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# An ion imprinted polymer for the selective extraction of mercury(II) ions in aqueous media

*A thesis submitted in fulfillment of the requirement for  
the degree of*

Master of Science in Chemistry

By

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December 2009

# Dedication

*To myself and my kids*

## Acknowledgements

Prof. Nelson Torto: Thanks, for giving me a chance and having a conviction in me that I can make it in research even when my first degree marks were not convincing. I guess you will pass for a talent scout, Prof! Thank you so much, I have rediscovered myself in the academia once again. For that I will forever be indebted to you.

Dr. Luke Chimuka: First and foremost, I should thank you for being a good host and probably more generous especially the first time I came to Witwatersrand University. You took care of two guests even though you were expecting `Me` only. That was so sweet of you. On a more serious note, thank you for your tutelage in the area of MIPS, an area I am now synonymous with in our lab (F12), here at Rhodes University.

Dr. Zenixole Tshentu: Thanks, for taking me through ICP-OES. Your academic suggestions regarding mercury(II) analysis were quite handy.

Tlotlo Belinda Baesi: Thank you so much for your emotional support and the encouragement right from University of Botswana where I started my MSc, through Wits, to here at Rhodes. Writing is limiting in the sense that it is one sided and does not allow one to act, otherwise a big hug will do. Thank you, Porty.

To my anchor in Grahamstown, Janes Mokgadi, you have been a source of hope and inspiration. At times I would want to quit for one reason or another but you would find a way around me. You have also been so resourceful. Thank you for your academic criticism and sarcasm. Always reminding me that I don't afford to relax and sleep like you do because I do not have a master's degree. I guess next year will be a different story.

Chemistry Departments: University of Botswana, Witwatersrand University and Rhodes University: Thank you for all the assistance and support.

To my colleagues in F12 (Prof Torto research group Rhodes University): Your professional critiques of my work during our weekly meetings have gone a long way in honing my oratory and presentation skills.

To all my family and friends: Thank you for being there!

***'Le ka moso багаetsho'***

## Abstract

This thesis presents the application of an imprinted mercury(II) polymer that we synthesized by copolymerizing the functional and cross-linking monomers, N'-[3-(Trimethoxysilyl)propyl]diethylenetriamine (TPET) and tetraethylorthosilicate (TEOS) in the presence of mercury (II) ions as template. A bulk polymerization method following a double-imprinting procedure and employing hexadecyltrimethylammonium bromide (CTAB), as a second template to improve the efficiency of the polymer was employed in the synthesis. The imprinted polymer particles were characterized by Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and their average size determined by screen analysis using standard test sieves. The relative selective coefficients ( $k'$ ) of the imprinted polymer evaluated from selective binding studies between  $\text{Hg}^{2+}$  and  $\text{Cu}^{2+}$  or  $\text{Hg}^{2+}$  and  $\text{Cd}^{2+}$ , were 10588 and 3147, respectively. These values indicated highly favored  $\text{Hg}^{2+}$  extractions over the two competing ions. Application of the polymer to various real water samples (tap, sea, river, pulverized coal solution, treated and untreated sewerage from the vicinity of Grahamstown in South Africa) showed high extraction efficiencies (EEs) of  $\text{Hg}^{2+}$  ions; (over 84% in all cases) as evaluated from the detected unextracted  $\text{Hg}^{2+}$  ions by inductively coupled plasma optical emission spectroscopy (ICP-OES). The limit of detection (LOD,  $3\sigma$ ) of the method was evaluated to be  $0.036 \text{ ng ml}^{-1}$  and generally the data ( $n=10$ ) had percentage relative standard deviation (%RSD) of less than 4%. These findings indicate that the double-imprinted polymer has potential to be used as an efficient extraction material for the selective pre-concentration of mercury(II) ions in aqueous environments.



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## List of Abbreviations

AA:	Acrylamide.
AMPSA:	2-acryl-amido-2-methyl-1-propanesulfonic acid
APTMS:	3-aminopropyltrimethoxysilane
ASE:	Accelerated solvent extraction
CEC:	Capillary electrochromatography
$C_i$ :	Initial concentration
CPMAS:	Cross polarization – magnetic angle spinning
CRM:	Certified Reference Material
CTAB:	Cetyltrimethyl ammonium bromide
CVAAS:	Vapour atomic absorption spectrometry
D2EHPA:	di-ethylhexylphosphoric acid
DEAEM:	Diethylamino ethyl methacrylate
DMF:	Dimethylformamide,
DNA:	Deoxyribonucleic acid
DOLPA:	Diolelphosphoric acid
DVB:	Divinylbenzene
EDMA:	Ethylene glycol dimethacrylate
EE:	Extraction efficiency
EHEHPA:	di-22-ethylhexyl-2-ethylhexyl phosphonic acid
FTIR:	Fourier transformer infrared spectroscopy
GC:	Gas chromatography

GC-MS:	Gas chromatography mass spectrometry
GMA:	Glycidyl methacrylate,
HCl:	Hydrochloric acid
HG-AFS:	Hydrogen Generator Atomic Fluorescence Spectrometer
HNO <sub>3</sub> :	Nitric acid
ICP-OES:	Inductively coupled plasma optical emission spectrometry
IIP:	Ion imprinted polymer
k':	Selectivity coefficient
K <sub>d</sub> :	distribution coefficient
LLE:	Liquid-liquid extraction
LOD:	Limit of detection
MAA:	Methacrylic acid
MAAM:	Methacrylamide
MASE:	Microwave assisted solvent extraction
MeCN:	Acetonitrile
MeOH:	Methanol
MIPs:	Molecularly imprinted polymers
NIP:	Non-imprinted polymer
NMR:	Nuclear magnetic resonance
PAH:	Polycyclic aromatic hydrocarbons
PCB:	Polychlorinated biphenyls
PETRA:	Pentaerythritol trimethylacrylate
PLE:	Pressurized liquid extraction

Py :	Pyrrole
RSD:	Relative standard deviations
SAX:	Strong anion exchange
SCX:	Strong cation exchange
SEM	Scanning electron microscopy
SFE:	Supercritical fluid extraction
SPE:	Solid phase extraction
SPME:	Solid phase micro-extraction
ST:	Styrene
TAIC:	Triallyl isocyanurate
TEM:	Transmission electron microscopy
TEOS:	Tetraethyl orthosilicate
TFMAA:	Trifluoromethyl acrylic acid
THF:	Tetrahydrofurane
TIBPS:	Tri-Isobutylphosphine sulfide
TRIM:	Trimethylolpropane trimethacrylate
UAE	Ultrasonic assisted extraction
UAEE:	Urocanic acid ethyl ester
UPS:	Ultrasound probe sonication
UV:	Ultra violet
VP:	Vinyl pyridine
XRF:	X-ray fluorescence

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# Chapter 1

## 1.0 Introduction

In recent years the release of various harmful heavy metal ions into the environment has attracted great attention worldwide because of their toxicity and widespread use. Mercury(II) is among the ones which are of great concern [1]. It is a widely distributed environmental pollutant in aqueous environments and its toxicity to humans and animals even at low concentrations is well known [2]. Together with its organic forms it may cause kidney toxicity, neurological damage, paralysis, chromosome breakage, and birth defects [3]. Mercury(II) is included in all lists of priority pollutants, and as a result different regulations and analytical methods have been developed for monitoring its level in water [4]. However, the current analytical monitoring methods have a challenge of selectivity. To address the challenge, sample preparation strategies relying on selective, robust, cheap and intelligent functional materials are needed prior to separation and detection. Such materials have recently been identified as molecular imprinted polymers (MIPs) or ion imprinted polymers (IIPs). Imprinted polymers are synthetic, nano - porous polymers possessing specific cavities designed for a target analyte(s). By mechanism of molecular recognition imprinted polymers are used as selective tools for the development of various analytical techniques such as liquid chromatography, artificial receptors, binding assays, biosensors and solid phase extraction (SPE).

Sample preparation is one step out of a series making up the overall analytical process (Fig. 1.1). It consists of several strategies which are all geared towards bringing the concentration of the analyte of interest to detectable levels before separation and detection. Over the last decade much progress has been made in the field of detection technology [5]. Nowadays there are commercially available hyphenated analytical instruments with quantification and detection limits down to femtogram levels. Despite these advances, direct determination of specific, toxic, heavy metal ions in natural waters is still a challenge due to the presence of the ions in trace quantities in highly complex matrices often coupled with sample to sample variability. Surprisingly, sample preparation strategies are still the ones neglected the most even though research has shown that they account

for more than 80% of the time spent on one analysis run and up to 50% of the possible error of the measurement [6,7]. This percentage may be considered to be even higher in metal speciation analysis, where the sample matrices are commonly more complex, the species concentration low and its distribution heterogeneous as well as having to selectively differentiate between closely related specie [7]. To address the challenges of selectivity and interfering matrices in heavy metal ion analysis, this thesis tends to have a bias towards metal sample preparation strategies. The thesis first gives an account of the conventional sample preparation strategies including the non selective extraction techniques followed by the selective ones, in particular molecular imprinting technology which was employed in experimental work. The thesis concludes with a report of the performance of a novel imprinted polymer synthesized for the selective extraction of the toxic mercury(II) ions from natural waters.

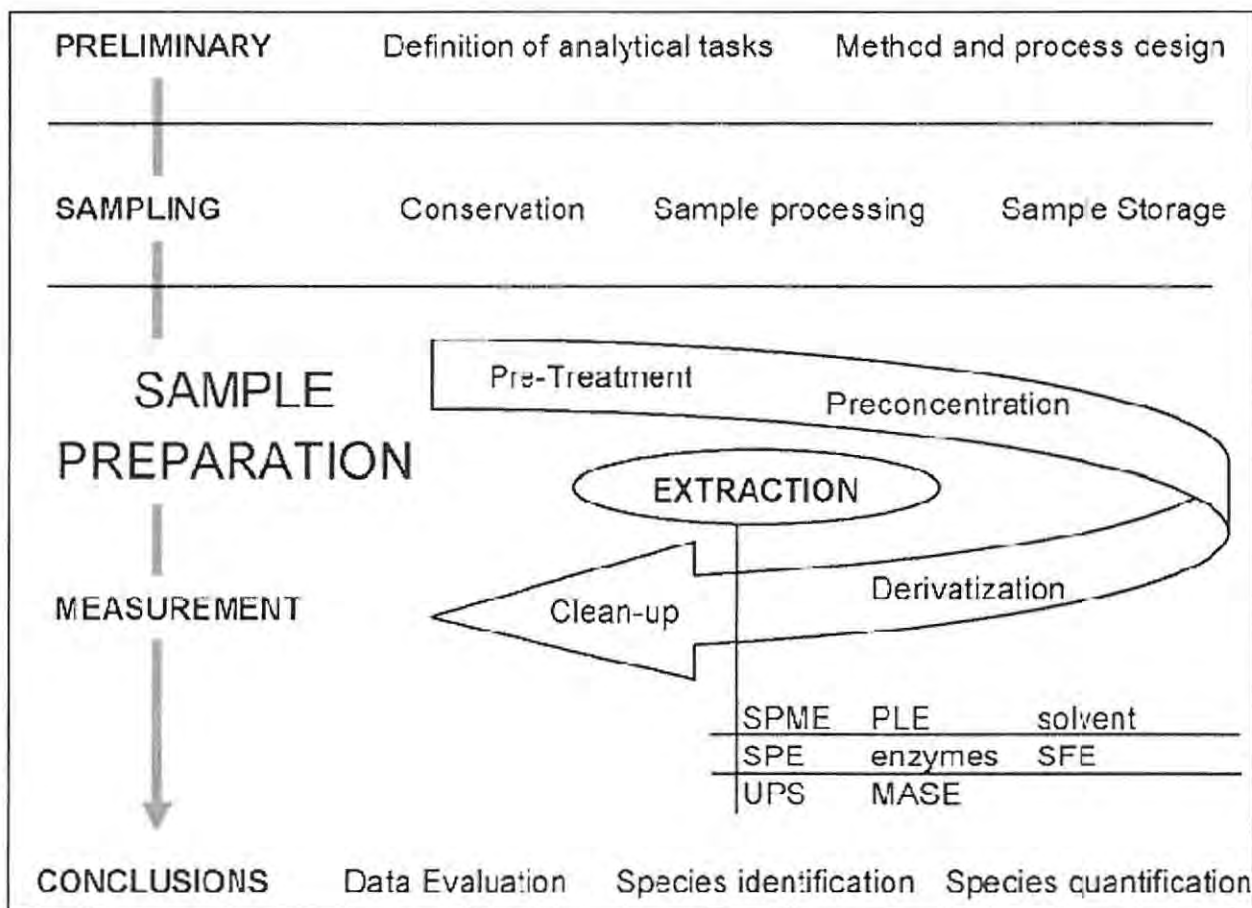


Fig. 1.1 Sample preparation strategies within the general steps of an analytical process in speciation [1].

## **1.2 Sample preparation strategies for heavy metal ion analysis**

### **1.2.1 Sample pre-treatment**

Sample pre-treatment consists of a whole series of manipulations. In the easiest case, only solubilization has to be applied to bring the entire sample to be analysed into solution. This can be achieved by acid, alkaline or enzymatic hydrolysis. The obtained solution may be fit for extraction techniques to be applied to isolate the analyte of interest or, in limited cases, for direct analysis. Leaching is the most widely used method for sample pre-treatment in metal analysis. Leached “species” do not reflect a well defined chemical form of a metal but a range of different compounds which are brought into solution following a specific analytical protocol [7]. In order to bring low analyte to detectable levels, sometimes a pre-concentration step is employed.

### **1.2.2 Pre-concentration**

Toxic heavy metal ions are usually present in natural waters at trace levels [5]. The sensitivity of the available detection system may not be adequate and detection limits of the method have to be improved. Pre-concentration, is often necessary to bring the concentrations to detectable levels. The techniques or sorbents involved in conventional pre-concentration, e.g. hot- or cold-trapping, amalgam formation, immobilized chelating agents, chelating sorbents or ion-exchange materials, are often combined with either the extraction or the derivatisation step [5,8].

### **1.2.3 Derivatisation**

Derivatisation may be necessary to protect species integrity through the whole analytical process, to produce extractable species or to convert originally present species into a form suitable for detection. For organometallic compounds, derivatisation is commonly related to chromatographic analysis in order to provide stable compounds prior to separation and in the form that can be detected by the chosen detector. The most common way is by hydride generation using tetrahydroborate, followed by alternative aqueous alkylation reactions such as those provided by sodium tetraethylborate, tetra(*n*-propyl)borate or tetrabutylammoniumtetrabutylborate in gas chromatography (GC) analysis of organometallic compounds[5]. Morabito *et al* have reported the



derivatisation methods for organotins which have been extended to other heavy metals like mercury in a variety of environmental samples [8]. Their method involved the use of non-aqueous alkylation, such as the use of Grignard reagents, which currently is less used due to a relatively tedious multi-step procedure. Alkylation assisted in thermally stabilizing the ions of interest by reducing their volatility and allowing for detection before they evaporate off.

#### **1.2.4 Clean-up**

Clean-up involves the removal of matrix components such as lipids, fats, and proteins usually with larger molecular weights than the analyte of interest from the sample. The large molecules may seriously affect derivatisation or extraction yields as well as prevent detection due to interference. A different clean-up procedure may be required after the extraction step, especially when a non-specific extraction procedure has been employed. The obtained extracts will not be fit for direct use in subsequent analyte determination hence a solvent with lesser elution strength will be used to wash off the loosely adsorbed matrix components [5] which might interfere with effective separation or detection.

### ***1.3 Extraction techniques for heavy metal ion analysis***

Extraction techniques are employed to selectively separate the target species from its matrix (e.g. water, soils, sediments, biological tissue or fluids). Several reviews have dealt with “classical” techniques of extraction [9–13]. Introduced in the mid 19<sup>th</sup> century, soxhlet extraction has been one of the classical extraction methods that have been extensively applied until the development of modern extraction techniques [9]. Classical extraction techniques, in particular soxhlet is slow (up to 24 – 48 h of extraction) with a very high consumption of organic solvents that have to be evaporated, although with very high recoveries and multiple extraction possibilities [9, 10].

To address the challenges associated with the classic techniques, efficient and fast extraction methods for toxic heavy metal species from water related samples, like ultrasonic assisted extraction (UAE) and pressurized liquid extraction (PLE) were developed. Both have reduced the

volume of extraction solvent required and shortened the sample preparation time (less than 1 h) when compared to conventional soxhlet extraction. The extraction recoveries attained were comparable to those of the conventional soxhlet, 80% or more for various heavy metal analysis [14-17].

### **1.3.1 Solvent extraction / Liquid-liquid extraction (LLE)**

Solvent extraction as a stand-alone technique is rarely used because the pre-concentration factors achieved are typically low (1:50–1:250) and often time consuming. However, it is relatively robust and can be directly applied to a non-filtered sample, even with complex matrices and allows the transfer of analytes into a nonpolar organic solvent. The organic phase is then ready for subsequent analyses, e.g. by gas chromatography (GC). Method development employing this technique is relatively simple for non polar species such as tetraalkyllead compounds [18], which may be directly extracted from natural waters by saturating them with salt and adding hexane. More polar but water immiscible solvents (ethylacetate, toluene) are recommended for quasi ionic species with covalent character [19] such as tributyl- and triphenylmercury and tin. Metallic and organometallic ions with a reduced number of organic substituents cannot be quantitatively extracted into such organic solvents as they require in general some kind of derivatisation or use of sorbent material. For example, the formation of chelate complexes such as carbamates [20] or tropolone [21]. The major disadvantage of bulk LLE is the need for large volumes of organic solvents. Also, due to limited selectivity particularly for trace level analysis, there is need for clean up or analyte pre-concentration prior to instrumental analysis. The need to reduce solvent consumption led to micro extraction forms of LLE [22-25] which have shown detection limits comparable to traditional LLE [26] but still with inadequate selectivity.

### **1.3.2 Pressurized liquid extraction (PLE)/Accelerated solvent extraction (ASE)**

PLE, also known as ASE, is an analyte- and matrix independent technique which provides cleaner extracts than the time-consuming classical procedures used for extraction of compounds from complex matrices. The process is based on applying increased temperatures, elevated pressures, and keeping the solvent below its boiling point, thus accelerating the extraction kinetics and enabling safe and rapid extractions. Since the first instruments became commercially available in

the mid 90's, this technique gained widespread acceptance for extraction of organic micro-pollutants such as pesticides, polycyclic aromatic hydrocarbons (PAH's), polychlorinated biphenyls (PCB's) and dioxins from a great variety of environmental and clinical matrices [27, 28]. Recently, metal speciation studies though rare [29, 30] have also employed this extraction technique. Organotin compounds were extracted with methanolic solution of 1 M sodium acetate and 1 M acetic acid from sediments [29], providing complete recoveries when applying four static cycles at 100 °C. In the case of the extraction of organoarsenicals from ribbon kelp, an extraction efficiency of 73% was achieved using a methanol–water mixture as solvent [30]. The limited use of this approach in speciation is due to the following challenges: (i) only fixed and relatively high volume extraction cells are available, this impedes working with small volumes of sample; (ii) no exact volume control of solvent used for extraction is provided; and (iii) commercial models are standalone instruments that cannot be hyphenated to the separation and detection step of an analytical method. In order to overcome these limitations, a miniaturized PLE device has been recently developed [31]. The system has a small thermostatic oven in which up to three extraction cells of variable size can be simultaneously mounted thus allowing for the control of the extraction fluid volume down to 0.05 mL. Furthermore the system can easily be coupled to capillary electrophoresis (CE), gas chromatography (GC) and liquid chromatography (LC).

