Fabrication of Nano-Receptors based Sensors for the Real-time and Online Monitoring of Environmental Pollutants

By

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Polymerization

Nano-Composite

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PhD Thesis

By

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6FBAS/PhD ES/F14

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It is certified that we have read the thesis submitted by Ms. Maimoona Yasinzai and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the fulfillment of Ph.D. Degree in Environmental Sciences

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A thesis submitted to Suleiman bin Abdullah Aba Al-Khail Center for Interdisciplinary Research for Basic Sciences, International Islamic University Islamabad, as a partial fulfillment of the requirement for the award of the degree of Doctorate in Philosophy in Environmental Sciences

Dedicated to

papa

DECLARATION

I hereby declare that this Ph.D. thesis entitled "Fabrication of Nano-Receptors based Sensors for the Real-time and Online Monitoring of Environmental pollutants" is an independent work based on the research and experimental work carried out by me at **Suleiman bin Abdullah Aba Al-Khail Center for Interdisciplinary Research in Basic Sciences, International Islamic University Islamabad,** Under the supervision of Dr. Ghulam Mustafa and the this has not been submitted anywhere else for any other degree.

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Maimoona Yasinzai

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LIST OF ABBREVIATIONS

Acronym	**Abbreviation**
AIBN	2, 2'-azobisisobutyronitrile
AFM	Atomic force microscopy
As	Arsenite
DMSO	Dimethyl sulphoxide
EtOH	Ethanol
EGDMA	Ethylene glycol di-methacrylate
FTIR	Fourier transform infrared spectroscopy
GO	Graphene oxide
IIP	Ion imprinted polymer
MeOH	Methanol
MAA	Methacrylic acid
MIP	molecular imprinted polymer
NIP	non-imprinted polymer
NVP	*N*-vinyl pyrrolidone
ppm	Parts per million
ppb	parts per billion
PS	polystyrene
Pb	Lead
Hg	Mercury

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ABSTRACT

Due to the uncontrolled industrialization, environment pollution has become a lethal challenge. Wherein, heavy metals contaminations are the most dangerous due to the high reactivities of these metals to the biological molecules that results into various diseases including cancers, damage to the various biological molecules, processes and organs. Therefore, to tackle these human health problems due to the reactive heavy metal contaminations, have become a global health priority. Wherein, efficient and cost-effective detection of these metals is of fundamental importance. Thus, during the recent decades, various approaches and techniques have been investigated and developed for the efficient detections of these lethal metals contaminations. Ion-imprinted polymers (IIPs) using the principle of molecularly imprinted polymers is considered to be most promising in term of efficiency, ease, cost and broader range of applications.

Therefore, this research was designed and aimed for developing ion-imprinted polymers for efficient detection of heavy metal ions and molecular imprinting polymers for efficient detection of reactive dyes using bulk imprinting technology. Our approach based upon incorporated templates (using mercury, chromium, arsenite, and lead) and a functional monomer in the presence of an excess of crosslinking agent (EGDMA), for achieving a three-dimensional polymer network. Multiple optimizations of different parameters such as, monomer to cross-linker ratio, type of porogenic solvent, amount of cross-linker, type of functional monomer were experimented to achieve highly sensitive and selective polymer. The morphological studies of IIP/MIP were done by AFM and SEM techniques respectively and they confirmed a porous structure of receptor. To further enhance sensitivity, graphene oxide-based nano-composite was prepared which provided better performance than the polymers mentioned above. Composites of mercury, chromium, arsenite and lead gave the highest sensitivity value. The lowest detection limit detected by this sensor system was found for mercuric iodide and was 97 ppb amongst Cr, Pb, As based polymers. Reactive dyes based molecularly imprinted polymer was also synthesized to determine reactive dyes and acid dye in the industrial wastewater. Furthermore, MIP based composites were also studied for the detection of reactive dyes.

These studies revealed that these synthetic polymers and nano-composite of graphene oxide are effective for the detection of Hg, Cr, Pb and As and azo dyes, even at very low concentrations. Furthermore, these receptors-based sensors can be used for online and real-time analysis of pollutants.

New procedures of alternative syntheses for bulk polymerization that can improve the properties of IIPs particles, should promote future development of MIPs and IIPs in different application fields.

CHAPTER 1:

INTRODUCTION

1.1 Introduction:

Extensive urbanization and industrialization of human civilization resulted in the discharge of hazardous contaminants and pollutants into the air, aquatic streams, and soil. These outflow wastes contain toxic substances such as metals, organic impurities, etc., which cause detrimental and toxicological effects on the natural biota. According to the World Health Organization (WHO), hazardous metals i.e. Zn_2C, Hg_2I_2, Pb_2C, $Ascl_3$, and Cr_2O_3 are needed to be removed from the environment (Chong 2010). The table below shows different sources of heavy metals into the environment.

Table1.1: Anthropogenic source of heavy metals (Dixit et al. 2015)

Source	As	Cr	Pb	Zn
Mining and ore processing	+	-	-	+
metallurgy	+	+	+	+
Chemical industry	+	+	+	+
Alloy industry	-		+	+
Paint industry	-	+	+	+
Glass industry	-	+	-	-
Paper mills	+	+	-	+
Leather tanning	+	-	+	+
Textile dyeing	+	+	+	+
Chemical fertilizer	+	+	+	+
Coal burning	+	+	+	-

Water pollution has become a universal threat during the past few decades. Industries and agriculture sector, have released a broad range of chemical contaminants into the environmental water (Chong, 2010). Heavy metals, such as arsenic, zinc, copper, nickel, mercury, chromium, and cadmium are released in wastewaters (Dickert 1998). Especially, unwanted and excessive heavy metals in the biological systems readily react with the biological molecules which provide the core reason of damaging the natural optimal biochemical processes of the biological systems. Copper although play an essential role in animal's metabolism but its excessive ingestion brings about serious toxicological concerns, such as vomiting, cramps, convulsions, or even death

(Paulino 2006). Nickel exists in DNA or RNA of our human body where it functions in association with nucleic acids but its exceeding level might bring creates lung problems and different complications in kidneys (Borba 2006). Lead is considered a cumulative toxicant that affects the reproductive system as well. Other lethal signs include anemia, sleeplessness, headache, faintness, irritability, and renal damages (Naseem 2001). Online and Real-time detection and analysis of aquatic pollutants are of potent prime importance as it gives quantitative as well as qualitative information regarding different water pollutants in real-time. Various analytical techniques are available for the monitoring of water pollutants but these techniques go through a number of processes before reaching sample analysis. Scientists are looking for such devices that are easy to handle and could be taken on field for online and real-time monitoring of environmental pollutants (FL. Dickert 2004). During recent years, sensors have received significant attention as one of the important devices in electronic systems with enormous capability for information processing developed within the electronics industry. A sensor is a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal. Chemical sensing is a process of getting information about the concentration of a specified analyte or the composition of target analyte in real time. So a chemical sensor is used to recognize and measure the specific analyte (both liquid and gases) in the mixture, more precisely an ideal chemical sensor should be selective and sensitive (W. Gopel 1998).

1.2 Chemical sensor

The chemical sensor is an analyzer that responds to a particular analyte selectively and reversibly and transforms input chemical quantity, ranging from the concentration of a specific sample component to a total composition analysis, into an analytically electrical signal (Janata. 2008). The chemical information may originate from a chemical reaction by a biomaterial, chemical compound, or a combination of both attached onto the surface of a physical transducer toward the analyte. The subject of chemical sensor is an emerging discipline formed by the multidisciplinary study among chemistry, biology, electricity, optics, mechanics, acoustics, thermology, semiconductor technology, microelectronics technology, and membrane technology (Janata. 2008). Chemical sensors are composed of three major parts,

- Receptor or sensitive layer
- Transducer
- Electronics and data storage system

The ***sensitive layer*** is a chemical recognition layer containing either a polymer or an antibody (synthetic or natural) that is used to selectively monitor the chemical constituents within the environment of the sensor. When this recognition layer is open to the analyte, it interacts with it. Upon this interaction, one of the physical properties of the layer i.e. masses, optical absorbance, reflectance, polarity, impedance, voltage, fluorescence behavior changes, and these changes are detected by the transducer system. A ***transducer*** converts energy from one form into another form. It measures physical variables that are linked with the resulting form of energy and allows it to estimate the physical variables which are associated with the input energy. ***Electronics and data storage*** systems detect, amplifies, and processes these signals (H .Yan, 2006).

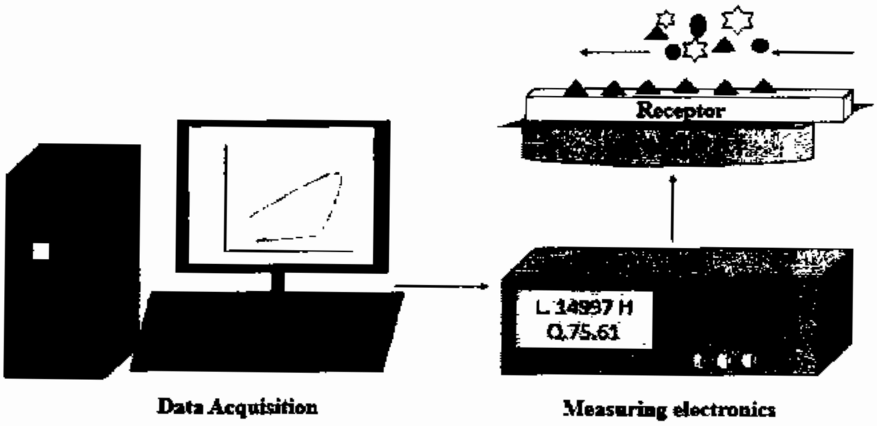


Figure 1.1: General layout of a chemical sensor

1.3 Classification of chemical sensors

The sensing system can be created when molecularly or ionic imprinted receptors are coated onto transducers that respond to any change occurring on the surface of materials or within its bulk. Sensors are normally designed to operate under distinct conditions for the specified analyte in certain sample types (Catterall 1997). Therefore, it is not always necessary that a sensor responds specifically to a certain analyte. Under fully controlled operating conditions, the analyte signal may be independent of other sample components, thus allowing the determination of the analyte without any major preliminary treatment of the sample. Contrarily unspecific but satisfactory reproducible sensors can be used in series for multicomponent analysis using multivariate calibration software and signal processing. Such systems for multicomponent analysis are called sensor arrays. Chemical sensors are generally allocated into three sub-groups created on the principle and operation of the transducer (Catterall 1997).

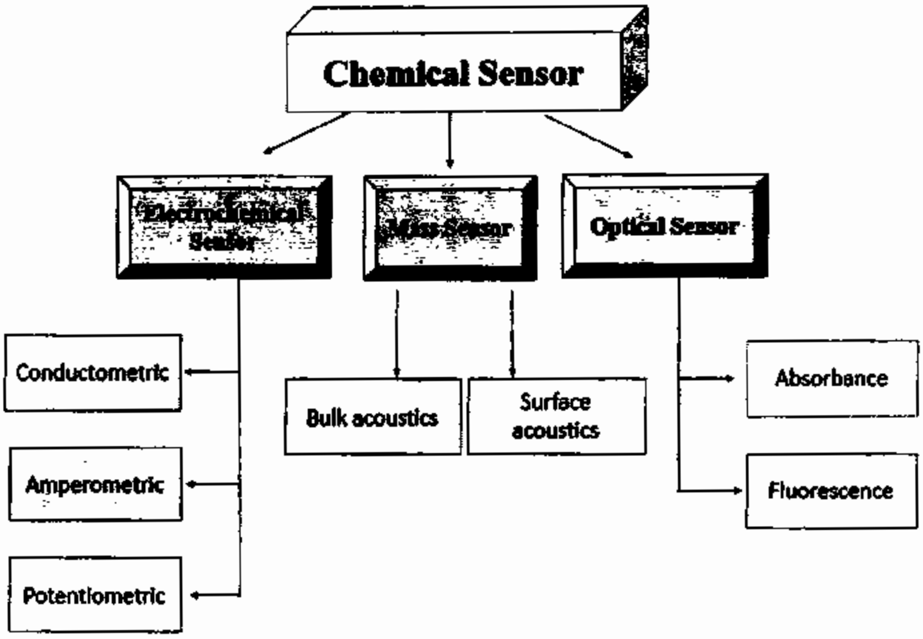


Figure 1.2: Classification of chemical sensor

1.3.1 Electrochemical sensor

Electrochemical sensing systems are highly sensitive and selective as well as cost-effective, occupying minimal space and low power requirements. The electrochemical interaction effects are transformed into beneficial electrical signals. An electrode is used in electrochemical sensors as the transduction part that is highly quailed to meet the power requirements and cost of on-site environmental monitoring (J. Wang, 2000) (E. Bakker and M. Telting-Diaz, 2002). Modern electrochemical sensors use several properties to detect various parameters in our everyday lives, whether physical, chemical, or biological parameters. Some examples are environmental monitoring, health and instrumentation sensors, and sensors related to machines, such as automobiles, airplanes, mobile phones, and technology media. In recent decades, modern sensing systems have benefited from advances in microelectronic and micro-engineering, mainly through the manufacturing of even smaller sensors with more sensitivity and more selectivity, and with lower production and maintenance costs (F.R. Simões 2017).

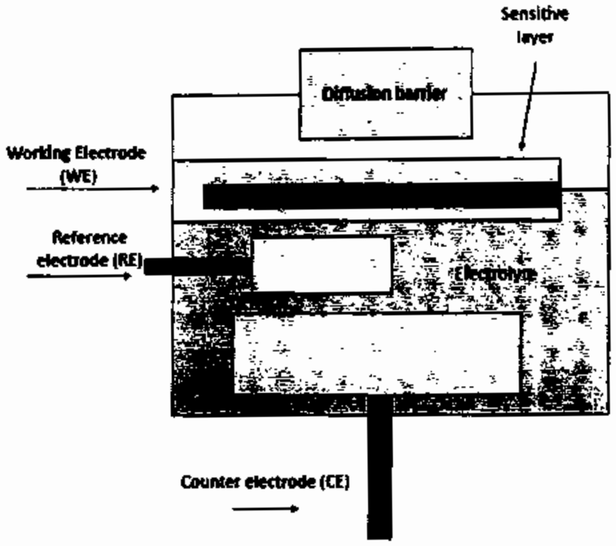


Figure 1.3: Systematic illustration of chemical sensor

The electrochemical sensors are further classified into three subgroups in according to the measured physical parameters which are briefed in below.

a) Conductometric: Conductometric devices measure the ability of an analyte (e.g. electrolyte solutions) or a medium (e.g. nanowires) to conduct an electrical current between electrodes or reference nodes (Albert 2000).

b) Potentiometric: Potentiometric devices measure the accumulation of a charge potential at the working electrode compared to the reference electrode in an electrochemical cell when zero or no significant current flows between electrodes (B. Eggins. 2002). In other words, potentiometric provides information about the ion activity in an electrochemical reaction (A. Chaubey, 2002), (E. Bakker, 2005).

c) Amperometric: Amperometric devices constantly measure the current that results from the oxidation or reduction of an electro active species during a chemical reaction (P. B. Luppa, 2001).

1.3.2 Mass sensors:

A mass-sensitive device utilizes the piezoelectric property of materials based on mechanical oscillations. It measures a change that occurs in a support material on specially modified surfaces. The mass change is due to the accumulation of the target material onto the mass sensitive piezoelectric crystal. The evolution of the oscillation frequency of the device depends on the adsorbed mass according to the following Sauerbrey equation:

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\rho_q \mu_q}}\Delta m$$

With no mass on QCM there is no change in frequency and is called as initial frequency as f_1, upon loading of mass the frequency decreases and that is called as final frequency f_2. The change in frequency is expressed as Δf and when the mass is removed the sensor response reverses to its initial frequency f_1. It has been experimentally found that with every 1ng increase in mass deposited onto the QCM surface changes the frequency change by 0.9 Hz.

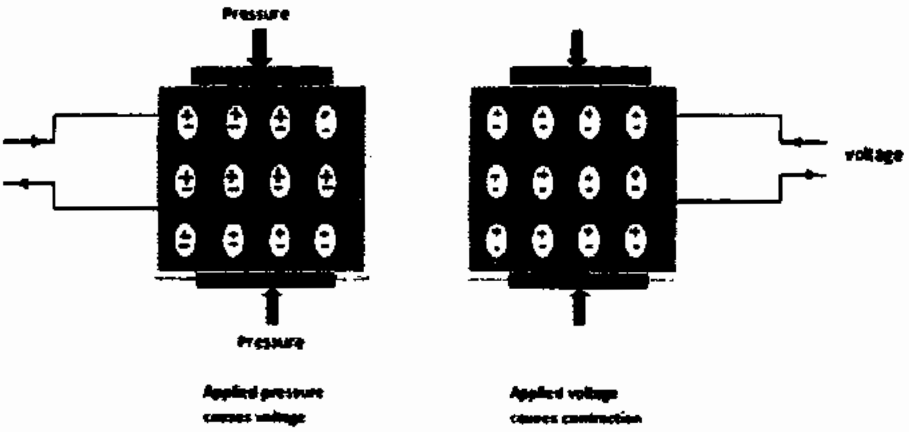


Figure 1.4: Piezoelectricity principle

Mass sensors are further classified into two sub groups are briefed in below.

a) Bulk acoustics:

The thin film resonators are the fundamental element of the bulk acoustic wave (BAW) device. A piezoelectric film is sandwiched between two metal films and the traveling wave propagate through the interior of the substrate. These waves identify vibrations individually where particle motion is parallel and perpendicular to the direction of wave propagation. Shear waves have comparatively lower velocity than longitudinal waves (A. Damhulalici, 1991).

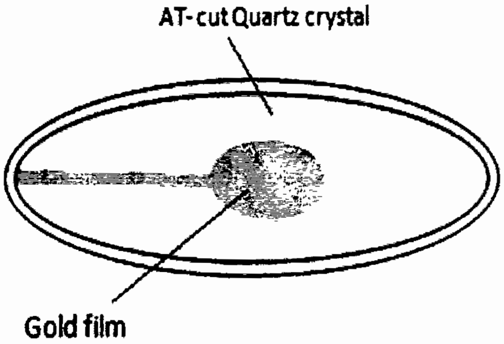


Figure 1.5: Quartz crystal microbalance chip

b) Surface acoustic

Such devices are layered with a receptor layer, and for sensing purpose are connected to a frequency defining element or component. A change in the recognition layers are responsible for selective interactions with different types of analyte material and considered as an essential part of SAW devices (D. Ahuja, 2012). Surface acoustic wave devices depend on the modification of the propagation velocity of a generated acoustical wave affected by the deposition of a definite mass of the analyte. SAW Oscillators are different from other **oscillator** types in that they use acoustic waves on a material's surface for signal processing. SAW Oscillators are comprised of an input transducer that converts an electric signal into acoustic waves and an output transducer that reconverts the waves into electrical signals (D. Schuetzle 1986).

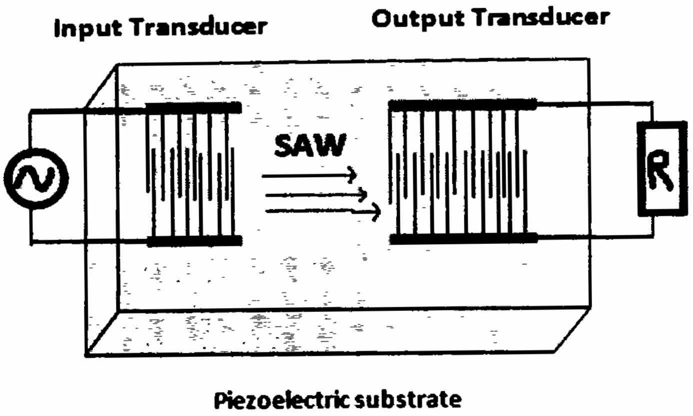


Figure 1.6: Surface acoustic wave sensor

1.3.3 Optical sensors

It is a class of sensor that alters light waves to electric signals and those read by the instrument. Optical sensor has the aptitude for measuring and detecting the change from a single or multiple light beam. This change is usually based around different alterations in the intensity of the light. These sensors can work on the distribution of points or these can also work through single point method (L. De Stefano, 2007).

1.4 Molecular imprinting polymers:

All cells and molecules in living organisms contribute to their functionalities in a predefined ordered fashion necessary for their functions and survival. These molecules show interactions very precisely with their counterpart in a highly selective and specific way and their interactions with other competing molecules are close to negligible. This sort of discrimination among the molecules and atoms is termed as molecular recognition (C. Alexander 2006).

This interaction between the natural binding partners has motivated the scientists to design or synthesized artificial receptors for the analyte of interest or pollutants. Molecular imprinting offers the most feasible synthetic approach to achieve biomimetic artificial recognition materials. Molecular imprinting polymer (MIP) is an accepted method in which template-assisted methods are used to generate selective binding sites in synthetic polymers (W.M. Mullett 1999).

The achievement of molecular imprinting polymer is to arrange functional monomer and cross-linker around a target analyte to create a cavity, which after removal of the template persists and rebinds the target analyte. Rebinding of an analyte to the polymer is dependent on different intermolecular forces. These intermolecular forces should be weak enough to remove the analyte from the synthesized polymer and at the same time, it should be strong enough for the polymer to perform its binding function (P. Turkewitsch 1998).

1.4.1 Synthesis of molecularly imprinted Receptors:

During the synthesis of the receptor layer, a template is allowed to interact with the functional monomer in the presence of a solvent (Porogen) through a non-covalent bond, followed by polymerization with crosslinking the polymer and at a fixed temperature. The template molecules are then detached by washing to interrupt the interactions and forming cavities behind. For that reason, the choice of the chemical elements making up the MIP is of crucial importance forming cavities for the template molecule (A. Poma, 2013).

Molecularly imprinted polymers are the artificial recognition material imparting the selectivity and increasing the sensitivity of a synthesized sensor for the desired template molecule. They work on the lock and key principle highly witnessed in nature as well such as antibodies. A lock is unlocked by its appropriate key, similarly, the analyte will selectively bind to its respective molecularly imprinted polymer (Kostrewa 2003) (F. Lanza 2001).

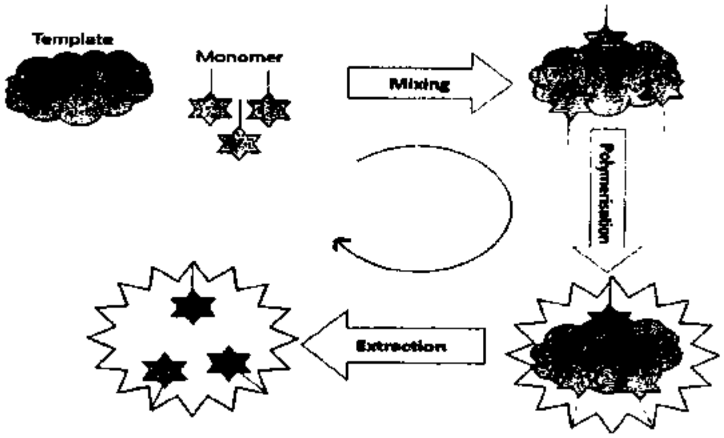


Figure 1.7: Molecular imprinting strategy

Molecularly imprinted polymer consists of four basic components.

a. Template, the material which is to be imprinted.
b. A monomer which provides the functional groups providing affinity for the analyte of interest.
c. A cross linker which impart robustness to the molecule. It has multi-functional groups. It gives the particular orientation to the cavities and this is why they are selective for the analyte.
d. An initiator which does not take part in the reaction but generates the radical which are assembled together.

1.5. Polymerization techniques:

Polymerization is the process to create polymers. Polymerization and material recognition are essential areas of molecular imprinting technology. Different polymerization techniques are applied to obtain polymers with unique properties suitable for various applications. Polymerization techniques are of different types depending upon the type and size of analyte.

Polymerization methods are of the following types:

- Bulk polymerization
- Solution polymerization
- Suspension polymerization
- Emulsion polymerization

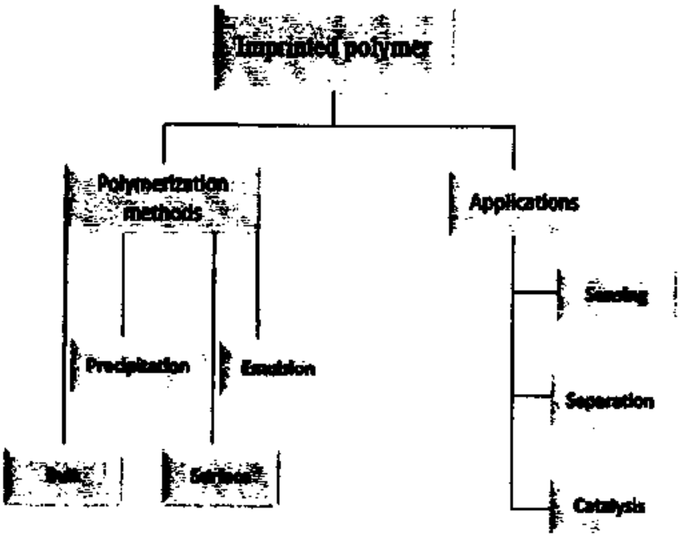


Figure 1.8: Polymerization and application fields of imprinting polymers

1.5.1 Bulk Imprinting

This method is usually applied for imprinting of low molecular mass template molecules i.e. metal ions, volatile organic compounds, reactive dyes, short-chain hydrocarbon, to prepare recognition materials for sensing purposes. Bulk polymerization is one of the most common methods of generating imprinting receptors, in which functional monomers are covalently or non-covalently bound to a template. During the polymerization step, the template (molecule or ion) is directly added to the monomer mixture due to which the interaction sites are distributed throughout the bulk of the polymer matrix. The resulting pre-polymer complex is then copolymerized with a cross-linking monomer and a free radical initiator which produces highly cross-linked insoluble polymer. Removing the template from the polymer will leave behind the imprints of the respective template. (B. Scho¨llhorn 2000)

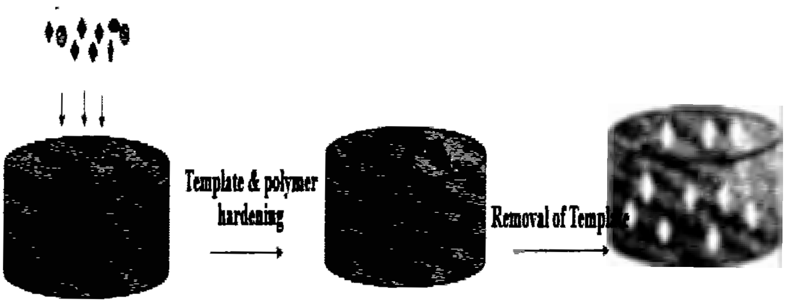


Figure 1.9: Bulk imprinting strategy

1.5.2 Surface imprinting:

As is already mentioned, bulk imprinting is used for small molecules and ion to produce imprints throughout the bulk of a polymer. But if the template is a large molecule (proteins, cells, MO) then surface imprinting approach is used. In this case, the specific template species are self-organized on a stamping surface as shown in figure 10. The oligomeric mixture is coated onto a transducer which is then hardened by high temperature. (Gizem Ertürk, 2017)

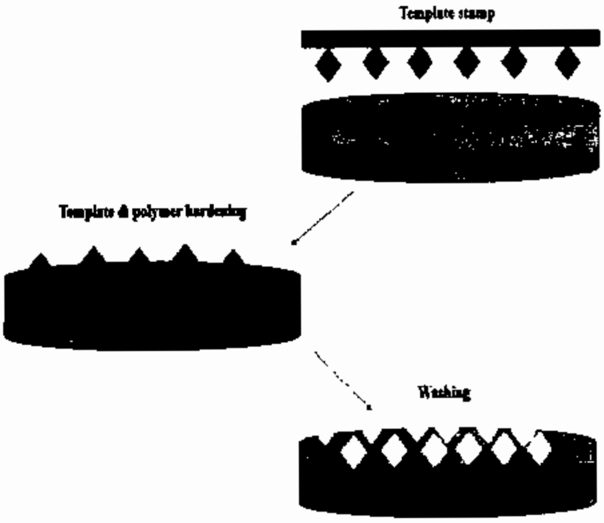


Figure 1.10: Surface imprinting strategy

1.6 Strategies for the creation of imprinted sites

The interaction or bonding formed between a template or analyte with a monomer is of utmost importance because these interactions are accountable for matching the functionality of binding cavities in a synthesized polymer.

