STUDIES ON DEVELOPMENT OF MEMBRANE SENSORS AND THEIR ANALYTICAL APPLICATIONS

A THESIS

submitted in fulfilment of the
requirements for the award of the degree
of
DOCTOR OF PHILOSOPHY
in
BIOSCIENCES AND BIOTECHNOLOGY

By

ANUPAM

DEPARTMENT OF BIOSCIENCES AND BIOTECHNOLOGY
UNIVERSITY OF ROORKEE
ROORKEE-247 667 (INDIA)

OCTOBER, 1996

CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **STUDIES ON DEVELOPMENT OF MEMBRANE SENSORS AND THEIR ANALYTICAL APPLICATIONS** in fulfilment of the requirement for the award of the degree of **DOCTOR OF PHILOSOPHY** and submitted in the **DEPARTMENT OF BIO-SCIENCES** and **BIO-TECHNOLOGY** of the University of Roorkee is an authentic record of my own work carried out during a period from November 1991 to October 1996 under the supervision of Dr. Ritu Barthwal and Dr. R. Sarin.

The matter presented in this thesis has not been submitted by me for the award of any other degree.

Anupam

Dated : Oct. 30, 1996 (**ANUPAM**)

This is to certify that the above statement made by the candidate is correct to the best of our knowledge.

R Sarin

Dr. R.Sarin
Head, BRT Division
NEERI, Nagpur

R Barthwal 30-10-96

Dr. Ritu Barthwal
Head, Deptt. of Bio-Sciences
and Bio-Technology
University of Roorkee, Roorkee

The Ph.D. Viva-Voce examination of Sri. Anupam has been held on March 18 1997 at 4.00 PM.

R Barthwal 18.3.97

Signature of Supervisors & Signature of H.O.D.

Professor & Head
Deptt. of Biosciences and
Biotechnology
University of Roorkee
Roorkee

Signature of
External Examiner
(Dr. K.G. VARSHNEY)

ABSTRACT

The development of high performance membrane sensors (ISEs) is a fast growing area and the electrodes now take their place among the several recent developments in analytical chemistry which can be claimed as spectacular. The devices find application in the analysis of raw materials, in quality control of the products, monitoring of environment and numerous other situations due to fast speed, sensitivity, cost, reliability and non consumption of sample in the process. ISEs are specially useful in field applications and also in clinical studies where a large number of samples need a rapid, cheap and reliable method of analysis. The device can be used irrespective of colour, viscosity etc., of the test solution and does not require any sample preparation.

Due to major advancement made in this field, efforts have continuously been directed to explore more selective ligands and there have been relatively few notable advances.

A survey of literature as well as market reveals the availability of electrodes for mono and bivalent cations. Sensors for polyvalent cations and anions are still not commercially available. Even those commercially available do not display ideal selectivity. As such investigations for tailor made electro active phase, having specific selectivity for a particular ion specially polyvalent ions are called for.

The development of biosensors is an outcome of the efforts of analytical, biological and clinical chemists involved in perfecting instruments and techniques capable of determining the identity and concentration of living things. Biosensors incorporate a biological sensing element either - intimately connected to or integrated

within a transducer. Biosensing applications are manifold - these devices apart from being concerned with clinical chemistry also find growing applications in fermentation monitoring and control, assessment of industrial and natural environment and in food industry for rapid methods of estimating shelf life, deterioration and contamination. Research interest in this direction is evident in terms of excellent review articles and monographs available on the subject. In spite of a significant development in various aspects of this technique - the device is yet to be commercially exploited and utilized and the field provides a rich area of further investigations.

The work included in this thesis deals with the development of sensors with the help of inorganic gels and bacterial strains. Inorganic gels taken up for investigations were selective to both cations as well as anions. Two inorganic gels viz., titanium ferricyanide and a mixed oxide of titanium-iron doped with cerium were found to exhibit promising cation exchange characteristics and their membranes have been tried for the estimation of potassium and barium ions. Thorium tellurite and stannic tellurite gels, prepared under specified conditions, were anion selective and their membranes were used for the estimation of sulphate and chromate ions. Besides this a glucose sensor has been developed by immobilising lactobacillus bulgaricus and a phenol sensor is fabricated with the help of pseudomonas cruciviae.

Before going into the detailed investigations with a membrane sensor, the amount of membrane ingredients was first optimized so that it demonstrates best performance in terms of detection limit and slope. The time and concentration of equilibrating solution were also ascertained so that the membrane develops

reproducible, noise free and stable potentials. These investigations were performed with all the gel membranes included in this dissertation.

For obtaining the inorganic gels possessing good exchange capacity and selectivity some preliminary experiments were performed to determine the optimum conditions of precipitation viz., the concentration of reactants, mixing ratio, mode of mixing, pH etc. The parameters were set to provide a material which should be stable and possess best exchange characteristics.

Titanium ferricyanide was obtained by mixing titanium tetrachloride (10^{-2} mol dm^{-3}) to ferricyanide (10^{-2} mol dm^{-3}) at pH ~ 1 and 90° temperature. The mixing ratio was 2.5:1. The black coloured product could be sieved to desired mesh size and was stable in acid and salt solutions but decomposes in alkaline medium. The exchange capacity of material for K^+ is 0.18 meg g^{-1}. The chemical formula of the compound, as determined by chemical and T.G. analysis etc., is K(TiO) $[Fe(CN)_6]$. Polystyrene based membrane of this compound has moderate swelling, water content and electrolyte uptake. Specific conductivity data of the membrane in various cationic forms exhibits the sequence:

$$Na^+ > K^+ > Li^+ > Tl^+ > Rb^+ > Cs^+ \text{ and } Zn^{2+} > Ca^{2+} > Mg^{2+}$$

Membranes equilibrated with KCl solution of 0.1 M concentration for 3 days were used for potential measurements with 10^{-1} to 10^{-5} M KCl solutions. 10^{-1} M KCl was used as reference solution. The proposed sensor can measure K^+ in the range of concentration 10^{-1} to 10^{-4} M although it is non-Nernstian in behaviour. The response time is 20 s and the potentials remain constant for three minutes. The sensor can be

used in the pH range 5 to 9 and also in non-aqueous medium up to 30% non-aqueous content.

Selectivity of the proposed sensor was estimated by Fixed interference method. Bi and trivalent cations do not interfere at all in the working of this electrode assembly. Among the monovalent ions Rb^+, Cs^+ and Ag^+ may cause some interference if the same are present at concentrations higher than 10^{-3} M. Na^+ and NH_4^+ interfere even at 10^{-4} M concentration. Changing anions do not cause any disturbing influence.

Other K^+ selective membrane sensors proposed in literature record very limited selectivity and life time of one month only whereas the one under consideration can be used up to four months time.

Mixed hydrous oxides are known to posses better sorption characteristics than simple oxides. Mixed oxides prepared with suitable dopants exhibit even better exchange capacity and selectivity. Ti(IV) - Fe(III) mixed and Ce(IV) doped oxide was prepared by using fresh solutions of ferric chloride and titanium tetrachloride 0.2 M each and 0.01 M solution of ceric ammonium nitrate. Coprecipitation of the oxide was performed with 1 M NH_4OH at pH $\sim$ 6.0. The brownish black product was stable in acid and salt solutions. DTA, TGA and IR has been used to characterise the product. Exchange capacity of the product was 0.57 meg g^{-1} of the material. Polystyrene based membranes were prepared and the values of porosity, water content and electrolyte uptake were found to be maximum for Ba^{2+}. The magnitude of these parameters is more for membranes loaded with monovalent ions.

The conductance data of the membranes in various cationic forms can be arranged in the following sequence:

$Cs^+ > Tl^+ > Rb^+ > K^+$ and $Ba^{2+} > Ca^{2+} > Mg^{2+} > Zn^{2+}$

It was observed that the doped - oxide membranes have higher conductance values than the respective undoped ones. This particular membrane was found to exhibit a much better selectivity for Ba^{2+} as compared to other mono - bi or trivalent ions.

These membranes were equilibrated with 0.1 M $BaCl_2$ solution for 48 h and a linear plot of potentials vs concentration was observed in 10^{-1} to 10^{-4} M concentration. The membrane sensor can measure Ba^{2+} in the range 7 x 10^{-5} to 10^{-1} M concentration as per IUPAC recommendations. The response time is 30 s over the entire working concentration range and the functional pH range is 5 to 9. The electrode system can be used in non-aqueous medium also having a maximum non-aqueous content equal to 25 per cent. Stable potentials are not obtained in solutions having more non-aqueous content. Most of the monovalent, bi and trivalent ions do not interfere with the working of the electrode assembly except Li^+, NH_4^+ and Cu^{2+} ions. These ions may interfere if the same are present at concentrations higher than 10^{-3} M. The membrane sensor has also been used as an indicator electrode in the titration of $BaCl_2$ with K_2SO_4.

Thorium tellurite was obtained by mixing thorium nitrate solution (0.033 M) to potassium tellurite (0.067 M) at pH < 1. Mixing ratio of thorium/tellurium was 0.49. White gel was washed and dried at 60°C and analyzed. Anion uptake capacity of the product, measured in terms of ^{36}Cl uptake was 1.12 meq per gram of the compound. On the basis of chemical analysis data the following formula was assigned to thorium salt:

$Th(TeO_3)_2, 4H_2O$

Functional properties of polystyrene based thorium tellurite membrane are higher than those of titanium ferricyanide and lesser than the hydrous oxide membranes. Conductivity measurements of the membrane in various anionic forms present the following sequence:

$$CrO_4^{2-} > Cl^- > SO_4^{2-} > Br^- = NO_3^- > MoO_4^{2-} > PO_4^{3-} > Fe(CN)_6^{4-}$$

This membrane was found to be quite selective to CrO_4^{2-} ions and so the potentials were measured by fixing the membrane in contact with K_2CrO_4 or $K_2Cr_2O_7$. Before measuring the potentials the membrane was equilibrated with chromium ion solution (0.1 M) for 48 h. The electrode assembly can be used for the estimation of chromium as chromate/dichromate in the concentration range 5×10^{-5} to 10^{-1} M. Response time of the membrane sensor was 20 s and the potentials remain constant for five minutes. The working pH range of the assembly is 3 to 6 if Cr^{6+} is taken as dichromate and 8 to 11 if it is taken as chromate. Electrode can be used in partially non-aqueous solvents also. Selectivity coefficient values for various anions at a level of interference of 10^{-3} M indicate that only Cl^-, Br^- and NO_3^- would interfere if present in large amounts. Among the various cations Pb^{2+}, Ag^+ and Ba^{2+} would interfere at levels at which these decrease the chromium concentration by precipitation. Apart from this the effect of anionic detergent on the electrode response has also been observed. Even small addition of the anionic detergent causes some shift in potentials. It is possible to over come this interference by treating the membrane with the surfactant solution (10^{-5} M) for one hour. The treatment conditions the membrane and it becomes immune to the disturbing effect of anionic detergent.

The membrane sensor has also been used for the titration of chromate ions with barium acetate.

Stannic tellurite gel has also been used for the fabrication of membranes selective to anions. This compound was prepared by slowly mixing 0.05 M solution of stannic chloride to 0.10 M solution of potassium tellurite at pH $\approx$ 1. The product was analysed and the compound can be expressed as Sn $(TeO_3)_2.3H_2O$ on the basis of chemical analysis data. Water content was obtained by the pyrolysis curve. Anion uptake capacity of the product is 1.26 meq per gram of the material (obtained by the uptake of ^{36}Cl). The membrane of this compound could be prepared with the help of araldite as binder. Except conductivity data, other characteristics of the membrane could not be determined due to the presence of araldite. The conductivity order of membrane in different anionic forms is:

$$SO_2^{2-} > Cl^- > NO_3^- > Br^- > CrO_4^{2-} > MoO_4^{2-} > PO_4^{3-} > Fe(CN)_6^{4-}$$

This membrane can be used for the estimation of SO_4^{2-} in the concentration range 10^{-1} to 7.5×10^{-5} M. The response time of the membrane is between 30 to 40 seconds and the useful pH range is 7 to 10. It can also be used in non-aqueous solvents. Among the various anions Cl^-, Br^- and NO_3^- may cause some interference. Cations like Na^+, K^+, Cd^{2+}, Zn^{2+} etc. do not interfere. Alkaline earth metal ions interfere only when these are present in significant amount. Ag^+ and Pb^{2+} affect the electrode assembly at all concentrations.

An anionic detergent SDS, if present, in the system would cause interference. The membrane can be conditioned to tolerate the presence of anionic surfactant by treating it with 5 x 10^{-4} M SDS for two hours.

The electrode assembly could also be used to indicate the end point in the titration of SO_4^{2-} with $BaCl_2$.

The two membrane sensors thorium tellurite and stannic tellurite have been successfully used for the estimation of Cr^{6+} and SO_4^{2-} in waste water from plating, tannery and pulp and paper industry.

Lactobacillus bulgaricus degrades glucose in very short duration of time and this is accompanied with a change in hydrogen ion concentration due to the production of lactic acid. Entrapment technique was used to immobilize the cells of above mentioned strain, for the preparation of membrane. The membrane was coated over a pH electrode with an O-ring and covered with a nylon net. Electrode response was measured with a pH meter. The biosensor can measure up to 2500 mg l^{-1} of glucose and the functional pH range is 5 to 7. The optimum temperature for the working of the electrode is 31°C. Among the various sugars, sucrose, fructose and lactose cause interference if the same are present at concentrations more than 1500 mg l^{-1}. The biosensor remains stable for eight days under operational conditions.

During the course of investigations to identify a suitable bacterial strain for the development of phenol biosensor, it was observed that Pseudomonas Cruciviae is able to degrade phenol in the presence of oxygen and the decrease in oxygen is found to be proportional to phenol concentration. As such the membrane was prepared by

immobilizing the cells with acrylamide, TEMED and ammonium persulphate etc. Membrane was attached to the surface of a gas permeable membrane on the oxygen electrode using an O-ring and was covered with a dialysis membrane. The electrode responds to phenol concentration in the range 0.25 to 4 millimols l^{-1}. Response time of the sensor is four minutes and maximum activity is in pH range 5.5 to 8.0. This sensor also records a positive response to m-cresol, chlorophenols and catechol. Thus these compounds would interfere in the working of this biosensor. Other substituted phenols do not interfere. Stability of the biosensor is one week under operational conditions.

ACKNOWLEDGEMENTS

No words are sufficient to acknowledge the didactic guidance rendered by my teachers **Dr. R. Sarin** and **Dr. Ritu Barthwal** during the tenure of my research work. I consider myself fortunate to have had the opportunity to work under their able guidance.

I owe a deep sense of gratitude to **Dr. R. Sarin** for fruitful discussions from time to time as well as his moral support when I was initially hobbing in research work. His clear cut ideas, an uncanny ability to look beyond the obvious and the urge to fathom the depths of science have often left me in awe and in turn provided me with great sagacity to go beyond myself.

I would like to express my sincere reverence to **Dr. Ritu Barthwal** for her invaluable erudite guidance, benevolent help, great judgement, and support during the entire work. Her intellectual inspiration and moral encouragement will have a niche in my memory as I continue to probe further and deeper in this field in the years to come.