### **1.3.3 Microwave assisted extraction (MAE)**

The efficiency of a microwave field for analyte extraction from different environmental matrices is well known in sample preparation [32–35] as considerably efficient and faster than conventional soxhlet extraction procedures. A low power focused microwave field, typically 20–90W which is about 10% of the maximum power provided by commercial systems, is usually employed to speed leaching of metallic specie [34]. At such powers the integrity of the specie is not affected while working at atmospheric pressure [35]. Microwave based strategies for speciation analysis of tin, arsenic, mercury and selenium from matrices such as urine, fruit juices, fish, mussel, and sediments were reviewed by N'obrega et al. [36]. They emphasized both its suitability for the leaching of labile species and to support derivatisation reactions. However, in order to avoid species losses or transformation, parameters such as extraction medium, applied microwave power and exposure time have to be carefully optimized. An intrinsic disadvantage is that usually polar solvents have to be used; often the extracted analytes have to subsequently be derivatised and transferred into a

non-polar solvent to be suitable for GC analysis, which introduces an additional step in the method to be developed. Nevertheless, this drawback can be overcome by combining leaching, derivatisation and MAE into one single step. When this was achieved it gave rise to a series of methods for the determination of tin and mercury species [37, 38]. Further the technique can be easily hyphenated with purge and trap devices [39], thus making microwave assisted procedures followed by solvent extraction and gas chromatographic separation an attractive alternative in environmental analysis for many metallic and organometallic species.

#### **1.3.4 Ultrasound probe sonification (UPS)**

The use of ultrasonic radiation is a well known extraction tool, commonly used to achieve removal of particles from a substrate (in general glassware) or to accelerate mixing processes. The use of standard ultrasonic baths operating at a frequency of 40 kHz is, in this context, easy to implement and efficient, though the standing wave pattern created in a bath is neither uniform nor of high density [40]. The driving force of sono-chemical action is the acoustic cavitations, provoked by bubbles formed by the sound wave in a liquid that continuously compresses and decompresses. The results are extreme local temperatures and pressures generated in the liquid as well as solute thermolysis and formation of hydroxyl radicals and hydrogen peroxide, the latter in case of aqueous solvents. As a consequence, when a solid is present in a solvent, compounds present in the solid may be partially or totally extracted into the liquid medium [41] faster than by other classical methods [42]. This technique has been successfully applied to the speciation of arsenic in the immediately mobilisable fraction of river sediments [43] by using focused ultrasound, followed by determination with high performance liquid chromatography–hydride generation–atomic fluorescence detection.

#### **1.3.5 Supercritical fluid extraction (SFE)**

A promising approach to reduce the consumption of and exposure to organic solvents is the use of fluids in the supercritical state. Among the properties attractive for extraction is the low viscosity and the low diffusion coefficients, contributing to a rapid mass transfer of solutions and enhanced interactions at molecular level, thus favoring solubilization processes. Unfortunately, fluids possessing critical points which are easily realizable are limited. So far CO<sub>2</sub> is the most prominent

supercritical fluid used for extraction. It is non-toxic, non flammable, relatively cheap and possesses a low critical temperature, enabling extractions under mild conditions, thus protecting thermally labile species. Applications in speciation are rare though, due to the low efficiency of the technique to extract highly polar or ionic compounds [44]. These drawbacks can be partly overcome by addition of complexing agents and/or modifiers in order to enhance extraction efficiencies [45–47]. Also the combination of solid phase extraction (SPE) followed by SFE of arsenic and mercuric derivatives have been successful. The inorganic arsenic specie can be extracted from CO<sub>2</sub> in the presence of thioglycolic acid methyl ester [48]. The derivatization reaction is carried out in supercritical CO<sub>2</sub>, leading to the formation of derivatives that can be determined reproducibly by GC. Reported recoveries under the optimum extraction conditions ranged from 90 to 103%. However, the results were not validated with CRMs [48].

### **1.3.6 Solid phase extraction (SPE)**

Solid phase extraction (SPE) involves the partitioning of selected analytes between a gas, fluid or liquid phase (sample matrix) into a solid (sorbent) phase [49]. The principal goals of SPE are trace enrichment (pre-concentration), matrix removal (separation) and medium exchange (transfer from the sample matrix to a different solvent or gas phase) [50, 51]. The distribution of analyte between an aqueous solution and the sorbent in SPE is based upon mechanisms such as adsorption, ion exchange, chelation, ion-pair or complex formation and other chemical reactions on or in the sorbents. A typical SPE procedure consists of three to four successive steps which include conditioning of the solid sorbent with an appropriate solvent, percolation of the sample through the solid sorbent, washing of the sorbent with a solvent of low elution strength to eliminate matrix components that have been retained on the solid sorbent (without displacing the analytes) and finally eluting the analytes of interest by employing an appropriate solvent with a higher elution strength (Fig. 1.2).

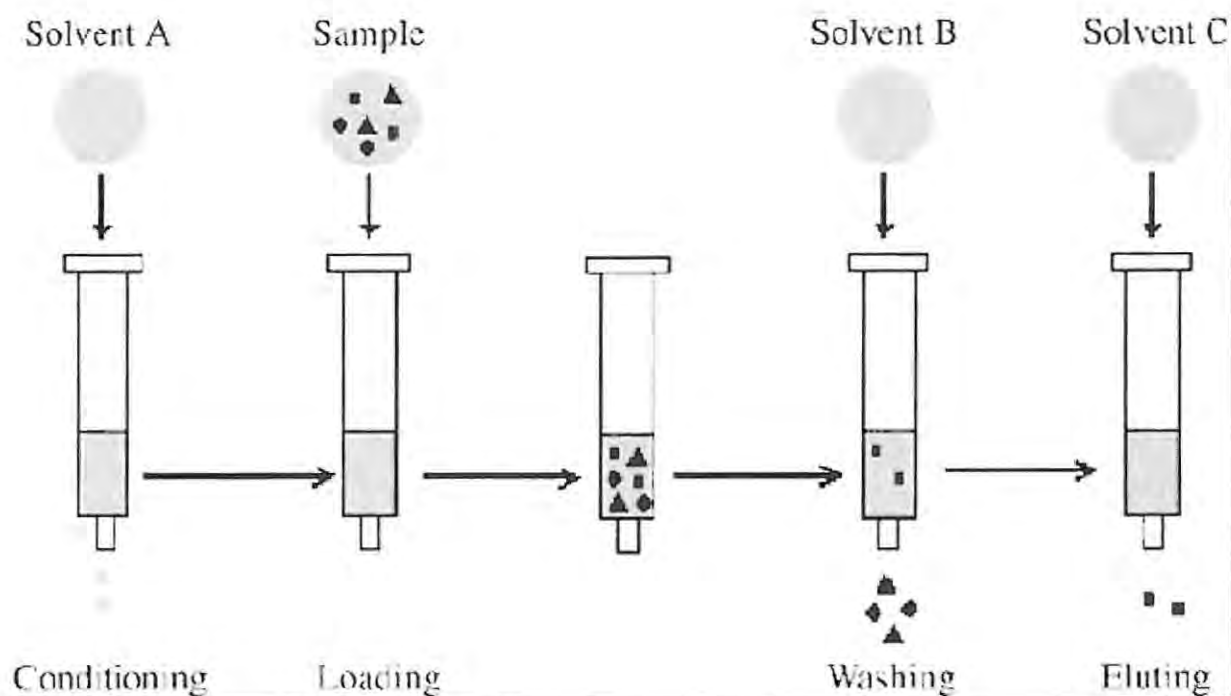


Fig. 1.2 Schematic diagram of SPE procedure [51]. The column is packed with sorbent. Solvent A, B and C are the solvents for conditioning, washing and eluting steps, respectively. The symbols of triangle and circle represent the impurities, and the symbols of square represent the target molecules.

Of the two modes of SPE, online flow injection SPE has several virtues over offline methods, viz. high sample throughput, improved precision, low sample and reagent consumption, reusability and green concept [51]. It is this mode of SPE that makes it even more, the method of choice for sample preparation. For instance, when compared to the commonly used LLE method, SPE can reduce the time required (especially the automated form), can handle small sample volumes (50 – 100  $\mu\text{L}$ ), and consumes small volumes of solvent [52]. Consequently, SPE is the most popular sample preparation method to date [53].

### 1.3.6.1 Conventional SPE sorbents/extractors

Solid phase extraction sorbents are based either on physical loading or chemical binding of selected chelating reagents to different solid supports such as ion exchange resins [54,55], modified silica [56,57], modified clay [58], and modified alumina [59], activated carbon [60,61], cellulosic derivatives [62,63] as well as polyurethane foam [64]. The selectivity of the solid phase extractors and pre-concentrators mainly depends on the structure of the immobilized organic compound as a

whole, the nature of the incorporated donor atoms (O, N, P and S), the positioning of the functional groups along the surface of the solid support and the steric requirements of the complex formed after uptake of the desired metal ion [65]. The most successful solid phase-extractors for Hg(II) and other soft metal ions are those immobilized by sulfur and nitrogen or phosphorous containing compounds [66]. Amongst these compounds are dithiozone [67] and dithiocarbamate derivatives [68] loaded to silica gel for selective extraction and pre concentration of Hg(II) ions. 2-Amino-1-cyclopentene-1-dithiocarboxylic acid is used for extraction of Ag(I), Hg(II) and Pd(II) [69]. 2-Mercaptobenzothiazole modified silica gel is used for flow injection on-line pre-concentration and separation of Ag(I) before determination by FAAS [70]. Tri-isobutylphosphine sulfide (TIBPS) sorbed on silica gel has been used for sorption and extraction chromatographic separation of Hg(II) [71]. 2-Aminoacetylthiophenol functionalized amberlite XAD-2 was utilized for pre-concentration and determination of Cd(II), Hg(II), Ag(I), Ni(II), Co(II), Cu(II) and Zn(II) in tap water and river water by ion coupled plasma atomic emission spectroscopy (ICPAES) [72]. Moreover, the uptake of inorganic mercury species by polysiloxane immobilized amino propyl and mercapto ligands have recently been studied by Walcarius *et al.* [73] as a function of pH and chloride concentration. The results showed higher recoveries, 99.5% (3.7% more) with low % relative standard deviation (%RSD) when compared to the functionalized amberlite.

Commonly used methods for organotin and arsenic extraction, providing pre-concentration factors up to 1000 use the bonded silica sorbents with a variety of functional groups, such as octadecyl (C18), octyl (C8) and ethyl (C2) to provide non-polar interactions [74-76]. C60 fullerenes have also been used to extract tin species from water [75]. They were complexed with sodium diethyldithiocarbamate and subsequently eluted from the column with ethyl acetate containing sodium tetra-*n*-propylborate as a derivatising reagent prior to detection by gas chromatography – mass spectrometry (GC–MS) [75]. This approach was found to be quite robust, sensitive (detection limits: 0.8 to 1.5  $\mu\text{g mL}^{-1}$ ), fast (2 min for extraction), did not suffer from interferences [As(III), Cd(II), Cu(II), Co(II), Fe(III), Ni(II), Mn(II), Sb(III), Pb(II) and Zn(II)] and was validated using certified reference materials (CRM's). Ionic compounds may selectively be pre-concentrated using anionic or cationic cartridges. This would avoid possible signal overlapping with related species in complex matrices when using atomic detectors. For example, the As(III) interference on arsenobetaine in arsenic speciation could be avoided by placing an anionic cartridge before the separation column, leading to the retention of arsenite, and arsenate [77,78]. Strong anion exchange- and reverse

phase-cartridges [79] were found to be suitable for separation of arsenate and arsenite in water analysis. The SPE cartridges could be reused up to 100 times and the method was relatively fast, speciation analysis was achieved within 1.5 min, with detection limits of 0.2 and 0.4 ng mL<sup>-1</sup>, for As(III) and As(V), respectively, using hydrogen generator atomic fluorescence spectrometer (HG-AFS) detection. The retained complex was eluted by 2 mL of 2 M HCl. A variety of silica-based SPE cartridges containing different types of sorbent materials, i.e. C8, C18, cyanopropyl, 2,3-dihydroxypropoxypropyl, quaternary amine (strong anion exchange, SAX), C8 and -NR, primary & secondary amine, aminopropyl, ethylbenzene sulfonic acid (strong cation exchange, SCX), propylcarboxylic acid & C18, -SO & -NR (mixed-mode), were tested for arsenic species retention and elution characteristics [80]. Selective elution of arsenic compounds was achieved with 1.0 M acetic acid or 1.0 M HNO<sub>3</sub> from both the SAX and SCX-3 material but not from the mixed mode cartridges.

#### ***1.4 New selective sorbents/extractors***

All the extraction methods including the classic SPE sorbents discussed in the previous sections, despite their attractive features retain targeted analytes by non-selective hydrophobic interactions that lead to a partial co-extraction of interfering specie. To enhance extraction selectivity, new selective materials based on molecular recognition were recently developed. They include immunosorbents (ISs) whose affinity and selectivity stem from antigen – antibody interactions. Therefore, they allow selective extraction of the targeted analyte. Several reviews have highlighted the interest in immunoextraction as a selective sample pretreatment method [81-83]. However, the development of ISs is time consuming, and relatively expensive. These drawbacks have contributed to the recent development of molecularly imprinted polymers (MIPs).

MIPs are synthetic polymeric materials with specific nano-sized cavities designed for a template molecule. The retention mechanisms involved is based on molecular recognition just like in ISs and as such are often referred to as synthetic antibodies in comparison to ISs. Indeed both have comparable selectivities but MIPs offer better handling and stability and are cheaper and easier to prepare [84] which make them attractive for numerous applications. Over 1450 references related to the use of MIPs in a large range of application areas have been recently collected [85]. Therefore, MIPs have already been successfully used as an alternative tool to these biological entities in



several analytical fields such as separation of enantiomers in LC or CEC [86, 87], binding assays [88, 89] and sensors [90–92].

In recent years the development of MIPs for solid-phase extraction (SPE) has been extensively reported [93–98] in the areas of environmental, food and pharmaceutical analysis including their use as selective sorbents for the extraction or for the clean-up of different classes of compounds from various complex matrices. Numerous papers have been reported on the application of SPE, Table 1.1 [99–137]. SPE is the most advanced application area of the MIP [138]. The principle of selective extraction on MIPs is the same as that of immunosorbents and follows the SPE procedure in Fig. 1.2. After a conditioning step, the sample is percolated through the MIP and then a washing step removes interfering compounds that were partially retained. The desorption of analytes is achieved by percolating a solvent able to disrupt the selective interactions involved between the MIP and the target analyte.

**Table 1.1 MIP for SPE of compounds from real matrices coupled off-line or on-line with analytical methods**

Target analytes	Template	Matrices	Monomer/CL/solvent	Sample pretreatment	Ref.
Alkyl-phosphonates	Pynacolyl-methylphosphonate	Soil	MAA/EDMA/MeCN	Extraction with pressurized hot water	[99]
		Water	MAA/EDMA/MeCN	SPE on Oasis HLB polymer	[100]
Alkyl-phosphonates and -phosphate	Diisopropylmethylphosphonate, tributylphosphate	Diesel fuel, gasoline, air extract	4-VP/TRIM/chloroform or MAA/TRIM/chloroform	Dilution in pentane	[101]
Alfuzozine	Alfuzozine	Soil	MAA/EDMA/CH <sub>2</sub> Cl <sub>2</sub>	Soil: solvent extraction	[102]
Benzo(a)pyrene	Benzo(a)pyrene	Waters	2-VP/DVB/CH <sub>2</sub> Cl <sub>2</sub>	Dilution with MeCN	[103]
Bisphenol A	Terbutylphenol	Surface water	4-VP/EDMA/**	No	[104]
Bisphenol A	Bisphenol A	Surface water	4-VP/EDMA/toluene + dodecanol	No	[105]
Bisphenol A	Bisphenol A	Tap water	APTMS/TEOS/MeOH	No	[106]
Bisphenol A	Bisphenol A-d16	Surface water	4-VP/EDMA/	Acidification	[107]
Carbamazepine	Carbamazepine	Waste water	MAA/DVB/CH <sub>2</sub> Cl <sub>2</sub>	pH adjustment	[108]
Carbaryl	Carbaryl	River water	AA/EDMA/MeCN	Filtration	[109]
Catechol	Catechol	Aqueous effluent	4-VP/EDMA/MeCN	No	[110]
		Tap water	4-VP/EDMA/MeCN	SPE on methacrylic polymer + C18	[111]
Chloro-, nitro-phenols	Chlorophenol	River water	4-VP/EDMA/MeCN	Acidification	[112]
Chlorophenoxy acetic acids	Trichlorophenoxy acetic acid	River water	4-VP/EDMA/MeOH-H <sub>2</sub> O	Acidification	[113]
17-βestradiol	6-Ketoestradiol	River water	4-VP/EDMA/toluene**	No	[114]
17-βestradiol	17-βestradiol	Waste water	4-VP/EDMA/acetonitrile	No	[115]
Fluoroquinolones	Ciproflaxin	Soil	MAA/EDMA/MeOH	Soil extract diluted in methanol	[116]
Methylxanthines	Caffeine	Water	APTMS/TEOS/aqueous solution	No	[117]
Metsulfuron-methyl	Metsulfuron-methyl	Drinking water	4-VP/EDMA/MeCN: Chloroform*	Addition of EDTA, filtration	[118]
Microcystin-LR	Microcystin-LR	Drinking water	AMPSA + UAEE/EDMA/DMSO	Addition of buffer (pH 4)	[119]
Monosulfuron	Monosulfuron	Soil	MAA/EDMA/DMF	Solvent extraction of soil	[120]
Naphtalene sulfonates	1-Naphtalene sulfonate	River water	4-VP/EDMA/MeOH, water	No	[121]
Nitrophenol	Nitrophenol	River water	4-VP or MAA/EDMA/ MeCN	Acidification	[122]
Non-steroidal anti-inflammatory drugs	Ibuprofen	River water	4-VP/EDMA/Toluene	No	[123]
Organotin compounds	Bu <sub>2</sub> SnO- <i>m</i> -vinylbenzoin	Certified mussel tissue	Sodium MA/EDMA/CAN	Solvent extraction of mussel tissue	[124]
Phenylureas	Isoproturon	Surface water	MAA/EDMA/toluene	SPE (PS-DVB)	[125]
		Surface water	MAA/EDMA/toluene	SPE on Oasis HLB polymer	[126]
Pirimicarb	Pirimicarb	Water surface	MAA/EDMA/CHCl <sub>3</sub>	No	[127]
Polyphenols	Caffeic acid p-Hydroxybenzoic	Olive mill waste water	4-VP/PETRA/THF 4-VP/EDMA/THF	pH control	[128]
		Water and soil	TFMAA/DVB/CH <sub>2</sub> Cl <sub>2</sub>	Addition of EDTA	[129]
Sulfonylureas	Metsulfuron-methyl	Soil	MAA/EDMA/DMF	Extraction by water-MeOH	[130]
		Soil	MAA/EDMA/DMF	Extraction by water-MeOH	[130]
Triazines	Irgarol	River water	TFMAA/EDMA**	Filtration	[131]
		Water surface and sediment	MAA/EDMA/CH <sub>2</sub> Cl <sub>2</sub>	Water: no. Soil: soxhlet extraction (MeOH)	[132]
Triazines and metabolites	Terbutylazine	Humic acid	MAA/EDMA/toluene	SPE (RAM)	[133]
		Surface water	MAA/EDMA/toluene	SPE (C <sub>18</sub> )	[134]
	Surface water	MAA/EDMA/CH <sub>2</sub> Cl <sub>2</sub>	No	[135]	
	Soil	MAA/EDMA/CH <sub>2</sub> Cl <sub>2</sub>	No	[136]	
	Propazine	Water, soil	MAA/EDMA/toluene	Water	[137]

where; AA: acrylamide. AMPSA: 2-acryl-amido-2-methyl-1-propanesulfonic acid. APTMS: 3-aminopropyltrimethoxysilane. DEAEM: diethylamino ethyl methacrylate. DMF: dimethylformamide, DVB:

divinylbenzene, EDMA: ethylene glycol dimethacrylate, GMA: glycidyl methacrylate, MAAM: methacrylamide. MAA: methacrylic acid. MeCN: acetonitrile; MeOH: methanol; PETRA: pentaerythritol trimethylacrylate, Py : Pyrrole, ST: styrene, TAIC: triallyl isocyanurate, TEOS : tetraethyl orthosilicate, THF: tetrahydrofurane; TFMAA: trifluoromethyl acrylic acid. TRIM: trimethylolpropane trimethacrylate, UAEE: urocanic acid ethyl ester, VP: vinyl pyridine. \*: Addition of reactants required for one or multi-step swelling. \*\*: Several solvents required for particular conditions of polymerization [138].

