

A Novel Multi-templated Cu-Fe-Ni Ion Imprinted Polymer for the  
Selective and Simultaneous Removal of Toxic Metallic Ions from  
Wastewater



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Chemistry

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Based on research carried out under the supervision of Dr.  
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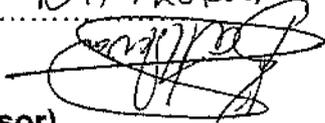
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# DEDICATION

This thesis is dedicated to my late beloved grandmother **Mrs. Sarah Y. Stevens** and my beloved father **Mr. Paul F. Stevens**

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## ABSTRACT

A novel multi-templated Cu-Fe-Ni ion imprinted powder was synthesized to demonstrate the selective and simultaneous removal of targeted ions; Cu(II), Fe(II) and Ni(II), employing molecular imprinting technology (MIT) via template (print ions) guided by bulk polymerization method reacting methacrylic acid (MAA) as the functional monomer and ethylene glycol dimethylacrylate (EGDMA) as cross linking agent in the presence of the initiator azobisisobutyronitrile (AIBN); Cu(II), Fe(II) and Ni(II) ions as template ions, and 1,10-phenanthroline as the complexing agent. The template ions were exhaustively removed from the synthesized polymer by rigorous, optimal template removal method involving, periodically increasing solvent (HCl) concentration from 1.0 M to 10.0 M. The physical and chemical properties of the sorbents were investigated using Fourier Transform Infrared Spectroscopy (FT-IR), X-ray Diffraction (XRD) and Atomic Force Microscopy (AFM). Operational parameters including time, pH and sorbent dosage for the rebinding batch experiments were optimized and found to be 15 min, 7.5 and 666.7 mg/L respectively. The optimized conditions were then applied to evaluate the effectiveness of the prepared Cu-Fe-Ni IIP through the percentage recoveries of the bound targeted metal ions in the real samples in the presence of competitive ions and complex matrices of waste and borehole water. For the wastewater and borehole water collected from Glen Valley Wastewater Treatment Plant and Extension Five, Gaborone, Botswana respectively. The percentage recoveries of the removed targeted ions from the spiked samples were:  $92.14 \pm 0.16\%$  ( $R^2 = 0.9997$ ),  $106.09 \pm 0.17\%$  ( $R^2 = 0.9993$ ) and  $99.86 \pm 0.04\%$  ( $R^2 = 0.9995$ ) respectively. The selectivity of the Cu-Fe-Ni IIP, competitive sorption studies between the template ions and similar ions were also carried out. The synthesized sorbents

showed good selectivity towards the targeted metal ion by removing 90% - 98% of the templated ions as compared to 58% - 62% of the competitive ions.

**Key Words:** Ion imprinted polymers, Multi-templating, Toxic heavy metal ions, and Recycled wastewater.

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# LIST OF ABBREVIATIONS

IIPs: Ion imprinted polymers

NIPs: Non-imprinted polymers

MAA: Methacrylic acid

EGDMA: Ethylene glycol dimethacrylate

AIBN: Azobisisobutyronitrile

ANOVA: Analysis of variance

FAAS: Force atomic absorption spectrometer

XRD: X-ray diffractometer

AFM: Atomic force microscope

FTIR: Fourier transform infrared

SPE: Solid phase extraction

WHO: World health organization

# CHAPTER ONE

## 1.0 Introduction

The use of treated wastewater is widely employed to compensate for the scarcity of safe and uncontaminated freshwater [1]. Wastewater treatment plants have been set up to clean and recycle wastewater for use in irrigation, animal consumption, groundwater recharge, non-potable reuse and domestic activities [2]. However, the existence of toxic heavy metal ions in the wastewater pose a health hazard to animals and the environment [3]. Agricultural activities, sewage, industrial disposal and even natural disasters have constantly increased the addition of heavy metal ions in the aquatic ecosystems [4]. Generally, heavy metals are stable under ambient environmental conditions. They are not easily degradable [5], thus, their tendency to accumulate in the environment, and eventually end up in the human food chain. Although the human body needs some of these metal ions in trace quantities, their existence in high concentrations result in serious human ailments [6]. Henceforth, it is essential that the concentration of heavy metal ions in recycled wastewater be considerably reduced to acceptable levels as set out by international monitoring agencies such as the World Health Organization (WHO) [7], Food Agriculture Organization (FAO) [7] and Environment Protection Agency standard (EPA) [7].

In the past, several methods such as membrane assisted liquid extraction (MALE) [10] [11] [12] liquid–liquid extraction [13] [14], membrane filtration [15] [16]; ion exchange [17], adsorption [18] [19] have been employed to remove toxic heavy metal ions from aquatic environments. However, these methods have been reported to have some challenges such as high cost [3] [10],

require a trained personnel [9], high energy consumption [20], use of large quantities of chemicals [3] [17], poor removal rate to meet the pollution control limits [7], low mechanical strength of adsorbents [20], weak hydrothermal stability [3], poor selectivity [20] and time consuming [3] [20].

In recent years, better extraction methods such as membrane extraction [3], use of bio adsorbents [18], use of solid-phase polymeric extraction sorbents (SPE sorbents) [21] or their combinations have been employed to eliminate the challenges of previous traditional methods [21] [16]. These methods exhibit numerous advantages such as high removal factors, high retention capacity, flexibility, robustness, speed and simplicity. However, these extraction methods are challenged as they are non-selective in removing/reducing the unwanted toxic metal ions to acceptable levels, thus other substances which may be needed to remain in the wastewater or pose no threat are co-extracted [22] [24]. Therefore, the development of new extraction materials, especially new adsorbents with high specific recognition sites and high affinity for a particular metal ion(s) is of great interest. Ion imprinted polymers (IIPs) are an example of such materials with high recognition sites and affinity for a targeted ion(s). Ion-imprinted polymers (IIPs) are nano-porous polymeric materials which on leaching the imprint ion can selectively rebind, sense or transport the target analyte in the presence of closely related inorganic ions.

In this thesis, a model, novel multi-templated IIP aimed at selectively removing several ions at the same time will be developed. It is common for IIPs to be developed for the extraction of one metal ion per time but this approach will not be that useful in most cases as waste water usually has contamination from several metal ions hence our proposal of developing a multi-templated IIP to simultaneously remove multiple toxic ions from waste water.

## 1.1 Sample Handling

Sample handling refers to all pre-analytical procedures that are carried out prior to instrumental analysis [28]. It includes sampling and sample preparation (Fig. 1).

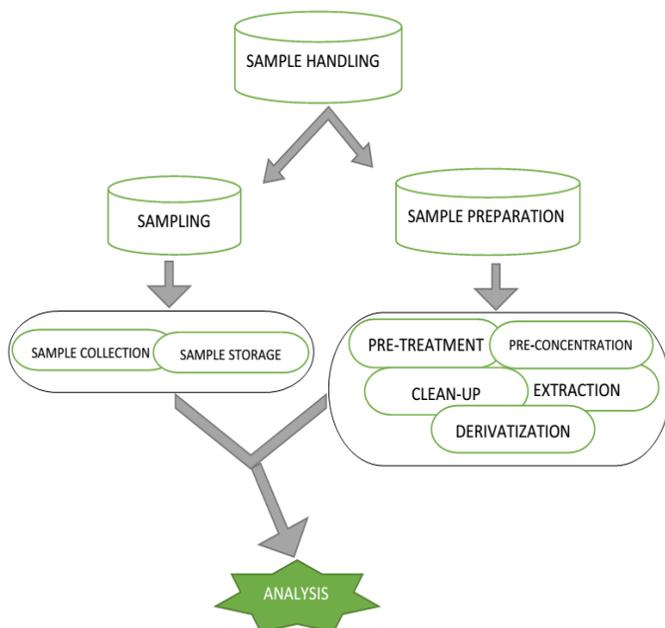


Figure 1.0: Schematic diagram of sample handling procedures

### 1.1.1 Sampling

Sampling is the first step of the sample handling procedures that an analyst undertakes in assaying populations. It involves selecting a representative portion from a population of interest so that by analysing the portion (sample), the results may fairly generalize the population from which it was chosen [5].

Sampling addresses the following; choice of sampling site, choice of the sampled population, sampling time/period, the form of samples one is dealing with, sample collection, environmental

conditions to consider during collection, the containers to use, sample management, sample preservation, storage and management of sample integrity [28].

For the analysis of water, four sampling collection methods are frequently employed; in-situ [10], on-site [15], grab [30] and automatic [14]. Amongst them, grab sampling is the most commonly applied due to its simplicity, inexpensiveness and robustness. It involves collecting a single sample randomly at different depths and points at a specific time [30]. Samples collected will then be mixed together in a single sampling vessel (polypropylene plastic or Teflon).

Examples of grab samples include dip, discrete, and pump samples. Dip samples are typically collected by dipping the collection container (of appropriate non-contaminating material) into the upper layer of the water body. Discrete samples are collected by either lowering a sampler to a specified depth and then collecting a sample by opening and closing the sampler or employing a single-stage sampler, which fills when stream stage rises to a predetermined height. Pump samples on the other hand, are collected with suction lift or submersible pump systems designed to collect water-quality samples. Pump systems can be permanently installed and automated for sampling [32].

### 1.1.2 Sample Preparation Procedures for Heavy Metal Ion Analysis in Water

In general, fresh waters are characterized by the existence of toxic heavy metal ions in very low concentrations in a very `dirty` matrix. In order to improve detectability and accuracy of their analysis, a series of work-up steps are necessary to perform on the sample after sampling and before instrumental analysis to improve detectability and reduce maintenance of the instruments.

The steps are referred to as sample preparation procedures. They include: sample pretreatment [37], pre-concentration [28] [37], extraction [36], clean-up [37] and derivatization [10] [36]. Sample preparation accounts for 80% of all the total assaying time, making it the most crucial step in analysis [5].

Wastewater on the other hand is characterized by the existence of toxic heavy metal ions in relatively high concentrations in a very `dirty` matrix. The challenge then, is to remove the `dirty` matrix as well as reducing the high concentrations of the toxic heavy metal ions to acceptable levels as per the monitoring agencies [7] in order to make it safe for use by both humans and animals. Sample preparation steps such as pretreatment and extraction techniques are usually employed in wastewater treatment plants in order to clean and recycle wastewater [37].

### 1.1.2.1 Pretreatment of Wastewater

Sample pretreatment accounts for all steps that are employed in removing the obvious dirt especially conditioning a sample to a definite state that allows further, subsequent treatment. It ensures that the sample retains its physical and chemical characteristics throughout the whole treatment procedure. Examples of pretreatment steps that are usually employed at wastewater treatment plants are; filtration, chemical dissolution, and disinfection [37]. Filtration, and disinfection are the most commonly employed to remove the obvious `dirt` especially debris and organic matters as well as to combat pathogenic microbial growth in the sample including to suppress biofouling of the storage container respectively.

For example, Ahmed *et al* investigated the effect of applying different disinfectants in water. They employed chlorinating agents including Ozone, chlorine dioxide, chloramines, peroxone to inhibit further microbial activities and to eliminate contaminants by allowing the chlorinating agents to form complexes with contaminants which were further removed by means of clean up procedures. The disinfected samples were stored for about four months and it was observed that the physical and chemical integrity of the different samples were upheld [34]. Bordas *et al* on the other hand, employed acidification to pretreat contaminated water before storage. They acidified the wastewater sample by adding 1.0 M HCl to obtain a pH of 4.0 and stored at 4 °C. The research was focused on preserving the nature and composition of the water before the intended use. Acidification prevented compositional reactions, including further microbial activities during the period of storage. It was observed that the integrity of the water was maintained for more than three months [38].

### 1.1.2.2 Extraction

Extraction refers to all sample procedures that bring out the analyte of interest from a sample matrix and make it available for further sample preparation and eventually detection. The matrix is usually brought into contact with a solvent that can easily solubilize the target analyte. [38]. The chemical properties of the analyte, the properties of the liquid medium in which it is dissolved (the solvent) in and the gaseous, liquid, supercritical fluid, or solid extractant employed to effect extraction are all essential for effective and efficient extraction. Five major chemical properties of the solute are fundamental to understanding the extraction theory: vapour pressure, solubility, molecular weight, hydrophobicity, and acid dissociation. These fundamental

properties determine the transport of analytes of interest between immiscible phases during extraction. For example, extraction of heavy metal ions from wastewater is accomplished by bringing the wastewater sample into contact with another substance (phase X) which could be a solid, liquid, gas or supercritical fluid that is immiscible with the wastewater sample distributed between the two phases. The extract will then be released and(or) recovered from phase X for subsequent extraction procedures or instrumental analysis [42] [32]. The most common extraction techniques in the removal of toxic heavy metal ions from water are: ion exchange [27], membrane extraction [43], membrane assisted liquid-liquid extraction (MALE) [37], and solid phase extraction (SPE) [44].

#### 1.1.2.2.1 Ion Exchange

Ion exchange is an extraction technique that requires the interchange of ions between the resin and the surrounding medium. Resins, are totally insoluble in most aqueous and organic solutions. They are usually constructed spherically, and are stress and strain free to resist physical degradation. They are stable at high temperatures and applicable over a broad pH range. Resins consist of cross linked polymer matrices to which charged functional groups are attached covalently. The ion exchangers which contain cations or anions as counter-ions are called cation exchangers or anion exchangers, respectively. In ion exchange extraction, a reversible interchange of ions between the solid and liquid phases occurs. A resin removes ions of like charge in a chemically equivalent quantity without any morphological alterations of the resin (see Fig.3) [39].

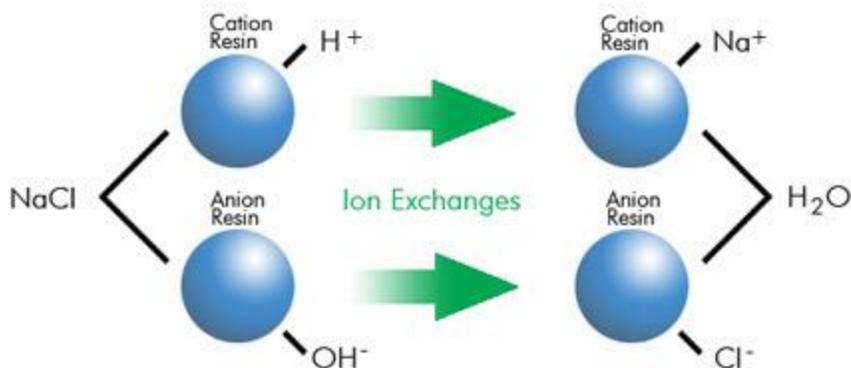


Figure 1.1: illustration of Ion Exchange extraction [39]

Ion exchange applications can be performed by column (flow continuous) [54] and batch [55] technique mode. After the application is completed, the resin can be regenerated in at the same place or transferred elsewhere for regeneration. In the batch mode the insoluble resin is sprinkled in the wastewater body and left for some time to equilibrate before it is reclaimed through filtration as a residue for regeneration and subsequent re-use [46] [55]. For example, Hubicki *et al*, evaluated the sorption of heavy metal ions;  $Cu^{2+}$ ,  $Zn^{2+}$ ,  $Cd^{2+}$  and  $Pb^{2+}$  in the presence of the complexing agents of a new generation Baypure CX 100 (IDS) and Trilon M (MGDA) on commercially available chelating ion exchangers; Dowex M 4195 and Diphonix Resin respectively, in comparison to reduction and precipitation removal technique. The chelating ion exchange resins Dowex M 4195 and Diphonix Resin were characterized and purified respectively prior to the experiment. The ion exchange applications were carried out employing batch experiments, in which,  $5000\text{ cm}^3$  of sample solution containing  $Cu^{2+}$ ,  $Zn^{2+}$ ,  $Cd^{2+}$  and  $Pb^{2+}$  and ion exchanger (0.5 kg) in a 10 L vessel were equilibrated. The experiments were conducted in three parallel series with the reproducibility of the measurements within a % relative standard

deviation (%RSD) of 5% for replicate measurements. In another experiment, 5000 cm<sup>3</sup> of the sample solution was acidified at pH > 6.3, digested and the original volume was reduced to 1000 cm<sup>3</sup> to allow the formation of complexes, hence, precipitates. It was observed that more than 90% of the metal ions were bound to Dowex M 4195 and Diphonix Resin within 10-20 min of the phase contact time relative to 67.43% of reduction and precipitation extraction techniques. On Dowex M 4195 about 95 %, 100 %, 99 % and 97.5 % of the Cu<sup>2+(II)</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup> and Pb<sup>2+</sup> complexes with IDS and 94 %, 98 %, 96 % and 95 % complexes with MGDA were sorbed respectively. The sorption for Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup> and Pb<sup>2+</sup> complexes with IDS and MGDA on Diphonix Resin were 94 %, 89 %, 97 % and 98 % and 97 %, 86 %, 99 % and 96 % respectively. The results indicated that the sorption process of metal ions in the presence of IDS and MGDA on Dowex M 4195 and Diphonix Resin were due to both external mass transfer and intra-particle sorption processes, whereas external mass transfer was the sole process involved in the sorption process of the reduction and precipitation extraction techniques [46].

Although ion exchange resins have higher capacity for the removal of undesired components, their usage may require several, further pre-treatments procedures and specialized apparatus, thereby incurring high installation and operation costs [47] [48].

### 1.1.2.2.2 Membrane Extraction

Membrane extraction techniques involve the employment of a membrane that is a selective barrier between two phases; the donor phase (sample) and the acceptor phase, which often consists of an aqueous or organic solvent [62]. Target analytes permeate from the sample through the membrane into the solvent. In this technique, a liquid sample is in contact with a

membrane made of semi-permeable material such as Teflon and silicone rubber [63]. There are two types of membranes that can be used: porous and non-porous. Porous membrane have the two phase solutions on either side of the membrane in physical contact through the pores of a membrane while the non-porous membrane forms a separate phase (polymeric or liquid) between the donor and acceptor solutions [64].

Maximous *et al* employed metal oxides/Polyether sulfone (PES); Al<sub>2</sub>O<sub>3</sub>/PES and ZrO<sub>2</sub>/PES, membranes for the extraction of Cr<sup>3+</sup>, Cd<sup>2+</sup> and Pb<sup>2+</sup> from water. The membranes were employed as a filter to trap competitive ions and undesired components thus preventing them from penetrating the pores of the membrane. The membrane demonstrated selectivity towards analytes of interest allowing them through the pore and retaining the competitive ions and undesirable components [65]. Qdais *et al* on the other hand, employed nanofiltration (NF), which is a membrane process, to extract copper and cadmium from water sample resulting into a similar outcome as Maximous *et al* [66]. However, selectivity and high production installation cost often hinder the full scale application of membrane extraction.

