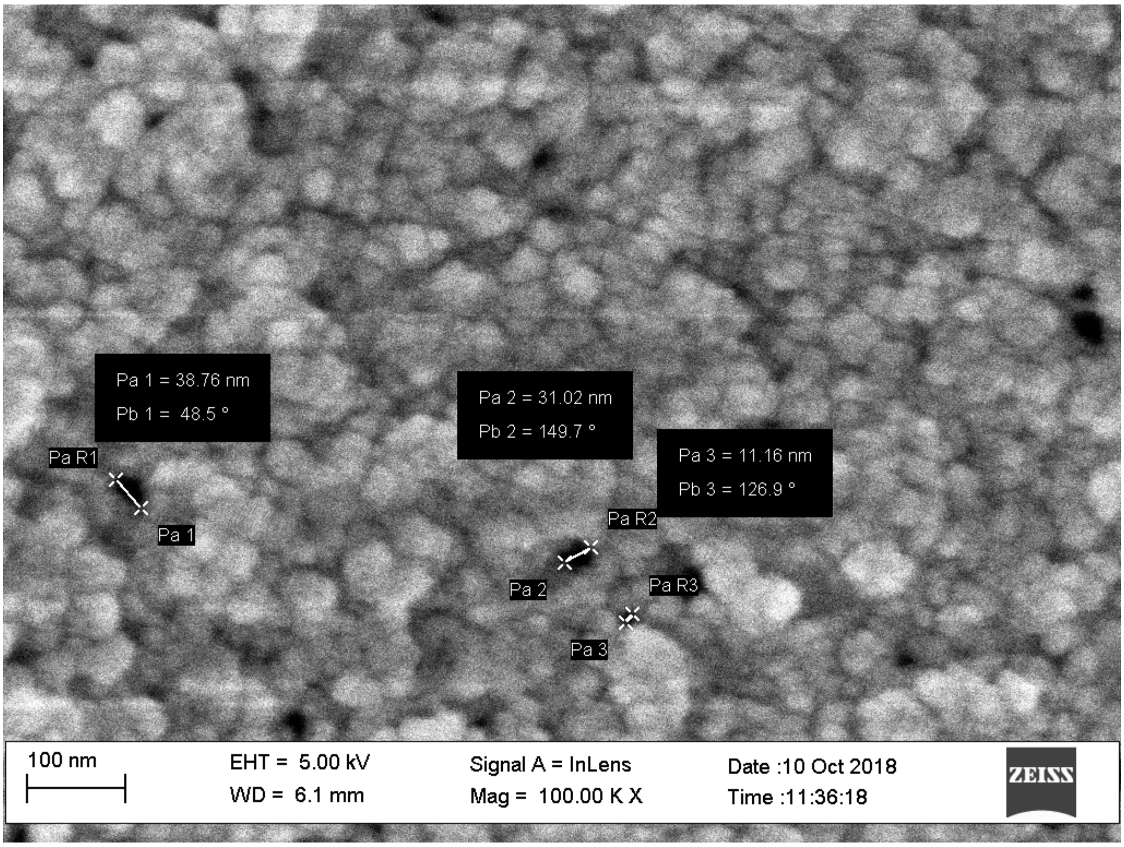


Figure 2.3: SEM image of dithizone-impregnated Amberchrom® CG300m, 10^5 magnification

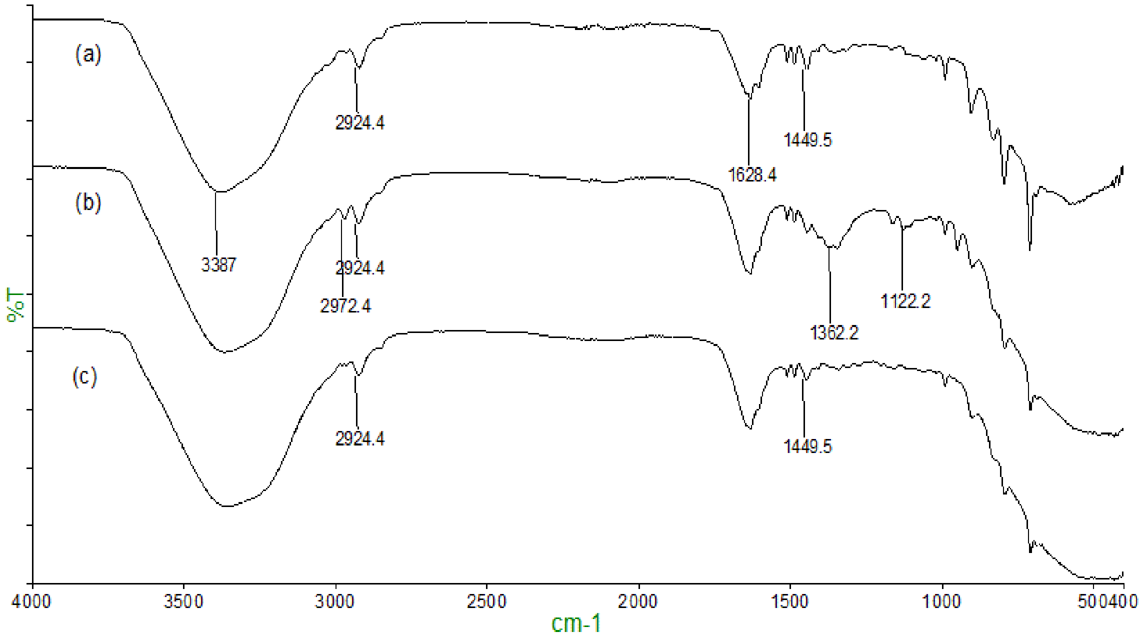


Figure 2.4: FTIR spectra of (a) ACG300m, (b) ACG300m-dithizone, and (c) ACG300m-dithizone-Cd

Behbahani et al. (2012) modified SBA-15 nanoporous silica with dithizone. The modified sorbent was then employed to determine the amount of cadmium, copper, nickel, and lead ions in various food and agricultural products. The effects of pH, flow rate, eluent, breakthrough volume, and interfering ions were studied. For pH studies, the pH of a 25 mL sample solution was adjusted over the range of 2 – 8. All metals were quantitatively sorbed (> 95 %) at pH 5. Metal ion retention at pH > 8 was not investigated because of the tendency of metal ions to hydrolyse at high pH. At lower pH, the quantitative recovery of the metal ions decreased due to electrostatic interactions between the positively charged metal species and the protonated active sites on the sorbent. To optimise the sample flow rate, a peristaltic pump was used to pass 25 mL of sample solution through a column at flow rates from 1 to 22 mL/min. Sample flow rates in the range 1 – 16 mL/min did not affect metal ion retention. A selection of eluent solutions, such as HNO_3, HCl, and CH_3COOH, at different concentrations, was used for the desorption of the metal ions. The metal ions were successfully desorbed by 5 mL of an HNO_3/HCl mixture with a concentration ratio of 2:1 and a flow rate of anywhere from 0.5 – 2.0 mL/min. The sorption capacities were found to be 189 mg/g for cadmium, 102 mg/g for copper, 91 mg/g for nickel, and 208 mg/g for lead.

The determination of cadmium in rice was studied using hydride generation-coupled atomic fluorescence spectroscopy (Lu et al., 2015). Activated aluminium oxide was modified by dithizone via impregnation. This method was used to separate and enrich the cadmium ion. Activated aluminium oxide is known for its huge surface area, effective chemical absorption, and great absorptive volume, all ideal characteristics for the separation and enhancement of cadmium ions.

Davarani et al. (2011) investigated the lead content in sugar using a C18-filled cartridge modified with dithizone. The type, concentration, and volume of the eluent were studied, as well as the pH, flow rate of extraction, and volume of the sample. The unmodified cartridge retained only 5 % of lead, whereas the sorption onto the modified sorbent increased significantly. The optimal pH for sorption was 7.4. An acidic solution was needed for the desorption of the lead ions because of the intrinsic properties of lead. HCl was chosen because of its high recovery yield compared to acetic acid and nitric acid.

Silica gel is regularly used as a solid-phase adsorbent because it is easily functionalised. It was used in the investigation of trace amounts of copper using FAAS (Yu et al., 2011). Dithizone was immobilised onto the surface of the silica gel via the following reaction:

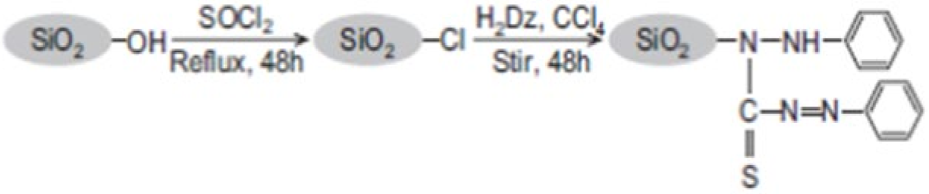


Figure 2.5: Reaction between silica gel and dithizone

This modification resulted in the formation of a strong metal complex (H_2Dz-SG).

An important factor taken into consideration during this investigation was the pH dependence of copper adsorption onto H_2Dz-SG. At pH = 1, H_3O^+ ions attach to the adsorption sites on H_2Dz-SG, making the H_2Dz-SG surface positively charged. This impedes sorption of copper because Cu^{2+} ions are repelled by the positive surface of H_2Dz-SG (Yu et al., 2011). On the other hand, precipitation occurs at pH > 6; therefore, a pH of 5.5 was used in this study. To investigate the effects of eluent type and eluent concentration, acids such as sulphuric acid, nitric acid, phosphoric acid, and hydrochloric acid were used. Favourable elution efficiencies were found for sulphuric acid, nitric acid, and hydrochloric acid within a range of 5 – 10 % v/v; however, phosphoric acid was not effective in this range. Nitric acid studies were conducted within a range of 2 –12 % v/v, where a greater elution efficiency was found at concentrations up to 10 % v/v. Copper recovery decreased at concentrations greater than 10 % v/v. An HNO_3 concentration of 100 g/L and elution time of 4 s were kept constant to determine the effect of flow rate within a range of 3.5 – 7 mL/min. A decrease in the recovery of copper was found at flow rates higher than 6.5 mL/min. Therefore, a flow rate of 6.5 mL/min was maintained.

A simultaneous SPE method was used to determine cadmium, cobalt, nickel, and copper using FAAS (Pourrez et al., 2010). Dithizone was fixed on naphthalene packed in a column. Sorption studies were performed in both acidic and basic mediums. Metal ions were best retained in a basic medium; therefore, 10^{-3} M NaOH was used as sorption medium. Hydrochloric acid, nitric acid, and thiourea were employed to optimise the eluent type. Nitric acid produced the highest recovery of retained metal ions. The highest recoveries were found for concentrations > 1.5 M; therefore, 2 M was chosen. Volumes between 3 – 5 mL were investigated; the best recovery was found when 3 mL was used.

Furthermore, the amount of dithizone loaded onto the sorbent was optimised, with results showing optimal recovery when 0.04 g of dithizone was used.

