FABRICATION OF MIPs BASED SENSOR FOR THE REAL-TIME AND ONLINE MONITORING OF FLUORIDES IN WATER

MPhil Thesis

By

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17/FBAS-MSNS/F-15

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(2017)

Fluoridation

Water Purification

Osteosarcoma

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بسم الله الرحمن الرحيم

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Dated: 26 oct 2017

FINAL APPROVAL

It is certified that the contents and form of the thesis entitled **"Fabrication of MIPs Based Sensor for the Real-time and Online Monitoring of Fluorides in Water"** submitted by Nausheen Ameer have been found satisfactory for the requirement of the degree of MS/M.Phil in chemistry.

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It is certified that I, **Nausheen Ameer,** registration number **17FBAS/MSNS/F15** have completed my Master of Philosophy in Chemistry from Sulaiman Bin Abdullah Aba Al-khail Centre for Interdisciplinary Research in Basic Sciences (SA-CIRBS), International Islamic University, Islamabad. The title of my thesis is **"Fabrication of MIPs based sensor for the real-time and online monitoring of fluorides in water"**. My thesis has been checked on Turnitin for similarity index and found that it lies within the limit provided by HEC.

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Declaration

The research work presented in this thesis was carried by me in the Centre for Interdisciplinary Research in Basic sciences laboratory, International Islamic University Islamabad. If anything found similar or copied, I will be responsible for that.

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A thesis submitted to Department of Sulaiman bin Abdullah Aba Al Khail Centre for Interdisciplinary Research in Basic Science (SA-CIRBS) Faculty of Applied and Basic Sciences, International Islamic University Islamabad, as a partial fulfillment of requirement for the award of the degree of MPhil.

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List of abbreviations

UV	Ultraviolet
nm	Nano meter
MIPs	Molecular Imprinting polymer
NIP	Non-imprinting polymer
AIBN	Azo-N-N‘-bisisobutyronitrile
EGDMA	Ethylene glycol dimethacrylate
DVB	Divinylbenzene
IDEs	Inter digital electrodes
µL	Micro liter
nF	Micro liter
abs	Absorption
WHO	World Health Organization
Hz	Hertz
Rev/min	Revolutions per minute
Ppb	Parts per billion
Ppm	Parts per million
IR	Infrared
THF	Tetrahydrofuran

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Abstract

Real-time and online monitoring of fluoride is of significant importance because of its adverse effects on human health. Numerous conventional techniques are used for the determination and detection of fluoride in water samples. However, these techniques have some limitations so it is of substantial interest to develop precise, robust and accurate devices which are capable of real-time and online monitoring of fluoride in water. This study focuses on the fabrication of molecular imprinted based electrochemical sensors for the sensing and quantification of fluorides in water. Three different polymer systems (styrene, acrylate and vinylpyrrolidone) were used for the generation of molecular imprinted polymers (MIPs) to assess the optimum imprinting of respective analytes. In case of sodium fluorides, all these three systems showed linear and reversible responses but polystyrene system showed better sensing responses as compare to acrylate and vinylpyrrolidone systems with a limit of detection of 220 ppb of fluorides in water. The sensor sensitivity behavior was investigated towards various concentrations of analyte from 0 ppm to 50 ppm. The fabricated sensor bears selectivity towards the imprinted template in the presence of other competing agents. In case of calcium fluoride, we used two different systems (styrene and vinylpyrrolidone) for the generation of MIPs to assess and optimize the analyte-MIPs binding. The imprinted vinylpyrrolidone system showed better sensitivity as compared to styrene system with limit of detection of 333 ppb. The sensor is highly selective towards the respective template which indicates that sensor can be employed for the highly specific and selective detection of target analyte in the presence of other competing ions with almost similar oxidation state, ionic radii and atomic weight. Furthermore, molecular imprinted polymers were used to generate sieves/filters for the selective removal and recovery of fluorides from water. The fluorides removal efficiency of newly fabricated sieves was asses and was cross validated by UV/Visible spectroscopic analysis. The molecular imprinted polymers coated sieves can be used as economically efficient and environment friendly tool for the selective removal and recovery of fluorides from water.

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Chapter 1

Introduction

Chapter 1

1. Introduction

1.1. Fluorine

Fluorine is the most electronegative and lightest element of halogens group. Beneath the earth's crust it is abundant that is 625 mg/kg, mobile where the temperature is high (Edmunds & Smedley, 2013) and is found in the form of fluorides in drinking water (Arif *et al.*, 2014). Fluorine gas forms fluoride by reacting many organic and inorganic substances, HF by reacting with water and forms fluorides when react with metals. Fluorine occurs in elemental state very rarely because it is highly reactive (Prystupa, 2011). Either it reacts and combines with different chemical minerals like cryolite, fluorapatite or fluorspar or it occurs in ionic form naturally. Primarily fluorine gas is used in making of different chemicals. The most important of those chemicals is UF6 (Uranium hexafluoride) which is used in the separation of uranium isotopes, which is further use in making of nuclear reactors and nuclear weapons (Weast *et al.*, 1989). Fluorides are salts/binary compounds of other elements with F^-. Calcium fluoride and sodium fluoride which are white solids are examples of fluorides. To prevent dental cavities sodium fluoride (NaF) is commonly added water supplies which is used for drinking and different dental products including mouth rinses and toothpaste. The compounds used for fluoridation are sodium fluorosilicate and fluorosilicic acid are other examples of fluorides compounds. In common mineral, i.e. fluorspar and fluorite consist of calcium fluoride.

For human health, there is a narrow range of fluoride concentration associated with harmful and useful impacts which are proved from credible evidences from scientific literature. The fluoride concentration which is below 1 ppm is useful to prevent tooth decay however, it causes incurable disease i.e. fluorosis, when its concentration exceeds 1.5 ppm. The most harmful effect of fluoride is fluorosis which plays chaos in more than 25 countries around the globe and continents. About two hundred million people around the world are at the risk of suffering from diseases of fluorosis which increases agony and global alarm. In drinking water fluoride does not change its odor, color and taste so usually fluoride can be only detected by testing it in laboratories. Patients suffering from skeletal and dental fluorosis continue to suffer without being diagnosed of their diseases, so for the treatment of their diseases they undergo with different kinds of treatment. Even patients suffering from fluorosis undergo surgical interventions, with no sign of relief or healing to the disability or pain (Susheela *et al.*, 1999) The common sight of endemic fluoride areas are paralyzing bone diseases, crooked legs and hands, stooped backs, stained teeth, blindness and other handicaps.

More than ten thousand people have already crippled by fluorosis and left thousands of others in constant misery (Ncube & Schutte, 2005). Fluorosis has crippled their hopes and limbs (Shivashankara *et al.*, 2000). United Nations promise of providing safe water in the areas of endemic fluorosis from the year 2005-2016 remained unfulfilled and it is worth mentioning because we are living in the era of 'water for life'. So, the study on the impacts of fluoride concentration when it's limit exceeds from recommended level in water on human beings are significant all over the world.

1.2. Physical structure of Fluorine

Fluorine is pale yellow green gas with in its elemental form. Fluorine has sharp odor. Atomic number of fluorine is 9 with a density of 1.8×10-3 g.cm-3 at 20°C. Melting and boiling point of fluorine is -219.6 °C and -188 °C respectively (Prystupa, 2011).

1.3. Chemical profile

Fluorine is most electronegative of all halogens gases. The fluorine gas reacts and combines with almost every element except that of noble gases forming strong electronegative bonds (Cotton & Wilkinson, 1988). As in water it forms hydrogen fluoride and sodium fluoride but when it dissociates it forms negative fluoride ion.

1.4. Availability and sources of fluoride

1.4.1. Natural sources

Fluorides enter the human body via different sources like air, water, food and medicaments. In soil the major source of fluorides is rocks. CaF_2 (fluorite) which is only one mineral of natural fluorine, presents as a mineral in granite rocks which contain 20-3600 ppm fluoride concentration. Fluorine bearing minerals which consists of amphiboles, apatite, and fluorite are geogenic sources of fluoride which are found in various sediments and rocks. Beyond that other atmospheric air and precipitation could be other sources of fluorides which consist of 0.0001-0.004 mg/L of fluorides. Natural coal additionally incorporates round 295 ppm of fluorine (Gupta *et al.*, 2005).

1.4.2. Anthropogenic

The primary sources of fluoride pollution are industries, specifically production of phosphate ore and mining, coal burning and aluminum manufacture. Without proper control on emission in those industrial areas, pollution of environment can be a major problem. Fluoride pollution is industrial hazard; however, adding fluoride to water is not a potential source of fluoride pollution (Reeves, 2000).

The phosphatic fertilizers which are used without any proper techniques is the main anthropogenic sources of contamination of fluoride in ground water. In addition, aluminium smelting stations, coal power stations, fertilizers and brick industries are also responsible for the fluoride pollution in the environment. The other major source of increased fluoride level in underground water is irrigation of fluoride enriched water (Pettenati *et al.*, 2013). Areas near the brick kiln industries have high fluoride concentration in groundwater. There are several hundred ppm of fluoride concentration in the clay that is used in the manufacture of bricks. Underground waters contain of high concentration of fluoride which exceeds from the recommended level beyond 3 mg/L (Thole, 2013).

1.4.3. Fluoride in food and beverages

As fluoride is ubiquitous in the environment almost all food stuffs contain trace amounts of fluoride. Fluoride enters human body by using spinach, tea, carrots and cabbage due to which its concentration increases in human body (Susheela *et al.*, 2005).

1.4.4. Other Sources

Huge number of industrial effluents which contain fluoride ions are produced from high technologies industries and factories which manufacture integrated circuit and semiconductors (Patra *et al.*, 2000). Fluoride enriched water used for irrigation is also one of the major source of fluoride (Pettenati *et al.*, 2013).

1.5. Fluoride in Human

Fluoride, as in the form of hydrogen fluoride (HF) is absorbed through the skins in animals and human. The 99% of fluoride which is deposited in teeth and bones are distributed via blood soon after its absorption (Hamilton, 1990) . Fluoride as hydrogen fluoride enter the intracellular fluid of some tissues as it does not accumulate in most soft tissues. Fluoride concentration is indicated and determined in the soft tissues of the blood. Fluoride has higher plasma content as fluoride is concentrated at elevated levels in renal tubes. Due to its exposure to comparatively high concentrations of fluoride, kidneys may be possible targets of endemic fluoride toxicity. Kidney secretion of fluorine is generally 35-70% of total consumption for adults. The biomarkers of chronic and acute exposure to fluoride are plasma, saliva and urine. The fluoride concentration reflects its intake just in three to six months before in nails (Godfrey *et al.*, 2010). For adults, living in the areas where fluoride concentration is 1.0 ppm in water the average consumption of fluoride is 0.02 to 0048 mg/kg/day and the areas which consists of below 0.3 ppm fluoride concentration in water the average consumption of fluoride is 0.014 mg/kg/day has been reported. For children's the recommended intake of fluoride ranges from 0.03-0.06 mg/kg/day in fluorinated water and 0.01-0.04 mg/kg/day in areas where there is no fluorinated water (FLUORIDES, 2003).