### 1.1.2.2.3 Membrane Assisted Liquid Extraction (MALE)

Membrane assisted liquid extraction (MALE) is a three-phase extraction procedure that mimics the principles of liquid/liquid extraction (LLE) [42] but with the addition of a membrane, partitioning the two liquid phases [69]. Membrane based separation techniques have drawn maximum attention in the last few years. The advantages of membrane based techniques are: high efficiency, low power consumption and easy scale-up due to a compact design etc. The membrane serves as a semi-permeable barrier separating two phases of different composition and

is often used in separation and purification schemes. Among the popular membrane processes, liquid membrane (LM) is one of the important configurations which is further classified into bulk liquid membrane (BLM), and solid liquid membrane (SLM). Due to industrial suitability for continuous processing, SLM with hollow fiber membrane is deployed in chemical, environmental and nuclear applications. LMs are particularly important in instances where solute concentrations are extremely low and large volumes of solutions need to be processed. The specific advantages of SLM based separation process are (a) high feed to strip volume ratio leading to large enrichment factors of the transported species, (b) very low extractant (carrier) inventory, (c) no phase separation problem occurs as the organic and aqueous phases never mix, (d) negligible organic phase entrainment in the feed and strip aqueous solutions, (e) no moving parts. In BLMs, two miscible aqueous liquids (feed and strip) are separated via a third immiscible organic liquid (carrier). The mass transfer from one liquid (feed) to the other liquid (strip) takes place via the carrier. Transport through the bulk takes place by convection, allowing the unstirred boundary layer to often form the largest transport resistance. BLMs are frequently used to investigate novel carriers, carrier systems or transport mechanisms; their main action is optimizing data for SLMs [70]. The principal disadvantage of traditional BLMs has been the low interfacial surface areas and mass transfer rates as compared to SLMs. Due to stability issues of liquid membrane, membrane contactors are often introduced and proved to be efficient contacting devices, due to their high surface area per unit volume that may results in high mass transfer rates. They are not only compact but also eliminate several of the challenges faced by conventional processes. Furthermore, they satisfy the requirements for process intensification [71]. Klaassen *et al* employed polyamide as a membrane contactor in a solid -liquid membrane to remove inorganic contaminants from industrial wastewater. They observed that the

membranes with polyamide as their skin material had a higher removal of inorganic contaminants and worked best in a wide range of temperatures (5 – 45 °C) [72]. However, the approach had a number of challenges that limited its effectiveness. It was challenged when it came to selectivity and had difficulty with automation. Meanwhile, other approaches, such as solid phase extraction in particular, are much easier to automate [73].

#### 1.1.2.2.4 Solid Phase Extraction (SPE)

Solid phase extraction (SPE) is an extraction method that employs a solid phase to isolate an analyte of interest from a solution. It is often employed to clean up samples prior to analysis [71] [73] [74]. Due to several challenges associated with the above mentioned techniques such as: poor selectivity of the targeted ion(s), high cost, use of large volumes of toxic solvents, low efficiency, and the need for special technical know-how, a more selective, simpler, cheaper convenient and efficient extraction technique referred to as Solid-Phase Extraction (SPE) was introduced in the mid-1970s [71].

##### 1.1.2.2.4.1 Theory of SPE

Solid Phase Extraction (SPE) is a practical sample preparation technique that involves the use of solid sorbents for the removal of targeted analytes [72][71] from aqueous environments. SPE is usually employed in industrial wastewater purification, specifically for the removal of contaminants such as toxic heavy metal ions [71]. It is able to isolate targeted heavy metal ions

from a wide variety of matrices, including water, urine, blood, beverages, soil, and animal tissue [74].

SPE employs the affinity of solutes (targeted analytes) dissolved or suspended in a liquid (mobile phase) for the selective separation. The sample containing the analytes of interest is passed through the sorbent and the liquid sample passes through the sorbent (the stationary phase). It separates into desired and undesired portions, where the desired analytes of interest or undesired impurities in the sample are retained on the sorbent. The portion that passes through the stationary phase is either collected or discarded, depending on whether it contains the desired analytes or undesired impurities. If the portion that is retained on the sorbent includes the desired analytes, it can then be removed from the stationary phase for collection in an additional step, in which the sorbent is rinsed with an appropriate eluent of higher strength [73]. However, if it is the undesired that is adsorbed, then the sorbent can be desorbed of the undesired impurity and be re-used [50].

#### 1.1.2.2.4.2 SPE mechanism

The mechanism of SPE is a non-equilibrium, exhaustive removal of chemical constituents from a liquid sample via retention on a solid sorbent and eventual recovery of the selected (adsorbed) constituents by elution from the sorbent [72]. There are three major separation mechanisms that are usually employed in SPE: normal phase SPE; where the sorbent material is equilibrated with a non-polar or slightly polar solvent, which wets the surface and penetrates the bonded phase, reverse phase; where extraction of the targeted analytes are based on their polarity, and where the

extraction of analytes is based on the electrostatic interactions between the targeted analytes of interest and the positively or negatively charged groups on the sorbent material [51].

### 1.1.2.2.4.3 SPE Sorbents

Solid phase extraction (SPE) especially sorbent technology research is still an advancing area with robust, selective and efficient sorbent being researched on and developed to meet the ever growing demands of extraction. Many of the modern phases/sorbents are developed and employed for specific applications which may sometimes prove challenging on conventional SPE sorbents [77]. In selecting the sorbent, it is required to consider the sample volume, the nature of the analyte, analyte concentration and the inherent properties of the sorbent itself [58].

#### 1.1.2.2.4.3.1 Conventional Sorbents

Conventional SPE sorbents include: silicas, hydrophobic polymeric sorbents, hydrophilic polymeric sorbents, ion exchange sorbents and mixed mode polymeric sorbents.

Silica and bonded silica are rigid supports that do not shrink/swell with solvents. The silica surface can be easily modified, creating a potential for many different selectivities for SPE from hydrophobic to hydrophilic interactions or their combinations. However, the pH stability of the bonded silica poses challenges. It is limited, typically within the range of 2 to 7.5 making silica sorbents specific to only certain group(s) of analytes. Thus, their selectivity is not one-analyte specific.

For hydrophobic polymeric sorbents, alkyl and aromatic chains, with high affinity for non-polar and mid-polar compounds are employed as functional groups on hydrophobic sorbent materials. This is referred to as reverse phase SPE. Extraction is achieved via Van der Waals forces. Hydrophilic polymeric sorbents on the other hand, employ compounds with polar functional groups like the cyano (CN) or hydroxyl (OH) groups, with high affinity for polar compounds is used to extract polar compounds. This mode of SPE is referred to as normal phase.

Among the polymeric sorbents, mixed mode polymeric sorbents, have proved to exhibit the greatest selectivity within the conventional sorbent type. They are made from ion exchange and hydrophobic chains bonded onto the surface of silica providing unique selectivities. The sorbents are employed for the extraction of basic compounds, of drugs and their metabolites within biological fluids [77][78]. The lack of high selectivity of conventional sorbents which always results in co-extraction of competing compounds is a major challenge that has over the years lead to the development and use of more selective type of sorbents.

#### 1.1.2.2.4.3.2 Selective Sorbents

Highly selective solid-phase extraction sorbents are desirable tools for the development of extraction materials/phases with high specificity for the targeted species acting as a contaminant and needing to be removed or reduced to acceptable levels [79]. Commonly employed selective sorbents are: restricted access material ((RAM), immuno-affinity ((IA) and molecularly imprinted polymer ((MIP) sorbents.

#### 1.1.2.2.4.3.2.1 Restricted access material (RAM) Sorbents

Restricted access material (RAM) sorbents are commonly employed when extracting analytes of biological origin especially proteins with large molecular weight, from aqueous environments. The sorbents permit small molecules to access the active or interaction sites, and exclude large molecules by allowing them to interact with the outer surface of the sorbent material, minimizing the adsorption of the matrix[79].RAMs combine both size-exclusion and reversed-phase separation mechanisms. Therefore, it can be described based on: size-exclusion mechanism, which could be a chemical or physical barrier with the chemical groups bound onto the surface. The inner surface usually lacks the same type of bonding on the surface. Consequently, smaller molecules will pass through and selectively bond to the inner functional groups. However, competitive adsorption/displacements and low sorption capacities are common with RAMs [79] [80]. Furthermore, the synthesis and development of RAMs take a long time, making them very expensive.

#### 1.1.2.2.4.3.2.2 Immuno-affinity Extraction (IAE) sorbents

Immuno-affinity extraction (IAE) sorbents, also known as immunosorbents (ISs) are based on molecular recognition and employ biological tools/mediums such as antibodies or enzymes to achieve better selectivity and affinity based on antigen-antibody or enzyme-substrate mechanisms. Immuno-sorbents are not readily available as they depend on the availability of antibodies or enzymes, which are rare to find [79] [80].

ISs allow selective extraction of a specific group of compounds from matrices, especially liquid matrices in just one single step [80].

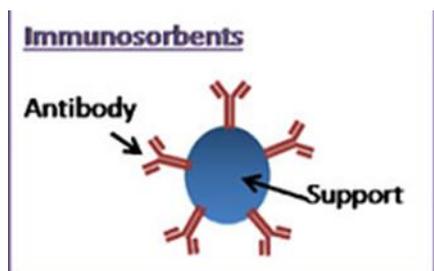


Figure 1.2: Immunosorbent

Several methods and examples have been used to illustrate IAE of antibodies [80], proteins enzymes [81], hormones peptides [82], viruses [83] and other subcellular constituents [83]. ISs are obtained through binding a specific antibody/enzyme to a solid support, which is then used as a selective sorbent.

For example, Ferguson *et al* employed a method, based on immuno-affinity extraction coupled with liquid chromatography/electrospray mass spectrometry (LC/ESI-MS) for the removal of  $\text{Fe}^{2+}$  from wastewater. It was employed specifically to overcome sample matrix-related interferences in order to achieve high-sensitivity analysis of iron bound compounds in wastewater effluents by HPLC-electrospray MS. The sample matrix included iron that bonded with analytes of interest and competitive ions which inhibited analysis of the analyte of interest. Immuno-affinity extraction removed much of the isobaric noise from the selected ion monitoring chromatograms, increasing the signal-to-noise ratios for analytes of interest, and contributing to the low detection limits of 0.07 ng/L. The method was applied to remove  $\text{Fe}^{2+}$  in two wastewater

effluents. Recoveries of the  $\text{Fe}^{2+}$  was excellent, >90%, while the competitive ions of nickel, lead, mercury and zinc were not retained (with recoveries <2%) from effluent extracts by the immunosorbent as compared to that of the use of magnetic ion exchange resins (MIEXs) that had a recovery of 86.48% of iron.

Despite their high selectivity abilities, ISs are ineffective in that antibodies/enzymes that can selectively extract small molecules are not readily available, thus the development of immunochemical methods that can extract low-molecular-mass analytes are greatly needed. Also, analyte –antibody/enzyme interaction can be influenced by the sample matrix and/or by additives within the sample which then lower extraction recoveries [80]. Furthermore, the parameters surrounding the synthesis of ISs are very intense and also, the restricted use of biological tools such as antibodies and enzymes remain a serious challenge [81].

### 1.1.2.2.4.3.2.3 Molecularly imprinted Polymer (MIP) Sorbents

These are manmade selective polymeric sorbents. Their selectivity matches that of their natural counterparts, immunosorbents [81]. MIP sorbents can be developed for nearly every analyte, making them some of the most sort after sorbents. MIPs are easily prepared through a template (analyte of interest) guided polymerization methods resulting with a highly cross linked polymer structure that is complimentary in shape, functionality and size to the template [82]. MIPs have a memory of the template that was employed during their synthesis [81]. Due to their high selectivity, they have found wide applications in biological analysis [83], residue detection [84], phyto-extraction [85], trace element detection [38], genotoxicity removal [86] and contaminant analysis and extraction [70].

In this thesis, a multi-templated ion imprinted polymer (IIP) targeting three model, toxic heavy metal ions will be synthesized to effectively remove the three heavy metal ions from wastewater at the same time, thus reducing the contamination and toxicity by heavy metal ions in treated wastewater.

The next chapters will cover the concept of molecular imprinting, the synthetic procedures of imprinting, and the different approaches of evaluating their physical and chemical characteristics as well as their selectivity. Their use as sorbent materials for the selective extraction or removal of toxic, heavy metal ions species in complex environmental matrices such as wastewater will be discussed.

## CHAPTER TWO

### 2.0 Molecular Imprinting technology

Molecular Imprinting technology is a science aimed at designing robust molecular recognition cavities in polymer matrices with memories of the template (print molecule/ion). The template used in molecular recognition could be a molecule; in which case the technology is referred to as a molecular imprinting or an ion; referred to as ion imprinting. This technology is based on a similar mechanism employed by enzymes or antibodies for substrate or antigens recognition respectively; often referred to as 'key and lock' model. [86] [96]. The substrate or antigen with a corresponding shape to the recognition site is recognized by selectively binding to the enzyme antibody, while a non-corresponding shaped molecule is not recognized. In a similar way, during molecular imprinting, functional monomers are self-assembled around the template via a template guided polymerisation interaction between the functional groups on both the monomer and the template. The self-assembly adduct is subsequently reacted with a second monomer referred to as the crosslinker, to form a highly cross linked, 3-dimensional imprinted polymer matrix. The template (print molecule/ion) is then removed from the matrix by employing a suitable extraction strategy or by a cleavage mechanism, leaving behind an imaginary cavity (reaction site) complementary in size, shape and/or charge to the template. [87].

## 2.1 The History of Molecular Imprinting

Molecular imprinting technology gained its significance back in the 1930s after a seminal work of Polyakov where he studied the polymerization of sodium silicate with ammonium carbonate. When he added additives such as benzene, toluene and xylene, the silica particles ultimately showed a higher uptake of the additive [87]. Later in 1949, Dickey reported polymerization of sodium silicate in the presence of methyl, ethyl, n-propyl and n-butyl orange dyes. The dyes were subsequently removed, and it was proven that silica prepared in the presence of any of the pattern molecules would bind the pattern molecules in preference to the other three dyes. This led to several research groups preparing specific adsorbents based on Dickey's method [88].

Another advancement in the technology was reported by Merck [89]. He patented a nicotine filter, consisting of nicotine imprinted silica and was capable of absorbing about 10.7% more nicotine than non-imprinted silica. His work sparked a wide interest in molecularly imprinting technology leading to over 5000 original publications in the field during 1931 – 2009 (from Scifinder). The new technique has caught the interest of the scientific world, especially with its effectiveness when targeting small molecules of molecular weight <1000 [86].

## 2.2 Types of Imprinting Strategies

Mechanistically, there are three major approaches that are usually employed during the synthesis of molecularly imprinted materials (MIMs) being; the covalent, non – covalent and semi – covalent imprinting strategies/method.

The covalent method was first introduced by Wulff and coworkers [91]. It is also referred to as ‘pre-organized’ or ‘stoichiometric’ imprinting methodology. This approach uses covalent reversible bonds providing a homogeneous population of active/binding sites, thus reducing the non-specific sites [86]. During the synthesis, the functional monomers form complexes with template monomers via reversible covalent bonds (such as boronate ester, ketal and acetal) prior to polymerization. The subsequent rebinding of the templates to the polymers also takes place via the formation of covalent bonds between them [91]. The semi – covalent approach, on the other hand, tends to combine the homogenous binding site created by covalent imprinting with the most widely used fast rebinding kinetics achieved via non-covalent approaches by using covalent linkages to create the binding site and on the other hand utilizing a non-covalent interactions to effect rebinding [90]. The Non – covalent method, also called the ‘self – assembly’ method was invented by Mosbach *et al.* in the 1980s [91]. It is the most widely employed method which implode non – covalent forces such as hydrogen bonds, ion or hydrophobic interaction, Van der Waals forces, metal-coordination and electrostatic interactions, to form a stable pre-polymerization complex of the functional monomers and the template molecules/ions in solution [86]. The polymerization initiators and the cross-linker and other complexing agents are added to form a polymer matrix around the complex. The template ions/molecules are subsequently removed after polymerization by extraction processes.

### 2.3 General Imprinting Process

The molecular imprinting process allows the synthesis of polymers with specific binding/active sites for target analytes, which is achieved by employing the target analytes as templates/print molecule/print ion during polymerization. Functional monomers with corresponding functional

groups to the template self-assemble around the template via a covalent, semi-covalent or non-covalent functional groups interactions and are eventually held in position by a cross linker (a second functional monomer). An initiator is subsequently introduced for the sole purpose of free radicalization thus initiating polymerization. Meanwhile, the formation of the polymeric framework is mostly initiated by heat [92] or ultraviolet irradiation of the reaction mixture. This is then followed by template removal by an appropriate solvent extraction method or cleavage reaction which leaves behind active/binding sites/cavities that are complementary to the template's morphology, charge and arrangement of its functional groups [93].

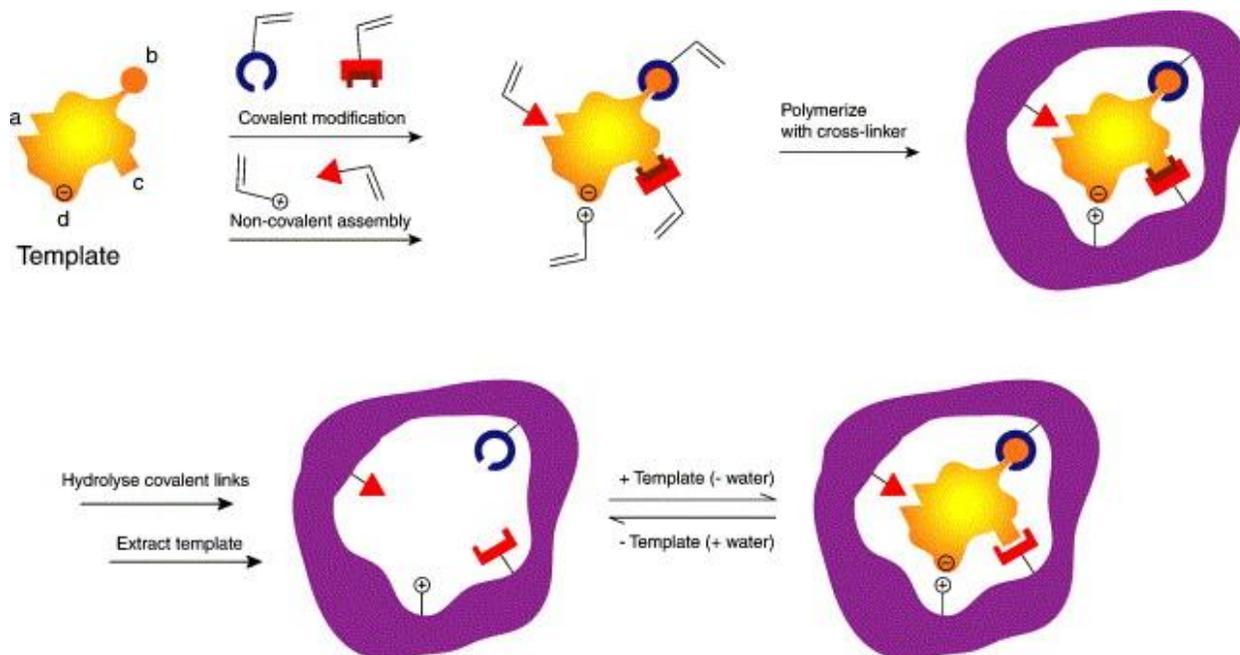


Figure 2.0: Molecularly imprinting procedure [99].

