MATERIALS AND METHODS

For any experiment, the materials adopted are the aspects, which decide and determine qualitatively the outcome of the research. In this chapter, the design of the present investigation consisted of the following steps.

I. Various reagents used for Electrode membrane preparation

II. Instruments used

III. Preparation of ionophore

IV. Design of membrane

V. Fabrication of ion selective electrode

VI. Selection of ion selective electrode based on electrode response

VII. Various parameters studied (Effect of pH, Medium & Interference study)

3.1. Various reagents used for electrode membrane preparation

3.1.1. Plasticizer and anion excluder for membrane preparation

For electrode membrane preparations generally Dibutyl Phthalate (DBP) and Dioctyl Phthalate (DOP) are used as plasticizer and sodium tetra phenyl borate (NaTPB) is used as an anion excluder.

3.1.2. a. Reagents used for ionophore preparation

To prepare different Ionophores, different reagents were used : Cinnamic acid, Methanol, Sodium carbonate, Cane sugar, Calcium carbonate, Aluminium hydroxide, Ethylacetate, Conc. Hydrochloric acid, Methylene chloride, Magnesium sulphate, Diethyl ether, Sodium hydroxide, Vinyl acetic acid, Glutaraldehyde, Salicyaldehyde, Glycine. High molecular weight Polyvinyl Chloride, Methylene chloride, Conc.sulphuric acid, Silicic acid, Tetra propyl ammonium bromide, Hexadecyl trimethyl ammonium bromide (HDTMA). (Analytical grade), Sodium tetra phenyl borate (Merck), Sodium hydride, 2-bromopropionic acid, Bisphenol-A (Loba), Ethanol. Polyurethane,

3.1.2. b. Different solvents used for membrane preparation

Tetrahydrofuran, Dry Tetrahydrofuran, Methanol, Diethyl ether, Ethanol, Ethyl acetate (Merck), Dimethyl Formamide (DMF).

3.1.3. Cations used for interference Study

In order to prepare different cation standard solutions, analytical grade reagents were used, Manganese chloride, Strontium chloride, Barium chloride, Sodium chloride, Potassium chloride, Magnesium chloride, Cadmium chloride, Nickel chloride, potassium nitrate, potassium iodide, potassium bromide.

3.1.4. Solvents used for electrode performance

To study the effect of solvents in electrode performance Ethanol, Acetone, Dimethyl acetamide (DMA), Dimethyl Formamide (DMF) (Merck) were used.

3.1.5. Buffer solution preparation

To prepare series of buffer solutions the following chemicals were used in analytical grade: Acetic acid, Sodium acetate, Di Sodium hydrogen phosphate, Potassium di-hydrogen phosphate.

3.1.6. Preparation of stock solution

All aqueous solutions were made using distilled water. The stock 1M $CaCl_2$ solution were prepared using distilled water and working solutions were made by dilution of the stock solution.

3.2. Instruments studied

i. UV spectroscopy

For recording the UV & Visible spectrum PC based UV Double beam spectrometer 2202 was used.

ii. FT IR spectroscopy

FT IR spectra were recorded on a FT IR spectrometer; (Model Shimadzu prestige – 21 series).

iii. X-Ray Diffractometry

X-Ray diffraction (XRD) analysis was carried out using PANanalytical X-Pert pro diffractrometer from CuKα radiation (X-Ray tube PW 3050/60 at a current of 30mA and voltage of 45 kV) and X'celerator detector. Continuous scanning was made in the range 10^0<2theta<80^0. The observed phases were identified by comparison of obtained data with those from PANanalytical X'Pert Highscore Plus software.

iv. Impedance measurements

Electrochemical impedance spectroscopy (EIS) is an efficient electrochemical technique for studying a variety of chemical, electrochemical and surface reactions. This technique is used due to the ability of the method to give information on both the bulk and interfacial properties of the polymer coated electrodes. Electrochemical impedance spectroscopy is the process of measuring impedance (complex resistance) response over a wide range of frequencies. The reason EIS provides so much information is due the response of the system changes with frequency. Thus, a broad range of information can be obtained in a single measurement. The electrochemical impedance spectroscopy response is often interpreted in terms of an equivalent circuit. Typically, the components in electronic equivalent circuits are modeled based on electrochemical process taking place at the electrode |electrolyte interfaces that correspond to the Impedance spectra.