I am grateful to **Professor P.Khanna,** Director, National Environmental Engineering Research Institute, Nagpur for providing me an opportunity to work in this famous institution. The exposure received, while moving through the portals of this institute and the help and guidance provided by Professor P.Khanna would go a long way in my future life and professional career.

I further extend my gratitude and deep-felt thanks to Sri Chandra Kant, Managing Director Chandra Lakshmi Safety Glass Ltd. Haryana who has helped me in every respect to complete this work. Without his encouragement and help it would not have been possible for me to submit this thesis.

I am in dearth of proper words to express my feelings for my friend and brother Dr. Dinesh Mohan, Research Associate, Chemistry Deptt. U.O.R. who has helped me at all times and in every possible manner throughout my research career, I heartily thank and

express my gratitude to Dr. Neeraj Jain for his help in making me aware of the techniques of handling bacterial strains. The help rendered by Dr. Manendra Singh and Vivek Bharti is also gratefully acknowledged.

On the personal fronts I must have an admiration for my all family members specially my mother for providing me the necessary ambience that sustained me during the course of my research work.

A critical assessment of my work and other help provided by my father, **Dr. S.K. Srivastava** (Ex-Professor & Head Chemistry Department, Roorkee University) is gratefully acknowledged.

I am also grateful to the Director National Environmental Engineering Research Institute Nagpur for providing the financial assistance.

Dated : Oct. 30, 1996

(ANUPAM)

CONTENTS

Chapter - 1
Introduction

1.1 INTRODUCTION

A general, strategic aim in analytical chemistry is the simplification of analytical methodology to a level where practical, routine measurement becomes possible with a minimal demand upon operator skills. For an applied scientist, certainly, any analytical system that allows a degree of de-skilling significantly widens the horizons for practical exploitation, must be regarded as an important advancement. An ideal method for the determination of substances in various fields viz. medicine, technology etc. requires the procedure to be technically simple, rapid, inexpensive, sensitive, precise and accurate, using stable reagents which are non-hazardous. Achievement of such practical goals has motivated much of the current research into membrane sensors and explains why these have captured the attention of basic and applied scientists alike.

Selective ion sensitive membrane sensors has been a subject of rapidly increasing interest over the last three decades and the development of membrane electrodes has opened up a large interesting field of potentiometry. The speed at which this subject has developed is a measure of the degree to which the membrane electrodes and probes meet the, requirements of the average analyst for rapid, accurate and low cost analysis. In situations where analysis is performed frequently, these probes are replacing the existing techniques. These probes are without parallel in fields and in situations where a fast trend analysis is required.

Analytical, biological and clinical chemists have for decades concerned themselves with the development of instruments and techniques capable of determining the identity and concentration of living things. This has led to the development of Biosensors - devices incorporating a biological sensing element either - intimately

connected to or integrated within a transducer. Biosensing applications, apart from being concerned with clinical chemistry also find growing applications in fermentation monitoring and control, assessment of industrial and natural environment and in food industry for rapid methods of estimating shelf life, deterioration and contamination. The study of biosensors has been motivated by a strong practical instinct with clear applications always in sight. Instant analysis of clinical samples, in vivo monitoring of metabolites, drugs and proteins using miniature highly portable systems has an obvious appeal. Research efforts made in this direction are evident in terms of some excellent review articles (1.1, 1.2) and monographs available on the subject. Although various aspects of this technique are quite well known, these devices are yet to be commercially exploited and fruitfully utilized. Advancing new strategies in this area of research and attempting to put the same into practical operation, are called for.

Membrane sensors (electrodes) now take their place among the several recent developments in analytical chemistry which can be claimed as spectacular. It is quite evident from the voluminous literature, piled up on this subject in the last two decades although very few electrodes, of this types, have, so far, been commercialised. In spite of tremendous research activity on this topic there are still some gaps which call for more intensive investigations on this technique.

1.2 LITERATURE SURVEY

A number of books, articles and reviews, in which the application and theory of operation of ion selective electrodes are discussed, have already been published and there is an abrupt increase in investigations in this field. It is absolutely impossible to

describe all the significant reports appearing on various types of membrane sensors in few paragraphs of this chapter. Therefore, only relevant references published in the last two decades have been included and in order to maintain a continuity in description some very old research papers have also been cited. It was necessary to do so while describing the chronology of events and significant lands marks on the subject of membrane and bio-sensors.

During the last century the attention of research workers was drawn more and more to the study and utilization of semi permeable membranes. An important step forward, in this field, was achieved by Nernst and Planck. By the end of the century they had defined for the first time the concepts of diffusion and electric potential at the liquid-liquid interface. Study of the electrochemistry of membranes was initiated by Ostwald who introduced the concept of semi-permeable membrane as a membrane impermeable to certain ionic species but permeable to others. These concepts were well established by the end of the last century and eventually led to the development of the theory of membrane potential of porous membranes.

From commercial point of view, the first significant event occurred around 1932 when Arnold Beckman developed the modern glass electrode and a vacuum tube voltmeter to be used with it. In every sense of the word, the glass pH electrode was the first true ion-selective electrode.

A few years later (1937), Kolthoff and Sanders (1.3) published a paper which might have started an ion - selective electrode industry. They prepared molten silver halide discs and showed that the resulting membrane, when placed between two silver solutions, obeyed Nernst law with respect to silver. Systematic investigations, however,

started only in 1940 when Pungor and Coworkers (1.4, 1.5) studied the behaviour of silver iodide precipitate as a model substance. Their paper dealt with the first heterogeneous precipitate based electrode. Thereafter a large number of papers, dealing with precipitate based ion sensors, appeared in literature thereby reflecting the interest generated in this area of research. All this is now a part of History.

The research activity in this field was very much boosted with the development of F^- selective membrane sensor developed by Frant and Ross (1.6). A year later this was followed by a liquid membrane electrode for calcium (1.7). The other contemporary significant development is the work of Stefanac and Simon (1.8) on naturally occurring macrocyclic antibiotics, nonactin homologues in CCl_4, which have been shown to be very selective for K^+.

Review articles by Koryta (1.9 - 1.11) provide details of the initial development, theory, methodology and applications of ion selective electrodes. Besides this some specialized reviews by Buck (1.12 - 1.14) also provide an exhaustive and detailed information on the subject.

Classically membrane sensors can be divided into three categories, viz. homogeneous solid membrane sensors, heterogeneous solid membrane sensors and liquid membrane electrodes. Besides this, the Gas sensors, Biosensors, Enzyme electrodes, Coated wire electrodes etc., formulate a separate class.

Among the homogeneous solid-state halide sensing electrodes, silver halide pellets have been used in titration involving halogen ions and for the determination of chloride in soil extracts, sea water etc., (1.15 - 1.17). Midgley (1.18) has reported a bromide selective electrode for bromine estimation.

Heterogeneous solid state membrane electrodes were fabricated by incorporating electroactive substance into flexible hydrophobic inert binder, silicone rubber. Other commonly used binders are polystyrene, hydrocarbon oil, shellac and poly vinyl chloride. Coetzee and Basson (1.19 - 1.21) fabricated epoxy resin based membranes of tungsto - and molybdo phosphate and found these quite selective to Cs^+ and Tl^+ ions. In this laboratory Malik et al., (1.22) tried tungstoarsenate membranes for the estimation of Tl^+ & Cs^+ whereas Jain et al., fabricated and reported some sensors for Rb^+ and Tl^+ (1.23, 1.24). Solid state electrodes for molybdate (1.25, 1.26) and sodium (1.27) ions have also been reported from this laboratory. Besides this Srivastava et al., have carried out exhaustive investigations on inorganic gels selective to Pb(II) and Cr(VI) ions (1.28, 1.29).

In general the solid membrane sensors have been developed using precipitates, single compound (LaF_3) or mixture AgI - Ag_2S. Rigid matrix (Na^+ glass electrode) and salts like quaternary ammonium compounds have also been used. All these materials share a common property when brought into contact with an electrolyte solution containing the suitable ions - their ability to set up an exchange equilibrium or ion exchange process across the phase boundary. A second requisite property is the ability of the active material to conduct electricity.

Inorganic ion exchange gels combine the above mentioned properties and these do shape quite well as prospective membrane materials for use as ion - selective electrodes. The possibility of using this type of membrane in potentiometric measurements for the determination of ion activities was envisaged in an article by

Geyer and Syring (1.30). Membrane electrodes have been tried for many ions, the only set back at present seems to be the question of selectivity.

Due to their diverse properties, not all the so called inorganic ion exchangers are suitable for use as sensors in the construction of ion selective electrodes. The major limiting factor in most cases is a lack of high degree of selectivity for single cations. These compounds, also, can not be easily pressed into pellets or discs and used as such in electrodes. These have to be incorporated into a membrane matrix to form a heterogeneous membrane. In general the active material is dispersed in silicon rubber, polyvinyl chloride, epoxy resin etc. The support material should be chemically inert, be hydrophobic, be tough and flexible and provide good adhesion for the sensor particles. In turn, the sensor material should be physically compatible with the matrix, have a low solubility product, be of right grain size and be capable of undergoing rapid ion exchange at membrane - sample interface. Hexacyanoferrates of potassium, zinc etc. have been used as ion exchangers. These compounds have also been tried as membrane electrodes. A novel type of potassium selective electrode was introduced by Engel and Graber (1.31). Copper hexacyanoferrate was deposited on glassy carbon. Kuwana and Siperco (1.32) characterized such membranes by voltametric and potentiometric measurements. These membranes show Nernstian response for K^+ and NH_4^+, Jain et al., (1.33) studied the use of chromium hexacyanoferrate (II) epoxy resin-matrix ion exchange membranes as indicator electrodes in potentiometric titrations.

Heteropolyacid salts are the other class of compounds which provide a highly suitable material for the fabrication of membrane electrodes. Coetzee and Basson

(1.20, 1.21) were the first to report the membranes of this class as sensors for alkali metal ions. Malik et al., (1.34) reported epoxy resin based membranes of zirconium molybdophosphate for the estimation of thallium (I) in the range of concentration 2.5 x 10^{-4} to 10^{-1} M. Response time was 25 seconds, the functional pH range was 3 to 6. Same workers (1.22) also reported the use of cesium and thallium tungstoarsenate araldite membranes for Cs^{+} & Tl^{+} ions. The response time of this membrane was quite large, of the order of 2 minutes. Jain et al. (1.35) used a 30% araldite based membrane of strontium - 12 - tungstoarsenate for the estimation of Sr^{2+} in the range 10^{-1} to 10^{-5} M. Functional pH range was 3-6. Silver selective membrane electrode was also proposed by Jain (1.36). This was developed using rubidium - 12 - tungstoarsenate. Srivastava et al. (1.37) estimated rubidium in the concentration range 10^{-1} to 4 x 10^{-5} with the help of titanium tungstoarsenate. Membranes were araldite based (40%). A copper (II) selective electrode was also proposed by Srivastava et al. (1.38). This was fabricated using copper tungstoarsenate and araldite in the ratio 7:3 (mass ratio). It could measure copper in the range 10^{-5} to 10^{-1} M. The response time was 20 s Pyridinium molybdoarsenate - araldite membrane was also proposed for pyridinium ions by the same group (1.39). The range of concentration was not very large (10^{-1} to 10^{-3} M). Response time was 1 minute and the useful pH range was 3 to 6. An ammonium - ion -sensing electrode was reported by Longhi et al. (1.40). The membrane was developed by sintering ammonium molybdophosphate with methacrylate powder at 130°C. The valid concentration range was 3 x 10^{-1} to 3 x 10^{-4} M and pH range was 2 to 9.

Besides hexacyanoferrates, the hydrous oxides of some metals have also been tried as electroactive phase for the fabrication of membrane sensors. Zirconium oxide membrane prepared by the method of Shor et al. (1.41) was found to respond to molybdate ions over the concentration range 0.5 to 10^{-3} M. Functional pH range was between 7 - 11 and the measurements were also possible in partially non-aqueous medium. Hydrous thorium oxide as a polystyrene based membrane shows promising selectivity for Sr^{2+} ions and can be used for its measurements. Even surfactants (up to certain percent) do not affect the selectivity of the membrane (1.42).

It was possible to measure molybdate ions with the help of zirconium molybdate membrane (1.25). Potential vs concentration plot was linear in the range 10^{-5} to 5 x 10^{-3} M and the operational pH range was 6.5 to 9.0. VO^{-}_{3}, NO^{-}_{3} and CNS^{-} caused serious interference in the working of this electrode assembly.

A few membrane sensors for mercury have also been tried. One of these is based on propylthiophosphoryl thiobenzamide (1.43). The other has been prepared by pressing Hg_2SO_4 at 1000 kg per cm^2 for five minutes (1.44). Recently Srivastava et al. have used polytungstoantimonate gel membrane for the estimation of Hg^{2+}. Normal interferents do not disturb the functioning of this membrane electrode (1.45).

Another ion for which no electrode has been commercialised so far is Zn^{2+}. In most of the reports Cu^{2+}, Cd^{2+} & Pb^{2+} cause interference and remain a problem. Some recent references (1.46, 1.47) describe the efforts made in this regard. They have tried a carbon support or graphite paste for the fabrication of membranes.

Some very recent references on solid membrane sensors deal with the flow injection analysis of sulphate (1.48), determination of K^+ at 10^{-5} M concentration (1.49) and subnanomolar detection of phenols (1.50).

In spite of a good deal of work already reported on solid state membrane sensors incorporating inorganic precipitates, crystals and inorganic gels, there are still some gaps which call for more specific investigations. Very few electrodes have been commercialized - to the satisfaction of customers. Even those marketed by M/S Orion, Beckman etc., suffer with the problems of sensitivity. Moreover sensors for polyvalent cations and anions are yet to be developed.

Of late substrates like conducting polymers, crowns, cryptands and calixarenes have been used for the fabrication of membranes and development of electrodes. Among the various neutral carriers, crown ethers are the most important class of compounds. The complexation between the crown ethers and metal ions is facilitated by the formation of ion-dipole bond of sufficient energy caused due to localized charge on the ethoxylate units in crown ethers. Long back Eyal and Rechnitz (1.51) had reported valinomycin to be a suitable neutral matrix for K^+ determination. Other ethers have been tried (1.52 - 1.55) for the estimation of alkali metal ions. Efficacy of crown ethers for the said purpose depends on the cavity size of the ligand, bond strength of the metal complex and exchange of bonded metal ions. Most of the work reported with such ligands deals with the development of sensors for alkali or alkaline earth metals.

Calixarenes and Cryptands are finding importance as active agents for chemical sensors because of their very selective complexation behaviour towards some ions. Forster and co-workers (1.56) incorporated calixarenes into plasticized PVC membranes

to produce ISEs. Change in functional groups, cavity size and molecular conformation allowed the selectivity of the corresponding electrodes to be controlled. Tetrameric calixarenes gave electrodes highly selective to alkali metal ions (1.57, 1.58).

It is well known that cryptands form very strong complexes with metal ions (1.59). As a result the ion exchange between membrane solution/interface is a little restricted in such compounds and probably this is the reason why cryptands so far have not been much exploited for the purpose. Even then some recent work includes the estimation of K^+ by cryptand 222B (1.60) and that of Rb^+ by cryptands having two thiourea fragments (1.61). Very recently Srivastava et al., have used 222 cryptand membrane for the estimation of Zn^{2+} (1.62).