In the next chapters, the concept of molecular imprinting, ionic imprinting, the principles of synthesis of imprinted polymers, and the different approaches of evaluating their physical characteristics as well as selectivity are discussed. Their use as sorbent materials for the selective extraction of toxic, heavy metal ion specie in complex environmental matrices is also discussed with special emphasis on their potential as SPE sorbents for the selective extraction of Mercury(II) ions from aqueous media.

## Chapter 2

### *2.0 Molecular imprinting*

Molecular imprinting can be dated back to the early 1930's when a Soviet chemist M.V. Polyakov [139] prepared silica gels and observed that when prepared in the presence of a solvent additive they demonstrated preferential binding capacity for that solvent. A similar study by a student of Linus Pauling, Frank Dickey, in 1949 published the results of experiments where silica gels had been prepared in the presence of dyes [140]. Dickey observed that after removal of the 'patterning' dye, the gels would rebind the same dye in preference to the others. Molecular imprinting did not progress much further thereafter until in the major breakthrough of 1966 when Mosbach and co-workers initiated a line of research in pursuit of binding cavities that expressed a memory for entrapped enzymes and smaller molecules following their removal from the matrix [141]. Research along this line ultimately led to the non-covalent molecular imprinting approach. It employed the non-covalent interactions for imprinting and rebinding of a template to the imprinted polymer [142, 143]. In 1972, the group of Guenter Wulff independently reported that they had successfully prepared a molecularly imprinted organic polymer [144]. Wulff used what is now termed as the 'covalent approach' to prepare an organic molecularly imprinted polymer capable of discriminating between the enantiomers of glyceric acid. The formation and cleavage of reversible covalent bonds are responsible for the imprinting and rebinding with this approach.

It was Mosbach's non-covalent approach, with its simple, seemingly trivial methodology, that triggered the explosion in molecular imprinting that was to occur during the 1990s. To date, the non-covalent versus covalent debate continues with both sides being championed. However, it is generally accepted that there are pros and cons to both approaches and in 1995 Whitcombe et al. reported an intermediate methodology that appeared to combine the advantages of the two main approaches [145]. It relies on covalent interaction during the polymerization stage but non-covalent interactions during rebinding. The non-covalent however is still by far the most widely used in imprinting polymer synthesis. Several of its drawbacks can be overcome by the use of

stoichiometrically associating monomer-template systems [146 - 149]. This has resulted in a range of receptors that exhibit high capacity and effective recognition properties in aqueous media.

## ***2.1 Ion imprinting***

The concept of molecular imprinting has been translated elegantly into that of ion imprinting technology which is still in its infancy [150]. Ion imprinting is a method of inducing ionic recognition properties in synthetic polymers in response to the presence of a template species (ions) during the formation of a three dimensional structure of the polymer [151]. The resulting polymeric materials will possess high selective and affinitive properties for the ion that was used as a template.

## ***2.2 The imprinting process***

The imprinting process involves complexation in solution of the template (normally target species, ions or molecules) with functional monomer(s), through either covalent or non-covalent bonds followed by a polymerization reaction with an excess of cross linking monomer. Removal of the template from the polymer by extracting with suitable solvents or by cleavage leaves specific recognition sites, which have a memory of the template and are complementary in shape, size and functionality in the polymer network (Fig 2.1). Hence imprinted polymers will recognize the template in rebinding even in the presence of closely related analogues [152 - 155]. If the template used during imprinting is an ion then the resulting polymer is referred to as an ion imprinted polymer (IIP) and a molecular imprinted polymer (MIP) if the template is a molecule. In an ion-imprinting process, the selectivity of the resulting IIP material would be based on the specificity of the ligand, the relative selectivity coefficients of the ion of interest to the competing ones, the coordination geometry & coordination number of the ions, their charges and sizes [156, 157].

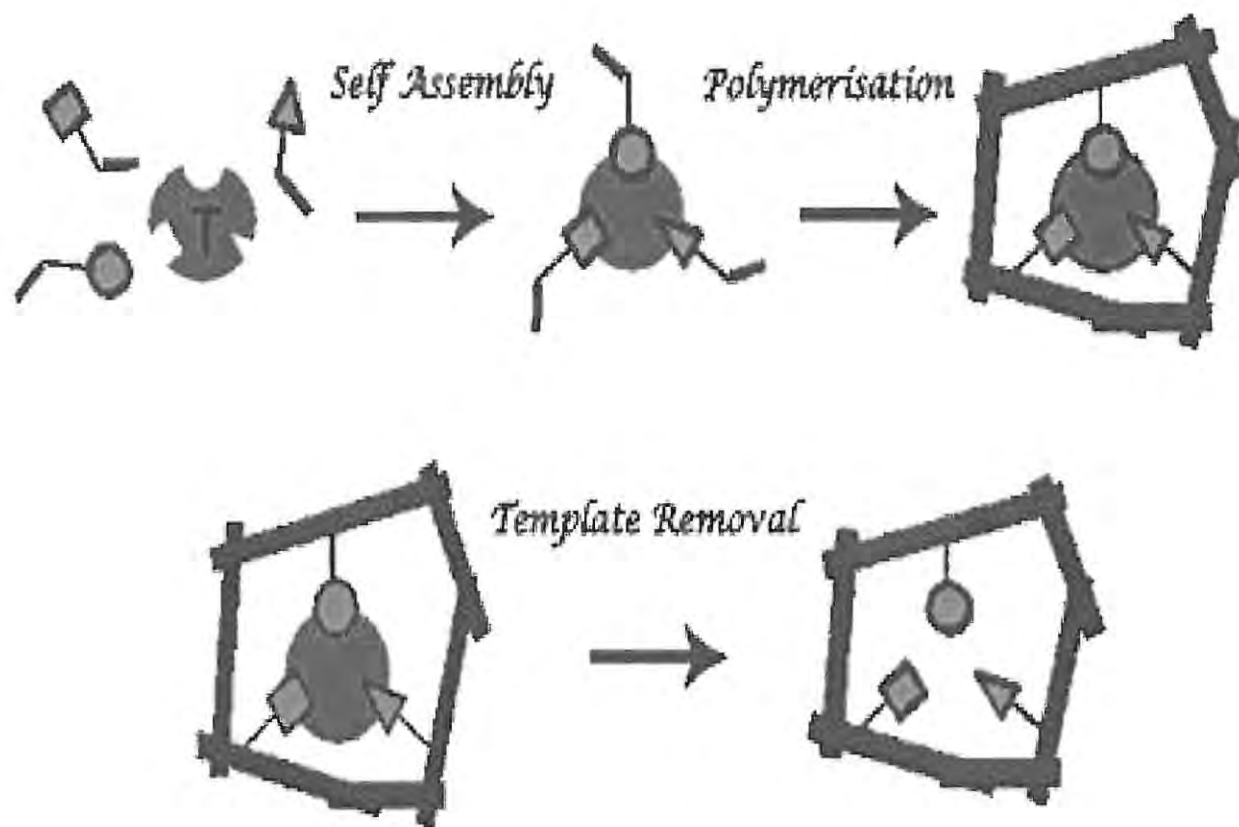


Figure 2.1 Schematic representation of the imprinting process [154], (1) Pre-assembly of functional monomer(s) guided by a template to form a firm or loose monomer-template adducts. (2) Polymerization in the presence of crosslinker, which subsequently 'glues' together the monomer-template adducts resulting in a highly networked construction. (3) Removing the template then leaves the construction with a binding site selective to the original template.

## 2.3 General reactants in the synthesis of imprinted polymers

### 2.3.1 Template (Print ion/molecule)

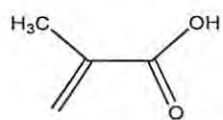
In all imprinting processes the template is of central importance in that it directs the organization of the functional groups pendent to the functional monomers. For practical applications, the analyte of interest is generally used as the print molecule/ion for preparing the imprinted polymer. The template should possess suitable functional groups for interaction with the ones on the functional monomer(s), to ensure stable complexation. The chemical structure of the template is

used as the starting point for selecting functional monomer candidates especially if the non-covalent approach is followed [158]. For example, if basic groups are present in the print molecule, an acidic functional monomer may provide strong ionic interactions between the two. When the print molecule is capable of forming complexes with certain metal ions, metal chelating functional monomers may be a better choice [159].

### 2.3.2 Functional monomers

The functional monomer ultimately becomes responsible for the binding interactions in the imprinted binding sites. Ideally it should form a stable complex with the print species during the pre assembly stage of the polymerization process. Functional monomers are usually used in excess relative to the number of moles of template to favor the formation of template-functional monomer assemblies (template to functional monomer ratios of 1:4 and upwards are rather common for non-covalent imprinting) [158]. It is very important to match the functionality of the template with the functionality of the functional monomer in a complementary fashion (e.g. H-bond donor with H-bond acceptor) in order to maximize complex formation and thus the imprinting effect. When two or more functional monomers are used simultaneously in “cocktail” polymerization [160], it is however also important to bear in mind the reactivity ratios of the monomers to ensure that copolymerization is feasible. If two or more functional monomers are taking part in the polymerization, different multiple interactions can be exploited simultaneously, provided that the different monomers do not interact with one another stronger than with the template. Some of the functional monomers widely used for non-covalent imprinting are shown in Fig 2.2.

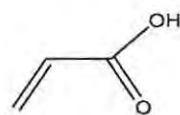
Acidic (a)



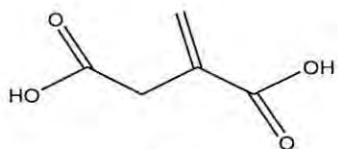
aI



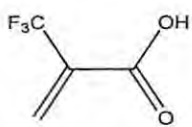
aII



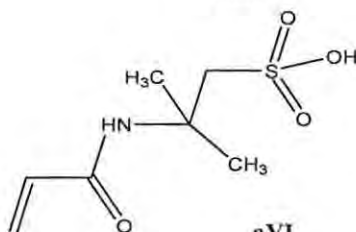
aIII



aIV

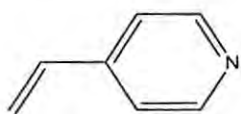


aV

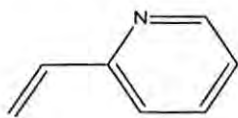


aVI

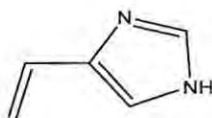
Basic (b)



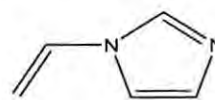
bI



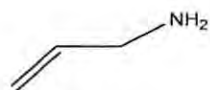
bII



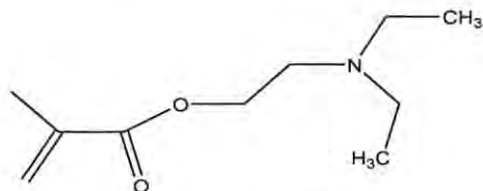
bIII



bIV



bV



bVI



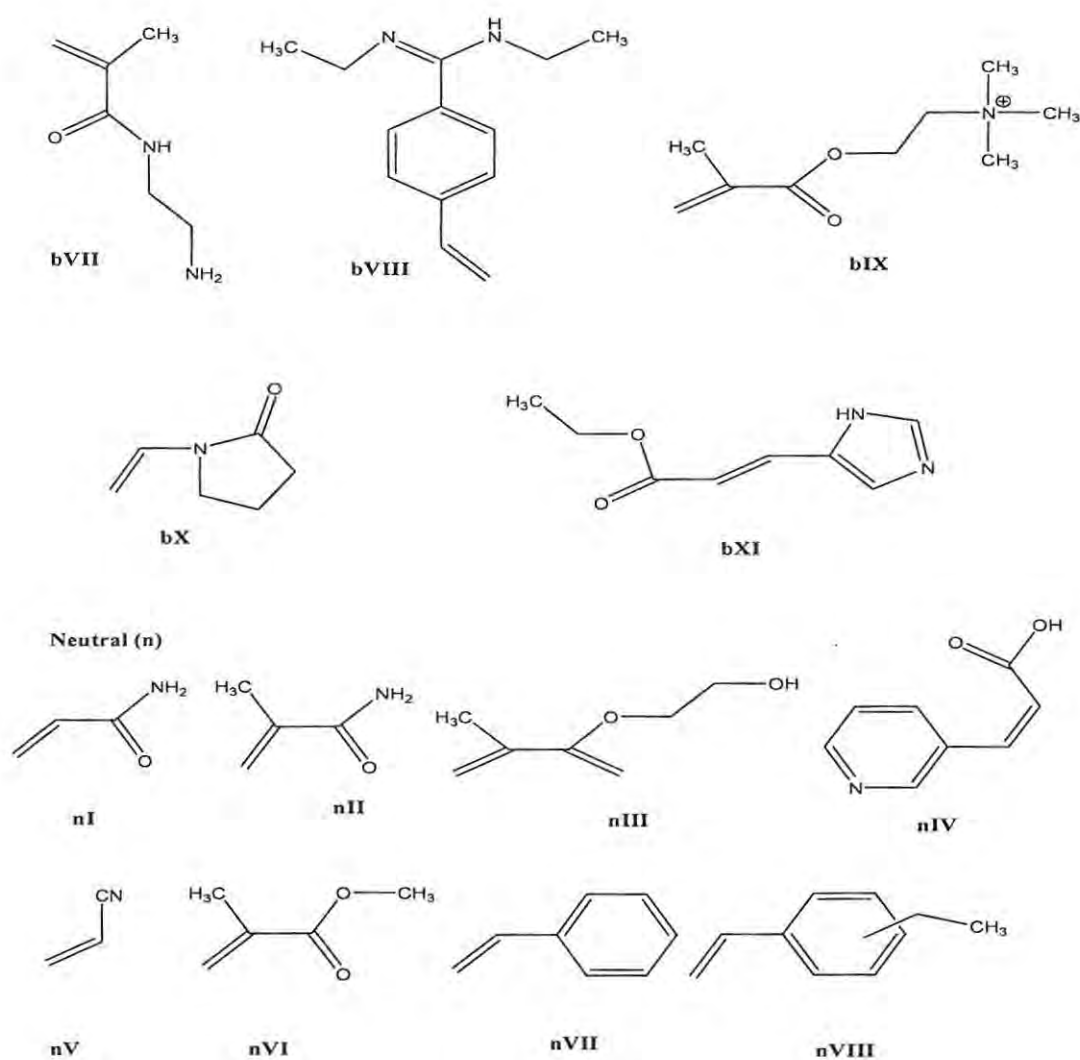


Fig 2.2 Selection of monomers used in the non-covalent approach. Acidic; aI: methacrylic acid (MAA); aII: p-vinylbenzoic acid; aIII: acrylic acid (AA); aIV: itaconic acid; aV: 2-(trifluoromethyl)-acrylic acid; aVI: acrylamido-(2-methyl)-propane sulfonic acid (AMPSA). Basic; bI: 4-vinylpyridine ; bII: 2-vinylpyridine (2-VP); bIII: 4-(5)-vinylimidazole; bIV: 1-vinylimidazole; bV: allylamine; bVI: N,N-diethyl aminoethyl methacrylamide, bVII: N-(2-aminethyl)-methacrylamide; bVIII: N,N-diethyl-4-styrylamidine; bIX: N,N,N-trimethyl aminoethylmethacrylate; bX: N vinylpyrrolidone; bXI: urocanic ethyl ester. Neutral; nI: acrylamide; nII: methacrylamide; nIII: 2-hydroxyethyl methacrylate nIV: trans-3-(3-pyridyl)-acrylic acid; nV: acrylonitrile (AN); nVI: methyl methacrylate (MMA); nVII: styrene; nVIII: ethylstyrene

### 2.3.3 Cross-linking monomer

In many cases high binding specificity is augmented by the rigid three-dimensional structure of the polymer, which in turn is ensured by the high cross-link density. For this purpose, a relatively large

volume of the cross-linking monomer is usually copolymerized with the functional monomer (cross linking to functional monomer ratios of 1:4 and upwards are common) [161]. In an imprinted polymer the cross-linker fulfils three major functions;

(1) The cross-linker is important in controlling the morphology of the polymer matrix, whether it is gel-type, macroporous or a microgel powder.

(2) It serves to stabilize the imprinted binding site.

(3) It imparts mechanical stability to the polymer matrix.

Fig. 2.3 shows some of the commonly used cross-linking monomers. Derivatives of divinylbenzene and acrylate cross-linkers are generally hydrophobic, while acrylamide ones are relatively hydrophilic.