There are generally two types of template imprinting: molecular imprinting and ion imprinting. Molecular imprinting involves the use of a molecule as a template, while ion imprinting employs an ion as a template. Both processes employ the general imprinting procedures as previously

explained. This thesis will be bias towards ion imprinting as the templates involved in the imprinting process are metallic ions.

Ion imprinting was introduced in 1976 by Nishide *et al.* They cross-linked poly (4-vinylpyridine) with 1,4-dibromobutane in the presence of a metal ion. The formed polymer showed a high uptake of  $Mg^{2+}$  [99]. In most cases, ion imprinting process requires a ligand to form a complex with the metal ion via radical polymerization. This is slightly different for molecular imprinting where there is a general interaction with template molecules via classical functional monomers through Van der Waals interactions [100].

Denizli *et al.* detailed the general procedure for ion imprinting into three steps: i) complexation of the metal ion to a polymerization ligand, ii) polymerization of the metal ion-ligand complex and iii) removal of the template ion after polymerization [100]. Functional monomers with vinyl groups are often employed in imprinting. The vinyl groups are used to further grow the polymer chain through elongation of the polymer backbone b repeating units build from the vinyl groups. For ion imprinting, polymerizable ligands are often referred to as ‘bi-functional reagents’; based on their chelating ability and their vinyl function [101]. Alternatively, a simpler approach for ion imprinting is the use of non-polymerizable ligand, where the ligand is embedded within the polymer matrix by means of entrapment. Coordinative bonds formed from some electrons donating heteroatoms (e.g. sulphur, oxygen or nitrogen) to the unfilled orbitals of the outer sphere of the metal ions accounts for the interactions between the polymer framework and the complexed ion.

The affinity of the ligand for the imprinted ion and the morphology of the imprinted cavities provides the high selectivity and robustness of ion imprinted polymers[100]. Recognition sites are generated based on the self-assembling of ligands around the template ion and eventually

crosslinking, which allows the active/binding sites to match the morphology, charge and coordination number of the ion. In addition, the geometry of the complex is preserved through crosslinking and washing steps thus providing a suitable environment for the template ion to be rebound.

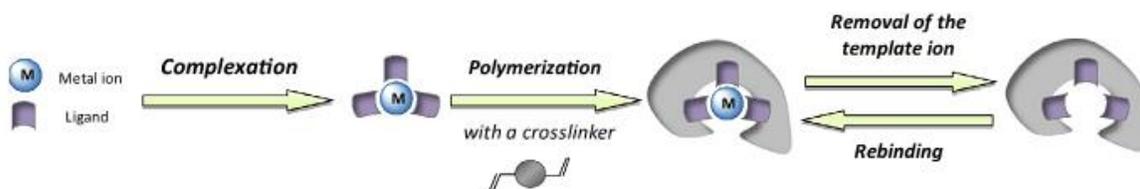


Figure 2.1: General Ion Imprinting procedure [100].

Crosslinking is one of the most essential step of ion imprinting that leads to the creation of binding sites. It is followed either in the presence of a ligand-functionalized monomer, or non-polymerized ligand or chain linear polymers. Rao et al. established a classification of the different mechanisms; crosslinking of linear chain polymers bearing metal ion-binding groups, chemical immobilization of vinylated ligands interacting with metal ions via crosslinking, trapping of non-functionalized ligands within the polymer matrix, surface imprinting conducted on aqueous-organic interface[102].

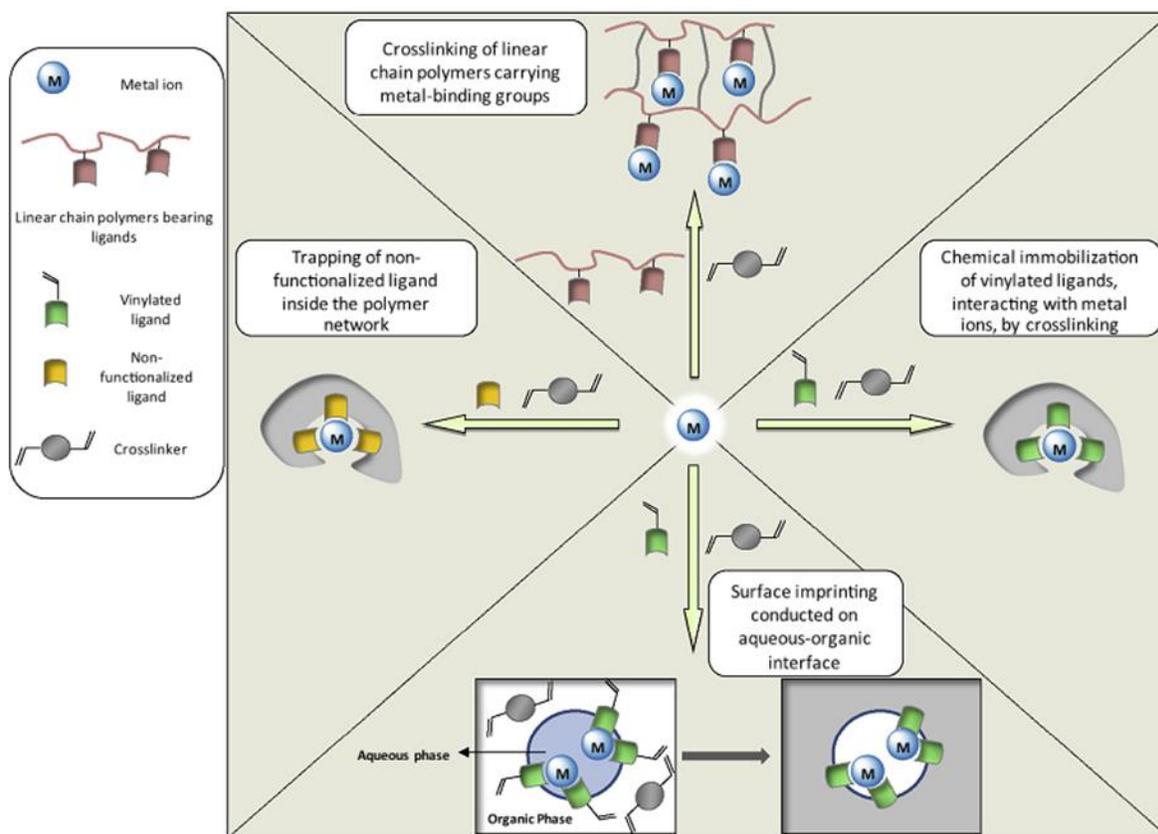


Figure 2.2 Different approaches for ion imprinting [100].

The formed polymer, which is referred to as ion imprinted polymer (IIP), is an artificial, tiny lock for a specific ion that serves as a miniature key, just like a plastic receptor. The imprinted polymer binds a specific ion [89]. In order to preserve the morphology of the cavity after the removal of the template ion, the polymer has to be rigid, and also it should possess high flexibility to enhance a fast equilibrium between the release and uptake of the template ion in the cavity [25]. Experimental variables such as: functional monomer(s), the nature and concentrations of template, cross-linking agent(s), porogen(s) and initiator and also the duration of the polymerization process poses a challenge in the synthesis and designing of the imprinted polymer [94]. Based on these challenges, optimization of the polymer is necessary and many

variables which can potentially impact the morphology, molecular/ionic recognition and chemical properties of the polymer are to be considered. However, a rational prediction of the effect of changing any one of the variables such as functional monomer to cross-linker ratio, is possible [25] [95] including MIP dialling [ref]. The IIPs can be in the form of lumps, gel or micro-beads [96] and can further be fabricated into probes, nano-particles [97], wipes, and mats [98]. Table 1 shows some advantages and disadvantages of IIPs.

Table 1: Advantages and disadvantages of IIPs

Advantages of IIPs	Disadvantages of IIPs
High Selectivity [14]	Collapsing of the cavity [94]
Low cost [96]	Distorting the binding points [100]
Wide range of applicability [3]	Incomplete removal of the template and rupture of the cavity (Template bleeding) [100]
High robustness and resistance to a range of temperatures, pH and solvents [32]	
Can be used in non-aqueous systems [12]	

## 2.4 General Reactants in the Synthesis of Ion Imprinting

The choice of chemical reagents for the synthesis of ion-imprinted polymers is the first and most essential part of the ion imprinting. The most relevant chemical reagents needed in the preparation of an ion-imprinted polymer are: template and ligand, functional monomer (including crosslinking agent), porogen and the initiator [103]

## 2.4.1 Template and Ligand

Based on the fact that the template used in ion imprinting is a metal ion, the interaction between the ion and the electron donating heteroatoms within the polymer matrix occurs through the unfilled orbital of the outermost shell of the metal ion [104]. The ligand plays a fundamental role because of the involvement or essentiality of ion chelation in the recognition process. Ligands possessing one or more chelating groups are generally used, but 4-vinyl pyridine, 1-vinylimidazole, acrylamide and acrylic acid are also often used in the chemical immobilization approach of ion imprinting. Unlike the chemical immobilization approach, ligands do not require a vinylated group (e.g. N,N'-ethylenebis (pyridoxylideneiminato), dithizone, thiosemicarbazide, acetaldehydethio-semicarbazone, 8-hydroxyquinoline ) [100]. Despite the formation of a ternary complex within the trapping approach or a functionalized ligand in a chemical immobilization approach, some other monomers are also introduced into the synthetic mixture.

## 2.4.2 Monomers

The ligand interacts with the monomer(s) with sufficient strength to form a stable ligand-metal complex. Monomers are chosen for several reasons based on the nature of the sample matrix. Some reasons include: hydrophilic character, good blood-compatibility, minimal non-specific protein interactions, high chemical and mechanical stability, and resistance towards microbial and enzymatic attacks.

The quantity of functional monomers that can be attached to the template is dictated by the template. Altering the template to functional monomer ratio is not necessary for covalent ion imprinting since the functional monomers are attached in a stoichiometric arrangement. Unlike

in non-covalent imprinting where optimal template monomers ratio is empirically achieved by evaluating different polymers synthesized with different template formulations; a concept governed by the Le Chatelier's principle (employing the principle to the complex prior to polymerization by increasing the concentration of components or binding affinity of the complex in the prepolymerization mixture will eventually predict an increase in the pre-polymer complex) [25]. Hence, for non-covalent imprinting protocols, the functional monomer is often used in excess to match the quantity of templates to favour the formation of template-functional monomer (ligands-monomer) assemblies [24].

### 2.4.3 Cross-linker

The kind and quantity of cross-linking agent used during the imprinting synthesis significantly influence the selectivity of the polymer. In general, the cross-linker performs three major functions: controls the morphology of the polymer matrix [58], provides stability for the imprinted binding sites [105], and provides mechanical stability to the polymer matrix [25]. The quantity of cross-linker used during imprinting should be high enough to maintain the stability of the recognition sites based on the fact that a high degree of cross-linker enables micro cavities to uphold a 3D (three-dimensional) structure complementary in both morphology and chemical functionality to that of the template after washing, hence the functional groups are held in an optimal configuration for rebinding [106]. There are few cross-linkers which are directly capable of simultaneously complexing the ligand and thus eliminates the need for a functional monomer.

## 2.4.4 Initiators

In ion imprinting, the initiator forms monomer radicals through thermal/photo decomposition in order to commence the polymer formation[1][107]. The monomer radicals are homolytically cleaved either by thermal decomposition, ionized radiation or photolysis. Azobisisobutyronitrile (AIBN) is a widely used initiator because of its compatible temperature of decomposition with most polymerization solvents [24]. Other used initiators are benzoyl peroxide (BPO) (Walas and Coll.), H<sub>2</sub>O<sub>2</sub>/ tetramethylethylene diamine(TEMED) (N, N, N', N'-tetramethylene diamine), potassium persulfate [24] [105].

Dam et al. worked on the effect of AIBN concentration during Ion imprinting synthesis, and observed the reduction in particle size with increasing AIBN concentration resulting from an increase of the amount of free radicals producing more nuclei in the polymerization process [108].

## 2.4.5 Porogen

Porogens are solvents that are employed as dissolving medium in polymerization reactions and can be classified as non-polar solvents, polar aprotic solvents and alcohols (polar protic solvents). The most employed porogens are acetonitrile and dimethyl sulfoxide (DMSO) as polar aprotic solvents and 2-methoxyethanol and methanol as alcohols and also toluene, chloroform, dichloroethane and deionized water [110][111].

In order to generate the porous structure of the polymer which provides a high number of binding sites accessible to the target ion, organic solvents are often incorporated during the ion imprinting process. The contradiction between chains (monomers and polymers) and the

solubility parameter accounts for the porogenic property of the organic solvent (porogen). In ion imprinting process a suitable porogen must be capable of completely dissolving all of the reagents; including the template ion, increasing the polar constituent. [100]. The selectivity of the IIPs, depends on the quality of the imprinting cavities which are hugely influenced by the polarity nature of the solvents (porogen). According to Godlewska-Zylkiewicz *et al.* the analytical features of a given IIPs are not significantly influenced by the porogen used during the polymerization of that IIPs rather it is essential for the separation of the target ion from an aqueous medium [109].

## 2.5 Types of Polymerization

With the exception of the use of poly-condensation for a very few types of IIPs, most IIPs especially organic IIPs, are mainly prepared via radical polymerization. The method varies based on their synthetic process, and includes bulk polymerization, suspension and emulsion polymerizations, dispersion or precipitation polymerization [112].

### 2.5.1 Bulk Polymerization

Bulk Polymerization which is also referred to as ‘solution polymerization’ is the easiest and convenient method of producing IIPs. It requires no specific skills, or modern sophisticated equipment. The direct mixing or combinations of required reagents and template in a solvent (porogen) is the central or key concept of bulk polymerization. With the exceptions of moulded polymers, the product of a bulk polymerization synthesis which is usually a monolith, is crushed,

grounded and sieved to desirable dimensions. The loading capacities of IIPs produced through bulk polymerization are sometimes low as a result of grinding which leads to destructions of binding sites [100] and also due to the tendency of grinding to be exothermic in nature. The challenge of sample overheating further restricts scaling-up of bulk polymerization.

## 2.5.2 Emulsion and Suspension Polymerization

Emulsion and suspension polymerization are considered to be heterogeneous polymerization methods. They require two non-miscible phases, a continuous and a dispersed phase. [113] [112]. The template ion, the porogen, the initiator and the functional monomer (along with the cross linkers in some instances) are the dispersed phase, and this is basically an organic phase in contact with an aqueous continuous phase, most often stabilized by polyvinylalcohol or hydroxyethyl cellulose (sometimes containing NaCl salt) or gelatin. The resulting polymer beads from suspension polymerization are formed via droplets of the dispersed phase acting as small sized reactors which make it easier for the morphology of the particles to be formed and also allows adjustments for porosity [100].

Emulsion polymerization starts with an emulsion using water, the monomer and a surfactant which is mostly employed for surface imprinting technique. Droplets of monomers are emulsified using surfactants in a continuous phase with an initiator for a heterogeneous emulsion polymerization. The most used emulsion polymerization is the oil-in-water emulsion in which the oil (droplets of monomer) is emulsified (with a surfactant) in a continuous phase of water/oil (W/O). Other emulsifiers/stabilizers sometimes used are water-soluble polymers such as polyvinyl alcohols or hydroxyethyl celluloses [114].



### 2.5.3 Precipitation or Dispersion Polymerization

Precipitation polymerization has some similarities to bulk polymerization with the difference being the amount of solvents/porogen involved (excess porogen for precipitation polymerization). It is arguably the second most widely used polymerization technique. Shamsiper *et al.* summarized the advantages of precipitation polymerization as; products are ready for application, easy removal of the template from the IIP, polymerized products are in particle form, unlike those of bulk polymerization procedure, and precipitation polymerization produces particles in nano, micro size or even submicron. Hence, the morphology of the prepared IIPs is a function of the monomer(s) / solvent ratio and also the mixing and stirring of the polymer matrix [117] [100].

The medium in the initiating steps of precipitation and dispersion polymerization is of a single phase [115][116]. After the dissolution of all necessary reagents (template ions, monomers and initiators) in the solvent (porogen), the polymerization process is then initiated in a homogeneous solution [100]. Due to the risk of coagulation and insufficient stability, the need for an alternative to a stabilizer is essential thus the need for proper mixing and stirring of the polymerisation reaction mixture.

In summary, precipitation polymerization and dispersion polymerization are both heterogeneous polymerization techniques. Precipitation polymerization starts off with a homogeneous mixture in a continuous phase, with the monomer and initiator completely soluble, after which the formed polymer becomes insoluble and hence precipitates out, followed by absorption of monomer and initiator into the polymer particles [114], while, dispersion polymerization is carried out in the presence of a polymeric stabilizer in the reaction medium . [118].

## 2.6 Characterization of Ion Imprinting Polymers

The characterization of an ion-imprinted polymer is mostly concerned with whether the ligands are successfully and correctly incorporated within the polymer matrix/materials as well as the successful removal of the template. Characterization of IIPs is normally achieved via elemental analysis where theoretical and experimental variables are compared. The size of the particles and their morphology is also covered.

### 2.6.1 Physicochemical Characterization of IIPs

The physical and chemical properties of the sorbent play an essential role in the efficiency and effectiveness of the sorbent. For example, the surface sponginess and its meso-porosity exemplify the absorptive nature of the sorbent. The bonding and compositional structures and arrangements on the other hand, explain the binding effect and the effectiveness of the sorbent. Atomic force microscopy, X-ray diffractogram, scanning electron microscopy and fourier transform infrared spectroscopy are among the most commonly employed instrumental analysis for physicochemical characterization of IIP sorbents.

#### 2.6.1.1 Fourier Transform Infrared Spectroscopy (FTIR)

FT-IR is employed to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas [114]. It is mostly used during the analysis of physicochemical properties of IIPs to establish the availability of the ligand in the IIP or non-imprinted polymer (NIP).

Complete removal of the template from the IIP is very essential especially because it determines the efficiency of the IIPs. Incomplete removal of template or incorrect template removal process results in bleeding of residual template thus posing a risk to the robustness of the IIPs [119] hence different FT-IR spectra are obtained to differentiate amongst; IIP with the template, template removed IIP and the non-imprinted IIP. employed to control leaching steps.

### 2.6.1.2 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis or thermal gravimetric analysis (TGA) is an instrumental method of thermal analysis where changes in the physical and chemical properties of the IIPs are measured as a function of increasing temperature (with constant heating rate), or as a function of time (with constant temperature and/or constant mass) [114]. This also gives idea about the stability of the IIPs as the temperature at which they decompose will be obtained.

TGA is often used to compare the behaviour of the leached IIPs with the unleashed IIPs and also the NIPs. In investigating the presence or absence of metallic ions within the polymer material, X-ray Diffraction (XRD) and Energy Dispersion X-ray (EDX) spectroscopy analysis proves very efficient.

