INTRODUCTION

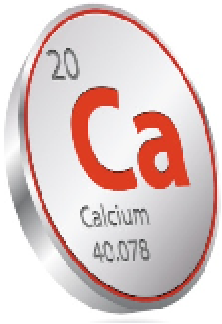
Electrochemistry is the branch of chemistry concerned with the interrelation of electrical and chemical effects. It deals with the study of chemical changes caused by the passage of an electric current and the production of electrical energy by chemical reactions. Electrochemistry is not merely physical chemistry, nor even chemistry, but it covers the field of science from biology through chemistry to physics and material science.

Electroanalytical chemistry is the field of electrochemistry that utilizes the relationship between chemical phenomena which involves charge transfer and the electrical properties that accompany some analytical determination.

Electroanalytical Chemistry plays a very important role in the protection of our environment. In particular, electrochemical sensors and detectors are very attractive for on-site monitoring of priority pollutants as well as for addressing other environmental needs. Sensor is a device that can detect and estimate an analyte of our interest when placed in a complex matrix. Significant technological advances are certain to facilitate the environmental applications of electrochemical devices.

1.1. Calcium

1.1.1. Function of calcium



- Calcium is the part of the architecture of the cell walls and membranes. It involves in cell division, cell growth, activation or inhibition of enzymes.
- Calcium is the major element in the body. Calcium plays an important role in formation of bone. It also plays a vital role in neuromuscular function, blood coagulation and membrane permeability.
- Calcium is also known to be essential plant nutrient. In plants, it helps in transpiration which leads to growth of the plant.
- They are present as soluble salts in all the fluids of the body and they supply the necessary electrolytes for the action of the muscles and the nerves.

Table 1.1

Rich sources of calcium

Food	Calcium level (mg)
Milk	303
Cheese	336
Bread	29
Green leafy vegetables	35
Soy products	310
Meat & Fish	180

Table 1.2

Daily recommended calcium allowances based on Indian Council of Medical Research (ICMR) and National Nutrition Committee
(Narasinga Rao, 2010)

Group	Recommended Calcium(mg/d)
Men	600
Women	600
Pregnant women	1200
Lactating women	
Post – menopausal women	800
Infants(1-12 months)	500
Children(1-12 years)	600
Boys and Girls(13-21 years)	800

Table 1.3

Impact of low and higher calcium levels

	Calcium level	Low calcium	High calcium
Human	1000 mg	• Nerve sensitivity,insomnia(Sleeping disorder It causes mental disorder like fear, stress, anxiety disorder), Palpitations(Abnormal heartbeat – It causes Asthma, Coronary heart disease) • Abnormal heart beat, Tetany, Cramps, Depression, tingling of hands leads to heart attack. • In children it causes rickets, growth retardation, and Bone deformities. **Osteoporosis** • Main deficiency of calcium in human is Osteoporosis this leads to curvature of spin and vertebral collapse.	• Stones and sclerosis in kidney. • Stomach related problems such as nausea, Vomiting and abdominal pain. • It also causes hyperirritability.
Blood	8.2mg/DL	**Hypocalcemia:(**An abnormally low level of calcium in the blood. It can limit blood clotting in-case of injuries so finally its leads to death).	**Hyperparathyroidism:(**An abnormally high level of calcium in the blood. It can be caused by high levels of parathyroid hormone (PTH) and may occur in hyperparathyroidism. It leads to a re-balance in the blood calcium levels. However, in the setting of absent, decreased, or ineffective PTH hormone, the body loses this regulatory function).
Lclic Cell	850mg	• Low level of calcium affects blood coagulation and signal transduction	• High calcium can damage cells.
Soil	400-500 ppm	• Low calcium causes acidic pH in soils. acidic soils are typically low in calcium, plants growing under these conditions are often subject to calcium deficiencies	• Excess amounts of calcium restrict the availability of several elements that are essential for healthy growth in plants
Water	75mg	• Low calcium level risks in heart failure.	• High calcium in water causes lethal (leads to death) conditions.

1.2. Ion selective electrodes

Ion selective electrodes are the type of analytical potentiometric electrochemical sensors. A chemical sensor can be defined as a small device that allows the transformation of chemical information into an optical or electrical signal that can be processed by an instrument. Ion-selective electrodes are used in biochemical and biophysical research, where measurements of ionic concentration in an aqueous solution are required, usually on a real time basis.