The speciation of ultra-trace inorganic and organic mercury was investigated in cereal and environmental samples using a dithizone-functionalised C-18 online SPE method (Wang et al., 2022). An online coupled SPE-HPLC-ICP-MS method was developed and used for the first time. 5 mL of 2.5 mg/L dithizone was used to functionalise the C-18 column at a flow rate of 5 mL/min in 84 s. 5 mL of analyte was subsequently loaded onto the column at the same flow rate. Thereafter, the mobile phase was introduced into the column reversely at a flow rate of 1.5 mL/min. 5 mL samples were loaded onto the functionalised column, where the Hg species coordinated with the sulphur groups of dithizone. The effects of eluent concentration and pH on Hg salt recovery were investigated. Hg ions were eluted with 20 µL of 2-mercaptoethanol as the mobile phase, and ultrapure water as the carrier. 1 % 2-mercaptoethanol (v/v) was used to desorb Hg(II), $C_2H_5Hg(II)$, and $CH_3Hg(II)$ effectively from the column. The method proved suitable for the determination of Hg in complex matrices with recoveries exceeding 80 %.

Uzcan et al. (2020) studied the preconcentration and separation of Pb (II) ions in environmental samples using a magnetic dispersive SPE method. Dithizone was the modifier and Fe_3O_4@XAD-8 the adsorbent. Variables such as pH, eluent type, and volume were studied. The pH was investigated from 2.0 – 6.0, where maximum recovery was found to be at a pH of 6 with quantitative recovery yields >90 %. For further studies, a pH of 6.0 was used, resulting in a recovery value of 97 ± 2. HCl and HNO_3 were used to study the effect of eluent concentration – all recoveries were quantitative (> 90 %). 1 M HCl was determined to be the ideal eluent with a range of 1 – 5 mL of acid being investigated.

The preconcentration of Zn and Cu water samples was investigated with dithizone-modified C-18 SPE sorbents (Adeyemi et al., 2017). Each C-18 SPE sorbent was pre-treated by saturating the sorbent with 10.0 mL of water, 5.0 mL of 1.0 mol/L HCl, and 5.0 mL of ethanol. To eliminate all possible impurities during the manufacturing process, the cartridge was washed with another 10.0 mL of deionised water and dried. To prepare the modified sorbent, 2.0 mL of a 1000 mg/L dithizone solution dissolved in chloroform was transferred into the cartridge to allow the dithizone to infiltrate the pores of the sorbent. 100 mL of 100 µg/L Zn^{2+} and Cu^{2+} samples at different pH levels were prepared to investigate the interaction between the metal and the unmodified sorbent.

Metal chelation was studied by combining 100 ml of each prepared sample with 2 mL of EDTA and phenanthroline ligands (1000 mg/L) at different pH values. In both studies, the sample solutions were passed through the cartridge at a flow rate of 5 mL/min. Each metal was quantified using FAAS, where adsorbed metal ions were eluted with 5.0 mL of 1.0 mol/L hydrochloric acid. The effect of pH in Zn samples saturated on the unmodified sorbent was conducted at pHs 3, 5, 7.5, and 10. This result showed that the retention capacity of the sorbent was ideal in acidic conditions where 39 %, 1.6 %, 10.1 % and 5.8 % of the metal was unchelated; a pH of 3 was favoured. However, the Cu^{2+} samples resulted in low recoveries ranging from 1.0 – 4.4 %. The recoveries for the dithizone-modified sorbent resulted in Zn^{2+}, producing percentages of 38.6 %, 5.4 %, 13.7 % and 7.6 % at pH 3, 5, 7.5, and 10, respectively, and 9.2 %, 28.5 %, 10.0 %, and 22.9 % at pH 3, 5, 7.5, and 10, respectively, for Cu^{2+}. For this investigation, recoveries were greatest at pH 3 for Zn^{2+} and pH 5 for Cu^{2+}. The inclination to retain Zn^{2+} grew noticeably at pH 5, 7.5 and 10 when compared with the unmodified sorbent. The formation of metal complexes developed after the addition of 2 mL EDTA solution before extraction. The results showed that an optimum recovery of 90.0 % was found for Zn^{2+} at pH 3. Copper does not form stable complexes with EDTA because EDTA contends with organic ligands for copper complexation, but its Cu chelate is not simply retained by the C-18 cartridges. In this investigation, the chelation seems to lower the sorption efficiency of Cu, possibly being the reason for the poor recovery between 0 – 0.4 %. An optimum recovery of 31.1 % for Zn^{2+} at a pH of 10 and a low recovery of ≤ 1.4 % at all investigated pHs for Cu^{2+} were found when the sorbent was modified with 1, 10-phenanthroline.

A sensitive and easy method was developed for the preconcentration and determination of Cd^{2+}, Cu^{2+}, Mn^{2+}, Pb^{2+} and Zn^{2+} by flame FAAS (Moalla et al., 2020). The study was based on the sorption between metal-dithizone complexes on activated carbon. The effect of several parameters like pH and eluent concentration was investigated. The effect of pH was studied by altering the solution to the pH range 2, 4, 6, 8, and 10 using universal buffer solutions. 1 g of activated carbon was added to the solution before it was shaken for 1 hr. The solution was filtered before it was eluted with 4 M nitric acid. The volume of solution obtained was lessened by evaporation to 5 mL, and the metal ion concentration was determined using FAAS. Results showed that the best pH for extraction for Cu^{2+} was 4, and for Zn^{2+} it was 6. The optimum pH for extraction for Cd^{2+}, Mn^{2+}, and Pb^{2+} was in a basic medium with pH values of 10 and 8, respectively. To investigate the effect of eluent concentration, the pH, the amount of activated carbon, and the shaking time were kept constant. The solution retained after metal recovery was evaporated to 5 mL after which it was analysed using FAAS.

10 mL of 1 M HNO_3 was used as eluent. Results showed that the successful elution of Mn^{2+} and Cd^{2+} complexes was achieved when 1 mol/L of HNO_3 was used. A 2 M HNO_3 solution successfully removed the Zn^{2+} complex, whereas 4 M HNO_3 successfully eluted Pb^{2+} and Cu^{2+}complexes.

Preconcentration and separation of trace amounts of cadmium in seawater were investigated (Wu et al., 2006) using Amberlite XAD-2 functionalised with dithizone. Mini columns were prepared by packing a laboratory-made 20 mm column with 0.08 – 0.09 g XAD2-H_2Dz resin and capped at both ends with glass wool. The column was conditioned with 2 mol/L HCl and washed with water until the eluent was neutral. For pH studies, 5 mL of cadmium solutions in a pH range 3 – 9 were pumped through the column. For the seawater samples, no pH adjustments were made. It was found that a pH of 8.19 was optimal for the elution of the cadmium ions in both samples. The effect flow rate had on sorption was investigated between the rates of 1 – 5 mL/min. Flow rates higher than 2.5 mL/min yielded lower analytical signals due to metal ions not fully attaining equilibrium with the resin bed. However, from 1.0 – 2.5 mL/min, the cadmium ions were absorbed quantitatively; therefore, a flow rate of 2.5 mL/min was used as the optimum flow rate. Nitric acid was used as eluent. Its concentration varied in the range of 1.0 – 4.0 mol/L. 3.0 mol/L was found to be the optimal concentration – concentrations higher than this damaged the spectrometer nebulizer.

The adsorption properties of cadmium ions were investigated (Cheng et al., 2009) in a system where a dithizone-modified sodium trititanate was used as a solid sorbent using an impregnation method. The sodium trititanate whisker modified with dithizone was planned by using an impregnation method, where 50 g of activated sodium trititanate whisker was dissolved in 200 mL of a 0.01 mol/L dithizone ammonia solution. Thereafter, it was soaked for 24 hours and then washed with water until the solution was neutral (to remove any excessive acid), filtrated, and dried at 50 ℃ ~ 60 ℃, before it was sifted out with a 100-mesh sieve, and stored until use. A percentage of the sample was moved to a 50 mL colourimeter cylinder where the pH was adjusted and diluted to 50 mL. Then, 0.2 g of the modified whisker was added to the cylinder which was vigorously stirred for five minutes. Before the sample was centrifuged it was allowed to rest for 12 hours to allow adsorption. Thereafter, the concentration of the liquid phase (unabsorbed ions) and the adsorbed ions, which were eluted with 10 mL 0.5 mol/L nitric acid, was analysed by FAAS.

For the effect of pH on adsorption, it was found that the adsorption rate increased with the increase in pH levels; however, stabilisation occurred when the pH level was higher than 5.0. Sedimentation of cadmium ions occurs under alkaline conditions; therefore, an optimum pH of 6.2 was used. Hydrochloric acid, sulphuric acid, and nitric acid were used to study the effect of eluent on desorption. The outcome of this study showed that nitric acid desorbed above 90 % of the cadmium ions; thus, it was selected as the optimum eluent. Ions were fully eluted with 10 mL 0.5 mol/L nitric acid.

Dithizone was used to functionalise an Amberlite XAD-2 resin for the preconcentration of copper at µg/L levels (Saxena et al., 2015). This online injection method was developed using FAAS for the analysis of industrialised water. Maximum recovery was ensured by optimising the pH, sample and eluent flow rates, and eluent concentration. To optimise the running of the online FI-FAAS method, 100 µg/L Cu(II) was used. The influence of sample pH on sorption was studied with pH levels of 2.0 – 10.0. Up to a pH level of 4, an increase in absorbance was observed; thereafter, a steady decrease occurred. Maximum sorption took place at a pH level of 4; hence, it was used as the optimum pH for the remainder of the study. The elution of the copper ions was investigated by using hydrochloric acid and nitric acid solutions for a concentration range of 0.01 – 2.0 mol/L. Nitric acid was selected as the optimum eluent because of its sharper and more enhanced signals compared to hydrochloric acid. Utmost elution was reached at 0.5 mol/L; therefore, 0.5 mol/L nitric acid was used in subsequent studies. A flow rate of 6.0 mL/min was found to be the optimum flow rate.

2.5 Techniques of heavy metals separation using dithizone-impregnated polymer resin

2.5.1 Static/batch method

2.5.1.1 Process description and mechanism

The batch method is an adsorption method in a specific type of container/reactor/tank. The adsorbate and the adsorbent are well mixed in a diluted solution at constant volume in a container (Patel, 2019). The container is utilised for various process factors, such as adsorbate concentration, pH, and dosage, stirring speed, particle size, temperature, and time duration. Once equilibrium is reached, the absorbent is removed (Patel, 2021). This treatment method is common and effective with wastewater and for the removal of various pollutants that occur in small effluent quantities with low pollution loads.

2.5.1.2 Advantages and limitations

The batch adsorption process is a cheap, easy and simple process used to study the practicality of adsorbent–adsorbate systems (Patel, 2019). The greatest limitation of this process is that it is not practical for industrial use, as small amounts of adsorbate are used for the removal of lower pollution loads (Patel, 2021). Another limitation is that the adsorbent is easily removed from the system (Patel, 2019).

2.5.2 Dynamic/column method

2.5.2.1 Process description and mechanism

The column (continuous pack bed or fixed bed) method is an adsorption method in which the adsorbent is packed into a column and the adsorbate solution is passed through the column at a certain flow rate, keeping them in constant contact. This method is used to remove large pollution loads from larger amounts of wastewater at a given time.

The capacity of the column is evaluated by analysing different parameters like bed height (adsorbent concentration), adsorbate concentration, particle size, breakthrough parameters, pH, and flow rate (Patel, 2021).