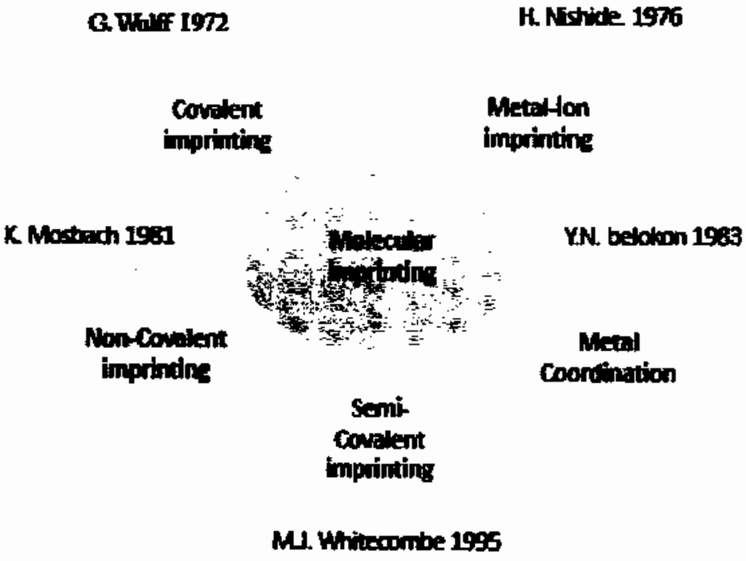


Figure 1.11: Scheme for classification of imprinting

1.6.1 Covalent approach:

It is is an imprinting strategy in which reversible covalent bonding is developed in a monomeric solution before proceeding to polymerization. The first time was introduced by G.Wulff and mainly deployed for the imprinting of compounds lacking functionalities (G. Wulff 1995). In the beginning, the covalent bonds are developed between template molecules and functional monomers whereas upon polymerization template is not released from the highly cross-linked polymer matrix (W. Lian, 2012). The bond breaking occurs on the removal of a template to observe recognition cavities. Generally, noncovalent imprinting can easily be achieved through a simple and straight forward way and also applicable to a wide range of templates. But with respect to the strictness of imprinting, covalent imprinting has some advantages over non-covalent imprinting because of two factors. Firstly, the template-monomer complex is more rigid and stable which leads to well defined and clear guest-binding. Secondly, as there is a strong interaction between monomer and template as compared to non-covalent binding, therefore imprinting at a wide range of temperature, pH, and solvent polarity is also feasible for covalent imprinting (L. Gasman).

1.6.2 Non-covalent imprinting:

In such type of molecular imprinting, a complex is made amongst template and monomer(s) on the basis of non-covalent, weak interaction. Generally, non-covalent imprinting can easily be achieved through a simple and straight forward way and also applicable to a wide range of templates. In the non-covalent approach specificity of binding sites depends upon the quantity of functional monomer; the more significant the amount of functional monomer the better the affinity of the template. On the other hand, if excess functional monomer is used in a process it will produce some non-specific binding sites which in return will affect the selectivity of the polymer. For optimization of monomer to Crosslinker ratios for the imprinting of analyte of interest active search should be conducted (E. Yilmaz, 2005).

1.6.3 Semi-covalent imprinting:

Semi-covalent imprinting is an advancement of covalent and non-covalent imprinting in which mainly recognition cavities are obtained via covalent bond formation between monomer-template complexes while a non-covalent binding occurs in rebinding of the template molecule. The template is typically washed out via hydrolysis from cross-linked polymer matrix[i]. Generally, two synthetic routes are adopted for semi-covalent imprinting: (i) by direct connection of template and monomer through an ester or amide linkage or (ii) introducing a spacer between the template and polymerizable recognition component in the imprinting step. There are some privileges associated with this type of binding, at first; association of functional monomers to template molecule, secondly; random distribution of functional monomers, thirdly; uniform recognition sites are produced and finally; re-inclusion of template molecule is free from any kinetic hindrance except diffusion (SH. Cheong 1997).

1.6.4 Ion imprinted polymers (IIPs):

The term molecular imprinting is mostly used for the MIPs synthesized for atoms, molecules, or microorganisms. A recent and a newly discovered branch of MIPs are the IIPs. The analyte for which these polymers are synthesized are the cations or anions. Being a recent technique, the work on IIPs is very little. Most of the time it is very difficult to achieve the imprinting of an ion, and study the interactions that it may undergo with the polymer. This is due to the reason that ions are the smallest possible analyte in the world of chemistry. Metal ion extraction from the water resource is a major issue because these ions are highly toxic and have carcinogenic effects (Catherine Branger., 2013).

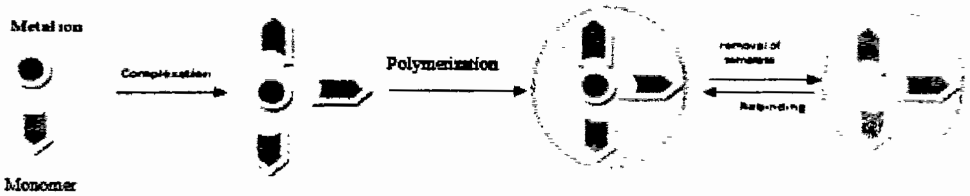


Figure 1.12: synthesis of metal ion imprinted polymer network

1.7 Chemicals and constituents required for MIP/IIPs:

1.7.1 Template

In every process of imprinting, the template molecule is the one that leads the arrangement of the functional groups drooping to the monomers. It should be remained chemically inert under polymerization conditions, provided that if the template molecule is not stable under polymerization conditions other imprinting approaches have to be consult.

1.7.2 Monomers

Monomer plays an important role as it creates specific cavities for the analyte of interest. During non-covalent imprinting, the monomers are utilized in a larger amount as compared to the template molecules to help in the foundation of template-monomer assemblages. Matching the functional groups of the template with that of the monomer in a paired way is important to maximize the effect of imprinting and formation of a complex. In covalent imprinting esters and amides of acrylic acid methacrylic acids are the most commonly used functional monomer and templates are linked to vinyl moieties by covalent bonding. For non-covalent imprinting, many functional monomers are commercially available but for better and efficient imprinting commercially available monomers must be distilled before use. As the fundamental forces in non-covalent imprinting may be Wander Waals forces, hydrogen bonding, ionic interactions, and hydrophobic forces. Most common monomers that are used in the synthesis of MIPs include acrylic acid (AA), Methacrylic acid (MAA), 2/4-vinyl pyridine, acrylamide and 2-hydroxyethyl methacrylate (HEMA), with the latter one being considered "universal monomer" due to its capacity to act as both hydrogen bond donor and acceptor (Hart & Shea, 2001).

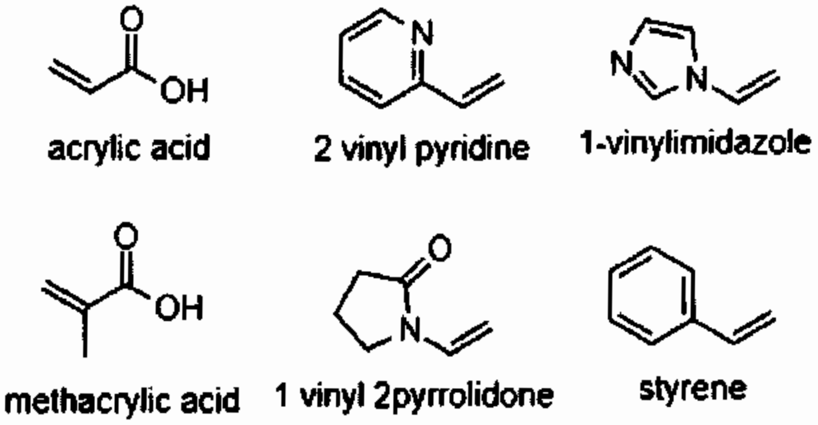


Figure 1.13: Chemical structures of different monomer

1.7.3 Cross-linker

The Cross-linker has a key role in IIP elaboration as it initiates the creation of binding sites. The selectivity of polymer is also affected by the type and how much the amount of Cross-linker is used during the synthesis process (N. Villoslada, 2004). The morphology of the imprinted receptor is also dependent upon the Cross-linker which directs whether the polymer is macro-porous, gel-type, or a microgel powder. The concentration of cross-linker should be adequate to form the voids with a three-dimensional structure which is like a template in both chemical functionality and shape (Sellergren, 1999).

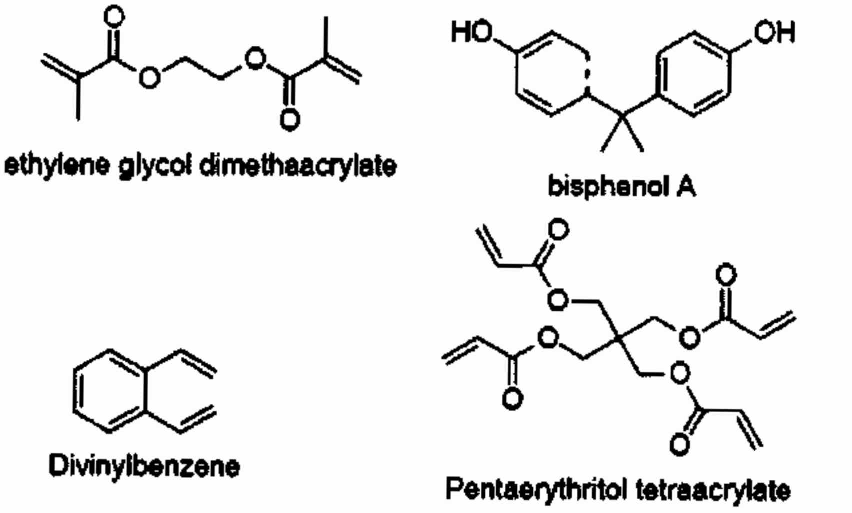


Figure 1.14: Chemical structure of different cross-linking monomer

1.7.4 Porogenic solvents:

Porogenic solvents help in the formation of macroporous polymer matrix. The nature of porogenic solvents directly influences selectivity of MIP. The porogen used during the synthesis of molecular imprinting polymer have to be solvable in the porogenic solvents. Such solvents should yield cavities within the polymer large enough to allow the passage of molecules or ions. Porogenic solvents that are highly soluble produce pores with small size and large surface area whereas solvents with less solubility will do the opposite. (Corrano, Longo, & Vasapollo, 2010)

1.7.5 Initiators

An initiator is an important reagent in the synthesis of MIPs as it helps in the rapid initiation of the polymerization reaction by providing free radicals. It, however, does not interfere in the non-covalent intermolecular interactions that take place during the polymerization process. The polymerization reaction could also proceed sometimes without initiator but it requires a lot of time. The usage of free radical initiators is widespread in the synthesis of MIPs due to the reason, that these are compatible with most of the polymer systems (F. Puoci 2010). Mostly, the free radicals that are used in the synthesis of MIPs are peroxides, azo compounds, and thermal inverters. In contrast to peroxides and azo compounds that require mild temperatures i.e., 80°C, thermal inverters require higher temperatures for their complete decomposition during the polymerization process. The choice of initiator is important in another way too as it may affect the binding capacity and morphology of the MIP. Mostly the method which is used for the synthesis of MIPs is the free-radical polymerization that utilizes the azo-initiators. The most common of the azo-initiators that is used is azo-*N*-*N*'-bisisobutyronitrile (AIBN). It can be initiated both by increasing temperature up to 60-70°C and in the presence of UV radiations (G. Vasapollo 2011).

1.7.6 Polymerization condition

MIPs polymerized at high temperature will produce polymeric material with low selectivity as compare to MIP that is synthesized at low temperature. Typically, 60 °C is used as the polymerization temperature. The stability of a polymer complex is highly affected by its temperature. Low temperature with long reaction time is highly preferable for a successful polymerization.

1.8 Fabrication of sensor

1.8.1 Immobilization of MIPs on transducer surfaces

MIP-based chemical sensors will be constructed by immobilizing MIPs onto the transducer surface. MIP-based recognition elements will be prepared on transducer platform.

1.8.2 In-situ polymerization

In-situ polymerization is one of the immobilization procedures which consist of an in-situ electro synthesis of imprinted polymer at the transducer surface. In-situ polymerization has the potential benefit of incorporating the immobilization step into an automatic mass-production process and technique will be applied to appropriate template molecules.

1.8.3 Surface coating of receptors onto transducer

Spin coating is a method of immobilizing MIPs onto a transducer surface. Surface-coating on a transducer surface at a specific speed helps in the production of a thin film of MIPs. Whereas in different circumstances while designing sensor imprinted receptor layers are manually deposited onto the transducer surface.

1.9 MIP-based composites

MIPs are integrated with the transducer component in a way that the binding sites and the conducting particles are in direct connection. The composites of affinity materials with MIPs will be prepared for the achievement of optimal recognition materials for the analyte of interest. Graphene oxide when integrated into IIP its sensitivity is enhanced than the IIP thin film. Ion imprinted polymers used in aqueous media for the detection of water-soluble analyte depends on its functionality whether the functional groups are hydrophilic or not. If hydrophilic groups are present on its surface e.g. OH groups are present on its surface it will attract the water molecules and will give high capacitance value. So if we look at the structure of GO there are several OH groups found on its surface attracting more water molecule thus increasing sensitivity (Chang.L.2011). Graphene oxide is a two-dimensional nanomaterial has gained world-wide interest in electronics, biomedical, sensor, etc. owing to its remarkable properties including large surface to volume ratio, high carrier mobility, good water dispersibility, outstanding thermal and electrical conductivity. GO is a promising material for fast and long-term chemical, bio-sensor applications owing to the existence of chemically reactive oxygen-containing (hydrophilic) functional groups like hydroxyl, epoxy, carboxyl, and carbonyl at the basal plane and edges (H. Seul Ki 2010).

1.10 Literature Review:

The first synthesis of Ion Imprinted Polymers (IIP), was reported 1976 and during the last decade an immense global R&D focus has been devoted to the design, and development of IIP, but publications have increased over the last decade. Ion imprinted polymers have exceptional recognition abilities that are categorized by great affinity, highly selective for the template ion, cost effective, can easily be stored and reuse (M. Yoshida, 1999). IIPs are prepared when a functional monomer, a Crosslinker, an initiator, and a template polymerized, a polymer is formed. Later, the template ions are washed out of polymer leaving behind sites or cavities paired to the analyte in shape, size and interaction. (M. Khajeh, 2011)

Nishide et al were the first to introduced IIPs by crosslinking the linear chain polymer with the metal-binding group. They used the ionic imprinted technique in the formation of chelating polymers then in1976. They copolymerized poly (4-vinylpirydyne) with 1, 4-dibromobutane with metal ions used as templates. The resins adsorb the metal ion (H. Nishide, 1976). Crosslinking is the main step in the synthesis of IIPs, it can be accomplished in a number of ways.

Nishide and coworkers in 1981 proposed another approach in which the ligand is chemically immobilized in a polymer matrix. They polymerized 1-vinyimidazole with 1-vinyl-2-pyrolidone (NVP) and divinyl benzene (DVB) to form a metal complex. After copolymerization and crosslinking with NVP the template metal ion was leached out. (M. Kato, 1981). Kim et al used this process with MMA and Cu (II) (A.H. Dam and D. Kim, 2008).

Alizadeh et al in 2011 also used the same procedure with Pb (II) and MMA (methacrylic acid) (T. Alizadeh and S. Amjadi, 2011). Fasihi et al used another approach of synthesis by mixing the metal ions, the chelating monomers, and the Crosslinker before copolymerization. They used 1-OH-9, 10-anthraquinone with an EGDMA (ethylene glycol dimethyl-acrylate) in presence of uranyl ions (J. Fasihi, 2011).

Ren et al removed Cu (II) from waste water. They discovered that functional monomer was efficaciously cross-linked into ionic imprinted polymer that resulted or formed a 3-dimensional net. The extreme adsorption capacity reported was 39.9 mg/g. (Ren, et al., 2018). Birlik et al arranged a IM adsorbent for Cr (III). First, the complex of Cr (III) with methacryloyl histidine was prepared; than Cr (III) was polymerized with *N*-methacryloyl-(L)-histidine and EGDMA. The achieved adsorption was 69.3 mg/g. Selectivity studies of Co (II), Cr (VI), Ni(II), and Hg(II) were also done. (Birlik, Ersöz, Açikkalp, Denizli, & Say, 2007).

Zhang and group made magnetic IM polymeric nanoparticles for adsorption of mercuric ions and the monomer used was allylthiourea. The adsorption capacity obtained was 78.3 mg/g. Relative selectivity coefficients were also calculated of the prepared for other metal ions, respectively. (Zhang, Wu, & Luo, 2016)

Namaskar et al. also made nickel (II) based ion-imprinted polymeric cryogels. Two different optimization recipes were synthesized for ion-imprinted polymeric cryogels with an altered template to functional monomer molar ratios. The Ac was 1.89 and 5.54 mg/g respectively (Tamahkar, Bakhshpour, et al. 2017). The Cu^{2+} ion-imprinted polymer/montmorillonite (IIP/Mt) nano-composites were prepared by a radical photo-polymerization process in visible light. The adsorption capacity of Cu^{2+} ions was 23.6 mg/g. (R Msaadi, 2019) Cerium (IV)-imprinting polymer (Ce-IIP) was prepared by using cerium metal with amidoxime ligand as the complexing agent. Aqueous form of HCl was used to percolate the cerium ions from polymer to make imprints of template. Adsorption capacity was 145 mg g^{-1}. (Rahman, et al., 2019).

During another study a core–shell magnetic ion-imprinted polymer was prepared through surface imprinting method. The polymer was made by using $Fe_3O_4@SiO_2$ as the support, with lead ion as the template, monomer used was AAPTS. High selectivity was observed for Pb (II) with selectivity factor over 3.75 in presence of other metal ions. (Minglei. Zhang, 2011). During another study again for the detection of Pb (II) in water and fruit juice a glass electrode of carbon was modified using IIP nanoparticles to generate a sensor of IIP-GCE. A new Ion Imprinted Polymer grafted onto Fe_3O_4 NPs used as converter to prepare the Pb based voltammetric sensor in which the 2-(2-aminophenyl)-1H-benzimidazole were used as a ligand for synthesizing IIP. The Adsorption capacity was increase by mixing IIPs and $Fe_3O_4@SiO_2$ nanomaterials respectively. (D. Zohreh, 2020)

During another work, ion imprinting were combined with ZnSe QDs on μPADs platform. Aim was to detect Cd^{2+} and Pb^{2+} ions. The ZnSe QDs were enfolded with ion imprinted polymers on 3D rotary μPADs. The 3D μPADs confirmed multiplexed detection, as well as pretreatment for the different water (Junrui Zhou., 2020). Z. Zhou et al. prepared Cr (VI)-IIPs by surface imprinting method with forming nanoparticles of Fe_3O_4 which were used as matrix. Fe_3O_4nanoparticles were synthesized by hydrothermal method and exposed to surface alteration. The concentrated adsorption capacity calculated was 129.36 mgg^1. (Zhiyong Zhou., 2020)

Z. Adibmehr et al., synthesized a cobalt ion imprinted adsorbent (Co (II)-MIIP) by use of magnetic SBA15 core-shell with max adp capacity 74 mg g^{-1} at about a pH = 8. (Zahra Adibmehr., 2017). Wang et al, synthesized IIPs for determination of Pb^{2+} by using carbon nanotubes as medium, hydroxyethyl methacrylate, chitosan, *N* isopropyl acrylamide as functional monomers for imprinting of Pb^{2+}. The synthesized IIP has thermo-sensitive properties (Haoqing Wang., 2020).

X.Zhang et al, during their work synthesized lead Pb-IIPs based on multi-walled carbon nanotubes (MWCNTs), and the monomer which was used during N-isopropyl acrylamide and methyl methacrylate (MMA) as the co-monomer respectively (Xing Zhang., 2019). Hamaguchi et al carried out the glycine-capped with Au NPs by photochemical initiator. The measurements were taken on QCM. Accumulation of glycine-stabilized Au NPs is observed through the colorimetric detection of heavy metals. The adsorption of the Hg^{2+} ions on the Au surface was 245 ng/cm^2 (Hamaguchi, 2010).

Sabri et al reported the adsorption and desorption of mercury over Au and Ag, therefore signifying that Au exteriors have the distinctive benefit to desorb Hg with a greater efficiency. They came to a conclusion that coarser surface not only has higher affinity for Hg but also retained their affinity. The sticking likelihood was reducing significantly at 40 °C (Sabri, 2009). H. Yu et al during their work, synthesized palladium ion-imprinted polymers with hydrophilic PHEMA polymer brushes by RAFT polymerization using different polymerization degrees (Haiyan Yu., 2019).

M. Khazaei et al developed an electrode for Hg^{2+} by adjusting a electrode with carbon paste using ion imprinted polymer. Nanoparticles of the Hg^{2+} imprinted polymer were formed through a two-step thermal precipitation polymerization method. For the preparation of Hg^{2+} -IP/g-C3N4/CPEs different quantities of Hg^{2+} -IP with different amounts of graphite powder, nujol oil, and g-C_3N_4 Nano-sheets were mixed to obtain a homogenous paste which was then filled in a plastic tube. Detection limit determined was of 4.3×10−10 M and the sensor showed found to be highly selective (Mohammad Khazaei, 2019).

G.-j. Zhu, et al in their study prepared a Fe (III) based IIP by radical copolymerization of Fe^{3+} ions as template. The performance and the removal speed of Fe (III) ions were 94.43%. The maximum capacity of adsorbing the Fe(III) ions was about 114 mg g^{-1} (Guang-jin Zhu., 2019). A conjugated polymer was synthesized by Wang and coworkers for lead ions detection. The sensing system exhibited quick fluorescent and colorimetric response with high sensitivity to Pb^{2+} and clear color change. Maximum limit for detection was 1 mM (Wang, 2015). M.Y. Zhang prepared a new fluorescence sensor for Cr (VI), in which ZnS Quantum dots (QDs) were covered with a coating of mesoporous SiO_2. During synthesis 3-(2-amino ethyl amino) propyl tri-methoxy silane (AAPTS) as a monomer, cross-linked by TEOS, and hexadecyltrimethylammonium bromide (CTAB) as the Porogenic agent. The synthesized polymer had a limit for detection i.e. 5.48 μg L^{-1} (M. H. Zhang, 2019).

During another study IIP for palladium ions were created by co-precipitation polymerization. Modified magnetic nanoparticles of Fe_3O_4 were used as support. During the process n, 8-aminoquinoline (AQ) as a monomer was applied to magnetic core i.e. Fe_3O_4/SiO_2 which was then followed by precipitation polymerization. 65.8 mg g^{-1} was the Max adsorption calculated (Shafizadeh, 2019). During a study, a magnetic graphene-based MIP composite was prepared for selective recognition of bovine hemoglobin. For this purpose, magnetic graphene was prepared in the first step. Ten, MIP layer selective to BHb was prepared using the functional monomer acrylamide (AAm) which has a high affinity towards BHb and cross-linker methylene bisacrylamide (MBA) (J. Guo 2015). During another study, a magnetic graphene-based MIP composite has been developed for the removal of 4-nitrophenol (4-NP) from aqueous solutions. Fe_3O_4 nanoparticles were immobilized on the surface of the graphene sheet and magnetic graphene (MGR) was prepared in the first step. Ten, MGR/MIPs composite was prepared by polymerization of phenyl-triethoxysilane and tetramethoxysilane in the presence of 4-NP (J. Luo 2016).

A cadmium imprinted receptor of allythiourea-urea was produced with an instantaneous segregation of humic acid (HA). During the process IM-polymer was measured by means of the co-monomer HEMA as well as by coating the surface with bovine serum albumin (L.G. Leandro 2019). Another ion imprinted polymers (IIPs) using composites was established for the colorimetric detection of Cd (II) ions in drinking water. Cadmium ions (template) joined on the external of low-cost print paper based on chain transfer polymerization. The adsorption capacity of Cd^{+2} composites was 155.2 mgg^{-1} whereas the limit of detection was 0.4 $ngmL^{-1}$ (Kai Huang., 2017).

To screen Cu ions in aquatic samples, an imprinted electro-synthesized film has been established on screen-printed electrodes. To develop a Sensor electro-polymerization of p-phenylenediamine with Cu^{2+} as template ion, joint with the screen-printed technology for the transducer element. The limit of detection achieved was 2.7 nM (Sabrina Di Masi., 2020). Ni (II)-IIP was created by bulk polymerization strategy by diphenylcarbazide (DPC) as ligand for exclusion of Ni (II) ions. Sound special effects of DPC on the performances of adsorption were calculated with many functional monomers. The maximum adsorption capacity achieved was 86.3 mgg^{-1} at pH 7.0 (ZhiyongZhou., 2018). A novel nanostructure Pb^{2+} based polymer was made. Lead ion-imprinted polymer with Pb $(NO_3)^2$ was used as template ion, through bulk polymerization strategy. Maximum adsorption capacity of the ion-imprinted polymer calculated was 75.4mg/g^1 (Mohammad Behbahani. 2013).

1.11 Scope of the present work:

Chemical sensors enable the detection of negligible quantities of chemical entities present in the environment even those that are not detected by modern equipment (Barnard 1991). Chemical sensors-based equipment is compact thus is more convenient for remote use and maintenance as well as are much easier to use. They also enable real-time remote using and mentoring. Because sensing devices are simple devices, they do not need too much maintenance and can also be operated by non-professional personnel. Sensing systems allow on-line and real-time monitoring of specified analyte/pollutants and therefore are highly appropriate for all kinds of applications, where it is important to trace a specified analyte over a longer period time e.g. for wastewater monitoring. Chemical sensors find their applications in industry for the monitoring of detrimental gases in the workplace to limit their exposure to workers. Chemical sensors are also used as electronic noses, which closely imitate human sense, for online quality control of cosmetics and food. Chemical sensors are used to measure the blood glucose and like a breath analyzer to detect the alcohol level in blood. They can also use to find out the patient's health by observing the quantity of oxygen in the blood and other components. Chemical sensors are very useful for the detection of pollutants and toxic chemicals in the environment for example monitoring of industrial effluents, wastewater, freshwater streams, detection of toxic warfare agents, etc.

1.12 Problem statement

Heavy metal contamination of water bodies has become a universal problem due to rapid industrialization (M. A. Hashem, 2017). Modern society has, therefore, become increasingly concerned by environmental issues related to industrial activity and so polluting industries have to follow to a more rigid environmental regulation (Hamdaoui, 2009). To achieve this, techniques such as cold vapor atomic absorption spectrometry, atomic fluorescence spectrometry, and inductively coupled plasma mass spectrometry, have been used intensively (k. J. Brodie1974) (E. Melek, 2006) (Eddaif 2019). These techniques are expensive and require trained personnel, and certain complicated processes are involved before final analysis. Electrochemical devices have been used for several decades for field monitoring of a variety of water quality parameters (e.g. conductivity, dissolved oxygen, or pH). MIPs based chemical sensors are compact robust devices with increased sensitivity and selectivity towards analyte of interest (E. Melek, 1995). The chemical sensors are highly sensitive and selective for respective environmental pollutants in the presence of other competing pollutants with almost identical molecular masses, atomic radii, functional groups, and oxidation states (Janssen L & Koene L., 2002). These unique properties of chemical sensors make them as promising tools for the recognition of pollutants in waste, drinking, and freshwater reservoirs.

Research aim and objectives:

- Screening of suitable polymer system for the molecular imprinting of analyte of interest i.e. environmental pollutants (heavy metals, water soluble dyes etc.), optimization of artificial receptors recipe and the optimization of coating protocols of Nano receptors onto transducer surface (immobilization of receptors).
- The fabrication of water-compatible MIPs based sensors for a variety of applications especially in environmental and clinical fields for real-time and online monitoring.
- The enhancement of sensitivity, selectivity and response-time of fabricated sensor by the use of nanotechnology (molecular imprinted polymers thin films, and nanocomposites receptor materials).

CHAPTER 2:

Ion-Imprinted Polymer-Based Receptors for Sensitive and Selective Detection of Mercury Ions in Aqueous Environment

2.1 Introduction

Mercury is considered as toxic pollutant (L.G.A. Barboza et al, 2018), (Batlokwa, 2012). In aqueous solutions mercury may transform chemically or biologically to its organic form as methyl mercury [Hg-CH_3] which is more toxic than Hg (II) (C.T. Driscoll, 2013), (Y. Huang 2015). Mercury (Hg) concentration if exceeds its limit, can induce congenital disabilities, cause damage to the brain, and various other diseases in humans and other species (M. McNutt, 2013). The main source of mercuric ion in water or soil is industrial processes and from chemical byproducts that are electronic materials, batteries, and fossil fuel combustion. An international agreement has been established to eradicate threats caused by mercury, which further motivates researchers to detect and remove Hg from contaminated aqueous media (D. Malakoff, 2013). According to US-EPA an acceptable limit of Hg in drinking water is 2ppb whereas much lower limit has been regulated for release in the ecosystem (B. Li, 2014). Therefore, an effective technique is required to efficiently detect Hg in wastewater in order to remediate the environment. Many methods have been established for ion quantification and detection (M. Resano, 2009). The sensors have certain complications and limitations regarding sensitivity and selectivity detection trace levels of mercury ions (*e.g.* ppt level) in environmental and food samples (Dan Song a, 2017). Molecular Imprinting is a targeted method to produce artificial receptors in the form of polymers (Haupt, 1997) as shown in Fig 1. Highly selective sieves are generated due to which MIPs could possibly be used as chemical sensors and assays. Due to the high binding selectivity metal ions, imprinting is a tedious task especially when selectivity is to be achieved, (R. Ito, 2009).