1.6. Fluoride geochemistry

Interaction of surface water and groundwater with stones creates a common health problem and acceptability of fluoride problems worldwide. Fluoride effects on human health and its geochemical distribution's effects are remarkably notices in tropics. In tropics, most of the people live in close contact with environment and they get their food and water from that area. The example to understand geochemistry of fluoride and its presence in groundwater which is related to cause dental fluorosis is that, most of the contribution of fluoride to human body is via drinking water.

In groundwater concentrations of fluoride generally depends on the fluoride concentration which also consists of minerals in rocks and depends largely on the dissolution and decompositions activities via water-rock interactions. For the dissolution of groundwater fluoride, alkaline environment with a pH range of 7.6-8.6 which consist of higher concentration of bicarbonate is more suitable (Saxena & Ahmed, 2001). So, leaching of minerals which consists of fluoride and the weathering of minerals in rocks produce F^- ions in solution (Saxena & Ahmed, 2003). In natural water fluoride concentration can be determined basically by fluorite. Carbonates like bicarbonate chloride and Na_2CO_3 has high fluoride concentration in groundwater (Armfield & Spencer, 2004).

1.7. Global scenario

In the mid-latitudes, around the globe the disease of fluorosis is endemic (Chen *et al.*, 1996) (Dissanayake & Chandrajith, 1999) while one of its chronic form that is skeletal fluorosis (Reardon & Wang, 2000) have been reported in many parts of the world (Hillier *et al.*, 2000). According to the latest report fluorosis which is a community health issues is endemic in more than 25 states around the world. Higher concentration of fluoride in water resulted more than 26 million and one million people which are suffering from skeletal and dental fluorosis.

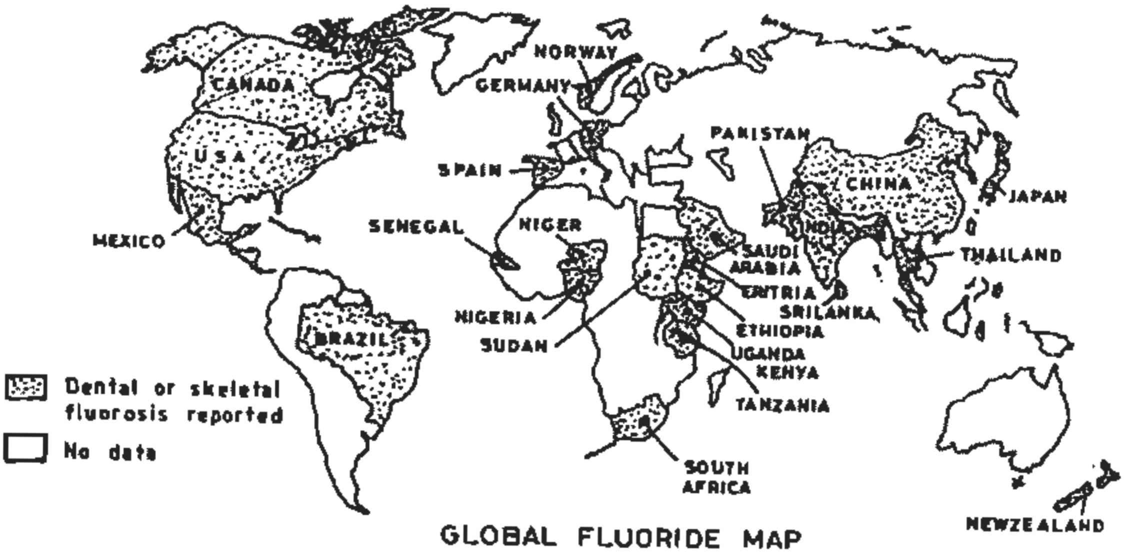

Figure 1.1. Map of fluoride concentrations around the world (Hillier *et al.*, 2000)

1.8. The Pakistan Scenario

Different studies have found that low and high fluoride concentrations in drinking water in Pakistan is apparently not a common problem. 84% of water samples for example, taken from different regions of the country have a fluorine concentration which is below than the recommended level for human health which is of 0.7 mg / l (Ayaz *et al.*, 2008). In addition, many studies have indicated fluoridation of water to prevent the harmful effects of fluoride deficiency in water (Rafique *et al.*, 2009). However, higher fluoride concentrations are also reported in different parts of country. However, throughout Pakistan the situations are not same. In southeast and northwest high concentrations of fluoride has been reported. The fluoride concentration in spring water was 13.52 mg/L in the province of Khyber Pakhtunkhwa (Shah & Danishwar, 2003). The average level of fluoride concentration in water sources in the desert of Thar in Pakistan was determined as 3.33 mg/L and maximum fluoride concentration as 7.85 mg/L which indicated that total 78% of water samples exceeded the standard limit of fluoride which was recommended by WHO (Naseem *et al.*, 2010). In Pakistan, higher concentrations of fluoride in eastern Punjab have been reported where its concentration exceeded to 21.1 mg/L (Farooqi *et al.*, 2007). In the country's major cities 6% and 25% of water samples of ground and surface water respectively have higher fluoride level beyond the criteria of standard quality. Across country studies have showed that there is large variation in concentration of fluoride. In 2012 a study has been reported in which 747 water sources from sixteen big cities of Pakistan has analyzed by Tahir and Rasheed and they discovered fluoride toxicity. It has been noted that in about 16 percent of controlled sources consist concentrations of fluoride more than recommended level. The study also revealed that Baluchistan and Punjab consists maximum concentrations of fluoride with ranges less than 0.05-19.07 mg/ L and 0.1-24.48 mg/L. The fluoride concentration in water sources of 29 cities of Pakistan is given in table.

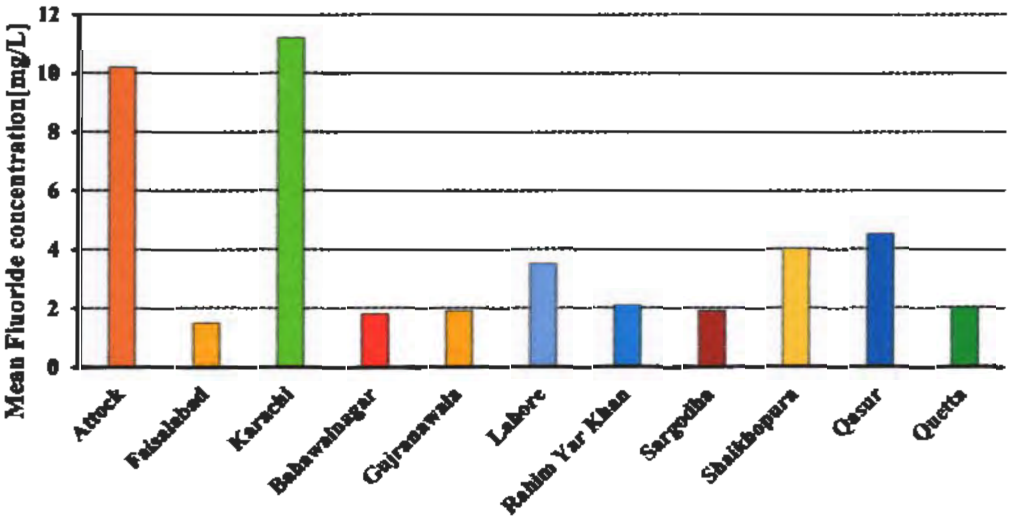

Figure 1.2. Fluoride concentrations in major cities of Pakistan (Azizullah *et al.*, 2011)

1.9. Efficacy and effectiveness

Prevention of tooth decay i.e. infection of teeth enamel is only indicator of the significance of fluoride on human health. Fluorapatite is formed by the reaction of fluoride with hydroxyapatite, less sensitive to erosion by the oral bacteria which produce acid. Fluoride absorbed by teeth and bones is approximately 50%, while remaining fluoride is excreted from the body in urine (Dhar & Bhatnagar, 2009). Fluoride is widely believed to help in prevention of dental caries through direct effects on enamel of tooth preferably after an outbreak and plaque (Warren & Levy, 2003).It has also been shown that inhibition of carcinogenic bacteria is done by fluoride. It is believed that fluoride affects the metabolism of sugars in bacteria, and decrease the ability of bacterial cell to maintain homeostasis, equilibrium, pH and prevent encholaze from acidity and as well as another enzymatic ATPase system (Iwami *et al.*, 1995).

The 2003 World Report on Oral Health and Dentistry concluded that tooth decay can be reduces by 15% by fluoridation of water. Water fluoridation also reduces the number of cavities in children which they receive in teeth up to 60% and reduces dental caries up to 35% in permanent adult teeth. Fluoridation of water continues to effectively reduce tooth decay by 20-40% is suggested by today's study (Association, 2005).

1.10. Fluoridation of water

Water fluoridation is controlled by adjusting the fluoride concentrations in community water networks at an optimal level to reduce the diseases of tooth decay and mottling of enamel which caused dental fluorosis. Fluoridation in water and other uses of fluorides such as in toothpaste greatly decreases incidence of tooth decay. In the literature review, Osterman has not found a single example of municipal water fluoridation which cause the concentrations of fluoride to b exceeded from recommended environmental levels, although there is high concentration of fluoride in some cases heavily contaminated with industrial water pollution but it is not associated with fluoridation of water. To ensure environmental safety the fluoride concentrations must be below the recommended level. Scientific data supports the adding fluoride to local supplies of water which is harmless to environment and healthy for people.

1.11. Impacts of fluoride on human health

Dental caries is most broad and obvious impact of fluoride. The process of demineralization of hard tissues of teeth which is caused by the formation of acids due to bacterial fermentation of glucose in human's diet is called dental caries.

Figure 1.3. Dental fluorosis

Skeletal fluorosis cases were reported in those areas where there is prolonged ingestion of fluoride i.e. 2 ppm or more than 2 ppm or where there is higher concentration of fluoride. In aluminium and fertilizers industries where workers are constantly exposed to fluoride or the areas where concentration of fluoride is above 2 ppm, skeletal fluorosis is common diseases which causes pain in lower and upper limbs, knock-knees and back pain in human body. Compression in spinal cord may also cause by vertebral osteosclerosis (Ayoob & Gupta, 2006) (Rochat & Severin, 2011).

The increasing level of fluoride is a great threat to human health (Ayoob & Gupta, 2006). Alzheimer's disease, allergic reactions, low intelligence, heart disease, fractures, cancer, Down syndrome and other diseases (Pollick, 2004). The optimum fluoride content in the first-year weight 0.045 mg/kg of body weight, tolerant to 0.073 mg/kg but toxic when its concentration increases from 0.150 mg/kg. The optimum fluoride dosage for an adult human range from 0.020 to 0.025 mg/kg/body weight. Fluoride wastewater can be extremely dangerous because in waste water it is present within in the form of HF which is extremely toxic and its toxicity is manifested by its penetrating characteristic in human's tissues because it moves quickly through the skin tissues as the size of HF molecules is very small and harm the human tissue permanently (Rajković & Novaković, 2007). Urolithiasis in humans, gastric renal disorders can also cause by fluoride and when the concentration of fluoride exceeds then it even causes death. Abdominal pain, faintness, gastro-intestinal disturbances and increased salivation are the adverse effects of fluoride on humans. Cardiac failure, hyperkalemia, tremors, cardiac arrhythmias, respiratory arrest and hypocalcemia are the other serious effects of Sodium fluoride on humans. Sodium fluoride single oral dose that is of 50-10 g can be fatal within two to four hours in human adult if he does not get treatment (Wang *et al.*, 2011). Sodium fluoride is also associated with nerve gases. This is why the greatest risk to human health is frequently increasing fluoride concentration in drinking water (Ayoob & Gupta, 2006).