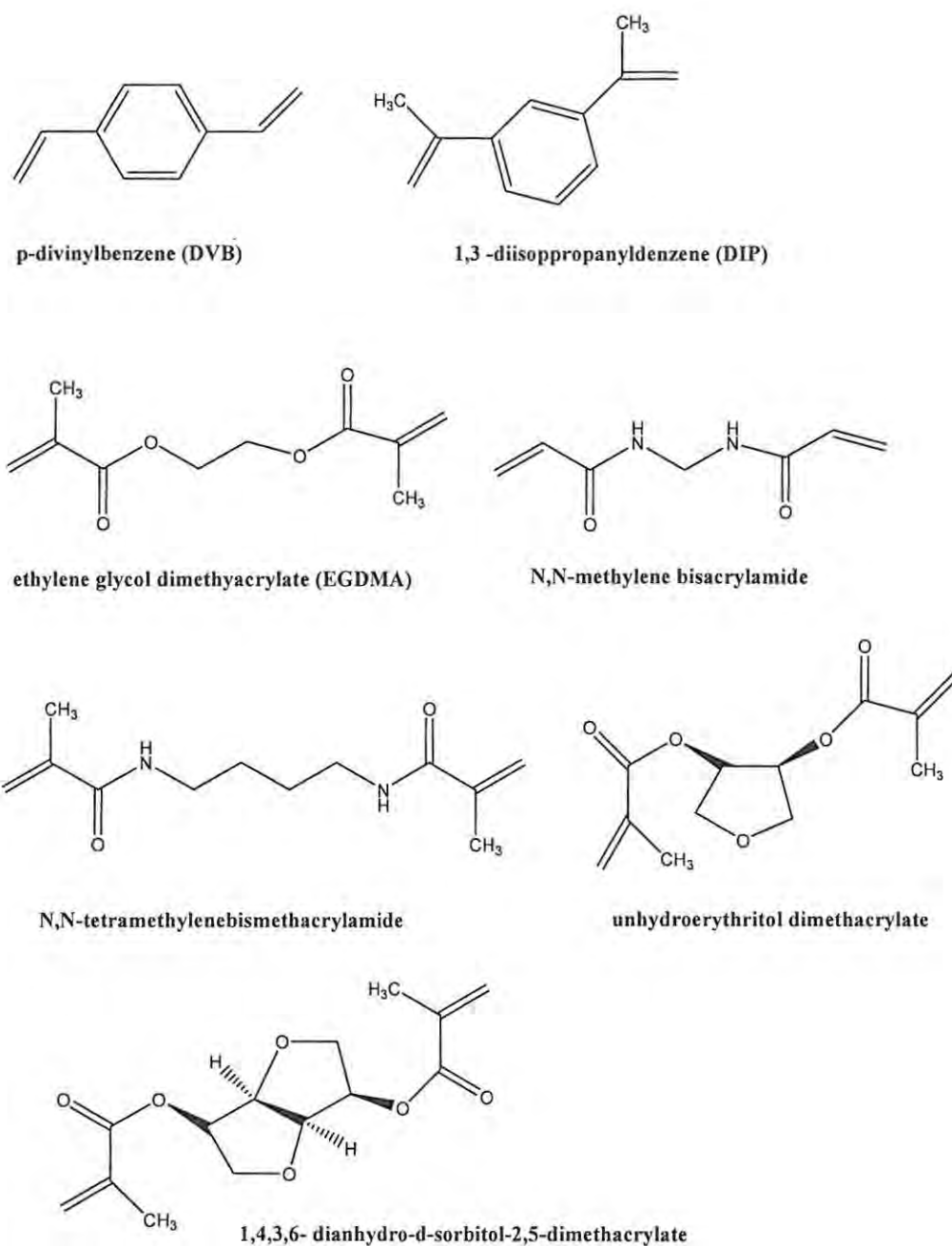


Fig. 2.3 Selection of cross-linkers used for molecular imprinting

### 2.3.4 Porogens

Besides their dual role as solvents and pore forming agents, they also help to homogenize the reaction components prior to polymerization. They must be judiciously chosen such that they maximize the likelihood of template-monomer adduct formation. Table 2.1 lists the imprinting

solvents commonly used in the non-covalent approach, together with their dielectric constants and hydrogen bond scales [162, 163, 164]. If solubility of template is satisfied, then solvents with lower dielectric constant, (thus apolar, non-protic solvents are preferred for imprinting using ionic interactions. When hydrogen bond interactions are utilized, solvents with lower hydrogen bond acidity or basicity generally result in a better imprinting effect. If hydrophobic interactions are being used to drive the complexation then water could well be used as the solvent of choice. Porogenic solvents play a role in determining the physical characteristics of the resulting polymer i.e. morphology, surface area and porosity [165, 166, 167].

**Table 2.1 Imprinting solvents commonly used in the non-covalent approach, together with their dielectric constants and hydrogen bond scales.**

Solvent	Dielectric constant $\epsilon$ (20°C)	Hydrogen-bond acidity $\alpha_2^H$	Hydrogen-bond $\beta_2^H$
Benzene	2.3	0.00	0.14
Toluene	2.4	0.00	0.14
Chloroform	4.8	0.200	0.02
Dichloromethane	9.1	0.13	0.05
Acetonitrile	37.5	0.09	0.44
Acetone	20.7	0.44	0.50
Tetrahydrofuran	7.6	0.00	0.51
Dimethylformamide	36.7	0.00	0.66
1-propanol	20.1	0.33	0.45
Methanol	32.6	0.37	0.41
Water	78.5	0.35	0.38

### 2.3.5 Initiators

Free radical polymerization is the most important synthetic method available to date for the conversion of monomer into polymer. Initiators (Fig. 2.4) are normally the sources of free radicals to start the propagation of the polymerization reaction. Their concentrations influence the formation or lack of formation as well as the morphology of the prepared polymer. The rate and mode of decomposition of an initiator in the first step of polymerization to produce radicals can be triggered and controlled in a number of ways including heat (thermolysis), light (photolysis), and chemical/electrochemical (chemo/electrochemolysis). For ion imprinting, sometimes an initiator is left out as a reaction between a metal ion (print ion) and a ligand (functional monomer) is normally spontaneous.

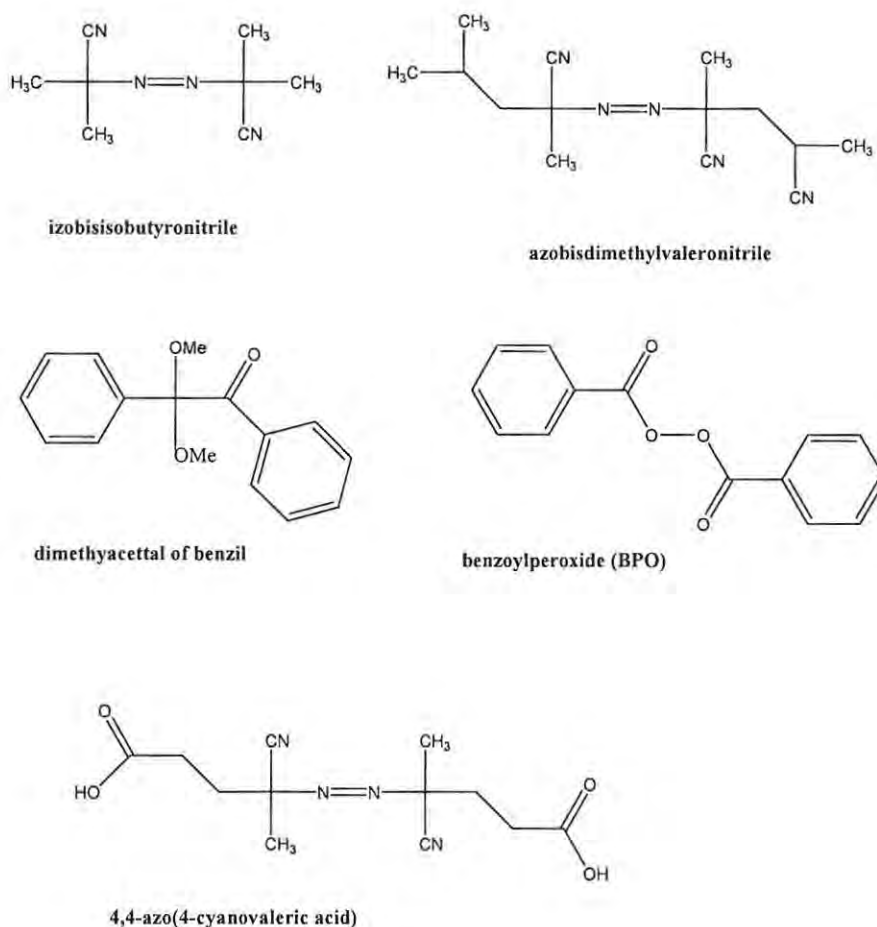


Fig. 2.4 Chemical structures of selected chemical initiators

## Chapter 3

### *3.0 Theory of imprinting technology*

The theory of imprinting polymers emanates from understanding the fundamentals of molecular recognition. Molecular recognition is a process involving both binding and selection of a substrate by a given receptor, which implies a structurally well defined pattern of intermolecular interactions [168]. All living processes rely on specific recognition at the molecular level and these include well known processes like DNA replication, transcription and translation. These processes involve the template guided coupling of various building blocks through specific binding and recognition of effector molecules to target macromolecular receptors [169, 170]. Supramolecular chemistry, which deals with the assembly of abiotic receptors and their interactions with specific substrates, explains the mechanisms and intermolecular interactions e.g. ionic interactions, hydrogen bonding, Van der Waals forces and hydrophobic effects, that underpin the important biological processes. These binding interactions are the same ones employed in molecular or ionic imprinting except that instead of concentrating on deciphering the fundamental mechanisms underlying recognition processes, imprinting technology aims to synthesize polymer materials for more practical purposes, using knowledge from related research areas [171].

### *3.1 Molecular interactions*

Common non-covalent interactions include ionic pairing, hydrogen bonding and hydrophobic interactions. These provide the required binding energy for a substrate to interact with its receptor. Even though the non-covalent interactions are weak if they act independent of each other they promote stable and specific complexation between substrate and receptor as they usually act in a polyvalent manner [172].

### 3.1.1 Ionic interactions

Effective binding between interacting charged particles  $q_1$  and  $q_2$  is given by the electrostatic potential,  $E$ ,

$$E = k \frac{q_1 q_2}{\epsilon r} \quad 3.1$$

where  $r$ , is the distance of separation between the reacting specie  $q_1$  and  $q_2$ ,  $\epsilon$  is the dielectric constant of the porogen and  $k$  is a predetermined constant based on specie interaction. For effective binding (large  $E$ ), therefore,  $r$  and  $\epsilon$  must be very small.

### 3.1.2 Hydrogen bonding

A hydrogen bond is a bond formed between a covalently bonded hydrogen atom on a donor group (e.g.  $-\text{OH}$  or  $=\text{NH}$ ) and a pair of non-bonding electrons on an acceptor group (e.g.  $\text{O}=\text{C}=\text{O}$  or  $\text{N}\equiv\text{C}-$ ). Hydrogen bonds show characteristics of both non-covalent and covalent interactions. They stabilize the secondary structures of biological macromolecules and are widely involved in recognition processes. Water, which is the medium in which most reactions take place, biological or synthetic, is both a hydrogen bond donor and acceptor and as such can readily interfere with hydrogen bond interactions between monomer and template [173].

### 3.1.3 Hydrophobic interactions

The hydrophobic effect reflects the tendency of non-polar molecules to form aggregates of like molecules in water. From thermodynamics, the hydrophobic effect is driven by the loss of hydrogen bonding and the higher entropic cost ( $\Delta S$ ) of forming a cavity around non-polar molecules. The formation of monomer-template complex requires a favorable energetic contribution, which is described by the free energy change as;

$$\Delta G = \Delta H - T\Delta S \quad 3.2$$

where the change in free energy ( $\Delta G$ ) determines if the process is favorable under specific conditions, the enthalpy term ( $\Delta H$ ), is due to the various electrostatic interactions while the hydrophobic effect comes from the entropy change ( $\Delta S$ ) as the non-polar parts of the monomer bind to the hydrophobic pockets of the template [174].



## Chapter 4

### *4.0 Synthesis of imprinted polymers*

#### *4.1 Free radical polymerization*

Free radical polymerization is usually the method of choice for preparing imprinted polymers. Vinyllic monomers are usually employed for this kind of polymerization. The monomers are commercially available at low prices and can be polymerized efficiently by this method. The method can be performed under mild conditions of ambient temperature and pressure in bulk or solution. There are three steps that characterize free radical polymerization; (1) initiation, (2) propagation and (3) termination.

In a typical free radical polymerization, the rate of propagation is usually much faster than the rate of initiation such that as soon as a new polymer chain starts to grow it propagates to high molecular weight in a relatively short period of time (seconds), before it terminates. It is often highly desirable, not only in imprinting to simultaneously polymerize (copolymerize) two or more functional monomers within the same reaction vessel to give linear chain copolymers as opposed to polymerizing one single polymer (homopolymerization). This allows the resulting copolymers to have chemical properties that are distinct to polymers that would have been formed if each of the monomers that were used in the copolymerization was allowed to homopolymerize. When multi-functional monomers (cross-linkers) are polymerized either on their own or in combination with a comonomer(s), the outcome is highly cross-linked, non-linear polymer networks. These materials may be soluble or insoluble, and can be classified as branched macromolecules, microgels or macroscopic networks [175], (Fig. 4.1). This is well described by the theory of radical cross-linking copolymerization (RCC) as defined by Funke *et al* [176]. For the copolymerization of mono- and poly-unsaturated monomers, there is a phase separation that occurs when the quantity of the cross-linker exceeds a critical value and the total monomer concentration is decreased to such an extent that a highly cross-linked network can no longer absorb all the porogen present in the

reaction mixture. Funke proposed that if the volume of the porogen was to be further increased, the growing polymer chains would be unable to occupy the entire volume available. A dispersion of macrogel particles in the porogen would then result (see Fig. 4.1). More dilute systems (with high volume of porogen) would lead to a decrease in the size of the gel particles. At infinite dilution, macromolecules consisting of intra-molecularly cross-linked primary chains are formed, which are known as primary particles or microgels (see Fig. 4.1).

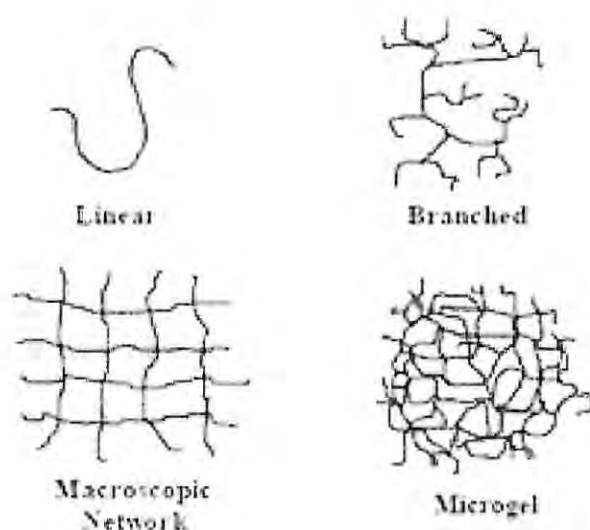


Fig. 4.1 Schematic representation showing polymers with different topologies: linear, branched macroscopic, network and microgel [176]

## ***4.2 Preparation techniques and physical configurations of imprinted polymers***

### **4.2.1 Bulk polymerization**

Depending on the preparation technique used, imprinted polymers can have various physical configurations [177]; monoliths, micro/nano beads, rods or membranes. Most reported imprinted polymers have been prepared by bulk polymerization method. It is the most widely used owing to its simplicity [178]. Most investigations still rely on it for the purpose of demonstrating the

imprinting effect and potential applications. The method involves mixing together in one reaction vessel the correct quantities of each reagent then applying the right conditions for the reaction to occur. The resultant polymer material is usually monolith which would then be ground and sieved to obtain polymer particles mainly in the size range of 25-50  $\mu\text{m}$  (Fig 4.2 A). Sedimentation then follows to remove the fine particles. Despite the ease of preparation by this polymerization technique, it is time consuming, labor intensive and wasteful as only 30-40% of the ground polymer is recovered as useable material [179]. The irregular shape of the polymer materials and the broad size distribution associated with the method can be avoided by using other methods which result in more regular, spherical and mono-dispersed particles which are good geometrical features characteristic of a potential sorbent. Other methods include suspension polymerization, dispersion/precipitation polymerization and seed polymerization [180].

#### **4.2.2 Suspension polymerization**

Suspension polymerization involves the dispersion of the polymerization mixture as droplets in a continuous phase (porogen) like water, mineral oil or perfluorocarbon. Each droplet acts like a mini bulk reactor producing spherical beads (Fig 4.2 C) in a broad size range of a few micrometers (10  $\mu\text{m}$ ) to millimeter (5 mm) size [181]. For this method, it has been reported that when the porogen is an aqueous phase and the weak non-covalent bonds like hydrogen bonds are the ones relied upon solely, the polymerization performed in aqueous media yield poorly imprinted polymer materials. However, Matsui *et al* has used the method successfully in an aqueous continuous media against atrazine, a lipophilic herbicide. They applied the synthetic receptor in the SPE of the triazine and reported group selectivity of the imprinted polymer for the triazine family [182]. In contrast, suspension polymerization in organic media like perfluorocarbon liquid continuous phases, yields good quality beads, with controlled particle sizes [183, 184,]. The beads have improved adsorption capacities as well as high selectivity coefficients [185].

#### **4.2.3 Precipitation polymerization**

When large quantities of the porogen are used than the ones used in bulk polymerization (2–10 times higher) [186, 187], the growing polymer chains become insoluble in the liquid phase and

precipitate out as the polymerization proceeds. If there is an accurate control of the polymerization conditions like the temperature and the cross-linker ratios [188] microspheres and nanospheres (Fig 4.2 B) are formed [189-194]. This kind of polymerization is referred to as precipitation polymerization method.

#### 4.2.4 Multistep swelling polymerization

In order to better control the size distribution and the shape of the particles as well as decrease the quantity of polymer material wasted, imprinted polymer layers have been grafted onto pre-formed, spherical supporting materials acting as casts or molds, for example silica particles [195, 196] or poly(TRIM) beads [197]. Polystyrene microspheres have also been used in a multistep swelling polymerization to produce uniformly sized beads against various substrates [198, 199]. The method is time consuming and sophisticated but it results in mono-dispersed spherical polymer particles of size ranges 5 – 100  $\mu\text{m}$ . A one step swelling approach was recently proposed by Liu *et al.* [200] to obtain MIP particles of 7.5  $\mu\text{m}$  for the selective extraction of metsulfuron - methyl from drinking water. Takagi *et al.* copolymerized surfactants with hydrophilic binding groups for the target metal ions with a cross-linking monomer using micro-emulsion polymerization. They referred to their approach as 'surface imprinting' because the complex between the metal ion and the polymerizable surfactant was formed at the aqueous – organic interface. In a similar manner, metal ion imprinted microspheres were prepared by seeded emulsion polymerization [201] allowing for controlled particle size. The small, uniform sized particles allowed for the regular packing of the particles in the SPE cartridges, hence improved kinetics and efficiencies in the percolation and extraction of the analyte(s).

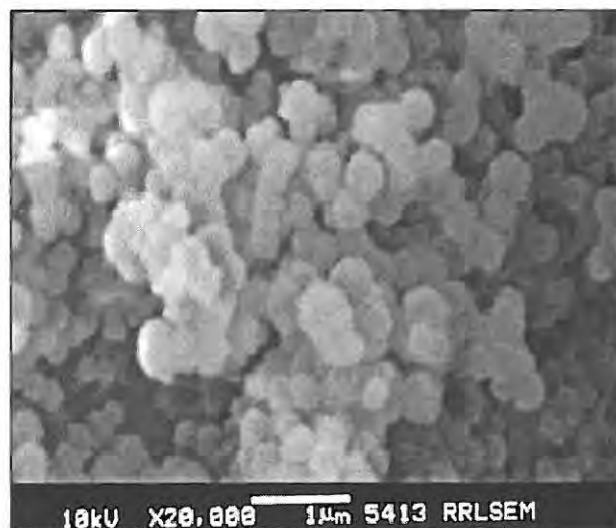
#### 4.2.5 In-situ polymerization

Imprinted polymers can also be prepared directly by filling the polymerization mixture into casts or moulds made from, for example, stainless steel, silica, or glass column. This results in different configurations like polymer rods that can be easily adapted to chromatographic applications including on line [202 - 204]. No grinding and sieving is required with this type of technique and the imprinted column can be used immediately following polymerization and washing. This technique

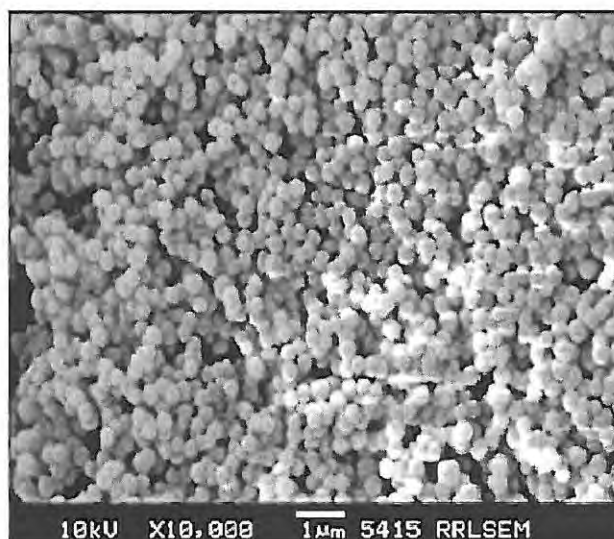
has been largely reported by Schweitz *et al.* who developed MIP monoliths for capillary electrochromatography (CEC), to combine the intrinsic high efficiency and enhanced flow dynamics of the CEC with the high selectivity of the MIP for the separation of enantiomers [205, 206]. It has been reported that strong interactions between the functional monomer and the template are essential for success, since specific porogens employed interfered with the desired hydrogen bond formation. This is said to limit the general applicability of this in-situ technique [207, - 210].