2.5.2.2 Advantages and limitations

The main advantages of the column method include that it is used for industrial purposes, where analysis of large quantities of wastewater containing high pollution loads and more adsorbates can be absorbed (Patel, 2021). This method is advantageous owing to the continuous contact between the adsorbate and fresh adsorbent in a fixed-column system (Patel, 2019). However, for this method to be commercially available, the design of the method and column system must be fully optimised – this is a major limitation. Furthermore, the adsorbent needed to be replaced after exhaustion.

2.6 Optimisation of separation parameters

2.6.1 Importance of optimisation in heavy metal separation

Traditional methods are not enough to show all the different factors that affect an experiment. Furthermore, optimisation of these techniques demand a significant amount of time. These restrictions can be fixed by using a statistical method that improves all important factors at the same time. This methodology examines the typical relationships between various factors to determine the most effective process conditions. By doing so, it enables the identification of interactions among optimized parameters, ultimately enhancing process efficiency and performance (Ince & Ince, 2020).

2.6.2 Flow rate and its impact on separation efficiency

The flow rate of the elution solvent must be carefully optimized to ensure efficient recovery of the target analyte while minimizing the duration of the process. Firstly, should be set low enough to achieve quantitative recovery, meaning that nearly all the target species are eluted without loss. Simultaneously, the flow rate should be sufficiently high to prevent prolonged processing times, which could lead to inefficiencies and potential degradation of the sample. Proper adjustment of the flow rate is crucial for balancing recovery efficiency and operational practicality, ultimately enhancing the reliability and throughput of the analytical procedure (Shamsipur & Mashhadizadeh, 2000). When considering flow rate, it is good to keep in mind that the larger the solvent volume, the higher the flow rate required for complete elution. This parameter directly effects the breakthrough volume. The flow rate must be carefully optimised to ensure it aligns with the duration required for the sampling process, thereby enhancing accuracy and efficiency (Poole et al., 2010). The time taken should be at a minimum with an optimal flow rate to ensure quantitative retention.

2.6.3 Influence of column length on separation process

The contact time between the stationary phase and the eluting compounds increases with column length, thus increasing the resolution and column efficiency. However, the increase in column length impacts the column head pressure and analysis time; therefore (Bhanot, 2013).

2.6.4 Selection and concentration of eluent: Effect on separation

The eluent may be organic, an acid or a complexing agent. To ensure a significant enrichment factor it would be appropriate to recover the analyte in small volumes of solvent. For efficient recovery, an eluting solution must be chosen to ensure recovery of the targeted species and quantitative recovery as far as possible (Compañó et al., 1993). The eluent solution must also be adaptable to the analysis technique (Yamini et al., 2002).

2.7 Summary and gaps in the literature

The purpose of this study was inspired by a recommendation of Dr Andre Spies in 2020, who suggested the quantitative separation of metal ions using the ACG300m-H_2Dz separation system. This is the first-ever study where the ACG300m-H_2Dz separation system has been used. Since there is only one study of this system, many unknown factors need investigating. In the paper “Sorption behaviour of heavy metals on a dithizone-impregnated polymer resin” (Spies, 2020), the author recommended further studies involving more heavy metals and their interaction with the ACG300m-H_2Dz.

2.8 Conclusion

In conclusion, the exploration of solid-phase extraction (SPE) presented in this study highlights its fundamental role as a versatile and effective technique in analytical chemistry. Through an in-depth analysis of various research sources, we have revealed the complex benefits that SPE offers, from its ability to enhance analyte concentration to its compatibility with diverse sample matrices. Different sorbent materials, methodologies, and the application in water treatment have been reviewed.

The significance of careful method development, altered to the unique characteristics of each analyte and sample type, cannot be ignored. This review emphasises the importance of optimising parameters, such as elution solvent and concentration, column height and flow rates to achieve maximum extraction efficiency.

As research continues to push the boundaries of knowledge, it is evident that SPE will remain a keystone in sample preparation strategies. Through the interaction of expertise and the exploration of uncharted territories, SPE is destined to revolutionise the landscape of analytical chemistry further, offering refined solutions to complex analytical challenges.

CHAPTER THREE

METHODOLOGY

The reagents, apparatus, analytical instrumentation, and separation methods employed in this study are described in this chapter. The methodologies and parameters utilized in this study are intricately connected to the findings presented in Chapters Four and Five.

3.1 Reagents and solutions

The resin Amberchrom® CG-300m, characterized by a surface area of 700 m^2/g, features a mean pore size of 300 Å and exhibits porosity ranging from 55 % to 75 %. Its dry bead diameter varies between 50 and 100 µm, with a dry polymer weight of 0.21 g/mL of wet volume. This resin was sourced from Sigma and Kimix. Notably, Amberchrom® CG-300m swelled by approximately 4 % when immersed in isopropanol, indicating its degree of expansion in this solvent.

Diphenyl thiocarbazone, commonly known as dithizone, was obtained from Sigma-Aldrich, a reputable supplier of chemical reagents.

Deionized water produced using a Milli-Q purification system was employed for sample preparation and dilution. All chemicals used in the procedures were of analytical grade or equivalent standard, ensuring their suitability without additional purification steps.

Standard solutions for inductively coupled plasma (ICP) and atomic absorption (AA) spectroscopy, with a concentration of 1000 mg/L ± 5, were prepared using solutions containing various metals dissolved in 5 % nitric acid (HNO_3). These stock solutions served as calibration references and were further diluted to generate working solutions with appropriate concentrations for the analysis.

Metal ions were obtained from their respective salts, including nickel(II) chloride hexahydrate ($NiCl_2·6H_2O$), cadmium oxide (CdO), and copper(II) nitrate trihydrate ($Cu(NO_3)_2·3H_2O$). All salts were supplied by Merck Chemicals (PTY) Ltd., except for CdO, which was sourced from BDH Chemicals Ltd. To prepare the 1000 mg/L stock solutions, 4.0502 g of $NiCl_2·6H_2O$, 1.1423 g of CdO, and 3.7980 g of $Cu(NO_3)_2·3H_2O$ were each dissolved in deionised water within 1000 mL volumetric flasks, then filled to the mark with deionised water, ensuring precise concentration standards for subsequent analytical procedures.

The buffer solutions were prepared as follows:

- Buffer 2: 0.3925 g potassium chloride (KCl) and 0.659 mL hydrochloric acid (HCl) in deionised water.
- Buffer 5: 0.5275 g sodium acetate ($C_2H_3NaO_2$) and 0.204 mL acetic acid (CH_3COOH) in deionised water.
- Buffer 6: 0.9550 g sodium acetate ($C_2H_3NaO_2$) and 0.029 mL acetic acid (CH_3COOH) in deionised water.
- Buffer 8: 16.2820 g of dipotassium phosphate (K_2HPO_4) and 0.8880 g of monopotassium phosphate (KH_2PO_4) in deionised water.

3.2 Procedure

3.2.1 Cleaning of Amberchrom® CG-300m resin

The resin was washed with 200 mL of 0.5 M NaOH solution. Thereafter, it was rinsed with deionised water, until the washings were neutral (pH 7). The pH of the wash was measured using a universal indicator solution. The resin was then dried overnight in an oven at 50 ℃.

3.2.2 Impregnation of Amberchrom® CG-300m resin

The resin (white) was impregnated with dithizone (green) in 0.5 M sodium hydroxide (NaOH). 1 M NaOH was prepared by dissolving 2 g of NaOH in a 50 mL volumetric flask. Thereafter, it was added to a known amount of resin in a 250 mL Erlenmeyer flask. NaOH was diluted by adding a further 40 mL of deionised water and 10 mL of alcohol (ethanol or 2-propanol) to the Erlenmeyer flask. The Erlenmeyer flask was then placed on an orbital shaker and shaken at 150 rpm for 30 min.

The impregnated resin, which was orange in colour, was titrated with 10.35 M HCl until it turned dark green. The impregnated resin was filtered under suction using a Buchner funnel and washed with deionised water to remove any excess acid. The column was then packed with the impregnated resin.

3.2.3 Column method

The impregnated resin was packed into a mini column (a modified 10 mL syringe), which was conditioned by running deionised water, an appropriate buffer solution, and the metal ion solution of interest against gravity at the desired flow rate. The column was then stripped with an appropriate acid at a concentration that was the best fit for the metal.

3.3 Optimisation and separation

3.3.1 Effect of eluent type

The effect of the eluent type was evaluated using 0.1 M ascorbic acid and 0.1 M hydrochloric acid. The 0.1 M ascorbic acid was prepared in a 100 mL volumetric flask by dissolving 1.7913 g in deionised water, and the 0.1 M hydrochloric acid was prepared in a 100 mL volumetric flask by diluting 0.97 mL of 10.35 M hydrochloric acid with deionised water.

Amberchrom® CG-300m resin (0.5 g) was impregnated with 0.0040 g of dithizone for each experiment. A 60 mL aliquot of a 2.3853×10^{-5} M (1.4 ppm) nickel solution was loaded onto the impregnated resin at a 6 mL/min flow rate. 60 mL of the eluent was run through the column to remove the sorbed metal from the column at a flow rate of 6 mL/min.

3.3.2 Effect of eluent concentration

Different eluent concentrations were studied by running a range of acid solutions at different concentrations through a column packed with impregnated Amberchrom® CG-300m resin. 60 mL of a 2.3853×10^{-5} M (1.4 ppm) nickel solution, 3.1470×10^{-5} M (2 ppm) copper solution, or 1.7792×10^{-5} M (2 ppm) and 4.4480×10^{-5} M (5 ppm) cadmium solution were loaded onto the impregnated resin.

The acid solutions were hydrochloric acid (0.1 M, 0.2 M, 0.3 M, and 0.4 M), ascorbic acid (0.1 M, 0.2 M, 0.4 M, and 0.5 M), hydrofluoric acid (0.05 M, 0.1 M, 0.2 M, and 0.3 M), and nitric acid (0.5 M, 2 M, and 5 M; the 5 M solution was heated to 50 ℃) along with ascorbic acid (0.01 M and 0.5 M). After the metal ion was loaded onto the column, an acidic solution was run through the column to remove the loaded metal ion. For each experiment, 60 mL of the eluent at the desired concentration was used. A fraction was collected every minute for up to 10 min.

3.3.3 Effect of flow rate

Flow rate experiments were performed using different flow rates (4, 5, 6, and 7 mL/min) to load and remove the metal ions. The flow rate was adjusted on the peristaltic pump by adjusting the rotation speed (rpm). For flow rates of 4 – 7 mL/min, the pump speed was adjusted to 20.6, 26.0, 31.0, and 36.4 rpm, respectively. Depending on the flow rate, 40 – 70 mL of the metal solution was loaded onto the impregnated resin. 0.1 M hydrochloric acid and 0.5 M ascorbic acid were used as eluents. Ten fractions of the samples were collected per test run and analysed using ICP or AA.

3.3.4 Effect of column height

The impact of column height was examined at a flow rate of 7 mL/min using 0.1 M hydrochloric acid and 0.5 M ascorbic acid. This investigation involved varying masses of Amberchrom® CG-300m resin (0.2 – 0.5 g) and impregnation with 0.0060, 0.0024, 0.0032, and 0.0040 g of dithizone. A metal solution (70 mL) was applied to the impregnated resin, followed by an eluent (70 mL) to extract the metal from the resin. Ten sample fractions were collected per test run and analysed using ICP or AA.

3.4 Apparatus

Impregnation was performed using an automatic orbital platform shaker (Labotec model 262) equipped with speed control.