### 2.6.1.3 Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) is an electron microscope that provides morphological images of the IIPs by scanning it with a focused beam of electrons [114]. Metallic-ion imprinted

polymers properties are mostly relay on its morphology, and thus the need for morphological analysis. SEM also provides information on the size and porosity of the IIP particles. A Transmission Electron Microscopy (TEM) & Atomic Force Microscopy (AFM) are other forms of microscopic techniques that are sometimes employed for such analysis. TEM is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through it. It operates on the same basic principles as the light microscope but uses electrons instead of light. TEMs use electrons as "light source" and their much lower wavelength makes it possible to get a resolution a thousand times better than with a light microscope [114]. AFM or scanning-force microscopy (SFM) is a type of scanning probe microscopy (SPM), that demonstrates resolution on the order of fractions of a nanometer, more than 1000 times better than the optical diffraction limit. The information is gathered by "feeling" or "touching" the surface with a mechanical probe.

## 2.6.2 Binding Studies

Achieving selectivity is a major challenge in ion-imprinting, therefore proving that the imprinting process was successful and useful is very essential. To do that, one must firstly ensure that the template ion is adequately removed. This step is of great significance because it results in exposure of binding sites within the polymer (IIP) matrix, for the purpose of rebinding experiments such as, optimization, selectivity and IIP application to real samples [3] [100] [104]. Recognition sites are the guiding forces in terms of IIP affinity and selectivity towards a variety of targeted ions, hence rebinding studies via batch experiments or frontal analysis have been

performed in order to characterize recognition sites, thus, their presence within the polymer matrix [23].

It has been reported that batch rebinding studies are a characterization method providing the first insight into the binding properties of a specific IIP sorbent. For example, in batch experiments, IIPs of known quantities are added to reaction vials containing targeted ions solutions of known concentration (initial), of which the whole mixture would be stirred and left to equilibrate for some time to allow for absorption by the employed IIPs [33]. The supernatant would then be collected by filtration and its concentration (final) determined using appropriate analytical instrument such as Force Atomic Adsorption Spectrometer (FAAS) for metal ion analysis as in this present work. From the results, the percentage removed would be calculated employing equation (1). The same experiments are usually performed using the non-imprinted polymer (NIP) for comparison and reference purposes, which is known to possess nonspecific recognition sites compared to the IIP. NIP synthesis follow the same procedure and incorporation of polymerization reagents except for the imprint ion. Both are then characterized in the metal solutions which involves either a mixture of interfering / competitive ions and target ion or the template ion alone [121], which can be achieved either in batch experiments or flow mode. The expectation is that the IIP would have a higher removal percentage, hence a large imprinting factor (IF) (see equation 2) than the reference material, thus, indicating selectivity and a greater affinity towards the targeted ion.

When investigating the metallic ion residual concentration in solution after analysing with the sorbent results in determining the percent recovery (%R).

$$\% \text{ Removal} = \frac{\text{Initial Concentration} - \text{Final Concentration}}{\text{Initial Concentration}} \times 100\% \quad (1)$$

$$IF = \frac{\% \text{ Removal of IIP}}{\% \text{ Removal of NIP}} \quad (2)$$

In presence of closely related competing species, characterizing binding sites on the basis of IIP and NIP selectivity has been performed employing the same batch rebinding experiments. The selectivity imprinting factor ( $IF_s$ ) would be calculated to investigate by what factor would the targeted ions be selectively recognized by the polymers than the competing species. Furthermore, the imprinting factor would depend on the distribution coefficients ( $K_d$ ) calculated for each bound ions and these are usually calculated employing the following equations,

$$Kd = \frac{C_i - C_f}{C_i} \times \frac{V}{m} \quad (3)$$

$$IFs = \frac{Kd \text{ Imprint ion}}{Kd \text{ Competing ion}} \quad (4)$$

Where  $K_d$  is the distribution coefficient,  $C_i$  is the initial concentration,  $C_f$  is the final concentration left unremoved in the supernatant,  $V$  is the volume employed and  $m$  as the mass quantity of the IIP or NIP employed in rebinding experiment [44].

The influence of pH on the sorbents' properties is one of the most common binding studies. The ligands often convey pH sensitive functions including phenolic, carboxylic acid groups or amino. There is always competition between  $H^+$  ion and the cationic template, leading to an increase of the extraction rate with increasing pH due to low pH, thus the optimization of pH [22].

The binding capacity of the template ion can be followed as a function of the ion initial concentration,  $C_o$ . The binding isotherms which measures the binding efficiency of the IIPs over

a specific measure of target ion concentrations, is often plotted as the binding capacity versus the concentration of free analyte remaining in the solution,  $C_e$  [54].

In order to investigate the time-dependence and controlling mechanism like mass transfer and chemical reaction, real time measurement analysis is employed. Thermodynamic parameters can be evaluated by leading binding experiments at various ranges of temperatures [122].

## 2.7 Analytical Application Studies

IIPs are often prepared for ion extraction and separation of metallic ions or for use as SPE sorbents. IIPs are generally used for treatment and analysis of water, blood, urine, solid samples such as food, soil, rocks, human hair. IIPs can also be mixed with carbon paste or incorporated in a PVC membrane for the preparation of ion-selective electrode (ISE). Ion imprinting methodologies have also been used/applied to electro-polymerized microporous poly(2-mercaptobenzothiazole) for fossil fuels purification [100]. The Ion imprinting technology is still being exploited and many areas are being studied and developed.

For this work, synthesized multi-templated IIPs will be employed for the simultaneous and selective removal of heavy metal ions from wastewater. The IIPs will be applied to a wastewater plant for effective removal, reducing the level of targeted metallic ions to acceptable levels.

## CHAPTER THREE

### 3.0 Synthesis of a Novel Multi-templated Ion Imprinted Polymer for the Selective and Simultaneous Removal of Metallic Ions from Wastewater

The main aim of this thesis was to synthesis a multi-templated ion imprinted polymer (IIP) powder to be applied as a solid phase extraction (SPE) sorbent material for the selective and simultaneous removal of the toxic heavy metallic ions;  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  from wastewater. The IIP powder was prepared by a multi-template guided free radical bulk polymerization method that was thermally initiated. The composition & extent of imprinting, structure and morphology & size of the IIP powder particles were characterized by employing FT-IR, XRD, and AFM, respectively. Optimized rebinding batch experiments were employed to demonstrate the efficiency of the prepared IIP powder in selectively and simultaneously removing the three toxic ions;  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  from waste water.

### 3.1 Materials and Reagents

Ultra-pure water, of 18.0 M $\Omega$ /cm resistivity, Type I, was prepared by a Millipore-Q purification system from Merck, (Darmstadt, Germany) and was used to prepare all solutions. Reagents used were: analytical grade HCl (32%) purchased from ACE (Johannesburg, South Africa), analytical grade Acetonitrile (99.9%), Nickel (II) Nitrate Hexahydrate (97.0%), Copper (II) Nitrate Trihydrate ( $\geq 98.0$  %) purchased from Sigma-Aldrich (Johannesburg, South Africa), analytical grade Ethanol (99.9%) purchased from Skylabs (Johannesburg, South Africa), analytical grade

Methacrylic acid, MAA (99%),  $\alpha,\alpha'$ -Azobisisobutyronitrile, AIBN (98%), analytical grade  $\text{HNO}_3$  (50%), 1,10-Phenanthroline (99%), analytical grade Ethylene glycol dimethacrylate (98%), ethylenediaminetetraacetic acid, EDTA (98%), Iron (III) Nitrate monohydrate (95%) purchased from Sigma-Aldrich (Johannesburg, South Africa), Apparatus/glassware used during the entire experiment were purchased from Pyrex companies (Frankfurt, Germany). Elemental standard solutions used (1000ppm – Fe, Ni, Cu, Pb, Zn & Hg) and NaOH (97%) pellets were purchased from Rochelle Chemicals (Johannesburg, South Africa). Mortar and pestle were purchased from Pyrex (Frankfurt, Germany). A 0.45  $\mu\text{m}$  pore sized Whatman filter papers (Hardened Ashless Circles 45 mm) that was used for all filtering processes were purchased from Sigma-Adrich (Johannesburg, South Africa), the pH meter was purchased from Crison (Liverpool, England), Thermo Scientific laboratory oven was purchased from Thermo Fisher Scientific Inc (New York, USA), Benchmark hot plate was purchased from Benchmark Scientific (New Jersey, USA), micropipette from BOECO (Berlin, Germany), a Tyler analytical balance from Mettler Toledo, A W.S. Tyler™ (Johannesburg, South Africa), and a Laval stainless steel sieve (5 – 20  $\mu\text{m}$ ) was purchased from Laval Lab (Minnesota, USA) was used for sieving the IIPs and NIPs.

## 3.2 Instruments

A Varian 220FS Atomic Absorption spectrometer (AAS) acquired from Varian (California, USA), operated with air/acetylene was used for the determination of metal ions (Pb, Ni, Hg, Fe, Zn, Cu) in all of the solutions including the unbound metal ions to the prepared IIP. A D8 Advance power X-ray Diffractometer purchased from Bruker (Karlsruhe, Germany) was used for phase identification of the polycrystallinity of the washed and unwashed IIP and NIP. A Nicolet

iS10 FTIR spectrometer purchased from Thermo Fisher Scientific (Johannesburg, South Africa) was used to identify the functional groups possessed on the surface of the washed and unwashed IIPs as well as the NIP. A Dimension edge with ScanAsyst® Atomic Force Microscope (AFM) purchased from Bruker (Karlsruhe, Germany) was used to determine the size and morphology of the washed and unwashed IIPs.

### 3.3 Synthesis of Multi-Templated Cu-Fe-Ni-IIP

The multi-templated Cu-Fe-Ni IIP powder was synthesized in two steps: i) preparation of the binary complex of Iron, Nickel and Copper with MAA as the ligand and 1, 10-Phenanthroline as the complexing agent; ii) copolymerizing the complexes with EDGMA as the crosslinking monomer, and a mixture of ethanol/acetonitrile employed as the porogen. The complex of iron (II), nickel (II) and copper (II) with the ligand was prepared with a mixture of 1 mmol of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ , 1 mmol of  $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , 1 mmol of  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ , 1.082 g of 1,10-Phenanthroline and 1.0179 mL of MAA dissolved in 35 mL of ethanol/acetonitrile (2:1 v/v) and stirred at 900 rpm for 30 min. 11.313 mL EGDMA and 0.24 g AIBN were added and mixed with the binary complex solution. The mixture was then put in a 100 mL round bottom flask, sealed after being sonicated for 15 min to degas it. The polymerization was then initiated, and proceeded at 60 °C for 24 h under continuous stirring at 600 rpm until completion. The obtained polymer monolith was ground in a mortar and dried in a hot air oven at 60 °C for 3 h. The dry, polymer powder was sieved to get particles smaller than 10 $\mu\text{m}$ . Unreacted materials were removed by washing the dry polymer particles with 40 mL of ultra-pure water for 3 h in three cycles. At the end of every cycle, the polymer particles were obtained and dried in the hot air

oven for 6 h. The Fe (II), Cu (II), and Ni (II) ions were removed from polymer particles by leaching with a solution mixture of a 1:1 volumetric ratio of 1.0 M HCl and ethylenediaminetetraacetic acid (EDTA). Non-imprinted polymer particles were synthesized in the same way except that the inclusion of Fe (II), Ni (II) and Cu (II) as print ions was skipped.

### 3.4 Optimal Template Removal

The iron (III), nickel (II) and copper (II) ions and other unreacted materials were exhaustively removed from the unwashed polymer powder in three steps: i) The unreacted materials were totally removed by refluxing the polymer powder with 40 mL of ultra-pure water for a total of 9 h of 3 h cycles. At the end of every 3 h cycle the solid IIP powder was recovered by centrifugation and dried in a hot air oven for 6 h; ii) The dry polymer powder was refluxed at 60 °C with 1.0 M of HCl (32%) for a total of 20 h of 5 h cycles. At the end of every 4 h cycle the solid IIP powder was recovered by centrifugation and dried in a hot air oven for 6 h, iii) Finally, the dry IIP powder was refluxed at 60 °C with 10.0 M HCl (32%) for a total of 6 h of 2 h cycles (applying same routines as in step ii). At every step and cycle, the metal ions intended to be removed, were determined by AAS in the supernatant washing solutions until at a certain point where the obtained concentrations for the removed ions remained the same in the washing solutions thus marking the completeness of the templates (print ions) removal. The three general steps resulted in a total of 35 h for optimal template removal. Furthermore, XRD was applied on the washed IIP powder to confirm the absence of the template ions.

## 3.5 Characterization of the IIPs

To evaluate and reveal details on the physical and chemical properties of the powder, FT-IR, X-ray diffractogram (XRD) and AFM were employed for the characterization.

### 3.5.1 FT-IR Analysis

Fourier transform infrared (FTIR) spectroscopy was used to identify the functional groups (responsible for the metal uptake and bonding) possessed on the surface of the washed and unwashed IIP as well as the NIP. The FTIR spectra were recorded in the wavenumber range 500-4000  $\text{cm}^{-1}$  on a Nicolet iS10 Thermo Scientific FTIR. The data were collected at 2.0  $\text{cm}^{-1}$  resolution, and each spectrum was a result of 256 scans.

### 3.5.2 X-Ray Diffraction Analysis

A powder D8 Advanced Powder X-Ray Diffractometer (XRD) analysis was employed to investigate the physical properties as it relates to the crystallinity of the washed and unwashed IIP particles and also to investigate the optimal template removal procedures applied during template removal. The XRD was operated with Cu  $K\alpha$  emission ( $\lambda = 1.54105\text{\AA}$ , 40 kV, 40 mA per sec) and with high efficiency linear detector of Lynx Eye type. The scanning mode used was coupled with  $2\theta/\theta$  on the scanning range  $10^\circ - 120^\circ$  values. The crystallite size of the sample was calculated by Deby-Scherrer method.

### 3.5.3 Atomic Force Microscopic Analysis

In order to determine the size and the morphology of the washed, unwashed IIP and the NIP, scans of each were taken in tapping mode using a Dimension edge with ScanAsyst® Atomic Force Microscope (AFM) with a piezo scanner that was set to scan the films at 1–1.5 lines/s. Commercially available tapping mode tips (TAP300- 10, Silicon AFM probes) with a resonant frequency in the range of 260–410 kHz were used as cantilevers. All AFM micrographs were filtered and analyzed using the Gwyddion Software (Ver. 2.19).

## 3.6 Sampling and Storage

To evaluate the intended applicability of the prepared multi templated Cu-Fe-Ni IIP powder to real samples, water samples were obtained from Gaborone waste water treatment plant (waste water) and Gaborone, Block 6 (Borehole water), Gaborone, Botswana. At the treatment plant five sampling sites were identified (Inlet point, primary settling, secondary settling point, aeration point and effluent point). Random sampling technique was employed to obtain the sample from the sampling sites. Grab sampling technique was employed to obtain the water samples from individual sampling sites. Each sample was put in labelled brown glass bottle and acidified to  $\text{pH} < 2$  in order to prevent further microbial activity which could have eventually affected the composition of the metal ions in the sample. Also acidifying the samples ensured that undissolved metals that remained after sampling were dissolved. The water samples were placed in the cold room at 4 °C to help immobilize any microbial activity or degradation of the sample. Samples were stored until analysis.

### 3.7 Optimization Rebinding Studies

Optimization of operational parameters such as time, pH, sorbent dosage, temperature and volume are often carried out to evaluate the effectiveness of sorbents. Optimization experiments reveal the most suitable working conditions for the sorbents; which enable the user to effectively and correctly employ the sorbents for their intended purpose. Time, pH and sorbent dosage were optimized in this study. The parameters affect the sorption capacity of heavy metals into the sorbent.

Table 2: Optimal Parameters

Parameters	Effect
<b>pH</b>	Increase in pH enhances chemical precipitation where there are formations of hydroxides and complexes and low pH results in H <sup>+</sup> competing with metallic cations for active/binding sites on the sorbent [130].
<b>Time</b>	It enhances sorptive removal rate of adsorption of metallic ions by minimizing its mass transfer resistance, but may also allow desorption [131].
<b>Sorbent Dose</b>	Increase of sorbent dose increases percentage removal, due to increase in the number of binding sites [132].

The removal efficiency of the IIP was determined by computing the percentage removal using the formular in equation (1) below

$$\% \text{ of Heavy metal ion removed} = \frac{C_i - C_f}{C_f} \times 100 \quad (4)$$

Where,

$C_i$  – is the initial concentration of the ion in a solution before a sorbent is added.

$C_f$  – is the concentration of the ion in the solution after removal of the metal ion by the added sorbent.

### 3.7.1 Optimization of Time

Quantities of 30 mg (literature value [22]) each, were weighed into 7 different conical flasks, to which 25 mL of 5 mg/L equimolar mixed standard solution of Fe(III), Ni(II) and Cu(II) was added. The mixture was shaken for 2 h and filtered using a 0.45  $\mu\text{m}$  pore size Whatman 45 mm filter paper and the filtrate was analyzed with AAS.

### 3.7.2 Optimization of pH

To optimize for pH, 25 mL of the 5 mg/L equimolar mixed standard solution was put into 7 different 100 mL conical flasks and the pH was adjusted to pH 2, 4, 6.5, 7.5, 8, 9, 10 respectively using 0.1 M HCl and 0.1 M NaOH. 30 mg of the IIP powder was added into each flask. The mixture was shaken for 15 min, then filtered with 0.45  $\mu\text{m}$  pore size Whatman 45 mm filter paper and put in a 50 mL volumetric flask, then later analyzed with AAS.

### 3.7.3 Optimization of Sorbent Dose

5 mg, 10 mg, 20 mg, 30 mg, 40 mg and 50 mg of the IIPs powder was weighed and put into different conical flasks. Then 25 mL of the 5 mg/L equimolar mixed standard solution at the optimized pH was added into each flask and the mixture was shaken for the optimized time, and

filtered off with a 0.45  $\mu\text{m}$  pore size Whatman 45 mm filter paper and the concentration of unbound ions analyzed with AAS.

### 3.7.4 Selectivity Studies

Selectivity of both the IIPs and NIPs were investigated by competitive adsorption of Fe(III), Fe(II), Ni(II), Cu(II), Pb(II), Zn(II) and Hg(II) from their mixture. 20 mg of the IIP sorbent and non-imprinted sorbent was added to a 25 mL of the 5 mg/L standard mixture solution containing all of the ions (each in a separate 100 mL conical flask) at optimal conditions and shaken for optimal time, filtered with a 0.45  $\mu\text{m}$  pore size Whatman 45 mm filter paper and analyzed with AAS.

## 3.8 Determination of Metallic Ions in Raw Waste and Borehole Water

50 mL of waste water from inlet, primary, secondary, aeration and effluent points were pulled in five 250 mL conical flasks. 1 mL of 50 %  $\text{HNO}_3$  and 0.5 mL of 37 %  $\text{HCl}$  was added to each flask. The mixture was heated until the initial volume was reduced to approximately 0.5 mL. The mixture was then filtered into a 50 mL volumetric flask and deionized water added to the mark. The sample was then analyzed with AAS to determine the concentration of three metals (Ni, Fe, & Cu). The same metals were determined in borehole water employing a similar procedure.