In this part of work, different ion imprinted polymeric receptors were synthesized for the determination of Hg^{+2} in water. For further optimization to achieve different parameters MIPs/functionalized graphene composite are to be used as receptors.

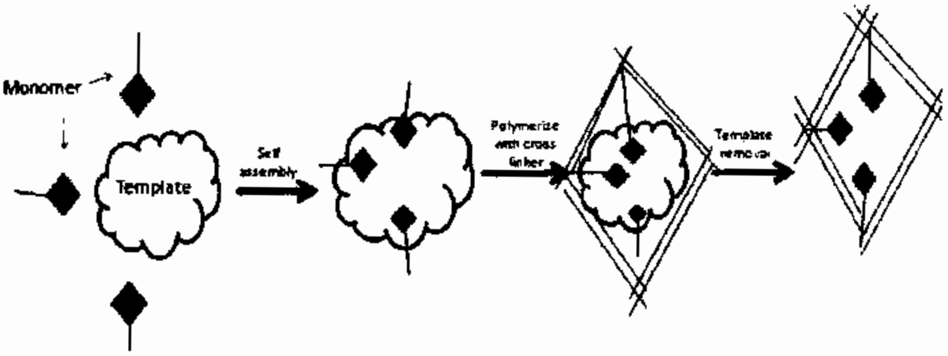


Figure 2.1: Molecular imprinting strategy

2.2 Experimental:

2.2.1 Chemicals and reagents:

Chemicals used during the synthesis process are styrene (99%), *N*-vinyl pyrrolidone (98%), ethylene glycol dimethacrylate (98%), azobisisobutyronitrile (AIBN; 99%), tetrahydrofuran (THF; 98%), methanol (98%- anhydrous) were purchased from Merck and Sigma-Aldrich.

Graphene used during our research work was purchased from Merck which was prepared using the hummer's method through oxidation of extra fine graphite oxide (B. Paulchamy and J. Jaya 2014). The synthesis procedure of GO is given in the figure below.

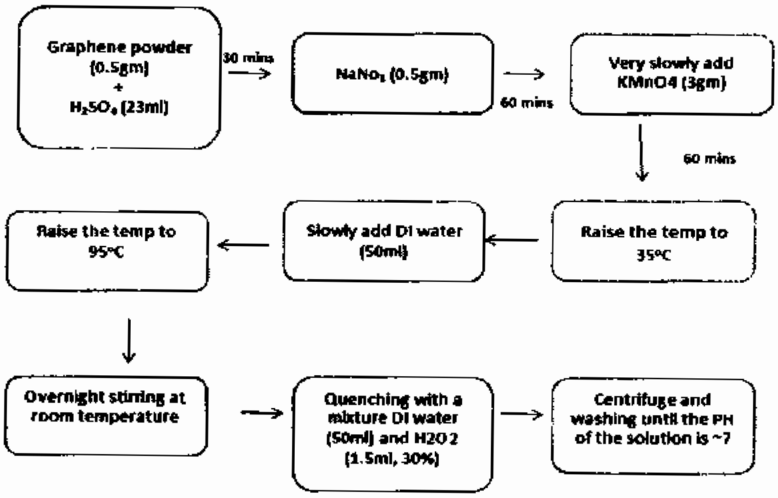


Figure 2.2: Synthesis procedure of Graphene oxide based on chemical oxidation of graphite using modified Hummers method (B. Paulchamy and J. Jaya 2014).

2.2.2 Synthesis of ion imprinted polymers:

2.2.2.1 Synthesis of ion imprinted *N*-vinyl pyrrolidone polymer:

N-vinyl pyrrolidone based Hg (II)-imprinted polymer was synthesized via bulk polymerisation method with mercuric iodide as template, NVP as the monomer, EGDMA as cross-linker and AIBN as the initiator at 60°C. Hg_2I_2 (2.00 mg) was dissolved in THF (200 µl), after thorough mixing *N*-vinyl pyrrolidone (30 µl), EGDMA (70 µg) were added. This mixture was vigorously stirred for 30 min. Afterwards AIBN (5 µg) was added to the solution to initiate polymerisation. The polymerization was carried out at 60°C for 30 minutes till a stable complex is formed between the Hg (II) ions, NVP, and EGDMA (Lucia Mergola1 2016).

2.2.2.2 Synthesis of ion imprinted co-polymer (NVP-Styrene):

A co-polymer based Hg (II)-imprinted polymer was synthesized via bulk polymerisation method with mercuric iodide as template, NVP and styrene as the monomers, EGDMA as cross-linker and AIBN as the initiator at 60°C. Hg_2I_2 (2.00 mg) was dissolved in THF (200 µl), after thorough mixing *N*-vinyl pyrrolidone (15 µl), styrene (15 µl), EGDMA (70 µg) were added. This mixture was vigorously stirred for 30 min. Afterwards AIBN (5 µg) was added to the solution to initiate polymerisation. The polymerization was carried out at 60°C for 30 minutes till a stable complex is formed between the Hg (II) ions, NVP, and EGDMA (Lucia Mergola1 2016).

2.2.2.3 Synthesis of ion imprinted polymer-graphene oxide composite

To prepare a composite, Hg_2I_2 (2.00 mg) was dissolved in THF (200 µl), after thorough mixing *N*-vinyl pyrrolidone (15 µl), styrene (15 µl), EGDMA (70 µg) were added. The mixture was vigorously stirred for 30 minutes. Afterwards graphene oxide (0.1 mg), were dissolved in the solution and was vigorously stirred to ensure thorough mixing. It was polymerized by cooking at 60°C for 30 minutes until the gel-point achieved.

2.2.2.4 Synthesis of non-imprinted polymer

NIPs were prepared with a similar procedure mentioned above (2.2.2.1), with the exception that analyte (Hg_2I_2) was not added during the polymerization.

2.2.3 Immobilizing receptors onto transducer surface:

Ion imprinted polymers and composite thin films were generated onto the interdigital electrodes (IDEs) and for this purpose, 10 μl of receptors (IIPs/composite) were coated by a spin coating method at 2500 rpm. After generating thin films, the IDEs were dried overnight at 80°C to obtain compact and dried layers. Then these IDEs were washed with excessive distilled water for 2h to generate ions cavities within polymers by washing out template ions.

2.2.4 Removal of template from ion imprinted polymer thin films:

To generate cavities with the polymer the imprint ion i.e. Hg^{+2} was leaked out from the above polymer coated on IDE by stirring with 100 ml of distilled H_2O for 2h. The resulting polymer after washing with distilled water (50 ml) was dehydrated in an oven at 85 °C overnight.

2.2.5 Apparatus:

The sonorex RK 102 H sonicator was used for sonication purpose; ultrasonic water bath was used for polymerization purpose. PH meter basic 20 was used to check the acidity and alkalinity of solutions. Ion quantification was achieved by LCR meter (IET 7600+ precision LCR meter).

2.2.6 Sensor Measurements:

Capacitance measurement of polymer coated on IDEs was taken with LCR meter at different concentrations of analyte of interest and competing ions at 20 μS and 1000 μS.

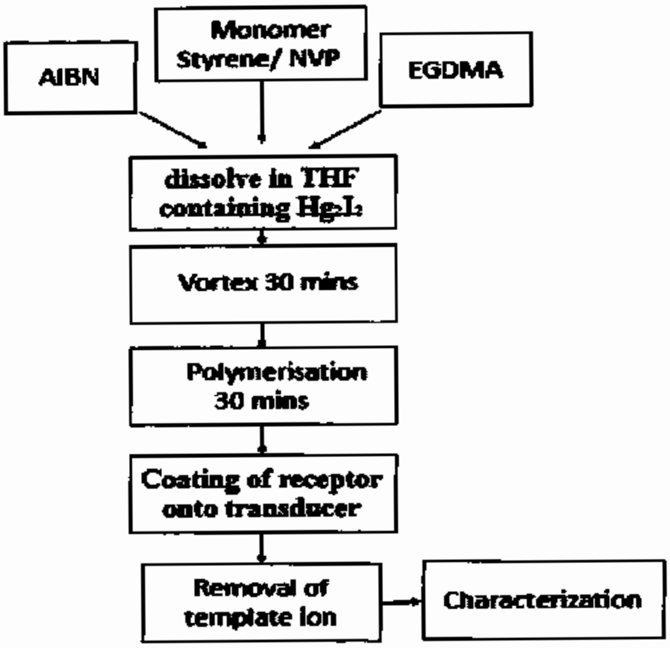


Figure 2.3: Scheme for preparation for ion imprinted polymer

2.3 Results and Discussion:

2.3.1 Fourier-transform infrared spectroscopy (FTIR):

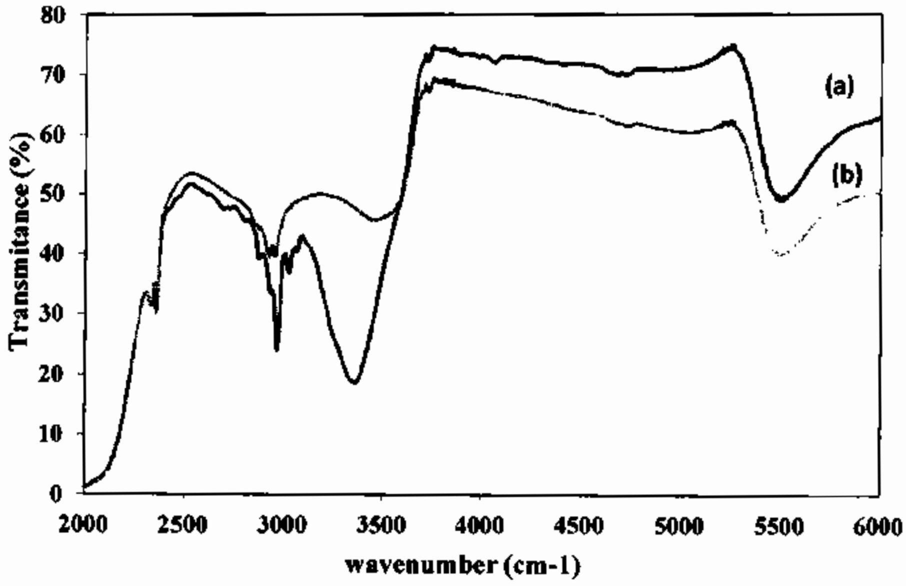


Figure 2.4: FTIR spectra of ion imprinted polymer (a) before leaching, (b) after leaching of mercuric iodide

The dynamics of the functional group presence or absence in the ion imprinted polymers (IIPs), before and after the removal of the template, was monitored by Fourier Transform Infrared (FT-IR) spectroscopy. The C–H stretching vibrations is shown by peaks at around (3000 cm^{-1}), also by broad band in the area of (2924 cm^{-1}). The band frequencies of N--H in unleached and leached IIP were 3420.7 cm and 3438 cm respectively.

2.3.2 Morphological study:

In our study the samples of ion imprinted polymers, co-polymer, and nan composite were evaluated using two different types of microscopy, namely the atomic force microscopy (AFM) and scanning electron microscopy (SEM). AFM proves advantageous over the other microscopy techniques when an accurate surface height measurement is required. While SEM can be used for even higher resolutions than AFM, it generates a two-dimensional image; accurate surface height is more easily obtained through AFM.

2.3.2.1 Scanning Electron Microscope: (SEM)

The morphology of Hg^{+2} IIP was investigated using scanning electron microscopy. Figure 2.5 indicates imprinted cavities generated on the surface of the polymer. The surface texture exhibited cavities on the surface of the leached IIP as a consequence of imprinting. The formed cavities further increase the sorption capacity of the sorbent. Fig.2.6 shows the distribution of GO on the polymer at 44.9 kx magnification. SEM image gives us the information about morphology, dimensions and crystallinity of a polymer and nano-composites.

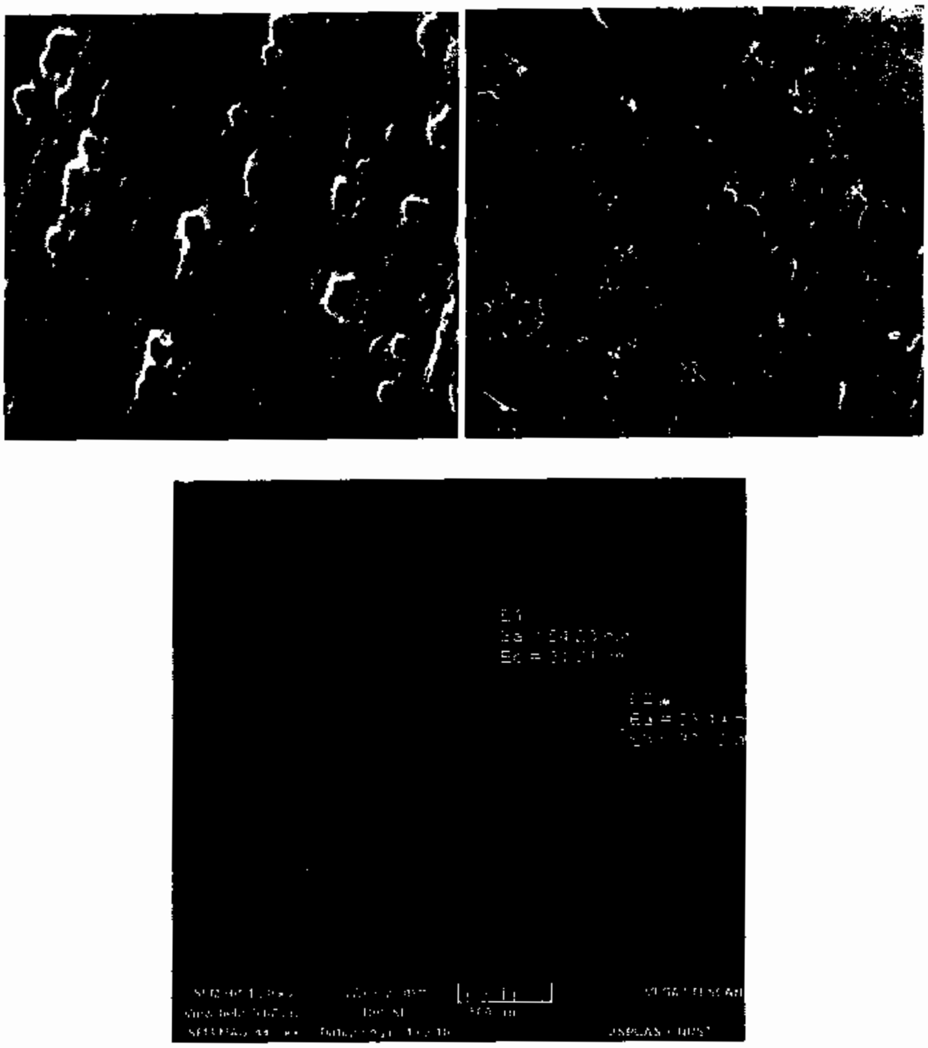


Figure 2.5: SEM image of mercuric iodide based polymer: a) polymer film with cavities b) polymer before washing: c) SEM image of the graphene composite

2.3.2.2 Atomic force microscopy: (AFM)

The 3-dimensional image of surface morphology of mercuric ion imprinted polymer and co-polymer is show in figure 2.7. Note that the pores created in the imprinted polymeric material are of the order of few meters. From this AFM image it can be revealed that the height of ion imprinted polymer thin film on the substrates is about 15nm. It reveals a uniform surface (RMS roughness of 3.7 nm). The roughness of the surface is important as it promotes the transfer of metal ions toward the polymer surface.

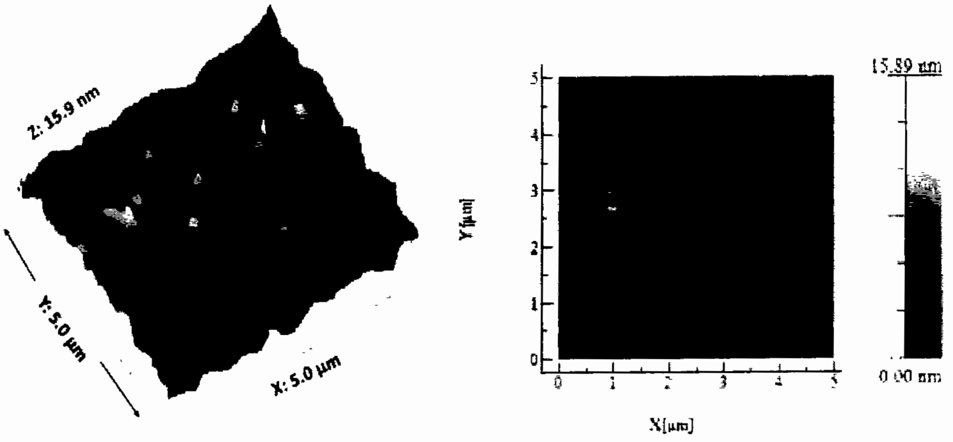


Figure 2.6: AFM topographical image of ion imprinted polymer surface study

2.3.3 Sensitivity study:

The ion-imprinted polymers were then spin-coated onto a silicon chip containing interdigital electrodes formed by lithography. The polymer films are 100-300 nm thin so that the adsorption event is detected and reported by the change in the conductivity of the device.

Coating IIP onto the transducer allows, mercuric ions in wastewater to incorporate into the cavities in the coated layer, thus, changing the electrical properties of the sensitive layer with increasing conductivity. During receptor synthesis, the monomer to the cross-linker ratio used was 30:70. The ethyleneglycol-dimethacrylate (EGDMA) that is used as a cross-linker during imprinting polymer synthesis is a hydrophobic di-functional methacrylate (Mohammed A.H. 2018). A higher ratio of cross-linkers in a polymer composition gives 3- dimensional spaces that are selective for the Hg^{2+} ions. In contrast, a low concentration of cross-linkers will collapse the cavities thus lowering its performance (Bajwa SZ 2014). The sensitivity of polymer systems was analyzed by exposing them to different analyte concentrations. *N*-vinyl pyrrolidone system was exposed to different concentrations of Hg^{+2} ranging 90-10 ppm and the sensor show a linear response. At 90 ppm the frequency shift is 103µS, at 70 ppm the frequency was reduced to 91 µS, at 50 ppm the frequency was further decreased to 78 µS. At 30 ppm and 10 ppm, the frequency falls to 67 µS and 54 µS respectively. Considering the noise level, the limit of detection is 163 ppb.

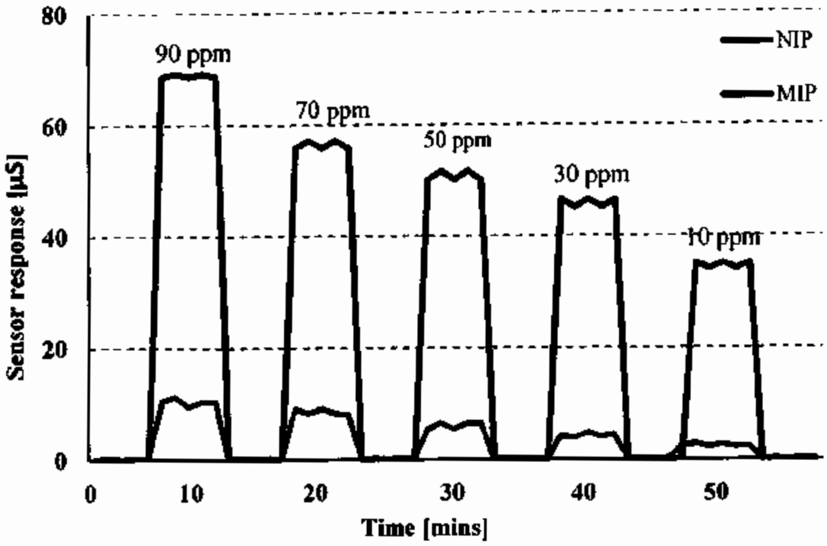


Figure 2.7: Sensitivity response of *N*-vinyl pyrrolidone based Hg^{2+} imprinted and non-imprinted polymer

N-vinyl-2-pyrrolidone (NVP) polymer has good properties such as low toxicity, biocompatibility, film-forming and adhesive characteristics (Beitz 2001), (Lee, Yu, K.H., & Kim, 2007). Mercuric ions due to its electron deficiency interact with the oxygen atoms of N-vinyl pyrrolidone and EGDMA and form weak inter molecular bonding Figure 2.8.

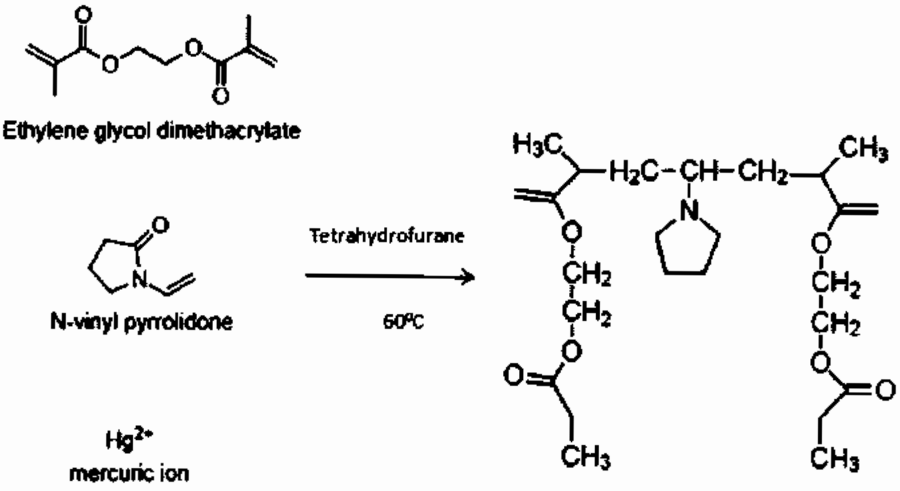


Figure 2.8: Expected imprinting and interactions of mercury in ion imprinted *N*-vinyl pyrrolidone based polymer

To investigate the sensitivity of polymer with the sensor a copolymer of styrene and NVP is formed (radical copolymerization). As is previously reported N-vinyl pyrrolidone can copolymerize with styrene and yields maximum sensor signal (N. Iqbal, 2013). N- Vinyl pyrrolidone has an amide group which is highly polar and has been copolymerized with a variety of monomers (Reddy, R., & George, 1995), (M. I.-9. Otagiri 1989). The presence of hydrophobic units (ST) along the chain will affect the solubility of polymer systems in aqueous media. The sensitivity of the co-polymer system was analyzed by exposing it to different analyte concentrations. The frequency shift of co-polymer system at 90 ppm was 96 μS which was decreased to 81 μS, 70 μS, 56 μS, 38 μS at 70 ppm 50 ppm 30 ppm and 10 ppm respectively. The lowest detection limit of the co-polymer system is 97 ppb. The sensitivity graph (Figure 2.9) of the sensor shows high linearity and concentration-dependent response.

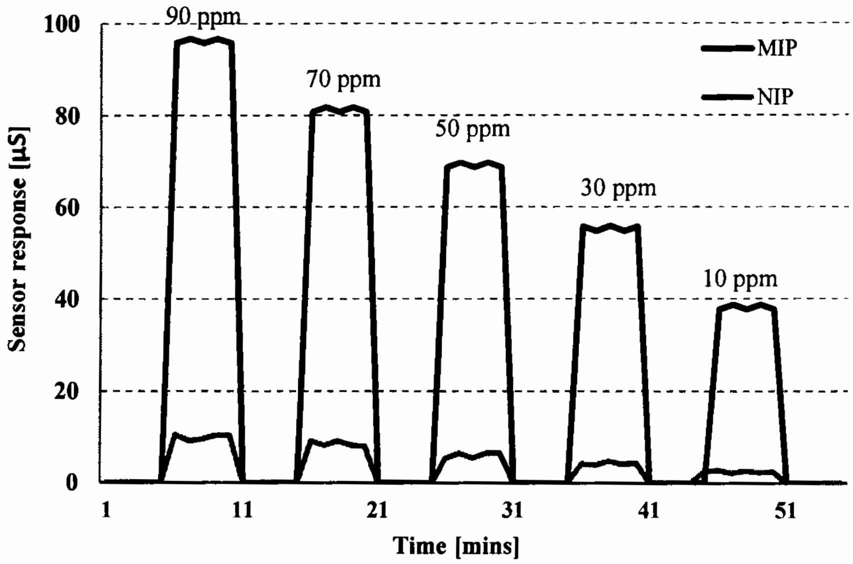


Figure 2.9: Sensitivity response of imprinted and non-imprinted Co-polymer.

To attain further sensitivity, the imprinted material should be adjusted in a way that the template ions should be positioned at the surface of imprinted materials. Graphene is an ideal supporting material for preparing MIPs because it has distinctive mechanical properties with a large surface area (S. Stankovich 2007).

The prepared MIPs would have a large surface area in case the polymerization occurring at the surface of graphene sheets (GS). The binding sites in the outer layer of the MIPs composite would provide complete removal of templates, improve the accessibility to target species, and also reduce the binding time. Graphene possesses functional groups including hydroxyls, epoxides, and carboxylic group (Y. Mao, 2011). Graphene oxide covalently and non-covalently binds small molecules and polymers to its reactive oxygen functionalities and is possibly used in polymer composites, paper-like materials, sensors, and drug-delivery systems. (D. R. Dreyer, 2010)

Due to electric and mechanical properties of graphene oxide blended with the polymer mixture will enhance the sensitivity of mercuric iodide polymer. At 90 ppm there is a drastic increase in the frequency which is 295 μS. Fig 2.10 gives us a linear response i.e. at 70 ppm 50 ppm 30 ppm and 10 ppm the frequency measured is 279 μS, 245 μS, 201 μS, and 167 μS respectively.

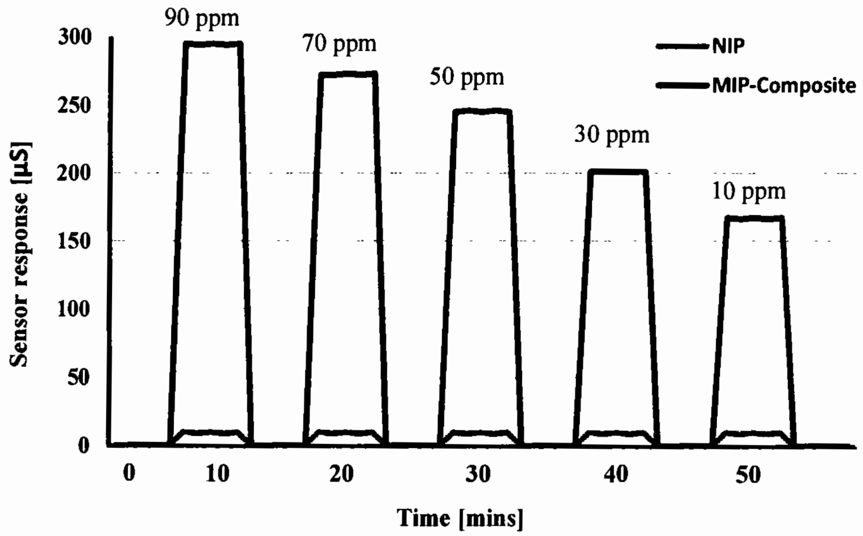


Figure 2.10: Sensitivity response of imprinted and non-imprinted Nano-Composite.

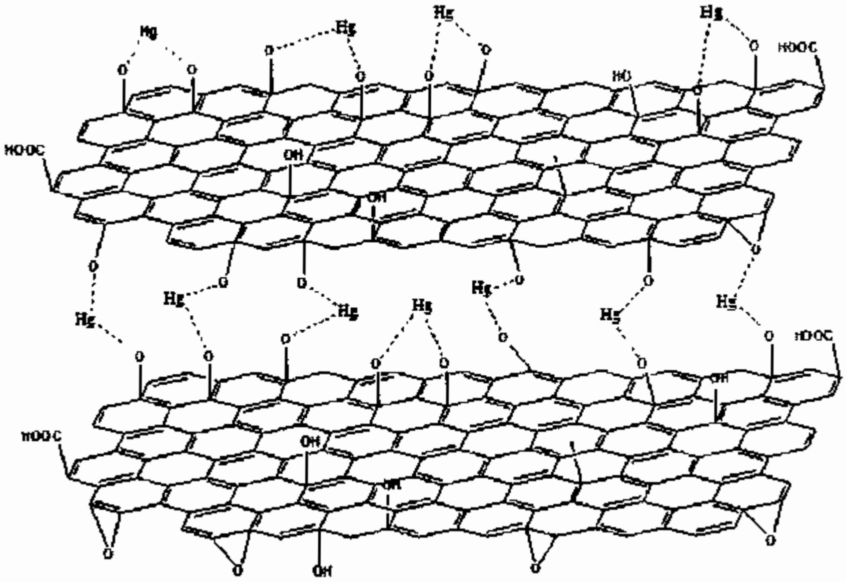


Figure 2.11: Intermolecular interaction of mercuric ions on the surface of graphene oxide

The key reaction of graphene oxide is its reduction reaction. Due to electron-deficient nature of mercuric ions it shows intermolecular interactions with the oxygen of the hydroxyl group and epoxide oxygen functionalities on graphene oxide surface.