1.11.1. Reproductive and Developmental Effects

Several reports have shown that exposure of fluoride may be related to variation in reproductive hormones. It also effects the development outcomes and fertility. Thus, determination of fluoride in the water is crucial because of its adverse effects on human health, animals and plants when their concentration in the body exceeds from the recommended level.

Development of methods of and means of detecting fluoride ions of high selectivity and sensitivity aroused considerable interest in chemists, analysts and scientists, and a significant amount of efforts have been made in recent decades (Kim *et al.*, 2009) (Tercier & Buffle, 1993). Over the last 2 decades Sensor technology has attracted considerable attention (Cammann, 1992). The phenomenon of molecular recognition, which are similar to biological recognition systems have been widely studied in designing robust chemical sensors with highly selective capabilities and its long-term stability. Impressive progress in artificial target molecules were obtained using molecularly imprinted polymers (MIPs).

1.12. Molecular imprinting polymer (MIPS)

Selection of appropriate functional groups which consists of monomers is the most important step in the synthesis of MIPs to obtain its desired results. Based on functional groups the monomers should be selected which depends on the affinity towards template molecules but their interactions must not be permanent.

1.12.1. Template

The amount of template which is used during the synthesis of molecular imprinting polymer (MIPs) is of great importance. Very small quantity of template molecules in turn results less interactions in generation of sites which in turn decrease sensitivity of sensor response while it leads to the dilution of monomer when larger amount of analyte is used so it is also not a good option. So, this is why template must be used in appropriate way (Lieberzeit & Dickert, 2008)·

1.12.2. Monomer

The interaction between template and monomer leads to the formation of a complex and we determine the resulting of molecularly imprinted polymer from the nature of that complex. If it is highly stable and complex, then it means that the MIP will have greater binding affinity towards template only (Alexander *et al.*, 2006)

1.12.3. Cross-linker

To secure the cavities and to set the functional group in a suitable orientation cross linker is used so that formation of specific spaces occurred which is responsible for generating selective sites even after the removal of template. To maintain the morphology of the polymer matrix cross linker is very important, whether it is a microgel powder or macroporous, gel-type. Cross linker stabilizes the binding sites and it also brings stability to the polymer matrix (Ramström & Ansell, 1998).

1.12.3. Porogenic solvents

For the process of polymerization to proceed the components need a matrix in which they might interact with one another. Solvent or porogen is used as a medium. It plays a crucial role in directing the affinities of the imprinted polymer. All the components must obviously be soluble in the porogen. The interactions of Porogenic solvent with both monomer and template must not be as strong as to hinder the affinity of template and monomer (Mayes & Whitcombe, 2005).

1.12.4. Initiators

The main role of initiator is in radical generation but it also affects binding sites and morphology as well. Chemicals initiator of different properties are used as a source in the process of free radical polymerization. Decomposition and rate of initiator could be activated in several ways which includes heat, chemical/light means, it also depends on its chemical nature. e.g. Azobisisiobutyronitrile (AIBN) can be easily decomposed by the process of thermolysis to give stable, carbon-centered radicals which are capable of initiating growth of monomers of vinyl.

1.13. Chemical binding

The process of molecular imprinting polymer can be done either by covalently or non-covalently.

1.13.1. Covalent Imprinting

The nature of bond between the monomer and template is covalent which indicates that the bonding is very specific and strong. It depends on template and monomer chemical binding through functionality which can be cleaved easily.

1.13.2. Non-covalent imprinting

It is very flexible and direct approach because it is used in a lot of compounds including chiral molecule that can also be imprinted (Kempe & Mosbach, 1995). Non-covalent approach is mostly used for organic molecules which have lower molar masses, e.g. Anesthetics or organic solvents, however species with large size can also be appropriate templates (Mustafa & Lieberzeit, 2012).

1.14. Imprinting process

The techniques of imprinting used in the process of polymerization is very important. Two commonly used methods are bulk and surface polymerization. (Beltran *et al.*, 2010).

1.14.1. Bulk-Imprinting

Cross-linking monomers and functional are polymerized in bulk-imprinting but in the presence of template, the template should be similar and identical to the target molecules. The self-assembly of a complex between the functional monomer and template is occurs by non-covalent intermolecular forces. After the process of polymerization, the cross-linked polymer structure held the functional groups in their place so that the rigid cavity engulfed the template. Template can be separated by following formation of a space which is empty and whose shape, chemical functionality and size are compliment to the original template. For polycyclic aromatic hydrocarbons bulk-imprinting technique is often used for sub-nanoscale imprinting.

1.14.2. Surface-Imprinting

The process of bulk-imprinting is not suitable for the imprinting of biological species like cells, microorganisms and protein due to their larger size and dimensions which significantly hinders their incorporation into the bulk polymer. First of all, transducer which is mass sensitive in nature is coated with an oligomeric mixture before polymerization followed by the pressing of stamp into the polymerizing material. So, during the process of polymerization around the bio-template oligomeric chains arrange themselves. The polymer mold layer is washed away after hardening which leaves cast that can be referred to as synthetic antibodies. Their selectivity depends on the shape and size of the template molecule along with their respective surface chemistry (Kempe *et al.*, 1995).

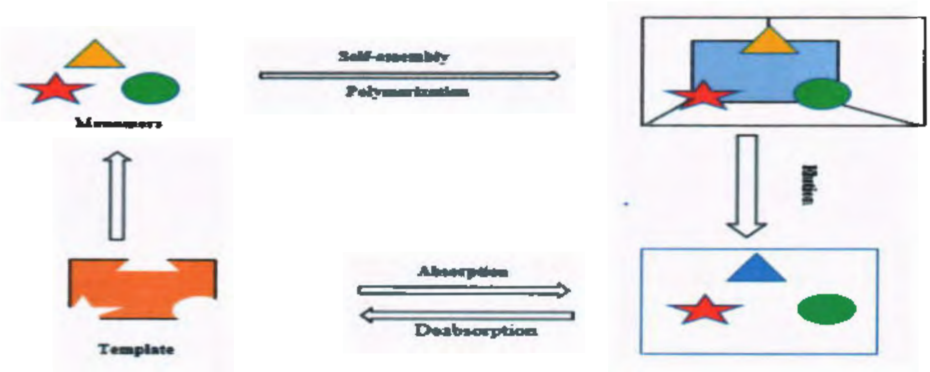

Figure 1.4. The process of molecular imprinting

MIPs were earlier used in the field of medical, diagnostic which includes artificial antibodies generation. MIPs is being used as stationary phases in the techniques of solid phase extractions and chromatography for separation of different analytes in analytical chemistry. Moreover, the advancements came as MIPs is being used as a sensor in environmental monitoring and the more advance concept of MIPs is wastewater treatment.

1.15. Sensors and its components

The development of sensitive and selective sensors for fluoride ions and their efficient separation techniques is of great importance due to great potential toxicity of fluoride ions. The fluoride which is present vastly not only in in water but also in tea leaves and different drugs and used for the treatment of osteoporosis and in toothpastes. This new platform offer an attractive alternative of low cost of the currently used methods which are reported in literature to have limitations such as inability to operate in competing and aqueous environments, low stability and selectivity, difficulty in reaching narrow spaces and high cost (Wade *et al.*, 2010).

The ability to perform detection, the new developed techniques should also have ability to separate fluoride ions. Up to now, based on absorbance or fluorescence measurements very few sensors have been developed for detection of fluoride ions because of their high detection limit and low selectivity. So, such devices that can be used in field for real-time and online monitoring of fluoride concentration in water is of great importance and scientists are working and looking for such devices which can also be easy to handle.

Chemical sensors are promising analytical tools that can monitor concentration of fluoride in real-time. It transforms chemical information ranges from specific sample concentration to total composition analysis, into a significance analytical signal. It can transmit online and real-time information in the presence of certain compounds or ions, even in samples which are complex by nature

1.15.1. Recognition material- receptor

Sensors, based on their receptor type, are classified into two major classes that are, biological or biosensors and the other being chemical sensors.

1.15.2. Bioreceptors

These have nucleotides or microorganisms as recognition element. Natural nucleotide materials like DNA and antigens/antibodies or enzymes are used as bio recognition materials. These receptor materials are extracted from the living organisms. Only its complementary part (analyte), having proper complementary sequences could bind to it through hybridization process. Thus, DNA is a highly selective recognition material for biological samples.

The principle on which antigens or antibodies and enzymes, as recognition material, function is the lock and key model. The antibody has a very special three-dimensional structure and shape that is highly selective for only one antigen. The antigen binds to the antibody in a way, that its three-dimensional looks do not change. It is due to this very attribute, antigens/antibodies are considered an excellent tool as a receptor in the biosensors for the detection of a variety of analytes form biological molecules to cells of microorganisms.

1.15.3. Chemoreceptors

Chemical receptors are of two basic types and include antibodies or artificially synthesized antigens and molecular-printed polymers (MIPS). These antibodies have been synthesized in laboratories and are very similar to the original antibody found in living organisms. Synthetic biological molecules fall under the category of chemical receptors because they are artificially synthesized in laboratories being not isolated from living cells. On the other hand, molecular printing is a completely different method, which involves the formation of very specific sites in the polymer matrix, which are complementary to specific analytes for which they were synthesized.

1.15.4. Transducer

The receptor material, upon interaction with the specific analyte, produces a signal that is converted into a measurable physical signal. When manipulating the analyte of interest, variation occurs in properties of the layer which is detected by transducer and turns it into useful analytical signal and thus quantifiable data.

1.15.5. Amplifier

The physical signal produced by the transducer is very small. The function of an amplifier is to enhance that signal so that it could be easily interpreted.

1.15.6. Data storage system

Last part of chemical sensor comprising is the data storage system. Sensor's response is stored in this data storage system and here the sensor's response can be analyzed later or directly. Usually these electronics signals recorded and amplified by the data processing unit which also includes assessment.

The sensor consists of materials that are receptor that interact with transducer and analyte which due to bindings gives response. If the receptor material has greater selectivity and sensitivity, then the response given by the transducer can be made more effective (Eggins, 2008).

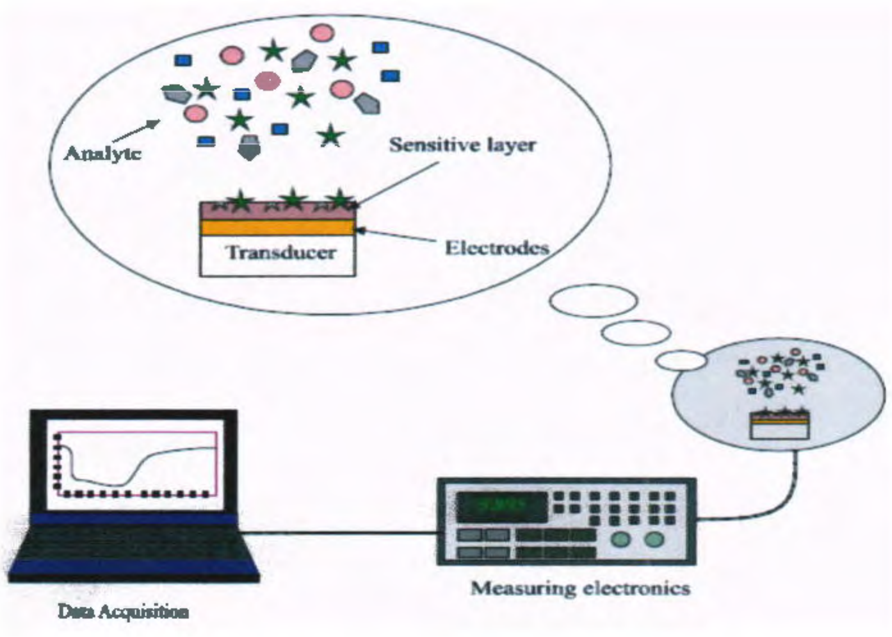

Figure 1.5. General layout of a chemical sensor

Molecular imprinted based sensors have many benefits like sensitivity, selectivity, robustness, easily transferrable to be carried to the field easily and it is cheap with less time.