Ion selective electrodes (ISE) have been widely used for more than 30 years in a wide range of applications for determining the concentration of certain ions in aqueous solution. Potentiometric methods using ISEs for determining the metal ion have been studied extensively due to their importance in biological process, easy handling and nondestructive analysis and inexpensive sample preparation, applicability to coloured sample and turbid solution.

1.2.1. Types of ion selective electrodes

- Glass membrane electrodes
- Solid-state membrane electrodes
- Liquid membrane electrodes
- Coated wire electrodes
- Modified-membrane electrodes
- Asymmetric electrodes

1.2.1.1. Glass membrane electrodes

The glass membrane pH electrode is an ultimate ion selective electrode. These are used to quantify the concentration of H^+ ion in solution, usually formed from silicate or multi composition glass. Different composition of glass membrane can cause the hydrated glass to acquire increased affinity for monovalent cation with a principle perpendicular to ion exchange mechanism. Thus causes increased cation action which results in electrode potential. The glass electrode gives an idea of specific sensing and it is often and successfully used as an electro chemical sensors. Since H^+ ion is interferent with other ISE the pH must be depending on the concentration of primary ion.

1.2.1.2. Solid state electrodes

The most successful solid state electrode is fluoride electrode. This is useful in determination of fluoride which is difficult in other methods. The potentiometric response of solid state membrane to the target ion is governed by the boundary potential at the membrane solution interference. The sensing ion is precipitated by the reaction with membrane component. Most of the crystalline materials can be used as membrane materials.

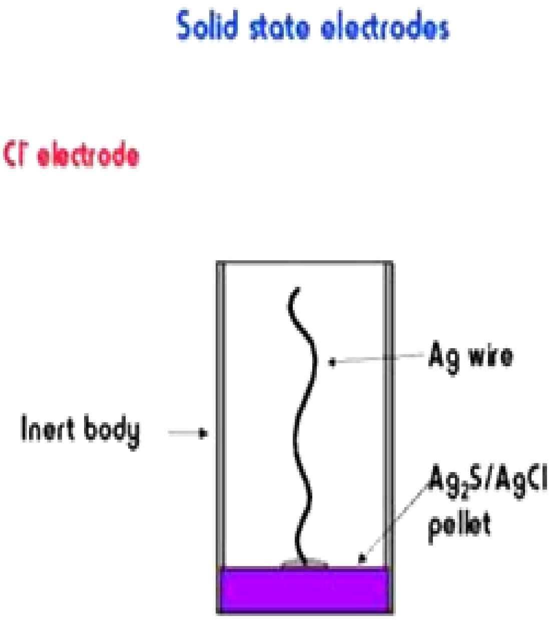


Figure 1.1. Solid state electrodes

The number of solid-state materials is limited. Among those used or considered for selective electrode applications are:

- Rare earth- and alkaline earth metal fluorides
- Halides of silver, lead, mercury and thallium
- Sulfides and other chalcogenides of silver, copper, lead, mercury, cadmium and zinc
- Silver thiocyanate, cyanide, azide, chromate and phosphate
- Mixtures of different halides or chalcogenides with silver sulfide

1.2.1.3. Liquid membrane electrodes

The membrane which determines the potential is made up of water immiscible liquid ion exchanger held by an inert or porous membrane. This can be synthetic, flexible or glass porous. The internal filling solution consists of a specific ion exchanger and an internal reference electrode. The best example is calcium selective electrodes. The sensitivity is seen by the solubility of ion exchange in the test solution. PVC is the most wide used matrix because of the simplicity in membrane preparation.

Among the ion carriers, electrically neutral ionophores have found a wide field of applications as components in ion-selective liquid-membrane electrodes, e.g. in clinical chemistry, electrophysiology, as detectors in ion chromatography, in highly selective transport processes through artificial membranes (also biological membranes). As a result of the introduction of natural as well as synthetic ionophores in ion-selective membranes, ISEs for direct measurement of various cations and anions were designed.

1.2.1.4. Plastic membrane ionophore electrodes

This is very versatile and easy type of electrodes. In this the neutral lipophilic ionophore selectivity complex of the ion is dissolved in a soft plastic membrane. The most important example of this type is potassium ion selective electrode. The plastic membrane is usually PVC based and consists of 33% PVC, 65% of plasticizer, 1.5% of ionophore and about 0.5 % of anion excluder to increase the conductivity and minimize interference from liphophilic anions. Solutions of these components are dissolved in Tetrahydrofuran, and the solvent was evaporated and mounted at the end of an electrode body.