The experiments were carried out using a standard three-electrode electrochemical cell. The counter electrode was a platinum electrode and the reference electrode was a silver electrode and working electrode was prepared calcium ion selective electrode.

All measurements were carried out using a Solartron SI 1280B frequency response analyzer. AC signal of 10 mV amplitude, at the open circuit potential (0.1 V) was used to sweap the variable frequencies for the different electrodes.

At each impedance study the frequencies were different because each electrode have different ionophore. So depending upon the ionophore the frequencies were varied.

v. Scanning Electron Microscopy(SEM)

SEM is applied for a numerous applications in various fields of science. SEM analysis was used to study the morphological appearance of the electrodes (using FEI Quanta 3D FEG focused ion beam/scanning electron microscope). In this the ionophore coating on the copper

wire surface was confirmed by this method. SEM allows studying the sample surface directly. The SEM images are obtained by scanning with a high-energy beam of electrons. The electrons interact with the atoms of the sample producing signals that contain information about topography and morphology of the surface, crystallography and elemental composition of the sample.

vi. Electro spinning instrument

Electro spinning is the process of uniaxial stretching of a viscoelastic solution under electrostatic forces for the purpose of nanoscale (diameter) fiber formation**. (**Prepared solution was electrospun into polyurethane nanofibers nonwoven mats by the variation of the initial technical parameters of the electrospinning process at room temperature of 25°C).

3.3. Potentiometry measurements

The electromotive force (EMF) was recorded using digital potentiometer (Equiptronics MODEL EQ 602) in a two electrodes system, working(ISE) and reference electrode(Ag/AgCl) at 25.0±0.5 ºC. The calibration curves for the different analytes were obtained by successive additions of different concentrated analyte solutions (addition method).

3.4. Preparation of Ionophore

i. Ester (Ionophore I)

10 g of Cinnamic acid was taken in a 100ml RB flask. 35 ml of methanol and 4 ml of conc.H_2SO_4 was added to cinnamic acid. It was attached into an air condenser and refluxed for 3 hours. The solution was cooled and added into crushed ice in a beaker. The obtained precipitate was filtered and washed with sodium carbonate solution and cold water. The precipitate was dried and powdered. It was recrystallized from methanol.

Yield : 5 g Melting point: 40^0C

ii. Vinyl acetic acid grafted polyvinyl chloride (PVC) (Ionophore II)

A Co ^{60} gamma irradiator (model GC 5000) with gamma dose rate of 2.5 KGY / hr was used for irradiation of PVC powder. 0.1g of PVC powder sample was irradiated in the dose of 150 KGY. 0.5 ml of Vinyl acetic acid was taken in a stoppered glass tube bubbled with nitrogen gas. The irradiated PVC powder was transferred into glass tube containing vinyl acetic acid and it was maintained at constant temperature (40^o C). The obtained vinyl acetic

acid grafted PVC powder was washed with methanol and water then dried in an oven for half an hour at 40^{o}C.

iii. Calcium lactate enzyme (Ionophore III)

To 10 ml of dilute solution of cane sugar, 5ml of sour milk was added. Temperature was maintained at 40 – 45^{0}C for six days. The bacillus acidi lacti brings forth fermentation. Methyl glyoxal forms as intermediate compound. Acid was removed by the addition of $CaCO_3$ which precipitates calcium lactate enzyme which can be used as an ionophore.

iv. Schiff base (Ionophore IV)

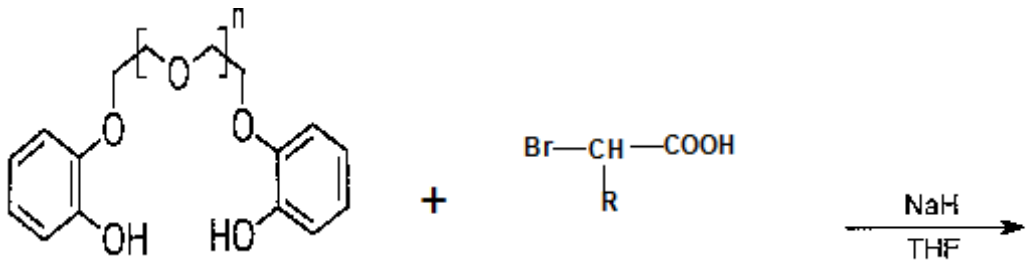
For preparing Schiff base ionophore, 1.1 ml of salicylaldehyde solution was mixed with 0.75g of glycine and the mixture was stirred. 0.4 g of Sodium hydroxide in 15ml of ethanol is added dropwise to the mixture and the final mixture was left for 15 minutes, filtered, washed with cold ethanol and dried.