Periodical reviews in Analytical Chemistry cover all the work appearing on this subject. The three review articles published in 1990, 1992 & 1994 include all the significant reports on this subject up to the year 1993 (1.63 - 1.65).

Biosensors, meaning sensors which incorporate biological materials in their structure were first described in 1962 at New York Academy of Sciences Symposium (1.66). In that presentation the use of enzyme transducers as membrane enclosed sandwiches was described to make electrochemical sensors more intelligent. These became specific for certain substrates by detection of a product of an enzyme catalysed reaction or a drop in a substance used in the reaction.

Application areas where biosensors are set to make a significant impact reach well beyond the established needs of medicine and veterinary science. These additional areas include environmental monitoring and control, food processing, bioprocessing, agriculture, pharmaceuticals and even defence and petrochemical industry (1.67).

Increased research effort in biosensors is certainly matched by a burgeoning literature, with excellent reviews and monographs (1.68 - 1.70). This dissertation includes the efforts made to develop glucose & phenol biosensors. As such only some recent & significant references, on these two species, are described here.

Urea measurement has been a popular target for basic researches; urease immobilized over a pH ISFET (1.71) used to follow the consumption of H^+ during enzymic hydrolysis or over an ammonia gas electrode to follow NH_3 generation (1.72) are two recent examples. For reasons of functionality and availability, enzymes have been the predominant bioreagent used in potentiometric devices. Besides this a number of biosensors have also been proposed for glucose. Cespedes and Valero (1.73) proposed an amperometric glucose biosensor based on a composite material made of graphite - epoxy - glucose oxidase. The analytical system showed a linear response for glucose in the range 10^{-5} to 10^{-2} M at pH 7.0. These authors also proposed a glucose electrode for amperometry (1.74). Karyakin (1.75) also proposed a prussian blue based biosensor for amperometric estimation of glucose. Cardosi (1.76) has described the manufacture of disposable strip-type electrode for glucose. Nyamsi Hendji (1.77) have immobilized glucose oxidase on aminosilanized Pt to develop a micro electrode of glucose. Besides this, a single shot reagentless biosensor for the amperometric determination of glucose has been proposed by Gilmartin et. al (1.78).

Methods for the determination of diamines and phenols have also been reported using tyrosinase (1.79). Tyrosinase - graphite - epoxy biocomposite has been utilized as an amperometric biosensor for phenols (1.80). Some workers have tried tyrosinase within a carbon paste matrix for the estimation of phenols (1.81, 1.82).

The importance of a reliable biosensor for glucose is quite well documented and there is ample scope of further work in this field. A fast and easy method of determination of phenol is of great importance for various industries and monitoring of environmental pollution and for this the use of phenol biosensors appears to be quite promising. Besides this new materials for constructing transducers or effecting links between the components of a sensor is an exciting avenue for research.

1.3 THE PROBLEM

Titanium ferricyanide gel and a mixed oxide of titanium - iron doped with cerium was prepared. The compounds are highly cation selective. As such their membranes were fabricated, characterized and tried for the estimation of potassium and barium. Thorium tellurite and stannic tellurite gels prepared under specified conditions are highly anion selective. Their membranes have been tried for the estimation of SO_4^{2-} and Cr^{6+} as CrO_4^{2-}. Where ever possible the membranes under investigation have been used to estimate the concerned species in waste effluents.

Besides this, efforts have also been made to develop a biosensor for glucose by immobilising lactobacillus bulgaricus, and also for phenol by immobilising pseudomonas cruciviae. The details are given in subsequent chapters.

1.4 REFERENCES

1.1. Vadgama, P. and Crump, P.W., "Biosensors: Recent Trends A review", "Analyst" 117, 1657 (1992).

1.2. Scheller, F., Schubert, F. & Moritz, W., "Clinical application of biosensors", "Analyst" 114, 635 (1989).

1.3. Kolthoff, I.M. and Sanders, H.L., "Electric potentials at crystal surfaces .....", J.Am. Chem. Soc., 59, 416 (1937).

1.4. Pungor, E. and Hollos-Rokosinyi, E., "The use of membrane electrode in the analysis of ionic concentrations" Acts Chim. Acad. Sci. Hung., 27, 63 (1961).

1.5. Pungor E., "Theory and applns. of anion-selective electrodes" Anal. Chem., 39, 28A (1967).

1.6. Frant, M.S. and Ross, J.W., "Electrodes for sensing fluoride ions" Science, 154, 553 (1966).

1.7. Ross, J.W., "Ca-selective electrode with liquid ion exchanger" Science, 156, 1378 (1967).

1.8. Stefanac, Z. and Simon, W., "Ion-specific electrochemical behaviour of macrotetrolides in membranes" Microchem J. 12, 125 (1967).

1.9. Koryta, J., Theory and Applns. of ion selective electrodes I. Anal. Chim. Acta, 61, 329 (1972).

1.10. Koryta, J., Theory and Applns. of ion selective electrodes II. Anal. Chim. Acta, 91, 1 (1977).

1.11. Koryta, J., Theory and Applns. of ion selective electrodes III. Anal. Chim. Acta, 111, 1 (1979).

1.12. Buck, R.P., "Ion selective electrodes, potentiometric titrations". Anal. Chem., 46, 28R (1974).

1.13. Buck, R.P., "Ion selective electrodes". Anal. Chem., 48, 28R (1976).

1.14. Buck, R.P., "Ion selective electrodes". Anal. Chem., 50, 18R (1978).

1.15. Dessouky, Y.M. and Pungor, E. "Appln. of anion-selective membrane electrode in pharmaceutical analysis" Analyst, 96, 442 (1971).

1.16. Lamm, G.C., Hansen, E.H. and Puzicka, J. "In situ use of ion selective electrodes ..." Anal. Lett., 5, 451 (1972).

1.17. Lerman, A. and Shatkay, A., "Degree of halide saturation ... measurement by electrode". Earth and Planetary Sci., Lett. 5, 63 (1968).

1.18. Midgley, D., "A bromide selective electrode - redox electrode for determination of bromine.Talanta, 30, 547 (1983).

1.19. Coetzee C.J. and Basson A.J., "Potentiometric determination of Cs^+". Anal. Chim. Acta., 56, 321 (1971).

1.20. Coetzee C.J. and Basson A.J., "Potentiometric determination of Cs^+". Anal. Chim. Acta., 57, 478 (1971).

1.21. Coetzee C.J. and Basson A.J., "Potentiometric determination of Tl^+". Anal. Chim. Acta., 64, 300 (1973).

1.22. Malik, W.U., Srivastava, S.K. and Kumar, S., "Tungstoarsenates as ion selective membranes for Cs^+ and Tl^+. J. Electroanal. Chem., 72, 111 (1976).

1.23. Jain, A.K., Singh R.P. and Agrawal, S., "Picolinium molybdate-membranes as Tl^+ selective electrode". Fresenius Z. Anal. Chem., 302, 407 (1980).

1.24. Jain, A.K., Singh R.P. and Agrawal, S., "Solid membrane for Rb^+" Indian J. Chem., 19(A), 1029 (1980).

1.25. Malik, W.U., Srivastava, S.K. and Bansal, A., "Potentiometric estimation of molybdate" Anal. Chem., 54, 1399 (1982).

1.26. Srivastava, S.K., Sharma, A.K. & Jain, C.K., "A polystyrene based membrane sensitive to molybdate ion". Talanta, 30, 285 (1983).

1.27. Srivastava, S.K., Kumar S. & Jain, C.K., "Determination of sodium by ion selective potentiometry", Analyst, 109, 667 (1984).

1.28. Srivastava, S.K., Kumar S. & Jain, C.K., "Ion exchange membrane selective to lead ions". Talanta, 33, 717 (1986).

1.29. Srivastava, S.K., Jain, C.K. & Kumar, S., "Estimation of Cr (VI) in water, tannery and plating wastes". Mikrochim Acta., 377, 111 (1986).

1.30. Geyer, R. and Syring, W., Z. Chemie., 6, 92 (1976).

1.31. Engel, D. and Grabner, E.W., "Studies with hexacyanoferrate membranes". Ber. Bunsenges. Phys. Chem., 89, 982 (1985).

1.32. Kuwana, T. an Siperco, L., "Studies with potassium selective membranes". J. Electrochem. Soc., 130, 396 (1983).

1.33. Jain, A.K., Srivastava, S.K. and Agrawal, S., "Potentiometric titrations with hexacyanoferrate membrane electrodes". Talanta., 25, 531 (1978).

1.34. Malik, W.U., Srivastava, S.K. and Bansal, A., "Studies with heteropolyacid salt membranes". Indian J. Chem., 22A, 221 (1983).

1.35. Jain, A.K., Srivastava, S.K. and Agrawal, S., "Tungstoarsenates as membrane sensors". J. App. Chem. & Biotech., 27, 680 (1977).

1.36. Jain, A.K., Srivastava, S.K. and Singh, R.P., "Silver selective membrane electrode". Anal. Chem. 51, 1093 (1983).

1.37. Srivastava, S.K. and Kumar, S., "Membrane electrode for Rb^+". J. Electroanal. Chem., 161, 345 (1984).

1.38. Srivastava, S.K., Pal, N. and Singh, R.P., "A copper sensitive membrane sensor". Indian J. Chem., 22A, 1033 (1983).

1.39. Srivastava, S.K., Jain, A.K. and Agrawal, S., "Heteropolyacid salt membrane electrode for pyridinium ions". Talanta., 25, 157, (1978).

1.40. Longhi, P., Mussini, T. and Rondinini, S., Nouv. J. Chim., 3, 649 (1986).

1.41. Shor, A.J., Kraus, K.A., Smith, W.T. and Johnson, J.S., J. Phys. Chem., 72, 2200 (1968).

1.42. Srivastava, S.K. and Jain, C.K., "Membrane sensor for Sr^{2+} ions". Bunsegi Kagaku, 33, E525 (1984).

1.43. Szczepaniak, W. and Oleksy, J., "Hg^{2+} electrode based on Isopropylthiophosphoryl thiobenzamide" Anal. Chim. Acta., 189, 237 (1990).

1.44. Zhang, S.X., Lu, P.Y. and Sun, N.Y., "Preparation of an ion selective electrode for Hg (II)". Yaowu Fenxi Zazhi., 14, 32 (1994).

1.45. Srivastava, S.K., Sahgal, V. and Vardhan, H., "A solid inorganic gel membrane sensor for mercury". Sensors and Actuators (B) 13, 391 (1993).

1.46. Rocheleau, M.J. and Purdy, W.C., "Investigations of materials for making a carbon supported zinc electrode". Talanta., 37, 307 (1990)

1.47. Raba, J., Fontan, C.A. and Cortinez, V.A., "Diaminobenzene-graphite paste electrode for Zn^{2+}". Talanta., 41, 273 (1994).

1.48. Tang, T.C. and Huang, H.J., "Flow injection analysis of SO_4^{2-}...". Anal. Chem., 67, 2299 (1995).

1.49. Bratov, A., Nataliya, A. and Bartroli, J., "Photocurable matrices sensitive to K^+". Anal. Chem., 67, 3589 (1995).

1.50. Vorlop, K., Strechlitz, B. and Stottmeister, U., "Detection of subnanomolar level of phenols". Anal. Chem., 67, 65 (1995).

1.51. Eyal, E. and Rechnitz, G.A., "Mechanistic studies on the valinomycin based potassium electrode". Anal. Chem., 43, 1090 (1971).

1.52. Lukayanenko, N.G. and Melink, O.T., "Sodium selective electrodes based on PVC membranes containing ether derivatives". Anal. Chim. Acta., 259, 145 (1992).

1.53. Tsingarelli, R.D. and Zolotov, Y.A., "Trans-cyclohexano crown ethers as sensors for metal ions". Analyst., 117, 853 (1992).

1.54. Moriarty, R.M., Rao, M.S.C. and Gilardi, R., "Crown ether ionophores. Construction of neutral carrier electrodes". J. Am. Chem. Soc., 115, 1194 (1993).

1.55. Bakeer, E., Pretsch, E. and Meyerhoff, M.E., "Selectivity of neutral career based membrane electrodes". Anal. Chem. 66, 3021 (1994).

1.56. Forster, R.J. and Harris, S.J., "Calixarenes as active agents for chemical sensors". Sensors and Actuators (B) 4, 325 (1991).

1.57. Domansky, K. and McKervery, M.A., "Sodium selective electrode based on p ter-butyl calix [4] arene" Analyst., 118, 341 (1993).

1.58. Shibutani, Y., Yoshinaga, H. and Shono, T., "Sodium selective electrode based on calix [4] aryl mixed ester type derivatives". Bunsegi Kagaku., 43, 333 (1994).

1.59. Gokel, G.W., "Crown ethers and cryptands". The Royal Soc. of Chemistry. Cambridge (1992).

1.60. Gojowski, J., Rieckemann, B. and Umland, F., "Potassium selective electrode based on cryptand 222B". Fresenius Z. Anal. Chem., 309, 343 (1991).

1.61. Lukayanenko, N.G., Titova, N.Y. and Scherbakov, S.V., "Cation selectivity of PVC membranes based on cryptands with thiourea fragment". Anal. Chim. Acta., 263, 169 (1992).

1.62. Srivastava, S.K. Gupta, V.K. and Jain, S., "PVC-based 2,2,2-Cryptand sensor for Zn^{2+}". Anal. Chem., 68(7), 1272 (1996).

1.63. Janata, J., "Chemical Sensors". Anal. Chem., 62, 33R (1990).

1.64. Janata, J., "Chemical Sensors". Anal. Chem., 64, 196R (1992).

1.65. Janata, J., and De Vaney, D.M., "Chemical Sensors". Anal. Chem., 66, 207R (1994).

1.66. Clark, L.C. Jr. and Lyons, C., "Electrode system for continuous monitoring in cardiovascular surgery". Ann. N.Y. Acad. Sci., 102, 29 (1962).

1.67. Jarvis, M.T., "Biosensors: To day's Technology". SEAI Tech. Pub. Madison, (1990).

1.68. Scheller, F., Schubert, F. and Moritz, W., "Fabrication of biosensors and applications". Analyst., 114, 653 (1989).

1.69. Thompson, M., and Krull, U.J., "Trends in biosensors". Anal. Chem., 63, 393 (1991).

1.70. Kraube, A.P.F. and Wilson, G.S., "Biosensors - Fundamentals and applications". Oxford Univ. Press, (1989).

1.71. Lehman, J., Biotechnology, 8, 729 (1990).

1.72. Narinesingh, D., Mungal, R. and Ngo, T.T., Anal. Chim. Acta., 49, 387 (1991).

1.73. Cespedes, F., Valero, E. and Alegret, S., "Fermentation monitoring by a glucose biosensor". Analyst., 120, 2255 (1995).

1.74. Cespedes, F., Martinez, E. and Algret. S., "Amperometric enzymic glucose electrode based on a graphite composite". Anal. Chim. Acta., 273, 409 (1993).

1.75. Karyakin, A., Gitelmacher, O.V. and Karyakina, E.E., "Prussian blue based first generation biosensor". Anal. Chem., 67, 2419 (1995).

1.76. Cardosi, M.F. and Birch, S.N., "Screen printed glucose electrode based on platinized carbon particles and glucose oxidase. Anal. Chim. Acta., 69, 276 (1993).