### 3.9 Application of the Prepared IIP to Real Samples: Waste and Borehole Water

The multi-templated Cu-Fe-Ni-IIP powder was applied to both the waste and borehole water samples employing optimal conditions for the selective removal of the targeted metallic ions from the water samples after rebinding batch experiment. The obtained data was analyzed and is discussed in the next chapter.

### 3.10 Method of Validation

Method validation is an evaluating process employed to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Its results can be used to judge the quality, reliability and consistency of analytical results. The objective of validating an analytical procedure is to demonstrate “suitability for its intended purpose”.

#### 3.11.1 Linearity

The linearity of the analytical method was evaluated by analyzing sample solutions spiked at different concentrations ranging from 0.1 mg/L to 10.0 mg/L using AAS and the linearity of the calibration curves were obtained and are discussed in the next chapter.

### 3.11.2 Detection Limits

Detection limit is the lowest quantity of an analyte that can be distinguished from the absence of a blank value within a stated confidence limit (often 1%). Several detection limits are considered in analytical chemistry.

The instrument detection limit (IDL) was calculated as the concentrations of the analyte giving signal blank equal to the blank signal plus three standard deviations of the blank. IDL is obtained using the equation:

$$IDL = Y_B + 3S_B \quad (5)$$

Where:

$Y_B$  is the analyte concentration giving signal equal to the blank signal.

$S_B$  is the standard deviation of the blank.

The limit of quantification (LOQ), which is the lowest concentration at which the analyte can reliably be detected for precise quantification, was calculated from the equation:

$$LOQ = Y_B + 10S_B$$

Where:

$Y_B$  is the analyte concentration giving signal equal to the blank signal.

$S_B$  is the standard deviation of the blank.

Ultra-pure water was used as the blank solution to calculate the detection limits. Three replicates were performed for all three selected metals (Ni, Fe, & Cu).

Method detection limit (MDL), which is the lowest concentration level that can be determined to be statistically different from the blank at 99% confidence level was calculated with the EPA 40 method (Environmental protection Agency (EPA) method). The equation used was:

$$MDL = T_{(n-1, 1-\alpha=0.99)} \times SD \quad (6)$$

Where:

$T_{(n-1, 1-\alpha=0.99)}$  is the student's t value appropriate for 99% confidence level.

$n$  is the number of replicates measured.

$SD$  is the standard deviation.

The standard deviation was calculated from measuring ten blank solutions. The blank was prepared from water samples that were passed through the same steps as the real samples and the optimized parameters.

### 3.11.3 Recovery Studies in Real Samples

Percent recovery of the metallic ions (Fe, Ni & Cu) were calculated for both waste and borehole water samples. The samples were spiked with 2 mg/L of the concentration of the analyte ions. The analysis was performed in triplicates employing FAAS. The percent recoveries were calculated using the equation:

$$\% \text{Recovery} = \frac{\text{Concentration of ions in spiked sample} - \text{concentration of ions in unspiked sample}}{\text{concentration of ions in added to the spiked sample}} \times 100\%$$

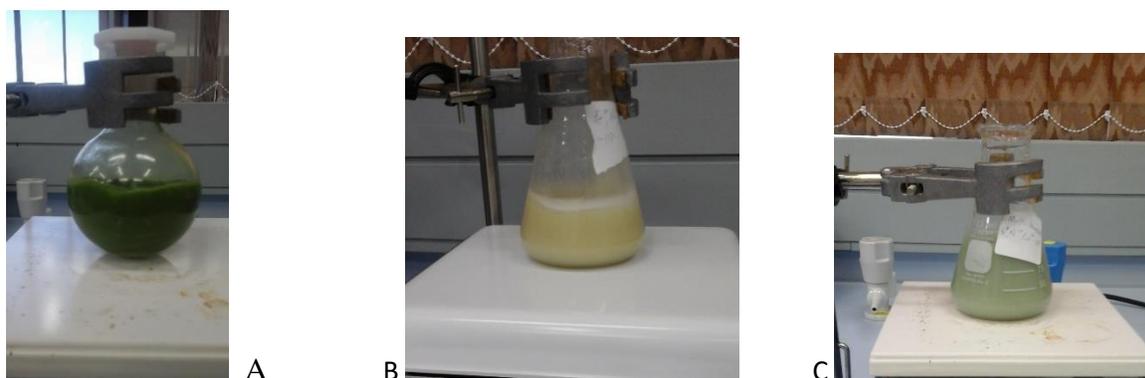
(7)

## CHAPTER FOUR

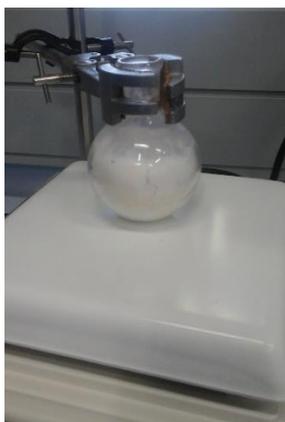
### Results and Discussion

#### 4.0 Synthesis of Fe-Ni-Cu Multi-templated IIP and the NIP

After polymerization as described in section 3.3, a forest greenish monolith (IIP) and a white monolith (NIP) were formed depicted in Figure 4.0A and 4.1 respectively. Continuous washing of the greenish IIP powder resulted in the gradual colour change with each succeeding washing cycle as seen in Figure 4.0B and 4.1C. The loss of colour of the mixture to almost colourless from Fig. 4.0A through to 4.0C was an indication that indeed the template ions (print ions) were indeed washed off (removed) from the prepared IIP powder. The print ions, Fe(III), Ni(II) and Cu(II) are characterized by colour hence their removal by a successful template removal procedure must result in a product with less colour (colourless/white), thus the obtained product after filtering the washing solvents was a whitish IIP powder as seen in Figure 4.0D.



*Figure 4.0: Products. A is the unwashed IIP, B & C are the IIPs during washing (C is the IIPs during the last wash) and D is the final product*



D

Figure 4.1: NIP Monolith

## 4.1 FT-IR Analysis

Analysis using FT-IR enables better understanding of the surface features of the powder and the evaluation of the optimal removal of template and unreacted materials. Figure 4.2, shows the FTIR of the Unwashed Multi-templated Cu-Fe-Ni IIP powder. The band around  $3400\text{ cm}^{-1}$  is very broad, indicating the presence OH groups from the likelihood of incomplete drying hence the presence of OH groups from water molecules within the structure. The two peaks at  $2973\text{ cm}^{-1}$  and  $2890\text{ cm}^{-1}$  are C-H stretch frequencies that belong to  $\text{CH}_3$  or  $\text{CH}_2$  groups from the backbone structure of the monomers. The large peak at  $1720\text{ cm}^{-1}$  is the stretch frequency of a carbonyl group. There is a similar strong peak at  $1153\text{ cm}^{-1}$ , which, together with the peaks at  $3396\text{ cm}^{-1}$  and  $1720\text{ cm}^{-1}$  evidence the presence of an ester. The minor peaks below  $900\text{ cm}^{-1}$  are the finger print region.

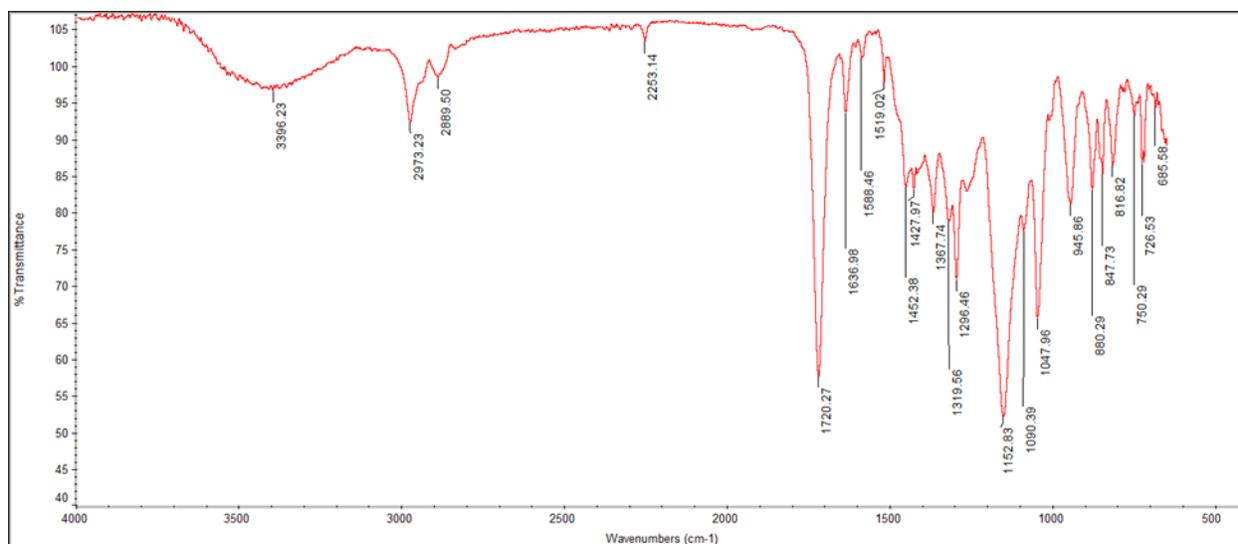


Figure 4.2: FT-IR for Unwashed Multi-Templated Cu-Fe-Ni IIP

Figure 4.3, the FTIR of the Washed multi-templated Cu-Fe-Ni IIP shows a shift and decrease in the peaks intensity in comparison to the FTIR of the unwashed multi-templated Cu-Fe-Ni IIP. There is the disappearance of peaks due to OH, CH<sub>3</sub> and CH<sub>2</sub> groups between 3300 and 2600 cm<sup>-1</sup> due to template removal by the washing procedure. The intensity of peaks at the region 1721 cm<sup>-1</sup> and 1146 cm<sup>-1</sup> by the C-O of the ester and carboxylic acid decreased after washing. Iron(III), nickel(II) and copper(II) are known for their strong coordinate bonding with carbonyl systems hence the strong peaks found in the spectrum of the Unwashed Cu-Fe-Ni IIP and their decreased intensity or their disappearance in the Washed Cu-Fe-Ni IIP due to the removed metal ions during the washing step [129].

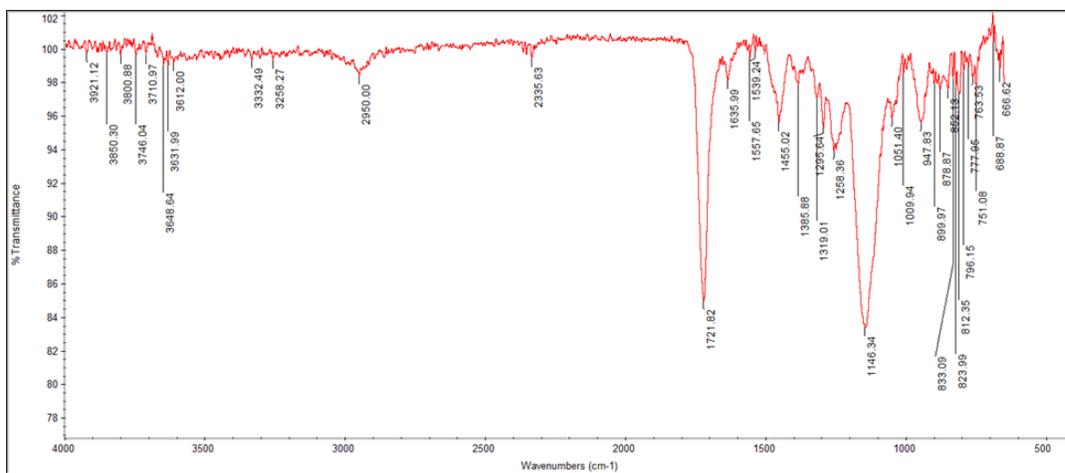


Figure 4.3: FT-IR for Washed Cu-Fe-Ni IIP

## 4.2 X – Ray Diffraction Analysis

Figure 4.4 presents the XRD patterns of iron, copper and nickel supported on the surface of the Unwashed Cu-Fe-Ni IIP powder.

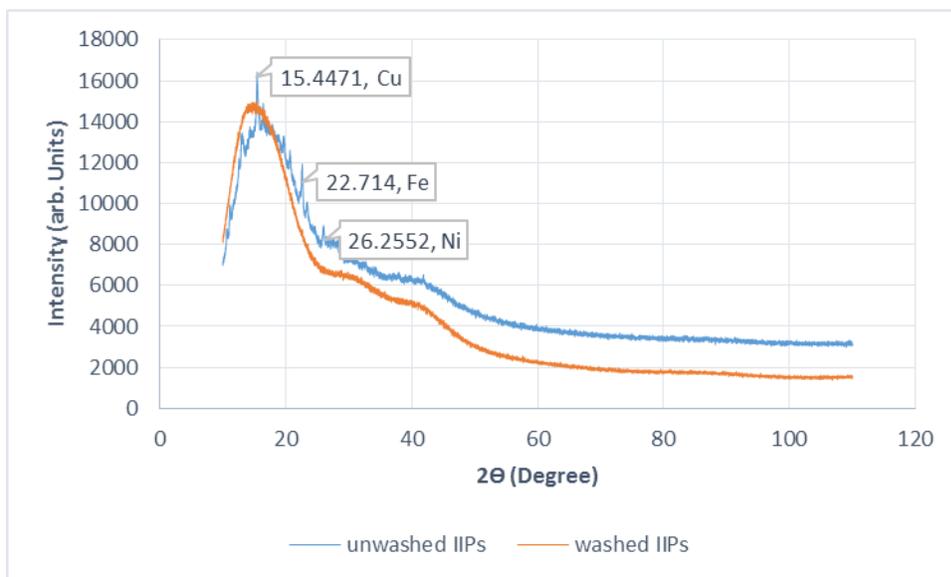


Figure 4.4: XRD Spectra for the Washed IIPs and Unwashed IIPs

Considering the relative maximum diffraction peaks for Ni ( $2\theta = 26.2552^\circ$ ) it was evident that nickel is present on the surface of the unwashed powder. The maximum diffraction peak ( $2\theta = 15.4471^\circ$ ) of the Cu crystalline phase presented a high number of counts, indicating a high crystallinity (JCPDS 4-0850). The XRD pattern of the pure iron reveals a weak and sharp peak at Fe (9731), indicating a poor crystallinity towards iron. The presence of the template ions on the surface of the powder indicated their presence throughout imprinting process prior to template removal.

The XRD spectrum of the washed IIPs in Fig. 4.4, (after optimal removal of the template ions) showed complete absence of nickel, iron and copper ions, thus reflecting optimal removal of the templated ions. The crystalline phase of the washed IIPs, as evident by the XRD, have absorption properties due to its mesoporosity and selective active sites, which are necessary in sorption activities. A mesoporous structure promotes percolation by increasing the permeability, thus a significant quantity of a targeted analyte(s) reactant can be diffused well into the particle interior to reach the active sites.

### 4.3 Atomic Force Microscopic Analysis

The AFM image (Fig 4.5) obtained revealed the sponginess of the powder, which reflects its absorptive nature. The surface roughness and its mesoporosity was also revealed. The AFM analysis provided the physical properties of the sorbents as shown in Table 2.

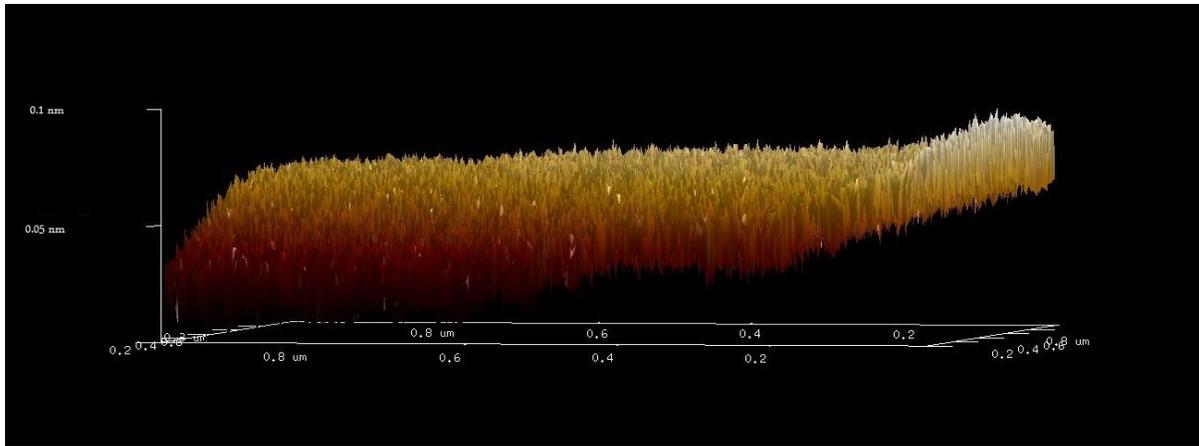


Figure 4.5: 3D AFM image of the Washed IIP

Table 3: IIPs Physical properties based on AFM analysis

Parameters	Minimum	Maximum	Mean/Average
<b>Total Count</b>	1844.000	1844.000	1844.000
<b>Density</b>	1844.000 (/μm <sup>2</sup> )	1844.000 (/μm <sup>2</sup> )	1844.000 (/μm <sup>2</sup> )
<b>Height</b>	3.903 (mV)	15.704 (mV/nm)	5.192 (mV/nm)
<b>Area</b>	15.259 (nm <sup>2</sup> )	14892.578 (nm <sup>2</sup> )	43.832 (nm <sup>2</sup> )
<b>Diameter</b>	4.408 (nm)	137.702 (nm)	5.723 (nm)

## 4.4 Optimization of Time

The percentage adsorption of the metallic ions on the sorbent increased with increase in contact time. Generally, more time allows the adsorbent particle to interact with the metal ion and increase chances of more adsorption [127]. When the washed Cu-Fe-Ni IIP powder was applied as described in section 3.7.1, the IIP optimal removal efficiency was reached within 15 minutes. Further increase in contact time did not translate into any further significant increase in the % removal efficiency of each of the three ions evaluated. The optimum time was as such, considered to be 15 minutes as marked by the plateaus in Fig.4.6 plot, starting at 15 sec for each of the ions. Consequently, subsequent experiments employing the washed Cu-Fe-Ni IIP powder were performed at the optimum contact time of 15 minutes as displayed by Figure 4.6 for each of the metal ions.