Chapter 2

Fabrication of MIPs based Electrochemical Sensor for the Detection of Sodium Fluoride (NaF) in Water

2. Fabrication of MIPs based Electrochemical Sensor for the Detection of Sodium Fluoride (NaF) in Water

Sodium fluoride is odorless compound mostly available in the form of white powder but can also be found in the form of solid dissolved in liquid or crystals. In water, it is soluble, dissociates into Na^+ and F^-ions. The molar mass of NaF is 41.99 g / mol. Boiling and melting point of sodium fluoride 993 °C. Sodium fluoride is hygroscopic solid with a density of 2.56 mg (Wells, 1984). Sodium fluoride is non-flammable but it is corrosive to aluminum metal. NaF under optimal temperature is stable but at high temperature it dissociates releasing corrosive and toxic Hydrogen Fluoride (HF) fumes. In very small amount it is found as a naturally occurring mineral namely villiaumite. Artificially sodium fluoride is prepared by neutralizing hydrogen fluoride (HF). It is also produced as a byproduct of the reaction of Fluorapatite reacted to the production of phosphate fertilizers. NaOH and Na_2CO_3 are used as neutralizing agents. To precipitate sodium fluoride alcohols are used.

$$HF + NaOH \rightarrow NaF + H_2O$$

The solutions which contain Hydrogen fluoride, $NaHF_2$ which is bifluoride salt, is formed because of precipitation of sodium fluoride.

$$HF + NaF \rightleftharpoons NaHF_2$$

The annual consumption of sodium fluoride was to be estimated to be several million tons around the globe published in a report in 1986 (Aigueperse *et al.*, 2005).

2.1. Industrial use of NaF

Since NaF^- is usually used in pesticides which includes fungicides and insecticides. In this type of substances, the range of sodium fluoride concentration varies from 15%-95%. As a preservatives NaF^- is used in several types of glues and adhesives. For the growth prevention of bacteria, mold and fungi and to manufacture aluminium and steel products sodium fluoride is used. According to Environmental Agency, when sodium fluoride is added to molten metal, it increases the process of deoxidation by producing more consistent metals.

Use of sodium fluoride in other industrial uses include wood preservatives, glass frosting and the pickling of stainless steel. Sodium fluoride is also used for the purpose of corrosion inhibitors and cleaning material. Other significant applications of sodium fluoride are imaging in nuclear medicine, while sodium fluoride (NaF^{18}), which is radioactive in nature is used as an indicator to detect different radioactive conditions (Metcalf, 1989).

2.1.2. Dental Caries

To prevent dental caries, maintain dental health the slat of sodium fluoride is added in local drinking water supply while sodium fluoride is also used in some states as certain food products. Fluoride increases durability of teeth due to the formation of fluorapatite, which is a natural component of tooth enamel. Sodium fluoride is also used in toothpastes for prevention of teeth cavities. This makes the teeth stronger and resistant to rotting caused by acid and bacteria (Selwitz *et al.*, 2007).

2.1.3. Osteoporosis

The density of bone is also enhanced by sodium fluoride while it is also used as a drug in the treatment of osteoporosis.

2.1.4. Osteosclerosis

In combination with $CaCO_3$, sodium fluoride can minimize the deterioration of sensorineural and conductive loss in those patients suffering from Osteosclerosis. To halt improvement in the early stages of Osteosclerosis, sodium fluoride seems to be more effective but at lower doses (Cruise *et al.*, 2010).

2.2. Effects

2.2.1. Teeth Staining and Fluorosis

There are also side effects of sodium fluoride when its concentrations exceed from 2.0 mg/L. It causes staining of teeth enamel which may lead to cause dental fluorosis which is irreversible condition of teeth.

Ashes and itching are the symptoms of allergic reactions. Sodium fluoride single oral dose that is of 50-10 g can be fatal within two to four hours in human adult if he does not get treatment.

2.2.2. General Side Effects

Common stomach disorder is another less serious but toxic side effects of sodium fluoride. Hypersensitivity reactions, i.e. itching, difficulty in breathing, swelling in mouth and tightness in the chest are other severe effects of sodium fluoride. It may also have accompanied by headaches and general weakness.

2.2.3. Osteosarcoma

According to American Cancer Society, Sodium fluoride in ingesting water has been associated to cause a disease called osteoarthritis and osteosarcoma (cancer of bones). When accumulation of fluoride occurs at that place where growth of bones is occurring and its concentration exceeds from required limits then it causes osteosarcoma. The presence of sodium fluoride in water increases the production of cancerous cells and quicken and accelerate the growth of cells. However, to fully explore these potential side effects further research is needed.

In the present scenario, there is a continuously increasing worldwide concern for the development of fluoride treatment technologies. Numerous conventional techniques are in practice for the detection and analysis of fluorides in water samples, however, these techniques are not applicable for real-time and online monitoring. It is of substantial interest to develop miniaturized robust, precise and accurate devices capable of real-time and online analysis and the sensor technology ultimately provides promising tools for this purpose. Chemical sensors are the analytical devices, constituting a recognition element (analyte) that is in direct connection with some physical or chemical transducer and which upon contact with the analyte produces a response in the form of signal showing the exact concentration of analyte of interest in the given sample (Coales, 2000). In the absence of that specific analyte the sensor would not show any response, even if competing agents bearing close resemblance with the analyte of interest are present in simple or complex media. When the specific analyte binds to receptors, the transducer detects the change, whether it can be optical, electrochemical, mass change, etc. and converts it into an electrical signal. Chemical sensors are usually designed for detection of low concentrations of chemical species and could not reach to the detection limits of modern analytical instruments but still have many desirable advantages over them due to their small size, low cost, ease of operation and their manufacturing by established technological methods (Dickert & Lieberzeit, 2000). Sensors have small size as compared to other analytical instruments and because of their small sizes and ruggedness; these can be used for remote measurements. These devices do not require too much maintenance because of their simplicity in design and can be easily operated by nonprofessional. Chemical sensors are promising candidate for on-line and in-time monitoring of specified analytes and are highly suitable for all kinds of applications. The fabrication of highly specific and simple molecular imprinted polymer (MIPs) based receptors capable for sensing fluoride anions has gained significant interest (Martinez-Manez & Sancenón, 2003).

2.3. Materials and methods

2.4. Chemicals and reagents

All chemicals including styrene, bisphenol A, 2,2'-Azobis-isobutyronitrile (AIBN), divinylpyrrolidone, ethanol (95%), Chloroform, ethylene glycol dimethacrylate (EGDMA), tetrahydrofuran (THF), methacrylic acid ($C_4H_6O_2$), sodium fluoride (NaF), potassium iodide (KI), potassium bromide (KBr), sodium chloride (NaCl), sodium carbonate (Na_2CO_3), potassium sulphate (K_2SO_4) were purchased from Sigma-Aldrich and Merck with maximum available purity.

2.4.1. Instrumentation

LCR (IET 7600 Plus Precision LCR Meter) is used to measure resistance (R), inductance (L) and capacitance (C) in sodium fluoride sensor. For the process of coating self-fabricated spin coater with a speed of 2500 rpm was used to generate thin film. Inter digital electrodes were used.

2.5. Synthesis of Molecularly Imprinted Polyvinylpyrrolidine

Polymer was synthesized by mixing 40 µl n-vinylpyrrolidone as a functional monomer, 60 µl EGDMA as a cross-linker, 0.008g Bisphenol A as a porogen, and 0.004g Azobisisiobutyronitrile (AIBN) as an initiator in eppendorf tube. This mixture was prepared in 300 µl of ethanol. This mixture was shaken on vortex and then polymerized it for 45 minutes at $70\overset{o}{C}$. After polymerization 7µl of this polymer was coated over on interdigital electrode by spin coating at a speed 2500 rev/sec for 3 minutes and dried in oven for overnight.

2.5.1. Synthesis of Molecularly Imprinted Methyl Methacrylate Polymer

Acrylate system polymer was synthesized by mixing 30 µl methacrylic acid as a functional monomer, 70 µl EGDMA, 0.008g Bisphenol A as a porogen and 0.004g Azobisisiobutyronitrile (AIBN) as a initiator. This mixture was prepared in 300 µl chloroform solution. This mixture was shaken on vortex. After then the mixture was polymerized for two hours at 60$\overset{o}{C}$. after polymerization 10 µl of this polymer mixture was coated over on interdigital electrode by spin coating at a speed of 2500 rev/min for 4 minutes and after then it was dried in oven for overnight at 70 $\overset{o}{C}$.

2.5.2. Synthesis of Molecularly Imprinted Polystyrene Polymer

Styrene system based molecular imprinted polymer is synthesized by mixing 30 µl styrene as functional monomer, 70 µl divinely benzene (DVB) as cross-linker, 0.008g Bisphenol A as a porogen and 0.004g Azobisisiobutyronitrile (AIBN) as free radical initiator followed by addition of 300 µl of ethanol as a solvent having 10 mg of sodium fluoride (NaF) is as a template in Eppendorf tube. This mixture was thoroughly shaken on a vortex and was cooked for two hours at 70$\overset{o}{C}$ until gel point is reached. After then polymer was coated on interdigital electrode by process of spin coating and was dried in oven for overnight.

2.5.3. Immobilization of MIPs onto Inter digital electrodes (IDEs)

After reaching the gel point that polymer matrix was coated on inter digital electrode using pipette. IEDs were clean with methanol and deionized water. 10 µl of polymer was spin coated onto IEDs at a speed of 2500 rpm to obtain thin film and dried in oven for overnight to completely adhere the polymer layer in IDEs. The monomer: crosslinker ratio was kept 40:60.

2.4.5. Washing (removal of template)

IEDs were placed in deionized water and thoroughly stirred by magnetic stirrer for 2 hours at room temperature for removal of template from imprinted polymer matrix.

Non-imprinted polymer (NIP) was prepared with the same procedure mentioned above with the exception that analyte i.e., NaF.

2.5.5. Preparation of ppm solution of sodium fluoride

0ppm, 10ppm, 20ppm, 30ppm, 40ppm and 50 ppm solutions of fluoride were prepared to check the sensitivity and selectivity of polymer by exposing the sensor to LCR meter. The sensor was exposed to various concentrations of sodium fluoride.

2.6. Results and discussion

2.6.1. Sensor measurements

Sensor measurement of sodium fluoride sensor was carried out at two different frequencies i.e. 20 Hz and 1000 Hz by using LCR meter for measurement of inductance, Capacitance and Resistance.