Among all the metal ions, especially alkali and alkaline earth ion, crown ethers are very much useful. Crown ethers are synthetic neutral cyclic ether compounds that can be combined to give a cage of right size of selectively complex ion. A long hydrocarbon of phenyl group is usually attached to make the given compound lipophilic.

1.2.1.5. Coated wire electrodes

The ion selective electrodes can be prepared by simply coating a wire with the PVC membrane to make an electrical conduct Fig.1.1. Thermo dynamically the wire which comes in contact should be well polished in order to make a metal electrode to work. These are very convenient to use. In this the solvent such as tetrahydrofuran (THF) solution of membrane ingredients is coated on the wire and the solvent was evaporated **(Christian, 2004).**

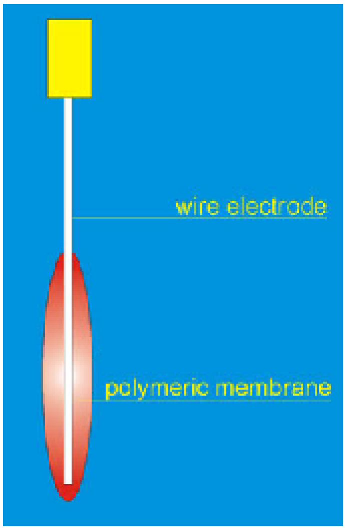


Figure 1.2. Coated wire electrodes

1.2.1.6. Modified electrodes

This is prepared by using composite membrane like an enzyme present in the outer part of the membrane which promotes the specific reaction to unite the ions. This is detected by internal ion selective membrane. This modified electrode otherwise called as potentiometric gas sensors. The important role of this is to detect hydrogen ion and acid base species which interface.

1.2.1.7. Asymmetric electrodes

Many gas-sensitive electrodes and enzyme electrodes have been developed with the potentiometric detection unit based on the conventional electrodes. These electrodes are constructed by immobilizing the ion-exchanger and ionophore in the tips of micropipettes. To eliminate the internal filling solution, the solvent polymeric membrane may be brought into direct contact with a metal wire or other conducting materials, semi-conductors / insulators. By coating thin wires with the solvent polymeric material, miniaturization of potentiometric sensors becomes possible **(Christian, 2004)**.

1.2.2. Properties of ion selective membrane

All of the ion selective membrane in the electrodes share common properties ,which lead to the sensitivity and selectivity of membrane electrodes towards certain cation or anions. These properties include,

1.2.2.1. Minimal solubility

A necessary property of an ion selective membrane is that its solubility in analyte solutions (usually aqueous) approaches zero . Thus ,many membranes are formed from large molecules or molecular aggregates such as silica glasses or polymeric resins. Ionic inorganic compounds of low solubility such as silver halides can also be converted into membranes.

1.2.2.2. Electrical conductivity

A membrane must exhibit some electrical conductivity although small. Generally this conduction takes the form of migration of singly charged ions within the membrane.

1.2.2.3. Selective reactivity with the analyte

A membrane or some species contained within the membrane matrix must be capable of selectively binding the analyte ion. Three types of binding are encountered; Ion –exchange, crystallization and complexation. The former two are the more common and the attention here will be largely focused on these types of bindings (**Anjaneyulu, 2006**).

1.2.3. Characterization of an ion-selective electrode

The properties of an ISE are characterized by parameters like:

i. Measuring range
ii. Detection limit
iii. Response time
iv. Selectivity
v. Lifetime
vi. Reproducibility
vii. Slope

1.2.3.1. Measuring range

The linear range of the electrode is defined as that part of the calibration curve through which a linear regression would reveal that the data points do not deviate from linearity by more than 2 mV. For many electrodes, this range can extend from 1 Molar down to 10^{-6}M.

1.2.3.2. Detection limit

The detection limit of an ISE is calculated using the cross-section of the two extrapolated linear parts of the ion-selective calibration curve, according to the IUPAC recommendation. In practice, the values of the detection limit for most selective ISEs are in the order of 10^{-5} –10^{-6} M.

1.2.3.3. Response time

The time which elapses between the instant when an ion-selective electrode and a reference electrodes (ISE cell) are brought into contact with a sample solution.