(A)



(B)



(C)

Fig. 4.2 SEM micrograph of IIPs synthesized via bulk (A), precipitation (B) and suspension (C) polymerization [149]

## Chapter 5

### ***5.0 Characterization and performance evaluation of imprinted polymers***

#### ***5.1 Monomer-Template characterization***

##### **5.1.1 Nuclear Magnetic Resonance (NMR)**

Spectroscopic techniques are usually the ones used to give evidence about any existence or the extent of pre-polymerization interactions between the monomer and template. Pioneering this was Sellergren *et al.* using NMR studies to show the minor presence of template self-association and higher order complexes [211]. In their studies, the extent of interaction of the monomer-template was predicted by determining the chemical shifts and relating them to the dissociation constants. The NMR characterizations of functional monomer–template interactions have also been applied to the study of the interactions between many functional monomers and templates [212 – 221]. In most of these studies, it was possible to determine the exact composition of the complex [217], dissociation constants [218] as well as predicting binding capacities of the overall polymer materials especially where NMR chemical shifts were determined [219, 222]. For example, Nicholls investigated in detail the characterization of complexes between nicotine and methacrylic acid [219].

##### **5.1.2 Fourier Transformer Infrared (FTIR)**

Fourier transformer infrared spectroscopy (FTIR) has been used with samples in solution and in the solid state to demonstrate if imprinting occurred or not. The spectra of the functional monomers and template (starting materials) before and after they have assembled during an imprinting process are compared. The imprinting process starts with the complexation of the functional monomer and the template, in most cases via hydrogen bonding in the non-covalent approach. The formation of the bond can be readily identified by FTIR since the stretching frequency of

hydroxyl or amino groups (hydrogen bond donors) and carbonyl groups (hydrogen bond acceptors) are displaced and an observable shift can be identified. The technique is not well applied to the pre-polymerization solution analysis [223] due to solvent interference on the determination of specific characteristics of complexation but it is a technique of choice for characterization when the imprinted polymer has already been formed.

### 5.1.3 Ultraviolet (UV)

Complexation or monomer-template self assembly has also been studied using UV spectroscopic titrations in order to calculate the dissociation constants for the solution adducts and the relative concentration of fully complexed templates in the polymerization mixture [224]. The main advantage of this technique is its simplicity and the possibility to control monomers–template complex formation in aqueous media [225, 226]. Ping *et al.* [227] synthesized a MIP from the photoinduced polymerization of acrylamide and butylenes diacrylate in the presence of l-2-chloromandelic acid (l-2-MDA) as a template. The MIP was used as a highly selective separation material for l-2-MDA. The UV spectra showed that the template l-2-MDA and the functional monomer acrylamide formed complexes before polymerization. The structures of the complexes were simulated using HyperChem and it was found that the binding capacity of the MIP to l-2-MDA was higher than that of the competing specie. Scatchard analysis suggested that the MIP recognized l-2-MDA with two classes of binding sites, which was in agreement with the complexes that were simulated using HyperChem [227].

## 5.2 Physical characterization of imprinted polymer materials

Imprinted polymers belong to a class of materials known as macro-porous polymers [228, 229]. Imprinted polymers are solids, and therefore cannot be characterized by more commonly employed polymer characterization methods that would require polymer solutions; e.g. gel permeation chromatography, solution NMR techniques, and UV measurements directly on the polymers. Furthermore, because they are amorphous, crystallographic or microscopy methods cannot be used to determine the structure of the imprinted polymer binding sites, although

microscopic techniques like scanning electron microscopy (SEM), atomic force microscopy (AFM) and transmission electron microscopy (TEM) have aided the macroscopic understanding of their morphology [230]. Therefore, there are only a limited number of direct physical characterization methods for imprinted polymers. These include surface area and porosity measurements, FTIR spectroscopy, solid state carbon – 13 nuclear magnetic resonance cross polarization – magnetic angle spinning (NMR <sup>13</sup>C – CPMAS) spectroscopy, and swelling experiments. The surface area, porosity, and swelling measurements characterize macroscopic features of MIPs; however, information provided by these on the binding site structure of MIPs is very limited but useful in some applications such as in drug delivery applications.

### 5.2.1 Surface area and porosity

The morphology of MIPs, as shown in Fig. 5.1, arises from the nuclei that form around the initiator and grow to 10 to 30 nm in diameter that then aggregate to form microspheres which further group themselves into larger clusters to form a body of beads. The porosity and resulting surface area in MIPs is formed from the irregular voids located between clusters of the microspheres (macropores, 50 nm in diameter), or from the interstitial spaces of a given cluster of microspheres (mesopores, 2 to 50 nm in diameter), or even within the microspheres themselves (micropores, of 0.6 - 2 nm in diameter). Typical values for the surface area of the imprinted polymers are in the range of 100 to 400 m<sup>2</sup>g<sup>-1</sup>. For pore size distribution there are both macropores and mesopores in the range 2 to 100 nm, and micropores of 0.6 to 2 nm in diameter [230]. The most effective variables that control surface area and pore distributions are the percentage of crosslinking monomer, the type and amount of porogen, and the reaction temperature. Although binding and selectivity by MIPs in chromatographic or batch rebinding mode are not dependent on macroporosity, applications in drug delivery may rely on mass-transfer kinetics related to porosity [174]. Surface area measurements in MIPs are primarily carried out by a nitrogen adsorption porosimeter employing a BET (Brunauer, Emmett and Teller) analysis routine that is standard to all instruments. For pore size distributions in MIPs, the same nitrogen adsorption data can be analyzed using BJH (Barret, Joyner and Halenda) methods also available on porosimetry instruments [230].



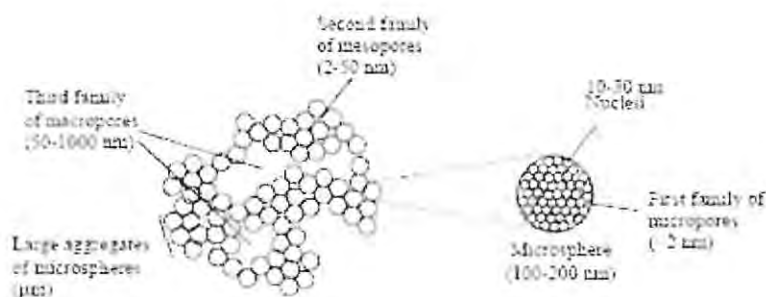


Fig. 5.1 Model of morphology formation that provides the porous network in imprinted polymers [230].

### 5.2.2 Characterization of MIPs by spectroscopic analysis techniques

FT-IR and solid state NMR techniques are useful for the measurement of functional group incorporation. They are also employed in the quantification of the degree of polymerization and reactivity for each type of the polymerizable groups on the monomers. For example, quantitative FT-IR can be used to measure the extent of unreacted double bonds using the C–H out of plane bend at  $900$  to  $950\text{ cm}^{-1}$  and the –C–C– stretch at  $1639\text{ cm}^{-1}$ . This would give an indication of the possible recognition sites in the formed polymer. A measure for the degree of polymerization is assessed from the number of unreacted double bonds, which are quantified by integration of the area under the peak corresponding to the wavenumbers [231]. Using computer aided programs like origin, the integrated value is converted to the number of double bonds using a calibration curve separately developed that correlates double bonds and integration areas. A more quantitative measure of overall unreacted double bonds in the different MIP materials can be obtained directly by  $^{13}\text{C}$  CP-MAS NMR without the need for calibration curves. All other functional groups of interest that are carbon-based can also be quantified using this technique [230].

### 5.2.3 Characterization of imprinted polymer swelling

Swelling in MIPs has most often been evaluated using volumetric methods as has been reported by Sellergren and Shea [230]. There are some difficulties, however, due to buoyancy (i.e. the polymers float) especially for the polymers in chlorinated solvents; and inaccuracy of the volumetric methods. A more accurate technique that can be used, measures changes in volume for a single

bead. It determines the size of the individual polymer particle. The size can be observed under a microscope in the absence and presence of solvent. The particles are then photographed in swollen and un-swollen states, and the ratios in surface area calculated to give the percent swelling [232]. In many cases the particles have irregular shape giving wide ranges of different sizes between the particles. Consequently, it is best to follow the same particle from the swollen state to the dry state.

#### **5.2.4 Binding studies**

Binding studies are experiments in which the presence of cavities in the imprinted polymer and their selectivity in recognizing the targeted species are assessed by methods such as the batch analysis [233, 234] and frontal analysis [235, 236]. In batch analysis experiments, a known concentration of the template is introduced in a vial with a specific quantity of the prepared imprinted polymer (IIP/MIP) and shaken for a known period of time to equilibrate. Once the system has equilibrated the concentration of the unextracted template in solution is determined so as to evaluate the bound concentration by the IIP/MIP. The binding studies are generally carried out in parallel on a non-imprinted polymer (NIP), also referred to as the control or blank polymer. The NIP is obtained by applying the same procedure of polymerization but in the absence of the template. Hence the NIP possesses the same chemical properties as the IIP/MIP but without any specific cavities. The difference between the values obtained for the MIP and NIP give the actual occurrence of selective cavities in the MIP.

In frontal analysis the imprinted polymer is packed into a column and a solution containing the target analyte is continuously percolated through the packed polymer material, which would now be acting as a sorbent [235]. Frontal analysis represents SPE studies more when compared to batch. Desorption and reusability experiments for the polymer are also easier to handle. A reproducible way of packing the polymer materials is usually a challenge especially if the particles were produced by the common bulk polymerization method. Both batch and frontal analysis methods allow for the determination of the number of binding sites and the equilibrium adsorption constants [236].

### 5.2.5 Selectivity studies

These are rebinding experiments performed in the presence of closely related, competing specie that co-exist and hence might interfere with the selective extraction of the targeted analyte. An estimation of the effect of imprinting on selectivity in the presence of competing specie is given by the relative selectivity coefficient ( $k'$ ), which in turn is calculated from the distribution coefficient ( $K_d$ ) values of the template (print ion or molecule) as per the following equations;

$$K_d = \frac{C_i - C_f}{C_f} \times \frac{V}{m} \quad 5.1$$

Where,  $K_d$  is the distribution coefficient,  $C_i$  and  $C_f$ , the initial and final concentrations respectively,  $V$ , the volume of the solution used for the extraction and  $m$ , the mass of the polymer used for extraction. The selectivity coefficient ( $k$ ), for the binding of a particular ion or molecule in the presence of competing specie can be obtained by;

$$k = \frac{k_{\text{print ion}}}{k_{\text{competing ion}}} \quad 5.2$$

The relative selectivity coefficient ( $k'$ ) is given by;

$$k' = \frac{k_{\text{imprinted}}}{k_{\text{non-imprinted}}} \quad 5.3$$

## Chapter 6

### 6.0 Ion imprinted polymers (IIPs)

Ion-imprinted polymers (IIPs) are similar to MIPs, except that they recognize metal ions after imprinting, while retaining all the virtues of MIPs. The number of papers, which feature ion-imprinted polymers, though initiated in 1976, has increased dramatically over the last few years ( see Fig. 6.1). Nishide and Deguchi [237], for the first time in 1976, cross-linked poly(4- vinylpyridine) with 1,4-dibromobutane in presence of metal ions; Cu(II), Zn(II), Co(II), Ni(II), Hg(II) and Cd(II) as templates contrary to small molecules, biological macromolecules, microorganisms or whole crystals as it was common to do in the case of MIPs during those years. Kabanov *et al.* [238] cross-linked a copolymer of diethylvinylphosphonate and acrylic acid with N,N - methylene diacrylamide in the presence of metal ions. Recently, Rao and colleagues reviewed, "Tailored ion-imprinted polymer materials for solid phase extraction of inorganics" [239]. In the review, various factors have been identified for the formation and the recognition properties of binding sites. Among them, multiple site interactions with functional monomers have been shown as a likely way to yield binding sites of higher specificity and affinity. The binding strength of polymer as well as the fidelity in the recognition depends on the number and type of interaction sites, the template shape and the monomer–template rigidity. Thus, better fit between the site and the template leads to less entropy loss due to the conformational changes in the site as well as in the template upon rebinding. Cosequently, the affinity and selectivity in the recognition will be enhanced. The metal ion templates are embedded rigidly in polymeric matrices which can result in IIPs with different configurations, viz. particles, beads or microspheres depending on the polymerization method. Some of the applications of the IIPs include solid phase extraction, metal ion sensors (selectrodes and optrodes) and membranes.

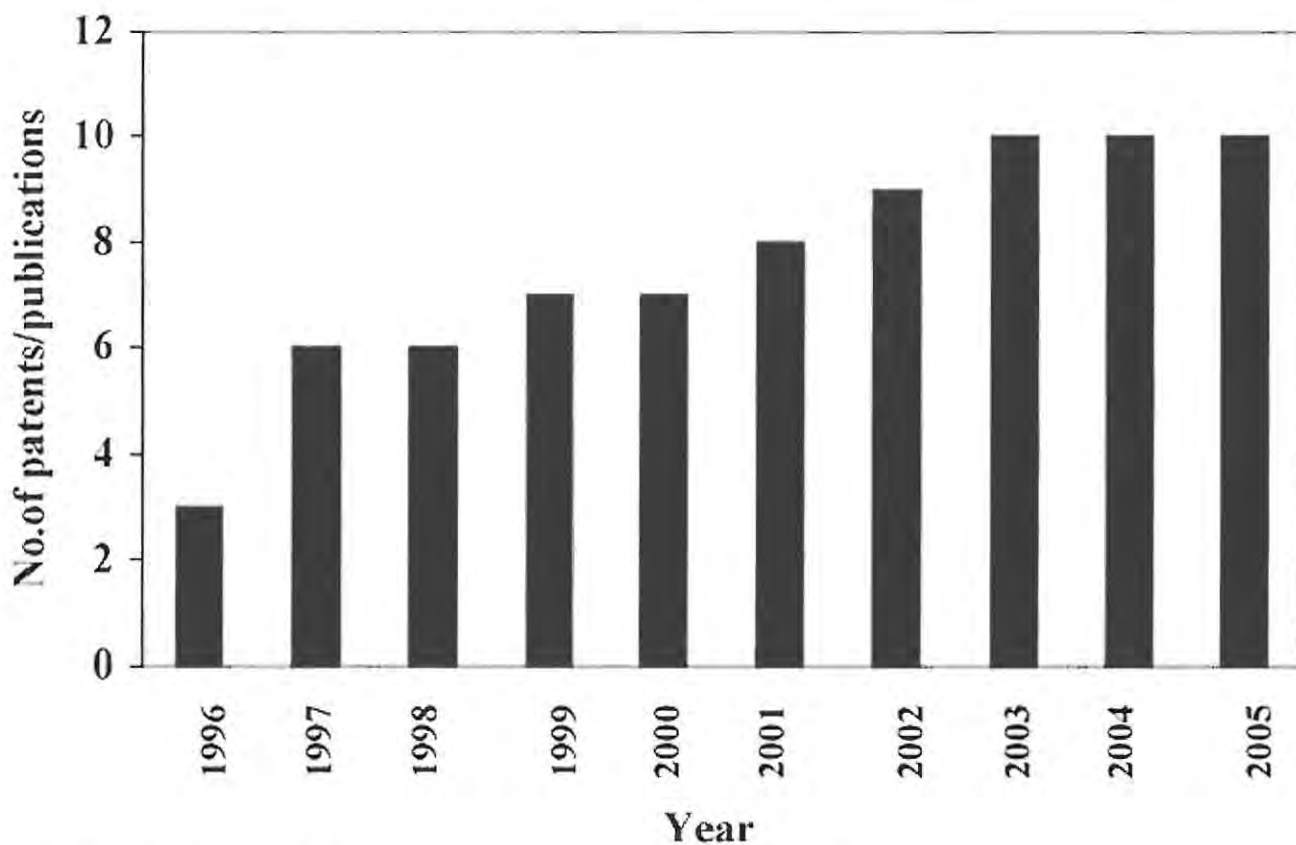


Fig. 6.1 Yearwise histogram of publications/patents of IIPs from 1996 [149].

### 6.1 Ion imprinted polymer – Solid phase extraction (IIP–SPE)

Ion-imprinted polymers (IIPs) are recently identified nano-porous polymeric materials which on leaching the imprint ion can rebind or sense selectively the targeted ion in the presence of closely related inorganic ions or its analogues [240]. A particularly promising application of IIPs is the solid phase extraction (SPE) of toxic, heavy metal ions present in trace quantities from other coexisting ions as well as from complex matrices. This may lead to selective environmental cleanup of analytes, not achievable by conventional methods [241].

A survey on the synthesis of metal ion-imprinted polymers show that these may be roughly classified into four approaches based on inclusion of the ligand in the polymer matrix. The four approaches are; (i) linear chain polymers carrying metal-binding groups being cross-linked with a

bifunctional reagent; (ii) chemical immobilization by preparation of binary complexes of metal ions with ligands having vinyl groups, isolation and then polymerization with matrix-forming monomers; (iii) surface imprinting conducted on aqueous–organic interface; (iv) trapping of non-vinylated chelating ligand via imprinting of binary/ternary mixed ligand complexes of metal ions with non-vinylated chelating agent and vinyl ligand.

### 6.1.1 Cross-linking of bifunctional reagent with linear chain polymers

Ion template effect in the synthesis of chelating polymers was first utilized by Nishide *et al.* in 1976. They cross-linked poly(4-vinylpyridine) with 1,4 dibromobutane in the presence of metal ions as templates [237,242,243]. The adsorption behaviour of the obtained resins for Cu(II), Zn(II), Co(II), Ni(II), Hg(II) and Cd(II) was studied. The resins preferentially adsorb the metal ion, which had been used as template. Kabanov *et al.* [238,244] cross-linked a copolymer of diethyl vinyl phosphonate and acrylic acid with N,N' - methylene diacrylamide (in the presence of metal ions,  $\text{Ca}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ). The metal ions were extracted selectively with high percentage extraction recoveries of over 95% and percentage relative standard deviation (% RSD) of less than 5 % in all cases. Ohga *et al.* cross-linked chitosan with epichlorohydrin to give an imprinted resin [245]. The column packed with cadmium(II) imprinted resin separated cadmium(II) from copper(II) and mercury(II) and this was reused six times with same performance.