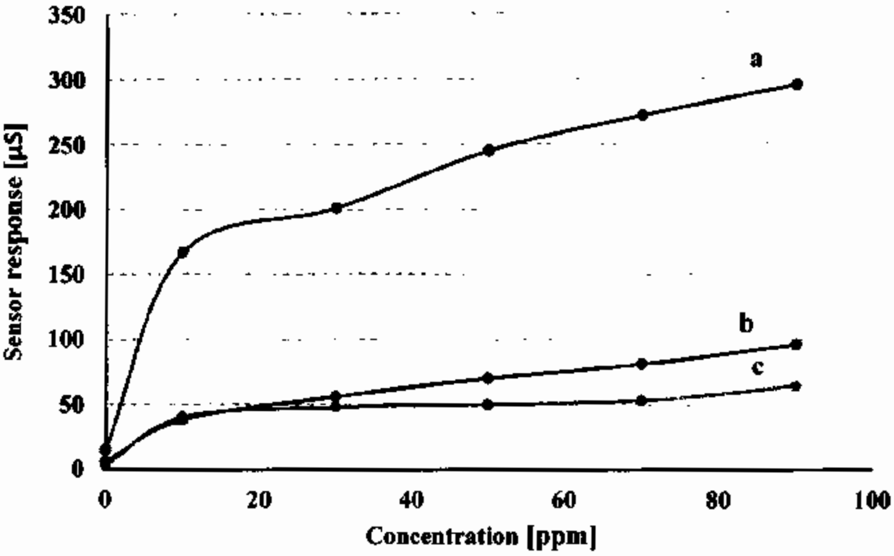


Figure 2.12: Comparison of three MIP receptors against different concentration of Hg a) MIP-composite, b) Co-polymer c) MIP-NVP

2.3.4 Selectivity Evaluation of MIPs Sensor:

Sensitivity analysis alone is not responsible for indicating the interactions between analyte and IIP. However, for obtaining such structural information, it is necessary to determine selectivity toward structurally related compounds or sub-structures of the mercury. After the removal of mercuric ions from the polymer matrix, the imprinted material was checked against other related metals ions chosen as competitors, such as Zn^{2+}, Mg^{2+}, As^{3-} and Na^{+}. As is evident in Figure 2.13 the Hg-IIP shows highly selective binding sites which could not accommodate other metal ions. The sensor shows 2-3 folds less response for other metal ions. Zn^{2+} and Mg^{+2} with the same oxidation state as Hg^{2+} shows 2 folds less response. The fabricated sensor proved to be ideal for waste water detection of mercury which contains a number of heavy metals competing for active sites.

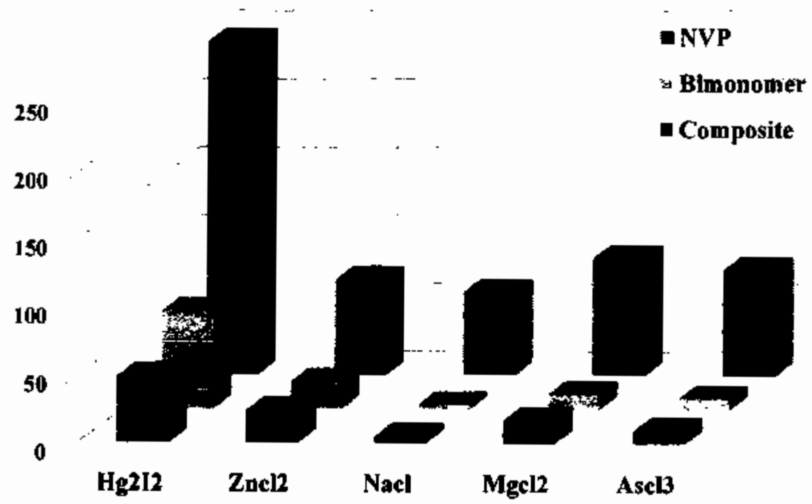


Figure 2.13: Selectivity of Hg^{2+} sensor against different analyte at 50 ppm concentration

2.4 Conclusion:

In this work, the influence of three different receptors on the efficiency of the sensor system was seen. One polymer system was generated using *N*-vinyl pyrrolidone as a monomer. In the second polymer system, both styrene and vinyl pyrrolidone were used as monomers thus forming a co-polymer. The third IIP/composite was generated with styrene and NVP used as a monomer in which graphene oxide was used during receptor synthesis. Graphene oxide blended with the polymer mixture has electric and mechanical properties which will enhance the sensitivity of mercuric iodide polymer. The order of MIPs sensitivity and selectivity observed was graphene composite >co-polymer > N-vinyl pyrrolidone. By comparing the response of composite with simple IIP the former shows high sensitivity and polymer stability with successive usage.

CHAPTER 3:

A novel ion-imprinted polymer based on graphene oxide Nano-composite for detection and removal of chromium (III) from aqueous solution.

3.1 Introduction:

Chromium Cr (VI) is a noxious heavy metal most probably present in Industrial wastewater that is released to the environment and causes high risk (B. Qiu 2014) (Q. Wang 2012). Chromium is considered as a highly toxic pollutant and enters water supply through industrial wastewater. Chromium is used in several processes i.e. electroplating, leather burning, and metal finishing from where these ions are released in the wastewater. Chromium considered as a lethal contaminant is known to cause epigastric ache, sickness, vomiting, carcinogen effect (Leonard, 1980), and severe diarrhea (F. Majone, 1979) several approaches have been introduced for the determination of Cr (III) and Cr (VI). Compared with other treatment techniques such as membrane separation (W.S.W. Ho, 2001) chemical precipitation (M. Erdem, 2004) photocatalytic method (F. Gode, 2005) and ion exchange (X.J. Liu, 2012) (S. Luo, 2017) (R.M. Wang, 2017) (G.F. Li, 2013), adsorption has advantages of being environmentally friendly and have a low cost. Several adsorbents have been applied for removing chromium (Z.Q. Ren, 2014) (F. Qin, 2012) (E.T. Liu, 2014). These sorbents have low capacity, extended time, and poor selectivity which decreased their practical application. Ion-imprinted polymer (IIP) is a synthetic material that is exceedingly choosy to target ions. Imprints of different targeted ions are synthesized and are used as adsorbents to remove particular ions from wastewater (D.L. Kong, 2014), (Y. Chen, 2016), (Wulff 2003).

Ion-imprinting of Cr^{3+} by bulk imprinting strategy is quite favorable to achieve a high sensitivity material. Graphene oxide (GO) a material resulting from graphene, has been used in many fields, such as sensors (S.R. Ng, 2011), (S. Roy, 2011). Due to this large surface area of GO, it is used as a support material in imprinting. Though, it is challenging to collect GO dispersed in aqueous solution because GO can reunite completely (I. Chowdhury 2013) which makes it inconvenient to apply GO in wastewater treatment. To successfully deal with this problem, GO was combined with other materials to produce hybrid composites (A. Islam, 2014), (Y. Fei 2016).

During this part of our work, chromium ion-imprinted polymers (IIP) for the selective determination of chromium in wastewater were synthesized to achieve significant sensitivity and selectivity. Additionally, the sensitivity parameter was further optimized by using IIPs/functionalized graphene composite as a receptor.

3.2 Experimental:

3.2.1 Chemicals & Reagents:

Pre-polymerization reagents, chromium chloride ($CrCl_3.6H_2O$), styrene (99%), N-vinyl pyrrolidone (98%), ethylene glycol dimethacrylate (98%), azobisisobutyronitrile (AIBN; 99%), tetrahydrofuran (THF; 98%), methanol (98%, anhydrous) were all purchased from Merck and Sigma-Aldrich. Stock solutions were prepared in double-distilled water. Additionally, deionized water was also prepared.

3.2.2 Synthesis of ion imprinted polymers:

3.2.2.1 Synthesis of ion imprinted *N*-vinyl pyrrolidone polymer:

To prepare an *N*-vinyl pyrrolidone based Cr (III)-imprinted polymer, NVP is used as monomer, and EGDMA is used as cross-linker and AIBN as the initiator at 60°C. $CrCl_3$ (2 mg), was dissolved in THF (200 µl) till a clear solution was formed. Afterwards, 30 µl of *N*-vinyl pyrrolidone, 70 µg of EGDMA, and 5 µg AIBN were added in the above solution and the resulting mixture was stirred vigorously. The polymerization was done at 60 °C for 30 minutes until a stable complex is formed between the Cr (III) ions, NVP, and EGDMA.

3.2.2.2 Synthesis of ion imprinted co-polymer (NVP-Styrene):

A co-polymer based Cr (III)-imprinted polymer was synthesized via bulk polymerisation method with mercuric iodide as template, NVP and styrene as the functional monomers, EGDMA as cross-linker, THF as solvent, and AIBN as the initiator at 60°C. $CrCl_3$ (2 mg) was dissolved in THF (200 µl), after thorough mixing N-vinyl pyrrolidone (15µl), styrene (15µl), EGDMA (70 µg) were added. This mixture was vigorously stirred for 30 min. Afterwards AIBN (5 µg) was added to the solution to initiate polymerisation. The polymerization was carried out at 60°C for 30 minutes till a stable complex is formed between the Hg (II) ions, NVP, and EGDMA (Lucia Mergolal 2016).

3.2.2.3 Synthesis of Ion Imprinted Polymer-Graphene Oxide Composite:

To prepare a polymer-graphene oxide composite, 2 mg of $CrCl_3$ was dissolved in 200 µl of THF till a clear solution formed. Afterwards 30 µl of styrene, 70 µg of EGDMA and 5 µg of AIBN were added in the solution mixture and stirred vigorously. 100 µg graphene oxide was added to the solution mixture and stirred for 10 minutes. It was polymerized by heating at 60°C for 30 minutes till the gel-point achieved. Graphene oxide was suspended in the solution mixture.

3.2.2.4 Synthesis of Non-imprinted polymer:

Non-imprinted polymer was synthesized with the same procedure mentioned above (3.2.1.1) respectively with the exception that analyte $CrCl_3$ was not added during polymerization.

3.2.3 Immobilizing of Receptors onto Transducer:

IIPs and composite thin films were generated onto the interdigital electrodes (IDEs) and for this purpose, 10 µl of receptors (IIPs/composite) were coated by spin coating method at 2500 rpm. After generating thin films the IDEs were dried overnight at 80°C to obtain compact and dried layers. Then these IDEs were washed with 50 ml distilled water for two hours to generate ionic cavities within polymers by washing out template ions. Capacitance measurement of IDEs were taken with LCR meter at different concentrations of analyte of interest and competing ions.

3.2.4 Removal of Template from Ion imprinted polymer thin films:

Chromium ion (Cr^{3+}) was extracted from the polymer synthesized above (3.2.2.1), coated on IDE by continuously stirring with distilled water for 2h to generate cavities. Resultant polymer with imprinted sites dried in an oven at 80°C.

3.2.5 Apparatus:

The sonorex RK 102 H sonicator was used for sonication purpose; ultrasonic water bath was used for polymerization purpose. PH meter basic 20 was used to check the acidity and alkalinity of solutions. Ion quantification was achieved by LCR meter (IET 7600+ precision LCR meter).

3.2.6 Sensor Measurements:

Capacitance measurement (20 µS and 1000 µS) of polymer coated on IDEs were taken with LCR meter at different concentrations of analyte of interest and competing ions.

3.3 Results and Discussion:

3.3.1 Fourier-transform infrared spectroscopy (FTIR):

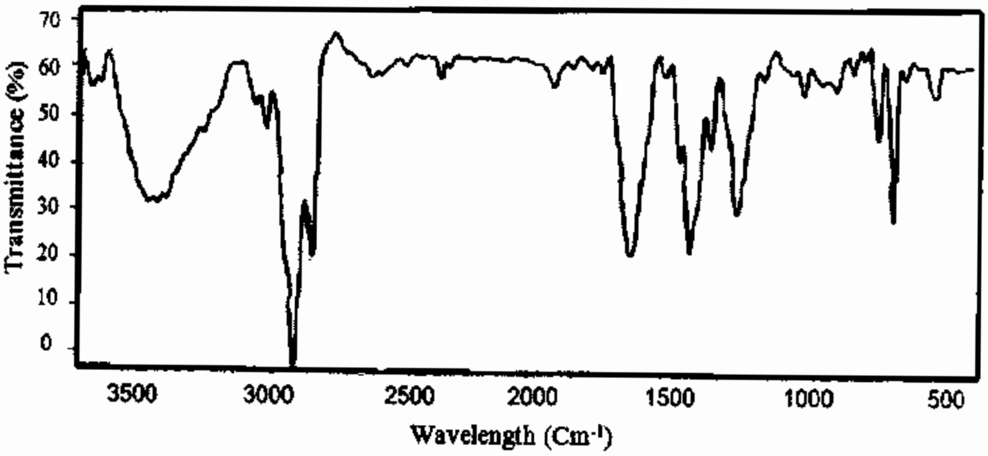


Figure 3.1: Ftir spectra of Co-Polymer (NVP-Styrene)

To further confirm the composition of obtained co-polymer, FT-IR measurement was carried out. Figure 3 shows the FT-IR spectra of co-polymer containing NVP-styrene and EGDMA. Absorption band at 1700 cm^{-1}, could be assigned to the C=O stretching vibration of EGDMA, used as a cross-linker. The NVP showed characteristic absorption bands at 1288 cm−1 for C−N stretching vibration. C-H stretching was observed at 2971 cm-1. Absorption band at 1644 cm^{-1}, could be assigned to the C=C stretching vibration.

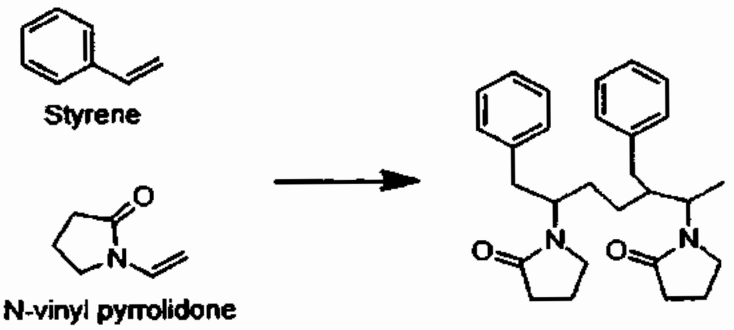


Figure 3.2: Interaction between styrene and NVP in co-polymer receptor system

3.3.2 Morphological characterization:

The SEM and AFM are powerful characterization tools in polymer science, capable of revealing surface structures. SEM provides a three-dimensional (3D) image with high resolution and is used to characterize the morphology of the sample surface, particle size, microorganism, and fragments. Analysis of polymer blends and composites morphology and surface changes (erosion) using AFM and SEM techniques gives a lot of information about various processes and phenomena.

3.3.2.1 Atomic force microscopy:

Figure 3.3 (a) indicates leached polymer and it clearly shows the pores formed by the leaching of the chromium ions from the polymer surface. From this AFM image it can be revealed that the height of ion imprinted polymer thin film on the substrates is about 15nm. It reveals a uniform surface.

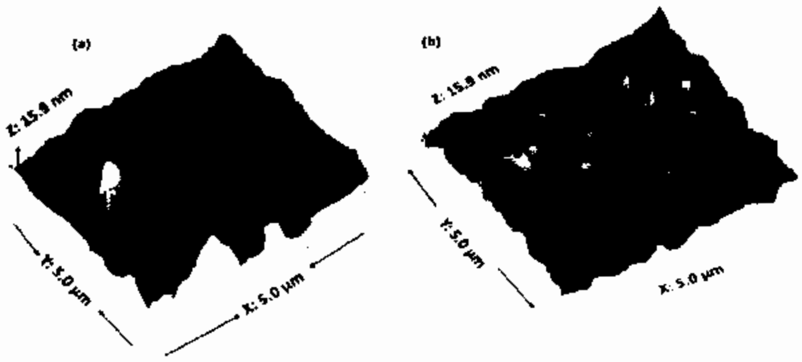


Figure 3.3: AFM image ion imprinted polymer (a) IIP- Composite (b) Ion imprinted polymer

3.3.2.2 Scanning electron micrograph:

The SEM imaging (Figure 3.4) results for the IIP films well correlated with those of the AFM observation. There was a clear difference in morphology of leached and unleached polymer surface. The washed, imprinted films show considerable surface roughness, presumably owing to the presence of numerous binding sites or cavities. Figure 3.5 shows image of NIP. As can be seen, from Figure 3.5 the coating surface is homogeneous even after washing.

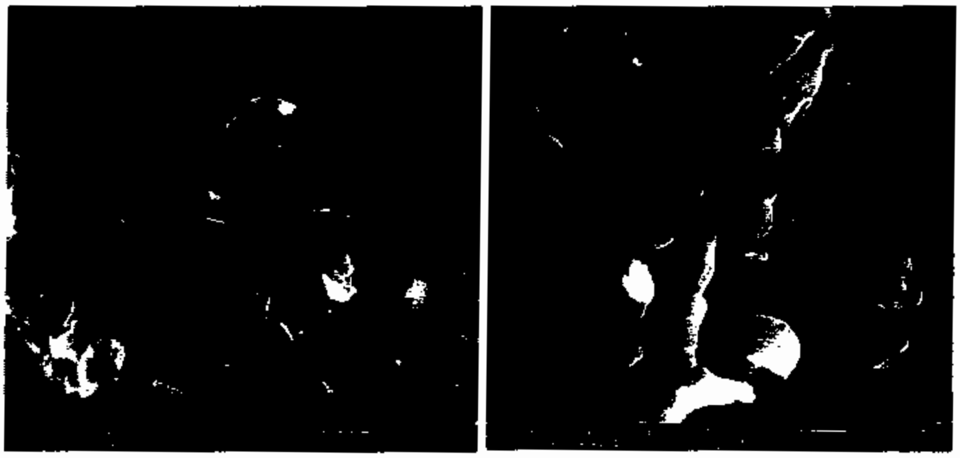


Figure 3.4: SEM image of ion imprinted polymers, (a) before leaching, (b) after leaching

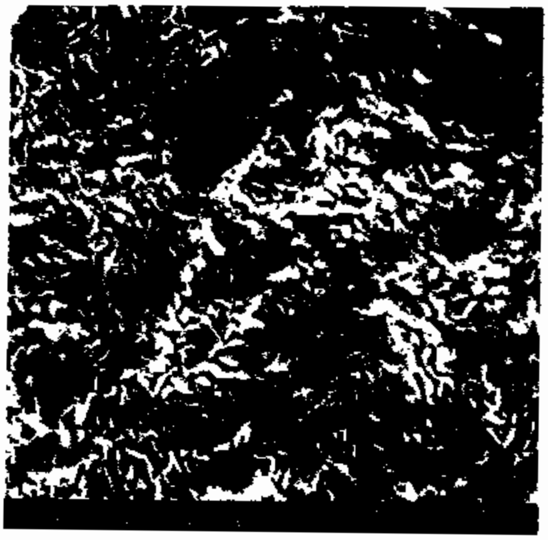


Figure 3.5: Scanning electron micrograph of NIP

3.3.3 Effect of pH on adsorption of Cr III:

PH of the aquoes solution containing 50 ppm of As (III) was adjusted between 6-7. Adsorption of metal ion is strongly pH dependent, because at low PH value H^+ ion may cause hindrance. Whereas high pH adsorption was affected by the presence of OH^- ions. Therefore, pH of the solution adjusted to pH 6 for the optimum extraction of Cr^{2+} ions.

3.3.4 Effect of temperature on adsorption of Cr (III):

Maximum adsorption was achieved at 25°C. High temperature cause metal ions to move easily in the solution thus affecting adsorption and the quantity of polymer which was applied on the electrode. Therefore, 25°C was taken as the optimized temperature used during our experimental work.

3.3.5 Optimization of the recipe:

To enhance the sensitivity and selectivity of IIP, different receptors were synthesized in accordance with table 3.1.

Table 3.1: Optimisation of different polymer compositions

Polymer	Template	Functional Monomer	Cross-linker	Monomer/ cross linker ratio	Polymerization conditions
P1	Chromium III	styrene/NVP	EGDMA	40:60	Thermal 60°C
P2	Chromium III	styrene/NVP	EGDMA	50:50	Thermal 60°C
P3	Chromium III	Styrene	EGDMA	30:70	Thermal 60°C
P4	Chromium III	NVP	EGDMA	30:70	Thermal 60°C
P5	Chromium III	styrene/NVP	EGDMA	30:70	Thermal 60°C
P6	Chromium III	Styrene/NVP/ graphene oxide	EGDMA	30:70	Thermal 60°C

In polymer system P1, P2 and P3 composition were optimized through using different ratios for monomer and cross-linker while keeping all the other parameters constant. The ratio of template, monomer, and cross-linker plays an important role in the selectivity of adsorption of the polymer towards the template. Polymer system with 50:50 monomer to cross-linker ratio shows a lesser sensitivity response as compared to the other two systems with 40:60 and 30:70 ratio. The response of the sensor having a monomer to the cross-linker ratio of 30:70 shows the highest sensitivity response than the other two sensors. Cross-linker provides structural rigidity to polymers and plays an important role in maximizing the quality of imprinting. It could be assumed that in the case of sensors having monomer to the cross-linker ratio of 50:50 and 40:60, due to less amount of cross-linker, enough rigid spaces are not formed. The high concentration of cross-linker will generate highly selective 3-dimensional cavities for imprinted ion however lower concentration of cross-linker will produce poor cavities.

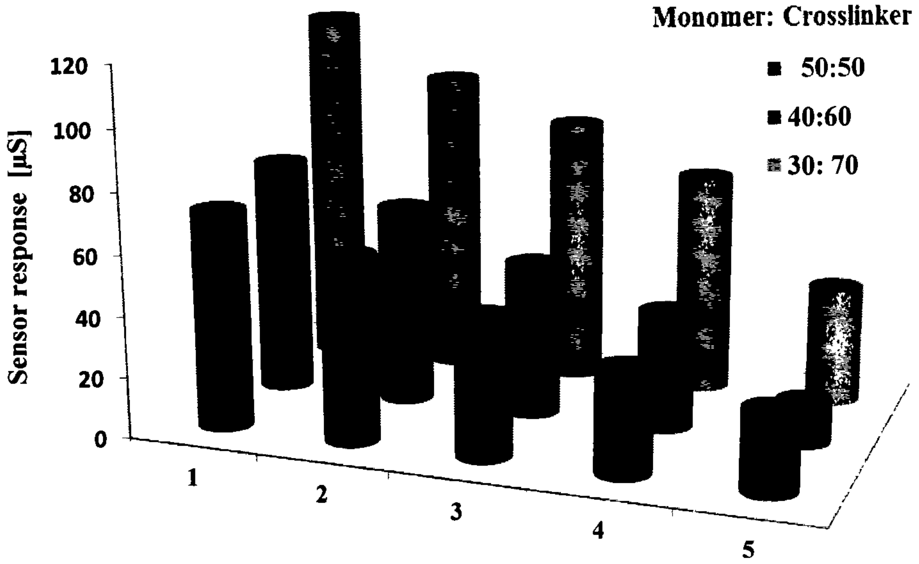


Figure 3.6: Shows Optimization response of the Cr (III) IIP sensors

During receptor synthesis monomer to the cross-linker ratio used was 30:70 which yield maximum sensor signal. In polymer system P4, P5, and P6 the type of monomer was analysed while keeping other parameters constant. The order of MIPs sensitivity and selectivity observed was P6≥P3≥P4≥ P1≥ P5≥ P2.

3.3.6 Sensitivity studies of Cr-IIP based sensor:

Coating IIP onto the transducer allow, chromium ions in waste water to incorporate into the cavities in the coated layer, thus changing the electrical properties of the sensitive layer with increasing conductivity. Sensitivity measurements of polymer systems were investigated through exposing them to different analyte concentrations. *N*-vinyl pyrrolidone system was exposed to different concentrations of chromium ranging from 90-05 ppm and sensor shows a linear response in Figure 3.7.

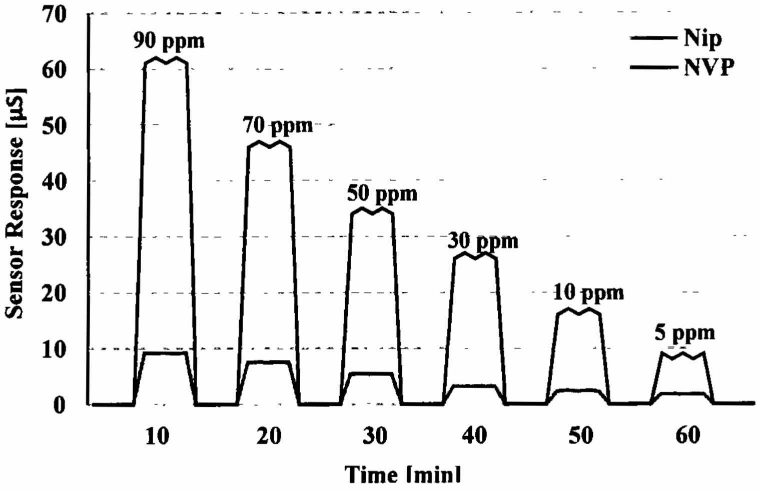


Figure 3.7: Sensitivity response of imprinted and non-imprinted N- vinyl pyrrolidone polymer

As shown in Figure 3.7 at 90 ppm the frequency shift is 61 µS, at 70 ppm the frequency was reduced to 46 µS, at 50 ppm the frequency was further decreased to 34 µS. At 30 ppm, and, 10 ppm the frequency falls to 26 µS and 17 µS and at 5 ppm the frequency was further reduced to 09 µS. Considering the noise level, the limit of detection was 0.14 ppm respectively.

The interaction of NVP with the cross linker might be through its vinyl group. Cr^{3+} ions with its lone pair of electrons binds with the oxygen atom bonded with the carbon on EGDMA. The amide group of NVP is highly polar, which acts as a hydrogen bond donor, thus confirming its hydrophobicity. Due to this quality the amide group binds with several small and large molecules. On the other hand the carbonyl group of NVP accepts hydrogen bond from water. The polymer system was tested for metal ion in waste water so NVP being a hydrophilic interact with water ions and capable of hydrogen bonding. Due to the hydrophilic nature of *N*-vinyl pyrrolidone the sensor response can be interfered with by humidity especially in aqueous media. To overcome this problem NVP was copolymerized with styrene. Styrene is a hydrophobic monomer and does not readily react with polar solvents.

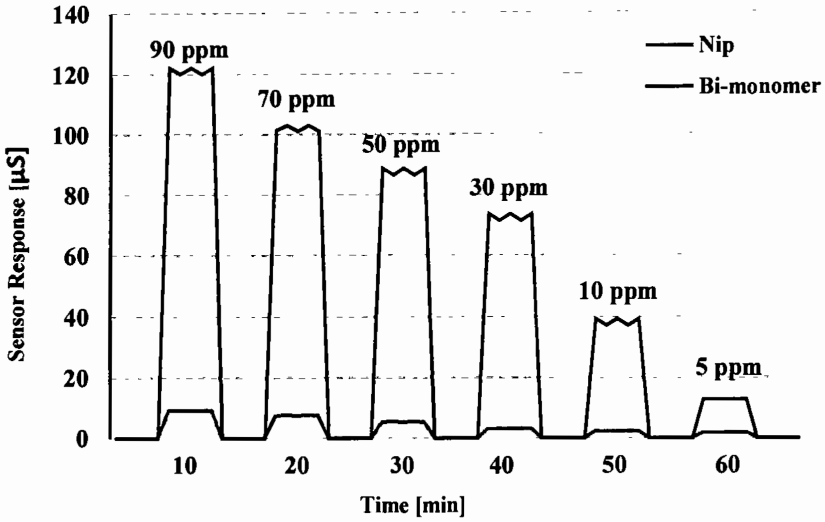


Figure 3.8: Sensitivity response of imprinted and non-imprinted co-polymer.