1.2.3.4. Selectivity

Selectivity is the most important characteristics of sensors. The smaller the selectivity coefficient is, the less is the interference. There are a number of different methods for the determination of the potentiometric selectivity coefficients namely,

1. The Separate Solution Method (SSM)
2. The Mixed Solution Method (MSM)
    a. Fixed Interference Method (FIM)
    b. Fixed Primary Method (FPM)
3. The matched potential method (MPM)
4. The Unbiased Selectivity Coefficients

Among this all method **fixed interference method** is a most familiar and easy method to calculate selectivity coefficient value.

The reduced form of Nicolksi-Eisenman’s equation given below is often used to calculate the selectivity co efficient K_{Ca}^{pot}

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

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 ions is kept constant in the test solution which is 1M, concentration of interfering ion in the present case.

1.2.3.5. Lifetime

The average lifetime for most of the reported ISEs is in the range of 4 - 10 weeks. After this time, the slope and the detection limit of the sensor will decrease and increase, respectively. It is accepted that the loss of plasticizer, carrier or ionic site from the polymeric film, as a result of leaching into the sample, is the primary reason for the limited lifetime of the carrier-based sensors.

1.2.3.6. Reproducibility

The standard deviation of E.M.F data collected in a series of E.M.F measurements in solutions of different concentrations (after removal and washing and/or wiping of the electrodes) is called reproducibility.

1.2.3.7. Slope

Slope is the linear part of the measured calibration curve of the electrode. The theoretical value according to the Nernst equation is: 59.16 [mV/log(a_x)] at 298 K for a single charged ion or 59.16/2 = 29.58 [mV per decade] for a double charged ion. A useful slope can be regarded as 50-60 [mV per decade] for single charged ions, (25-30 [mV per decade] for double charged ion respectively). However, in certain applications the value of the electrode slope is not critical and worse value does not exclude its usefulness.

1.2.4. Advantages of ion-selective electrodes

1) When compared to many other analytical techniques, Ion- Selective Electrodes are relatively inexpensive and simple to use and have an extremely wide range of applications and wide concentration range.

2) The most recent plastic-bodied all-solid-state or gel-filled models are very robust and durable and ideal for use in either field or laboratory environments.

3) Under the most favorable conditions, when measuring ions in relatively dilute aqueous solutions and where interfering ions are not a problem, they can be used very rapidly and easily (e.g. simply dipping in lakes or rivers, dangling from a bridge or dragging behind a boat).

4) They are particularly useful in applications where only an order of magnitude concentration is required, or it is only necessary to know that a particular ion is below a certain concentration level (semi-quantitative analysis).

5) They are invaluable for the continuous monitoring of changes in concentration: e.g. in potentiometric titrations or monitoring the uptake of nutrients, the consumption of reagents, or point of care monitoring.

6) They are particularly useful in biological/medical applications because they measure the activity of the ion directly, rather than the concentration.

7) With careful use, frequent calibration, and an awareness of the limitations, they can achieve accuracy and precision levels of ± 2 or 3% for some elements and thus compare favorably with analytical techniques which require far more complex and expensive instrumentation.

8) ISEs are one of the few techniques which can measure both positive and negative ions.

9) ISEs can be used in aqueous solutions over a wide temperature range. Crystal membranes can operate in the range of 0°C to 80°C and plastic membranes from 0°C to 50°C.

1.2.5. Applications

The technology of ISE's has been implemented to analyze substances in a wide range of scientific fields. A selection of applications is shown here to emphasize the versatility of the method.

1.2.5.1. Analytical chemistry

Within analytical chemistry, the ability to measure single ion activity at practically any concentration has tremendous influence. Modern ISE's have been shown to span a staggering 8 orders of magnitude in concentration. The pH-electrode is typically of this construction. With these sensors, it is possible to measure on a wide range of different solutions, from aqueous samples and oil to beer or curded milk.

1.2.5.2. Biomedicine and Medico-Technical

The ability to measure on whole blood, instead of having to separate the plasma, add buffer, etc., has had a tremendous influence on the applicability within biomedicine. Technology and engineering has made it possible to put several different sensors in an array, where they measure simultaneously on the same blood sample. Miniaturization has even made it possible to measure intracellular concentrations.

In biomedicine, the detection range is not as important as the reproducibility of the results, since physiological concentrations seldom vary more than one order of magnitude.