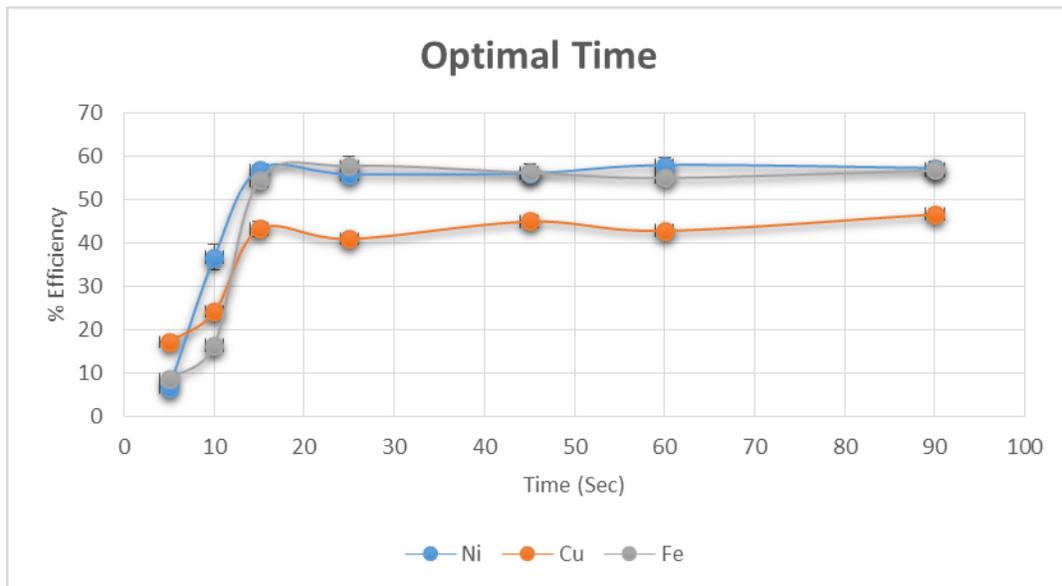


Figure 4.6: Percent removal of Fe, Ni & Cu ions at optimal time

## 4.5 Optimization of pH

In the same manner as time and as describe in section 3.7.2, pH was optimized by adjusting the pH from 2 to 9.5. The percentage removal increased up until pH 7.5 after which there was a gradual decrease as displayed by Figure 4.7. At low pH, the cations compete with the Hydrogen ion in the solution for the binding sites and hence lower adsorption. At higher pH, the surface of the adsorbent has a higher negative charge which results in higher attraction of metallic cations [115]. However, at  $\text{pH} > 7.5$ , precipitation of metal hydroxide is most likely to occur including the formation of unintended complexes. Therefore, the optimum pH was taken to be 7.5 for all metals since there was the highest percentage removal before the pHs at which there is a likelihood of precipitation.

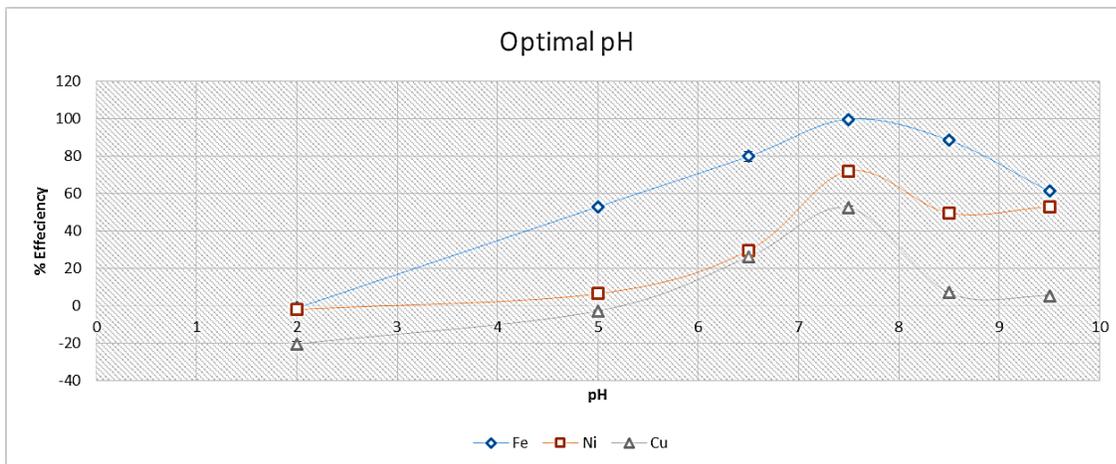


Figure 4.7: The plot of optimization of pH for the removal of Fe, Ni & Cu ions.

## 4.6 Optimization of Sorbent Dose

As the mass of the washed Cu-Fe-Ni IIP was increased, there was increased uptake of the targeted ions due to more availability of the binding sites from the increased IIP mass. An optimal sorbent dose was found to be 20 mg which was marked by the point at which Fig. 4.8 plot for each of the targeted ions, started to plateau at a maximum %removal. At the optimum mass of 20 mg of sorbent, the removal trend was Fe > Cu > Ni. The removal for Fe, Cu and Ni ions was 100%, 91%, 78.8%, respectively. There was no significant difference when more than 20 mg was applied to remove all specific ions.

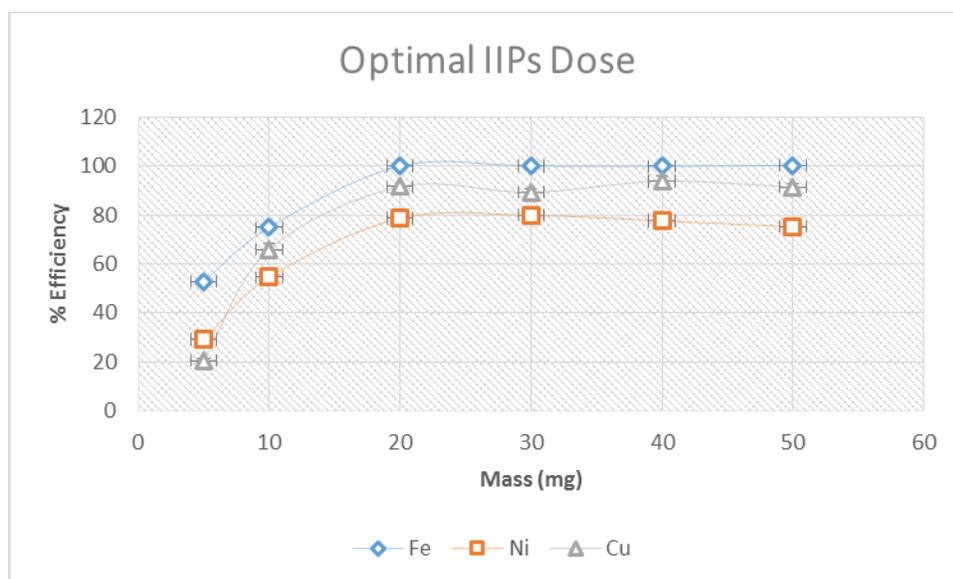


Figure 4.8: The plot of optimization of sorbent dose for the removal of Fe, Ni & Cu ions.

The optimal conditions were also applied to the control powder (NIP) to evaluate uptake of metal ions in comparison to the Cu-Fe-Ni IIP, see Fig. 4.9.

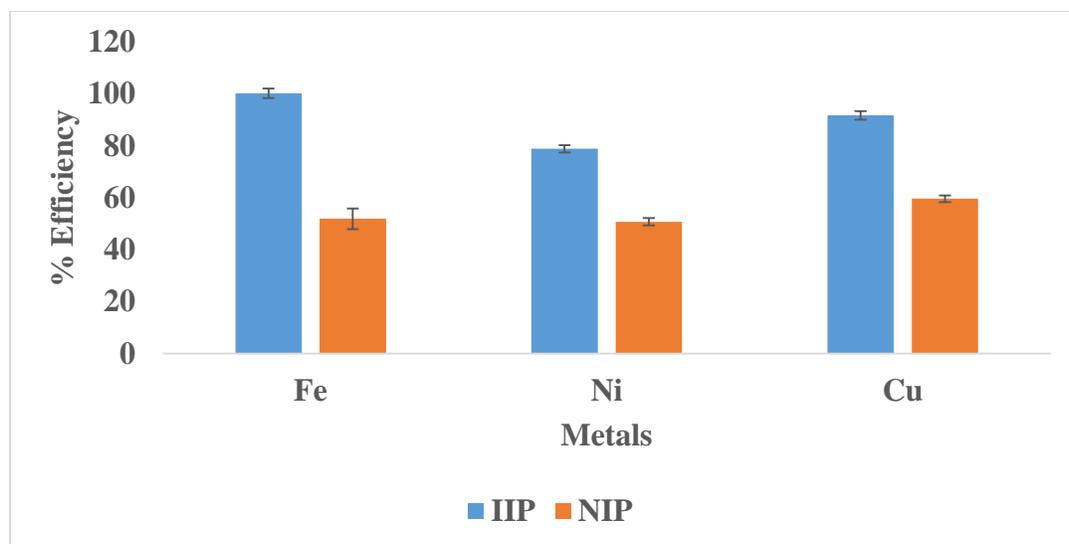


Figure 4.9: Percent removal by the IIPs and NIPs

## 4.7 Selectivity Studies

Pb (II), Zn (II) and Hg (II) were chosen as the competitive ions, due to the similar charge, nearly identical size to the analytes of interest in this thesis; (ionic radius e.g. Zn (II) – 74 pm, Ni (II) – 72 pm, Cu (II) – 69 pm). The percent removal of Fe (II), Ni (II) and Cu (II) was higher than that of the competing ions; Pb (II), Zn (II) and Hg (II) as displayed in Fig 4.10. It was observed that there was low selectivity and removal efficiency (on the average of 45%) of the NIP towards all the metallic ions that were involved in the selectivity studied and high selectivity and removal efficiency (on the average of 85%) of the washed Cu-Fe-Ni IIP powder towards the target ions (Fe (II), Ni (II) and Cu (II)). The reason for the low selectivity of the IIP towards the competitive ions was due to the fact that the cavities imprinted by the templated ions were not suited to the competing ions; Zn (II), Pb (II) and Hg (II) in size, shape and spatial arrangement. For example, the ionic radius of Zn (II) is larger than that of Ni (II) and Cu hence cannot fit at the imprinted sites created by templating with an ion other than it.

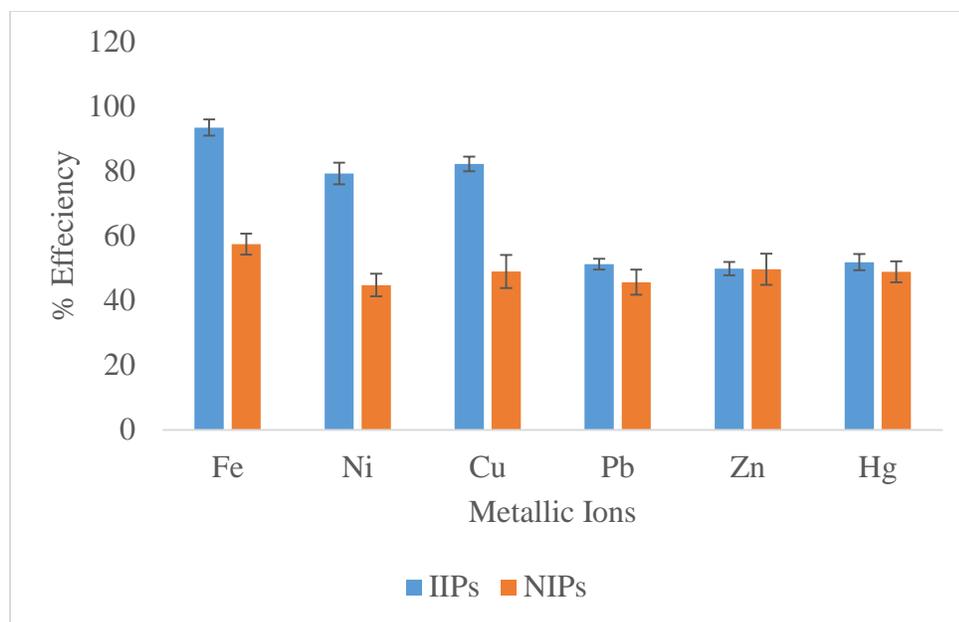


Figure 4.10: Selectivity studies.

## 4.8 Determination of Metallic Ions in Waste and Borehole Water

The major source of water for local people in Botswana is ground waters from local boreholes but most often goes dry quickly especially during winter. The supply does not meet the demands for irrigation. To facilitate irrigation, the Glen Valley Wastewater treatment plant, irrigation schemes using the wastewater from the plant have been set up.

Hence, borehole and wastewater samples were used as the real water samples in this work. The total metal concentrations from the digested wastewater samples and borehole water samples were determined as shown in Table 4. Five sampling sites from Glen valley wastewater treatment plant were considered while analysing for heavy metals in the wastewater samples. The samples were collected from I) inlet point, which is the point where the waste water is

received into the wastewater plant from majority of the Gaborone regions and where the water is screened, ii) primary settling tank where organic and Inorganic solids are allowed to settle out in these large sedimentation tanks, iii) secondary settling tank, the stage where biological treatment is used to remove the dissolved organic matter that escapes primary treatment, iv) aeration tank, at which stage where the water is put into large rectangular tanks and v) effluent storage tank where the water is directed to a tank where it is stored before being released from the plant to the maturation ponds. The metal concentrations in wastewaters were observed to be lower than the toxic levels which are set by wastewater specification-(BOBS 93:2012) in Botswana and US. Likewise, the metal concentration in the borehole water samples showed that the water sample was safe for drinking according to WHO 1999. The instrumental detection of metallic ions in both raw samples were unsuccessful due to their very low concentrations.

Table 4: Metallic ion concentration (mg/L) in waste water sample

Concentrations (mg/L)			
Waste Water			
Points	<b>Fe</b>	<b>Ni</b>	<b>Cu</b>
<b>Inlet Point</b>	1.361 ± 0.020	0.285 ± 0.021	0.085 ± 0.005
<b>Primary Settling Tank</b>	1.074 ± 0.023	0.245 ± 0.007	0.078 ± 0.001
<b>Secondary Settling Tank</b>	0.288 ± 0.046	0.230 ± 0.042	0.029 ± 0.002
<b>Aeration Tank</b>	2.432 ± 0.046	0.390 ± 0.003	0.127 ± 0.012
<b>Effluent Storage Tank</b>	0.255 ± 0.028	0.265 ± 0.021	0.023 ± 0.002
Borehole	0.344± 0.004	1.288± 0.032	0.012± 0.001

## 4.9 Application of Method

The applicability of the prepared IIP was evaluated in selectively removing the targeted metallic ions, Cu(II), Fe(II), Ni(II) from real water samples, in this thesis being waste water and borehole water. The optimized parameters were applied on both water samples and the obtained percentage removals for each of the targeted ions in waste water and borehole were as shown in Fig. 4.11. Ni recorded the lowest percentage removal (79.274% in waste water sample and 83.462% in borehole water) as compared to Cu (82.213% in waste water and 91.67% in borehole) and Fe (93.472% in waste water and 96.148% in borehole water) with the highest percentage removal. This could be attributed to saturation of binding sites for Ni ions in the sorbents.

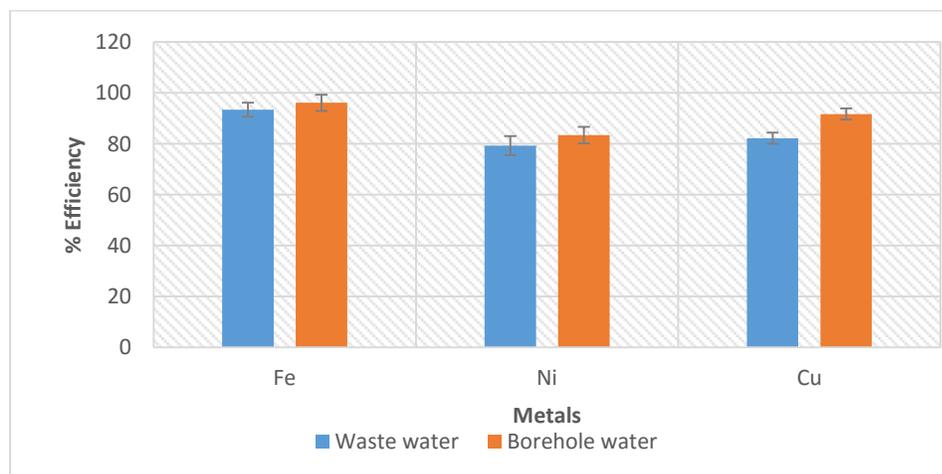


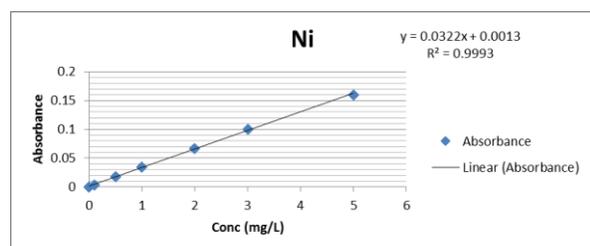
Figure 4.11: Percentage removal of metallic ions from waste water sample and borehole water sample use IIPs

## 4.10 Linearity Studies

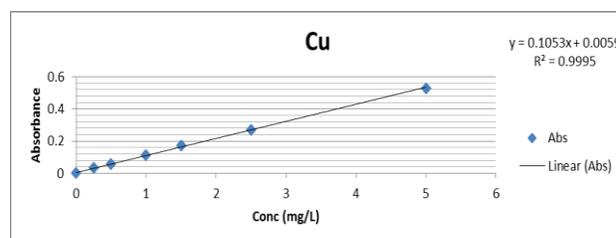
The linearity ( $R^2$ ) for the metals concentration ranging from 0 – 5 mg/L ( $n = 3$ ) was found to be  $>0.998$  for all metals as shown in Table 5 below.

Table 5: Linearity data for selected metallic ions using FAAS

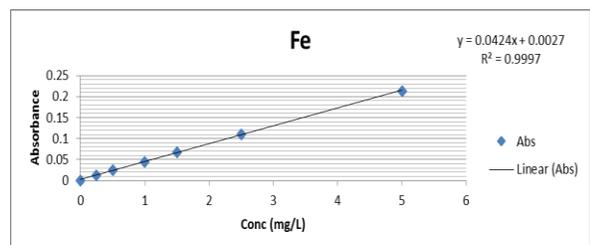
	Regression Eqn.	R2
metal	$y=0.086x + 0.0092$	0.9982
Fe	$y=0.0424x + 0.0027$	0.9997
Cu	$y= 0.1053x + 0.0059$	0.9995
Ni	$y=0.0322 + 0.0013$	0.9993



A



B



C

Figure 4.12: Linearity line graph for A) Ni ions B) Cu ions and C) Fe ions using FAAS

## 4.11 Detection Limits

The limit of detection (LODs) and Limit of quantification (LOQs) were both investigated.

The limit of detection and quantification of the selected metals using FAAS is shown on Table 6 below.