1.77. Hendji, N. and Bataillard, P., "Covalent immobilization of glucose oxidase on a silanized Pt. micro-electrode". Sensors and Actuators (B), 15, 127 (1994).

1.78. Gilmartin, A.T., Hart, J.P. and Patton, D., "Prototype solid phase glucose biosensor". Analyst., 120, 1973 (1995).

1.79. Macholan, L. and Jilek, M., "An enzyme electrode for the determination of aromatic diamines and phenols". Collection Czechoslovac Chem. Commun. 49, 752 (1988).

1.80. Wang, J., Fang Lu. and Lopez, D., "Amperometric biosensor for phenols based on Tyrosinase - graphite composite". Analyst., 119, 455 (1994).

1.81. Bonakdar, M. and Mottola, H., "Biosensor based on tyrosinase immobilized in carbon paste". J. Electroanal. Chem., 49, 266 (1991).

1.82. Ortega, F., Cuevas, J.and Dominguez, E., "Estimation of phenol with biosensors". J. Pharm. Biomed. Anal., 10, 789 (1994).

Chapter - 2

Methodology & Materials

2.1 SYNTHESIS, CHARACTERIZATION AND PROPERTIES OF INORGANIC GELS

During the last three decades inorganic ion exchangers have attracted much attention as these exhibit better selectivity and stability towards temperature and ionizing radiations. Among the different class of compounds of this type, the insoluble salts of polybasic metal ions, heteropoly acid salts and hydrous oxide of some metals like Zr, Sn, Fe, Al etc. have been studied intensively and these show important ion exchange characteristics. Some of the compounds, having good ion exchange properties have been reported from the chemical labs of this institution (2.1-2.4).

Preparation of tailor made materials for the fabrication of "Ion-selective electrodes" possessing ideal selectivity is an ongoing research activity. In the process, inorganic ion exchangers have also been tried and some good sensors have been proposed for the estimation of cations and anions of analytical importance (2.5-2.8).

Electroactive phase used for the fabrication of membranes, included in this dissertation, consists of thorium ferricyanide, cerium doped titanium-iron mixed oxide, thorium and stannic tellurite. The first two substances are cation exchangers while the other two are anion selective materials. The compounds were prepared by the procedure already reported. Methodology was optimised to provide substances with optimum exchange characteristics.

(a) Preparation, characterization and properties of titanium ferricyanide gel

For obtaining the product in gel form, showing a good exchange capacity (2.1) and selectivity some preliminary experiments were performed to determine the optimum conditions of precipitation viz., the concentration of titanium and

potassium salt solutions, mixing ratio, pH of precipitation and the order of mixing. The material having best exchange capacity, stability etc. was obtained by mixing titanium tetrachloride (concentration 10^{-2} mol dm^{-3}) to potassium ferricyanide (concentration 10^{-2} mol dm^{-3}) at pH ~ 1 and 90°C temperature. The mixing ratio of the two solutions was Ti^{4+}: ferricyanide as 2.5:1. The precipitate was left overnight in contact with mother liquor at room temperature and was then filtered, washed and dried at 90°C.

The product is of black colour, can be ground and sieved to a desired mesh size, is fairly stable in acids and salt solutions but decomposes in highly alkaline medium. It was converted to hydrogen form by immersing in a solution of 1.0 mol dm^{-3} HCl for 48 hours. The exchange capacity of the material (for K^+) is 0.18 meq g^{-1}. Exchange capacity does not change up to 100°C and a fall in the same was observed on further heating the compound. At 400°C the compound did not exhibit any exchange characteristics.

For analysis a weighed sample of the gel was fused with an excess of sodium peroxide in a platinum crucible. The fused product was dissolved in hydrochloric acid and a known volume of solution was made up with double distilled water. A suitable amount was reduced with hydroxylamine hydrochloride and analysed spectrophotometrically for iron at 510 nm using 1,10-phenanthroline. Ti^{4+} was estimated spectrophotometrically (2.9) with the help of H_2O_2 and K^+ was determined by flame photometry (2.10).

The chemical analysis of the product (Ti, 15.86; Fe, 17.96; K, 12.60; H_2O, 28.40%) gives the molar ratios K:Fe:Ti of 1:1:1. The TG analysis showed a complete loss of water at 350°C and the decomposition of the product started at 500°C. The proposed chemical formula of the compound is:

$$K\ (Ti\ O)\ |Fe(CN)_6|.$$

This was further confirmed by infrared spectroscopy. The I.R. spectra of the product exhibited sharp cyanide, Fe-C and Ti-O stretching frequencies.

The uptake of various cations, on this product, follow the sequence:

$$Na^+ > K^+ > Ag^+ > Li^+ > Rb^+ > Cs^+ \quad \text{(for monovalent ions)},$$

$$Pb^{2+} > Mg^{2+} > Ni^{2+} > Mn^{2+} > Zn^{2+} > Ca^{2+} > Hg^{2+} > Cd^{2+} \quad \text{(for bivalent ions)},$$

and $$Fe^{3+} > Cr^{3+} > Al^{3+} > La^{3+} \quad \text{(for trivalent ions)}$$

The series is different from the one normally reported for conventional organic resins but similar behaviour has been reported for a number of inorganic ion exchangers (2.11).

(b) Preparation, characterization and properties of cerium doped titanium-iron mixed oxide

Hydrous oxides have also been used for the uptake and separation of metal ions. Mixed oxides are found to possess more specific selectivity and a good deal of work on such systems has been reported by Venkateswarlu and coworkers (2.12,2.13). Besides this the mixed oxides prepared with suitable dopants exhibit even better exchange capacity and selectivity. Ti(iv) -Fe(iii) mixed and Ce(iv) doped oxide has been prepared and the electro analytical performance of the membrane of this compound has been investigated.

A good deal of preliminary investigations (2.14) were performed to fix up the concentration of reactants and mode of preparation so that the compound possesses optimum exchange characteristics. Doped mixed hydrous oxide was prepared by using fresh 0.2M solution of ferric chloride and 0.2M solution of titanium tetrachloride and 0.01M solution of ceric ammonium nitrate. 62.0 ml of ceric ammonium nitrate solution was added to one litre solution of titanium and

iron salts (Ti:Fe ~ 9:1 ratio). Coprecipitation of the oxide was performed with 1M NH_4OH at pH ~ 6.0.

The product was obtained in the form of brownish black granules which was sieved easily to desired particle size. It is stable in acidic solutions (above pH 2.0) and in salt solutions up to 1M concentration.

DTA curves of the product had endothermic peaks at 130, 270 and 330°C corresponding to the removal of adsorbed water molecules and the removal of structural hydroxyl groups. The splitting of endothermic effect of dehydration into distinct peaks possibly point to the structural inhomogeniety of the sample suggesting for more than one type of phases possessing distinct features simultaneously present in the gel.

TG plots indicate that the loss in weight takes place in different steps at temperatures corresponding to the endothermic and exothermic peaks in DTA curves. I.R. spectra of the compound provided evidence for water and structural hydroxyl groups and metal oxygen lattice vibrations etc.

The exchange capacity of the doped product is 0.57 meq g^{-1} of material as against the exchange capacity of the mixed oxide [Ti(iv)-Fe(iii)] which is equal to 0.22 meq g^{-1}.

The doped-mixed hydrous oxide exhibits very high uptake of Ba^{2+}, Cu^{2+} and Ni^{+} ($Ba^{2+} > Ni^{2+} > Cu^{2+}$). Besides this the selectivity sequence of various ions is:

$Cs^{+} > Li^{+} > Rb^{+} > Ag^{+}$;

$Ba^{2+} > Ni^{2+} > Cu^{2+} > Mg^{2+} > Zn^{2+} > Sr^{2+}$;

$La^{3+} > Fe^{3+} > Cr^{3+} > Al^{3+}$

(c) Preparation, characterization and properties of thorium tellurite

Again a wide range of experiments were performed to determine the optimum

reactant concentration and precipitation conditions so that the resulting product possesses good exchange characteristics (2.15).

Thorium nitrate solution (0.033M) was slowly added to potassium tellurite (0.067M) at pH < 1. Mixing ratio of thorium/tellurium was 0.49. The white gel, so obtained, was washed thoroughly with distilled water and dried in an air oven at 60°C for six to eight hours till it was completely dry. The product was converted to homoionic form, washed with deionized water and dried again.

The compound was fused with excess of sodium peroxide in a platinum crucible and extracted with HCl. Tellurite was estimated as thiourea complex and thorium was measured by titration with EDTA at pH 2.0 using Xylenol orange indicator.

The compound is fairly stable in acids, bases and salt solutions. On the basis of chemical analysis data (Th- 39.6, Te-42.6, H_2O-10.6%) the formula assigned to the product is Th $(Te\ O_3)_2$. $4H_2O$. The theoretical values calculated on the basis of this formula are (Th-39.8, Te-43.7, H_2O-11.0%). Water content was calculated by the method of Alberti et.al. (2.16) from the pyrolysis curve obtained in thermal analysis. A weight loss up to 310°C was observed for this compound. I.R. spectra of the compound depicted stretching vibration of co-ordinated water and deformation oscillations of free water molecules, besides the broad characteristic band of tellurite ion.

The cation exchange capacity, as determined by routine method was 0.52 meq g^{-1} of the compound. Anion exchange capacity of the product, measured in terms of ^{36}Cl uptake, is 1.12 meq per gram of the compound. The exchange capacity, however, decreases at higher temperatures due to loss of water molecules. Since the compound has significant anion exchange capacity the uptake of anions was investigated on this product and the same exhibits the following affinity series:

$$PO_4^{3-} > SO_4^{2-} > CrO_4^{2-} > SCN^- > VO_3^- > Cl^- > Br^-.$$

The metallic tellurite does not exhibit much of affinity for monovalent ions and also there is no proper gradation with respect to ionic size etc. in polyvalent anions.

(d) Preparation, characterization and properties of stannic tellurite

This compound was prepared by slowly mixing 0.05M solution of stannic chloride to 0.10M solution of potassium tellurite (Sn^{4+}/Te^{4+} = 0.50) at room temperature and at pH $\approx$ 1. The gel formed was digested with mother liquor for 20 hours at 60°C. It was then washed with distilled water and dried in an air oven at 60°C till completely dry. The conditions to provide a product with optimum exchange characteristics had been ascertained earlier. The compound was converted to homoionic form and then washed and dried in an air oven.

For analysis 0.2g of stannic tellurite was treated with aqua regia for 24 hours and then with concentrated NaOH to get stannic precipitated as hydroxide. Tellurite was estimated in filterate as thiourea complex at 360 nm spectro photometrically (2.17). The precipitate was again dissolved in concentrated HCl and tin was determined spectrophotometrically at 530 nm using dithiol as reagent (2.18).

The compound is quite stable in acid, base and salt solutions and on the basis of chemical analysis data (Sn-24.6, Te-53.8, H_2O-10.6%) can be expressed as $Sn(TeO_3)_2$. $3H_2O$. The theoretical values as calculated on the basis of this formula are (Sn-25.2,Te-54.3, H_2O-10.9). Water content was obtained from the pyrolysis curve. A weight loss up to 279°C was observed for this compound. I.R. spectra had some common peaks, as obtained in the case of thorium tellurite, corresponding to water and tellurite ion.

The cation exchange capacity is 0.54 meq per gram of the compound and the anion uptake capacity (obtained by the uptake of ^{36}Cl) is 1.26 meq g^{-1}. In this case also the exchange capacity decreases with temperature. In view of the significant anion uptake shown by the compound its affinity for other anions was also investigated and the following affinity series was obtained.

$$SO_4^{2-} > PO_4^{3-} > MoO_4^{2-} > CrO_4^{2-} > SCN^- > VO_3^- > Cl^- > Br^-.$$

In this case also the monovalent ions are least preferred and other anions do not follow any proper gradation.

2.2 REQUIREMENTS OF THE PRECIPITATE BASED SOLID MEMBRANE ELECTRODES

There are certain stringent requirements which any material must satisfy before it can act as a successful membrane in a solid state electrode device. Moody and Thomas (2.19) have summarized the properties of the solids to be incorporated in solid membrane sensors.

The active sensor material must preferably be non-porous, that is, imporous, solicit the minimum photoelectric response, must have good mechanical strength and not be readily scratched or abrazed. Besides this, it is ideal if the substance is available as a large crystal or else it should have sufficient tenacity to be mouldable as a membrane. During the pressure process, stress may cause the decomposition of metal. This can be a serious drawback, since the electrode could then be subjected to interference from redox systems. Further it is also important that the membrane material should be water insoluble. In addition to this, it must also exhibit a good selectivity as well as good response time characteristics. For practical manouvering it should be capable of

easy tight sealing to the electrode body, otherwise leak paths would arise between the internal reference solution and the sample solution. For ideal conditions it should be capable of producing a Nernstian response.

For the construction of heterogeneous membranes a suitable matrix support is required. In addition to the above requirements, the active material should be physically compatible with the matrix. Further, it should be of appropriate grain size (1- 15 nm) which is a function of precipitation technique. It is of utmost importance that the sensor material must be mixed with the matrix support in the right proportion. This is essential in order to maintain physical contact between sensor particles, and provide electrical conduction through the membrane, otherwise the resistance may be too high. The material should undergo rapid ion exchange at the membranes sample interface.

Virtually all commonly available ion-exchanging solids, used as single crystals or pressed pellets, have been successfully tried in heterogeneous form and translated into practical and commercial reality. Commonly used binders are PVC, polystyrene, silicon rubber and araldite. The presence of binder does not affect the mode of operation.

2.3 PREPARATION OF POLYSTYRENE SUPPORTED MEMBRANES

Solid state electrodes fall conveniently into two classes, homogeneous and heterogeneous. Pungor has emphasized that the terms homogeneous and heterogeneous relate to the composition, and not function of an electrode. Any theoretical discrimination between the two classes is undesirable owing to their similar mechanisms. The membrane of a typical solid state electrode is an "all-in one" sensor-cum-support matrix, whereas a heterogeneous membrane comprises of at least two components, one the active sensor such as a simple insoluble salt, a chelate or ion exchange resin, and the other an inactive matrix support.

It was in 1950, with the advent of ion exchange resins, that heterogeneous membranes incorporating the commercially available ion exchangers into thermoplastics polymers were prepared by hot pressing a mixture of ion exchange granules and polystyrene. Since then heterogeneous membranes have been prepared by using a number of methods.

Heterogeneous electrodes are those where the active materials are dispersed in the widely used inactive support e.g., polystyrene (2.20,2.21) and poly vinyl chloride (2.22,2.23).

The suitable matrix support must be chemically inert, provide good adhesion to the sensor particles, be hydrophobic, tough, flexible, yet nonporous and crack resistant to prevent leakage of internal solution. The matrix should not swell in sample solutions. Any extensive swelling disrupts the active chain of sensor particles.

Heterogeneous membranes, obtained by embedding polystyrene in respective gels, were found to be quite satisfactory. Various physico-chemical properties could easily be studied with these membranes. The following method based on U.S. Patent No. 2,614, 976(20) was employed to prepare the polystyrene supported membranes.