Figure 3.8 shows the sensitivity response of the co-polymer system in which styrene and NVP are copolymerized with EGDMA. At 90ppm concentration the sensor showed a response of 122 µS. By lowering the concentration to about 5 ppm the sensor response decreased to 12 µS. As the concentration of Cr^{3+} was reduced, the sensor response gradually decreases, thus showing linearity in all polymer systems as shown by Figure 3.8.

Styrene used as monomer has no special functionality thus making it a hydrophobic. However it forms a specific 3-dimensional imprint of the Cr^{3+} ion where it could specifically coordinate with the crosslinking monomer. It thus provides the IIP its geometric selectivity for the imprinted ion. To achieve further sensitivity the structure of IIP was further optimized with graphene oxide. At 90 ppm there is a drastic increase in the frequency which is 365 µS. Figure 3.10 gives us a linear response at 5 ppm the frequency is decreased to 12 ppm. Graphene oxide is a typical pseudo-planar solid in bulk form, owns functional groups including hydroxyls, epoxides and carboxyl (Haupt H., 2000) (Suryanarayanan, 2010).

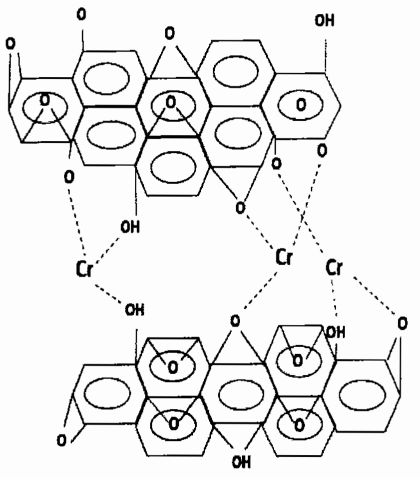


Figure 3.9: Interaction of chromium ions on the surface of graphene oxide

Graphene, with extremely large surface area, is therefore used as support material for preparing imprinted polymer and recognition sights are available at the surface of graphene sheets (GS). The binding sites in the outer layer of the MIPs composite would provide complete removal of templates, improve the accessibility to target species, and also reduce the binding time. The reduction is the critical reaction of graphene, as lone pair of electrons of chromium ions interacts with the hydroxyl and carboxyl groups on the graphene oxide surface. Graphene oxide covalently and non-covalently attached polymer to its reactive oxygen functionalities or potential use in polymer composites, paper-like materials, sensors, and drug-delivery systems (Ramström, 2005). Graphene is hydrophobic, thus, producing water-dispersible graphene. Nano-composites have always been of great interest, which can be achieved by modification with water-soluble polymers.

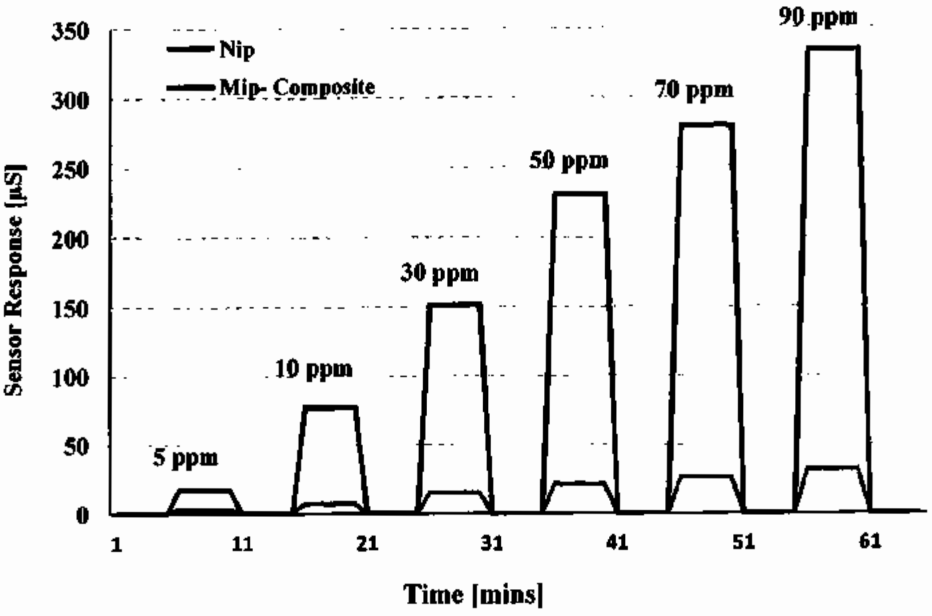


Fig 3.10: Sensitivity response of imprinted and non-imprinted composite.

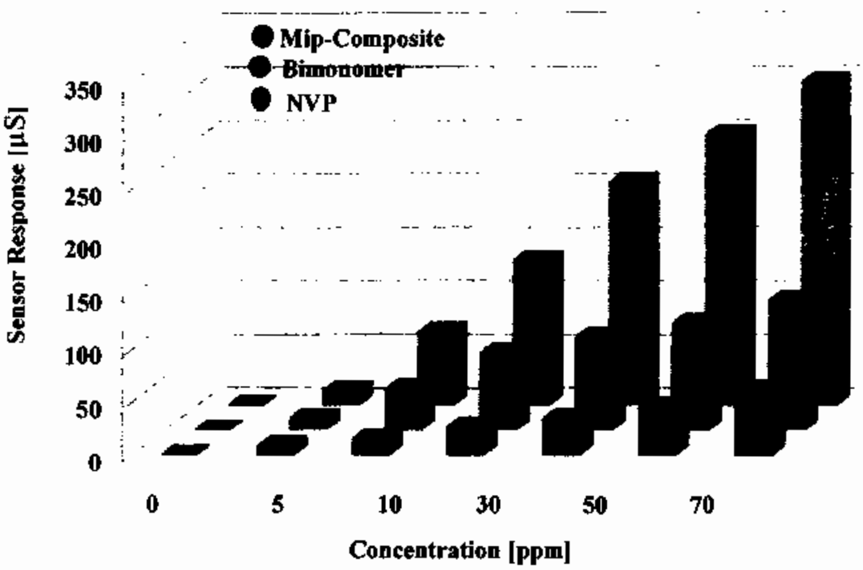


Figure 3.11: Comparison of three MIP receptors against different concentration of Cr ions. (a) IIP-Composite, (b) Co-polymer (c) IIP-NVP.

3.3.7 Selectivity:

To yield further evidence the sensor should be tested for its systematic selectivity. For gaining structural information, it is necessary to determine selectivity toward structurally related compounds of chromium chloride. Selectivity measurements of different heavy metals were checked with LCR meter. With LCR meter (Figure 3.12) the chromium ion imprinted polymer was checked against Mg^{2+}, As^{3+} and Hg^{2+}. This indicates that the sensor could show remarkable selectivity for Cr^{3+}, in complex environments where other highly similar analyte species are present.

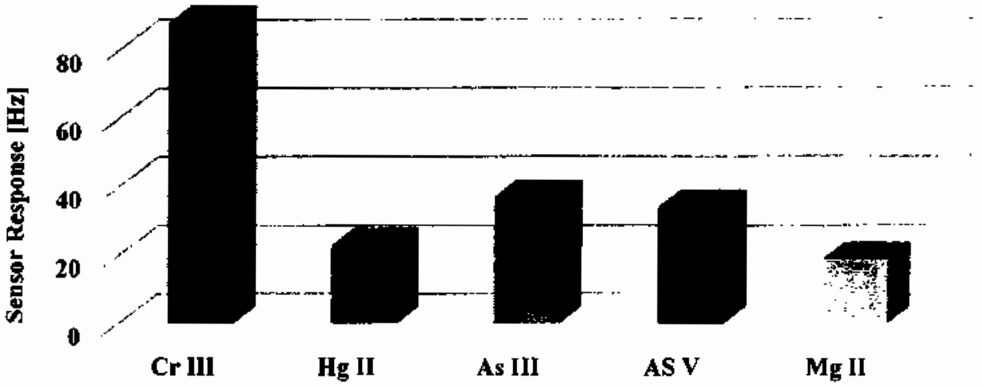


Figure 3.12: Selectivity response of the sensor for different heavy metal ions at a concentration of 50 ppm

3.4 Conclusion:

The sensitivity of chromium ion-imprinted polymer was analyzed with IDE as transducers. Chromium ion-imprinted polymer was optimized through using different ratios for monomer and cross-linker while keeping all the other parameters constant. Three different ion-imprinted receptors were generated, polymer system with monomer and cross-linker ratio 30:70 gave better response as compared to the polymer with monomer and cross-linker ratio 50:50 and 40:60 respectively. Thus results showed that a high concentration of cross-linker will generate highly selective 3-dimensional cavities for imprinted ion however lower concentration of crosslinker will produce poor cavities. The monomer used for the synthesis of Cr ion-imprinted polymer was styrene and *N*-vinyl pyrrolidone. Styrene system being hydrophobic does not readily dissolve in water which in turn proves itself to be ideal for wastewater treatment. The addition of graphene oxide further enhances the sensitivity response of the polymer. The sensor shows good selectivity even for the most similar ions like As^{3+} which indicates that it could be used for the selective as well as sensitive online monitoring of Cr^{3+} in real-life drinking or wastewater samples.

CHAPTER 4:

Detection of Arsenite from water samples using As-ion imprinted polymer

4.1 Introduction:

Among a variety of metalloid pollutants, arsenic is of supreme environmental significance due to its carcinogenicity and severe toxicity (C.O. Abernathy 1999). It is released from some natural processes such as weathering and volcanic emission, as well as anthropogenic activities i.e. mining, use of pesticides, and fossil fuel combustion directly into the environment (P.L. Smedley 2002). Arsenic in drinking water is a global threat as it is highly toxic for humans and other living organisms (Sharma 2009). Arsenite (AsO_3^{3-}) and arsenate (AsO_4^{3-}) are the major problems causing species of arsenic in groundwater (Thomas 2001), (M.B. Baskan 2001). The quantification and extraction of metal ions in water is a tedious task. World Health Organization (WHO) recommended 10μg/L as the guideline value in drinking water for arsenic (World Health Organization 1996). The most common treatments for As (III) removal from contaminated water include flotation (Y. Zhao 1996), precipitation with sulfide (D. Bhattacharya 1979), and coagulation (M.B. Baskan 2001), filtration and ion exchange (J. Kim 2004). Recently, a lot of novel materials have been developed and is still in progress such as alumina (Han 2013), activated carbon (Oliveira 2009) (Zhang 2007) iron oxides (Sun 2013), (Giménez 2007), zeolite (Swarnkar 2012), clays (Anjum 2011), etc. to adsorb As^{3+} from wastewater. These Conventional techniques often fail when it comes to lowering a concentration of arsenic. On the other hand, electrochemical techniques offer us the advantages of minimal sample pretreatment and ease of analysis (T. Ndlovu 2014). To achieve sensitivity and selectivity ion-imprinted polymer system is used in this study (K. Haupt 2001). The general principle of MIP is a process where synthetic polymers are created. The analyte is extracted from the polymer forming cavities (D. Lièvremont 2009).

During our work, we have synthesized ion-imprinted polymers for As (III) determination in wastewater using interdigital electrodes (IDEs) as a transducer. Different receptor layers were optimized with different monomers to access its performance. The ion-imprinted polymer system was further modified using graphene-based composite.

4.2 Experimental:

4.2.1 Materials and Methods:

Chemicals used during synthesis were arsenic chloride ($AsCl_3$), styrene (99%), *N*-vinyl pyrrolidone (98%), ethylene glycol-dimethacrylate (EGDMA; 98%), azobisisobutyronitrile (AIBN; 99%), graphene oxide (GO), chlorides of magnesium, lead and zinc were all purchased from Aldrich and Merck. For standard solutions, a stock solution of Arsenic (III) was prepared by dissolving 01 g of $AsCl_3$ in 5 ml of deionized water and dilution to 50 ml with distilled water.

4.2.2 Ion imprinted polymers (IIP)

4.2.2.1 Ion imprinted polystyrene system

As (III) imprinted polymer was prepared by bulk polymerization method where styrene used as monomer, EGDMA as cross-linker, AIBN as initiator and methanol as porogenic solvent. $AsCl_3$ (2 mg), was dissolved in 200 µl of methanol. The solution was sonicated for 10 minutes as arsenic do not readily dissolve in methanol. After the formation of a clear solution, 30 µl of styrene, 70 µg of EGDMA, and 5 µg of AIBN were dissolved in 200 µl of the above solution and the resulting mixture was stirred vigorously. It was polymerized by heating at 60°C till it reaches a gel point.

4.2.2.2 Ion imprinted poly (*N*-vinyl pyrrolidone)

As (III) imprinted polymer was prepared by bulk polymerization method where *N*-vinyl pyrrolidone used as monomer, EGDMA as cross-linker, AIBN as initiator and methanol as porogenic solvent. To prepare a NVP based As (III)-imprinted polymer, $AsCl_3$ (2 mg), was dissolved in 200 µL of methanol. Afterwards 30µl of NVP, 70 µg EGDMA and 5 µg of AIBN were added to the above solution and the resulting mixture was stirred vigorously. The polymerization was carried out at 60-65$^{+\circ}$C to reach its gelation point.

4.2.2.3 Ion imprinted Methacrylate system:

As (III) imprinted polymer was prepared by bulk polymerization method where methacrylic acid (MAA) used as monomer, EGDMA as cross-linker, AIBN as initiator and methanol as porogenic solvent. $AsCl_3$ (2 mg), was dissolved in 200 µl of methanol. The solution was sonicated for 10 minutes as arsenic do not readily dissolve in methanol. After the formation of a clear solution, 30 µl of MAA, 70 µg of EGDMA, and 5 µg of AIBN were dissolved in 200 µl of the above solution and the resulting mixture was stirred vigorously. It was polymerized by heating at 60°C till it reaches a gel point.

4.2.2.4 Ion imprinted co-polymer-graphene oxide composite:

To prepare graphene oxide composite, $AsCl_3$ (2 mg), was dissolved in 200 µl of methanol. The solution was sonicated for 10 minutes as arsenic do not readily dissolve in methanol. After the formation of a clear solution, 20 µl of styrene, 10 µl of NVP, 70 µg of EGDMA, 100 µg of graphene oxide, 5 µg of AIBN were added to the solution and was stirred vigorously. It was polymerized by heating at 60°C till it reaches a gel point.

4.2.3 Immobilizing receptors onto transducer:

IIPs and composite thin films were generated onto the interdigital electrodes (IDEs) and for this purpose 10 ul of receptors (IIPs/composite) were coated by spin coating method at 2500 rpm. After generating thin films the IDEs were dried overnight at 80°C to obtain compact and dried layers. Then these IDEs were washed with excessive distilled water for two hours to generate ions cavities within polymers by washing out template ions. Capacitance measurement (20 µS and 1000 µS) of IDEs were taken with LCR meter (IET 7600 plus precision LCR meter) at different concentrations of analyte of interest and competing ions.

4.2.4 Removal of template from ion imprinted polymer thin films:

The imprint ion i.e. As (III) was leached out from the above polymer coated on IDE by stirring with 50 ml of distilled H_2O for 2h to generate cavities. The polymer once washed was dried in an oven at 80°C overnight.

4.2.5 Sensor measurements:

Capacitance measurement of polymer coated on IDEs was taken with LCR meter at different concentrations of analyte of interest and competing ions at 20 µS and 1000 µS.

4.3 Results and Discussions:

4.3.1 Characterization:

Morphological studies were done using scanning electron microscope.

4.3.1.1 SEM Analysis:

Figure 4.1 shows the surface morphology of the ion imprinted polymer and ion imprinted nano-composite. In (figure 4.1) the SEM images of As-IIP and GO-IIP are shown and it was found that the particle size were in the range of 1–3 µm. As can be seen, IIP has shown smaller cavities and accumulation was carried out. The rough surface as observed may provide more specific recognition sites for the target

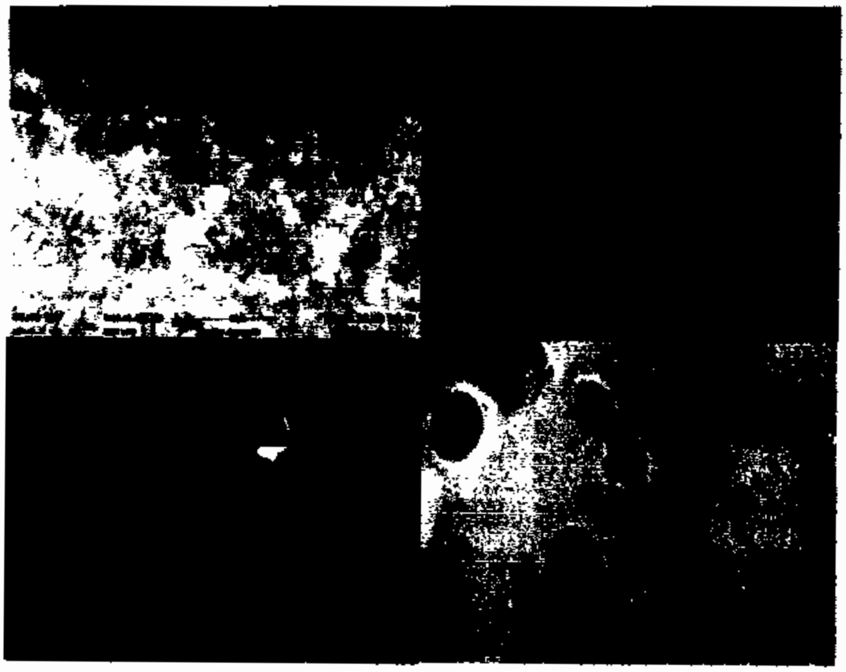


Figure 4.1: Scanning electron micrograph showing polymer a) after washing showing pores b) size of polymer particles (C) (IIP composite)

4.3.2 Effect of pH on adsorption of As (III):

PH of the aqueous solution containing 50 ppm of As (III) was adjusted between 6-7. Adsorption of metal ion is firmly pH dependent, because at low pH value H^+ ion may cause hindrance. The same is the situation at high pH adsorption was affected by the presence of OH^- ions. Therefore, the pH of the solution adjusted to pH 6 for the optimum extraction of As (III) ions.

4.3.3 Effect of temperature on adsorption of AsIII:

Maximum adsorption was achieved at 25 °C. High temperature cause metal ions to move easily in the solution thus affecting adsorbtion and the quantity of polymer which was applied on the electrode. So, 25°C was taken as the optimized temperature used during our experimental work.

4.3.4 Optimization of recipe

To enhance the sensitivity and selectivity of As-IIP, different receptors were synthesized in accordance with table 4.1.

Table 4.1: Synthesis of metal ion imprinted polymers

Polymer	Template	Functional monomer	Cross-linker	Monomer/ cross linker ratio	Polymerization conditions
P1	Arsenic III	MAA	EGDMA	30:70	Thermal 60°C
P2	Arsenic III	styrene	EGDMA	30:70	Thermal 60°C
P3	Arsenic III	NVP	EGDMA	30:70	Thermal 60°C
P4	Arsenic III	Styrene/NVP/ graphene oxide	EGDMA	30:70	Thermal 60°C

Different monomers were used in the polymer system to assess their performance compared to one another. As shown in (table 4.1) monomer to cross-linker ratio was kept constant in all synthesized polymer. It was observed in the last chapter polymer with the monomer to cross-linker ratio 30:70 shows high sensitivity and better stability than other systems. The physical properties of IIP are influenced by the percentage of cross-linked, amount of porogenic solvent, and polymerization conditions (Holland 2010). Recognition cavities on Ion-imprinted polymer (IIP), coated on transducer (IDE) surface, selectively binds arsenic ions. The transfer of template ions from the sample solution, into these recognition cavities results in changing electrical properties of the IIP-coated layer therefore, its conductivity is increased. Thus any change in conductivity is directly proportional to template ions added or removed from the polymer.

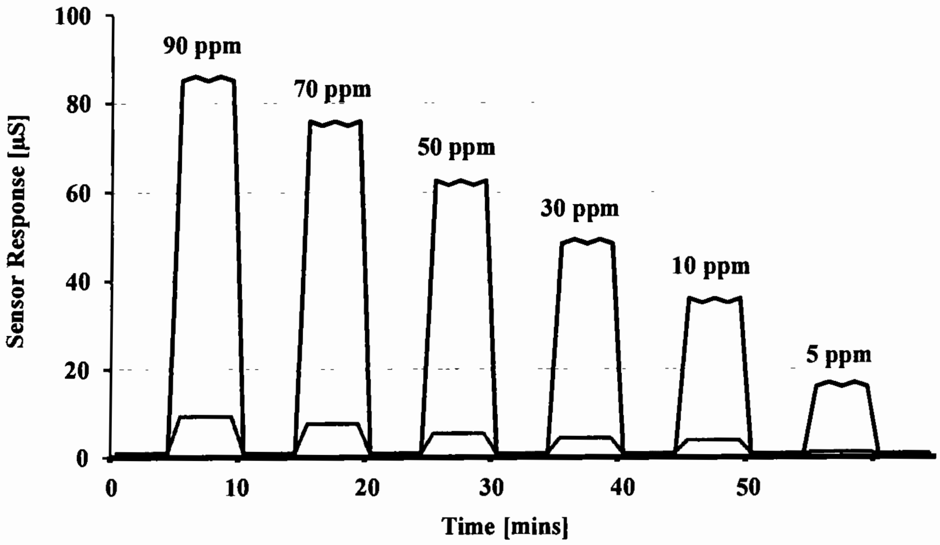


Figure 4.2: Sensitivity response of imprinted and non-imprinted N-vinyl pyrrolidone polymers towards different concentration of arsenic solutions

In the first experiment, the NVP-based As III-imprinted receptor system was synthesized. Polymer system interacts with As^{+3} ions via electron-enriched entities present in their structure. The sensitivity of polymer systems was analyzed by exposing them to various concentrations of template ion ranging from 90–5 ppm as shown in Figure 4.2. The conductance of the IIP layer at 90 ppm was 86 µS which reduces to 75, 62, 48, 36, and 17 µS at 70, 50, 30, 10, and 5 ppm, respectively.

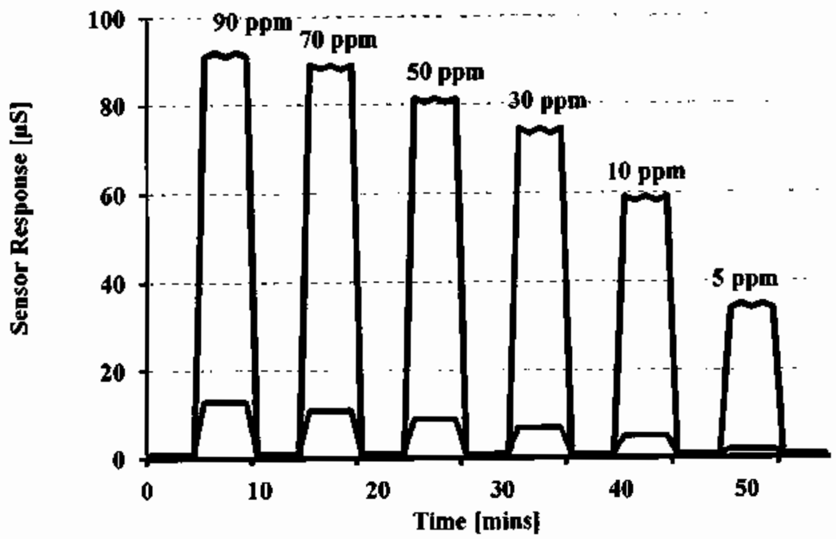


Figure 4.3: Sensitivity response of imprinted and non-imprinted styrene polymer towards different concentrations of arsenic solutions

The sensitivity of the styrene-based polymer system was investigated through exposing it to different arsenic concentrations as shown in Figure 4.3. The styrene-based polymer showed better sensor response than NVP due to its hydrophobicity.

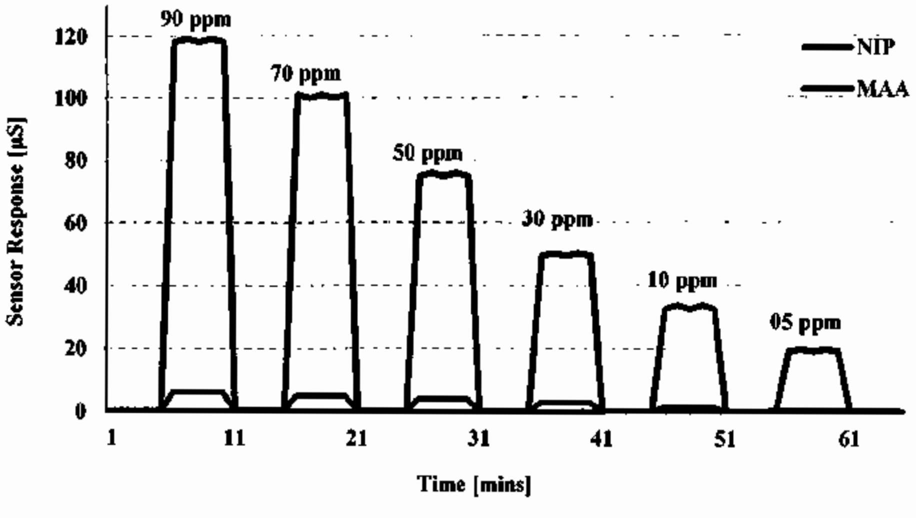


Figure 4.4: Sensitivity response of imprinted and non-imprinted methacrylic acid polymer towards different concentrations of arsenic solutions.

In our third experiment, Methacrylic acid was used as a monomer during polymer synthesis. Functional monomers like methacrylic acid are commonly selected for polymer synthesis to mimic biological interactions because of their anionic acidic functional groups which allow non-covalent interactions. Additionally, with a non-covalent imprinting approach, it is easy to fabricate the polymer, which helps in template removal (washing) and rapid template rebinding (Boonpangrak. S 2006). A graphical representation of MAA-IIP is given in Figure 4.5 (a). The conductance value of IIP at 90 ppm was 112 µS which decreases to 96, 71, 46, 32, and 19 µS at 70, 50, 30, 10, and 5 ppm, respectively. As observed MAA based polymer showed better sensitivity then P1 and P2 respectively.

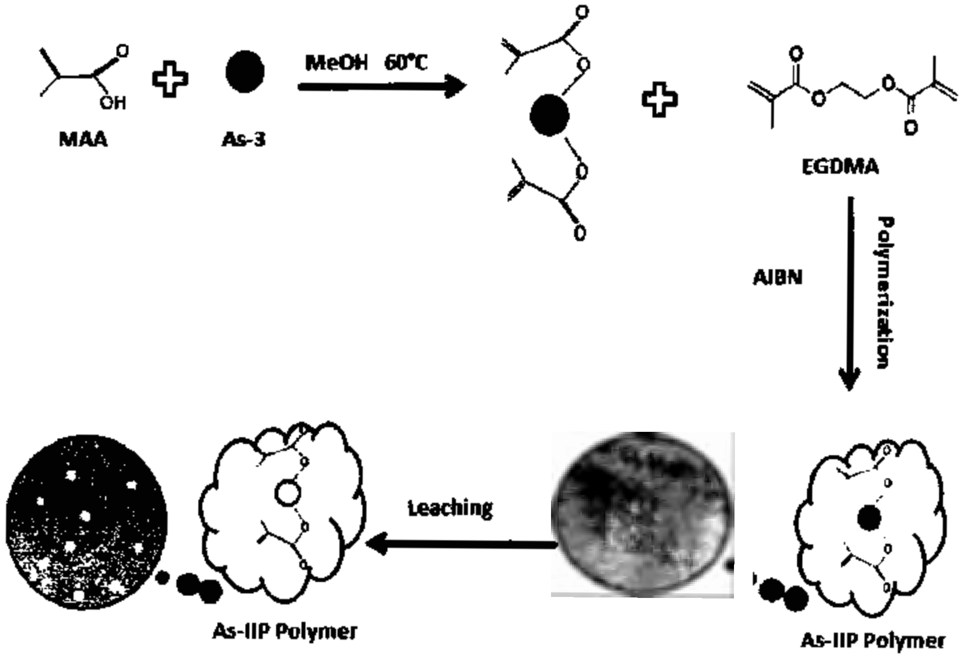


Figure 4.5 (a): Graphical representation of As-IP

To further enhance the sensitivity of the polymer IIP-graphene composite system was also investigated. Graphene, with an extremely large area, and distinctive mechanical properties are extensively used as supporting material for preparing imprinted receptors. The binding sites in the outer layer of IIP composite give complete removal of templates, thus reducing its binding time. Graphene oxide binds both small and large molecules and ions to its surface through covalent or non-covalent interaction (K.S. Novoselov 2004). Graphene exhibits promising potential in energy storage applications (B. Wang 2011), (Y. Chen 2011), (B. Wang 2011).