For the column experiments, a Gilson MINIPULS Evolution peristaltic pump was employed, and a customized syringe served as the column.

A Basic 20+ CRISON pH meter, calibrated every 20 hours with standard buffer solutions (4.01, 7.00, and 9.00), was utilized to measure the pH.

3.5 Instrumentation

3.5.1 ICP

The metal ion concentrations were measured using a SpectroTM ARCOS ICP-OES spectrometer.

Table 3.1: Operating parameters of the ICP-OES spectrometer

RF Power	1400 W
Argon Flow	12 L/min
Auxiliary Flow	1.0 L/min
Nebuliser Flow	0.8 L/min

3.5.2 AA

The metal ion concentration was measured using a Thermo Fisher Scientific SOLAAR S-Series Atomic Absorbance spectrometer.

Table 3.2: Operating parameters of the FAAS spectrometer

Spectrometer Parameters	
Measurement Mode	Absorbance
Wavelength	232.0 nm
Background Correction	Deuterium lamp
Bandpass	0.2 nm
Lamp Current	75 %
Flame Parameters	
Flame Type	Air-Acetylene
Fuel Flow	0.9 L/min
Burner Height	7.0 nm

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter depicts the data for the optimisation of the separation parameters namely, the effect of eluents, the concentration of the eluent, the flow rate, and the effect of column height for cadmium and nickel ions. The results for the separation of nickel and copper are also displayed in this chapter. Each experiment was performed in triplicate, and the results expressed are the average of those experimental results. All experimental work was based on the work of Spies and Wewers (2020), where the effect of pH, sorption, and kinetic studies were conducted.

4.2 Optimisation of metal ions

4.2.1 Cadmium

4.2.1.1 Effect of eluent and eluent concentration

When selecting an eluent for this investigation, low concentrations of acids were considered owing to the harmful effects of acids on the environment. The study on cadmium was conducted at a flow rate of 5 mL/min and a pH of 8, using a buffer solution to condition the impregnated resin. Initial studies used ascorbic acid, but it was found to have no effect on the removal of cadmium ions. Subsequently, hydrochloric acid and hydrofluoric acid were investigated at concentrations as low as 0.01 M. When comparing hydrochloric acid to hydrofluoric acid, the latter produced a higher percentage recovery of up to 100 % in the removal of cadmium ions. However, owing to its high toxicity levels and harmful effects on the environment and humans, hydrochloric acid was chosen for further investigation.

Table 4.1: The influence of eluent and eluent concentration

Eluent	Concentration (M)	Cd loaded (mg)	Cd recovered (mg)	Percentage recovery (%)
Hydrochloric acid	0.01	0.24	0.130	54.92
	0.10	0.24	0.100	41.82
	0.20	0.24	0.070	27.53
	0.30	0.24	0.010	4.17
Hydrofluoric acid	0.05	0.10	0.019	20.15
	0.10	0.10	0.096	96.00
	0.20	0.10	0.134	139.48
	0.30	0.10	0.127	132.52

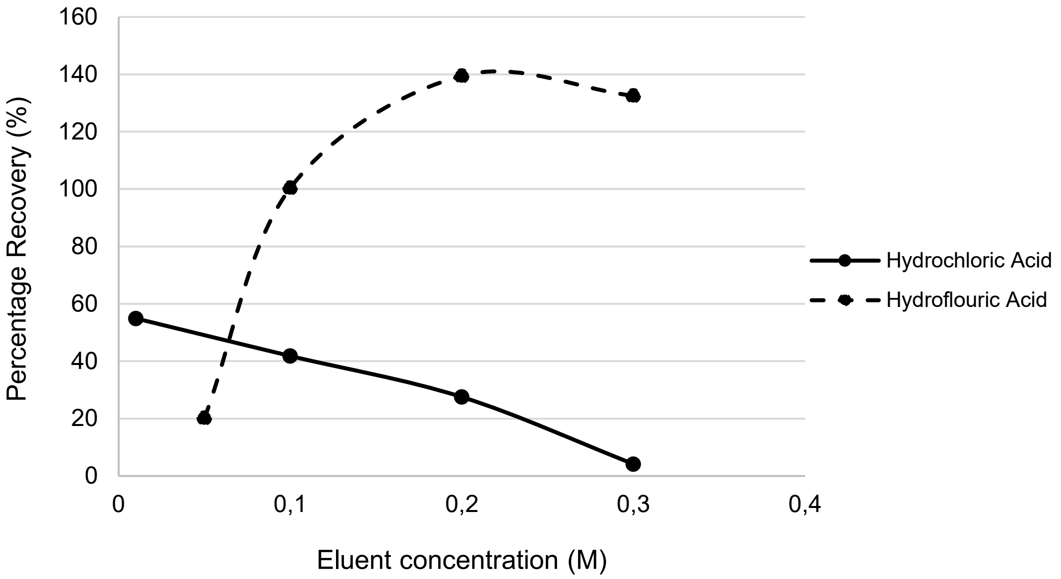


Figure 4.1: The influence of eluent and eluent concentrations of Cd^{2+} using ICP spectrometry

The percentage recovery of cadmium ions decreased sharply with increasing concentration of hydrochloric acid, with a pronounced tail produced in the elution curve (see Figure 4.2). This observation could be a result of the removal of cadmium ions being more effective at low pH levels, making acids suitable eluents for cadmium ions. However, dilute concentrations of hydrochloric acid can raise the pH, reducing their efficiency in breaking the complexes formed between metal ions and the sorbent surface (Chatterjee & Abraham, 2019). However, when using 0.1 M hydrochloric acid as eluent, a sharper peak was produced.

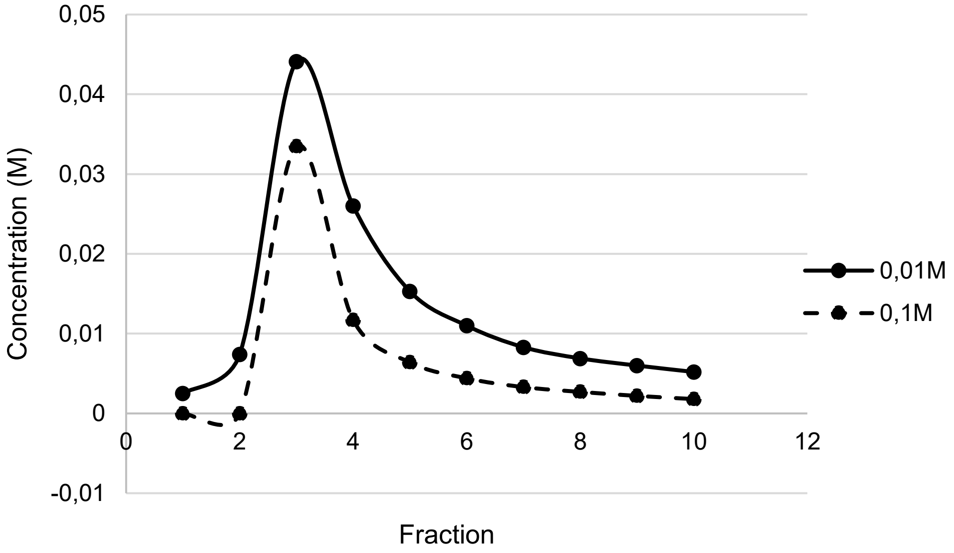


Figure 4.2: The comparison between 0.01 M and 0.1 M HCl for the removal of Cd^{2+} ions

4.2.1.2 Effect of flow rate

The influence of flow rate was examined within the range of 4 – 7 mL eluent per minute. The concentration of the sample solution was the same for each test, however, because at different flow rates a different amount of sample ran through the column different amounts of Cd in mg was loaded. Both the loading and removal of the metal ion were carried out at the same flow rate. Within the range studied, a low percentage recovery was observed, with the highest percentage recovery reaching 24.14 % at a flow rate of 7 mL/min. An outlier was observed at a flow rate of 4 mL/min, where a trend emerged showing that an increase in the percentage recovery occurred as the flow rate increased. Interestingly, at 4 mL/min, a higher percentage recovery was observed compared to a flow rate at 5 mL/min.

Table 4.2: The influence of flow rate

Flow rate (mL/min)	Cd loaded (mg)	Cd recovered (mg)	Percentage recovery (%)
4	0.20	0.02	11.84
5	0.25	0.01	5.732
6	0.30	0.02	7.034
7	0.35	0.08	24.14

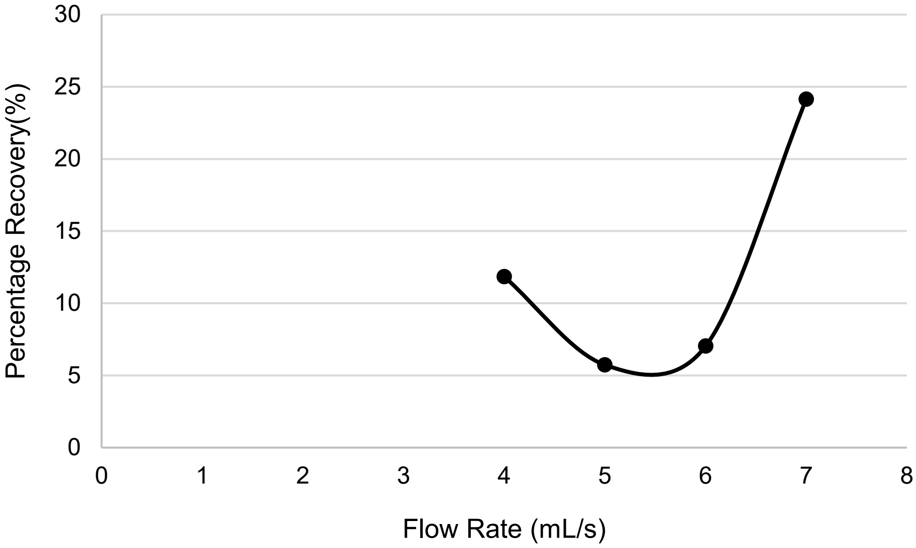


Figure 4.3: The influence of flow rate on the removal of Cd^{2+} ions

4.2.1.3 Effect of column height

The effect of column height (expressed in terms of the mass, in grams, of packed resin) was investigated within the range of 0.2 – 0.5 g of resin, after which the resin was impregnated following the procedure outlined in Chapter 3, Section 3.2.2. The column heights tested in this study did not yield high percentage recoveries. However, the percentages were within a close range of each other, suggesting that the column height range did not affect the removal of cadmium ions from the impregnated resin significantly.