Preparation

Polystyrene granules were heated in a glass tube in sulphuric acid bath at 200°C. The molten mass was allowed to cool down to room temperature and the polystyrene rod was taken out after breaking the glass tube. The polystyrene rod was ground to fine particles by putting it against a lathe. It was then finally ground to get a 50 mesh sieve product.

Membranes were prepared by mixing an appropriate amount of polystyrene and inorganic gel and heating the homogeneous mass in a die kept in a metallurgical

specimen mount press at particular temperature and pressure. The optimum quantity of polystyrene needed to prepare a stable membrane was determined by trials.

The optimum temperature, pressure and other conditions such as rate of cooling etc., were fixed up after a great deal of preliminary investigations and are mentioned for each membrane at appropriate place. Membranes prepared in this way were quite stable and did not show any dispersion in water or in different electrolyte solutions. It took almost two months to ascertain the best conditions which provide stable membranes capable of generating reproducible potentials. These were also examined under an electron microscope for any cracks and homogeneity of the surface.

2.4 PREPARATION OF ARALDITE BASED MEMBRANES

Sometimes the heterogeneous membranes prepared by embedding the exchanger in polystyrene or PAC matrix are not of adequate strength and get broken during the use or do not show adequate electrochemical performance. In such cases araldite can be used as the binder. Some araldite based membrane electrodes (2.24,2.25) have already been reported from this laboratory.

Preparation

The powdered samples of the electroactive material and araldite were mixed up thoroughly in the required ratio, on a watch glass and the paste was made homogeneous. This was then spread out thinly over a piece of filter paper and gently pressed and left as such in air for 24 hours. When the membranes get dried up, circular pieces of ≈ 2.0 cm diameter were cut manually with the help of razor and adhering filter paper was also removed, by immersing it in water. A number of membranes were thus prepared and only those having smooth surface showing no cracks and undulations (checked under optical microscope) and having a regular

thickness (≈ 0.5 mm) were selected for further studies. The minimum amount of araldite required to give adequate stability to membrane was determined by trials and is mentioned for each membrane at appropriate place.

2.5 DETERMINATION OF FUNCTIONAL PROPERTIES OF MEMBRANES

The pre-requisite for understanding the performance of an ion exchange membrane, particularly the perm-selective one, is the complete physico-chemical characterization. A survey of the existing literature reveals that this particular aspect of membrane phenomena has received lesser attention. Comprehensive and critical studies on the characterization of the ion exchange membranes, is therefore justified. Normally the process involves the determination of those parameters which affect the electrochemical properties of membranes e.g. porosity, electrolyte absorption electrical conductance etc.

The first major attempt to establish standard methods for membrane characterization was reported on the dried collodion membrane (2.26). Hale and co-workers (2.27) examined the effect of resin content and the degree of crosslinking of the resin on the physical and electrochemical properties of membranes while Wyllie and Kannan (2.28) reported that if a rigid plastic such as polystyrene is used, the properties of the ion exchange membrane get modified. Gregor (2.29,2.30) and Kawabe (2.31) studied the characterization of ion exchange membranes in a number of different exchange states and correlated this information with the structure. From this laboratory Jain and co-workers (2.32) have reported functional properties of pyridinium molybdoarsenate and rubidium and copper (II) tungstoarsenate membranes while Srivastava and co-workers investigated the functional properties and permeability of chromium ferricyanide and cerium (IV) molybdate membrane (2.33).

The electrical conductance of system are also quite important (2.34). The ohmic resistance of a membrane of high ionic selectivity measured under standard conditions provides an accurate and quantitative measure of relative ionic permeabilities. Generally, larger the conductivity, greater is the ionic premeability and vice versa. A number of techniques have been used by various workers (2.35) for the measurement of membrane conductance. Barrer (2.36) measured the resistance with and without a membrane in 0.01M solution of chloride or nitrate of the ion under study. The difference between the two is taken as the membrane resistance. Lakshminarayanaiah (2.35) has given a direct method of measuring the membrane resistance which gives better results as compared to the methods followed by earlier workers.

Standard methods used for the determination of functional properties (water content, porosity, swelling, electrolyte absorption and electrical conductance) are given below:

a. Water content

The membrane was soaked for 24 hours in a solution of 1.0M concentration of electrolyte. It was then washed several times with distilled water, wiping out the adhering liquid with a blotting paper. The soaked membrane was weighed and later dried to a constant weight in vacuum desiccator at 60°C. The difference in the two weighings, divided by the weight of the wet membrane was taken as the water content.

b. Porosity

It was determined by the method followed by Mizutani and Nishimura (2.37) and was calculated using the formula:

$$\Sigma = \frac{\text{Water content}}{\text{A.L. } \rho_w}$$

Where, A is the area of the membrane, L the thickness and ρ_w is the density of water.

c. Swelling

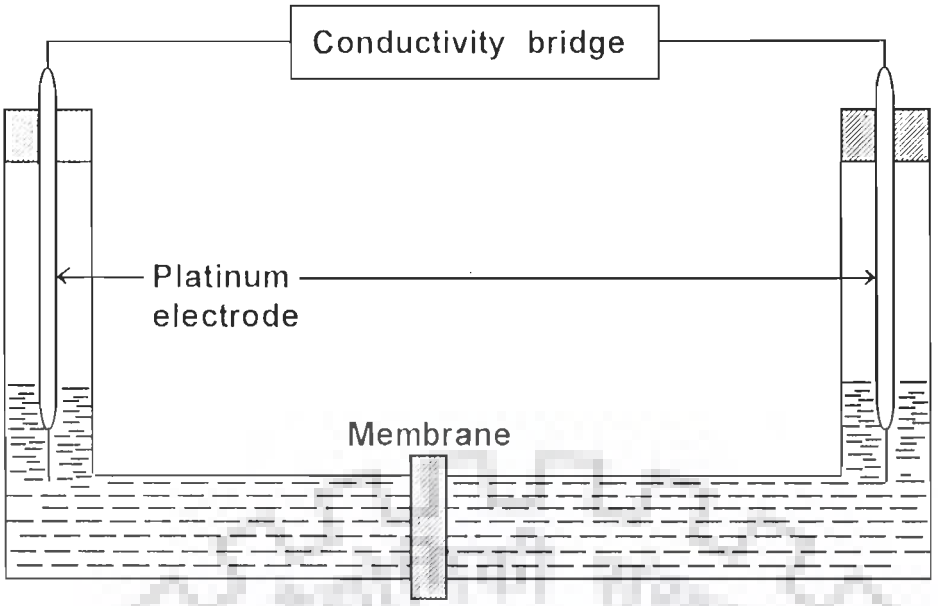
After measuring thickness of the dry membrane it was immersed in a solution of electrolyte for one day and the thickness was measured again after wiping it out with blotting paper. Difference between the thickness of dry and swollen membrane was taken as a measure of swelling.

d. Electrolyte absorption

The membrane after attaining equilibrium in electrolyte solution was wiped free of adhering electrolyte and dipped in 20 ml of distilled water. It was intermittently shaken and left as such for a few hours. The solution was transferred to a 100 ml measuring flask. The whole process was repeated three to four times and the entire solution was collected in a measuring flask. It was finally made upto the mark by distilled water and the strength was measured conductometrically.

e. Conductance

The modified method adopted by Lakshminarayanaiah and Subrahmanayam (2.35) which makes use of mercury pool on both sides of the membrane to minimize polarization at the membrane interface, was employed for conductance measurement. The experimental setup is shown below :



The membrane was cemented in between the two half cells with the help of araldite and kept in contact with electrolyte solution of 0.1M concentration. The solution was then replaced by mercury previously equilibrated with the electrolyte solution of the same concentration and the conductance was measured by connecting the platinum electrodes to a conductivity bridge.

2.6 THEORY AND MEASUREMENT OF MEMBRANE POTENTIALS

The use of ion-selective electrodes depends on the determination of membrane potentials. These potentials cannot be determined directly, but can be easily derived from the e.m.f values for the complete electro-chemical cells, which comprise the membrane separating solutions 1 and 2 as well as the two reference electrodes (2.38).

When the ion exchange membrane separates two solutions 1 and 2 both containing the same counter ion A, a membrane potential (E_m) is developed across the membrane due to the diffusion of counter ions from higher to lower concentration. The membrane potential (E_m) which is the sum of the diffusion and Donnan potentials, is given by the expression (i)

$$E_m = \frac{RT}{ZF}\left[\ln \frac{\left(a_A\right)_2}{\left(a_A\right)_1} - (Z_y - Z_A)\int_1^2 \bar{t}_y \, d\ln a_{\pm}\right] \qquad \text{(i)}$$

where, A = counter ion, Y = co-ion, Z = charge on ions and $\bar{t}_y$=transference number of the co-ions in the membrane phase; $\left(a_A\right)_1$ and $\left(a_A\right)_2$ represents activities of the counter ions in solutions 1 and 2 and $a_{\pm}$ is the mean ionic activity of the electrolyte.

The right hand side of expression (i) consists of two terms. The first term gives the thermodynamic limiting value of the concentration potential while the second term denotes the diffusion potential due to co-ion flux in membrane.

If the membrane is considered to be an ideally perm-selective one ($\bar{t}_y$=0), then the equation (i) takes the forms of the Nernst Equation.

$$E_m = \pm \frac{RT}{Z_A F} \ln \frac{\left(a_A\right)_2}{\left(a_A\right)_1} \qquad \text{(ii)}$$

This equation simply represents Donnan potential for an ideally perm-selective membrane or it can be said that it gives the thermodynamic limiting value of concentration potential, Equation (ii) takes positive sign for cation and negative sign for anion .

The membrane potential measurement is carried out using a cell setup of the following type:

Reference Electrode (SCE)		Test Solution (2)			Internal Solution (1)		Reference Electrode (SCE)
	$E_1(2)$		Membrane			$E_1(1)$	

As a general practice, the concentration of one of the solution (say 1) is maintained constant (usually 0.1M) and this solution is referred to as internal or reference solution and a saturated calomel electrode in contact with this solution is used as a reference electrode. The membrane together with internal solution and the connected reference electrode is one compact unit which, as a whole, is called as membrane electrode. This membrane electrode is then immersed in solution 2, usually referred as external solution or test solution, having SCE as the reference electrode., Some other electrode like silver/silver chloride electrode may also be used as reference electrode. The e.m.f. of this potentiometric cell is given by the following expression :

$$E_{cell} = E_{cal} + E_L(2) + E_m + E_L(1) - E_{cal} \qquad \text{(iii)}$$

Where E_{cal}, E_L and E_m refer to calomlel electrodes, junction and membrane potentials, respectively. Combining equation (ii) and (iii), the equation (iii) takes the form

$$E_{cell} = E_{cal} - E_{cal} + E_L(2) + E_m + E_L(1) \pm \frac{RT}{Z_A F} \ln \frac{\left(a_A\right)_2}{\left(a_A\right)_1} \qquad \text{(iv)}$$

For cation exchange membrane ,

$$E_{cell} = \left[E_L(1) + E_L(2) - \frac{RT}{Z_A F} \ln (a_A)_1\right] + \frac{RT}{Z_A F} \ln (a_A)_2 \qquad \text{(v)}$$

As activities of internal solution are kept constant and the values of $E_L(1)$ and $E_L(2)$ are also almost constant, their term in parenthesis may be taken

as equal to a constant factor E^{o}. Further, the values of $E_L(1)$ and $E_L(2)$ are negligibly small (due to salt bridge being used), the cell potential in the above equation may approximately be taken as membrane potential.

The equation (v) reduces to

$$E_{cell} = E^{o} + \frac{RT}{Z_A F} \ln (a_A)_2 \qquad \text{(vi)}$$

It is obvious (from this equation) that the cell potential would change with a change in concentration (or activity) of the cation in external or test solution 2. At 25°C, value of $\frac{RT}{Z_A F}$ comes out to be equal to $0.059/Z_A$ volt. If the plot between cell potential and log activity has a slope of 0.059/Z volt, the membrane is said to give a Nernstian response. These plots are called Nernst plots and the slope as Nernstian slope. It has been generally observed that only few membranes of ion exchanging materials show Nernstian response (2.39,2.40) and that too within a limited concentration range (say 10^{-4} to 10^{-1} M). Outside this range potential vs log activity plots deviate from linear Nernstian behaviour. At lower concentrations, deviation originates due to H^+ ion (available by the dissociation of water) competing with electrolyte counter ion and at higher concentrations due to co-ion transference (2.40).

2.7 ION-SELECTIVE ELECTRODES

If the ion exchange membrane exhibits a linear variation in potential with changing log concentration of external solution (in contact with it) it can be used to determine the ionic activity in test solution, if the activity in the other one is known. If the membrane electrode responds selectively to a particular ion, it is usually referred to as an ion-selective electrode (ISE).

(a) Selectivity of electrodes

No ion-selective electrode responds exclusively to the ion for which it has been designed, although it is often more responsive to this primary ion in comparison to others. If another interfering ion is present in significant concentration with respect to the primary ion, the electrode response will have contributions from both the primary as well as interfering ions. The degree of selectivity of the electrode for the primary ion, A, with respect to an interfering ion B, is expressed by the potentiometric selectivity coefficient, $K^{pot}_{A,B}$. The selectivity coefficient is defined by the general Nikolskii equation for the electrode potential:

$$E = E_o \pm \frac{RT}{Z_A F} \ln \left[a_A + \sum_B K^{pot}_{A,B} a_B^{Z_A/Z_B} \right] \qquad \text{(vii)}$$

Where Z_A and Z_B are the charges on ions A and B, E^o is the standard potential of the electrode. This equation is applicable to nearly all the electrodes (2.41).

When an electrode is ideally selective to A in comparison to B, value of $K^{pot}_{A,B}$ should be much less than unity. Conversely, if the electrode responds preferentially to B rather than A, $K^{pot}_{A,B}$ will be greater than unity. Further, for ions A and B of same charge and activity, a value of $K^{pot}_{A,B} = 1$ shows that the membrane responds equally to both the primary and interfering ions. For different values, of Z_A and Z_B, the $K^{pot}_{A,B}$ values showing equal membrane response, both to ion A and B would be different and these have been worked out using equation (vii) for same activity of primary and interfering ions and are shown in Table 2.1. When the $K^{pot}_{A,B}$ values are smaller than the limiting values listed in

Table 2.1 the interfering ion is said to cause less interference, Ideally a zero value of $K^{pot}_{A,B}$ indicates no interference. However, in practice, $K^{pot}_{A,B}$ values are about 1000 times smaller than the limiting values (Table 2.1) and are taken to indicate that the interfering ions do not cause any significant interference and the electrode is said to be sufficiently selective.

TABLE 2.1

$K^{pot}_{A,B}$ values for interfering ions indicating equal response to the membrane as that of primary ion.

Charge on primary ion(A) Z_A	Charge on Interfering ion (B) Z_B	$K^{pot}_{A,B}$ value for equal response of A and B
1	1	1.00
1	2	0.10
1	3	0.05
2	1	100.00
2	2	1.00
2	3	0.20

Thus, in order to assess the selectivity of a membrane, it is necessary to determine selectivity coefficient values. Several methods (2.41) have been described for the experimental determination of selectivity coefficients. These methods fall in two categories:

(i) Separate solution method, and

(ii) Mixed solution method

(i) Separate solution method

In this method, the potential of the electrode under investigation is measured first in solution containing the primary ion A with no B present (E_A) and then in solutions containing the interfering ion B with no A present (E_B) .