### 6.1.2. Chemical immobilization

This synthetic procedure involves the use of vinylated ligands only so that after polymerization, the ligands are chemically immobilized in the polymer matrix. Hence, the ligand is firmly anchored to the cross-linked polymer. The first example of this method of imprinting was reported by Nishide and co-workers [246] who polymerized a metal complex of 1 – vinyl - imidazole with 1 - vinyl-2-pyrrolidone and divinyl benzene (DVB). The metal – vinyl imidazole complex was copolymerized and cross linked with 1 – vinyl – 2 - pyrrolidone by  $\gamma$  - irradiation and the template metal ion was removed by treating the polymer complex with an acid. Gupta and Neckers [247] have prepared poly(4 – methyl – 4' – vinyl - 2,2' - bipyridine) copolymers with DVB in the presence of metal ions. These polymers on removal of metal ions retained some memory of the original metal ion. IIP–SPE methods have been described for the separation of lanthanum and gadolinium using cation-

imprinted polymers, which have been synthesized with diethylene triamine pentaacetic acid (DTPA) and DVB in the presence of respective metal salts [248]. In another study, the functionalized monomer of DTPA was copolymerized with commercially available DVB containing 45% ethylstyrene in the presence of gadolinium salts [249]. The resulting IIP resins were found to be more selective to gadolinium over lanthanum when compared to untemplated ones. Subsequently, the same group [250] extended the study to determine selectivity coefficients of Gd/Nd, Gd/Eu, Gd/Lu and evaluated the results obtained with gadolinium imprinted and blank polymeric resins. IIP materials based on vinyl pyridine, acrylic, or a DTPA derivative with gadolinium ion were prepared and the influence of the structure of cross-linking agents and the use of monomeric-complexing agent on the SPE behaviour of the material was studied [251]. The results showed a significant increase in the selectivity of complexation when the polymers were prepared in the presence of target metal ion.

The invention by John *et al.* [252] relates to the detection and extraction of uranyl ions by polymer imprinting, wherein the complexable functionality has the formula  $CTCOOH$ , where T is a hydrogen, any halogen (preferably chlorine), methyl, halogen-substituted forms,  $CCOOH$  or  $PhCOOH$ . The preparation of mesoporous sorbent materials were described in John's patent; embedding binary complex templates of uranyl ion with bifunctional ligands such as amines, sulphonic acids and phosphonic acids [252]. The patents by John *et al.* [252] and Dai *et al.* [253] do not describe the extraction characteristics of other competitor metal ions. Saunders *et al.* [254] reported a modified method for the preparation of uranyl IIP material by copolymerizing with chloroacrylic acid and EGDMA, which after removal of template selectively extracts uranium from dilute aqueous solutions containing +2, +3 and +4 competitor metal ions. The polymer particles had better selectivities than previously reported. Bae *et al.* [255] have also synthesized IIP resins for uranyl ion based on the isolation of binary uranium–vinyl benzoic acid complex and copolymerizing in the presence of styrene and DVB. The resins were used for preconcentration of uranyl ion from aqueous and seawater solutions. They authors did not show the extraction ability of the polymeric resins for the control, i.e. imprinting effect was not been clearly demonstrated. In another study, Dai *et al.* investigated the properties of sol–gel glasses templated with uranyl ions [256]. They observed a significant increase in the affinity and selectivity of the imprinted sol–gel sorbents over control polymer-based sorbents, while the surface areas remained equal. An IIP material was prepared with methacryloylamidoglutamic acid (MAGA) and EGDMA and was used for selective

separation of uranyl ions from aqueous solutions [257]. MAGA was chosen because the glutamic acid molecules are linked to the polymer backbone through primary amine groups and the pendant carboxyl groups are responsible for the uranium binding as a strong complex formation is expected for the complexing of uranyl ion with carboxyl groups. In another study they prepared thorium IIP particles with MAGA-EGDMA and selectively preconcentrated thorium in the presence of uranyl, cerium and lanthanum ions [258].



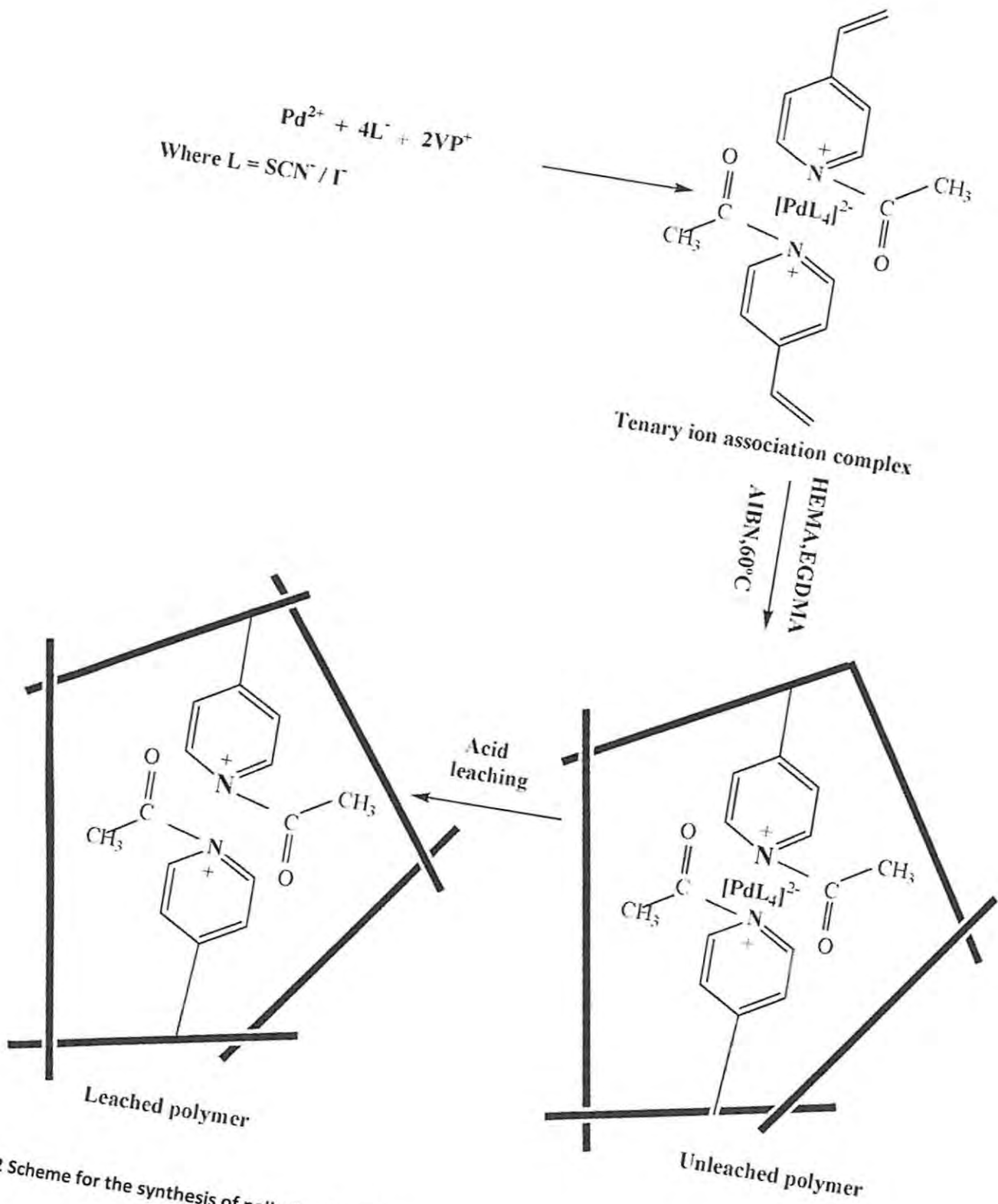


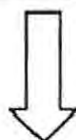
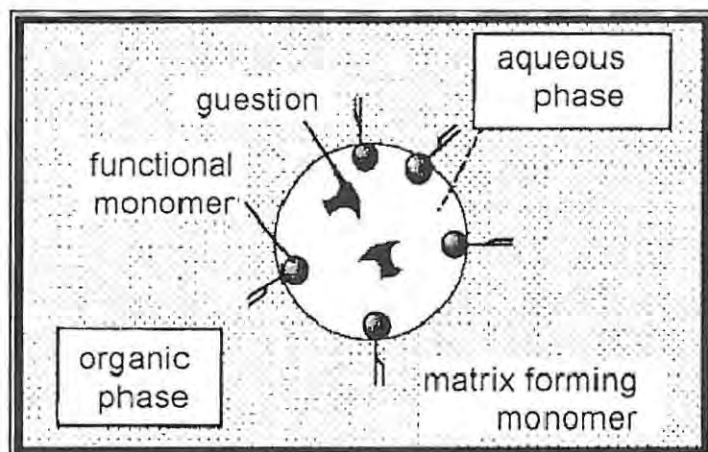
Fig. 6.2 Scheme for the synthesis of palladium(II) IIP [149].

A new IIP material was synthesized by bulk polymerization using palladium-iodide/thiocyanate–4-vinyl pyridinium ternary ion association complex in the presence of 2-hydroxyethylmethacrylate (HEMA) and EGDMA (Fig. 6.2). The imprinted anionic species  $[\text{PdI}_4]^{2-}$  or  $[\text{Pd}(\text{SCN})_4]^{2-}$  were removed by leaching the materials for 18 h with 6 M HCl to obtain leached IIP particles [259]. The leached IIP particles were used for rebinding the analyte both in the offline and online mode for the selective separation of palladium from aqueous solutions containing inorganic species that coexist with palladium in mineral ore. The developed procedure was successfully applied to the analysis of street/fan blade dust samples collected from the busy cities of India for palladium [259]. For the first time, the authors synthesized platinum IIP particles employing the same methodology [260]. The polymer materials have been used for the selective SPE of platinum from dilute aqueous solutions containing other noble and transition elements. Chemically immobilized IIPs do not have the limitation of the ligands being leached out during the washing off of the template. However, it is difficult to get or synthesize the vinylated ligands all the time. Also, the vinylation procedure is too tedious for most of the ligands because the magnitude of imprinting effect is rather low.

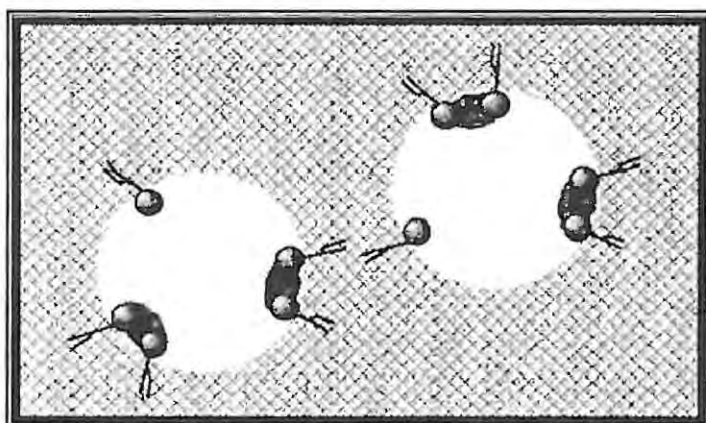
### 6.1.3. Surface imprinting

The surface-imprinting technique proposed by Takagi's group in 1992 involves the emulsion polymerization utilizing a functional monomer, an emulsion stabilizer, a polymer matrix-forming comonomer and a print molecule. A functional monomer, which is amphiphilic in nature, forms a complex with the print molecule at the interface of the emulsion and the complex so formed remains stationary at the reaction surface. After polymerization, the template is removed which results in polymeric resins, that carry the functional groups spatially arranged on their surface, giving recognition sites with preferential rebinding ability for the template ion. Subsequently, a number of variants of surface imprinting; water-in-oil or oil-in-water emulsion have been reported essentially by Japanese [261-264 and very recently by Chinese researchers [265 – 268]. The concept of the surface-imprinting technique using w/o emulsions is illustrated in Fig. 6.3.

Formation of  
w/o emulsion



Complexation  
and radical  
polymerization



Removing  
of guest  
ions and  
grinding

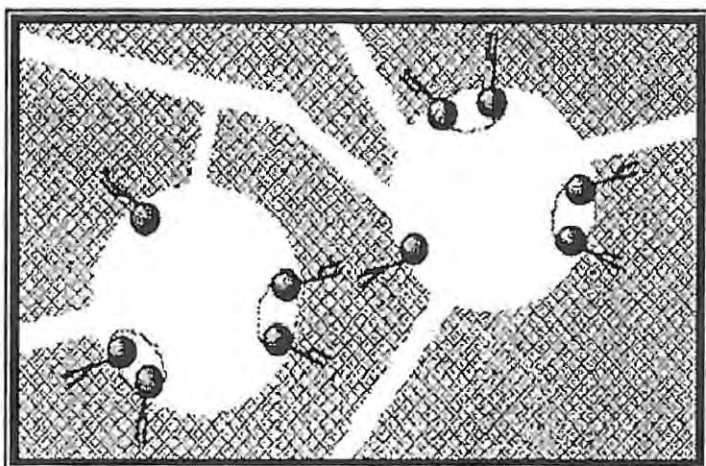


Fig. 6.3 Scheme of surface template polymerization with w/o emulsion [149]

Metal ion {Cu(II), Ni(II) and Co(II)} imprinted microspheres were prepared by employing seed emulsion polymerization [261–268]. Seed emulsion was obtained by the polymerization of styrene, butylacrylate and methacrylic acid in water. DVB, butyl acrylate and water were added to the polymerization mixture (seed emulsion) and the emulsion was left for 1 h so that the seed microspheres became swollen. The emulsion was combined with a metal ion solution to achieve complexation between the metal ion and carboxyl group on the surface and then polymerized by  $\gamma$  - rays at room temperature. Maeda and coworkers [269–273] carried out surface imprinting in oil–water emulsion. This procedure relies on a cooperative interaction of multiple functional groups with a target—the approach involves the preorganization of functional surfactants (amphiphilic host monomers) on an oil/water emulsion surface by cooperative interactions with target (template guest) in the aqueous phase. The organized structure involving the template and the functional surfactants is then immobilized by polymerization of the oil phase, which is made up of a vinyl monomer. Removal of template results in polymeric resins which carry the functional groups spatially arranged on their surface, giving recognition sites that preferentially rebind template ions, Cu(II), Zn(II) or Cd(II). 1,12 - Dodecane diol - *O,O'* - diphenyl phosphonic acid (DDDPA) having two functional groups in the molecular structure was employed as a complexing agent by Nakashio and co-workers [274–276] for surface imprinting in a water/oil emulsion. Employing the complexing agent, novel zinc ion-imprinted resins were prepared by surface template polymerization with water-in-oil emulsion using dioleoylphosphoric acid (DOLPA). A comparison of DOLPA with di-2-ethylhexylphosphoric acid (D2EHPA) suggests that interfacial activity is a substantial factor in choosing an ion-binding amphiphile [274].

Yoshida *et al.* [275] have clearly demonstrated that  $\gamma$  - irradiation of IIPs, which have been obtained by surface template polymerization resulted in more rigid polymer matrices and also allowed the functional monomer to attach firmly to the matrices. The irradiated and imprinted resin exhibit a tremendously high selectivity for zinc over copper ions using DDDPA, a bifunctional monomer when compared to conventional LLE [276]. Monomer-type functional surfactants [2-(*p*-vinylbenzylamino)-alkanoic acid and *N,N*-dialkyl derivatives] have been used as both ligand and as emulsifier for the preparation of surface template resins [277]. The surfactants were adsorbed at the water–toluene interface and emulsified DVB-styrene in a Cu(II) or Zn(II) solution. Both resins showed an imprinting effect for Cu(II) or Zn(II), respectively. Yoshida *et al.* [278] showed that incorporating aromatic rings

and a suitable straight alkyl chain in the functional monomer (organophosphorus compound) provided high binding affinity to zinc, the target metal ion. Fang *et al.* [279] synthesized a new ion-imprinted thiol-functionalized silica gel sorbent by the surface-imprinting technique in combination with a sol-gel process for selective online SPE of Cd(II). All competitive ions studied did not interfere with the determination of Cd(II) and the IIP has been applied to environmental and biological samples. Goto [280], Uezu *et al.* [281,282] and Tsukagoshi *et al.* [283] reviewed the progress of surface imprinting in various books/conferences/journals and can be referred for more details. Since, the recognition sites are residing on the surface of the polymer, in the case of surface imprinted polymers, this offers faster mass transfer kinetics. However, literature survey shows that the separation factor of these types of imprinted polymers are not much higher than the non-imprinted polymers, i.e. the imprinting effect is not that much significant in these type of polymers. Also, the retention capacity obtained with these polymers were found to be very low [277,283,284].

#### 6.1.4. Trapping

Trapping involves the use of a vinylated and a nonvinylated chelating agent. Polymerization results in the chemical bonding of the vinylated ligand, while the non-vinylated ligand is trapped inside the polymer matrix. This explains a typical trapping approach. Dysprosium [285] erbium [286] and neodymium [287] IIP particles were prepared via thermal polymerization by copolymerizing ternary mixed ligand complex [Ln-5,7-dichloroquinoline-8-ol (DCQ)<sub>3</sub>-4-vinylpyridine (VP)<sub>2</sub>] with styrene (monomer), divinyl benzene (DVB) (crosslinking monomer) in presence of 2,2' - azobisisobutyronitrile (initiator). The lanthanide imprint ion was removed by leaching with 6.0 M HCl. The preparation of leached lanthanide IIP particles is shown schematically in Fig. 6.4. The leached IIP particles were found to offer better selectivity coefficients over adjacent lanthanides compared to the separation factors reported with D2EHPA or 2-ethylhexyl-2-ethylhexyl phosphonic acid (EHEHPA) extractants using liquid-liquid extraction (LLE). This observation is particularly interesting as the selectivity coefficients with control polymer particles are lower than LLE extractants mentioned above. Kala *et al.* [288] have filed US and Indian patents for synthesis of erbium IIP particles polymerized by thermal, photochemical and radiochemical means. The patent relates to the synthesis of IIP particles for solid phase extractive preconcentration of erbium ions from other

lanthanide ions in monazite sand which is processed by a series of beneficiation processes to produce lighter, middle and heavier rare earth chloride fractions. The last fraction contains 55 – 60 %  $Y_2O_3$  along with Dy, Gd, Er as impurities. The preparation of 99.9 – 99.999%  $Y_2O_3$  gains importance as it is widely used in the manufacture of lasers, superconducting materials and colour television phosphorus. Hence, the separation of Dy, Gd and Er is an essential prerequisite to prepare such high purity  $Y_2O_3$ . Thus the three different polymerization processes described in the patent enables the separation of Er from  $Y_2O_3$ .