Table 4.3: The influence of column height on the removal of Cd^{2+} ions

Column height (g)	Cd Loaded (mg)	Cd Recovered (mg)	Percentage recovery (%)
0.2	0.22	0.030	13.64
0.3	0.22	0.038	17.27
0.4	0.22	0.040	18.18
0.5	0.22	0.030	13.64

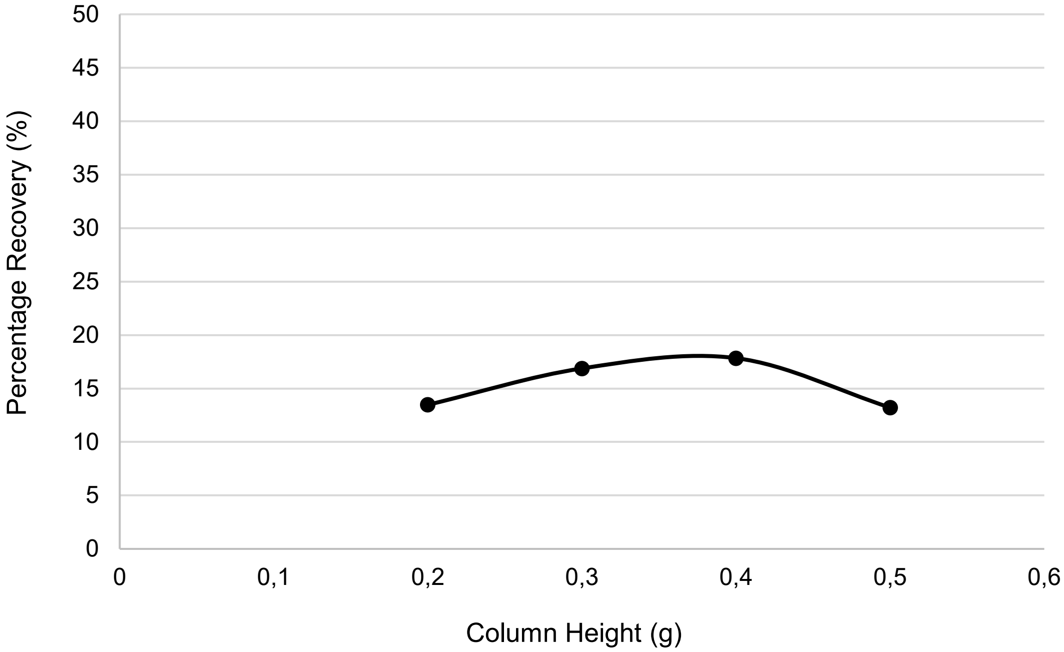


Figure 4.4: The influence of column height on the removal of Cd^{2+} ions

4.2.2 Nickel

4.2.2.1 Effect of eluent and eluent concentration

The influence of eluent and eluent concentration was investigated by conducting experiments with various acid concentrations to remove nickel ions from the dithizone-impregnated resin. This study on nickel was carried out at a flow rate of 6 mL/min and a pH of 8, with the impregnated resin being conditioned, using a pH 8 buffer solution before loading the metal onto the column. Hydrochloric acid and ascorbic acid were used at very low concentrations to minimise environmental impact. Hydrochloric acid was studied in the concentration range of 0.1 – 0.4 M, and ascorbic acid was studied in the concentration range of 0.1 – 0.5 M.

Table 4.4: The influence of eluent and eluent concentration on the removal of nickel ions from a dithizone-impregnated resin

Eluent	Concentration (M)	Ni Loaded (mg)	Ni Recovered (mg)	Percentage recovery (%)
Hydrochloric acid	0.1	0.085	0.071	83.18
	0.2	0.085	0.071	83.88
	0.3	0.085	0.071	83.18
	0.4	0.085	0.072	84.61
Ascorbic acid	0.1	0.088	0.005	5.79
	0.2	0.088	0.019	21.0
	0.4	0.088	0.028	31.83
	0.5	0.088	0.010	11.83

Based on the results, ascorbic acid yielded a significantly lower recovery rate compared to hydrochloric acid. The highest recovery achieved with ascorbic acid was 31.83 % at 0.4 M concentration. In contrast, hydrochloric acid produced the highest recovery, reaching 84.61 % at a concentration of 0.4 M, which was subsequently used for the remaining investigation.

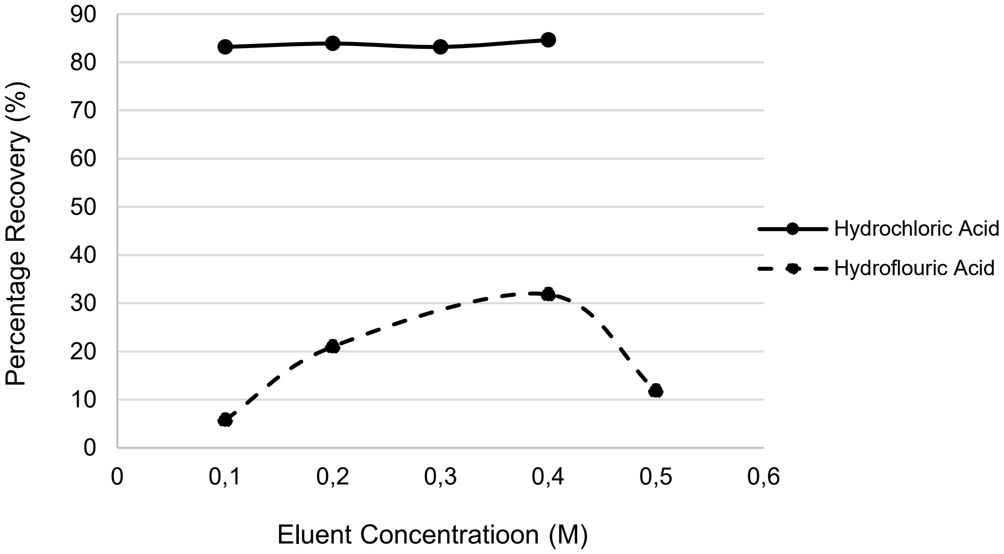


Figure 4.5: The influence of eluent and eluent concentration on the removal of nickel ions from a dithizone-impregnated resin

4.2.2.2 Effect of flow rate

The effect of flow rate was investigated within the range of 4 – 7 mL/min. The amount of metal added for each experiment was determined by calculating the number of nickel ions loaded onto the impregnated resin, based on the volume passed through the column. The highest recovery, 73.88 %, was achieved at a flow rate of 6 mL/min. However, within the studied range, recoveries did not differ by more than 4 %. For flow rates of 5 – 7 mL/min, recoveries increased, but at 4 mL/min, a higher flow rate was observed compared to at 5 mL/min.

Table 4.5: The effect of flow rate on the removal of nickel ions from a dithizone-impregnated resin

Flow rate (mL/min)	Ni Loaded (mg)	Ni Recovered (mg)	Percentage recovery (%)
4	0.064	0.047	72.78
5	0.081	0.058	71.93
6	0.097	0.071	73.88
7	0.113	0.083	73.84

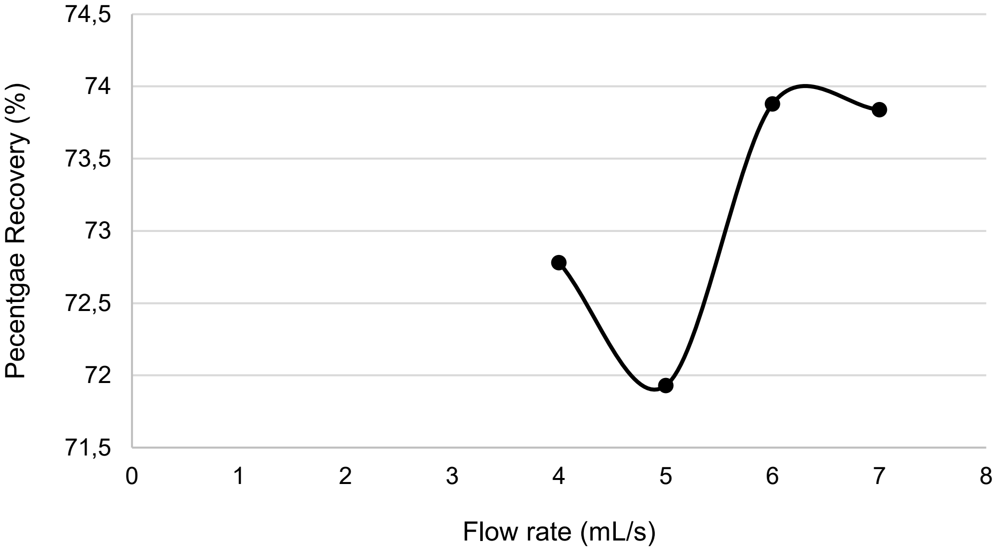


Figure 4.6: The influence of flow rate on the removal of nickel ions from a dithizone-impregnated resin

4.2.2.3 Effect of column height

The influence of the amount of impregnated resin packed into the column was studied within the range of 0.2 – 0.5 g. The results indicated that the lowest recovery occurred at 0.2 g of resin, suggesting that there were significantly fewer loading sites when compared to the higher range of 0.3 – 0.5 g. In the higher range, recoveries exceeded 90 %, with the highest recovery of 95.30 % achieved at 0.4 g.

Table 4.6: The impact of column height on the removal of nickel ions from a dithizone-impregnated resin

Column height (g)	Ni Loaded (mg)	Ni Recovered (mg)	Percentage recovery (%)
0.2	0.091	0.069	75.97
0.3	0.091	0.083	91.12
0.4	0.091	0.087	95.30
0.5	0.091	0.083	91.61

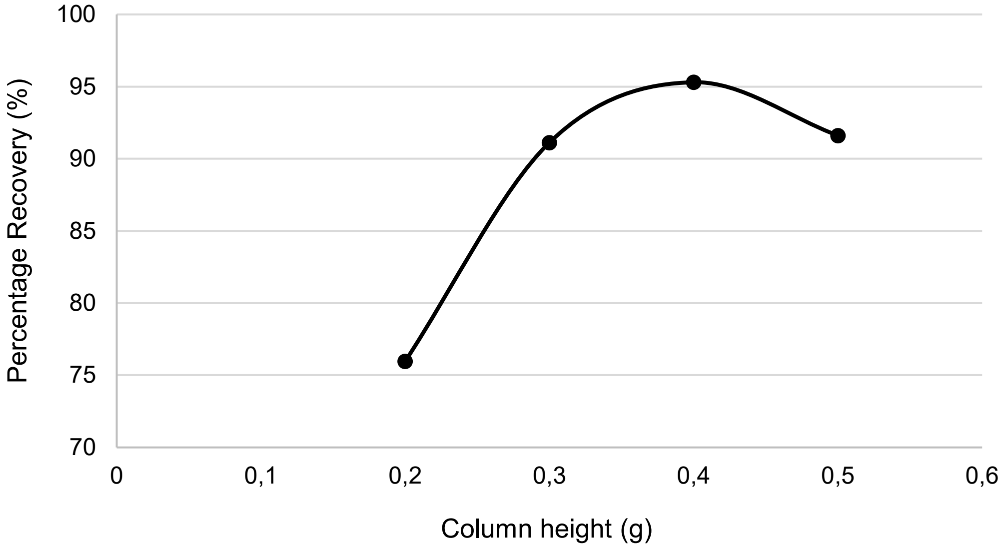


Figure 4.7: The impact of column height on the removal of nickel ions from a dithizone-impregnated resin

CHAPTER FIVE

APPLICATION

5.1 Separation of nickel–copper binary solution

For the separation of nickel from copper in a binary solution, the optimal conditions for nickel removal were applied. The pH was adjusted to 3 to facilitate the passage of nickel through the column while retaining copper. Despite trying hydrochloric acid, nitric acid, hydrofluoric acid, and ascorbic acid, copper could not be removed from the impregnated resin within the selected pH ranges, as determined by visual observation of the resin's colour change. Flow rate and column height were not investigated for copper removal, as a suitable eluent was not found based on the eluents chosen for this investigation. The results presented below represent the average of three experiments conducted for both metal ion loading and removal at a pH of 8.