$K^{pot}_{A,B}$ is calculated from the activities of A and B in different solutions and the electrode potentials

$$E_A = E^o + \frac{RT}{ZF} \ln a_A \qquad \text{(viii)}$$

$$E_B = E^o + \frac{RT}{ZF} \ln K^{pot}_{A,B} (a_B)^{Z_A/Z_B}$$

From these equations

$$\log K^{pot}_{A,B} = \frac{E_B - E_A}{2.303\ RT/ZF} + \log \frac{a_A}{a^{Z_A/Z_B}_B} \qquad \text{(ix)}$$

The term 2.303 RT/ZF is the slope of the Nernst plot. As most of the solid membranes exhibit deviation from Nernstian behaviour, the experimental slope 'S' differs from the theoretical slope, i.e., 2.303 RT/ZF. Thus, it is desirable to use 'S' instead of Nernstian slope for the calculation of $K^{pot}_{A,B}$ (2.42) and so equation (ix) becomes

$$\log K^{pot}_{A,B} = \frac{E_B - E_A}{S} + \log \frac{a_A}{a^{Z_A/Z_B}} \qquad \text{(x)}$$

The separate solution method for measuring $K^{pot}_{A,B}$, although simple to perform, cannot be recommended as this reflects selectivity in separate solutions and the selectivities in mixed solution (containing both A and B) are likely to be slightly different.

(ii) Mixed solution method

Mixed solution method is always preferred to separate solution method.

This entails the measurement of the electrodes potential in a range of solution containing different activities of A and B both. For simplicity in interpreting the results, it is usual to prepare solutions either with a constant a_B and varying a_A or with a constant a_A and varying a_B. Conventionally, the first method is preferred as it usually corresponds more closely to the situation in samples. The second method has been used particularly when H^+ is the interfering ion. In this case curves are produced showing the electrode potential in solution of constant a_A but varying pH.

If a range of solutions with constant a_B and varying a_A are prepared and the electrode potentials measured in these solutions are plotted against pa_A, a curve of the type shown in Fig. 2.1 is usually obtained.

In the region PQ the electrode is responding in a Nernstian manner to the primary ion, A. As a_A decreases, the electrode potential is increasingly affected by the constant activity of B and in the region QR the electrode shows a mixed response to both A and B. From R to S the potential is constant as the electrode is responding entirely to the constant a_B and the effect of the decreasing a_A is not detectable. There are several methods for calculating $K^{pot}_{A,B}$ from the data presented in Fig. 2.1. Some of these are described here:

Method 1

The first method depends on finding graphically the point T. The potential corresponding to point T can be produced either by a_B or by a_A. Thus, $E_A = E_B$ at point T. Therefore, from equation (ix) :

$$\log K^{pot}_{A,B} = \log \frac{a_A}{(a_B)^{Z_A/Z_B}} \qquad \text{(xi)}$$

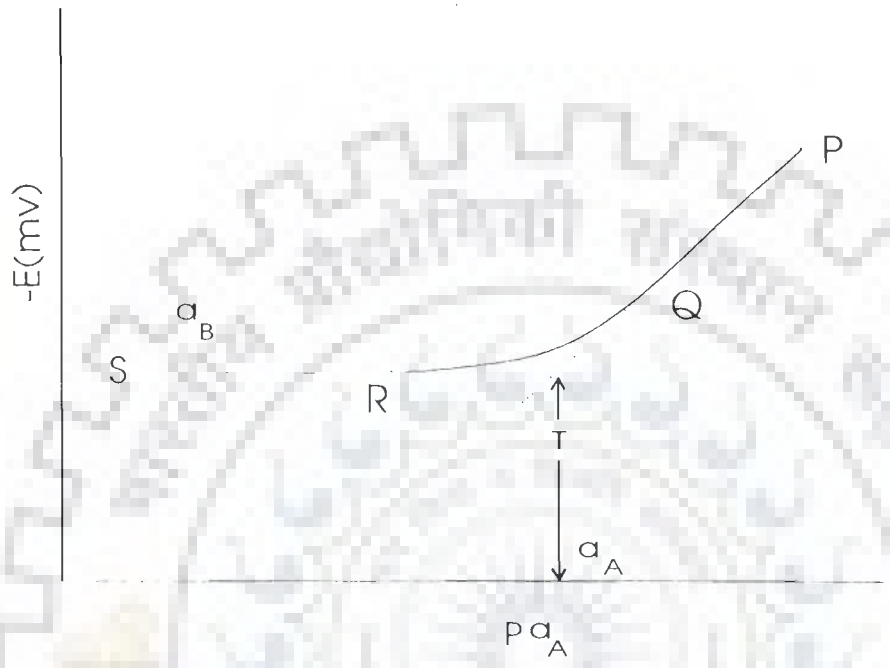


Fig. 2.1. Calibration graph illustrating Method 1 for determination of selectivity coefficients

Thus, knowing the value of a_A and a_B from the figure corresponding to point T. $K^{pot}_{A,B}$ can be calculated from equation (xi). This method is known as Fixed Interference Method and is most widely used as per IUPAC recommendations. However, this method is only suitable if RS is a straight line (2.41,2.42).

Method 2

In this method, potentials, E_A and E_{AB} are measured using solutions of primary ions only and a mixture of primary and interfering ions, respectively. From equation (vii) and (viii), we get,

$$K^{pot}_{A,B} = \frac{a_A}{(a_B)^{Z_A/Z_B}} \left[10^{\frac{E_{AB} - E_A}{2.303\ RT/Z_F}} - 1 \right]$$

or

$$K^{pot}_{A,B} = \frac{a_A}{(a_B)^{Z_A/Z_B}} \left[10^{\frac{E_{AB} - E_A}{S}} - 1 \right] \qquad \text{(xii)}$$

where S is the slope of the plot.

2.8 BIO-SENSORS

Methodology used for the fabrication of bio-sensors etc. is described along with the details of glucose and phenol sensors (Last Chapter).

2.9 REFERENCES

2.1. Srivastava, S.K., Saraswat, I.P. and Shardanand, Selective separation of rubidium on titanium(IV) ferricyanide gel columns. J. Anal. Sci. (Japan), 2,434 (1986).

2.2. Srivastava, S.K., Kumar, S. and Agrawal S., Ion Exchange properties of Chromium ferrocyanide gel, J. Radio. Anal. Chem., 33,4, 69(1977).

2.3. Srivastava, S.K. Singh, A.K. and Khanna, R., Anion exchange properties of zerconium tellurite, Indian J. Chem., 24, 254(1985).

2.4. Malik, W.U., Srivastava, S.K. and Vidyarthi, A., Appln. of molybdophosphate gel for the separation of thallium ions, 'The Analyst', 108, 340 (1983).

2.5. Srivastava, S.K., Kumar, S and Amla, B., Estimation of Rb^+ by a solid membrane sensor, J. Electroanal. Chem., 161, 345(1984).

2.6. Srivastava, S.K. and Jain, C.K., Estimation of sulphate by a solid membrane electrode, The analyst, 109, 667 (1984).

2.7. Srivastava, S.K. and Amla, B., Estimation of molybdenum as molybdate by ion selective potentiometry, Anal. Chem., 54, 1399 (1982).

2.8. Srivastava, S.K., Anupam and Singh M., Solid membrane sensor for potassium., Mikrochim Acta, 116, 123 (1994).

2.9. Sandell, E.B., Colorimetric determination of metals, p. 870 (1959).

2.10. Vogel, A.I., Quantitative Inorganic analysis, p. 884(1964).

2.11. Beastle, L.H. and Van Deyck D. Synthetic inorganic ion exchanges, J. Inorg and Nucl. Chem. 26, 1329 (1964).

2.12. Venkateswarlu, K.S. and Mahal, H.S., Ion adsorption properties of some mixed oxides, Indian J. Chem., 16A, 712 (1978).

2.13. Ventateswarlu, K.S. and Shankar, J., Sorption properties of some mixed hydous oxides, J. Colloid and Int. Sci., 70, 1 (1980).

2.14. Srivastava, S.K., Saraswat, I.P. and Shardanand, Characterization of some mixed-doped hydrous oxides, J. Material Sci. Letters, 5, 795 (1986).

2.15. Srivastava, S.K. and Khanna, R., Anion exchange characteristics of some tellurites, Ind. J. Chem., 24(A), 254(1985).

2.16. Alberti, G., Costantino, U. and Zsinka, L., Synthesis and ion exchange properties of microcrystalline phosphate; J. Inorg. Nucl. Chem., 34, 3549 (1972).

2.17. Snell, F.D. and Snell, T.C., " Colorimetric methods of analysis", D.Van. Nostrand, New York, p. 683 (1959).

2.18. Charlot, G., "Colorimetric determination of elements", Elsevier Pub. Co. New York, p. 405 (1964).

2.19. Moody, G.J. and Thomas, J.D.R., "Selective ion-sensitive electrode", selective Ann. Rev. Anal. Sci., 3, 56(1973).

2.20. Srivastava, S.K. and Jain, C.K., " Determination of sodium by ion-selective potentiometry", Analyst, 109, 667(1984).

2.21. Govindan, K.D. and Narayanan, P.K., "Interpolymer membranes", Ion Exchange Symposium U.K. 255 (1978).

2.22. Lima, J.L. and Machado, A.A., "Procedure for the determination of all solid state PVC membrane electrodes", Analyst.111, 799(1986).

2.23. Moody, G.J. and Thomas, J.D.R., "Epoxy based all solid-state PVC matrix membrane electrode", Analyst, 111, 611(1986).

2.24. Srivastava, S.K. Jain, A.K., Agarwal, S. and Singh, R.P., "Studies with chromium ferrocyanide epoxy resin based membrane", J.Chem. Tech. Biotechnol., 29, 379 (1979).

2.25. Srivastava, S.K.and Jain, C.K., "Estimation of Sulphate ions in water and industrial wastes", Water Research, 19, 53 (1984).

2.26. Michaelis, L., "Permeability of ions across plant membranes", Proc. International, Cong., Plant Sci., Ithaco., 2, 1139 (1962).

2.27. Haley, D.K. and McCaule, M.J., "Str. and Prop. of heterogeneous cation exch. membranes" Trans. Faraday Soc., 57, 135 (1971).

2.28. Wyllie M.R.J. and Kannan, S.L., "Membrane properties in relation to bi-ionic potentials", J. Phys. Chem., 58, 73 (1974).

2.29. Greger, H.P., Jacobson, H. and Shir, R.C., "Inter Polymer ion - selective membranes", J. Phys. Chem., 61, 141 (1957).

2.30. Greger, H.P. and Kawabe, H., "Functional properties of ion exchange membranes", J.Colloid and Int. Sci., 21, 79 (1976)/

2.31. Kawabe, M., Tanagita, M., Takamatus, T., "Cation exchange membranes", Japan, 21, 157(65) 1962.

2.32. Jain, A.K., Singh, R.P. and Agrawal, S., "Selectivity of heterogeneous ion exchange membranes", J. Ind. Chem. Soc., 39, 106 (1982).

2.33. Srivastava, S.K. Jain, C.K. and Kumar, S., " Functional Properties of chromium ferrocyanide membranes", Ind. J. Chem., 22A, 395 (1983).

2.34. Toshima, V., Vyasa M. and Fujita, H., "Electrical resistance of membranes". Trans. Faraday Soc., 63, 2803(1967).

2.35. Lakshminaraya naiah, N and Subrahmanyam., J.Phys. Chem., 72, 4314 (1968).

2.36. Barrer, R.M., Barric, J.A. and Rogers, M.G., "Permeation through a membrane" Trans. Faraday Soc., 58, 2473(1962).

2.37. Mizutani, V. and Nishimura, M., Heterogeneity in ion exchange membranes ". J.Appl. Polymer. Sci., 14, 1847 (1970).

2.38. Baiulescu, G.E and Cosofret, V.V., Appln. of I.S.E.S in organic analysis". Ellis Horwood Ltd. (1977).

2.39. Moody G.J. and Thomas J.D.R." Development and Publication of work with ISE's. Talanta., 19, 623(1972).

2.40. Hellferrich, F., "Ion Exchange " McGraw Hill, N.Y.376(1972).

2.41. Bailey, P.L, "Analysis with I.S.E's". Heydon, London, 45(1980).

2.42. Levins, R.J., "Barium ion selective electrode based on a neutral complex". Anal. Chem., 44, 1045(1971).

Chapter - 3

Ion - Selective Electrodes

3.1 INTRODUCTION

One of the newer and very elegant tools of analytical chemistry are ion-selective electrodes. These are developed and designed to respond to a particular ion in solution. The development of ion selective electrodes blew new life into potentiometry and the manufacturing of equipment for potentiometric measurements.

A good number of ion selective electrodes are commercially available for cations and anions but still there is a need for the development of sensors for multivalent cations and anions. Efforts made in this direction have, so far, not met with much success. Even the existing sensors suffer with problems of selectivity and durability on their prolonged use.

In an ever increasing search for suitable materials which can be used for the fabrication of membrane sensors - a variety of substances have been tried. The use of inorganic ion exchangers has been looked into by various workers. A review by (late) Professor Coetzee (3.1) deals with various aspects of membrane electrodes based on inorganic gels exhibiting ion exchange characteristics. Even the first membrane electrode which stimulated research in this field was based on lanthanum fluoride (3.2).

Due to their diverse properties not all the so called inorganic ion exchangers are suitable for use as sensors in the construction of ion-selective electrodes. For being used as a membrane sensor the electroactive material should be capable of undergoing a rapid exchange at the membrane - sample interface, should be of right grain size, should have a low solubility product and be physically compatible with the binder material used for casting the membrane.

Inorganic gels being used as ion exchangers are solids which are relatively insoluble. These cannot be easily pressed into pellets or discs and used as such in electrodes. These are to be incorporated into a membrane matrix to form a heterogeneous membrane. Substances used as binder material are silicone rubber,

PVC, epoxy resin, paraffin etc. Individual properties and transport mechanisms may vary, but, in any case, presumably involve the transfer of ions between crystals that are in physical contact within the matrix. The support material should be chemically inert and provide good adhesion for sensor particles, be hydrophobic, be tough, flexible yet non porous and crack-resistant.

Bailey (3.3), in his book on analysis with ion selective electrodes has mentioned a number of characteristics against which the performance of an electrode is to be assessed. These include the response range and slope, selectivity, stability and reproducibility, response time, cost and life time.

Fogg et. al., (3.4) prepared two membrane electrodes from finely ground potassium zinc hexacyanoferrate (II). These were tried for the estimation of potassium ion. The same salt was again tried by Rock et al., (3.5) with different binder but the response time of the sensor was quite high. Calibration curves with the above mentioned electrodes compared favourably with those of an Orion potassium ion selective electrode and a Beckman glass electrode under similar experimental conditions. D'Olieslager and Heerman (3.6) employed this compound in a PVC matrix as a cesium ion selective electrode. Previous studies (3.7,3.8) demonstrated a high selectivity of this compound for cesium ion over other alkali metal ions.