The solid-contact polymer-membrane electrodes are durable enough to last for several weeks of use, or several hundred blood measurements. The new generation of apparatus from Radiometer, contains 8 different sensors, making it able to measure the concentration/activity of up to 8 different analytes in a blood sample of less than 0.2ml, in less than two minutes. These platforms are now a standard feature of emergency rooms and intensive care units, where the first measurement is taken to establish a baseline for a patient, and subsequent measurements are taken to detect changes.

1.2.5.3. Environmental

In environmental science, the low detection limit makes it possible to detect trace amounts of analytes. This has been implemented in sea-water and waste-water analyzers. Electrodes have even been constructed that detect non-ionic substances through their interactions with, and influence upon, the regular membrane components.

1.2.5.4. Network application

The Chemical Nose is an example of a different approach. It consists of an array containing a large number of ISE's, with only slightly different compositions. Instead of predicting a response theoretically, the Nose is subjected to a large number of different chemical samples (e.g. 10000) and a neurological network is trained to relate the resulting potentials to substances and their concentrations in the samples.

1.3. Potentiometry

Potentiometry, an analytical technique, emerged at the beginning of the last century. The measuring instruments have undergone many improvements from the old devices, which did not display enough resolution. As a consequence, potentiometers with much higher performance characteristics are available nowadays. Together with the measuring instruments, research on electrochemical sensing mechanisms and their components have been evolving continuously.

In potentiometric analysis, the potential between two electrodes (working and reference electrodes) is measured while the electric current between these electrodes is maintained usually nearly at zero values. Potentiometric sensors offer many benefits due to their versatility and ease of use. Another advantage is the monitoring facilities, using simpler and less expensive devices than other techniques. As a consequence, potentiometric measurements are frequently recorded as routine essays in control and research laboratories. Nevertheless, this kind of sensors also presents some drawbacks such as the difficulty to achieve very low limits of detection and low selectivity in some cases.

Potentiometry with ISM (Ion Selective Membrane) is the method to measure Electromotive Force (E.M.F) associated with the difference in ion concentration between a working electrode and a reference electrode. Theoretically, the E.M.F is governed by Nernstian equation that predicts 30mV increase in E.M.F when ion concentration increases 10 times at room temperature.

1.4. Impact of various additives in ion selective electrode

Sensitivity and selectivity of Pure copper electrode:

From literature study **(Thomas, 1982)** the copper electrode is a highly sensitive and the most successful of the heavy metal electrodes. But it is prone to interference. Example by traces of sulphide, silver and mercury (II ions) and also by iron (II) and cadmium ions and by moderate levels of chloride and bromide. It is particularly sensitized to oxidizing media. Further literature study indicated that the effect of ionophore content in pure copper electrode increases the sensitivity and selectivity **(Mahajan and Sood, (2007)).** To increase the selectivity of pure copper electrode various additives are essential

1.4.1. Plasticizers or Membrane solvent

An additive is essential for the construction of an ion- selective electrode with high analytical performances such as sensibility, selectivity, fast response, and long lifetime.

The membrane solvent reduces the viscosity and ensures a relatively high mobility of the membrane constituents. In order to provide homogeneity of the organic phase it must be compatible with the other membrane components and acts as a plasticizer. Depending on its polarity and dielectric constant, it can influence the ion-exchanger characteristics of the membrane and, as a consequence, the selectivity. The plasticizers that are usually used in ion selective electrode membranes are bis (2-ethylhexyl) sebacate (DOS, apolar) or ortho-nitrophenyl octyl ether (o-NPOE, polar), Dioctyl Phthalate (DOP), Dibutyl Phthalate(DBP).

Plasticizers represent a category of organic substances that can be added to polymers in order to improve some of their characteristics like elasticity and mechanical properties. Plasticizers are very important for the design of the selective polymeric membrane for potentiometric sensors, an important class of the electrochemical sensors. They fix the dielectric constant of the membrane and improve the selectivity of those devices by choosing an appropriate plasticizer as well as an adequate electrode component.

1.4.2. The selection of a proper plasticizer

The desirable properties of a plasticizer used in the membrane preparation of the ion selective electrodes are: compatibility with the polymer, low volatility and low solubility in aqueous solution, low viscosity, low cost and low toxicity (**O'Rourke *et al.*, 2011).** In order to select the best plasticizer usually some tests are necessary. Electrodes with different compositions are built, in which both the nature of the ionophore and of the plasticizers are modified and their proportions in the membranes are changed. The properties of the ion selective electrodes with different membrane compositions are tested. The electrode which has the proper characteristics is selected. The most important considered characteristics are: linear response range, slope (sensitivity), and also selectivity towards the ions that can be present in the analyzed solution. An electrode used to determine species *i* can respond to species *j*. The selectivity coefficient shows the electrode sensitivity ratio for different species. A low value of the selectivity coefficient shows a low interference toward certain chemical species.