5.1.1 Loading of the metal ions

Ten samples were collected during the metal loading process. These samples were analysed using ICP to determine the amount of metal that did not adhere to the resin; thus, providing an accurate measure of the loaded amount. Of the samples collected, 13.19 % of the copper ions and 14.93 % of the nickel ions were found in the analysis, indicating that 86.81 % of copper and 85.07 % of nickel ions were retained on the column.

Table 5.1: The percentage recovery for the loading of metal ions in a binary solution

	Loaded onto the resin (mg)	Recovered (mg)	Percentage recovery (%)
Nickel	0.147	0.022	14.93
Copper	1.059	0.140	13.19

Table 5.2: The mass of metal ions loaded from a binary solution

Fraction	Ni	Cu
1	0.0003	0.0044
2	0.0008	0.0091
3	0.0011	0.0112
4	0.0012	0.0124
5	0.0019	0.0138
6	0.0019	0.0150
7	0.0026	0.0163
8	0.0034	0.0165
9	0.0041	0.0186
10	0.0049	0.0224

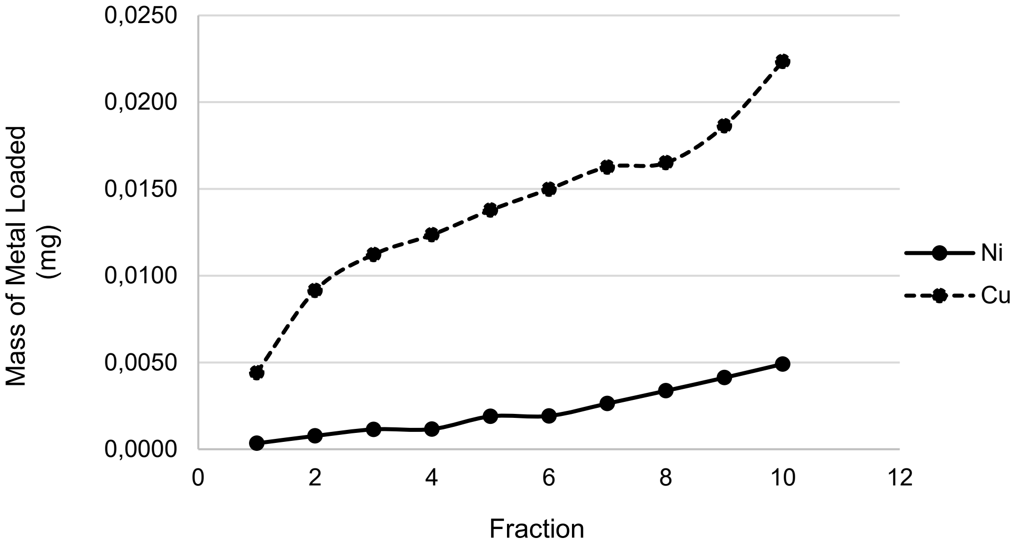


Figure 5.1: The mass of metal ions loaded from a binary solution

5.1.2 Stripping of the metal ions

To remove the metal ions from the resin, 0.4 M hydrochloric acid was employed. In the ten samples collected, 39.72 % of copper was found, leaving 50.27 % of copper ions on the resin. For nickel ions, 94.40 % was removed from the column using 0.2 M hydrochloric acid, leaving 5.96 % on the resin.

Table 5.3: The percentage recovery for the removal of metal ions in a binary solution

	Loaded onto the resin (mg)	Recovered (mg)	Percentage recovery (%)
Nickel	0.125	0.118	94.40
Copper	0.919	0.365	39.72

Table 5.4: The mass of metal ions removed in a binary solution

Fraction	Ni	Cu
1	0.0874	0.3339
2	0.0090	0.0148
3	0.0051	0.0039
4	0.0037	0.0025
5	0.0031	0.0023
6	0.0025	0.0019
7	0.0023	0.0016
8	0.0019	0.0016
9	0.0017	0.0014
10	0.0018	0.0014

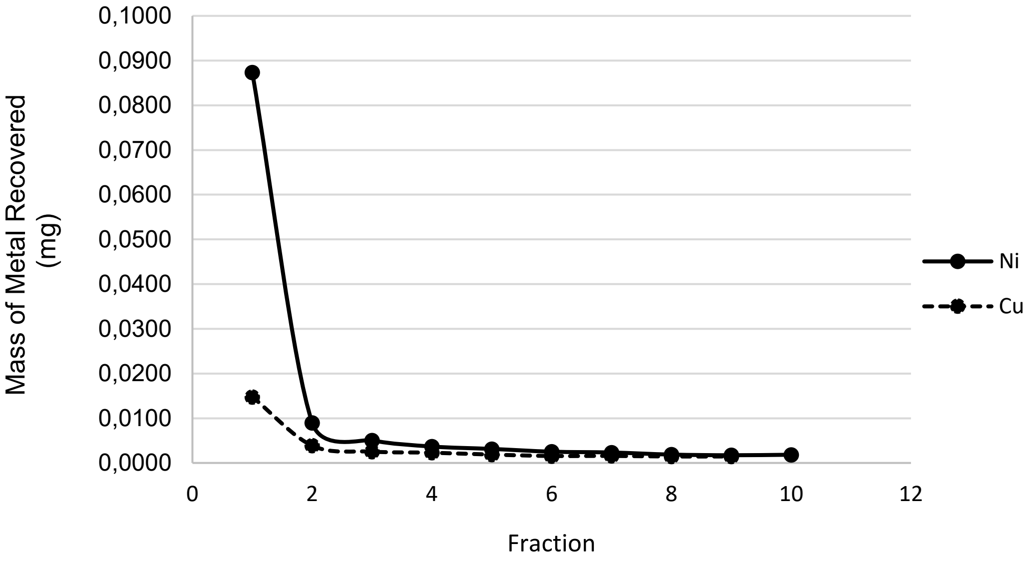


Figure 5.2: The mass of metal ions removed in a binary solution

5.2 Separation of cadmium–tin binary solution

Based on the experimental work done by Spies, 2020 only Cd ions are retained on the column while the tin ions (Sn^{4+}) passed through (unsorbed).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

A dynamic investigation was conducted to optimise the removal of metal ions from a dithizone-impregnated resin. Amberchrom® CG-300m resin was modified using dithizone, a potent chelating agent, by adding measured amounts of dithizone to the resin, which was dissolved with sodium hydroxide, deionised water, and alcohol. The resin changed from white to orange in colour, indicating a successful impregnation, after shaking the mixture on an orbital shaker for 30 minutes and titrating it with hydrochloric acid.

The optimisation of metal ion removal involved the study of factors such as eluent and eluent concentration, flow rate, and column height. For the removal of cadmium ions from the impregnated resin, low percentage recoveries were observed for the ranges chosen in each condition. The optimal conditions were found to be a flow rate of 7 mL/min with a column height of 0.4 g of impregnated resin, using 0.1 M hydrochloric acid as the eluent.

In the investigation of the optimal conditions for removing nickel ions from the impregnated resin, the best results were achieved at a flow rate of 6 mL/min with a column height of 0.4 g using 0.4 M hydrochloric acid, resulting in percentage recoveries of 73.88 %, 95.30 %, and 83.88 %, respectively.

The investigation of the separation of a copper nickel binary solution yielded a very low recovery rate owing to the difficulty in removing copper ions from the resin using various acids at different concentrations. However, using the optimal conditions for the removal of nickel ions, only 5.96 % of nickel ions remained on the column.

6.2 Recommendations

The following aspects can be investigated further:

- Larger ranges for each parameter.
- Exploring the removal of metal ions with other eluting agents.

REFERENCES

Adeyemi, D.K., Johnson, O.O. & Ayoola, G., 2017. Investigation of dithizone modified c-18 SPE sorbents for pre-concentration of zn and cu in water samples. https://ir.unilag.edu.ng/items/c19a9a5b-398a-4550-a78d-bb37ef94b202

Adonis, S., 2022. *A comparative study of the efficiency of ion exchange and extraction chromatography for the separation of milligram amounts of scandium from gram amounts of calcium* (Doctoral dissertation, Cape Peninsula University of Technology).

Ali, H., Khan, E. & Ilahi, I., 2019. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry*, *2019*. https://doi.org/10.1155/2019/6730305

Augusto, F., Hantao, L.W., Mogollón, N.G. & Braga, S.C., 2013. New materials and trends in sorbents for solid-phase extraction. *TrAC Trends in Analytical Chemistry*,*43*:14-23.

Bao, S., Tang, Y., Zhang, Y. & Liang, L., 2016. Recovery and separation of metal ions from aqueous solutions by solvent-impregnated resins. *Chemical Engineering & Technology*, *39*(8);1377-1392.

Behbahani, M., Najafi, M., Amini, M., Sadeghi, O., Bagheri, A. & Salarian, M., 2013. Dithizone-modified nanoporous fructose as a novel sorbent for solid-phase extraction of ultra-trace levels of heavy metals. *Microchimica Acta*, *180*(9-10):911-920.

Behbahani, M., Salarian, M., Amini, M., Sadeghi, O., Bagheri, A. & Bagheri, S., 2012. Application of a new functionalized nanoporous silica for simultaneous trace separation and determination of Cd(II), Cu(II), Ni(II), and Pb(II) in food and agricultural products. *Food Analytical Methods*, *6*(5):1320-1329.

Bhanot, D., 2013. *Why are HPLC columns shorter than GC columns?* Lab-training.com Available at: https://lab-training.com/why-are-hplc-columns-shorter-than-gc-columns/ (Accessed: 09 August 2023).

Camel, V., 2003. Solid phase extraction of trace elements. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 58(7):1177-1233.

Castillo, E., Beltrán, J., Prat, M., Granados, M. & Cortina, J., 2001. Simultaneous determination of Cd(ii), Cu(ii) and Pb(ii) in surface waters by solid phase extraction and flow injection analysis with spectrophotometric detection. *The Analyst*, 126(7):1149-1153.

Chatterjee, A. and Abraham, J., 2019. Desorption of heavy metals from metal loaded sorbents and e-wastes: A review. *Biotechnology letters*, *41*, pp.319-333.

Chaplin, B.P., 2019. The prospect of electrochemical technologies advancing worldwide water treatment. *Accounts of Chemical Research*, *52*(3):596-604.

Cheng, Y., Miao, Y., Zhu, L., Pei, Z., Xia, F. & Song, S., 2009. Study on adsorption behavior of Cd(II) on dithizone modified sodium trititanate whisker and its application to wastewater treatment. *2009 International Conference on Energy and Environment Technology*.

Chieh, C., 2019.*17.6: Precipitation and separation of ions*. [online] Chemistry Libre Texts. Available at: 17.6: Precipitation and separation of ions - Chemistry Libre Texts [Accessed 31 Jan 2020].

Compañó, R., Granados, M., Leal, C. & Prat, M., 1993. Solid-phase extraction and spectrofluorimetric determination of triphenyl tin in environmental samples. *Analytica Chimica Acta*, 283(1):272-279.

Crini, G. & Lichtfouse, E. 2018. Advantages and disadvantages of techniques used for wastewater treatment. *Environmental Chemistry Letters*, 17(1): 145-155.