Table 6: Limit of detection and quantification of targeted metallic ions

Metal Ions	Instrument Detection Limits (mg/L)	Instrument Quantification Limits (mg/L)	Method Detection Limit (mg/L)
Cu	0.019 ± 0.004	0.050 ± 0.004	0.010 ± 0.003
Fe	0.051 ± 0.006	0.095 ± 0.006	0.036 ± 0.010
Ni	0.054 ± 0.004	0.086 ± 0.005	0.015 ± 0.005

## 4.12 Recoveries Studies

In order to do method validation, the water samples were spiked with a solution of 2 mg/L concentration of the selected metals (Ni, Cu and Fe). The analyses were done in triplicate using FAAS. Table 7 shows the percentage recoveries for the selected metallic ions. Using the below equation, percentage recoveries were obtained for each of the metallic ions.

$$\% \text{Recovery} = \frac{\text{Concentration of ions in spiked sample} - \text{concentration of ions in unspiked sample}}{\text{concentration of ions in added to the spiked sample}} \times 100\%$$

Table 7: Percent recoveries for selected metallic ions

	Raw Values	Spiked	Unspiked	Difference	Recoveries
<b>Wastewater</b>					
<b>Metallic Ions</b>					
<b>Ni</b>	ND*	2.167571429	0.0456	2.121971429	106.09857
<b>Cu</b>	ND	2.024714286	0.0275	1.997214286	99.860714
<b>Fe</b>	ND	2.097728571	0.255	1.842728571	92.136429
<b>Borehole</b>					

<b>Ni</b>	ND	2.116142857	0.1133	2.002842857	100.28429
<b>Cu</b>	ND	2.052385714	0.0123	2.040085714	104.00857
<b>Fe</b>	ND	2.509814286	0.344	2.165814286	116.58143

ND\*---not detected.

## CHAPTER FIVE

### 5.0 Conclusions

A novel multi-templated Cu-Fe-Ni IIP via a template guided bulk polymerization approach to selectively and simultaneously remove the targeted ions; Cu(II), Fe(II) and Ni(II) from waste and borehole water in the presence of competing ions, was successfully prepared. The prepared Cu-Fe-Ni IIP demonstrated to be selective, effective, needed less time and was easy to apply towards the removal of the three targeted, model ions (Fe, Ni & Cu). It also demonstrated the ability to differentiate between closely related structural analogues.

Atomic force micrograph revealed that the powder had both micro spherical, spongy, and micro particular morphologies which are sizes, charges, geometrical shape and morphological artefacts suitable for optimal adsorption. The development of this polymeric powder resulted in the compilation of detailed knowledge for the preparation of such polymer and the affecting polymerization factors.

The Cu-Fe-Ni-IIP exhibited both group and specific target selectivity. The study showed that the effort to enhance the interaction between the template ions and the functional monomer in the pre-polymerization complex, where 1,10-phenanthroline was used as the complexing agent led to the high degree of affinity for the target metallic ions during uptake.

The prepared polymer has demonstrated a potential for a cheap and efficient sorbent material that can replace some of the sorbent materials that are expensive & non-selective and are currently

employed in removing toxic heavy metal ions from wastewater that is recycled and treated for use in irrigation.

## 5.1 Future Work

Future work for this powder will focus on increased control over particle morphology and their use as probes for screening and warning in different environments. Future investigations will also include studies on the effect of template bleeding; its occurrence and control and the use of more metal ions as template ions to increase the number of selective binding sites for the simultaneous uptake of several heavy metals.

This study has highlighted the urgent need in the field of ion imprinting to focus on the development of multi-templated, imprinted polymer extraction, sample clean-up, purification and even the determination of several metallic ions at once in a complex sample matrix. The ability to design and synthesize IIPs capable of exploiting all of the possible metallic ion extractions or determination in samples with a wide range of heavy metals ion uptake, will widen the applicability of ion imprinting, not just in terms of target ion choice but also in the realisation of their full analytical and commercial potentials.

## REFERENCES

- [1] M. Javed and N. Usmani, "Assessment of heavy metal (Cu, Ni, Fe, Co, Mn, Cr, Zn) pollution in effluent dominated rivulet water and their effect on glycogen metabolism and histology of *Mastacembelus armatus*," *Springerplus*, vol. 2, no. 1, p. 390, 2013.
- [2] a K. Chopra, C. Pathak, and G. Prasad, "Scenario of heavy metal contamination in agricultural soil and its management," no. 2007, pp. 99–108, 2009.
- [3] M. Rammika, G. Darko, and N. Torto, "Incorporation of Ni ( II ) -dimethylglyoxime ion-imprinted polymer into electrospun polysulphone nanofibre for the determination of Ni ( II ) ions from aqueous samples," *Water SA*, vol. 37, no. 3, pp. 539–546, 2011.
- [4] F. . Ekpo, N. . Agu, and U. . Udoakpan, "Influence of Heavy Metals Concentration in Three Common Fish, Sediment and Water Collected within Quarry Environment, Akampa L. G. Area, Cross Rivers State, Nigeria," *Eur. J. Toxicol. Sci.*, pp. 1–11, 2013.
- [5] P. N. Librel, "Determination of Trace Metalconcentration In Liver , Muscle , Kidney and Gills Of *Oreochromis mossambicus* and *Labeo rohita* Exposed to Plant Nutrient," no. 2013, pp. 94–116, 2012.
- [6] A. a Ali, E. M. Elazein, and M. a Alian, "Investigation of Heavy Metals Pollution in Water , Sediment and Fish at Red Sea – Jeddah Coast- KSA at Two Different Locations," vol. 1, no. 12, pp. 630–637, 2011.
- [7] R. a. Wuana and F. E. Okieimen, "Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation," *ISRN Ecol.*, vol. 2011, pp. 1–20, 2011.
- [8] O. Akoto, F. Bismark Eshun, G. Darko, and E. Adei, "Concentrations and health risk assessments of heavy metals in fish from the Fosu Lagoon," *Int. J. Environ. Res.*, vol. 8, no. 2, pp. 403–410, 2014.
- [9] Department of Health and Human Services, "Copper," p. 2, 2004.
- [10] D. E. Güven and G. Akinci, "Comparison of acid digestion techniques to determine heavy metals in sediment and soil samples," *Gazi Univ. J. Sci.*, vol. 24, no. 1, pp. 29–34, 2011.
- [11] G. L. Long, H. M. McNair, M. R. Anderson, P. a Deck, and A. M. Dietrich, "Metal Extraction From Soil Samples By," 2000.
- [12] C.-Y. Kuo, C.-H. Wu, and S.-L. Lo, "Leaching efficiency of copper from industrial sludge with traditional acid extraction (TAE) and microwave assisted treatment (MAT).," *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.*, vol. 40, no. 12, pp. 2203–2214, 2005.

- [13] T. T. Teng, Y. Yusup, and L. W. Low, "Heavy Metal Ion Extraction Using Organic Solvents : An Application of the Equilibrium Slope Method," 2011.
- [14] D. Villemin and M. Didi, "Extraction of rare earth and heavy metals, using ionic solvents as extraction medium (A Review)," *Orient. J. Chem.*, vol. 29, no. 4, pp. 1267–1284, 2013.
- [15] P. Moslehi, J. Shayegan, and S. Bahrpayma, "Performance of Membrane Bioreactor in Removal of Heavy Metals from Industrial Wastewater," *Iran. J. Chem. ...*, 2008.
- [16] F. Fu and Q. Wang, "Removal of heavy metal ions from wastewaters: A review," *J. Environ. Manage.*, vol. 92, no. 3, pp. 407–418, 2011.
- [17] R. W. Gaikwad, V. S. Sapkal, and R. S. Sapkal, "Ion exchange system design for removal of heavy metals from acid mine drainage wastewater," *Acta Montan. Slovaca*, vol. 15, no. 4, pp. 298–304, 2010.
- [18] K. Ahmad, I. a Bhatti, M. Muneer, M. Iqbal, and Z. Iqbal, "Removal of heavy metals ( Zn , Cr , Pb , Cd , Cu and Fe ) in aqueous media by calcium carbonate as an adsorbent," vol. 2, pp. 48–53, 2012.
- [19] S. a Shama, M. E. Moustafa, and M. a Gad, "Cd 2 + from Aqueous Solutions by Using Eichhornia Crassipes," vol. 28, no. 2, pp. 125–133, 2010.
- [20] E. Najafi, F. Aboufazeli, H. R. L. Z. Zhad, O. Sadeghi, and V. Amani, "A novel magnetic ion imprinted nano-polymer for selective separation and determination of low levels of mercury(II) ions in fish samples," *Food Chem.*, vol. 141, no. 4, pp. 4040–4045, 2013.
- [21] M. a. Barakat, "New trends in removing heavy metals from industrial wastewater," *Arab. J. Chem.*, vol. 4, no. 4, pp. 361–377, 2011.
- [22] S. G. Ozcan, N. Satiroglu, and M. Soylak, "Column solid phase extraction of iron(III), copper(II), manganese(II) and lead(II) ions food and water samples on multi-walled carbon nanotubes," *Food Chem. Toxicol.*, vol. 48, no. 8–9, pp. 2401–2406, 2010.
- [23] C. Ricardo, T. Tarley, M. Del, P. Taboada, and L. Tatsuo, "Divulgação," vol. 28, no. 6, pp. 1087–1101, 2005.
- [24] J. Otero-Romaní, A. Moreda-Piñeiro, P. Bermejo-Barrera, and A. Martin-Esteban, "Synthesis, characterization and evaluation of ionic-imprinted polymers for solid-phase extraction of nickel from seawater," *Anal. Chim. Acta*, vol. 630, pp. 1–9, 2008.
- [25] H. Yan and K. H. Row, "Characteristic and Synthetic Approach of Molecularly Imprinted Polymer," *Int. J. Mol. Sci.*, vol. 7, pp. 155–178, 2006.
- [26] B. Y. X. E. Spectroscopy, "SAMPLE PREPARATION TECHNIQUES IN TRACE ELEMENT ANALYSIS BY X-RAY EMISSION SPECTROSCOPY," 1983.

- [27] Agilent, "Sample Preparation Fundamentals for Chromatography," *Agil. Present.*, 2013.
- [28] S. Preservation and S. H. Times, "CHAPTER 8 SAMPLE HANDLING PROCEDURES," pp. 1–8.
- [29] S. Koning, H.-G. Janssen, and U. a. T. Brinkman, "Modern Methods of Sample Preparation for GC Analysis," *Chromatographia*, vol. 69, pp. 33–78, 2009.
- [30] D. of W. Government of Western Australia, *Surface Water Sampling Methods and Analysis*. 2009.
- [31] M. N. Sarrion, F. J. Santos, and M. T. Galceran, "In situ derivatization/solid phase microextraction for the determination of haloacetic acids in water," *Anal. Chem.*, vol. 72, no. 20, pp. 4865–4873, 2000.
- [32] F. Bordas and A. C. M. Bourg, "A critical evaluation of sample pretreatment for storage of contaminated sediments to be investigated for the potential mobility of their heavy metal load," *Water. Air. Soil Pollut.*, vol. 103, no. 1979, pp. 137–149, 1998.
- [33] P. De Zorzi, S. Barbizzi, M. Belli, G. Ciceri, A. Fajgelj, D. Moore, U. Sansone, and M. Van Der Perk, "Terminology in soil sampling (IUPAC Recommendations 2005)," *Pure Appl. Chem.*, vol. 77, no. 5, pp. 827–841, 2005.
- [34] G. Qu, S. Zheng, Y. Liu, W. Xie, A. Wu, and D. Zhang, "Metal ion mediated synthesis of molecularly imprinted polymers targeting tetracyclines in aqueous samples," *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*, vol. 877, pp. 3187–3193, 2009.
- [35] J. W. Hooijmans, "Knowledge Based Bio-based Products Overview of current relevant sampling and biogenic carbon standards on global level Public."
- [36] I. Fujiwara, "Preparation of anion-imprinted resin by a surface-imprinting polymerization method and its use for chemical separation," *Bunseki Kagaku*, vol. 54, pp. 243–244, 2005.
- [37] J. F. E. Giho, "Analysis of Chemical States of Heavy Metals in Environmental Samples Using XFAS †," vol. 9, no. 9, pp. 37–42, 2007.
- [38] M. Welna, A. Szymczycha-madeja, and P. Pohl, "Quality of the Trace Element Analysis : Sample Preparation Steps," *Wide Spectry Qual. Control*, pp. 53–70, 2011.
- [39] E. Turiel and A. Martín-Esteban, "Molecularly imprinted polymers for sample preparation: A review," *Analytica Chimica Acta*, vol. 668. pp. 87–99, 2010.
- [40] National Research Council, *Toxicological effects of methylmercury*. 2000.
- [41] D. Majors, Ronard E., Wilmington, *Sample preparation fundamentals for chromatography*. Canada: Agilent Technologies, 2013.

- [42] F. G. Tamayo, E. Turiel, and a. Martín-Esteban, “Molecularly imprinted polymers for solid-phase extraction and solid-phase microextraction: Recent developments and future trends,” *J. Chromatogr. A*, vol. 1152, pp. 32–40, 2007.
- [43] K. J. Elnabris, S. K. Muzyed, and N. M. El-Ashgar, “Heavy metal concentrations in some commercially important fishes and their contribution to heavy metals exposure in palestinian people of Gaza Strip (Palestine),” *J. Assoc. Arab Univ. Basic Appl. Sci.*, vol. 13, no. 1, pp. 44–51, 2013.
- [44] S. L. Goddard and R. J. C. Brown, “Investigation into Alternative Sample Preparation Techniques for the Determination of Heavy Metals in Stationary Source Emission Samples Collected on Quartz Filters,” no. ii, pp. 21676–21692, 2014.
- [45] M. Khajeh, Z. Shamohammadi, and E. Sanchooli, “Synthesis , characterization and removal of lead from water samples using lead-ion imprinted polymer,” *Chem. Eng. J.*, vol. 166, no. 3, pp. 1158–1163, 2011.
- [46] S. Mitra and R. Brukh, *Sample Preparation: An Analytical Perspective*, vol. 162. 2003.
- [47] “Author ’ s personal copy Sample Preparation Techniques for the Electrochemical Determination of Metals in Environmental and Food Samples,” pp. 0–10, 2013.
- [48] G. M. Ogendi, G. M. Maina, J. W. Mbutia, H. K. Koech, C. M. Ratemo, and J. C. Koskey, “Heavy Metal Concentrations in Water , Sediments and Common Carp ( *Cyprinus carpio* ) Fish Species from Lake Naivasha , Kenya,” vol. 6, no. 8, pp. 416–423, 2014.
- [49] H. Shir Khanloo, Z. Mousavi, and A. Rouhollahi, “Preconcentration and determination of heavy metals in water, sediment and biological samples,” *J. Serbian Chem. Soc.*, vol. 76, no. 11, pp. 1583–1595, 2011.
- [50] M. R. Ras, F. Borrull, and R. M. Marcé, “Sampling and preconcentration techniques for determination of volatile organic compounds in air samples,” *TrAC - Trends Anal. Chem.*, vol. 28, no. 3, pp. 347–361, 2009.
- [51] M. Ghaedi, a. Shokrollahi, a. H. Kianfar, a. S. Mirsadeghi, a. Pourfarokhi, and M. Soylak, “The determination of some heavy metals in food samples by flame atomic absorption spectrometry after their separation-preconcentration on bis salicyl aldehyde, 1,3 propan diimine (BSPDI) loaded on activated carbon,” *J. Hazard. Mater.*, vol. 154, pp. 128–134, 2008.
- [52] F. J. M. J. Maessen, “Separation and preconcentration of trace substances. I— preconcentration for inorganic trace analysis.,” *Spectrochim. Acta Part B At. Spectrosc.*, vol. 35, no. 1, p. 55, 1980.

- [53] G. a. Zachariadis, “In situ derivatization of metals and organometallics using borate reagents in gas chromatographic speciation studies,” *J. Chromatogr. A*, vol. 1296, pp. 47–69, 2013.
- [54] S. N. Atapattu and J. M. Rosenfeld, “Solid phase analytical derivatization as a sample preparation method,” *J. Chromatogr. A*, vol. 1296, pp. 204–213, 2013.
- [55] R. Cornelis, J. Caruso, H. Crews, and K. Heumann, *Handbook of Elemental Speciation II - Species in the Environment, Food, Medicine and Occupational Health*. 2005.
- [56] R. Cornelis, *Handbook of Elemental Speciation : Handbook of Elemental Speciation :* 2003.
- [57] R. W. Hoffmann, “The quest for chiral Grignard reagents.,” *Chem. Soc. Rev.*, vol. 32, no. 4, pp. 225–230, 2003.
- [58] V. Vatanpour, S. S. Madaeni, S. Zinadini, and H. R. Rajabi, “Development of ion imprinted technique for designing nickel ion selective membrane,” *J. Memb. Sci.*, vol. 373, pp. 36–42, 2011.
- [59] H. Kataoka and K. Saito, “Recent advances in SPME techniques in biomedical analysis,” *Journal of Pharmaceutical and Biomedical Analysis*, vol. 54, no. 5. pp. 926–950, 2011.
- [60] C. Sparr Eskilsson and E. Björklund, “Analytical-scale microwave-assisted extraction,” *Journal of Chromatography A*, vol. 902, no. 1. pp. 227–250, 2000.
- [61] B. Jurado-Sánchez, E. Ballesteros, and M. Gallego, “Comparison of microwave assisted, ultrasonic assisted and Soxhlet extractions of N-nitrosamines and aromatic amines in sewage sludge, soils and sediments,” *Sci. Total Environ.*, vol. 463–464, pp. 293–301, 2013.
- [62] M. Hyder and J. Å. Jönsson, “Aerosols , Sampling and Sample Treatment Methods : A Review and State of the Art,” pp. 295–316.
- [63] J. Å. Jönsson, L. Mathiasson, L. Chimuka, and E. Cukrowska, “Chapter 7 Membrane Techniques for Analysis , Sampling and Speciation in Environmental Measurements.”
- [64] J. Pawliszyn, “Sample Preparation : Quo Vadis ?,” vol. 75, no. 11, pp. 2543–2558, 2003.
- [65] T. P. Rao, R. Kala, and S. Daniel, “Metal ion-imprinted polymers-Novel materials for selective recognition of inorganics,” *Analytica Chimica Acta*, vol. 578. pp. 105–116, 2006.
- [66] A. M. Carro-diaz and R. A. Lorenzo-ferreira, “Molecularly Imprinted Polymers for Sample Preparation,” pp. 87–97.

- [67] A. Ersöz, R. Say, and A. Denizli, “Ni(II) ion-imprinted solid-phase extraction and preconcentration in aqueous solutions by packed-bed columns,” *Anal. Chim. Acta*, vol. 502, pp. 91–97, 2004.
- [68] T. Alizadeh, “Development of a molecularly imprinted polymer for pyridoxine using an ion-pair as template,” *Anal. Chim. Acta*, vol. 623, pp. 101–108, 2008.
- [69] G. Tan and L. Abdula’uf, “Recent Developments and Applications of Microextraction Techniques for the Analysis of Pesticide Residues in Fruits and Vegetables,” *Pestic. - Recent Trends Pestic. Residue Assay*, 2012.
- [70] G. H. C. M.-K. Tan, “Sample Preparation in the Analysis of Pesticides Residue in Food by Chromatographic Techniques,” *Pestic. - Strateg. Pestic. Anal.*, pp. 29–58, 2011.
- [71] M. Mode, “The Chemistry of Solid- Phase Extraction.” 2013.
- [72] “Theory of SPE,” *Sep. Sci.*, pp. 1–31, 2014.
- [73] M. Biziuk, “Solid Phase Extraction Technique – Trends , Opportunities and Applications,” vol. 15, no. 5, pp. 677–690, 2006.
- [74] D. State, J. O. Y. F. Akintujoye, C. I. Anumudu, and H. O. Awobode, “Assessment of heavy metal residues in water , fish tissue and human blood from Ubeji , Assessment of heavy metal residues,” 2013.
- [75] J. T. Baker (JTBAKER.COM TECHNICAL LIBRARY), “20110310184418.Jtb\_Folleto-Spe.Pdf.” .
- [76] M. Zhang, Z. Zhang, Y. Liu, X. Yang, L. Luo, J. Chen, and S. Yao, “Preparation of core-shell magnetic ion-imprinted polymer for selective extraction of PB(II) from environmental samples,” *Chem. Eng. J.*, vol. 178, pp. 443–450, 2011.
- [77] “SorbentSelection @ [www.interchim.eu](http://www.interchim.eu).” .
- [78] “chromatography-Modern-SPE-Sorbents @ [www.chromacademy.com](http://www.chromacademy.com).” .
- [79] M. Cruz-Vera, R. Lucena, S. Cárdenas, and M. Valcárcel, “Highly selective and non-conventional sorbents for the determination of biomarkers in urine by liquid chromatography,” *Anal. Bioanal. Chem.*, vol. 397, no. 3, pp. 1029–1038, 2010.
- [80] M. Hennion, “Immuno-based sample preparation for trace analysis,” vol. 1000, pp. 29–52, 2003.
- [81] F. Puoci, G. Cirillo, M. Curcio, and F. Iemma, “Molecularly Imprinted Polymers ( MIPs ) in Biomedical Applications,” 2008.