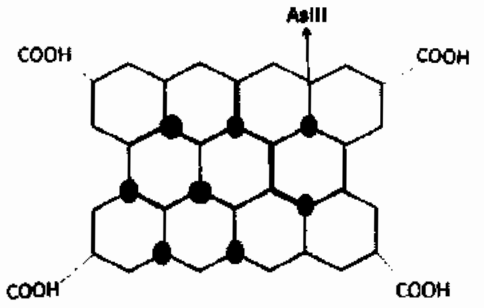


Figure 4.5 (b): Intermolecular interaction of arsenite ions on the surface of IIP-GO composite

Due to the excellent mechanical and electric properties of GO, the sensitivity of IIP-graphene composite is enhanced incredibly as shown in Figure 4.6. At 90 ppm of analyte concentration, there is a drastic increase in the conductance which is 233 µS, compare to styrene and NVP polymer systems. The graph shows a linear response of 199, 143, 76, 38, and 21µS while exposing to 70, 50, 30, 10, and 5 ppm template concentration.

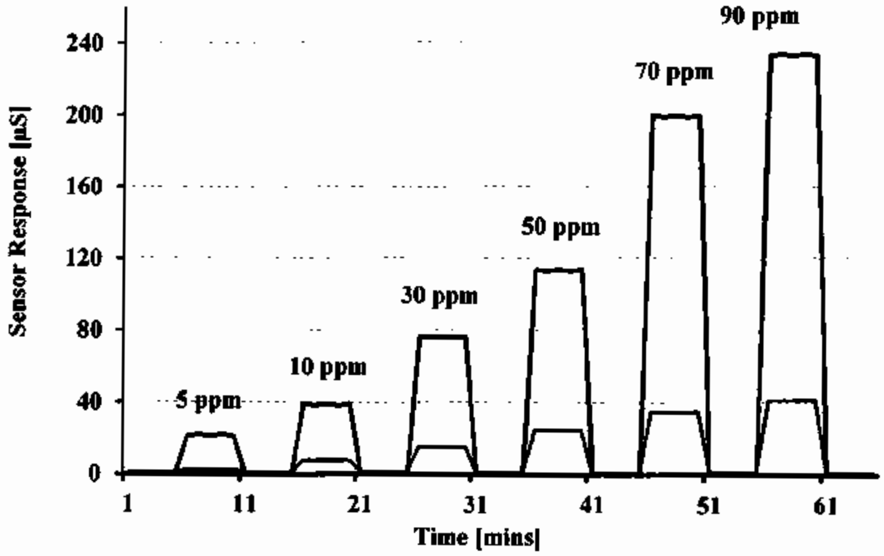


Figure 4.6: Sensitivity response of imprinted and non-imprinted graphene composite towards different concentrations of arsenic solutions

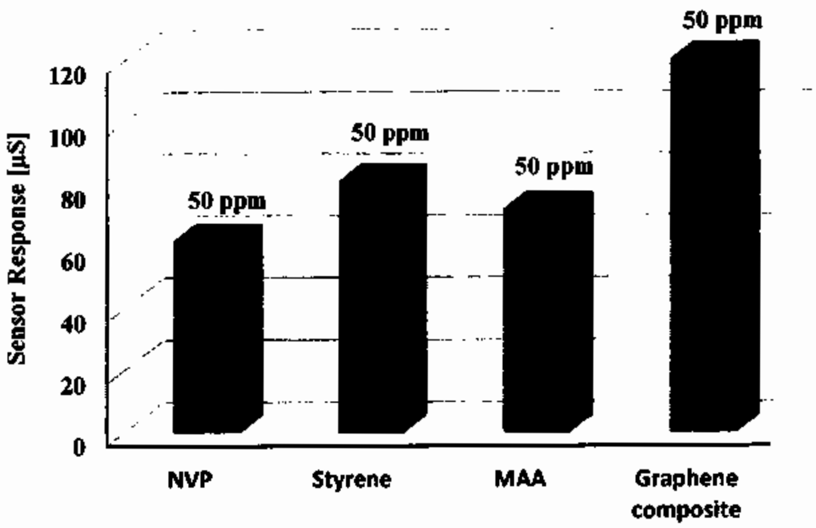


Figure 4.7: Comparison of four different As-imprinted polymer based receptors at 50 ppm concentration

4.3.5 Selectivity:

The Sensor system was tested further for systematic selectivity. To obtain structural information, toward structurally related compounds of arsenic chloride, selectivity measurements of different heavy metals were checked with LCR meter. As the result shows arsenite- based polymer was highly selective towards arsenite ions.

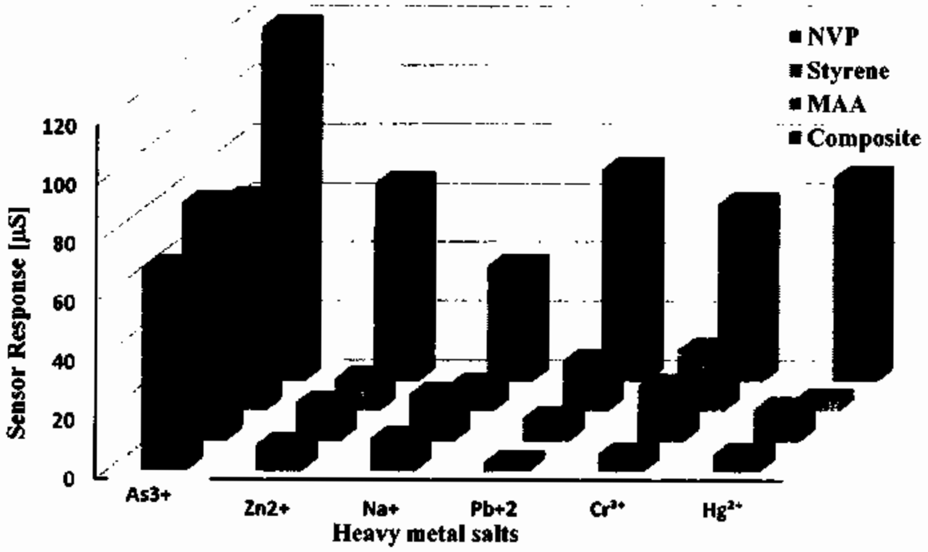


Figure 4.8: Selectivity response of the sensor for different heavy metal ions at a concentration of 50 ppm

4.4 Conclusion:

Arsenite based ion- imprinting polymer was synthesized using methanol used as a solvent. Five different polymers using different functional monomers were created. Polymer P_1 contains MAA as a functional monomer, P_2 comprises of styrene, P_3 involved NVP, and P_4 was a composite that contains co-polymer of sty and NVP with 50:50 ratio. Out of four monomers used the bi-monomer system with styrene and NVP used as monomers showed better sensitivity and selectivity. GO-based composite gave the highest sensitivity value because of its excellent electrical conductivity. The sensitivity sequence of respective polymers is as follows $P4 \geq P2 \geq P1 \geq P3$.

CHAPTER 5:

Effect of porogenic solvent on efficiency of Pb (II) ion-imprinted polymer in selective removal from wastewater

5.1 Introduction:

Lead is known to be one of the most toxic heavy metal produced as a result of different processes such as fossil fuels burning, mining and manufacturing (H. Parham 2009)(M. Ronteltap 2007). Lead ion concentration if exceeds its limit may cause lead poisoning, once considered to be a classic disease seen in children and adults (Markowitz, 2000).

Soil and aquatic systems are most possibly affected by toxic heavy metals like lead that move through the food chain as soil-plant–animal-human, causing severe contamination (Liu 2003), (Krishnamurthy 1992). Lead if present in the environment is non-biodegradable, and accumulates through binding with organic and inorganics matter respectively (Anthemidis 2008), (Boonamnuayvitaya 2004). Moreover, the lead ion is considered to cause serious threats to human health, such as affecting the liver and kidneys, blood composition, nervous system, and reproductive system (K. Yetilmezsoy 2008).

As already mentioned different problems are caused by lead therefore, strict handling and treatment solutions to eradicate lead concentrations in the environment are required (A. Gunay 2010). Several studies are reported on the abatement of lead from drinking water (W.L. dos Santos 2004). Especially, separation and pre-concentration measures are frequently used. Conventional methods are adopted such as ion exchange (J.O. Agbenin 2012), electrolysis (J.Q. Pan 2012), chemical precipitation (F.L. Fu 2012) membrane separation (M. Soylak 2007), biological treatment (J.E. Morgan 1988), bio-sorption (W.L. Yan 2005), and adsorption (Z. Al-Qodah 2006). Most of the adsorption processes are non-specific towards a specific metal ion (Y.K. Lu, 2006). Since there are several competing metal ions in waste-water therefore, it is of considerable significance to selectively remove or detect the ions of a particular metal. Molecular imprinting technology (MIT) is a method to create artificial locks that can recognize molecular keys. By using this technology, molecular imprinting polymers (MIP) with selective recognition sites of the particular molecule (template or imprinted molecule) can be created. Recognition of metal ions in aqueous solutions is of great importance and a lot of progress has been made in this field during recent years.

In this study, the ion-imprinted polymer was synthesized for trace monitoring of Pb (II) in water samples using different monomers and porogenic solvents. The performance of imprinted polymer was further optimized by using IIPs/functionalized graphene composite as receptors.

5.2 Experimental:

5.2.1 Chemicals and Reagents:

Pre-polymerized reagents used during the synthesis were lead (II) chloride ($PbCl_2$), styrene (99%), *N*-vinyl pyrrolidone (NVP; 98%), ethylene glycol-dimethacrylate (EGDMA; 98%), azobisisobutyronitrile (AIBN; 99%), graphene oxide (GO), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO; 98%), chlorides of magnesium, arsenic, and zinc were all purchased from Aldrich and Merck. For standard solutions, a stock solution of Pb (II) was prepared by dissolving 01 g of $PbCl_2$ in 5 ml of deionized water and dilution to 50 ml with distilled water.

5.2.2 Synthesis of ion imprinted polymers:

5.2.2.1 Synthesis of ion imprinted MAA polymer:

Lead ion imprinted polymer was prepared by bulk polymerization method where methacrylic acid was used as monomer, EGDMA as cross-linker, AIBN as initiator and THF as porogenic solvent. $PbCl_2$ (2 mg), was dissolved in 200 µl of tetrahydrofuran (THF). After the formation of a clear solution, 30 µl of *N*-vinyl pyrrolidone, 70 µg EGDMA and 5 µg of AIBN were added in the above solution and the resulting mixture was stirred vigorously for 5 minutes. It was polymerized by heating at 60°C till it reached a gel point. Other MAA based ion imprinted polymers were prepared using the same way but by using different porogenic solvents i.e. DMSO and methanol.

5.2.2.2 Synthesis of ion imprinted co-polymer (NVP-Styrene):

Lead ion imprinted co-polymer was prepared by bulk polymerization method where styrene and NVP were used as functional monomers, EGDMA as cross-linker, AIBN as initiator and THF as porogenic solvent. $PbCl_2$ (2 mg), was dissolved in 200 µl of tetrahydrofuran (THF). After the formation of a clear solution, 30 µl of *N*-vinyl pyrrolidone, 70 μg EGDMA and 5 μg AIBN was added in the above solution and the resulting mixture was stirred vigorously. It was polymerized by heating at 60°C till it reached a gel point. Other co-polymers were prepared using the same reagents and procedures but by using different porogenic solvents i.e. DMSO and methanol.

5.2.2.3 Synthesis of ion imprinted polymer-graphene oxide composite:

Lead ion imprinted composite was prepared by bulk polymerization method where styrene and NVP were used as functional monomers, EGDMA as cross-linker, AIBN as initiator and THF as porogenic solvent. $PbCl_2$ (2 mg), was dissolved in 200 μl of tetrahydrofuran (THF). After the formation of a clear solution, 30 μl of N-vinyl pyrrolidone, 70 μg EGDMA, 100 μg of graphene oxide and 5 μg AIBN were added in the above solution and the resulting mixture was stirred vigorously. It was polymerized by heating at 60°C till it reached a gel point. Other composites were prepared with the same reagents, quantities and procedure but by using different porogenic solvents i.e. DMSO and methanol.

5.2.2.4 Synthesis of non-imprinted polymer:

A non-imprinted polymer (NIP) was prepared as the control. It was synthesized under the same solution conditions as those used for the IIP, however, in the absence of analyte (Pb^{2+}).

5.2.3 Immobilization of receptor layer onto transducer:

IIPs and composite thin films were generated onto the interdigital electrodes (IDEs). For this purpose 10 ul of receptors (IIPs/composite) were coated by spin coating method at 2500 rpm. After generating thin films the IDEs were dried overnight at 80 °C to obtain compact and dried layers. Then these IDEs were washed with excessive distilled water for two hours to generate ions cavities within polymers by washing out template ions. Capacitance measurements of IDEs were taken with LCR meter at different concentrations of analyte of interest and competing ions at 20 Hz and 1000 Hz.

5.2.4 Removal of template from Ion imprinted polymer thin films:

The imprint ion i.e. Pb^{+2} was leached out from the above polymer coated on IDE by stirring with 100 ml of distilled H_2O for 2h to generate cavities.

5.2.5 Apparatus:

SEM measurements were carried out on a JEOL JSM 6500F microscope equipped with a field emission source. Sonication was performed using a Sonorex RK, 102 H ultrasonic water bath. Batch rebinding experiments were done using a pH meter basic 20. Ion quantification was achieved by LCR meter (IET 7600 plus precision LCR meter).

5.2.6 Sensor Measurements:

Capacitance measurements of polymer-coated on IDEs were taken using LCR meter at different concentrations of analyte of interest i.e. lead ion and other competing ions at 20 Hz and 1000 Hz.

5.3 Results and Discussion:

5.3.1 Characterization:

In our study the samples of lead ion imprinted polymers, co-polymer, and nano composite were assessed using two different types of microscopy, namely the Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM). AFM proves advantageous over the other microscopy techniques when an accurate surface height measurement is required. While SEM can be used for even higher resolutions than AFM, it generates a two-dimensional image; accurate surface height is more easily obtained through AFM.

5.3.1.1 Scanning Electron Microscopy:

The morphology of Pb-IIP was investigated using Scanning Electron Microscopy. The surface texture exhibited cavities on the surface of the leached IIP as a consequence of imprinting figure 5.1 (b). The formed cavities further increases the sorption capacity of the sorbent. It is clearly seen that leached Pb (II)-IIP has more adsorption sites for the target Pb (II) ions in the solution.

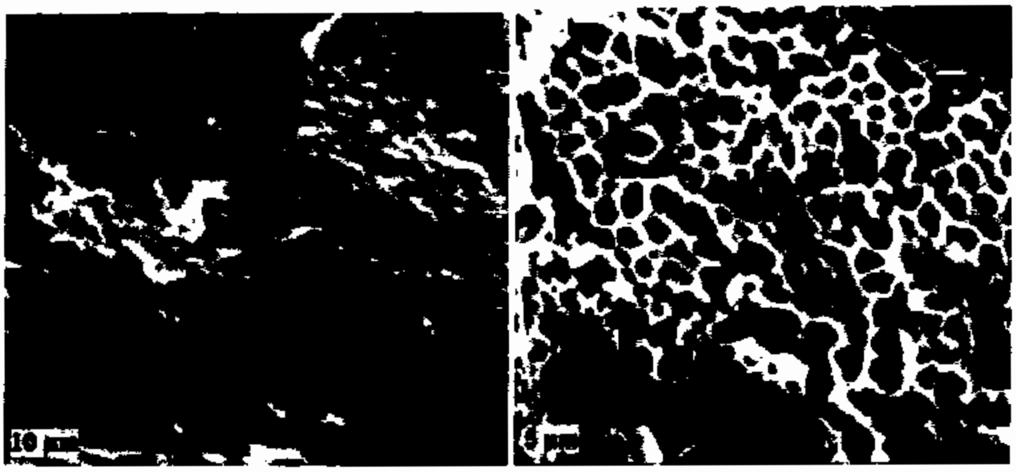


Figure 5.1: (a) SEM image of the polymer having Lead ion as a template molecule at the scale of 10 µm, and (b) SEM image of ion imprinted polymer after removing the lead as a template molecule at the scale of 5 µm.

5.3.1.2 Atomic Force Microscopy:

The formation of IIPs is characterized by significant change in polymer morphology, which is observed, using AFM techniques on the nano-scale. As shown in figure 5.2, atomic force microscopy (AFM) images of our non-imprinted and Pb^{2+} ion-imprinted polymers. The surface roughness shows higher roughness for the leached imprinted surface than for the unleached surface. The roughness of the surface is important as it promotes the transfer of metal ions toward the polymer surface.

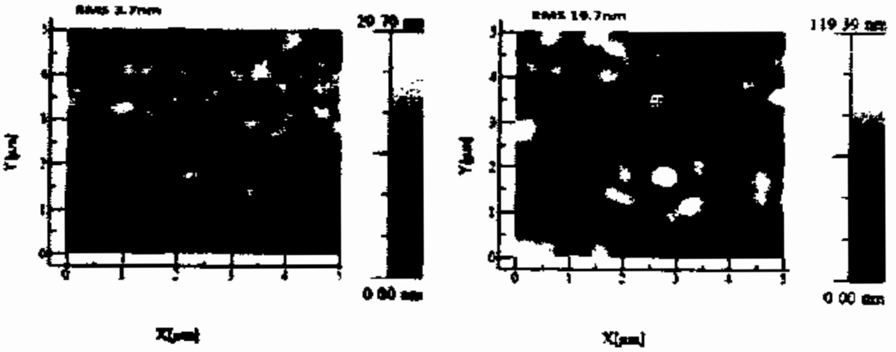


Figure 5.2: A two-dimensional AFM image of leached and unleached Pb ion imprinted polymer.

5.3.2 The effect of pH on adsorption of pb II:

PH of the aquoes solution containing 50 ppm of Pb^{2+} was adjusted between 6-7. Adsorption of metal ion is strongly pH dependent, because at low pH value H^+ ion may cause hindrance. Same is the situation at high pH adsorption was effected by the presence of OH^- ions. Therefore, pH of solution adjusted to pH 6 for the optimum extraction of As (III) ions.

5.3.3 The effect of temperature on adsorbtion of pb II:

Maximum adsorbtion was achieved at 23°C. High temperature cause metal ions to move easily in the solution thus affecting adsorbtion and the quantity of polymer which was applied on electrode. Therefore, 25°C were taken as the optimized temperature used during our experimental work.

5.3.4 Optimization of recipe:

Arsenite ion-imprinted polymer was optimized to assess its performance. A detail description of synthesis is given in table 5.1.

Table 5.1: Lead (II) imprinted polymers – conditions of synthesis.

Polymer	Template	Functional monomer	Cross-linker	Monomer/ cross-linker ratio	Porogenic Solvent	Polymerization conditions
P1	Pb^{2+}	styrene/NVP	EGDMA	30:70	DMSO	Thermal 60°C
P2	Pb^{2+}	styrene/NVP	EGDMA	30:70	THF	Thermal 60°C
P3	Pb^{2+}	styrene/NVP	EGDMA	30:70	Methanol	Thermal 60°C
P4	Pb^{2+}	MAA	EGDMA	30:70	DMSO	Thermal 60°C
P5	Pb^{2+}	MAA	EGDMA	30:70	THF	Thermal 60°C
P6	Pb^{2+}	MAA	EGDMA	30:70	Methanol	Thermal 60°C

The porogenic solvent plays an important role in making an effective IIP's because the solvent used interacts significantly with template molecules and with functional monomers. These interactions in return control the structure and formation of molecular or ion-imprinted polymer. The solvent provides a medium for MIP formation and its polarity will affect its interaction with template molecule and functional monomer. The association and bonding between different components in polymer synthesis have a strong impact on the structure of imprinting sites in MIP. To evaluate the effect of the porogenic solvent on the formation of IIP, three porogenic solvents were selected having different polarities in the presence of different monomers. In polymer P1 P2 and P3, the styrene and NVP were used as bi-monomer with EGDMA as cross-linker in the presence of three different solvents. 2, 2'-Azo-bisisobutyronitrile (AIBN) was used as free radical initiator as at a temperature of 60 °C its half-life time is about 10 hours which is considered as a proper time for polymerization (Xingliang Song 2009).

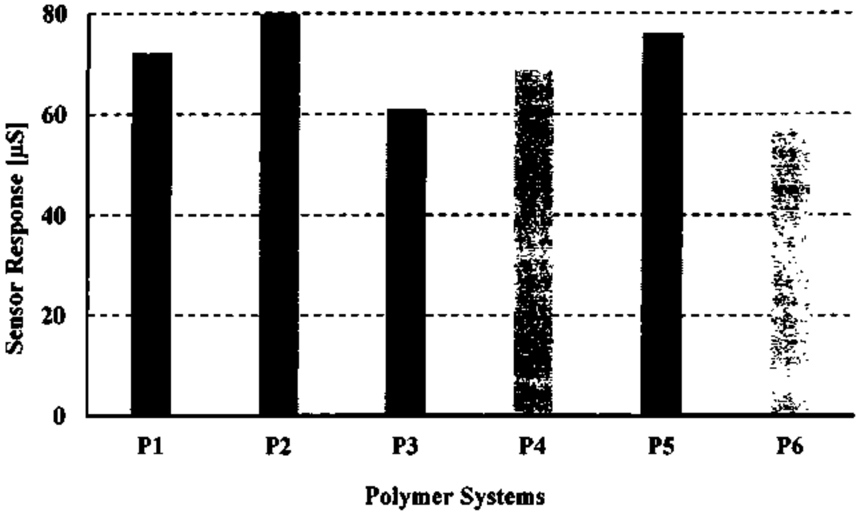


Figure 5.3: Optimisation of different polymer compositions

Since the solubility of Pb^{2+} in DMSO (Polarity 0.44) was more than other solvents, but THF (polarity 0.207) as a Porogenic solvent showed higher sensitivity and selectivity. The solubility of $PbCl_3$ was poor with methanol used as a porogenic solvent. So it is proved that the low polar solvent such as THF is a better solvent for preparing IIP's for metal ions. The order of sensitivity and selectivity was as P2>P5>P1>P4>P3>P6. The polarity of the solvent affects the structure and number of imprinted sites formed on IIP. Solvents with high polarity will form a strong bond with metal ion and monomer thus allowing less interaction between metal ion and monomer. The reason that THF as a porogenic solvent produced better results as compared to other solvents. The fast adsorption in the first few hours was due to the rapid recognition of metal ions onto imprinted sites on the surface of the polymer. When these imprinted sites were occupied, it was becoming hard for these metal ions to penetrate the IIP and find imprinted sites inside thus causing cause adsorption to slow down. The strength of interactions between the template and the monomers affects the affinity of the imprinted polymers and determines the accuracy and selectivity of the recognition sites (Mohammad Behbahani. 2013), (Xuewen Fu 2015).

In our first experiment with lead ion as a template, methacrylic acid (MAA) was used as a monomer. Possible interaction scheme of lead ions with the methacrylic acid is shown in figure 5.4. At 90 ppm the MAA system with THF as solvent showed a sensitivity of 99 μS which was reduced to 28 μS at 5 ppm.

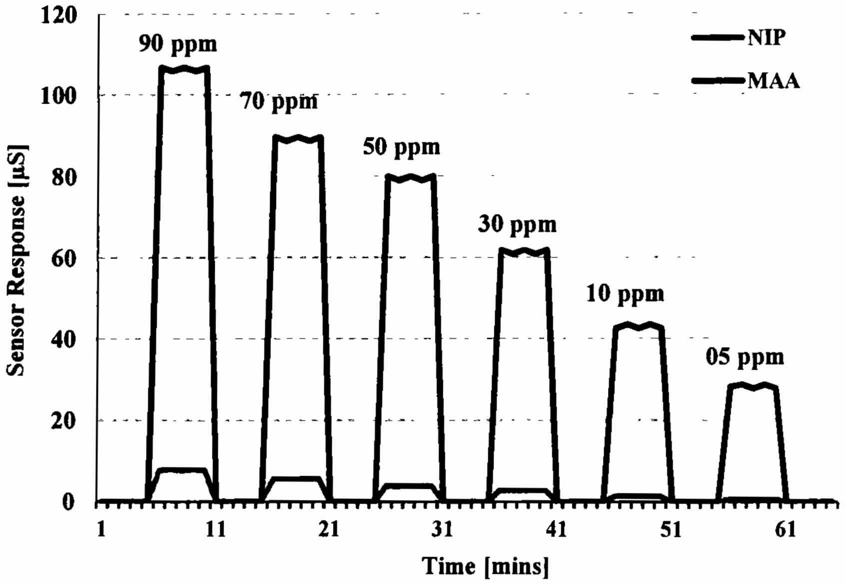


Fig 5.4: Sensitivity response of imprinted and non-imprinted MAA polymers towards different concentrations of lead solution.

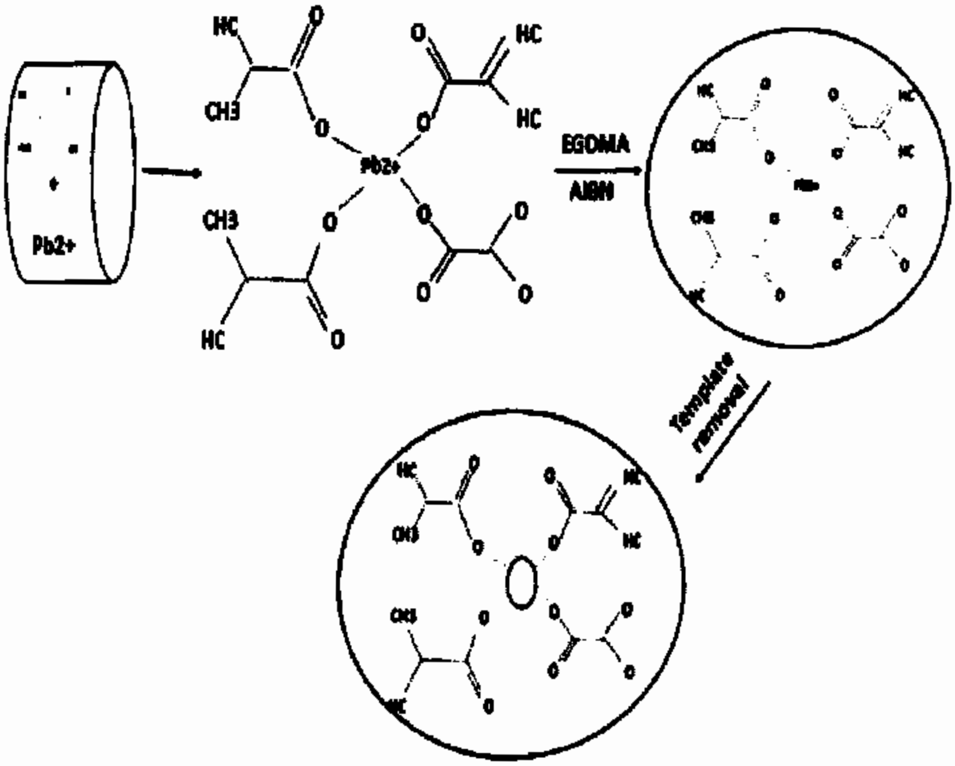


Figure 5.5: The fabrication process for the preparation of MAA based lead-ion imprinted polymer.

In our second experiment with lead ion as a template, styrene and NVP were used as monomers. The co-polymer system with styrene and NVP give hydrophobic interactions and thus proved to be ideal for metal ion detections in waste water. At 90 ppm the co-polymer system with THF as solvent showed a sensitivity of 102 µS which was reduced to 33 µS at 5 ppm. Although there is a very slight difference in sensor response towards MAA and co-polymer system which shows that both IIP systems if synthesized in THF give a good response.