2.7. Sensitivity studies of NaF fabricated sensor

Sensitivity response of the NaF fabricated and NIP fabricated sensors was studied through exposing them to various concentrations of the analyte i.e., NaF. The change in sensor response was noted in capacitance (CP) at 20 Hz. Increase in capacitance has occurred linearly as the concentration of analyte solution increases. At a concentration of 10 ppm, the sensor shows a response of 45.36628 nF approximately at 20Hz. However, the concentration of analyte increased the response of sensor also increased. At 20 ppm, it gives response of 94.864 nF and as for 30 ppm, 40ppm and 50ppm concentration the response of sensor was 143.65 nF, 191.653 nF and 240 nF respectively as shown in figure 2.1. As for the NIP, fabricated sensor it was 5.1 nF with the same concentration of analyte. This response is almost negligible as compared to the response of the fluoride imprinted sensor. This significance difference of responses between MIPs coated IDEs and NIPs is because of the availability of selective molecular imprints/cavities in the molecular imprinted polymer.

This increase in the response of sensor by increasing the concentration of analyte was almost same with a difference of 50 nF interval for all the concentrations of analyte. As the concentration of analyte increased the response of sensor also goes on increasing gradually showing a linearity (fig 2.1). The sensor shows excellent sensitivity even at low concentration which indicates generation of cavities. The cavities produced capture the template molecules by the process of adsorption, thus shows increase in response of sensor. The sensor shows an excellent reversibility when deionized water was injected having zero concentration of the analyte which indicates that it could be used repeatedly after washing because after washing with water the sensor response again reaches to its initial value which indicates that sensor shows complete reversibility and regenrability. On the other hand, NIP does not show any significant change in its response due to lack of binding capacity towards analyte. The response time of this sensor is 1-3 seconds. The limit of detection of the fabricated sensor is 525 ppb which is good detection limit.

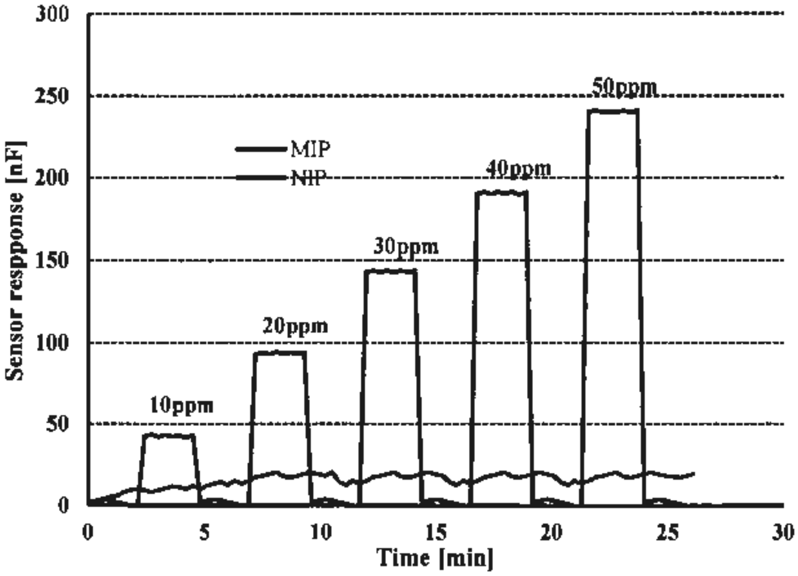

Figure 2.1. Sensor response of MIP and NIP using Acrylate system at 20 Hz (CP)

The results obtained indicated that by increasing fluoride concentration the response of fabricated sensor increased linearly.

Similarly, when the same sensor response for capacitance (CP) was checked at 1000Hz, the response at 10ppm was 41.13201 nF approximately which shows increase in response with increase in concentration of analyte i.e. sodium fluoride.

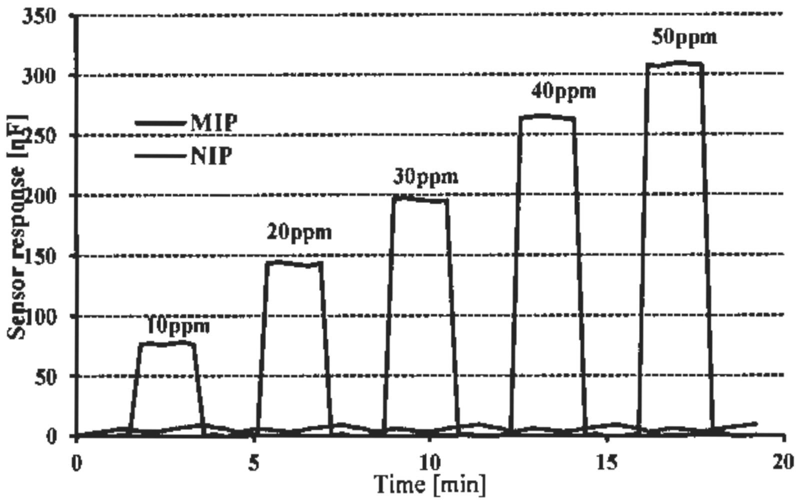

Figure 2.2. Sensor response of NIP and MIP using Acrylate system at 1000 Hz

The response of sensor was 93.638, 143.876, 189.764 and 240.887 nF for 20, 30, 40 and 50 ppm respectively which shows an increase in sensor response with almost equal difference 40 nF showing linear response as shown in fig 2.2. for the NIP, fabricated sensor, the response was 3.8768 nF as the concentration of analyte was kept same.

The sensor response at 1000 Hz also showed increased while increasing the concentration of analyte solution. The sensor showed linear response while increasing analyte solution.

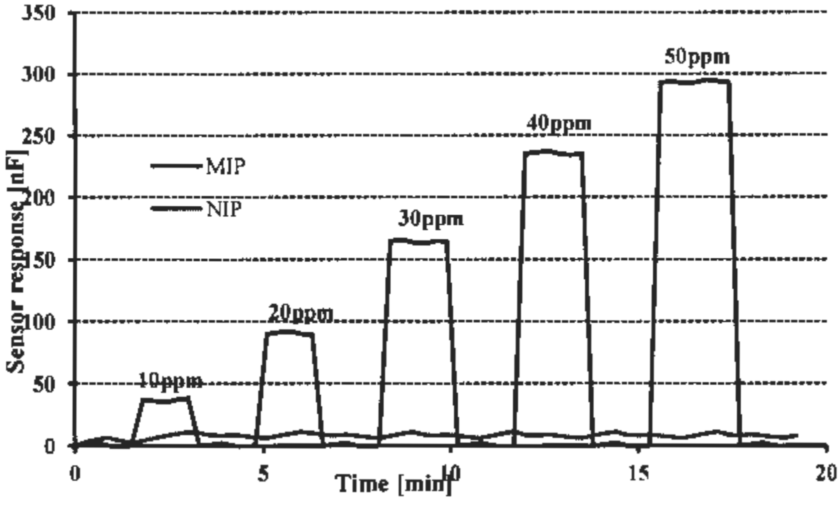

Figure 2.3. Sensor response of NIP and MIP using Vinylpyrrolidone system at 20 Hz (CP)

When Vinylpyrrolidone system was used for the fabrication of sodium fluoride sensor it showed almost same response for capacitance like Acrylate system at 20 Hz. At 10 ppm, the sensor response was 35.865 nF which increased to 92.463 nF at 20 ppm. Similarly, (fig 2.3) shows that response of sensor is 292.753 nF at 50ppm, which indicates that gradual increase in sensor response occurs upon increasing concentration of sodium fluoride showing linear relation between analyte concentration with sensor response. Sensor response was 2-3 seconds. The detection limit of this sensor was 510 ppb.

While using polystyrene system, the sensor shows excellent response as compare to acrylate and vinylpyrrolidone system. Th capacitance (CP) has been increased linearly with the increasing analyte concentration. The response of sensor at 10 ppm is 111.757 nF which shows gradual increase up to 50 ppm where it gives a response of 598.674 nF.

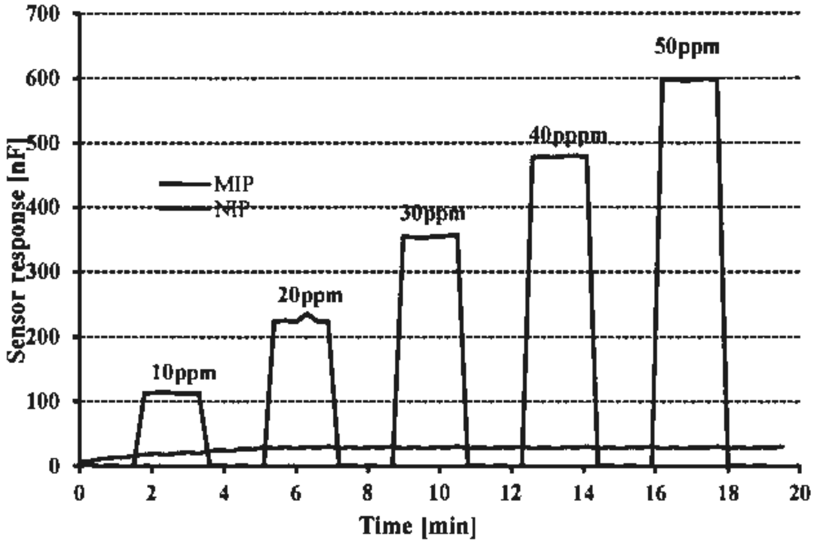

Figure 2.4. Sensor response of MIPs and NIPs using polystyrene system at 20 Hz (CP)

The sensitivity graph of the sensor shows high linearity as well as reversibility which indicates that it could be used repeatedly after washing (fig. 2.4). The sensor shows excellent sensitivity even at low concentration which indicates that generation of cavities occurs which capture template molecules via adsorption in the polymeric matrix. The response time of sensor is 1-2 seconds. Detection limit of this fabricated sensor is 220.2 ppb which is low as compare to vinylpyrrolidone and acrylate system. The excellent response of styrene system as compare to vinylpyrrolidone and acrylate system might be due to the cavities produced in styrene system were more stable than acrylate and vinylpyrrolidone system. Strong interaction of template with styrene system might be another possible reason. Cavities produced in styrene system may be stronger which results in strong binding with template molecules i.e. sodium fluoride. Another possible reason of good response of styrene system is that it may interact with template molecules with strong intermolecular forces while acrylate and vinylpyrrolidone systems may interact with template molecules through weak inter molecular forces.

2.8. Selectivity studies

Sensor's sensitivity response could not tell us whether a fabricated sensor is suitable for the sample analysis in real environment, in which thousands of other similar ions with highly related mass and radii, are present along with the ion of interest. Therefore, it is of significant importance to check if the sensor is selective for that ion of interest for which it has been fabricated or not. To check the selectivity of the sensor, solutions of different competing ions including Bromine, Chlorine, Iodine, ate, Carbonates and as well as imprinted NaF at a concentration of 50ppm at 20 Hz were prepared. It is obvious from figure 2.5 that the sensor response (CP) was selective for fluoride ions. But for Cl^-, Br^- and I it showed two folds less response than fluoride ions. Although they all have similar oxidation state i.e. -1 to that of fluorine but the response was selective for fluoride ions. When the selectivity of same sensor checked for SO_4^- and CO_3^- which have different oxidation states then fluoride showed much less response than the Cl^-, Br^- and I i.e. 3 to 4 folds less response than fluorine which indicates that with a different oxidation state, the sensor is selective only for the imprinted ion. The response of Cl^- and Br^- is higher as compared to other ions, this is because both of them are ions and these ions are more geometrically and chemically similar to fluoride ions as compared to sulphate and carbonates or other groups elements. This indicates that the sensor could show remarkable selectivity for NaF, in complex environments where other highly similar analyte species are also present. The high selectivity of this sensor for fluoride ions indicates that the cavities generated in the MIP were specific only for fluoride ions.