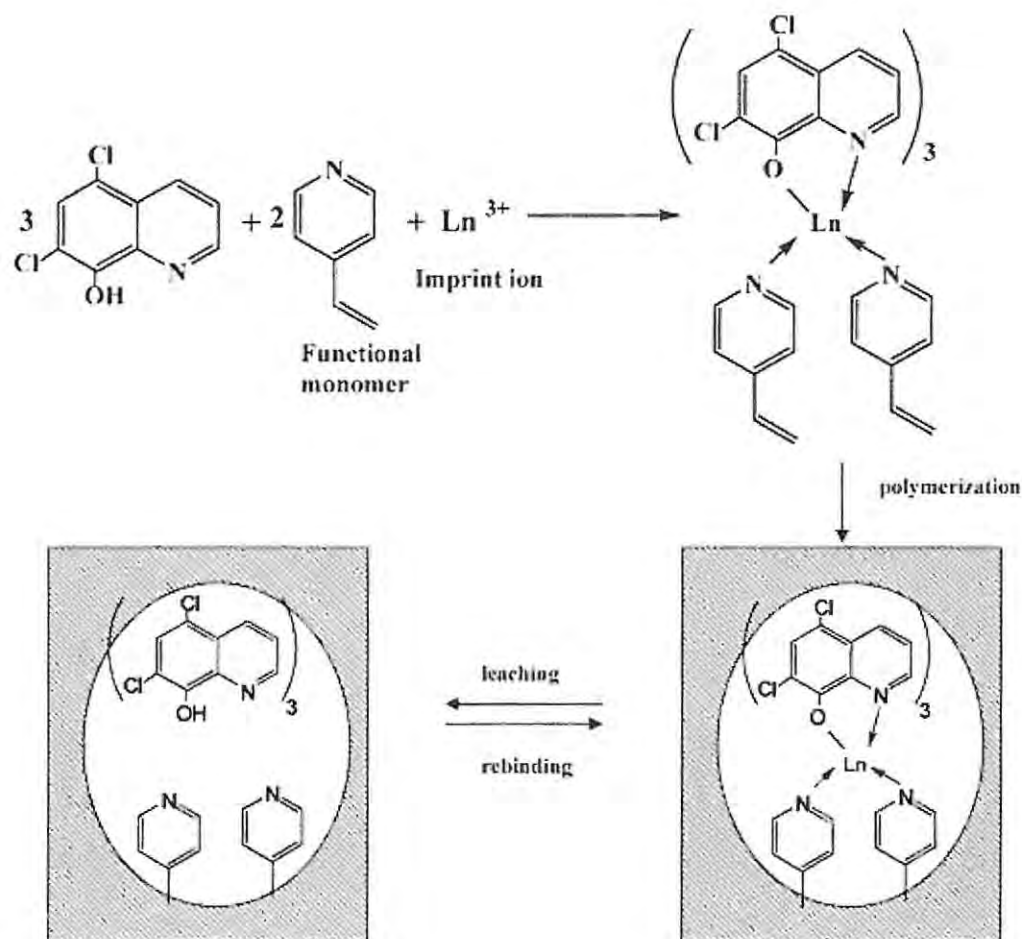


Fig. 6.4 Schematic representation of IIP synthesis via trapping [149]

Liu *et al.* [289] have prepared mercury(II) IIP particles by using  $Hg(II)$ –diazaminobenzene–VP ternary mixed ligand complex as the template in presence of EGDMA as crosslinking monomer and

AIBN as initiator. The IIP has been used for offline batch column SPE studies. These particles on leaching with 0.1M HCl + 0.5M of thiourea rebind selectively to mercury(II) compared to several transition metal ions. The accuracy of the IIP–SPE procedure was tested by analyzing certified river sediment sample (GBW08603). A similar ternary mixed ligand complex prepared with cadmium(II) was employed during the preparation of cadmium(II) IIP particles [290]. The IIP–SPE procedure was good enough for the selective recovery of cadmium(II) from matrices containing components with similar chemical property such as Cu(II), Zn(II) and Hg(II). Lu and Yan [291] prepared organic–inorganic sorbent for selective separation of cadmium through self-hydrolysis, self-condensation and co-condensation of cross-linking agent (tetraethoxy silicate) and the functional precursor {3-(2-aminoethylamino)-propyl trimethoxy silane} in an alkaline media followed by gelation. The selectivity of the sorbent was investigated by a batch competitive ion-binding experiment using Cd(II) and Zn(II) mixture. The hierarchical double imprinting concept in the presence of cetyltrimethyl ammonium bromide (CTAB) offers higher uptake capacity and selectivity coefficients than that in the absence of CTAB. The sorbent was found to possess fast kinetics for the removal of cadmium(II) from aqueous solutions [291]. Metilda *et al.* have shown that the quantitative enrichment of uranium is possible only with the IIP based on ternary complex, but not with the IIP based on binary complex [283] (i.e., without the nonvinylated ligand). Research schools from China have proposed trapping-based IIPs, essentially for transition metals. The trapping approach enabled the preparation of the imprinted polymer using simple chelating ligands, without going for the vinylation step. The IIPs prepared by this method were proved to have higher retention capacity and selectivity coefficients compared to those obtained by other methods [284].

## Chapter 7

In this chapter a mercury(II) IIP, that was synthesized and applied to water samples collected in the vicinity of Grahamstown in eastern cape, South Africa is discussed. The synthesis procedure employed was the hierarchical double imprinting approach proposed by Wu *et al* [292]. The idea was to use a monomer with more nitrogen (N) donor atoms (3 in the triamine as opposed to the 2 in the diamine they used) to improve coordination during the pre-assembly step in the imprinting process. In order to improve selectivity, a more rigorous method was used to leach out the template ion thus resulting in more available cavities for rebinding. This was carried out to improve on the relative selectivity coefficients ( $k'$ ), {300 – 500}, that were reported by Wu *et al*. A large  $k'$  value means selectivity of the prepared polymer material relative to the competing ions is high.

### 7.1 Objectives

The objectives of this thesis are;

- (1) To synthesize Mercury(II) ion imprinted polymer materials.
- (2) To characterize the polymer materials using SEM and FTIR.
- (3) To evaluate the performance of the prepared polymer materials using binding studies in batch analysis by applying them to real water samples.

### 7.2 Experimental





### 7.2.1 Chemicals

N'-[3-(trimethoxysilyl)-propyl]diethylenetriamine (TPET) and tetraethylorthosilicate (TEOS), hexadecyltrimethylammonium bromide (CTAB), sodium hydroxide, nitric acid, sodium acetate and acetic acid were supplied by Sigma – Aldrich (Saint Louis, MO, USA), mercury(II) nitrate monohydrate,  $\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$  by BDH AnalR (London, England). Reagents used were of analytical grade. All water used was purified using Direct Q 3UV millipore system.

### 7.2.2 Instrumentation and Apparatus

ICP-OES, ICAP 6000 series, Thermo Electron Corporation, (Waltham, MA, USA) was used to measure the concentration of the unextracted  $\text{Hg}^{2+}$  as well as that of  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  in aqueous media (at 194.4, 214.438, 324.754 nm respectively). To ensure that the imprint ions,  $\text{Hg}^{2+}$ , were thoroughly washed off the imprinted polymer, XRF EDX 900 spectrometer Pan Analytical, Shimadzu, (Kyoto, Japan) was used to detect the concentration of mercury from the washings of the polymer as well as in the dried polymer itself.

For morphology and characterization, scanning electron microscope (SEM) micrographs for the imprinted (washed & unwashed) and the non-imprinted polymer powders were obtained at 20 kV on a JSM 840 field emission scanning electron microscope JEOL, (Tokyo, Japan). FTIR spectra ( $4000 - 400 \text{ cm}^{-1}$ ) were recorded by a Bruker Tensor 27 FTIR spectrophotometer (Ettlingen, Germany).

The polymer particles were obtained by centrifuging with MSE Mistral 1000 centrifuge, Sanyo Gallenkamp, (Loughborough, England), at 45000 rpm for 10 min. A Jenway 3510 pH meter, (Dunmow, England) was used to measure the pH values. Standard Test sieves Retsch GmbH & Co., (Haan, Germany), were used to obtain the average size of the polymer particles by screen analysis.

### 7.3.3 Preparation of the mercury(II) ion-imprinted polymer

The mercury(II) ion-imprinted polymer was prepared by mixing  $\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$  (print ion), CTAB (surfactant micelle as second print species), TPET (monomer), TEOX (cross linking agent), 1 M NaOH (pH modulator) and ultrapure water (porogen) according to the following optimal molar ratios; 1:2:2.5:10:4:1500 respectively. The mixture was magnetically stirred at 900 rpm for 4 h. Off-white gels were yielded. The gels were mixed with more water and refluxed at 90 °C for 1 h. The gels that formed were recovered by centrifuging and subsequently refluxed with a mixture of 3 M  $\text{HNO}_3$  and 99.99% ethanol in a 1:1 v/v ratio to leach out the mercury(II) ions and CTAB template from the gels, respectively. The resulting mixture was then centrifuged and the supernatant liquid was kept so that the concentration of mercury(II) ions could be measured. The process of refluxing and centrifuging was repeated until there was no further change in the quantity of mercury(II) ions detected in the supernatant liquid. The resulting gels were washed with 3 M NaOH until the pH of the washings were at pH 7.5, then washed three times with water, before drying in the oven at 60 °C for about 4 h. The resultant granules were ground and wet sieved to a homogenous off white powder to yield the mercury(II) ion-imprinted polymer of 25 – 30  $\mu\text{m}$  as measured by standard test sieves. The unwashed polymer was obtained by washing the gels with copious amounts of water only. For the non imprinted polymer the same procedure as for the imprinted polymer was followed except that the mercury(II) nitrate was not included in the reaction mixture.

### 7.2.4 Removal of the print specie, mercury(II) ions and CTAB from the imprinted polymer

Mercury(II) ions and CTAB were exhaustively removed by refluxing seven times for 1 h with a mixture of 3 M nitric acid and 99.99% ethanol. The cooled reaction mixture was centrifuged and the supernatant liquids obtained between the several centrifuging steps were kept separately. Concentrations of mercury in these supernatant liquids were analyzed by an XRF spectrophotometer. The procedure was repeated until constant concentrations of mercury from the supernatant liquids were recorded. The solid polymer materials from the centrifuge were dried, ground, sieved and analyzed for the quantity of mercury by XRF as well.

### 7.2.5 Binding studies

### 7.2.5.1 Optimization of IIP quantity needed for maximum extraction of Hg<sup>2+</sup>

20 ml aliquots of 1 µg ml<sup>-1</sup> Hg<sup>2+</sup> spiked water, each containing increasing concentrations of the Hg<sup>2+</sup> imprinted polymer (5, 10, 15, 20, 30, 35, 25 and 40 mg) as well as the sodium acetate/acetic acid buffer, were mechanically shaken and kept for 24 h. The mixture was then filtered and the concentration of the unextracted Hg<sup>2+</sup> measured by ICP-OES. The experiment was carried out in triplicates. Statistical methods were used to determine the mean values and standard deviations. From the values, the extraction efficiencies (EEs) were evaluated using equation 7.1;

$$EE (\%) = \frac{\text{spiked concentration} - \text{unextracted concentration}}{\text{spiked concentration}} \times 100 \quad 7.1$$

### 7.2.5.2 Optimization of time needed for maximum extraction of Hg<sup>2+</sup>

Following the procedure for optimization of quantity, unextracted Hg<sup>2+</sup> ions (at 5 min intervals) were determined until a constant value was reached. This marked the optimum time needed for the polymer to bind.

### 7.2.5.3 Effect of pH on extraction of Hg<sup>2+</sup>

The optimal time and quantity of the IIP were used in the evaluation of the effect of pH by performing binding experiments at different pH. The pH of the solutions was adjusted using sodium acetate/nitric acid for pH 1-3, sodium acetate/acetic acid for pH 4-7.5, ammonium hydroxide/ammonia for pH 8-10 and ammonium hydroxide/sodium hydroxide for pH 10-12.

### 7.2.5.4 Selectivity experiments

Using the optimized conditions, competitive and selectivity experiments were performed. Both the imprinted and non-imprinted polymers were used to obtain two sets of experimental data. 25 mg polymer material was added to 20 ml aqueous solutions containing 1 µg ml<sup>-1</sup> Hg<sup>2+</sup>/Cd<sup>2+</sup> and 1 µg ml<sup>-1</sup> Hg<sup>2+</sup>/Cu<sup>2+</sup>. The pH was then adjusted accordingly to pH 7.2. These were placed in sealed

containers and stirred magnetically at 900 rpm for 15 min. After the adsorption-equilibrium, the mixtures were filtered and the concentration of each ion in the remaining solution was measured by ICP-OES. The measured values gave the concentrations of the unextracted ions, from which extraction efficiencies (EEs) were evaluated. The experiments were performed in triplicates and the results subjected to statistical analysis at 95% confidence limit.

#### 7.2.5.5 Application to real samples

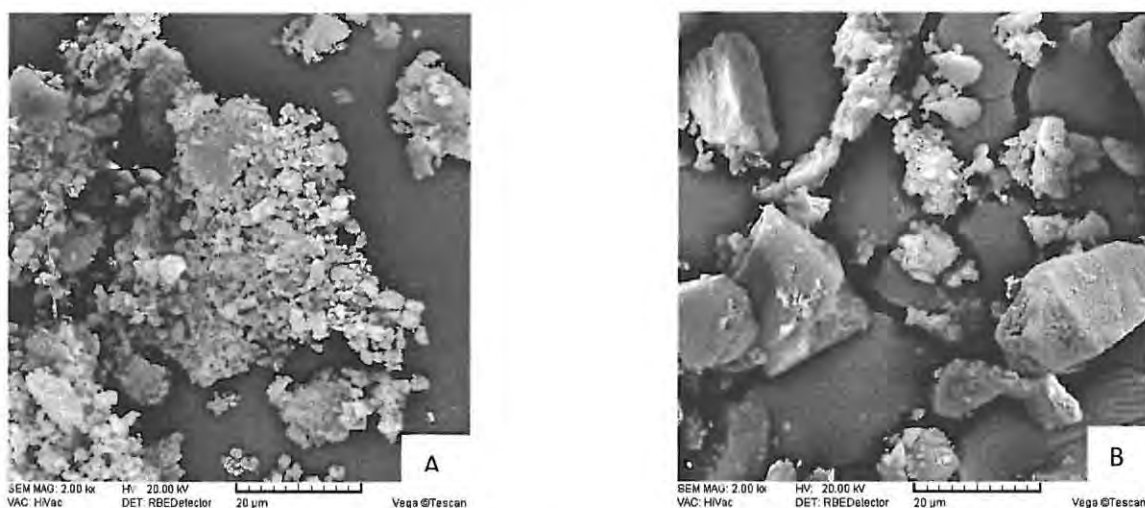
Real water samples (tap, sea, river, pulverized coal solution, treated and untreated sewerage from the vicinity of Grahamstown in South Africa) were filtered through the Millipore Millex-HV hydrophilic PVDF 0.45  $\mu\text{m}$  filter and refluxed for 1 h with 1%  $\text{H}_2\text{O}_2$  to oxidize the organic matter. The pHs of the resulting water samples were adjusted accordingly to pH 7.2. For each of the samples the concentration of  $\text{Hg}^{2+}$  was determined by ICP-OES in 100 ml aliquots for background,  $1\mu\text{g ml}^{-1}$  spiked and  $1\mu\text{g ml}^{-1}$  spiked sample equilibrated with 75 mg IIP. The samples with the IIP were continually shaken for 1 h to allow for equilibration after which the unextracted concentration of the ions was determined and EEs calculated. The imprinted powder with rebound  $\text{Hg}^{2+}$  was then eluted with millipore water, 3 M  $\text{HNO}_3$ , millipore water in sequence and the desorbed  $\text{Hg}^{2+}$  was subsequently determined with ICP-OES.

## Chapter 8

### 8.0 Results and discussion

#### 8.1 Characterization of the polymers

The SEM micrograph of the imprinted polymer displays a regular, spherical morphology with numerous pores on the spherical surface (Fig. 8.1A), which is a suitable geometrical and textural property for a potential adsorbent. This indicates that there are many well defined binding sites on the imprinted polymer. The irregular, amorphous morphology exhibited in the non-imprinted SEM micrograph (Fig 8.1B) show no well defined binding sites hence unsuitable to act as an adsorbent.



**Fig. 8.1** SEM images of the ion imprinted polymer (A) and the non imprinted (B)

Characterization of the imprinted (unwashed & washed) and non-imprinted polymers by FTIR showed similar locations and appearances of major bands (Fig 8.2). The observed peaks around  $1055$  and  $951\text{ cm}^{-1}$  are attributed to the Si-O-Si and Si-O-H stretching vibrations respectively, while the peak at  $788\text{ cm}^{-1}$  is assigned to Si-O stretching. Of particular interest is the peak at  $1472\text{ cm}^{-1}$  due to the existence of the N-Hg-N stretching vibrations. It is relatively strong in the unwashed

polymer, relatively weak in the washed polymer and not present in the non-imprinted polymer. The strong peak in the unwashed polymer spectrum indicates the abundant existence of a coordination complex,  $[\text{Hg}(\text{TPET})]^{2+}$  in its polymer structure. The weak peak in the washed polymer is due to the removal of most of the  $\text{Hg}^{2+}$  ions from the polymer hence very little coordination remaining between  $\text{Hg}^{2+}$  and TPET after washing. The  $\text{Hg}^{2+}$  ions were not included during the synthesis of NIP hence the obvious absence of the characteristic peak in its spectrum.

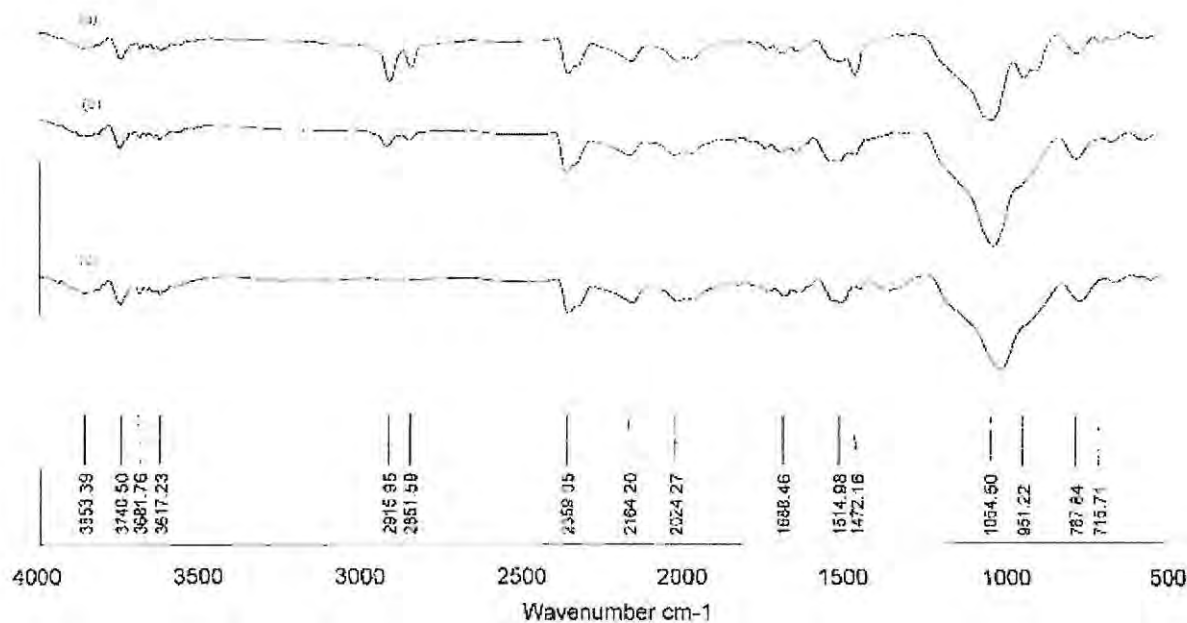


Fig. 8.2 FTIR spectra of (a) unwashed, (b) washed and (c) non imprinted polymers

## 8.2 Washing off the mercury (II) ions and CTAB

The relatively weak N-Hg-N stretch at  $1472\text{ cm}^{-1}$  in the FTIR spectrum of the washed polymer as well as the low concentration of mercury (0.111%) determined by XRF in the final washing of the IIP suggest that  $\text{Hg}^{2+}$  ions were thoroughly washed out. Contrarily, the direct determination of mercury concentration of the washed IIP by XRF was 13.158 % (Table 8.1). This is noted to be very high for a polymer, which was thought to be thoroughly washed, and for a material intended to be used in trace analysis. A logical explanation to these discrepancies is that, maybe some of the mercury got bound to the structure of the polymer during synthesis to the extent that it could not

be removed by the methods that were used for washing in this study. Another assumption is that since XRF is a very sensitive technique and measures total mercury, it could be that the mercury that was detected in the washed IIP even after thorough washing was not the one involved in the selective nano-pore formation of the IIP. Hence of little concern to our study as it would not affect the performance of the polymer if it was not involved in the initial binding of the template.