- [82] F. G. Tamayo, J. L. Casillas, and A. Martin-Esteban, "Evaluation of new selective molecularly imprinted polymers prepared by precipitation polymerisation for the extraction of phenylurea herbicides," *J. Chromatogr. A*, vol. 1069, pp. 173–181, 2005.
- [83] L. Zhao, L. Ban, Q. W. Zhang, Y. P. Huang, and Z. S. Liu, "Preparation and characterization of imprinted monolith with metal ion as pivot," *J. Chromatogr. A*, vol. 1218, pp. 9071–9079, 2011.
- [84] Z. Luo, A. Zeng, P. Zheng, P. Guo, W. Du, K. Du, and Q. Fu, "Analytical Methods Preparation of surface molecularly imprinted polymers as the solid-phase extraction sorbents for the specific recognition of penicilloic acid in," *Anal. Methods*, vol. 6, pp. 7865–7874, 2014.
- [85] E. Baltussen, C. a. Cramers, and P. J. F. Sandra, "Sorptive sample preparation - A review," *Anal. Bioanal. Chem.*, vol. 373, pp. 3–22, 2002.
- [86] G. Vasapollo, R. Del Sole, L. Mergola, M. R. Lazzoi, A. Scardino, S. Scorrano, and G. Mele, "Molecularly imprinted polymers: Present and future prospective," *International Journal of Molecular Sciences*, vol. 12, pp. 5908–5945, 2011.
- [87] C. Alexander, H. S. Andersson, L. I. Andersson, R. J. Ansell, N. Kirsch, I. a. Nicholls, J. O'Mahony, and M. J. Whitcombe, "Molecular imprinting science and technology: A survey of the literature for the years up to and including 2003," *J. Mol. Recognit.*, vol. 19, no. 2, pp. 106–180, 2006.
- [88] Wikipedia, "Molecular imprinted polymer," *Academic, wikipedia*. Wikipedia, 2010.
- [89] G. B. Sellergren, University of Mainz, Mainz, *Molecularly Imprinted Polymers Man-Made Mimics of Antibodies and their Application in Analytical Chemistry*, 23rd ed. Mainz: Elsevier B.V., 2000.
- [90] L. Fitzhenry, "Development of Molecularly Imprinted Polymers for Corticosteroids By," 2011.
- [91] S. Eppler, "Advanced Strategies for Characterizing Molecular Imprinted Polymers," 2014.
- [92] M. Bompart, K. Haupt, and C. Ayela, "Micro and nanofabrication of molecularly imprinted polymers," *Top. Curr. Chem.*, vol. 325, pp. 83–110, 2012.
- [93] A. Hault, "Molecularly Imprinted Polymers," *Research Group Karsten Haupt*, 2015. [Online]. Available: [http://www.utc.fr/~wmpi/index.php?option=com\\_content&view=article&id=4&Itemid=8](http://www.utc.fr/~wmpi/index.php?option=com_content&view=article&id=4&Itemid=8). [Accessed: 16-Jun-2015].

- [94] E. M. A. Dourado, C. Herdes, P. R. van Tassel, and L. Sarkisov, "Molecular recognition effects in atomistic models of imprinted polymers," *Int. J. Mol. Sci.*, vol. 12, pp. 4781–4804, 2011.
- [95] C. He, Y. Long, J. Pan, K. Li, and F. Liu, "Application of molecularly imprinted polymers to solid-phase extraction of analytes from real samples," vol. 70, pp. 133–150, 2007.
- [96] T. Takeuchi and J. Haginaka, "Separation and sensing based on molecular recognition using molecularly imprinted polymers," *J. Chromatogr. B Biomed. Sci. Appl.*, vol. 728, no. 1, pp. 1–20, May 1999.
- [97] J. O'Mahony, A. Molinelli, K. Nolan, M. R. Smyth, and B. Mizaikoff, "Anatomy of a successful imprint: analysing the recognition mechanisms of a molecularly imprinted polymer for quercetin.," *Biosens. Bioelectron.*, vol. 21, no. 7, pp. 1383–92, Jan. 2006.
- [98] A. Mollnelli, J. O'Mahony, K. Nolan, M. R. Smyth, M. Jakusch, and B. Mizaikoff, "Analyzing the mechanisms of selectivity in biomimetic self-assemblies via IR and NMR spectroscopy of prepolymerization solutions and molecular dynamics simulations," *Anal. Chem.*, vol. 77, no. 16, pp. 5196–5204, 2005.
- [99] W. Mayes, "Molecular Imprinted Polymers (MIP) | NICE DB," *Nanotechnology in City Environments (NICE) Database*, 2005. [Online]. Available: <https://nice.asu.edu/nano/molecular-imprinted-polymers-mip>. [Accessed: 02-Jul-2015].
- [100] C. Branger, W. Meouche, and A. Margailan, "Recent advances on ion-imprinted polymers," *Reactive and Functional Polymers*, vol. 73, pp. 859–875, 2013.
- [101] T. Queila O. dos Santosa, Marcos A. Bezerraa, Giovana de Fátima Limab, Kristiany M. Dinizc, Mariana G. Segatellc and V. da S. S. and C. R. T. T. O. Germinianoc, "SYNTHESIS, CHARACTERIZATION AND APPLICATION OF ION IMPRINTED POLY(VINYLMIDAZOLE) FOR ZINC ION EXTRACTION/PRECONCENTRATION WITH FAAS DETERMINATION," *Artigo*, vol. 37, no. 1, pp. 63–68, 2014.
- [102] T. P. Rao, R. Kala, and S. Daniel, "Metal ion-imprinted polymers—Novel materials for selective recognition of inorganics," *Anal. Chim. Acta*, vol. 578, pp. 105–116, 2006.
- [103] M. Ahamed, X. Y. Mbianda, A. F. Mulaba-Bafubiandi, and L. Marjanovic, "Ion imprinted polymers for the selective extraction of silver(I) ions in aqueous media: Kinetic modeling and isotherm studies," *React. Funct. Polym.*, vol. 73, pp. 474–483, 2013.
- [104] J. Terao and N. Kambe, "Cross-coupling reaction of alkyl halides with Grignard reagents catalyzed by Ni, Pd, or Cu complexes with  $\pi$ -carbon ligand(s)," *Accounts of Chemical Research*, vol. 41, no. 11, pp. 1545–1554, 2008.
- [105] J. O'Mahony, A. Molinelli, K. Nolan, M. R. Smyth, and B. Mizaikoff, "Towards the rational development of molecularly imprinted polymers:  $^1\text{H}$  NMR studies on

- hydrophobicity and ion-pair interactions as driving forces for selectivity,” *Biosens. Bioelectron.*, vol. 20, pp. 1884–1893, 2005.
- [106] S. Yan, Z. Gao, Y. Fang, Y. Cheng, H. Zhou, and H. Wang, “Characterization and quality assessment of binding properties of malachite green molecularly imprinted polymers prepared by precipitation polymerization in acetonitrile,” *Dye. Pigment.*, vol. 74, pp. 572–577, 2007.
- [107] M. Firouzzare and Q. Wang, “Synthesis and characterization of a high selective mercury(II)-imprinted polymer using novel aminothiols monomer,” *Talanta*, vol. 101, pp. 261–266, 2012.
- [108] A. H. Dam and D. Kim, “Metal ion-imprinted polymer microspheres derived from copper methacrylate for selective separation of heavy metal ions,” *J. Appl. Polym. Sci.*, vol. 108, pp. 14–24, 2008.
- [109] B. Godlewska-Yłkiewicz, B. Leśniewska, and I. Wawreniuk, “Assessment of ion imprinted polymers based on Pd(II) chelate complexes for preconcentration and FAAS determination of palladium,” *Talanta*, vol. 83, pp. 596–604, 2010.
- [110] J. feng He, Q. hong Zhu, and Q. ying Deng, “Investigation of imprinting parameters and their recognition nature for quinine-molecularly imprinted polymers,” *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, vol. 67, pp. 1297–1305, 2007.
- [111] P. Metilda, J. M. Gladis, G. Venkateswaran, and T. Prasada Rao, “Investigation of the role of chelating ligand in the synthesis of ion-imprinted polymeric resins on the selective enrichment of uranium(VI),” *Anal. Chim. Acta*, vol. 587, pp. 263–271, 2007.
- [112] S. Daniel, P. Prabhakara Rao, and T. Prasada Rao, “Investigation of different polymerization methods on the analytical performance of palladium(II) ion imprinted polymer materials,” *Anal. Chim. Acta*, vol. 536, pp. 197–206, 2005.
- [113] J. Chen, L. Y. Bai, K. F. Liu, R. Q. Liu, and Y. P. Zhang, “Atrazine molecular imprinted polymers: Comparative analysis by far-infrared and ultraviolet induced polymerization,” *Int. J. Mol. Sci.*, vol. 15, pp. 574–587, 2014.
- [114] S. Slomkowski, J. V. Alemán, R. G. Gilbert, M. Hess, K. Horie, R. G. Jones, P. Kubisa, I. Meisel, W. Mormann, S. Penczek, and R. F. T. Stepto, “Terminology of polymers and polymerization processes in dispersed systems (IUPAC Recommendations 2011),” *Pure Appl. Chem.*, vol. 83, no. 12, pp. 2229 – 2259, Jan. 2011.
- [115] R. Say, E. Birlik, A. Ersöz, F. Yilmaz, T. Gedikbey, and A. Denizli, “Preconcentration of copper on ion-selective imprinted polymer microbeads,” *Anal. Chim. Acta*, vol. 480, pp. 251–258, 2003.

- [116] P. Li, F. Rong, and C. Yuan, "Morphologies and binding characteristics of molecularly imprinted polymers prepared by precipitation polymerization," *Polym. Int.*, vol. 52, pp. 1799–1806, 2003.
- [117] M. Shamsipur and A. Besharati-Seidani, "Synthesis of a novel nanostructured ion-imprinted polymer for very fast and highly selective recognition of copper(II) ions in aqueous media," *React. Funct. Polym.*, vol. 71, pp. 131–139, 2011.
- [118] S. Kawaguchi and K. Ito, "Dispersion Polymerization," *Adv Polym Sci*, vol. 175, pp. 299 – 328, 2005.
- [119] F. G. Tamayo, E. Turiel, and A. Martín-Esteban, "Molecularly imprinted polymers for solid-phase extraction and solid-phase microextraction: Recent developments and future trends," *Journal of Chromatography A*, vol. 1152, pp. 32–40, 2007.
- [120] E. Birlik, A. Ersöz, E. Açikkalp, A. Denizli, and R. Say, "Cr(III)-imprinted polymeric beads: Sorption and preconcentration studies," *J. Hazard. Mater.*, vol. 140, pp. 110–116, 2007.
- [121] Y. Ren, M. Zhang, and D. Zhao, "Synthesis and properties of magnetic Cu(II) ion imprinted composite adsorbent for selective removal of copper," *Desalination*, vol. 228, pp. 135–149, 2008.
- [122] A. S. Chauvin, J. C. G. Bünzli, F. Bochud, R. Scopelliti, and P. Froidevaux, "Use of dipicolinate-based complexes for producing ion-imprinted polystyrene resins for the extraction of yttrium-90 and heavy lanthanide cations," *Chem. - A Eur. J.*, vol. 12, pp. 6852–6864, 2006.
- [123] X. Zhu, Y. Cui, X. Chang, X. Zou, and Z. Li, "Selective solid-phase extraction of lead(II) from biological and natural water samples using surface-grafted lead(II)-imprinted polymers," *Microchim. Acta*, vol. 164, pp. 125–132, 2009.
- [124] J. Zolgharnein, N. Asanjarani, and S. N. Mousavi, "Optimization and Characterization of Tl(I) Adsorption onto Modified *Ulmus carpinifolia* Tree Leaves," *CLEAN - Soil, Air, Water*, vol. 39, no. 3, pp. 250–258, Mar. 2011.
- [125] H. Huang, Z. Huang, and X. Wang, "Selective solid-phase extraction of Ni(II) by an ion-imprinted polymer from biological samples," *Asian J. Chem.*, vol. 23, pp. 1527–1530, 2011.
- [126] W. B. Knighton and E. P. Grimsrud, "Linearization of electron capture detector response to strongly responding compounds," *Anal. Chem.*, vol. 55, no. 4, pp. 713–718, Apr. 1983.
- [127] E. R. Ziegel, *Statistics and Chemometrics for Analytical Chemistry*, vol. 46, no. 4. 2004.

- [128] K.Chalapathi, "ISSN-2231-5012 Analysis of Heavy Metals in Fish Samples after Preconcentration on Activated Carbon OH," vol. 2, no. 1, pp. 122–128, 2012.
- [129] S. Mishra, "Synthesis and characterization of Fe ( III ) -ion imprinted polymer for recovery of Fe ( III ) from water samples," vol. 69, no. October, pp. 767–772, 2010.
- [130] E. Zambrzycka, D. Roszko, B. Leśniewska, A. Z. Wilczewska, and B. Godlewska-Zyłkiewicz, "Studies of ion-imprinted polymers for solid-phase extraction of ruthenium from environmental samples before its determination by electrothermal atomic absorption spectrometry," in *Spectrochimica Acta - Part B Atomic Spectroscopy*, 2011, vol. 66, pp. 508–516.
- [131] E. Birlik, A. Ersöz, A. Denizli, and R. Say, "Preconcentration of copper using double-imprinted polymer via solid phase extraction," *Anal. Chim. Acta*, vol. 565, pp. 145–151, 2006.
- [132] P. Sikiti, T. A. Msagati, B. B. Mamba, and A. K. Mishra, "Synthesis and characterization of molecularly imprinted polymers for the remediation of PCBs and dioxins in aqueous environments.," *J. Environ. Heal. Sci. Eng.*, vol. 12, no. 1, p. 82, 2014.
- [133] X. Luo, S. Luo, Y. Zhan, H. Shu, Y. Huang, and X. Tu, "Novel Cu (II) magnetic ion imprinted materials prepared by surface imprinted technique combined with a sol-gel process," *J. Hazard. Mater.*, vol. 192, pp. 949–955, 2011.

# APPENDIX

## Appendix I

Analysis of variance (ANOVA) for optimal extraction time of selected metals using IIPs

Table 7 ANOVA for Ni ions

Anova: Single Factor		Ni-Time				
SUMMARY						
Groups	Count	Sum	Average	Variance		
15	3	170.5933	56.86444	1.008742		
25	3	161.893	53.96433	3.354444		
45	3	161.099	53.69967	4.285056		
60	3	166.9927	55.66422	3.63836		
90	3	166.54	55.51333	2.585217		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	20.53392	4	5.13348	1.725909	0.220428	3.47805
Within Groups	29.74364	10	2.974364			
Total	50.27756	14				
Fcalc < Fcrit, i.e., there is NO significant difference in the measurement						

Table 8 ANOVA for Fe ions

Anova: Single Factor		Fe - Time						
SUMMARY								
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>				
15	3	164.645	54.88167	0.189502				
25	3	167.823	55.941	4.774917				
45	3	166.962	55.654	0.371043				
60	3	163.435	54.47833	0.498408				
90	3	167.8533	55.95111	2.581753				
ANOVA								
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>		
Between Groups	5.331889	4	1.332972	0.791963	0.556436	3.47805		
Within Groups	16.83125	10	1.683125					
Total	22.16314	14						
Fcalc < Fcrit, i.e., there is NO significant difference in the measurement								

Table 9 ANOVA for Cu ions

Anova: Single Factor		Cu - Time				
SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
15	3	130.821	43.607	2.868229		
25	3	127.2267	42.4089	1.794946		
45	3	131.032	43.67733	2.310086		
60	3	128.6037	42.86789	0.175143		
90	3	136.2547	45.41822	1.48256		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	15.79975	4	3.949937	2.288236	0.131507	3.47805
Within Groups	17.26193	10	1.726193			
Total	33.06168	14				
Fcalc < Fcrit, i.e., there is NO significant difference in the measurement						

## Appendix II

Analysis of variance (ANOVA) for optimal sorbent dosage of IIPs

Table 10 ANOVA for Ni ion

Anova: Single Factor		Ni					
SUMMARY							
Groups	Count	Sum	Average	Variance			
20	3	234.956	78.31867	1.71623			
30	3	234.261	78.087	3.421179			
40	3	230.542	76.84733	0.821956			
50	3	226.4973	75.49911	0.387756			
ANOVA							
Source of Variation	SS	df	MS	F	P-value	F crit	
Between Groups	15.16502	3	5.055008	3.185701	0.084429	4.066181	
Within Groups	12.69424	8	1.58678				
Total	27.85927	11					
Fcalc < Fcrit, i.e., there is NO significant difference in the measurement							

Table 11 ANOVA for Cu ions

Anova: Single Factor		Cu				
SUMMARY						
Groups	Count	Sum	Average	Variance		
20	3	273.6677	91.22256	0.148198		
30	3	264.8317	88.27722	1.438507		
40	3	275.1697	91.72322	4.140569		
50	3	270.83	90.27667	1.591633		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	20.83918	3	6.946395	3.79641	0.058305	4.066181
Within Groups	14.63782	8	1.829727			
Total	35.477	11				
Fcalc < Fcrit, i.e., there is NO significant difference in the measurement						

Table 12 ANOVA for Fe ions

Anova: Single Factor		Fe				
SUMMARY						
Groups	Count	Sum	Average	Variance		
20	3	300.026	100.0087	0.018825		
30	3	298.7847	99.59489	0.634525		
40	3	298.4747	99.49156	0.268807		
50	3	299.1963	99.73211	0.488224		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.451855	3	0.150618	0.427171	0.739033	4.066181
Within Groups	2.820763	8	0.352595			
Total	3.272619	11				
Fcalc < Fcrit, i.e., there is NO significant difference in the measurement						