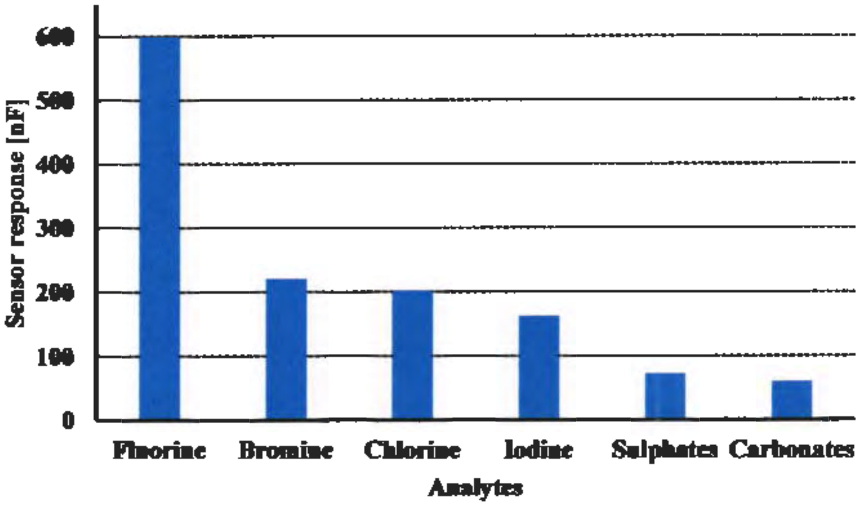

Figure 2.5. Polystyrene based sensor selectivity response at 50 ppm of each analyte at 20 Hz (CP)

Low response of these anions as compare to fluoride ions might be due to the fact that molecular geometry and molecular structure of those interfering anions do not resembles to the molecular structure and geometry of fluoride ions. The cavities produced were complimentary to the size of template molecules i.e. sodium fluoride. This sensor can be used for the real-time and online monitoring of fluorides in water

2.9. Conclusion

In this experiment, we used three different polymer system i.e. polystyrene, vinylpyrrolidone and acrylate systems. The results of polystyrene system were much efficient as compared to vinylpyrrolidone and acrylate system. This fabricated sensor was then analyzed using LCR meter to check the sensitivity and selectivity response. NaF fabricated sensor shows highly reversibility and linear response linearity with increase in concentration of analyte. The detection limit is 220.2 ppb. The response time of fabricated sensor is 2 seconds. The sensor also shows high selectivity towards sodium fluoride even in the presence of other competing ions which are chemically and geometrically identical to sodium fluoride. This fabricated MIPs based sensors can be used for the detection of fluoride ions in aqueous environment and it can be used for the removal of fluoride ions from aqueous media efficiently in future.

Chapter 3

Fabrication of MIPs based Electrochemical Sensor for the Detection of Calcium Fluoride (CaF_2) in Water

Chapter 3

3. Fabrication of MIPs based Electrochemical Sensor for the Detection of Calcium Fluoride (CaF_2) in Water

The molecular formula of calcium fluoride is CaF_2 and is an inorganic compound. Calcium fluoride is found in isometric crystals with cubic in shape. When calcium fluoride is pure it is transparent and colorless with a glassy luster. The impurities cause the stone color and its different varieties emit fluorescence. Calcium fluoride usually occurring in pure veins or related with silver, lead or ores of zinc but it is common in dolomites and limestone. It is hygroscopic white powder mainly occur in nature as fluorspar or fluorite. The main source of fluoride and its compounds is calcium fluoride. Molecular weight of calcium fluoride is 78.075 g/mol. In metallurgical industries fluorite is mainly used as a flux, and very pure fluorspar can be used to manufacture special lenses. Water contains specific amount of calcium fluoride can prevent dental problems. Moreover, it can be used to produce iron ore, steel, glass, chemicals and ceramic. Pure product can be used for the dehydration of catalyst and also for the dehydrogenation. The main source of calcium fluoride in water is its release during the production and industrial use to waste water due to which its precipitation occurs mainly when its $pH > 4.5$. Even when calcium fluoride is released as free ions in wastewater, it is not expected to result in large HF emission in the atmosphere, since only a small fraction of fluoride occurs as HF, especially when the pH is above 5. Moreover, calcium fluoride also released as dust during production and downstream use, which is expected to settle both near by the production and downstream user sites and in surface water as soil. Calcium fluoride deposits in within the surface layer in soil but moves throughout the root region even in the calcareous soil. Typically, the lowest levels of fluoride found in herbivores have levels higher than predators.

3.1. Uses of CaF_2

Calcium fluoride can be used in electronics, instrumentation industry, for the making of hydrofluoric acid and fluoride, can also be used for enamel, metallurgical flux, ceramic and it is widely used in cement and chemical industries. Calcium fluoride is also used in medicine. In chemical industries for the preparation of HF and fluorine the main raw material is fluorite and for the production of polymers of fluorine, synthetic cryolite, chemicals containing fluorine and Freon cryolite is also used as basic raw (Khalaf *et al.*, 2012). The colorless and transparent crystals of fluorite can also be used as in fluorite process and as a bulk optical fluorite. By the action of concentrated sulfuric acid HF is emitted from the mineral (Martinez-Manez & Sancenón, 2003).

$$CaF_2 + H_2SO_4 \rightarrow CaSO_4\,(solid) + 2\,HF$$

To manufacture optical components such as lenses and windows, spectroscopy and thermal imaging system calcium fluoride is used. Over a broad range of UV and IR frequencies calcium fluoride is transparent and it minimize the need for anti-reflection coatings due to its low refractive index. It has also dental applications.

Calcium fluoride has potential health risks. The toxicity of calcium fluoride is due primarily to the hazardous effects of its silica and fluorine contents. Severe inhalation can cause problems with the digestive system, circulatory system and nervous system. Chronic inhalation or ingestion can lead to weight loss, appetite, anemia, and bone and dental damage. Pulmonary lesions were reported among people who inhaled dust containing 92-96% calcium fluoride and 3.5% silica. Apparently, calcium fluoride enhances the action of silica fibrosis in the lungs. Also, the cases of silicosis and bronchitis have been reported among the miners of fluorspar. The chronic exposure of calcium fluoride can cause bone fluorosis when its concentration is high. Its dust can irritate eyes, skin and respiratory tract. Prolonged contact with the skin can cause skin irritation. Acute intake of calcium fluoride causes skeletal as well as dental fluorosis.

3.2. Materials and methods

3.2.1. Chemicals and reagents

All chemicals including Styrene, bisphenol A, 2,2'-azobis-isobutyronitrile (AIBN), divinylpyrrolidone, Ethanol, chloroform, Ethylene glycol dimethacrylate (EGDMA), tetrahydrofuran (THF), Methacrylic acid (C_4H6O_2), Calcium Fluoride (CaF_2), Potassium iodide (KI), Potassium Bromide (KBr), Sodium chloride (NaCl), Sodium Carbonate (Na_2CO_3), Potassium Sulphate (K2SO4) were purchased from Sigma-Aldrich and Merck with maximum available purity.

3.2.2. Synthesis of molecularly imprinted vinylpyrrolidone polymer

Imprinted polymer was synthesized by mixing 30 µl n-vinylpyrrolidone as functional monomer, 70 µl Divinylbenzene as a cross-linker, 0.008g Bisphenol A as a porogen and 0.004g Azobisisiobutyronitrile (AIBN) as an initiator. 0.01g calcium fluoride (CaF_2) was added to this mixture as an analyte. After then this mixture was prepared in 300 μl of ethanol. This mixture was thoroughly shaken on a vortex and was cooked for 2 hours to polymerize the mixture until reaching of gel point. Non-imprinted polymer (NIP) was polymerized by same method without adding template.

3.2.3. Synthesis of molecularly imprinted Polystyrene polymer

Styrene system based molecular imprinted polymer is synthesized by mixing 30 μl styrene as functional monomer, 70 μl divinely benzene (DVB) as cross-linker, 0.008g Bisphenol A as a porogen and 0.004g Azobisisiobutyronitrile (AIBN) as free radical initiator followed by addition of 250 μl of ethanol as a solvent having 10 mg of calcium fluoride (CaF_2) as a template in Eppendorf tube. This mixture was thoroughly shaken on a vortex and was cooked for two hours at $70\overset{o}{C}$ until gel point is reached. Non-imprinted polymer (NIP) was polymerized by same method without adding template.

3.2.4. Immobilization of MIPs onto IDEs

After reaching the gel point that polymer matrix was coated on inter digital electrode using pipette. IEDs were clean with methanol and deionized water. 10 μl of polymer was spin coated onto IEDs at a speed of 2500 rpm to obtain thin film and dried in oven for overnight to completely adhere the polymer layer in IDEs. The monomer: crosslinker ratio was kept 30:70.

3.2.5. Removal of template

IEDs were placed in deionized water and thoroughly stirred by magnetic stirrer for 2 hours at room temperature for removal of template from imprinted polymer matrix. Non-imprinted polymer (NIP) was prepared with the same procedure mentioned above with the exception that analyte i.e. CaF_2 and coated onto IDEs with similar methodology

3.3. Sensor measurements

Sensor measurement of sodium fluoride sensor was carried out at two different frequencies i.e. 20 Hz and 1000 Hz by using LCR meter for measurement of inductance, Capacitance and Resistance. 0 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm solutions of fluoride were prepared to check the sensitivity and selectivity of sensor by exposing the fabricated sensor to LCR meter.

3.4. Results and Discussion

3.4.1. Sensitivity studies of CaF_2 sensor

Sensitivity response of the CaF_2 fabricated and NIP fabricated sensors was studied through exposing them to various concentrations of the analyte. The increase in capacitance (CS) has been noted in a linear manner with increasing analyte concentration. At a concentration of 10 ppm, the sensor showed a response of 59.6503 nF approximately at 20Hz as shown in figure 3. But as the concentration of analyte increases the response of sensor also increases. At 20 ppm, 30ppm, 40ppm and 50 ppm the sensor shows response of 118.846 nF, 169.647 nF, 227.746 nF and 280 nF respectively. There is increase in response with increase in concentration of fluoride which shows that there is a linear relationship between sensor response and analyte concentration.