**Table 8.1 Percentage of Hg in the washings and the corresponding dried polymer measured by XRF.**

<b>Number of washings</b>	<b>% of Hg remaining in each washing (<math>\pm 0.002</math>)</b>	<b>% of Hg remaining in corresponding dried polymer (<math>\pm 0.002</math>)</b>
1	6.335	35.486
2	0.873	33.347
3	0.675	26.343
4	0.426	24.562
5	0.111	13.158
6	0.108	13.153
7	0.109	13.155

### ***8.3 Extraction behaviors of the polymer***

The percentage of  $\text{Hg}^{2+}$  extracted increases with the quantity of polymer from 5 mg up to 25 mg, after which further increase in the quantity of the polymer does not yield any increase as shown in Fig. 8.3. This marks the optimum amount of the polymer powder (25 mg) needed to bind maximally. The highest extraction efficiency (EE in %) of  $\text{Hg}^{2+}$  achieved was calculated as  $88.8 \pm 0.1\%$ .

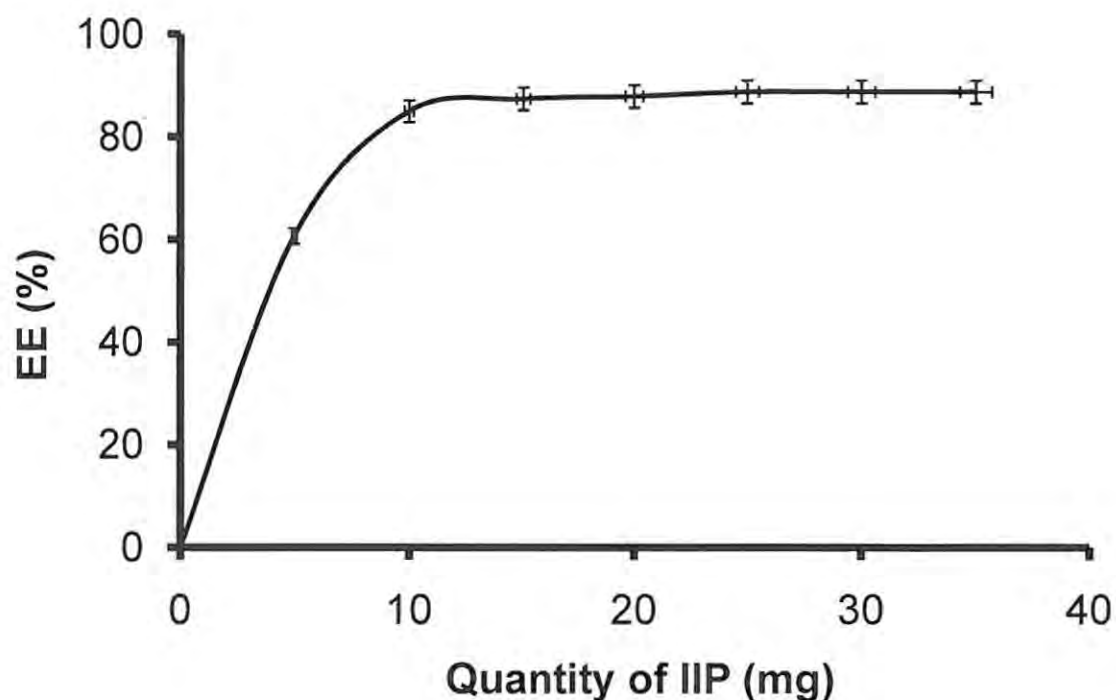


Fig. 8.3 Optimization of the quantity of IIP needed to extract maximum  $\text{Hg}^{2+}$ .

Fig. 8.4 shows the time dependence of the adsorption capacities of  $\text{Hg}^{2+}$  ions on the polymer powder as a function of time.  $\text{Hg}^{2+}$  ion adsorption increases with time during the first 15 min after which it levels off (Fig. 8.4.), exhibiting fast kinetics for binding the  $\text{Hg}^{2+}$  ions. A good EE (%) of the bound ions was recorded ( $89.9 \pm 0.1\%$ ) even at these fast equilibration-adsorption times.



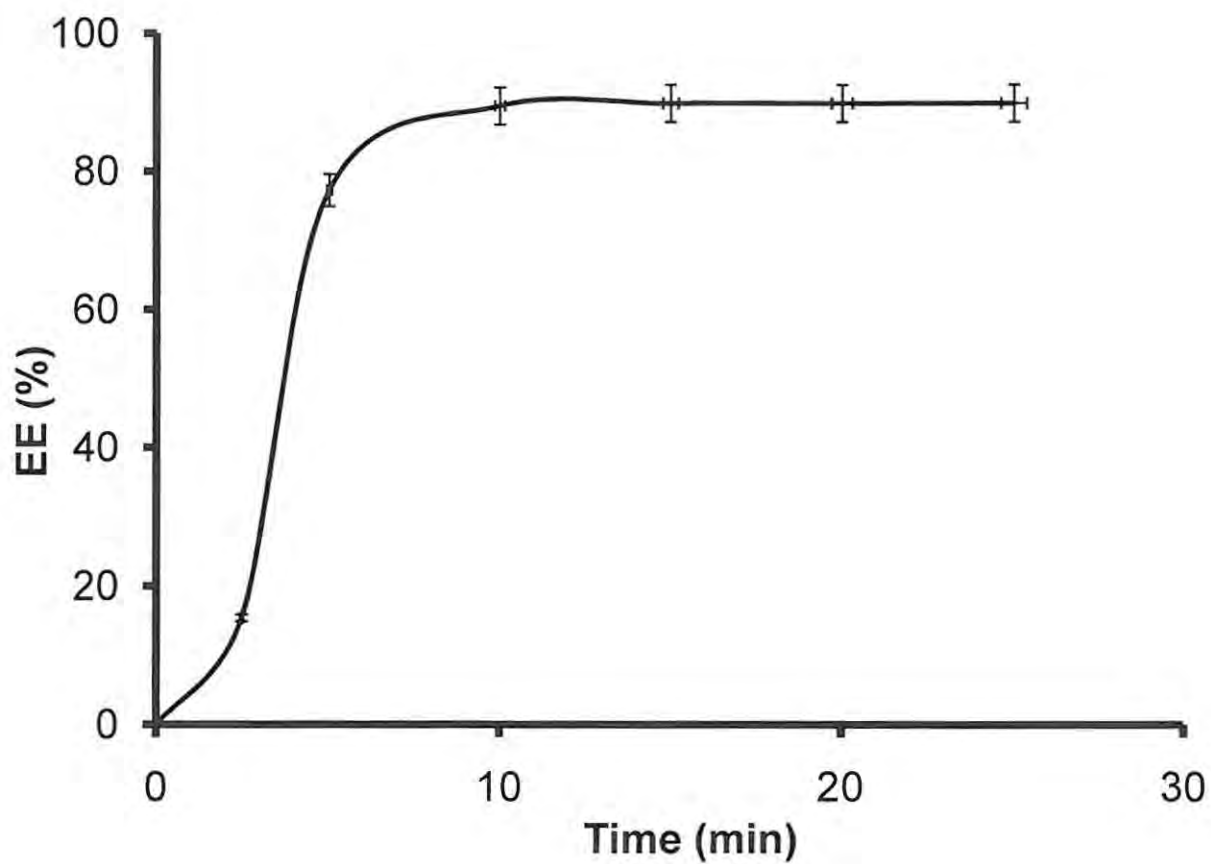


Fig. 8.4 Optimization of the time needed for 25 mg of IIP to extract maximum  $\text{Hg}^{2+}$

The effect of pH on  $\text{Hg}^{2+}$  ion adsorption by the polymer powder is shown in Fig. 8.5. The polymer exhibited low affinities for the  $\text{Hg}^{2+}$  ion uptakes in very acidic and alkaline conditions as indicated by the low EEs, and the highest calculated being  $87.5 \pm 0.1\%$  at  $\text{pH } 7.2 \pm 0.2$ .

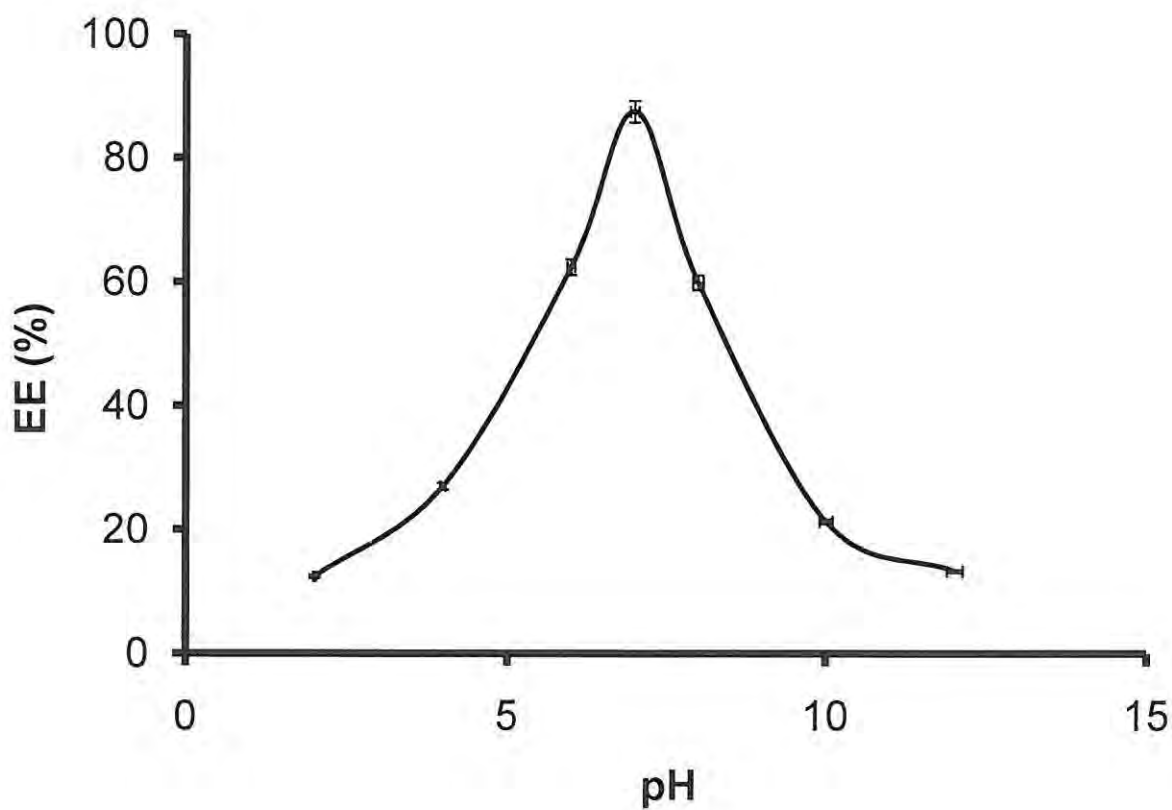


Fig.8.5 Effect of pH on  $\text{Hg}^{2+}$  extraction.

#### ***8.4 Selectivity studies of the imprinted polymer powder for $\text{Hg}^{2+}$ versus closely related ions, $\text{Cd}^{2+}$ and $\text{Cu}^{2+}$***

Competitive adsorptions of  $\text{Hg}^{2+}/\text{Cd}^{2+}$  and  $\text{Hg}^{2+}/\text{Cu}^{2+}$  couples were investigated in an equilibration-adsorption batch system (Table 8.2).

**Table 8.2 Concentration (%) of the ions extracted by the IIP and NIP from the spiked 1  $\mu\text{g ml}^{-1}$  aqueous solutions as individual ions or couples.**

Spiked ultrapure water (1 $\mu\text{g}$ $\text{ml}^{-1}$ )	% of $\text{Hg}^{2+}$ extracted		% of $\text{Cd}^{2+}$ extracted		% of $\text{Cu}^{2+}$ extracted	
	IIP	NIP	IIP	NIP	IIP	NIP
$\text{Hg}^{2+}$	99.89 (1.33)	27.48 (0.92)	–	–	–	–
$\text{Cd}^{2+}$	–	–	36.41 (0.44)	23.11 (0.26)	–	–
$\text{Cu}^{2+}$	–	–	–	–	13.81 (0.47)	22.04 (0.21)
Mixture of $\text{Hg}^{2+} / \text{Cd}^{2+}$	88.88 (0.65)	11.79 (0.09)	21.79 (0.37)	13.06 (1.03)	–	–
Mixture of $\text{Hg}^{2+} / \text{Cu}^{2+}$	96.11 (1.02)	14.64 (0.58)	–	–	7.36 (0.16)	6.83 (0.47)

NB: The values in parenthesis are % RSD values.

$\text{Cd}^{2+}$  ion was chosen as a competing ion because just like  $\text{Hg}^{2+}$  it binds well with amine ligand types while  $\text{Cu}^{2+}$  has higher affinity for the same types of ligand. Additionally both of the competing ions have the same charge, comparative ionic radii and they often coexist with  $\text{Hg}^{2+}$  ions, exhibiting certain interference properties in aqueous environments. Table 8.3, summarizes the distribution

coefficient ( $K_d$ ) the selectivity coefficient ( $k$ ) and the relative selectivity coefficient ( $k'$ ) values of the competing ions with respect to the target ions,  $Hg^{2+}$  ions.

Table 8.3  $K_d$ ,  $k$  and  $k'$  values for individual ions and ion couples for the IIP and NIP

Ion	$K_d$ ( $ml\ g^{-1}$ ) $\times 10^3$		K		$k'$	
	IIP	NIP	IIP	NIP	IIP	NIP
$Hg^{2+}$	908	0.1516	-	-	-	-
$Cd^{2+}$	0.2290	0.1202	-	-	-	-
$Cu^{2+}$	0.0640	0.1131	-	-	-	-
Mixture of $Hg^{2+}/Cd^{2+}$	-	-	3965	1.26	3147	-
Mixture of $Hg^{2+}/Cu^{2+}$	-	-	14188	1.34	10589	-

As indicated in equation 5.1 the  $K_d$  values are the ratios of the concentration of a particular ion between the imprinted polymer and the aqueous environment. The values signify the extraction ability of a unit quantity of the polymer for a particular ion in a unit volume of solution.  $K_d$  values for the imprinted polymer for all ions are higher than those of the non-imprinted indicating that even the non-imprinted polymer has non-selective sites in which any ion may be bound to. The  $K_d$  value for the imprinted polymer used to extract  $Hg^{2+}$  ions in the spiked samples is significantly high ( $908.0909 \times 10^3\ ml\ g^{-1}$ ), more than three folds higher than that of  $Cd^{2+}$  and  $Cu^{2+}$  (152 and 120  $ml\ g^{-1}$

respectively). This demonstrates the ability of the imprinted polymer to bind  $\text{Hg}^{2+}$  ions as they perfectly fit the fabricated recognition sites far more than the competing ions. The selectivity coefficient ( $k$ ) for  $\text{Hg}^{2+}$  binding in the presence of  $\text{Cd}^{2+}$  ions was found to be 3965, that is the polymer extracts  $\text{Hg}^{2+}$  3965 times more than it does with  $\text{Cd}^{2+}$  ions. For  $\text{Cu}^{2+}$  it is 14189 times making it the less interfering ion as the  $\text{Hg}^{2+}$  ion out-competes it by a large  $k$  value.

The  $k$  values for the non-imprinted polymer are of the same order of magnitude; 1.26 for the  $\text{Hg}^{2+}/\text{Cd}^{2+}$  and 1.34 for the  $\text{Hg}^{2+}/\text{Cu}^{2+}$  competition systems. The closeness of these values shows that the non-imprinted polymer has similar if not the same affinity for all the ions as it does not bears no recognition sites that were originally created for any particular ion in its structure. From the  $k$  values the effect of imprinting on selectivity can be estimated. The high values of  $k$  exhibited by the prepared imprinted polymer powder between  $\text{Hg}^{2+}/\text{Cd}^{2+}$  (3147) and  $\text{Hg}^{2+}/\text{Cu}^{2+}$  (10588) indicate that the prepared polymer is highly selective to  $\text{Hg}^{2+}$  even in the presence of its closely related analogues.

### **8.5 Application to water samples**

High extraction efficiencies, over 84% in all cases (Table 8.4), were evaluated even when the prepared IIP was used to extract mercury(II) ions from spiked real water samples. This demonstrates the suitability of the IIP to selectively extract mercury(II) ions from aqueous complex matrices. Ten calibration standards of different concentrations of  $\text{Hg}^{2+}$  in the range 0-1  $\mu\text{g ml}^{-1}$  were used for calibration and a correlation coefficient ( $r$ ) of 0.9997 was obtained with limit of detection (LOD,  $3\sigma$ ) of 0.036  $\text{ng ml}^{-1}$  which is far below the maximum contaminant level of 2  $\text{ng ml}^{-1}$  set by the United States of America environmental protection agency (USA-EPA) [293]. The accuracy and precision of the method were determined using ten replicate samples of the smallest standard (0.100  $\mu\text{g ml}^{-1}$ ) and the highest standard (1.000  $\mu\text{g ml}^{-1}$ ). The standards were pre-concentrated with the sorbent and the unextracted mercury concentration was determined with ICP-OES. The mean of the extracted  $\text{Hg}^{2+}$  as calculated was 0.089 and 1.004  $\mu\text{g ml}^{-1}$  with relative standard deviations (RSD) of 3.6 and 1.8 % respectively. The data indicate that the accuracy and

reproducibility of this method is satisfactory for the pre-concentration and analysis of  $\text{Hg}^{2+}$  in aqueous solutions.

**Table 8.4 Determination of  $\text{Hg}^{2+}$  in real water samples**

	Concentration of $\text{Hg}^{2+}$ measured in $\mu\text{g ml}^{-1}$					
	Tap	Sea	River	Treated sewage	Untreated sewage	Pulverized coal solution
Background	-	-	-	-	-	~0.0056
$1 \mu\text{g ml}^{-1}$ $\text{Hg}^{2+}$ spiked	1.0002 (0.02)	0.9998 (0.02)	1.0009 (0.03)	1.0019 (0.002)	1.0017 (0.005)	1.0056 (0.003)
Calculated values	0.0002	-0.0002	0.0009	0.0019	0.0017	0.0056
$1 \mu\text{g ml}^{-1}$ $\text{Hg}^{2+}$ + IIP spiked	0.1338 (0.002)	0.1403 (0.002)	0.1400 (0.002)	0.1479 (0.001)	0.1516 (0.002)	0.1459 (0.002)
EE (%)	86.62 (0.99)	85.97 (0.47)	86.01 (1.02)	85.24 (0.68)	84.87 (0.94)	85.49 (1.06)

NB: The values in parenthesis are % RSD values

## Chapter 9

### *9.0 Conclusion*

A mercury(II) ion imprinted functionalized polymer with exceedingly high performance as marked by the fast equilibration-adsorption kinetics (15 min), the very large relative selectivity coefficients (over 3147), high extraction efficiency percentages (over 84%) of the targeted ion ( $\text{Hg}^{+2}$ ) even in the presence of other closely related ions was successfully prepared. The polymer is simple and relatively easy to prepare. To the best of our knowledge, this is the first time such relative coefficients ( $k'$ ), in the ranges of several thousands are reported. Experimental results obtained show that the polymer has high analytical potential for the selective extraction and pre-concentration of mercury(II) ions in the presence of closely related ions. Its use as a solid phase extraction sorbent as well as performing kinetic and breakthrough studies will be evaluated in further studies. A different type of polymerization that can result in smaller, spherical particles without grinding is proposed in future work. The polymerization would allow us to be able to control the particle size and shape of the polymer material without losing some of the usable particles. Grinding the particles to narrow size ranges may be adding to the total cost of making them and surely the yield of usable particles is reduced.

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