1.4.3. Anion excluder or Ionic additives

The permselectivity of the ISE membranes is a prerequisite in order to attain a theoretical response. The permselectivity ensures that no significant amount of the counter ions may enter the membrane phase. To achieve this so-called Donnan exclusion with the electrically neutral carriers, the counter ions (ionic sites) confined to the membrane must be present. Despite the fact that the neutral-carrier based ISE membranes could function properly, even when they contain only a very small amount of ionic sites, the addition of a lipophilic ion salt is advantageous for various other reasons. The anionic interference decreases, observed in the presence of lipophilic anions (e.g. thiocyanate) is the basic reason for the addition of a tetraphenyl borate salt to the membrane of a cation-selective electrode. Simultaneously, the electrical resistance of the membrane is diminished, which is especially important in microelectrodes. The ionic additives are ion exchangers, which themselves induce a selective response if no or only an insufficient ionophore amount is present. It becomes pretty obvious that their concentration must be carefully adjusted.

The most important salts used as lipophilic additives are potassium tetrakis(*p*-chlorophenyl) borate (KTPClPB), sodium tetrakis-[3,5-bis(1,1,1,3,3,3-hexafluoro-2-methoxy-2-propyl)phenyl] borate (NaHFPB), sodium tetraphenyl borate (NaTPB), tetrakis(4-fluorophenyl)borate (cesibor), tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (TFPB) as cationic additive and hexadecylpyridinium bromide (HDPB), hexadecyltrimethylammonium bromide (HTAB), trioctylmethylammonium chloride (TOMACl) as anionic additive.

1.4.4. Importance of cross linking of enzymes

This method involves attachment of biocatalysts to each other by bi- or multifunctional reagents or ligands. In this way, very high molecular weight typically insoluble aggregates are formed. Cross-linking is a relatively simple process. It is not a preferred method of immobilization as it does not use any support matrix. So they are usually gelatinous and not particularly firm. Since it involves a bond of the covalent kind, biocatalyst immobilized in this way frequently undergoes changes in conformation with a resultant loss of activity. Still it finds good use in combination with other support dependent immobilization technologies, namely to minimize leakage of enzymes already immobilized by adsorption. The most commonly used bifunctional agent for cross-linking is glutaraldehyde (GA). The reactive aldehyde groups at the two ends of glutaraldehyde react with free amino groups of enzymes through a base reaction and have been extensively used in view of its low cost, high efficiency, and stability. The enzymes or the cells have been nominally cross-linked in the presence of an inert protein like gelatin, albumin, and collagen and can be applied to either enzymes or cells. The main disadvantages are the undesirable activity losses that can arise from the participation of catalytic groups in the interactions responsible for the immobilization. The cross-linking reaction is not easily controlled and so it is very difficult to obtain large enzyme aggregates with high activity retention. The gelatinous physical nature of the immobilized enzyme preparations is a great limitation in many applications.

The final general method of immobilization, cross-linking enzyme molecules is often brought about by the action of glutaraldehyde. This is a bifunctional reagent (ie).one with two functional groups. In the case of glutaraldehyde, both functional groups are aldehyde, which form linkages with free amino groups on enzymes.

For example, it may be employed to prevent the leakage of enzymes from polymerized gel, which might otherwise occur if the enzyme molecules were too small to be constrained by some of the larger pores. Alternatively, glutaraldehyde might be used to link enzyme molecules to amino groups on a carrier, as well as for causing cross-linking.

Objectives

- The main objective of this study is to prepare new ionophore based calcium ion selective electrodes and utilize them for potentiometric studies.

- To study the principal analytical parameters such as the linear response range, calibration slope, detection limit, effect of pH and non aqueous media, and selectivity for all the electrodes.
- Characterization of the prepared ionophores using UV, FT IR, SEM and XRD.
- To carry out the Electro chemical impedance study to know about the charge transfer process that occurs in electrode/substrate interface.
- To evaluate the analytical application of the prepared electrode in real samples like milk, blood, Hardness of water and some pharmaceutical product by direct potentiometry.
- To apply the developed ion selective electrodes as an indicator electrode in potentiometric titrations.