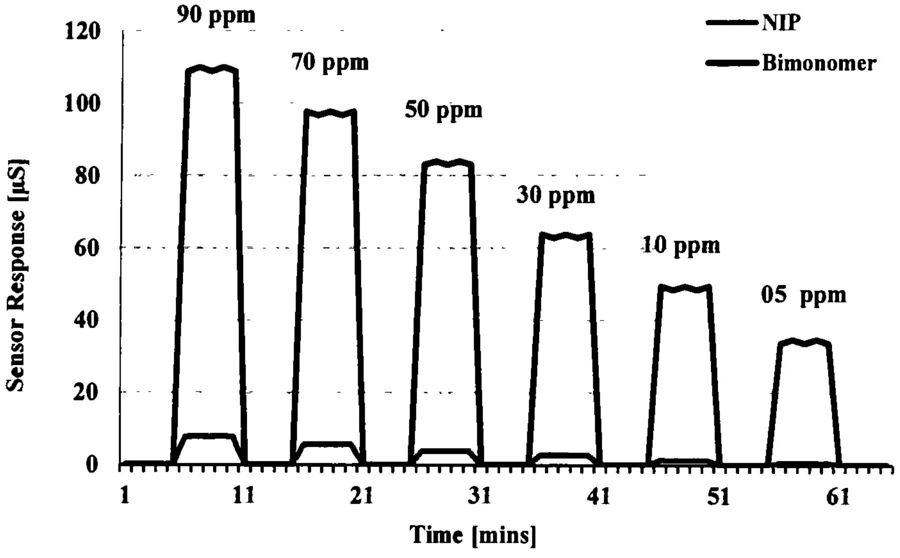


Fig 5.6: Sensitivity response of imprinted and non-imprinted co-polymer towards different concentrations of lead solution

In order to further enhance the sensitivity, IIP was modified with graphene oxide. *N*-vinyl pyrrolidone and styrene were used as functional monomers during polymer synthesis and THF was used as porogenic solvent. Graphene oxide prepared by chemical oxidation of graphite has a lot of oxygen-containing groups on its surface, including carboxyl group, epoxy group, and hydroxyl group which make GO hydrophilic and easier to be modified into an ideal solid support for Ion imprinted polymer. (S. Sreejith 2012) (Y. Liu 2016) (M. Ramezanzadeh 2018) At 90ppm the graphene nano-composites with THF as solvent showed a sensitivity of 211 µS which was reduced to 52 µS at 5 ppm respectively. With Graphene oxide the sensor response was doubled because it attached metal ions to its reactive oxygen functionalities located on the exterior of the polymer.

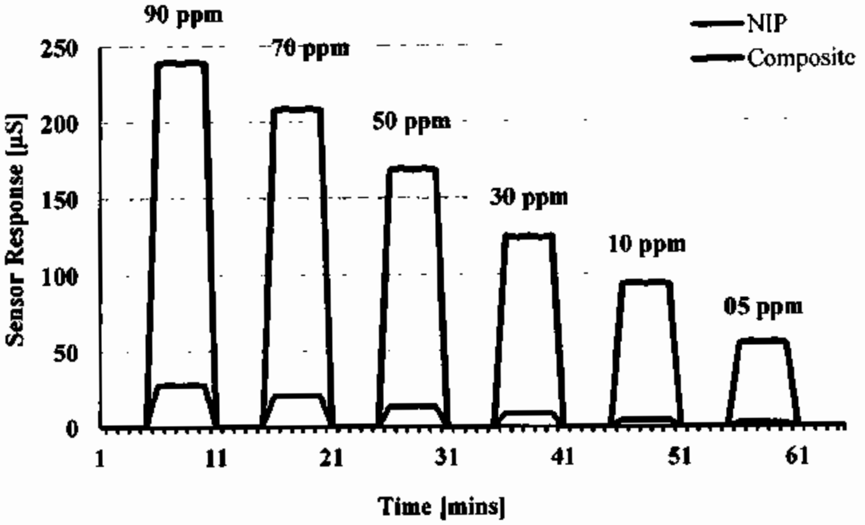


Figure 5.7: Sensitivity response of imprinted and non-imprinted graphene composite towards different concentrations of arsenic solutions

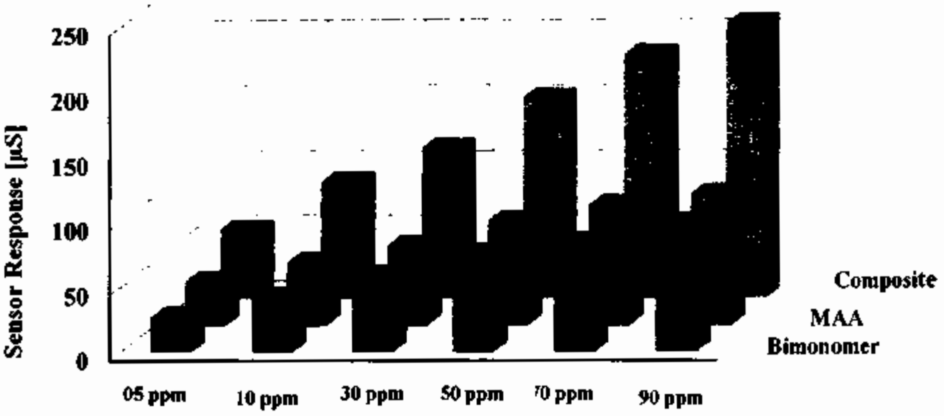


Figure 5.8: Comparison of three IIP receptors against different concentration of P A) MIP-Composite, b) Co-polymer c) MIP-NVP

5.3.5 Selectivity:

Selective adsorption experiment was conducted with the same concentration of binary mixtures including Pb(II)/Zn(II), Pb(II)/Na(I), Pb(II)/As(III), Pb (II)/Cr(III) and Pb(II)/Hg(II) solutions. The IIP showed good selectivity towards Pb^{2+} over other metal ions such as Hg^{2+}, Cr^{3+}, $As^{3+,}$ Zn^{2+} and Na^{+} respectively as shown in Figure 5.9.

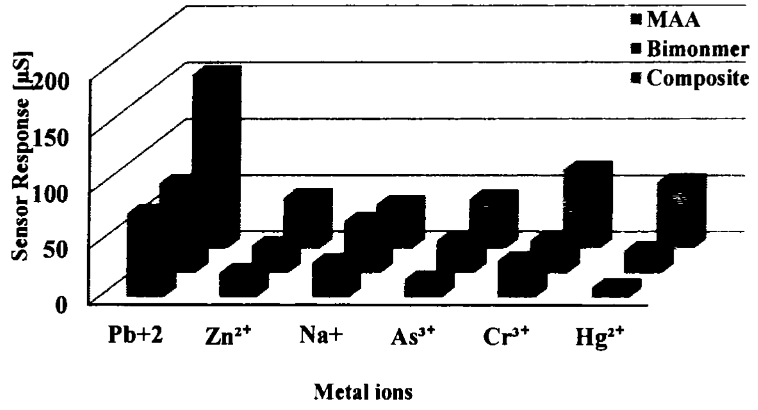


Figure 5.9: Selectivity response of the sensor for different heavy metal ions at a concentration of 50ppm

5.4 Conclusion:

Lead ion-imprinted polymer was synthesized using Pb^{2+} as a template, styrene, *N*-vinyl pyrrolidone, and methacrylic acid as functional monomers, EGDMA as cross-linker respectively. Optimization of the composition was done by using three different porogenic solvents i.e. DMSO, tetrahydrofuran (THF), and methanol. Each polymer was tested for DMSO, tetrahydrofuran (THF), and methanol individually. The results gave a better prove that the type of porogenic solvent used had an impact on the formation of IIP and its Pb^{2+} adsorption. THF gave good results as compared to the other two solvents. Methanol being highly polar and hydrophilic does not prove to be an ideal porogenic solvent. The IIP obtained in a medium polar solvent showed better molecular recognition ability. The type and amount of the porogenic solvent used had a great influence on the adsorption effectiveness of IIP for metal ions (Tan TW 2001).

CHAPTER 6:

Graphene oxide-based molecularly imprinted polymeric receptor for detection of reactive dyes from industrial water

6.1 Introduction:

The textile industries are the supreme producers of liquid discharge containing pollutants. Because of various processes in the textile industry, the composition of wastewater released varies constantly (T. Kim 2004). Worldwide the textile dyes discharged every year in industrial effluents are estimated to be 280,000 tons (X. Jin 2007), (Kalyani 2009). Dyes are said to have a toxic effect on plants and affect its germination rates and Biomass production. These dyes dissolve readily even a small quantity in water is visible and exceedingly undesirable. (Nigam 2000) (W. Wang 1991). Activated carbon is used for the removal of color but it is too expensive (Roy D 1993). The efficacy of various wood charcoals (Marmier D, 1991), commercial activated carbon (CAC) (Streat 1995), Alumina (Saleem M 1993), china clay (Gupta GS 1992) were different sorbents for the removal of Dyes. The selective absorption method is necessary to isolate the toxic or costly reactive dyes from wastewater which will facilitate the environment protection and the reuse of precious water-soluble reactive dyes. Molecular Imprinting Polymer (MIP) is an accepted method that produces artificial receptors in the form of polymers for specific molecular recognition (W.M. Mullett 1999) (P. T. Vallano 2000). Molecular imprinting is an effective method and has a lot of advantages such as selective recognition, separation, and purification of pollutants in wastewater (Dan-Lian Huang et al. 2015). Few studies have been reported on the removal of dyes from wastewater using MIPs.

In this study, MIPs of three textile dyes Reactive Red 24, Acid Orange 7, Reactive Blue 221 was prepared. Styrene and N-vinyl pyrrolidone were used as monomers for the selected dyes. Methanol (MeOH) was used as a porogenic solvent. Bulk imprinting technology was used to prepare MIPs for the detection of various reactive dyes from textile industrial wastewater. Further sensitivity was achieved by graphene-based nano-composites.

6.2 Experimental:

6.2.1 Chemical and Reagents:

Pre-polymerized reagents used during synthesis were, styrene (99%), *N*-vinyl pyrrolidone (NVP; 98%), ethylene glycol-dimethacrylate (EGDMA; 98%), azobisisobutyronitrile (AIBN; 99%), graphene oxide (GO), were all purchased from Aldrich and Merck.

The template material used were reactive red 24, acid orange 7, reactive yellow 160, reactive blue 221. Blank solutions during the work were prepared using freshly deionized water.

6.2.2 Instrumentation

SEM observations were taken from JEOL JSM 6500F microscope (Peabody, MA, USA). Sonorex RK 102H ultrasonic water bath was used to sonicate the solutions. PH meter basic 20 was used to check a respective pH of any solution. Ion quantification was achieved by LCR meter (IET 7600 plus precision LCR meter). Absorbance was measured using T80 series of UV-Visible Spectrophotometer.

6.2.3 Synthesis of molecular imprinted polymer (MIP):

Molecular imprinted polymer for reactive dyes was prepared by bulk imprinting methodology. Two different polymer systems i.e. polystyrene system and *N*-vinyl pyrrolidone systems were generated to assess the analyte binding efficiency of MIPs receptor.

6.2.3.1 N-vinyl pyrrolidone polymer synthesis:

Methanol was used as a solvent to dissolve 500 μl solution of the analyte (red, blue and orange dye). 200 μl of the resulting solution was mixed with 30 μl of the monomer, *N*-vinyl pyrrolidone. This mixture was thoroughly shaken on a vortex followed by the addition of 70 μg of the cross-linker, EGDMA and 5 μg of the initiator, Azobisisobutyronitrile (AIBN) in the pre-polymerized mixture. This mixture was then polymerized in a hot water bath for 15 minutes.

6.2.3.2 Polystyrene polymer synthesis:

Polystyrene polymer was prepared with the same procedure as above except for the addition of styrene as a monomer instead of NVP.

6.2.3.3 MIP-composite:

Again 500 µl solution of the analyte (red, yellow, blue and orange dye) were prepared in methanol as a porogenic solvent. 200 µl of this solution was mixed with 15 µl of the n-styrene and 15 µl of *N*-vinyl pyrrolidone with 50:50 ratio. This mixture was methodically shaken and then 70 µg of the cross-linker, EGDMA 5 µg of the initiator, azobisisobutyronitrile (AIBN) and 100 µg graphene oxide were added in this mixture. This mixture was then polymerized.

6.2.3.4 Non-imprinted polymer:

NIP was synthesized using the same procedure without the addition of analyte (dye).

6.2.3.5 Applying Receptors on Transducer surface:

Dye-MIPs and composite thin films were generated onto the interdigital electrodes (IDEs) and for this purpose 10 ul of receptors (MIPs/composite) were coated by spin coating method at 2500 rpm. The resulted thin film ranges from 80-100 nm in thickness. After generating thin films, the IDEs were dried overnight at 80°C to obtain compact and dried layers.

6.2.3.6 Generating Cavities:

The imprint molecules i.e. reactive dyes were leached out from the above polymer coated on IDE by stirring with 100 ml of distilled H_2O for 2h to generate cavities. The resultant polymer after washing was dehydrated in a vacuum oven. Capacitance measurement of IDEs were taken with LCR meter (IET 7600+) at different concentrations of analyte of interest and competing dye molecules at 20 Hz and 1000 Hz.

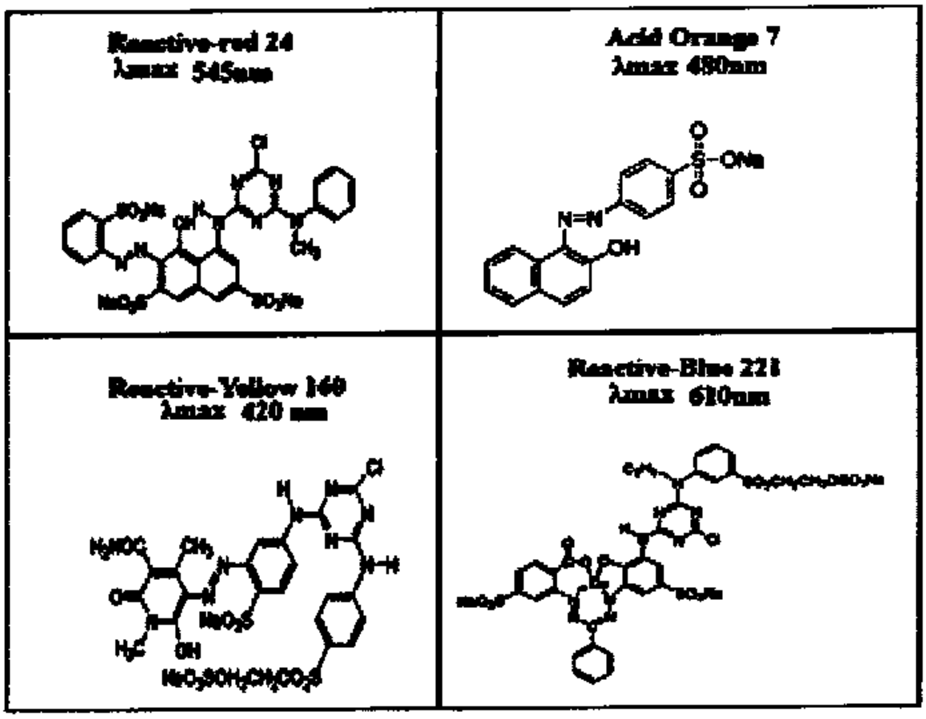


Figure 6.1: Structure of different dyes.

6.3 Results and Discussion:

6.3.1 Characterization:

6.3.1.1 Morphological study:

Electron microscopy was conducted to characterize the surface morphology of the molecular imprinted polymer of reactive dyes. Figure 6.2 shows the SEM images of reactive dye. As can be seen in Figure 6.2 there are a substantial number of pores on the polymer surface. These pores or cavities play a significant role during the adsorption process and the specific recognition sites can match with the reactive dyes in size shape charge and coordination geometry by molecular imprinting.

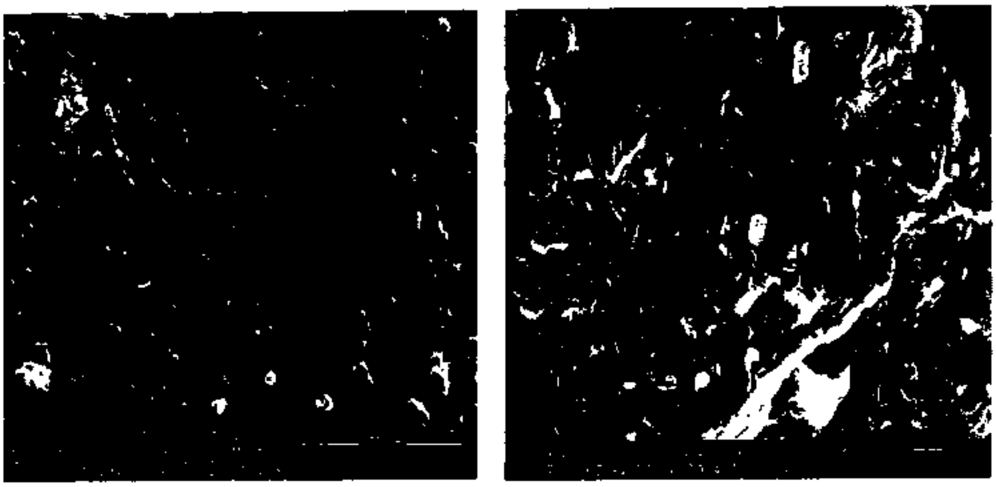


Figure 6.2: Scanning electron micrographs of polymer showing pores

Surface topography was characterized by Atomic force microscopy. AFM image further confirms the formation of imprinted pores as shown in Figure 6.3.

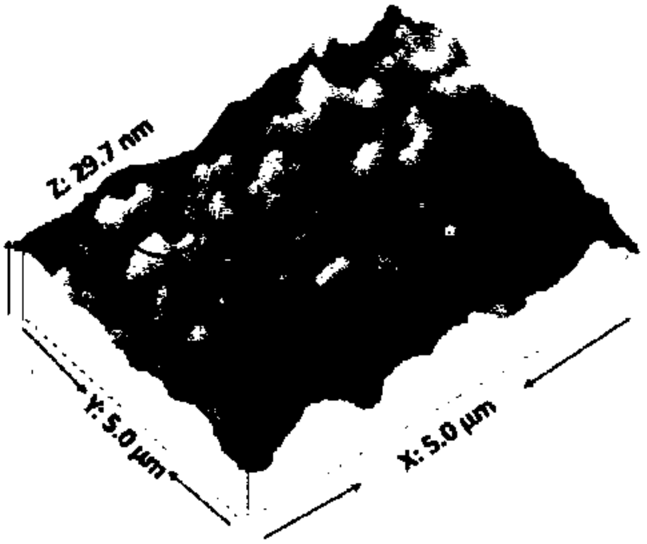


Figure 6.3: AFM image for dye-MIP

Bulk imprinting polymerization strategy was used to produce a polymer. Bulk imprinting approach creates cavities throughout the bulk of a polymer and is suggested for templates molecules with low molecular weight. (P.A. 2014). The template (dyes) were hydrophilic so a straightforward approach was to make a hydrophobic polymer system. An organic polymer system for the water soluble reactive dyes has been optimized. To achieve that purpose methanol was used as a porogenic solvent. Two polymer systems were compared, one having styrene as monomer and the other having NVP as monomer.

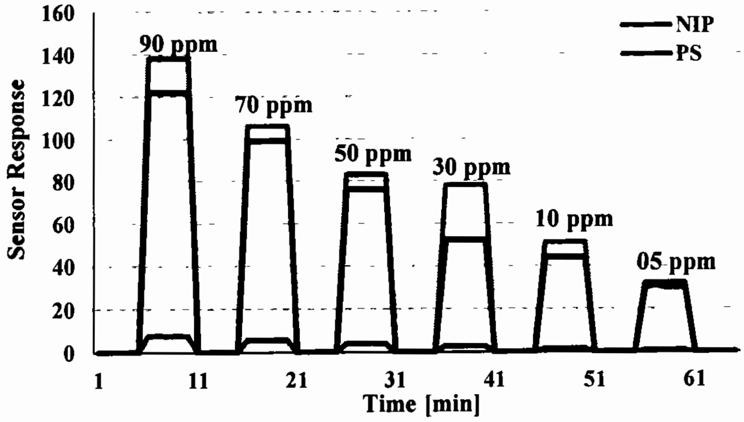


Figure 6.4 Sensitivity response of styrene system and *N*- vinyl pyrrolidone based red dye- imprinted and non-imprinted polymer.

Different standards having concentrations from 1-100 ppm dye/ml were made and preceded for analysis. MIP receptors were subjected to wastewater containing reactive dyes for analysis. When the detection ability of these receptors were analyzed so polymer with styrene as functional monomer gave better response than the NVP polymer system. As evident from Fig 4, there is a linear response of polystyrene and NVP polymer systems towards different analyte concentrations. At about 90 ppm the response of the sensors for styrene system was 138 µS which was reduced to 106 µS, 83 µS, 78 µS, 51 µS, and 32 µS at 70 ppm, 50 ppm, 30 ppm, 10ppm, and 05 ppm respectively. Sensor response for NVP system at 90ppm was 122µS which was reduced to 99 µS, 76 µS, 52 µS, 44 µS, and 30 µS respectively.

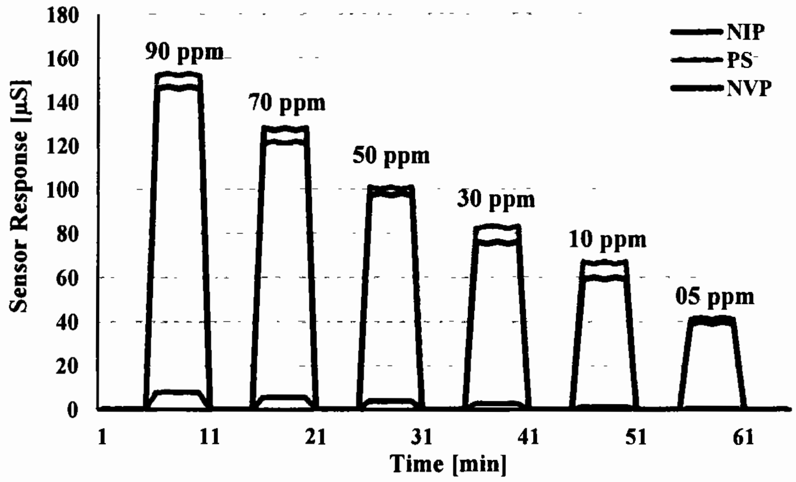


Figure 6.5: Sensitivity response of styrene system and N- vinyl pyrrolidone based blue dyes-imprinted and non-imprinted polymer.

During our next experiment blue dye was used as a template for both the polymer systems one with styrene as the monomer and the other with NVP as a functional monomer respectively. As is evident from Figure 5, the polystyrene system is better than that of the *N*-vinyl pyrrolidone for reactive blue also. At about 90ppm the sensor's response for styrene system was 152 μS which was reduced to 121 μS, 101 μS,82 μS,67 μS, and 41 μS at 70 ppm, 50 ppm, 30 ppm, 10 ppm, and 05 ppm respectively. Sensor response for NVP system at 90ppm was 146μS which was reduced to 128 μS, 97 μS,76 μS,59 μS, and 39 μS respectively (Figure 6.7).

Styrene and *N*-vinyl pyrrolidone polymers are both organic systems. The only possible interactions between the template (dye) and the *N*-vinyl pyrrolidone are because of van der Waal's forces. As vinyl pyrrolidone is hydrophilic it will interact eagerly with water. The pyrrolidone ring on NVP has a dipolar imide group on it which gives polymer its hydrophilic nature, while methylene groups in the ring provide sites for hydrophobic interactions. In the case of polystyrene, the presence of the hydroxyl group allows hydrogen bonding, therefore, leading to greater binding. The monomer styrene having a solubility of less than 1% in water and is hydrophobic, so interacts with the cross-linker and allows it forms the specific imprinted sites, required for the template. The possible predicted interactions for reactive red and polymer systems are given in Fig 6.6.

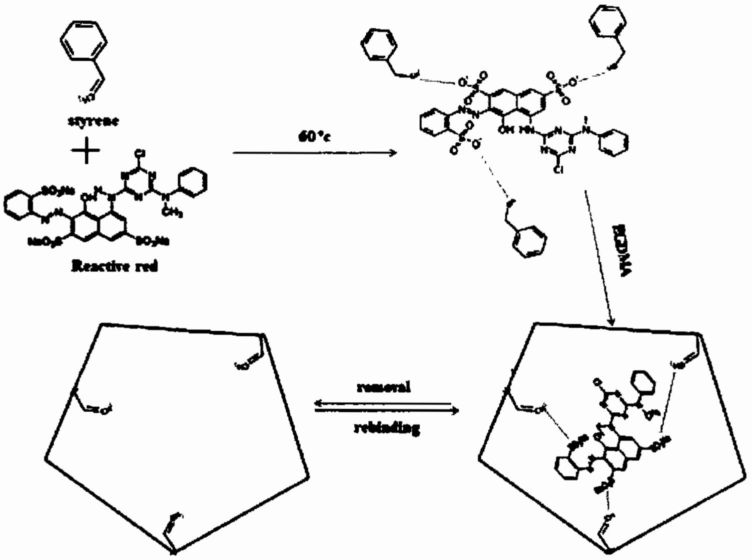


Figure 6.6: Synthesis process of reactive red

The absorption and detection ability of MIP was also check against water soluble acid dyes i.e. acid orange.

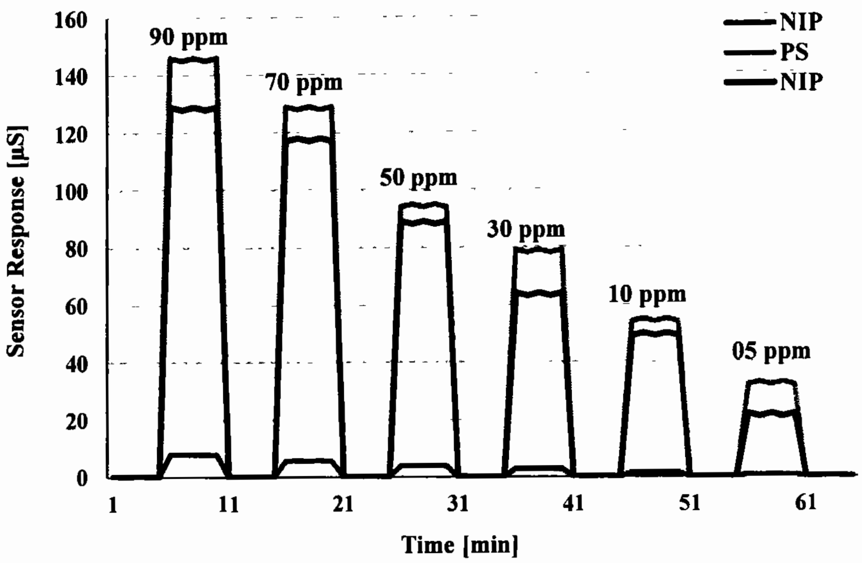


Figure 6.7: Sensitivity response of styrene system and N- vinyl pyrrolidone based orange dye- imprinted and non-imprinted polymer.

Figure 6.7 shows that at about 90 ppm the sensor's response for styrene system was 147 µS which was reduced to 129 µS, 94 µS,79 µS, 55 µS, and 33 µS at 70 ppm, 50 ppm, 30 ppm, 10 ppm, and 05 ppm respectively. Sensor response for NVP system at 90 ppm was 129 µS which was reduced to 117 µS,89 µS,64 µS,49 µS, and 21 µS respectively. Mostly, the selectivity of MIP is less in the water as the polar solvents form hydrogen bonds with the polymer, therefore, affecting the imprinted cavities (Spivak 2004). Reactive dyes contain sodium salt of sulphonic acid which makes them highly water-soluble. Methanol, despite being polar had the advantage of being highly volatile as well. It acted as a medium for the dissolving of the template but did not react with the monomer system as it evaporated quickly. The EGDMA used as crosslinker also ensured the stability and strength of the imprinted receptor.

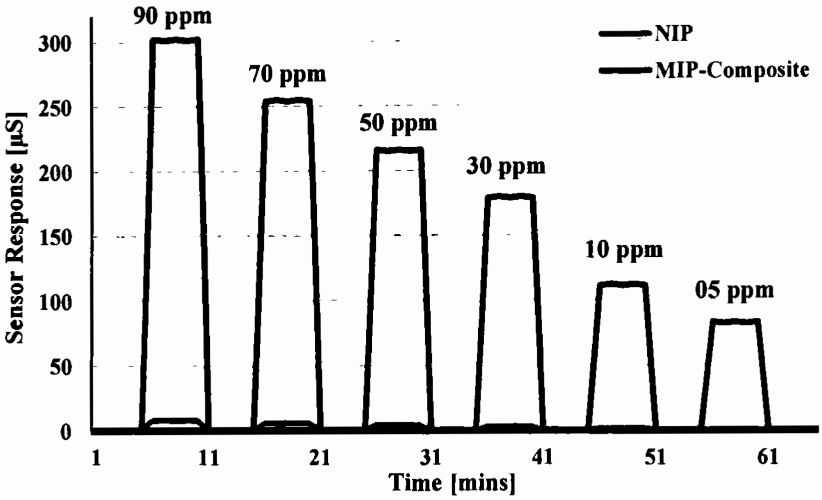


Figure 6.8: Sensitivity response of MIP-composite based red dye- imprinted and non-imprinted polymer.

To synthesis highly sensitive molecular imprinted receptor further optimization was achieved with graphene oxide. Styrene and N-vinyl pyrrolidone were used as functional monomers with a 50:50 ratio. Methanol was used as a porogenic solvent and EGDMA was used as a crosslinker.