Yield : 3g Melting point: 198^{o}C

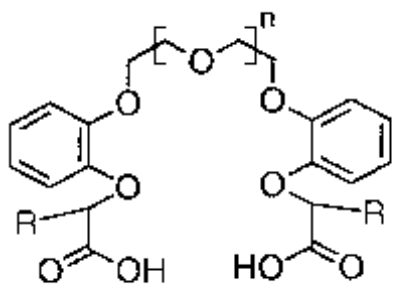
v. Acylic poly ethers:(Ionophore V)

NaH (53% dispersed in mineral oil) present in the mineral oil was removed by using nitrogen atmosphere. A solution of Bisphenol A was prepared by 7.861 g bisphenol A is mixed with 150ml of dry tetrahydro furon and the solution was added to the above solution. 13.2 ml of 2- bromoproponic acid was added dropwise to the reaction mixture and it was stirred for 2h at room temperature, after the addition of acid, the mixture was stirred for 10h at room temperature. Then 9 ml of water is added carefully to the above mixture and ice bath is maintained to destroy the unreacted excess NaH, it gave a homogeneous solution. The aqueous layer was separated and the solution was poured into 50 ml of ethyl acetate, to remove unreacted bisphenol A and organic impurities. The aqueous layer was washed with ethyl acetate. It was acidified with Conc.HCl to pH 1, then the crude was extracted with methylene chloride. The methylene chloride solution was dried over $MgSO_4$ and evaporated in vacuum. The colorless oil was obtained, which was recrystallized from 50 ml of diethyl ether to give white crystals.

The synthetic route for the preparation of acyclic polyether dicarboxylic acid is shown below **(Kim *et al*, 1999).**



Bisphenol A **R: 2-bromoproponic acid**



Acyclic polyether dicarboxylic acid

Yield : 1g Melting point:192^{o}C

vi. Surface Modified Zeolite (Ionophore VI)

2 g of silicic acid was mixed with 3.5g of tetrapropyl ammonium bromide and 0.514g of NaOH and 50ml of water. The above mixture was heated at 100^{o} C for 5 hours. The white precipitate was obtained. The silica content of the precipitate was determined by evaporation method and found to be 1.1ML^{-1}. 1g of the above precipitate was dissolved in 2 ml of water and was mixed with a 1.15 M aluminate solution (1 ml) to adjust the Si/Al molar ratio to 0.5M.The mixed solution was heated at 80^{o}C for 24 hours. The resulting reaction mixture was dried in a oven at 60^{o}C overnight. The surface modified Zeolite was prepared by mixing the above mixture with 100 mM HDTMA solution and stirred for 24 hours. The mixture was then centrifuged at 5000 rpm for 20 min and finally the mixture was dried.

Yield : 2.5 g Melting Point: 201^{0}C

vii. Polyurethane fibres

0.4 g of Polyurethane was dissolved in 2 ml of DMF, the solution was kept overnight in stirring to obtain a homogeneous solution. Then it was subject to electro spinning process. The fibers were coated on copper wire that was polished and irradiated using UV light.

Table 3.1

Conditions involving electro spinning of polyurethane

Conditions	Optimum condition used in this study
Polymer Concentration	12 wt%
Volume Flow Rate	2 ml/hr
Tip to collector distance	20 cm
Electrical Voltage	24 KV
Needle Size	24 gauge

3.5. Design of membrane

3.5.1. For membrane electrodes

The membranes were prepared using varying amounts of ionophore (0.1, 0.2, 0.3 g) and predetermined ratio of PVC, DOP and NaTPB was dissolved in 3 ml of THF and a clear solution was evaporated slowly. Then it was mixed with araldite and spread uniformly over the whatmann filter paper No.42 so that 0.3 mm thicknesses of the electro active membrane were obtained. The membrane was air dried for overnight.