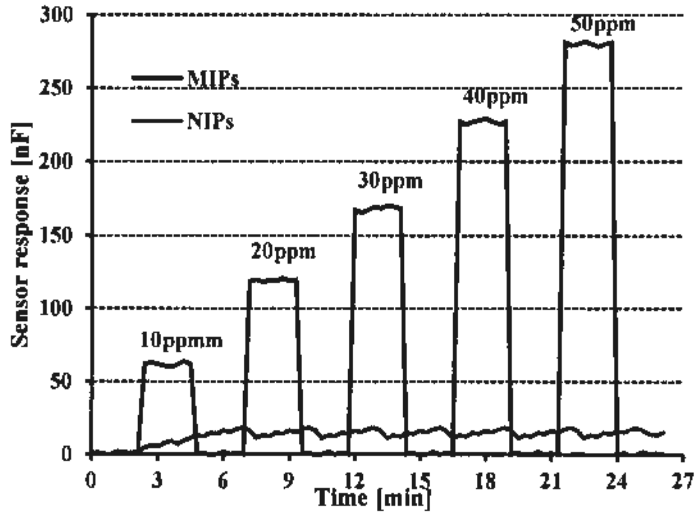

Figure 3.1. Sensor response of MIPs and NIPs using polystyrene system at 20 Hz (CS)

The sensor shows an excellent reversibility and regenrability because when the sensor was washed with deionized water the sensor response gives its initial value again which indicates that sensor can be used repeatedly. As for the NIP, fabricated sensor it was 23.981 nF at 50 ppm which is almost negligible and it does not show any significance response due to lack of binding capacity towards fluoride. The response time of this fabricated sensor is 3-5 seconds approximately. The detection limit for the F^- sensor has been calculated as 535 ppb which is a good detection limit.

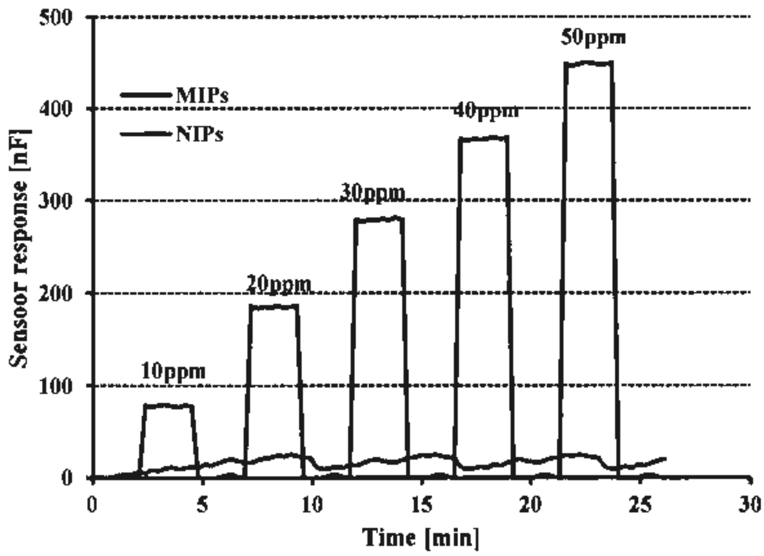

Figure. 3.2. Sensor response of MIPs and NIPs using vinylpyrrolidone system at 20 Hz (CS)

Similarly, the while using vinylpyrrolidone system the sensor shows better result than polystyrene system with greater sensor response. In vinylpyrrolidone system increase in analyte concentration results an increase in capacitance (CS) at 20 Hz. As shown in figure 3.1. The sensor response is 80.738 nF at 10 ppm which goes on increasing gradually as we increase concentration of fluoride. At 20, 30, 40 and 50 ppm the sensor shows a response of 186.743, 278,744, 366.131 and 450.643 nF respectively, it indicates that there is increase in response almost 100 nF at each concentration thus showing a linear relation between analyte concentration and sensor response. The sensor is high sensitive for fluoride ions even at low concentration which is due to the formation of cavities which by the process of adsorption captures template molecules. This results in increase in sensor response when the concentration of analyte increases. On the other hand, NIP fabricated coated sensor shows a response of 24.764 nF at 50ppm with same concentration of analyte. This response is almost negligible when compare with molecular imprinted sensor. The sensor also shows excellent reversibility after washing with deionized water which means that sensor can be used again and again. The response time of this sensor is 1-4 seconds. The limit of detection for this fabricated sensor is 333. 3ppb which is indeed a good a good detection limit.

The results of vinylpyrrolidone system are better in comparison of styrene system for calcium fluoride. This might be due to the differences in the extent of intermolecular attractive forces. These forces might be strong for vinylpyrrolidone system resulting in strong binding sites and stable cavities as compare to styrene system with weak binding sites with template molecules i.e. calcium fluoride.

3.4.2. Selectivity

Fluorides may show similar interaction with different anions so to check whether the sensor is selective for fluoride ions we checked the sensor response of different anions which are quite similar to fluoride ions in their shape and geometry. We checked the sensor response (CP) for chlorine (Cl^-), bromine (Br^-), iodine (I^-), sulphate (SO_4^-) and carbonates (CO_3^-) with a same concentration of 50 ppm at 20 Hz (figure 3.2). As Cl, Br and I are in same group with same oxidation number and quite close atomic weight so they show three to four folds less response as compare to F^- ions but the sulphate and carbonate which belong to different groups with different oxidation states shows less response which is almost negligible. From this it indicates that the sensor fabricated for fluoride ions is selective for F^- ions even if the other ions have same oxidation state and almost same ionic radii are present in water. High selectivity of this fabricated sensor shows that the cavities generated in the MIP were specific for fluoride ions and their shape is complimentary to the shape of fluoride ions.

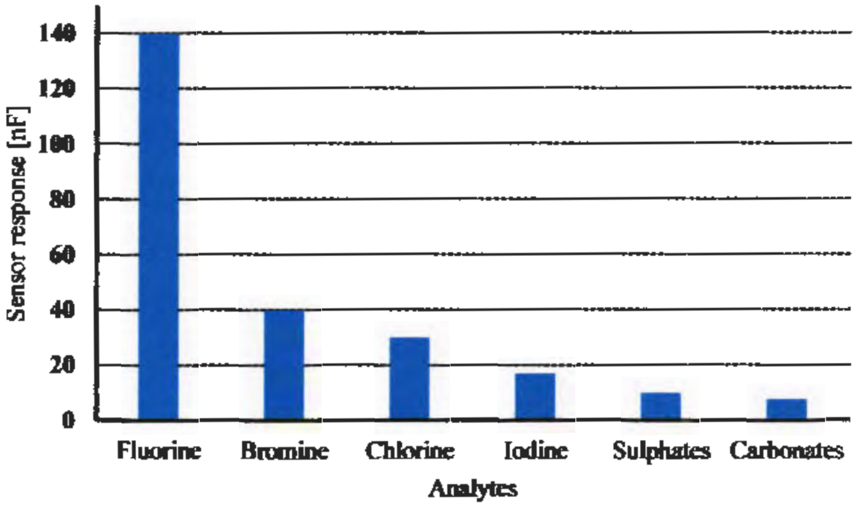

Figure 3.3. vinylpyrrolidone based sensor selectivity response at 50 ppm of each analyte (20 Hz)

Hence it indicates that the F- sensor is only selective for fluoride ions in aqueous environment and can detect and determined fluoride ions in water. Low response of these anions as compare to fluoride ions might be due to the fact that molecular geometry and molecular structure of those interfering anions do not resembles to the molecular structure and geometry of fluoride ions. The cavities produced were complimentary to the size of template molecules i.e. calcium fluoride and can be used for the real-time and online monitoring of fluoride in water.

3.5. Conclusion

In this experiment, a sensor for the calcium fluoride was prepared using two different polymer systems i.e. polystyrene and n-vinylpyrrolidone system. The results of vinylpyrrolidone system were much better as compared to polystyrene system. The fabricated sensor was then analyzed for its selectivity and sensitivity. F^- fabricated sensor shows highly reversibility and linear response linearity with increase in concentration of analyte. The detection limit is 333.3 ppb and its response time is 3 seconds. The sensor also shows high selectivity towards calcium fluoride even in the presence of other competing ions which are chemically and geometrically identical to calcium fluoride. This newly fabricated sensor can be used for the real-time and online monitoring of calcium fluoride in water.

Chapter 4

Generation of Sieves for the Selective Removal of Sodium Fluoride from Water

4. Generation of Sieves for the Selective Removal of Sodium Fluoride from Water

Fluoride is an important element of human health and its overexposure and deficiency resulting adverse effects on bones and teeth. In terms of its effect on human health, the optimal range of concentrations of fluoride in ingesting water is very narrow. In the variety 0.7-1.2 mg/l, it is necessary to prevent enamel and tooth deterioration, however when its concentration exceeds from 1.5 mg/l then it causes skeletal fluorosis and dental fluorosis. According to WHO, in drinking water the maximum limit of fluoride should not exceed from 1.5 mg/L and Pakistan Environmental Protection Agency has also recommended the same limit (Azizullah *et al.*, 2011). Water contaminated with fluoride is a global problem as fluoride ions are toxic for health when its concentration exceeds or decreases from normal level. In the present scenario, there is a continuously increasing worldwide concern for the development of fluoride treatment technologies. One of them is molecular imprinting polymer technology (MIPs). Sodium fluoride has toxic for human health as it causes Teeth Staining and Fluorosis, Allergic Reaction and its single oral dose that is of 50-10 g can be fatal within two to four hours in human adult if he does not get treatment. According to American Cancer Society, Sodium fluoride in ingesting water has been associated to cause a disease called osteoarthritis and osteosarcoma (cancer of bones. The presence of sodium fluoride in water increases the production of cancerous cells and quicken and accelerate the growth of cells. So, to capture fluoride ions MIPs has been synthesized for sodium fluoride.

When molecular imprinted polymer is used as adsorbents it increases the selectivity for the template molecules. Its adsorption can be specific or nonspecific. The nonspecific nature of adsorption of molecular imprinted polymer is due to the hydrogen bonding and electrostatic interactions and its specific nature is due to the imprinting of template molecules. The removal of template molecules is much higher with molecular imprinted polymers. The imprinting sites are favorable adsorption in aqueous environment.

4.1. Materials and methods

4.2. Chemicals and reagents

Chapter 4

All chemicals including Styrene, bisphenol A, 2,2'-azobis-isobutyronitrile (AIBN), divinylpyrrolidone, Ethanol, chloroform, Ethylene glycol dimethacrylate (EGDMA), tetrahydrofuran (THF), Methacrylic acid ($C_4H_6O_2$), were purchased from Sigma-Aldrich and Merck with maximum available purity.

4.3. Instrument and apparatus

Instrument and apparatus that were used in the research project were as follows

4.3.1. Instrument

1. UV-Visible spectrophotometer (T80+ UV/VIS spectrometer)
2. LCR meter
3. Magnetic stirrer
4. Vortex
5. Weight balance machine

4.4. Molecularly Imprinted Polystyrene Synthesis

Styrene system based molecular imprinted polymer is synthesized by mixing 30 μl styrene as functional monomer, 70 μl divinely benzene (DVB) as cross-linker, 0.008g Bisphenol A as a porogen and 0.004g Azobisisiobutyronitrile (AIBN) as free radical initiator followed by addition of 300 μl of ethanol as a solvent having 10 mg of calcium fluoride (CaF_2) is as a template in Eppendorf tube. This mixture was shaken on a vortex and was cooked for two hours at $70\overset{o}{C}$ until gel point is reached. The prepared polymer was coated on glass slides using micropipette. The slides were polymerized in UV for 45 minutes and is further dried in oven at $70\overset{o}{C}$ for overnight for complete adherence of polymer matrix on glass slides. The monomer:crosslinker ratio was 30:70.

4.4.1. Removal of Template - Washing

After then these slides were washed in deionized water for two hours to remove template molecules for the purpose of formation of cavities of template molecules that is fluoride ions. The sodium fluoride is soluble in water that's why it was easily washed. The slides were put in water and stirred on a magnetic stirrer for two hours.