Davarani, S., Sheijooni-Fumani, N., Najarian, A., Tabatabaei, M. & Vahidi, S., 2011. Preconcentration of lead in sugar samples by solid phase extraction and its determination by flame atomic absorption spectrometry. *American Journal of Analytical Chemistry*, *02*(05):626-631.

Dave, S.R., Kaur, H. & Menon, S., 2010. Selective solid-phase extraction of rare earth elements by the chemically modified Amberlite XAD-4 resin with azacrown ether. *Reactive & Functional Polymers*, *70*(9):692-698.

Esmaeilzadeh, M., 2019. Ultrasound-assisted dispersive magnetic solid phase extraction based on metal–organic framework/1-(2-pyridylazo)-2-naphthol modified magnetite nanoparticle composites for speciation analysis of inorganic tin. *New Journal of Chemistry*, *43*(12):4929-4936.

Feng, Y., Yang, L., Liu, J. & Logan, B., 2016. Electrochemical technologies for wastewater treatment and resource reclamation. *Environmental Science: Water Research & Technology*, 2(5):800-831.

Ferreira, S., dos Santos, W. & Lemos, V., 2001. On-line preconcentration system for nickel determination in food samples by flame atomic absorption spectrometry. *Analytica Chimica Acta*, 445(2):145-151.

Ince, M. & Ince, O.K., 2019. Heavy metal removal techniques using response surface methodology: water/wastewater treatment. In *biochemical toxicology-heavy metals and nanomaterials*. IntechOpen.

Kim, J.J., Kim, Y.S. & Kumar, V., 2019. Heavy metal toxicity: an update of chelating therapeutic strategies. *Journal of Trace Elements in Medicine and Biology*,*54*:226-231.

Laureano-Anzaldo, C.M., González-López, M.E., Pérez-Fonseca, A.A., Cruz-Barba, L.E. & Robledo-Ortíz, J.R., 2021. Plasma-enhanced modification of polysaccharides for wastewater treatment: a review. *Carbohydrate Polymers*, 252:117195. doi:10.1016/j.carbpol.2020.117195.

Lenntech.com. (n.d.). *Membrane technology*. [online] Available at: https://www.lenntech.com/membrane-technology.htm [Accessed 31 Jan. 2020].

Liška, I., 2000. Fifty years of solid-phase extraction in water analysis – historical development and overview. *Journal of Chromatography A*, 885(1-2):3-16.

Lu, X., Lu, J., Zhang, Z. & Xue, M., 2015. Determination of cadmium in rice by activated aluminum oxide immobilized with dithizone-solid phase extraction-hydride generation coupled atomic fluorescence spectrometry. *Asian Journal of Chemistry*, 27(12):4383-4386.

Mahurpawar, M., 2015. Effects of heavy metals on human health. *International Journal of Research Granthaalayah*, *530*(516):1-7.

Masoodi, K.Z., Lone, S.M. & Rasool, R.S., 2020. *Advanced methods in molecular biology and biotechnology: a practical lab manual*. Academic Press.

Moalla, S., Rashed, M., Aziz, A. & Maghazy, M. 2020. Determination of Cd2+, Cu2+, Mn2+, Pb^{2+}, and Zn2+ in aqueous solution after their separation and preconcentration as metal-dithizone complexes on activated carbon. *Aswan University Journal of Environmental Studies*, *1*(2):157-170.

Nezhadali, A. & Nazari, S., 2009. Graphite furnace atomic absorption spectrometric determination of cadmium after solid-liquid extraction with dithizone. *Bulletin of the Chemical Society of Ethiopia*, 23(2):257-261.

Nyamunda, B., Chivhanga, T., Guyo, U. & Chigondo, F., 2019. Removal of Zn (II) and Cu (II) Ions from industrial wastewaters using magnetic biochar derived from water hyacinth. *Journal of Engineering*, 2019:1-11.

Patel, H., 2019. Fixed-bed column adsorption study: a comprehensive review. *Applied Water Science*, *9*(3):45.

Patel, H., 2021. Comparison of batch and fixed bed column adsorption: a critical review. *International Journal of Environmental Science and Technology*, *19*(10):10409-10426.

Płotka-Wasylka, J., Szczepańska, N., de La Guardia, M. & Namieśnik, J., 2016. Modern trends in solid phase extraction: new sorbent media. *TrAC Trends in Analytical Chemistry*, *77*:23-43.

Poole, C., Gunatilleka, A. & Sethuraman, R. 2010. ChemInform abstract: Contributions of theory to method development in solid-phase extraction. *ChemInform*, *31*(46):17.

Pourrez, N., Fathi, M. & Ardan, Z. 2010. Flame atomic absorption spectrometric determination of Cd(II), Ni(II), Co(II) and Cu(II) in tea and water samples after simultaneous preconentration of dithizone loaded on naphthalene. *Journal of the Iranian Chemical Society*, *7*(4):965-971.

Ritter, K.R., Sibley, P., Hall, K., Keen, P., Mattu, G. & Linton, B.L., 2002. Sources, pathways, and relative risks of contaminants in surface water and groundwater: a perspective prepared for the Walkerton inquiry. *Journal of Toxicology and Environmental Health Part A*, *65*(1):1-142.

Saxena, R., Meena, P. & Tiwari, S., 2015. Determination of copper in industrial water by innovative flow injection flame atomic absorption spectrometry. *Instrumentation Science & Technology*, 44(2): 210-222.

Samer, M., 2015. Biological and chemical wastewater treatment processes. *Wastewater Treatment Engineering*,*150*:212.

Shamsipur, M. & Mashhadizadeh, M., 2000. Preconcentration of trace amounts of silver ion in aqueous samples on octadecyl silica membrane disks modified with hexathia-18-crown-6 and its determination by atomic absorption spectrometry. *Fresenius' Journal of Analytical Chemistry*, *367*(3):246-249.

Sunder, G.S.S, Adhikari, S., Rohanifar, A., Poudel, A. & Kirchhoff, J.R., 2020. Evolution of environmentally friendly strategies for metal extraction. *Separations*, *7*(1):4.

Sonone, S.S., Jadhav, S., Sankhla, M.S. & Kumar, R., 2020. Water contamination by heavy metals and their toxic effect on aquaculture and human. *Letters in Applied NanoBioScience, 10*(2):2148-2166.

Spies, A.R.L., 2020. *Sorption behaviour of heavy metals on a dithizone-impregnated polymer resin* (Doctoral dissertation, Cape Peninsula University of Technology).

Spies, A.R. and Wewers, F., 2020. Equilibrium, kinetics and thermodynamics studies of Cd sorption onto a dithizone-impregnated Amberchrom CG-300m polymer resin. *Arabian Journal of Chemistry*, *13*(4), pp.5050-5059.

Sultana, F. 2018. Water justice: why it matters and how to achieve it. *Water International*, *43*(4):483-493.

Tabrizi, A., 2007. Development of a cloud point extraction-spectrofluorimetric method for trace copper(II) determination in water samples and parenteral solutions. *Journal of Hazardous Materials*, *139*(2):260-264.

Tang, Y., Bao, S., Zhang, Y. & Liang, L., 2017. Effect of support properties on preparation process and adsorption performances of solvent impregnated resins. *Reactive and Functional Polymers*,*113*:50-57.

Tiandho, Y., 2019. Quality improvement of water from post tin mining based on a wasted cockle shell. In *IOP Conference Series: Materials Science and Engineering*, 496:012058.

Tranter, T., 2011. Solid-phase extraction technology for actinide and lanthanide separations in nuclear fuel reprocessing. In *Advanced separation techniques for nuclear fuel reprocessing and radioactive waste treatment.* Amsterdam: Elsevier: 377-413

Türker, A.R., 2012. Separation, preconcentration and speciation of metal ions by solid phase extraction. *Separation & Purification Reviews*, *41*(3);169–206.

Tyagi, R. & Jacob, J., 2020. Design and synthesis of water-soluble chelating polymeric materials for heavy metal ion sequestration from aqueous waste. *Reactive and Functional Polymers*, *154*:104687.

Urasa, I., Macha, S. & El-Maaty, W. 1997. Application of solid-phase extraction in metal speciation. *Journal of Chromatographic Science*, *35*(11):519-524.

Uzcan, F., Shah, S. & Soylak, M. 2020. Magnetic dispersive solid phase extraction of lead(II) as dithizone chelates in food and environmental samples on Fe_3O_4@XAD-8 prior to its flame atomic absorption spectrometric detection. *International Journal of Environmental Analytical Chemistry*:1-12.

Wang, L.K., Vaccari, D.A., Li, Y. & Shammas, N.K., 2005. Chemical precipitation. In *Physicochemical treatment processes* (pp. 141-197). Totowa, NJ: Humana Press.

Wang, Y., Zhu, A., Fang, Y., Fan, C., Guo, Y., Tan, Z., Yin, Y., Cai, Y. & Jiang, G., 2022. Dithizone-functionalized C18 online solid-phase extraction-HPLC-ICP-MS for speciation of ultra-trace organic and inorganic mercury in cereals and environmental samples. *Journal of Environmental Sciences*, *115*:403-410.

Wu, D., Wang, A. & Guo, L. 2006. Synthesis and application of Amberlite XAD-2 functionalized with dithizone for field preconcentration and separation of trace cadmium in seawater. *Analytical Sciences*, 22(9):1245-1248.

Yamini, Y., Hassan, J., Mohandesi, R. & Bahramifar, N. 2002. Preconcentration of trace amounts of beryllium in water samples on octadecyl silica cartridges modified by quinalizarine and its determination with atomic absorption spectrometry. *Talanta*, 56(3):375-381.

Yebra, M., Carro, N., Enríquez, M., Moreno-Cid, A. & García, A. 2001. Field sample preconcentration of copper in sea water using chelating minicolumns subsequently incorporated on a flow-injection–flame atomic absorption spectrometry system. *The Analyst*, 126(6):933-937.

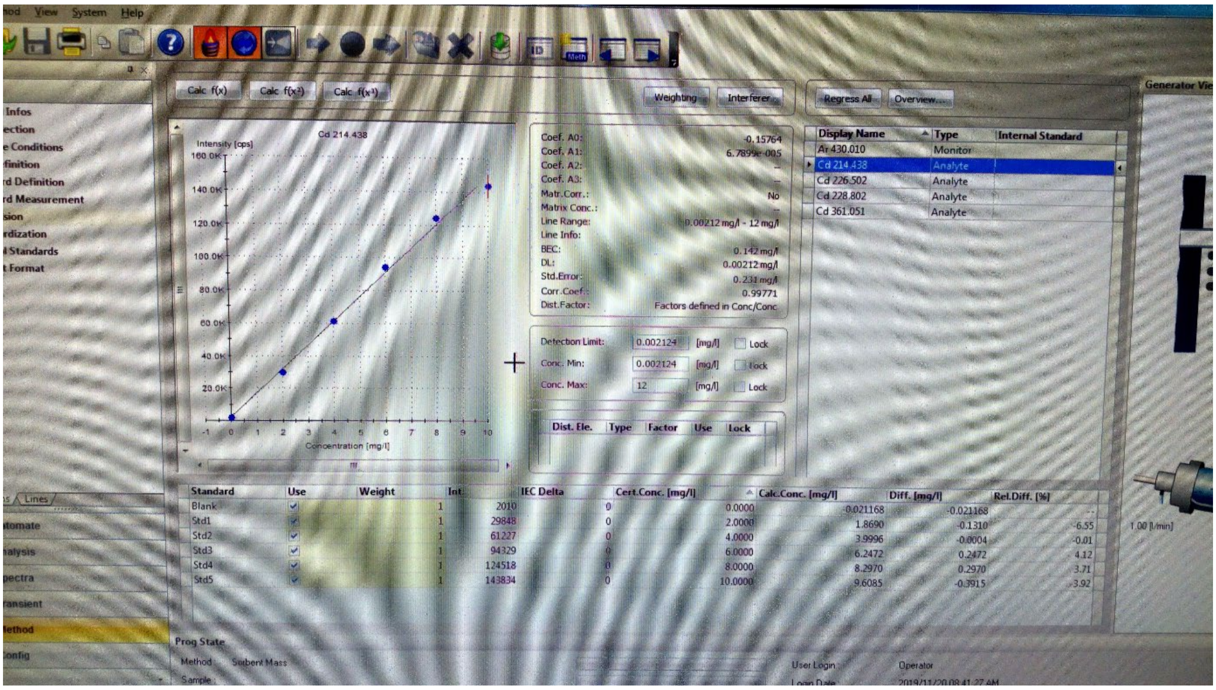
Yu, H., Song, H. & Chen, M. 2011. Dithizone immobilized silica gel on-line preconcentration of trace copper with detection by flame atomic absorption spectrometry. *Talanta*, 85(1):625-630.