As shown in figure 6.8 there is a linear response of MIP-composite towards different concentrations of reactive red dye. At about 90 ppm the sensor's response for MIP-composite was 302 µS which was reduced to 254 µS, 216 µS,178 µS, 111 µS and 82 µS at 70 ppm, 50 ppm, 30 ppm, 10 ppm, and 05 ppm respectively. Graphene is usually used as support material in MIPs because it has an extremely large area and polymerization occurs at the exterior of graphene sheets (GS). The binding sites in the outer layer of the MIPs composite that ensure complete removal of templates, improve the availability to target species, thus reducing the binding time (G. Mayer. 2005).

6.3.2 Selectivity:

To investigate the selectivity of the synthesized MIP against other dyes 50 mg of MIP was immersed in 100 ml of different dyes solution. For example, red dye-MIP was immersed in the blue and orange dye solution and vice versa to access its binding adsorption. MIP of reactive red has a lower affinity for orange and blue dye compared to red dye while MIP of reactive blue absorbs more blue dye than MIPs for red and orange whereas MIP of acid orange had strong interaction for orange dye.

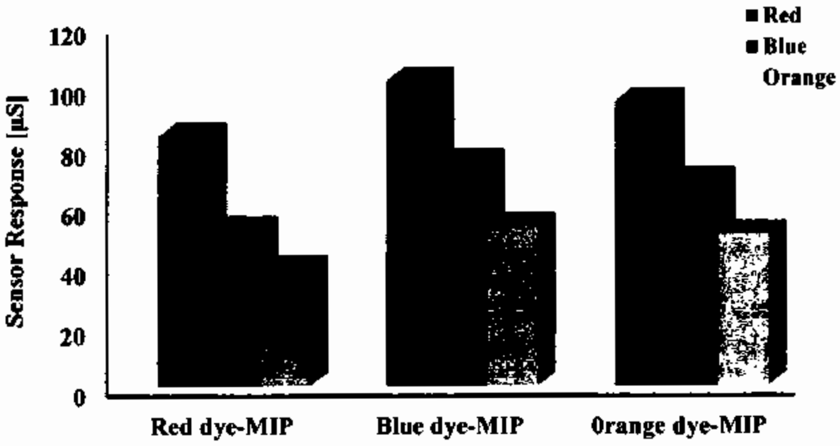
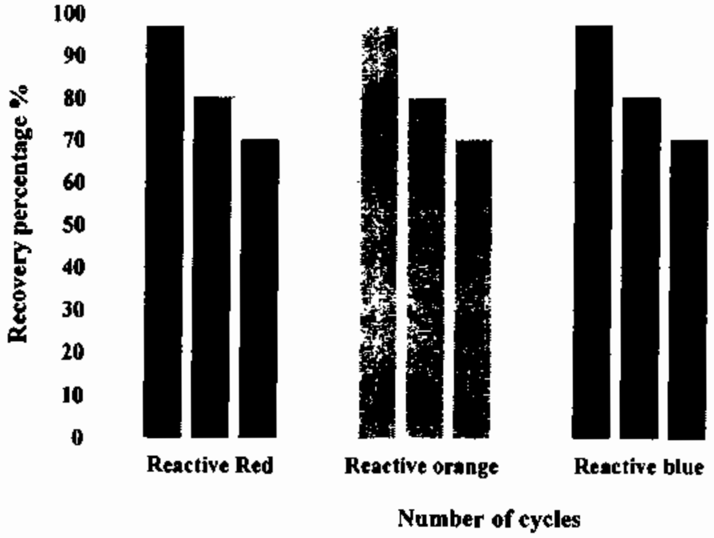


Figure 6.9: Selectivity evaluation of reactive and acid dyes

6.3.3 Reusability:

MIP once used can be regenerated without much loss of the receptor. The ability of a MIP to be reused depends on its robustness or strength. The MIP prepared during our work was used thrice which means for three cycles the MIP has good binding efficiency which began to decrease after that. To solve this problem a greater amount of cross-linker should be used to provide strength to the polymer structure.



6.4 Conclusion:

The experiments showed that the technology of molecularly imprinted polymers can be utilized for wastewater treatment with efficient results for reactive and acid dyes. The MIPs generated were used as the sensing material. For successful polymerization, a suitable cross-linker should be used for the appropriate functional monomer and should be distributed uniformly throughout the polymer. The molar ratio between functional monomer and cross-linker should be optimized otherwise the efficiency or deficiency of cross-linker damage the imprinting especially in case of non-covalent imprinting. During our work, the monomer to cross-linker ratio was kept 30:70. On the other hand, monomer plays an important role in the stability and sensitivity of a respective receptor as it interacts with the template molecules (dyes).

This experiment is but a proof of this MIP to be utilized for sensing of effluents contaminated with reactive red blue and acid orange dyes. Further work is needed for its commercialization and practical application. The suggestions for this purpose are the use of this technology in combination with the other techniques to generate better results.

Overall Conclusions and Future Outlook:

The primary focus of my research was to synthesize an ion-imprinted and molecular imprinted polymer for heavy metal ions and reactive dyes in the industrial wastewater. The aim was to detect these heavy metals and dyes in minute concentration in aqueous media. Several polymeric receptors were generated during my experimental work based on bulk imprinting to detect heavy metals such as Hg, Cr, As, Pd, and reactive dyes.

The first polymer was synthesized for mercuric iodide. The effect of three different receptors on the efficacy of the sensor system was seen. One polymer system was generated using N vinyl pyrrolidone as a monomer, in the second polymer system both styrene and vinyl pyrrolidone were used as monomers. The third IIP/composite was generated. The order of MIPs sensitivity and selectivity observed was graphene composite >co-polymer> *N*-vinyl pyrrolidone. By comparing the response of composite with simple IIP the former shows high sensitivity and polymer stability with successive usage.

During our second experiment chromium ions were used as a template. Again NVP and styrene were used as functional monomers. Styrene system being hydrophobic does not readily dissolve in water which in turn proves itself to be ideal for wastewater treatment. Different polymers were evaluated by changing the ration of monomer to cross-linker and the polymer with 30:70 ratio of monomer: cross-linker showed good results.

The third experiment was to synthesize arsenite based molecular imprinting polymer with methanol used as a solvent. Five different polymers with different functional monomers were created. Polymer P1 contains MAA as a functional monomer, P2 comprises of styrene, P3 involved NVP, and P4 was a composite that contains co-polymer of sty and NVP with 50:50 ratio. Out of four monomers used the co-polymer system with styrene and NVP used as monomers showed better sensitivity and selectivity. GO-based composite gave the highest sensitivity value because of its good electrical conductivity. The sensitivity sequence of respective polymers is as follows P4≥ P2 ≥P1 ≥P3.

The fourth experiment was to synthesized receptors for lead ion. During this experiment different porogenic solvents were used to assess its effect on the polymer system. Three types of porogenic solvents were used that is THF, DMSO, and MeOH. Methanol has high polarity and is hydrophilic. THF with medium polarity produced a much stable polymer.

The fifth experiment was to synthesized molecular imprinted polymer for reactive dyes. Red dye, blue dye yellow, and orange dyes were used as templates respectively. Again co-polymer gave better polymer. Graphene-based MIP for dyes was also generated with high sensitivity and selectivity.

Characterization was done through SEM and AFM. Therefore morphological study showed imprints of different ions and molecules on respective polymer systems. The receptors generated were used for several times to assess its strength. The generated receptors could be employed on an industrial scale for the assessment of wastewater treatment and drinking water. The current urge in research is for providing a clean and non-toxic environment. Ion imprinted polymers can play an effective role in achieving the goal.

Based on the present preparation technologies, for high scale production of IIPs selectivity is a factor that is often sacrificed. For batch production, cost effectiveness is always the primary goal, but selectivity is a secondary requirement. The remarkable difference of conditions between industry and a lab definitely will decrease the selectivity of IIPs. Hence, more work should be done to apply suitable existing preparation technologies to produce large amounts of IIPs with atleast moderate selectivity.

Therefore, more related accomplishments and explorations should be uninterruptedly made to introduce various technologies and strategies into IIPs preparation in order to rapidly obtain high amounts of IIPs for industrialization applications.

CHAPTER 7:
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Research Article

Ion-Imprinted Polymer-Based Receptors for Sensitive and Selective Detection of Mercury Ions in Aqueous Environment

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Interdigital electrodes (IDE) coated with ion-imprinted polymers (IIP) as recognition materials have been tested for screening and ion quantification. For screening of receptors, three polymer systems based on styrene (Sty), N-vinylpyrrolidone (NVP), and Sty-co-NVP were examined to identify an efficient recognition system for mercury ions in an aqueous environment. Results showed that all these polymeric systems can detect analyte even in very low concentration, that is, 10 ppm. Ion-imprinted polystyrene system proved to be an ideal receptor for detecting mercury ions in solution with a detection limit of 2 ppm. The sensitivity of ion-imprinted copolymeric system was further enhanced by making its composite with graphene oxide, and estimated detection limit of composite system was around 1 ppm. Ion- imprinted Sty-co-NVP graphene composite-based sensor system exhibits 2 to 5 times higher sensor response towards templated analyte in comparison to other polymer-based sensor systems. Moreover, the composite-based sensor shows very low or negligible response to competing metal ions with similar or different oxidation states such as Zn, Mg, Na, and As metal ions.

1. Introduction

Heavy metals are widely distributed pollutants in aqueous environment, and these are highly toxic to humans and animals even at trace levels [1]. Mercury (II), among other heavy metals, becomes more toxic in aqueous solution when it transforms [2] biologically or chemically to its organic form as methyl mercury, due to its bioaccumulation in living bodies [3, 4]. Mercury is one of the most hazardous elements for human health because of its relative solubility in water and its tendency to accumulate in human body tissues [5] and causes weakness, neurological damage, and chromosomal mutation [6, 7]. Therefore, its detection and quantification in water are of substantial importance. Numerous analytical methods and techniques, such as cold vapor atomic absorption spectrometry, atomic fluorescence spectrometry, and inductively coupled plasma mass spectrometry, have been intensively developed for its quantification [8–10]. These techniques are expensive and require trained personnel, and certain complicated processes are involved prior to final analysis. Therefore, development of cost-effective, simple, and robust tools for the detection of heavy metals has attracted considerable interest of analytical chemists which leads to the fabrication of sensors. Limited numbers of reports are available in this field [11–14], and these sensors bear complications and limitations regarding sensitivity, selectivity, and detection of ultra-low concentration of mercury ions (e.g., ppt level) in environmental and food samples [15]. Molecular imprinting offers a straight forward and targeted method for producing such polymeric artificial receptors which contains analyte-specific molecular recognition properties [16–18]. The general principle of molecular imprinting is based on procedures (as shown in Figure 1) where functional monomer and crosslinkers are polymerized in the presence of a target analyte (the imprinting molecule), which acts as a template. The polymerization process can be performed by reversible covalent and noncovalent interactions between monomers and imprinting molecules.

The imprinting of smaller ionic molecules like metal ions [19] is a tedious task especially from selectivity point of view. In this manuscript, we designed ion-imprinted polymers for Hg determination in water with remarkable selectivity by using interdigital electrodes (IDEs) as transducer. Furthermore, the sensitivity and selectivity parameters were optimized by using a MIPs/functionalized graphene composite as receptors.

2. Materials and Methods

2.1. Chemicals and Reagents. Mercuric iodide (HgI_2), styrene (Sty), N-vinyl pyrrolidone (NVP), ethylene glycol dimethacrylate (EGDMA), azobisisobutyronitrile (AIBN), graphene oxide (GO), sodium chloride (NaCl), magnesium chloride ($MgCl_2$), Arsenic chloride ($AsCl_3$), and zinc chloride ($ZnCl_2$) were purchased from Sigma-Aldrich and Merck.

2.2. Synthesis of Ion-Imprinted Polymers and Composites

2.2.1. Ion-Imprinted Polystyrene. Hg(II)-imprinted polystyrene was synthesized by using styrene as monomer, EGDMA as crosslinker, and AIBN as initiator while using HgI_2 as template. At first, Hg^{2+} solution was prepared by dissolving 0.002 g of HgI_2 in 200 μL of THF. Then, 30 μl of styrene, 70 mg of EGDMA, and 5 mg of AIBN were added in HgI_2 solution. The resultant mixture was stirred vigorously and polymerized by heating at 60°C until its gelation point.

2.2.2. Ion-Imprinted Poly(Vinylpyrrolidone). Hg(II)-imprinted poly(vinylpyrrolidone) was synthesized by following the above-mentioned procedure using NVP as monomer instead of styrene while keeping all other parameters constant.

2.2.3. Ion-Imprinted Poly(Styrene-Co-Vinyl Pyrrolidone). Hg(II)-imprinted poly(styrene-co-vinyl pyrrolidone) was synthesized by following the abovementioned procedure using both styrene and NVP as monomers in (2 : 1) while keeping all other parameters constant.

2.2.4. Ion-Imprinted Copolymer-Graphene Oxide Composite. Hg(II)-imprinted copolymer-graphene oxide composite was prepared by adding 30 μL of styrene, 10 μL of NVP, 70 mg of EGDMA, and 5 mg of AIBN and graphene oxide in 200 μL solution of HgI_2 (0.002 g). The resultant mixture was stirred vigorously and polymerized by heating at 60°C until its gelation point.

2.2.5. Nonimprinted Polymer. Nonimprinted polymer (NIP) was prepared by following the above-mentioned procedure but without adding any template (HgI_2).

2.2.6. Immobilization of Receptors onto Transducer and Measurements. Thin films (80–100 nm thickness) of receptors were prepared onto the interdigital electrodes (IDEs) by spin coating 10 μL of solution at 2500 rpm. Afterward, thin films were dried overnight at 80°C and then washed with distilled water to remove the template. LCR meter (IET 7600 Plus precision LCR meter) was used to measure various concentrations of analyte and competing ions at 20 Hz and 1000 Hz. The synthetic procedure and measuring setup are shown in Figure 2.

3. Results and Discussions

Ion-imprinted polymer (IIP), coated on transducer (IDE) surface, contains recognition cavities to selectively bind mercuric ions. The incorporation of templated ions, from sample solution, into these cavities results in changing electrical properties of IIP-coated layer. Conductivity of IIP layer increases with incorporation of metal ions into recognition sites. Thus, conductance of IIP layer is directly proportional to metal ion concentration incorporated into the cavities. NVP-based Hg^{2+}-imprinted receptor system was synthesized by using EGDMA as crosslinker. Polymer system interacts with Hg^{2+} ions via electron-enriched entities present in their structure. Sensitivity of polymer systems was analysed through exposing them to various concentrations of templated ion ranging from 90–10 ppm as shown in Figure 3. The conductance of IIP layer at 90 ppm was 69 μS which reduces to 56, 50, 46, and 35 μS at 70, 50, 30, and 10 ppm, respectively. The sensor system shows a quite linear response: the conductivity decreases with decreasing concentration of analyte. Nonimprinted polymer expressed almost very negligible response while exposing to various analyte concentrations. The estimated value of LOD, measured from calibration curve, is around 4 ppm.

The sensitivity of styrene-based IIP was investigated while exposing to various concentrations of templated ions. The metal ion undergoes coordination or chelation with electron-rich entities present in polymer material. The sensor showed a response of 103 μS at 90 ppm of analyte concentration. By lowering analyte concentration from 90 to 10 ppm, the sensor response decreased to 54 Hz. At 70, 50, and 30 ppm of analyte concentration, the conductivity of material shifts from 92, 78, to 67 μS, respectively. The response of NIP is almost negligible as compared to ion-imprinted-based sensor system as shown in Figure 4. The lowest detection limit of styrene-based polymer was around 2 ppm, estimated from calibration curve.

Styrene is a hydrophobic monomer with no special functionality. The role it may play in polymer formation is to form an imprint of the Hg^{2+} ion where it could specifically fix and coordinate with the crosslinking agent, that is, EGDMA. It thus induces geometric fit cavities in IIP, which enable polymer matrix for selective incorporation of templated ions. It is notable from the above results that

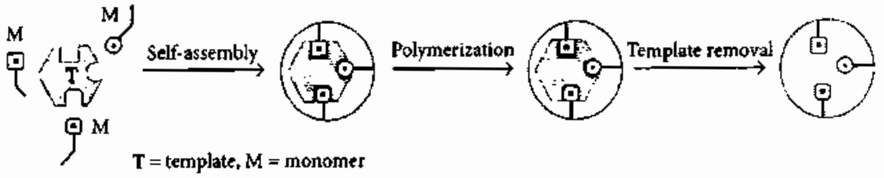

Figure 1: Molecular imprinting strategy.

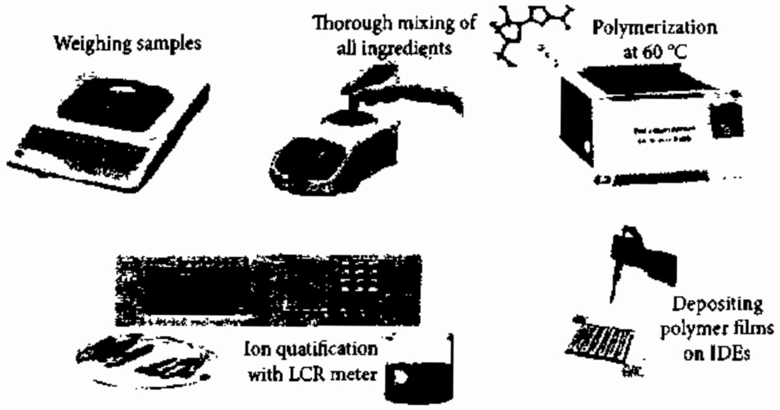

Figure 2: Receptor synthesis and sensor setup.

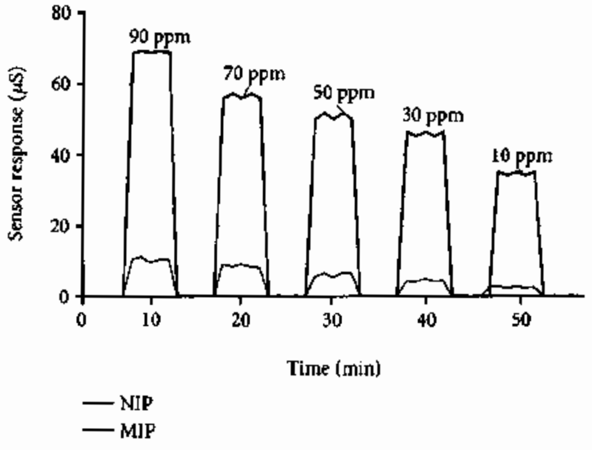

Figure 3: Sensitivity response of NVP-based Hg^{2+}-imprinted and nonimprinted polymers towards different concentrations of mercury solutions.

polystyrene-based polymer produces higher sensor effect than NVP system due to its aromaticity and functionality.

Ion-imprinted copolymer system based on Sty and NVP was also synthesized (radical copolymerization) to analyse its interaction towards templated analyte. The amide group of NVP has a high binding affinity for several molecules due to its polar nature and has been copolymerized with a variety of monomers [20–24]. N-vinylpyrrolidone can copolymerize with styrene and yields maximum sensor signal [25].

Sensitivity of copolymer system was investigated through exposing it to different analyte concentrations as shown in Figure 5. The sensor response based on copolymer system towards 90 ppm analyte concentration was 96 µS which was decreased to 81, 70, 56, and 38 µS at 70, 50, 30, and 10 ppm, respectively. The graph depicts a linear- and concentration-dependent sensor response. The estimated lowest detection limit of ion-imprinted copolymer sensor system is around 2 ppm.

To achieve higher sensitivity and excellent performances, the structure of imprinted material was optimized in such a way that recognition sites should be situated at the surface or in the proximity of material surface. Graphene, with unique mechanical properties and extremely large area, is an excellent candidate as a supporting material for preparing imprinted materials. The prepared IIPs would be in possession of large surface area in case the polymerization occurs at the surface of graphene sheets (GS). The binding sites in the outer layer of IIP composite provide complete removal of templates, improve accessibility to target species, and reduce its binding time. Graphene oxide is a typical pseudo two-dimensional oxygen containing solid in bulk form, which possesses functional groups including hydroxyl, epoxide, and carboxyl [26–28]. Graphene oxide, covalently and noncovalently, is known to attach both small molecules and polymers to its reactive oxygen functionalities [29]. The sensitivity of IIP-graphene composite system substantially enhanced towards its templated analyte attributed to the excellent electric and mechanical properties of graphene oxide as shown in Figure 6. At 90 ppm of analyte concentration, there is a drastic increase in the conductance which is 295 µS, in comparison to Sty, NVP, or Sty-co-NVP polymer-based sensor systems. The graph shows a linear response of 272, 246, 201, and 167 µS while exposing to 70, 50, 30, and 10 ppm analyte concentration, respectively.

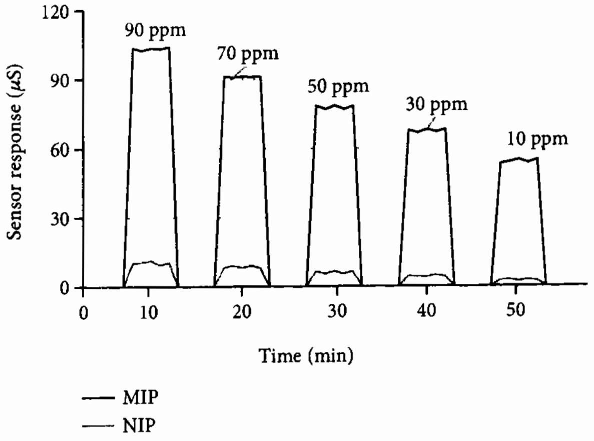

Figure 4: Sensitivity response of styrene-based Hg^{2+}-imprinted and nonimprinted sensors towards different concentrations of mercury solutions.

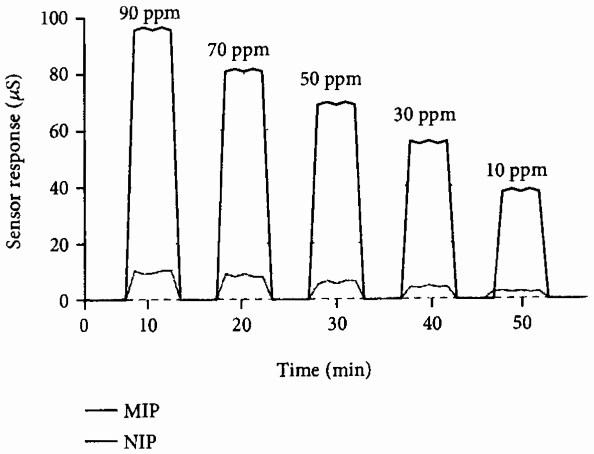

Figure 5: Sensitivity response of Hg^{2+}-imprinted and nonimprinted Sty-NVP copolymer systems.

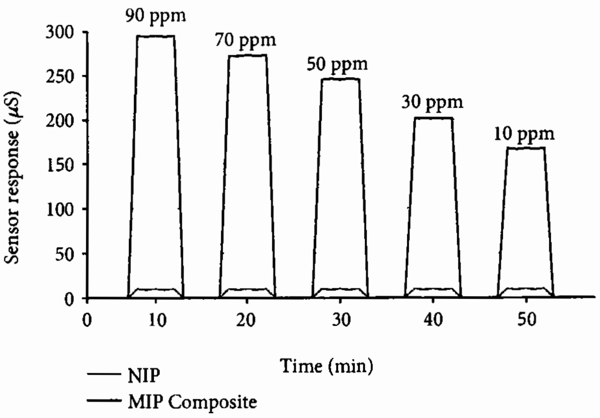

Figure 6: Sensitivity response of Hg^{2+}-imprinted Sty-co-NVP graphene composite and nonimprinted composite sensor while exposing to various concentrations of analyte ranging from 10–90 ppm.

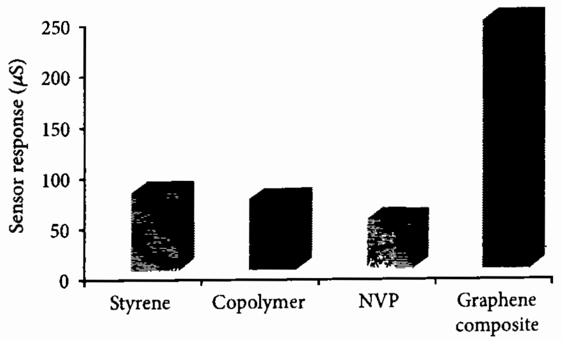

Figure 7: Comparison of four different Hg^{2+}-imprinted polymer-based receptors at concentrations 50 ppm of templated ion solution.

The ratio of monomer to crosslinker was 30 : 70. High crosslinker ratio gives three dimensional spaces that are selective for Hg^{2+} ions whereas lower crosslinker ratio in a polymer composition will cause imprinting sites to collapse. The composite-based sensor system is highly sensitive to templated analyte and is the only material responding to concentrations down to 0.9 ppm (estimated by calibration curve) in comparison to simple ion-imprinted polymer-based sensor, as shown in Figure 6. One of the most important reactions of graphene oxide is its reduction. Electron-rich hydroxyl group and oxygen functionalities on graphene oxide surface interact with electron-deficient mercuric ions present in sample solution.

All ion-imprinted polymeric- and composite-based sensor system exhibits a linear response towards templated analyte, which enhances with an increase in analyte concentrations. All polymer-based systems show sensor response in a similar range; however, an almost 3-fold increase in sensitivity was achieved by making template-specific recognition sites on graphene sheets as shown in Figure 7. This increase in conductivity of graphene sheets is due to its high surface area in comparison to all other polymer thin films, and secondly, it contributes to sensitivity because of its oxygen and mercuric ion interaction. N-vinylpyrrolidone (NVP) shows good biocompatibility due to their hydrophilic nature and low cytotoxicity [30–37]. Electron-deficient Hg^{2+} ions interact with electron-rich moieties of NVP/ EGDMA. Aqueous environment affects the performance of NVP-based receptors because of their hydrophilic nature. The quality of NVP-based receptors was further improved by copolymerizing it with hydrophobic monomer (styrene).

Sensitivity analysis alone is not sufficient to evaluate a sensor system. Thus, it is very necessary to investigate sensor selectivity by exposing it to structurally related analogues. After removing the template molecules by extraction, a molecularly imprinted polymer was analysed against other metal ions typically, such as Zn^{2+}, Mg^{2+}, As^{3+}, and Na^{+}. These ions were chosen as competitors to evaluate selectivity performance for Hg^{2+}-imprinted polymeric and composite systems.

It is evident from Figure 8 that Hg^{2+}-imprinted polymers and composites comprise binding sites which are highly

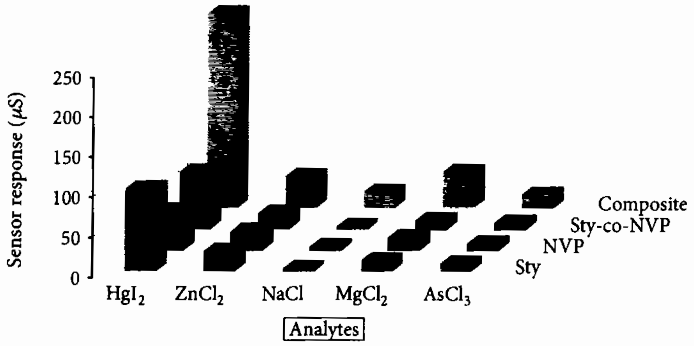

FIGURE 8: Selectivity of Hg^{2+}-imprinted polymer-based sensor systems against other competing metal ions at 50 ppm concentrations.

selective to templated ion. The monomers and crosslinkers arrange themselves around template ions during polymerization, in such an efficient way, to produce highly selective recognition sites which can accommodate only specific analyte. All these ion-imprinted polymer-based receptors show concentration-dependent linear response which reduces with decrease in analyte concentration. The Hg^{2+}-imprinted Sty-co-NVP-graphene composite-based sensor shows 2-5-folds higher sensor response to Hg^{2+} in comparison to other polymer-based systems. Moreover, it remains very less responsive to other metal ions with similar oxidation state as Hg^{2+}, and it further reduces towards metal ions having lower or higher oxidation states.

The fabricated sensor proved to be suitable for heavy metal ion detection in aqueous environment even in the presence of other competing metal ions.

4. Conclusion

In this work, Hg(II)-imprinted polymeric- and composite-based sensor systems were fabricated by following molecular imprinting approach. Four different receptors were synthesized based on different polymeric systems such as (i) polystyrene, (ii) poly(vinylpyrrolidone), (iii) Sty-co-NVP polymer, and IIP composite of Sty-co-NVP polymer and graphene sheets. Efficiency of these sensor systems was investigated while exposing to their templated analytes. The sensitivity and selectivity of ion-imprinted polymers and composite were in the following order: composite > Sty > copolymers > NVP. The results indicate that a very sensitive, selective, low-cost, and user-friendly sensor system can be fabricated for any metal by combining molecular imprinting approach and interdigital transducers.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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