3.5.2. For coated electrodes

These types of electrodes were prepared by dissolving 0.3g of ionophore, predetermined ratio of PVC, NaTPB and dioctyl phthalate in THF. The solvent was evaporated slowly until an oily concentrated mixture was formed.

3.6. i. Fabrication of ion selective electrode (with internal solution)

A circular piece from each of the membrane were cut and fixed with resin at one end of hollow glass tube of diameter 2 cm and length 10 cm. The tubes were filled with 1M solution of calcium chloride and a reference copper metal wire of diameter 0.5 mm and

length 12 cm was inserted through the other end of tube in such a way that it remains dipped in the 1 M solution of calcium Chloride. The electrodes was conditioned for 2 days to attain equilibrium in 1M $CaCl_2$ solution and later the E.M.F measurements were carried out using the following cell assembly,

Internal Reference (1M $CaCl_2$ Solution)	Electro Active Membrane	Sample Solution	External Reference Electrode (Ag/AgCl)

ii. Fabrication of ion selective electrode (Without internal solution)

A copper wire was polished with emery paper and it was washed with distilled water and THF. The copper wire was dipped into the concentrated solution (0.3 g of ionophore+ 0.1g of DOP + PVC + 3 ml of THF) for some minutes, so that a non-transparent coating was formed. The wire was taken out from the mixture and dried overnight. The electrode was finally conditioned to attain stable equilibrium for 10 days by dipping in 1M $CaCl_2$.

iii. Cross linking and Immobilization of copper wire with enzyme

In this part, we developed calcium electrode by copper wire immobilizing with calcium lactate and cross linking molecule (Glutaraldehyde (GA)). We prepared 2.5 % glutaraldehyde solution in 0.1 mM phosphate buffer solution (PBS) as well as calcium lactate solution in PBS having a concentration of 2 mg/ ml of enzyme. After mixing GA and enzyme solution in one bottle, we dipped the copper wire into the solution of enzyme for 15 min to ensure the surface of the copper wire with the monolayer of enzyme and repeated the process for 20, 25, 30 min. We found that all time interval shows the same electrode response and other characteristic features. The immobilized copper wire was taken as working electrode and silver–silver chloride electrode as a reference electrode and the wire was conditioned for 2 days to attain equilibrium in 1M $CaCl_2$ solution.

For this type of electrodes, the E.M.F was measured using the following cell assembly,

Immobilized coated copper wire (working electrode)	Sample Solution (1M $CaCl_2$ Solution)	External Reference Electrode (Ag/AgCl)

3.7. Experimental process

The ion selective electrode was connected to one terminal of a digital potentiometer. The other terminal was connected to the reference electrode (Ag/AgCl electrode). The ISE was immersed in the sample solution calcium chloride and the reference Ag/AgCl is immersed in deionozed water. The solutions are connected using Potassium nitrate agar-agar salt bridge. Before the measurements were started, the electrode was preconditioned in deionozed water until a steady potential was obtained.