Ion selective electrodes based on the membranes of inorganic ion exchangers, have been extensively studied in this laboratory also and the use of cesium and thallium (3.9) tungstoarsenate membranes have been reported by Malik et al. (3.9) for the estimation of Cs^+ and Tl^+ ions. Besides this strontium tungstoarsenate membrane (3.10) was fabricated for the estimation of Sr^{2+} and the same electrode was also used for the measurement of activity and CMC of cationic surfactants (3.11). Srivastava et al. (3.12) prepared araldite based chromium hexacyanoferrate (II) membranes and found these sensitive to hexacyanoferrate

ion. Recently an inorganic gel membrane sensor for mercury (3.13) has also been proposed by Srivastava et al.

An electrode made of a mixture of lead molybdotungstate and perrhenate has been used for the estimation of molybdate, phosphate, chromate and hydroxide (3.14). Purdy (3.15) used zinc orthophosphate and zinc mercuric thiocyanate as an inorganic exchanger for making a zinc selective electrode.

Various workers (3.16-3.20) have tried to develop sensors for the estimation of phosphate using nickel phosphate, silver phosphate etc.

Tang and Huang (3.21) have performed "Flow injection analysis" of SO_4^{2-} with the help of a lead ion selective electrode.

Most of the investigations reported on this subject are periodically reviewed by Janata (3.22-3.25) in "Analytical Chemistry" journal.

Investigations carried out with four different inorganic gel membranes are reported in this chapter. Titanium (IV) ferricyanide gel was found to show very specific selectivity for some cations (3.26). As such the membrane of this compound was tried for the estimation of K^+. Besides this a doped and mixed hydrous oxide (Ti-Fe-Ce (IV) doped) was prepared and found to possess good cation exchange characteristics. Its membrane has also been investigated for electroanalytical applications. Some inorganic gels exhibiting very good anion exchange characteristics were for the first time prepared and reported from this lab. (3.27). Two of these compounds stannic and thorium tellurite have been tried as electroactive material for the development of membrane sensors showing response to anions.

3.2 FUNCTIONAL PROPERTIES OF MEMBRANES

Heterogeneous membranes of titanium ferricyanide, Ti-Fe mixed-Ce(IV) doped hydrous oxide and that of thorium tellurite were prepared with the help of

polystyrene, while an epoxy resin Araldite was used for preparing the membrane of stannic tellurite.

Water content, porosity, swelling and electrolyte absorption data of titanium ferricyanide membrane is given in Table 1 and the same for the mixed hydrous oxide and doped membrane (Ti-Fe mixed with Ce(IV) doped) is given in Table 3. Conductance data of the two membranes is given in Tables 3.2 & 3.4.

There seems to be some gradation in water content (at least in monovalent ions) data. With the exception of K^+, the water content decreases with increasing radii of the ions present in membrane (Table 3.1). In this case porosity has been taken as the volume of water incorporated per unit membrane volume and has been calculated from the water content data with the help of the expression given earlier in chapter 2.

The values (Table 3.1) do not show any significant or regular change. When a dry membrane is immersed in water or electrolyte solution, swelling takes place. The solution penetrates into the membrane structure occupying interstices which are already present or which develop as a result of different swelling properties of membrane material and binder. The interstices act as a film and the permeation of different ions also become possible by film diffusion, besides normal diffusion across the membrane material.

The magnitude of swelling depends on the gel structure as well as the amount of binder present in membrane. A complete swelling of gel particles does not take place in titanium ferricyanide membrane due to significant amount of binder used in their preparation. This may be due to some restraints imposed by the binding material and consequently some portion of the gel becomes inaccessible to water. Titanium ferricyanide membrane has lower porosity and the extent of swelling is also small. This suggests that diffusion through the membrane would be dependent more on the exchange sites rather than porosity. The membrane, under

investigation, may therefore be expected to show a better selectivity.

The electrolyte uptake is also poor (Table 3.1) and this is compatible with the water content and swelling data of this membrane.

The conductance of membrane in different cationic forms is recorded in Table 3.2. It was observed that the conductance changes slowly in initial stages of experiment and becomes constant after four to six minutes. This naturally points to an almost negligible polarization at the mercury membrane interface. Specific conductivity data (Table 3.2) for titanium ferricyanide membrane in various cationic forms exhibits the following sequence:

$$Na^+ > K^+ > Li^+ > Tl^+ > Rb^+ > Cs^+ > \text{ and } Zn^{2+} > Ca^{2+} > Mg^{2+}$$

Conductivity data is supposed to predict the selectivity pattern of a particular membrane but this does not seem to be true for a heterogeneous membrane where the electroactive phase is embedded in an inert binder material. In this case also the membrane sensor exhibits much better selectivity for K^+ in comparison to Na^+.

Functional characteristics viz., water content, porosity, swelling etc. of mixed-doped oxide membrane (Ti-Fe mixed and Ce(IV) doped) are given in Tables 3.3 & 3.4. Preliminary investigations revealed that the doped membranes have lower water content than the undoped one. Similarly the doped one were found to have low porosity than the undoped membranes (data for the undoped ones has not been included in the text due to a very poor electroanalytical performance observed with these membranes). Among the bivalent ions (Table 3.3) the water content, porosity and electrolyte uptake is maximum for Ba^{2+}. The magnitude of these parameters, however, is more for membranes loaded with monovalent ions.

Conductance of membrane in different cationic forms is reported in Table 3.4. Before doing measurement the membranes were equilibrated with appropriate electrolyte solutions. Constant readings were obtained in three minutes time and no drifting was observed for a sufficiently long time. This is indicative of negligible polarization at the mercury membrane interface. The conductance data of the membranes in various cationic forms can be arranged in the following sequence :

$$Cs^{+} > Tl^{+} > Rb^{+} > K^{+} \quad \text{and} \quad Ba^{2+} > Ca^{2+} > Mg^{2+} > Zn^{2+}$$

It was also observed (preliminary investigations not recorded here) that the doped-oxide membranes have higher conductance values than the respective undoped ones. This particular membrane was found to exhibit a much better selectivity for Ba^{2+} as compared to other mono, bi or trivalent ions. As such it was tried for the estimation of barium ion.

Thorium and stannic tellurite membranes have more dominant anion exchange characteristics. These have been tried for the estimation of anions. Functional properties of these membranes in various anionic forms are given in Tables 3.5 to 3.7. Heterogeneous membranes of stannic tellurite were prepared with the help of an epoxy resin Araldite. The inert binder Araldite has been very widely used for the preparation of heterogeneous membranes of a variety of electroactive substances (Ref. 2.5-2.7). This material is highly hydrophobic and the membranes prepared with its help exhibit negligible swelling, water uptake etc. As such, it has not been possible to measure the conventional parameters with stannic tellurite membranes except conductance, which has been recorded in Table 3.7. Functional parameters of thorium tellurite are given in Tables 3.5 & 3.6. The water content of the membrane in various anionic forms is maximum in the case of

chromate loaded membrane and the values in general are higher than those of titanium ferricyanide (Table 3.1) and lesser than the hydrous oxide membrane (Table 3.3). Porosity of thorium tellurite is almost the same as that of titanium ferricyanide membrane.

Swelling takes place when a dry heterogeneous membrane is immersed in water or electrolytic solution. The magnitude of swelling depends on the gel structure and also on the amount of binder present in membrane. The extent of swelling in thorium tellurite is higher (Table 3.5) in comparison to titanium ferricyanide (Table 3.1) and mixed oxide membranes (Table 3.3). Excessive swelling, however, is not a desirable characteristic but the presence of swelling and porosity, within reasonable limits, assures the diffusion of ion, across the membrane, through exchange sites. The magnitude of the two parameters, for the membrane under consideration, are comparable to those reported for other inorganic ion exchange membranes (3.10,3.12). The uptake of electrolyte is compatible to swelling and porosity data (Table 3.5).

Conductance of the two membranes thorium tellurite and stannic tellurite are given in Tables 3.6 & 3.7. Obviously the conductance would depend on swelling as well as electrolyte uptake of membrane. Although the swelling and electrolyte uptake of thorium tellurite (Table 3.5) is more than that of titanium ferricyanide and mixed oxide membranes, the conductance of tellurite membrane in various ionic states is quite comparable to the other two membranes. The values are slightly higher in case of stannic tellurite membrane (Table 3.7). Conductivity data of the two membranes follows the sequence :

$$CrO_4^{2-} > Cl^- > SO_4^{2-} > Br^- = NO_3^- > MoO_4^{2-} > PO_4^{3-} > Fe(CN)_6^{4-}$$

(Thorium tellurite membrane)

$$SO_4^{2-} > Cl^- > NO_3^- > Br^- > CrO_4^{2-} > MoO_4^{2-} > PO_4^{3-} > Fe(CN)_6^{4-}$$

(Stannic tellurite membranes)

Although the membrane should be conducting if the same is to be used as ion-sensor but highly conducting membrane is not desirable as it may lead to a larger transport of ions and the membrane may respond to a large number of anions in varying degree.This would naturally make the sensor less selective.

TABLE 3.1

Water content, porosity, swelling and electrolyte absorption data of titanium ferricyanide membrane in different cationic forms

Cations	Water content g H_2O/g wet membrane	Porosity	Swelling (mm)	Electrolyte absorbed/g of wet membrane (10^{-5}M)
Li^+	0.134	0.252	0.06	44.90
Na^+	0.130	0.248	0.08	36.90
K^+	0.133	0.250	0.06	43.80
Tl^+	0.128	0.249	0.04	42.20
Rb^+	0.126	0.248	0.05	41.40
Cs^+	0.124	0.248	0.05	40.20
Mg^{2+}	0.130	0.245	0.04	39.10
Ca^{2+}	0.128	0.242	0.02	38.60
Zn^{2+}	0.127	0.240	0.04	37.90

TABLE 3.2

Specific conductance of titanium ferricyanide membrane in different cationic forms

Cations	Specific conductance (mhos cm^{-1})x10^4
Li^+	18.23
Na^+	19.18
K^+	18.78
Tl^+	17.22
Rb^+	17.11
Cs^+	16.78
Mg^{2+}	16.99
Ca^{2+}	17.23
Zn^{2+}	17.94

TABLE 3.3

Water content, porosity, swelling and electrolyte absorption data of Ti-Fe mixed Ce(IV) doped hydrous oxide membrane in different cationic forms

Cations	Water content g H_2O/g wet membrane	Porosity	Swelling (mm)	Electrolyte absorbed/g of wet membrane (10^{-5}M)
Li^+	0.180	0.340	0.09	44.50
Na^+	0.214	0.403	0.01	45.40
K^+	0.214	0.402	0.01	44.80
Tl^+	0.210	0.396	0.08	44.30
Rb^+	0.182	0.342	0.06	43.00
Cs^+	0.182	0.342	0.06	43.10
Ba^{2+}	0.192	0.386	0.07	44.20
Ca^{2+}	0.174	0.328	0.08	41.90
Mg^{2+}	0.180	0.339	0.08	42.50
Zn^{2+}	0.172	0.326	0.06	40.30

TABLE 3.4

Specific conductance of Ti-Fe mixed Ce(IV) doped hydrous oxide membrane in different cationic forms

Cations	Specific conductance (mhos cm^{-1})x10^4
Li^+	26.54
Na^+	25.42
K^+	28.44
Ti^+	29.52
Rb^+	28.58
Cs^+	29.61
Ba^{2+}	33.72
Ca^{2+}	31.46
Mg^{2+}	29.82
Zn^{2+}	29.58

TABLE 3.5

Water content, porosity, swelling and electrolyte absorption data of thorium tellurite membrane in different anionic forms

Anions	Water content g H_2O/g wet membrane	Porosity	Swelling (mm)	Electrolyte absorbed/g of wet membrane (10^{-3}M)
Cl^-	0.161	0.220	0.15	4.0
Br^-	0.156	0.214	0.15	4.0
NO_3^-	0.158	0.212	0.15	3.6
SO_4^{2-}	0.160	0.224	0.16	4.5
CrO_4^{2-}	0.168	0.246	0.16	4.8
PO_4^{3-}	0.146	0.164	0.14	3.6
MoO_4^{2-}	0.160	0.224	0.18	4.8
$Fe(CN)_6^{4-}$	0.142	0.142	0.14	3.2

TABLE 3.6

Specific conductance of thorium tellurite membrane in different anionic forms

Anions	Specific conductance (millimhos cm^{-1})x10
Cl^-	1.80
Br^-	1.42
NO_3^-	1.42
SO_4^{2-}	1.60
CrO_4^{2-}	1.82
PO_4^{3-}	1.14
MoO_4^{2-}	1.35
$Fe(CN)_6^{4-}$	1.12

TABLE 3.7

Specific conductance of stannic tellurite membrane in different anionic forms

Anions	Specific conductance (millimhos cm^{-1})x10^2
Cl^-	9.80
Br^-	9.60
NO_3^-	9.62
SO_4^{2-}	9.82
CrO_4^{2-}	8.62
PO_4^{3-}	5.20
MoO_4^{2-}	6.80
$Fe(CN)_6^{4-}$	4.56

3.3 ELECTROANALYTICAL PERFORMANCE OF TITANIUM FERRICYANIDE MEMBRANE (ESTIMATION OF K^+)

As mentioned earlier (Chapter-2) the compound can be represented as $K(TiO)|Fe(CN)_6|$. It exhibits very promising selectivity for Na^+, K^+; Pb^{2+},Mg^{2+}; Fe^{3+} and Cr^{3+}.

Homogeneous membranes of this compound could not be prepared and so heterogeneous membranes were prepared using polystyrene as binder. The minimum amount of binding material required to give adequate stability to membranes was determined by trial and 15.0 per cent binder was found to be sufficient to provide mechanically and chemically stable membranes generating reproducible potentials. The procedure adopted for preparing the membranes is already described in Chapter 2.

An intimate and finely ground mixture of titanium ferricyanide plus polystyrene (15 percent by weight) was heated in the metallurgical specimen mount press at $90^{O}C$ under a pressure of 6500-7000 psi. After the required temperature was attained, the sample was allowed to cool under normal conditions. Membranes thus obtained were quite stable, did not dissipate in water or salt solution and generated reproducible potentials. A good deal of preliminary investigations were performed to fix up the optimum quantity of binder, the temperature, pressure etc. necessary for casting membranes having optimum electroanalytical properties.

Table 3.8 records the effect of the amount of binder on the working of titanium ferricyanide membrane sensor. A perusal of the data (Table 3.8) depicts the amount of binder necessary for the fabrication of membrane with optimum performance.

Membranes were equilibrated with KCl solution of 0.1M concentration for 3 days. Equilibrated membranes were washed with deionized water, several times, to remove absorbed electrolyte from the surface and dried in air. Concentration of

solution used and time of equilibration were optimized after a good deal of preliminary investigations. Equilibrated membranes must have short response time, generate significant and reproducible potentials and exhibit Nernstian or near Nernstian behaviour. 0.1M concentration of the equilibrating solution and a contact time of 72 h provides membrane possessing good electrochemical characteristics.

Equilibrated membranes were used as electrodes and potentials measured with 10^{-1} to 10^{-5} M KCl solution show a linear pattern in the concentration range 10^{-1} to 10^{-4}M with a slope of 53.33 mV/decade of concentration (Fig.3.1). The concentration of reference solution (KCl) used was 10^{-1}M. This was also fixed up by trial. Experiments run with 10^{-2} and $5x10^{-1}$M reference solution resulted in plots with smaller linear range and much divergent slope values (plots not shown here).