APPENDICES

Appendix A

Figure 7.1: Fractions collected during experimental work.

Appendix B:



Coef. A0: -0.15764
Coef. A1: 6.7899e-005
Coef. A2: --
Coef. A3: --
Matr.Corr.: No
Matrix Conc.: --
Line Range: 0.00212 mg/l - 12 mg/l
Line Info:
BEC: 0.142 mg/l
DL: 0.00212 mg/l
Std.Error: 0.231 mg/l
Corr.Coef.: 0.99771
Dist.Factor: Factors defined in Conc/Conc

Detection Limit: 0.002124 [mg/l] ☐ Lock
Conc. Min: 0.002124 [mg/l] ☐ Lock
Conc. Max: 12 [mg/l] ☐ Lock

Dist. Ele.	Type	Factor	Use	Lock

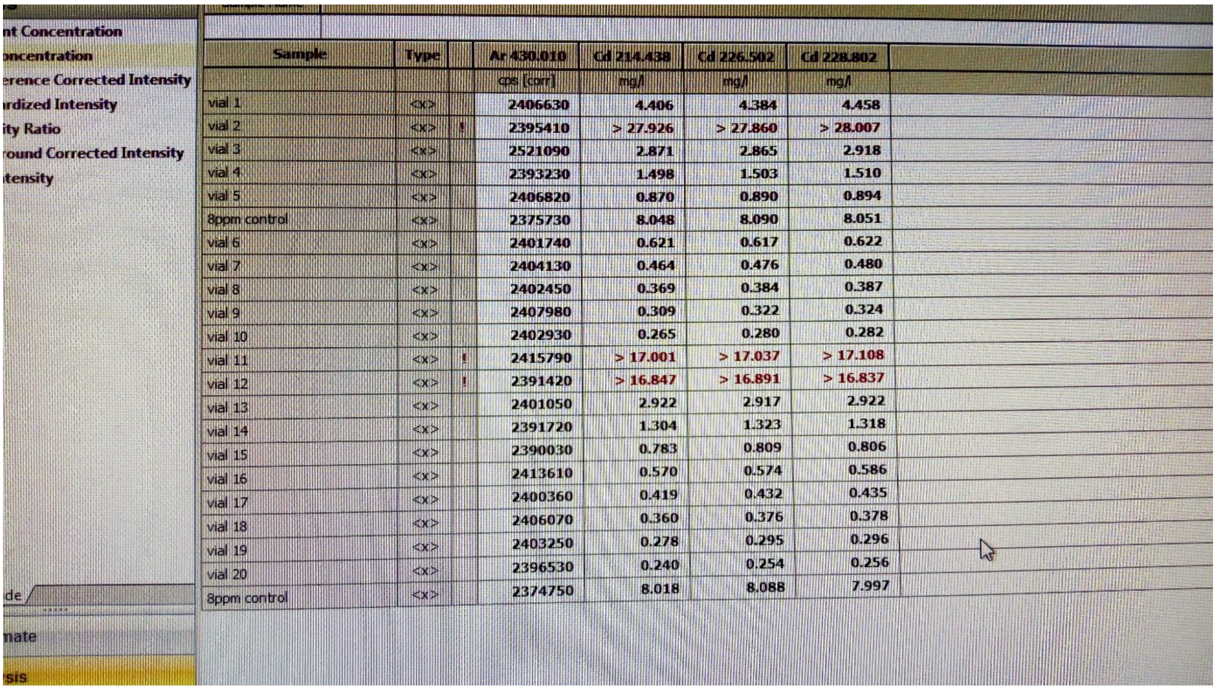
Display Name	Type	Internal Standard
Ar 430.010	Monitor	
Cd 214.438	Analyte	
Cd 226.502	Analyte	
Cd 228.802	Analyte	
Cd 361.051	Analyte	

Standard	Use	Weight	Int.	IEC Delta	Cert.Conc. [mg/l]	Calc.Conc. [mg/l]	Diff. [mg/l]	Rel.Diff. [%]
Blank	☑	1	2010	0	0.0000	-0.021168	-0.021168	--
Std1	☑	1	29848	0	2.0000	1.8690	-0.1310	-6.55
Std2	☑	1	61227	0	4.0000	3.9996	-0.0004	-0.01
Std3	☑	1	94329	0	6.0000	6.2472	0.2472	4.12
Std4	☑	1	124518	0	8.0000	8.2970	0.2970	3.71
Std5	☑	1	143834	0	10.0000	9.6085	-0.3915	-3.92

Prog State
Method: Sorbent Mass
Sample:
User Login: Operator
Login Date: 2019/11/20 08:41:27 AM

Figure 7.2: An example of the ICP calibration for cadmium studies

Appendix C:



Sample	Type		Ar 430.010	Cd 214.438	Cd 226.502	Cd 228.802
			cps [corr]	mg/l	mg/l	mg/l
vial 1	<x>		2406630	4.406	4.384	4.458
vial 2	<x>	!	2395410	> 27.926	> 27.860	> 28.007
vial 3	<x>		2521090	2.871	2.865	2.918
vial 4	<x>		2393230	1.498	1.503	1.510
vial 5	<x>		2406820	0.870	0.890	0.894
8ppm control	<x>		2375730	8.048	8.090	8.051
vial 6	<x>		2401740	0.621	0.617	0.622
vial 7	<x>		2404130	0.464	0.476	0.480
vial 8	<x>		2402450	0.369	0.384	0.387
vial 9	<x>		2407980	0.309	0.322	0.324
vial 10	<x>		2402930	0.265	0.280	0.282
vial 11	<x>	!	2415790	> 17.001	> 17.037	> 17.108
vial 12	<x>	!	2391420	> 16.847	> 16.891	> 16.837
vial 13	<x>		2401050	2.922	2.917	2.922
vial 14	<x>		2391720	1.304	1.323	1.318
vial 15	<x>		2390030	0.783	0.809	0.806
vial 16	<x>		2413610	0.570	0.574	0.586
vial 17	<x>		2400360	0.419	0.432	0.435
vial 18	<x>		2406070	0.360	0.376	0.378
vial 19	<x>		2403250	0.278	0.295	0.296
vial 20	<x>		2396530	0.240	0.254	0.256
8ppm control	<x>		2374750	8.018	8.088	7.997

Figure 7.3:Concentration results using the ICP during cadmium investigations

Appendix D:

Example of statistical data for the separation of nickel and copper

Table 7.1: The statistical data for the loading of nickel ions in a binary solution

Fraction	Experiment 1	Experiment 2	Experiment 3	Average	Standard Deviation
1	0.0004	0.0000	0.0006	0.0003	0.0003
2	0.0006	0.0005	0.0011	0.0008	0.0003
3	0.0011	0.0005	0.0019	0.0011	0.0007
4	0.0009	0.0008	0.0019	0.0012	0.0006
5	0.0012	0.0016	0.0029	0.0019	0.0009
6	0.0010	0.0016	0.0032	0.0019	0.0011
7	0.0012	0.0024	0.0037	0.0026	0.0010
8	0.0021	0.0029	0.0051	0.0034	0.0015
9	0.0029	0.0039	0.0056	0.0041	0.0014
10	0.0033	0.0047	0.0067	0.0049	0.0017

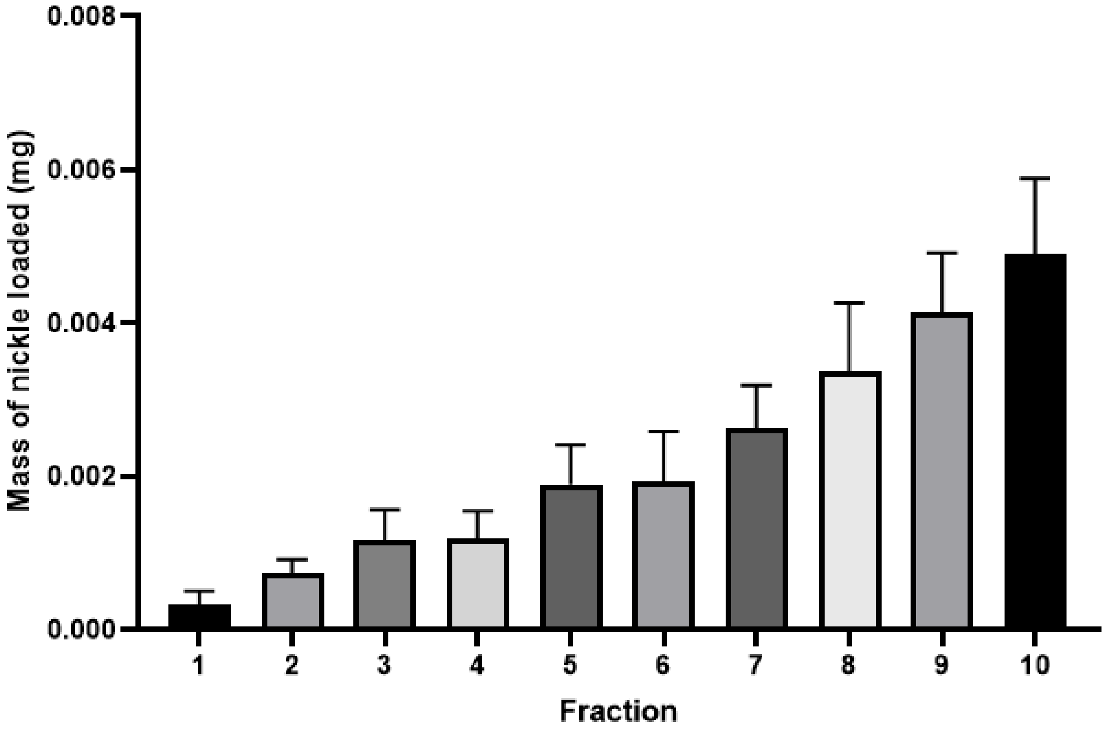


Figure 7.4: Error bar for the for the loading of nickel ions in a binary solution