Figure 3.1

Experimental set up for direct potentiometric measurements

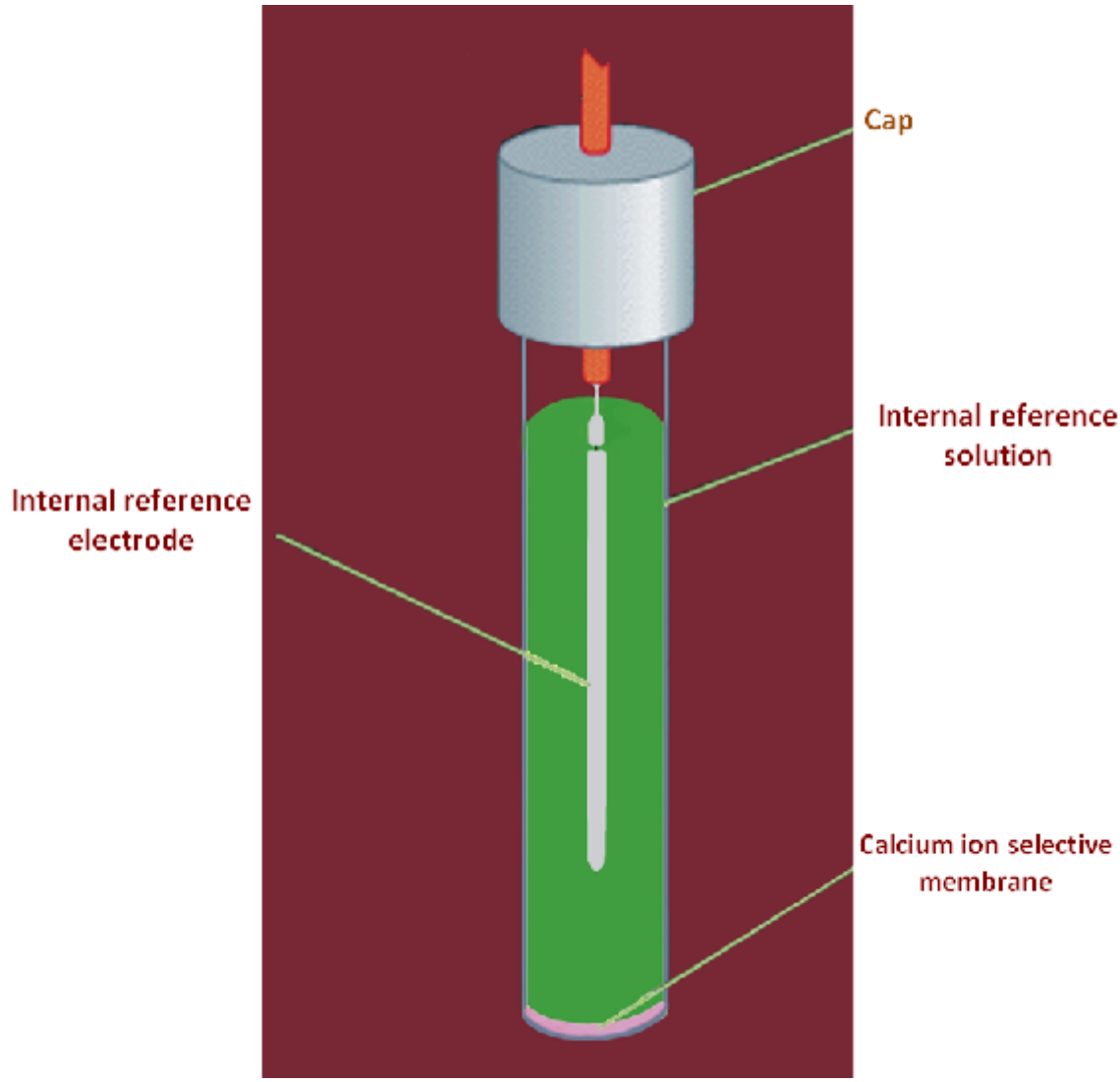


Figure 3.2

Schematic diagram of membrane calcium ion selective electrode

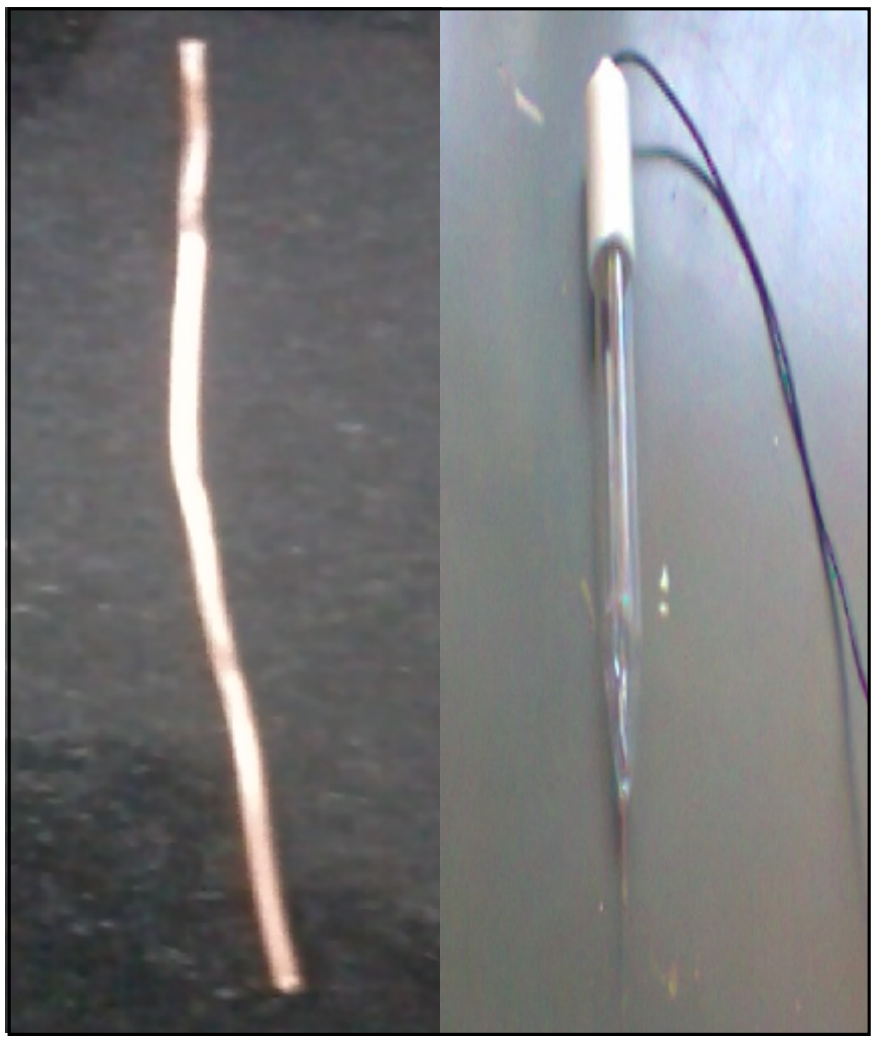


Figure 3.3

Coated calcium ion selective electrodes

3.8. Selection of electrode

According to IUPAC recommendations, the response time is the length of time between the instant at which the ISE and a reference electrode are brought into contact with sample solution and the first instant at which the potential of the cell becomes equal to its steady state value with in 1 mV or has reached 90% of the final value. The response times of all the ISEs prepared were measured using 1M calcium chloride solution. By comparing the response time, the electrode with smaller response time was selected for further analysis.

3.9. Various parameters studied

- Effect of pH
- Effect of medium
- Interference study

3.9.1. Effect of pH

The buffer solutions were prepared using the following buffer mixture. Buffer solutions with pH values of 3.42, 4.63, 5.57, 6.24, and 7.17 were prepared by using 0.02 M acetic acid, 0.02 M sodium acetate, N/15 M disodium hydrogen phosphate, N/15 M potassium dihydrogen phosphate. The effect of pH of the test solution on the response of the membrane electrode was examined at various concentration of $CaCl_2$ solution.

3.9.2. Effect of medium

The functioning of the electrode was also investigated in a partially non-aqueous media using 25%, 50%, 75% of water– acetone, water–ethanol, water-DMA, and water – DMF.