Stock solution of 100ppm of the fluoride was prepared using water as a solvent. Although the polymer was prepared in an organic media yet the solution was prepared in the water. The use of organic media was to avoid the water breaking effect because of hydrophobicity. The glass slides coated with the washed MIP were dipped into the carefully prepared 100 ppm of fluoride solution. After then these polymers coated glass slides were washed with 100 ppm solution of fluoride for 30 minutes. The slides were removed from the above setup and washed with water and then dried. The polymer was still present on it and was reused in the same manner as before. It was then again removed, washed, dried and used. Three times the experiment was repeated

After washing with template solution, the analysis was done on spectrophotometer where the solution was analyzed before and after each treatment for checking the initial and final concentrations and to check the formation of cavities of target molecules that is fluoride ions. Non-imprinted polymer (NIP) were synthesized under similar conditions and procedure but without adding template molecules.

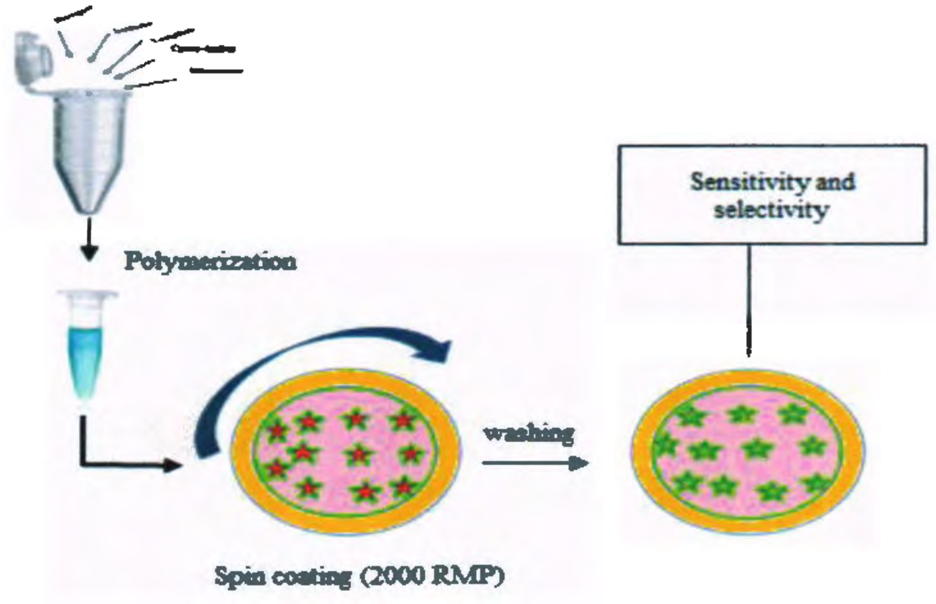

Figure 4.1 MIPs synthesis

4.5. Results and Discussion

4.5.1. UV visible spectroscopic analysis

The solutions were taken at spectrophotometer immediately after preparation in case of initial concentration and after treatment with polymer. The wavelength at which the optical density for sodium fluoride was checked was 420 nm.

The spectra and absorbance of untreated sample taken by UV spectrophotometer was higher absorbance of treated UV and oven sample was less than the untreated sample. The decrease in absorption indicated that polystyrene based molecular imprinted polymer (MIPs) synthesized for fluorides ions has entrapped the fluorides ions because of the formation of sieves which are complimentary to the size and shape of fluorides ions.

Table 1. UV/VIS absorption data

Test solution	**Solution concentration**	**Peak**	**Absorbance**
Untreated solution of sodium fluoride	100 ppm	420 nm	0.155
Polymerized at 70 $\overset{o}{C}$	100 ppm	420 nm	0.055
Polymerized under UV	100 ppm	420 nm	0.054

After the spectrophotometric analysis, the final concentration was decreased. Concentration of treated samples were decreased from untreated samples. The concentration of fluoride solution at 420nm of untreated sample was 0.1555 which was decreased to 0.054 for UV treated sample and when it was polymerized at a temperature of 70$\overset{o}{C}$ it was decreased to 0.055 at the same wavelength.

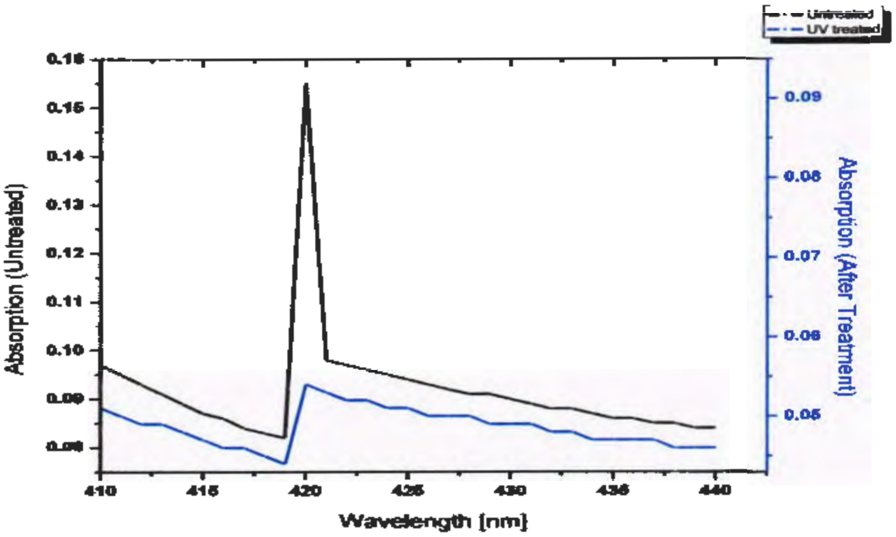

Figure 4.2 Spectrophotometric analysis of treated (polymerized under UV) and untreated NaF solution

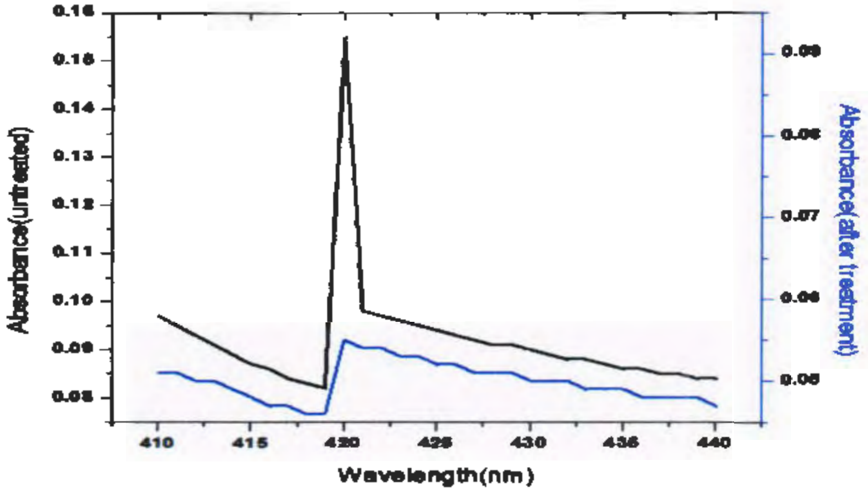

Figure 4.3. Spectrophotometric analysis of treated (polymerized at 70°C) and untreated NaF solution

The UV analysis and the difference between the absorption of untreated and after treatment samples showed that the formation of sieves has been occurred. The size of sieves is complimentary to the size of template of interest i.e. fluoride ions. This molecular imprinted based polymer is thus can be used to determine and remove fluoride ions from water due its great efficiency and capacity to remove and capture fluoride ion from water.

Difference between treated and untreated samples of sodium fluoride are clearly indicated from figure 4.3. there is a linear relationship between concentration of absorbance species and absorbance explained by Beer lambert law. Absorbance of treated sample was much less than the untreated samples which clearly shows that concentration of fluoride is decreased in solution. This indicates that decrease in absorbance was due to the generation of cavities in polymer matrix which captured the fluoride ions. The size cavities were complimentary to the size of template molecules i.e. fluoride ions.

4.5.2. Removal efficiency

14 cm area was covered by the MIPs which was used for treatment of sodium fluoride solution of 100 ppm. The final concentration was decreased after taking the spectrophotometric analysis. As the experiment was repeated for three time, so the mean of these three replicates gave a value of 32.82 ppm for UV polymerized imprinted polymer and 35.76 ppm for the polymer which was polymerized at 70 C̊.

The decrease in concentration was when converted into percentage it was calculated that about 68% removal of sodium fluoride has been occurred for UV polymerized and 65% for polymer polymerized at 70 C̊

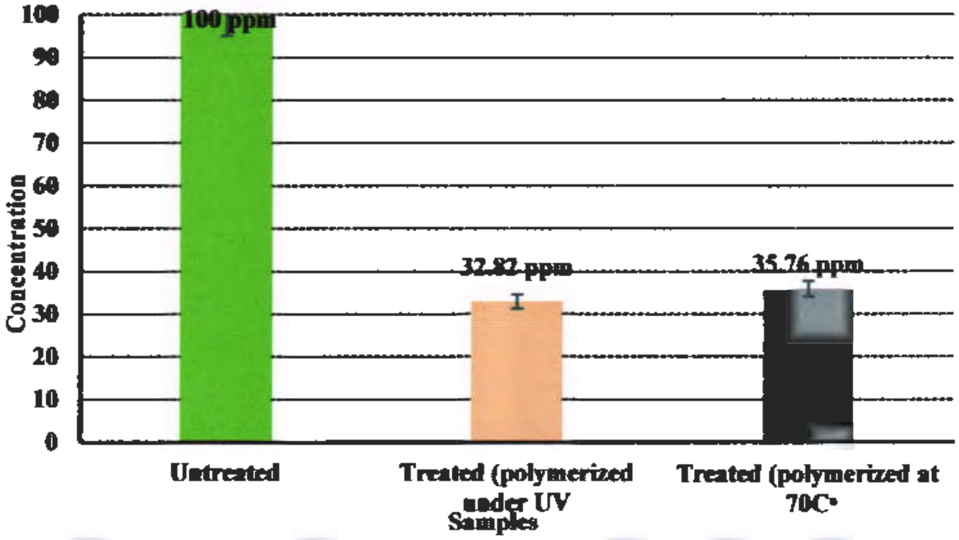

Figure 4.4. Removal of sodium fluoride from the solution using imprinted polymer

The decrease in concentration was when converted into percentage it was calculated that about 68% removal of sodium fluoride has been occurred for UV polymerized and 65% for polymer polymerized at 70 C̊.

The results obtained indicate that synthesized MIPs can be used for the removal of fluorides from water.

4.6. Conclusion

In this work, we fabricated a polystyrene based sieve membranes for the purpose of removal of fluoride ions from water. The polystyrene based sieves membrane is efficient in removing fluoride ions from water and it is also obvious in the shift of absorbance of sodium fluoride solution treated with polystyrene based sieves membrane. The difference in absorption indicates that formation of sieves has been occurred which captured the fluoride ions, thus decreasing the concentration of fluoride solution. This experiment also proved that the technique of molecular imprinted polymers can be utilized for the treatment of water with efficient results for fluoride removal. The molecular imprinted polymers act as an absorbent for this purpose due to the reason that they are selective for fluoride ions and can be reused.

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