3.9.3. Selectivity

An important performance parameter for an ion-selective electrode is its selectivity towards target ion over other ions. In the design of sensors for environmental determinations, the interference due to other species in the sample poses a challenge to the analysts. Depending on the proposed end-use of the sensor, interference from other species can cause problems for measurement. The selectivity of the method was determined by adding different amounts of potential interfering ions, such as Cu^{2+}, Co^{2+}, and Pb^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Na^{+}, K^{+}, Sr^{2+} etc.

The concentration level of the interfering ion, which an electrode can tolerate, depends on the numerical value of selectivity coefficient. The smaller the value of selectivity coefficient, the higher is the concentration of interfering ion (s), which can be tolerated by the sensor.

The reduced form of Eisenman's equation given below is often used to calculate the selectivity co efficient $logK_{Ca,B}^{pot}$

$$\boldsymbol{logK_{Ca,B}^{pot} = \frac{(E_1 - E_2)}{S} - \left(\frac{n}{Z} - 1\right) * log[a]}$$

Where E_1 and E_2 are the potentials measured in 1 M solutions of interfering ion and Ca^{2+}; S is the calibration slope, n and z are the charges of Ca^{2+} and interfering ions respectively and [a] is the concentration of the ions used. In this method, the concentration of primary ion, Ca (II) ion is varied where as the concentration of secondary interfering ion is kept constant in the test solution which is 1 M.