The proposed sensor, as such, can measure K^+ in the concentration range 10^{-1} to 10^{-4}M, although it is non-Nernstian in behaviour. A non-Nernstian slope, in membrane sensors, is nothing exceptional and is found in commercially available electrodes as well.

The electrode response (in the range 10^{-1} to 10^{-4}M) is fast, stable potentials being obtained within 20 s. The response time being same whether one goes from dilute to concentrated solutions or vice versa and potentials were reproducible over a period of two months with a standard deviation of 0.2mV. Membranes could be used for two months without any distraction but thereafter a change in potential of about 2mV/decade of activity, throughout the range, was observed. This can, however, be overcome by treating the membrane again with 0.1M KCl solution for 36 h and with this treatment one membrane can be used for four to five months time and thereafter it can be replaced by a new one.

The variation of membrane potential (of 10^{-2}M KCl) with pH is depicted in Fig.3.2. Potentials almost stay constant from pH 5 to 9 which may be taken as the working pH range of the electrode system.

Quite often the electrode is required to be used in partially non aqueous system as well and so its utility was explored in non aqueous solvents having upto 30 percent (v/v) methanol, ethanol and acetone media. Potential vs log concentration plots in solvent systems with 30% non aqueous content are given in Fig.3.3. The slopes of these plots decrease to 46.5 mV with increasing non-aqueous content and an increase in response time from 20 to 45 s is also observed. In spite of this, the sensor can safely be used in solutions having a maximum of 30 per cent non-aqueous content, as the linearity range of the plot (Fig.3.3) matches well with the original calibration plot (Fig.3.1).

Interference by other ions in the working of the proposed electrode system has been assessed by both the fixed interference method (suggested by IUPAC), and the separate solution method. This has been estimated at low levels of interference viz., 10^{-3} and 10^{-4} M concentration by the suggested fixed interference method and at 10^{-3}M concentration (of interfering ions) by separate solution method. The values are recorded in Table 3.9. A perusal of the data reveals that the bi and trivalent cations do not interfere at all. Among the monovalent ions Rb^+, Cs^+ and Ag^+ may cause a little disturbance in the functioning of this electrode assembly if the same are present at concentrations higher than 10^{-3}M - situation which is not likely to be faced quite frequently. Na^+ and NH_4^+ ions would cause interference even at 10^{-4} M concentration. In general lower values of selectivity coefficients are obtained by the separate solution method, but these do not reflect a real picture as the method is not based on the working conditions of a membrane electrode system.

Effect of anions was also investigated by observing the electrode response with KCl, KNO_3 and K_2SO_4. Apparently no disturbing influence was noticed with changing anions and the potential VS. $[K^+]$ plot with all the three salts was almost same (not depicted here).

It is worth mentioning that synthetic zeolite membranes fabricated by Evmirids et.al. (3.28) have very limited selectivity while the one proposed by Engel and Grabner (3.29)and Hartmann et.al. (3.30) have serious interferences by Rb^+, Cs^+, Ag^+ and Na^+. Moreover the maximum life time of their sensor is one month. The sensor under investigation can be used without any treatment for two months and the total life time is four to five months. Besides this, the samples do not need any pretreatment except the pH adjustment.

TABLE 3.8

	Amount of polystyrene (Percent by weight)	Detection limit (M)	Slope mV/decade of concentration
1.	10.0	unstable	membranes
2.	12.0	$6x10^{-4}$	48.0
3.	15.0	$1x10^{-4}$	53.3
4.	20.0	$1x10^{-3}$	64.3

TABLE 3.9

Selectivity coefficient ($K^{pot}_{A,B}$) values of interfering ions for titanium ferricyanide membrane sensor at various levels of interference

Ions	$K^{pot}_{A,B}$ values as estimated by		
	Fixed Interference Method		Seperate solution Method
	10^{-3}M Concn.	10^{-4}M Concn.	10^{-3}M Concn.
Na^{+}	1.20	1.12	0.85
NH_4^{+}	0.35	0.22	0.15
Rb^{+}	0.73×10^{-1}	0.23×10^{-1}	0.20×10^{-1}
Cs^{+}	0.12	0.11	0.11
Ag^{+}	0.52×10^{-1}	0.21×10^{-1}	0.12×10^{-1}
Mg^{2+}	0.72×10^{-2}	0.63×10^{-2}	0.31×10^{-2}
Zn^{2+}	0.69×10^{-2}	0.56×10^{-2}	0.35×10^{-2}
Ca^{2+}	0.65×10^{-2}	0.52×10^{-2}	0.32×10^{-2}
Ba^{2+}	0.60×10^{-2}	0.49×10^{-2}	0.26×10^{-2}
Hg^{2+}	0.56×10^{-2}	0.50×10^{-2}	0.22×10^{-2}
Mn^{2+}	0.50×10^{-2}	0.42×10^{-2}	0.32×10^{-2}
Ni^{2+}	0.38×10^{-2}	0.32×10^{-2}	0.20×10^{-2}
Cd^{2+}	0.32×10^{-2}	0.26×10^{-2}	0.16×10^{-2}
Sr^{2+}	0.30×10^{-2}	0.22×10^{-2}	0.12×10^{-2}
Al^{3+}	0.17×10^{-2}	0.14×10^{-2}	0.12×10^{-2}
Fe^{3+}	0.15×10^{-2}	0.12×10^{-2}	-
Cr^{3+}	0.16×10^{-2}	0.12×10^{-2}	-

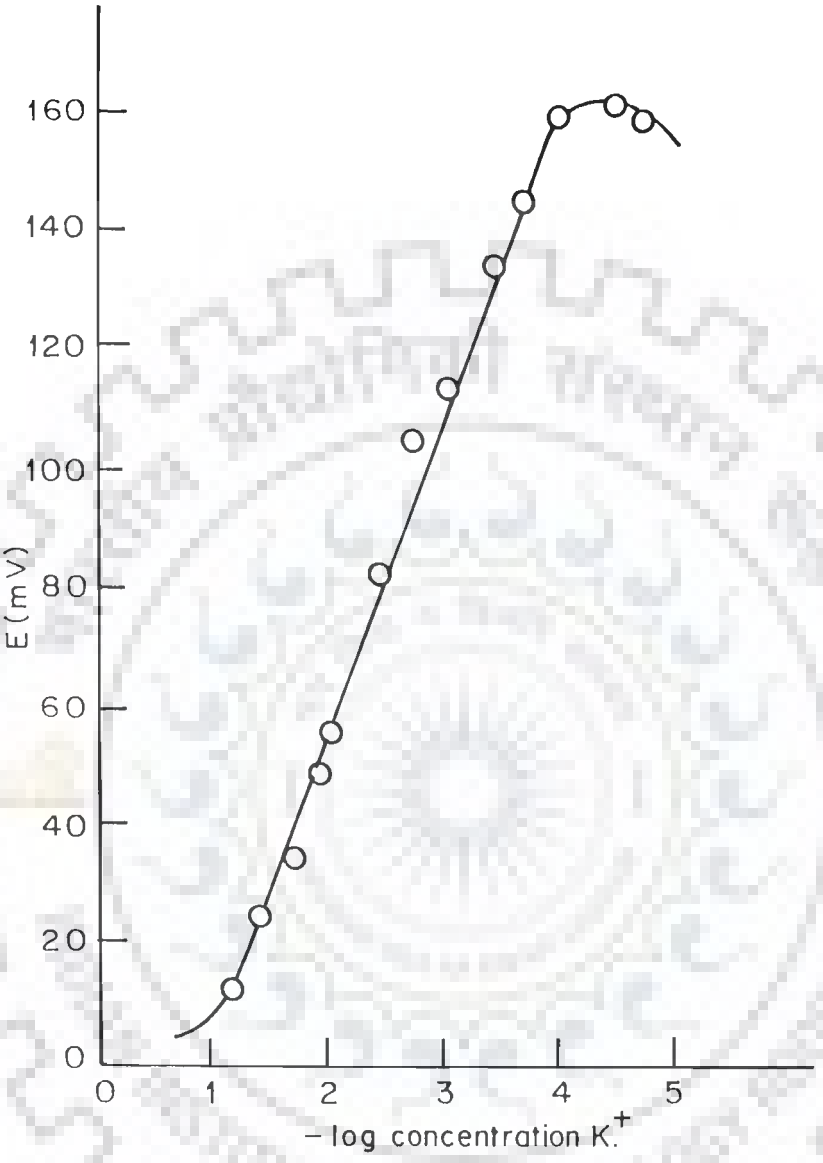


Fig. 3.1 Potenial versus [K^+]plot.

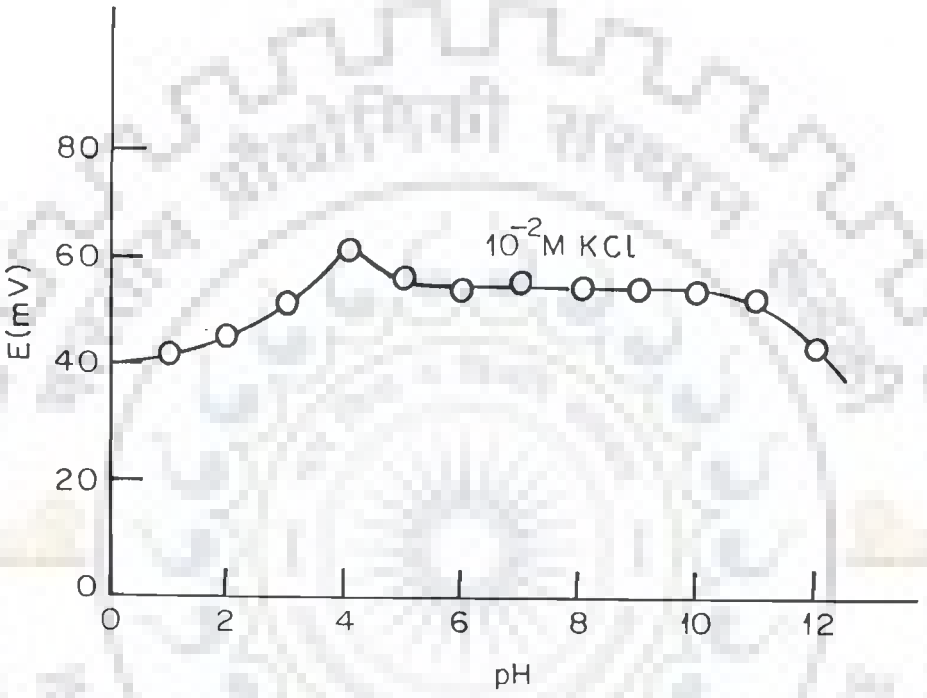


Fig. 3.2 Variation of membrane potential with pH.

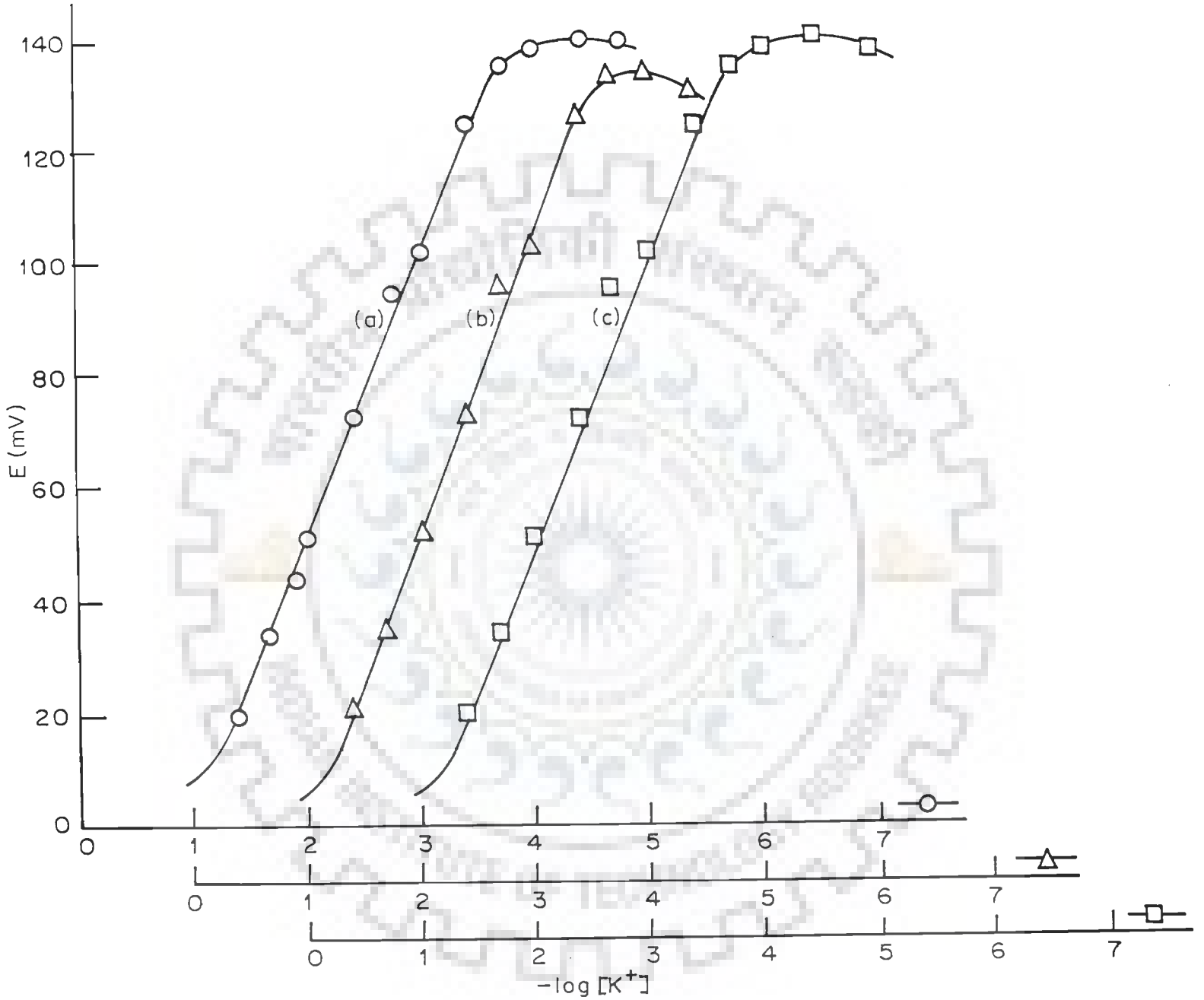


Fig. 3.3 Potential versus $[K^+]$ plot in 30% (a) methanolic, (b) ethanolic & (c) acetonic solutions

3.4 ELECTROANALYTICAL PERFORMANCE OF A MIXED-DOPED [Ti- Fe MIXED Ce(IV) DOPED] HYDROUS OXIDE MEMBRANE (ESTIMATION OF Ba^{2+})

Mixed hydrous oxides are known to possess better sorption characteristics than simple oxides. Mixed oxides prepared with suitable dopants exhibit even better exchange capacity and selectivity. Cerium doped titanium iron mixed oxide has been prepared and is found to have higher exchange capacity than the simple undoped mixed oxide. The membrane of this compound was initially tried for monovalent ions but its performance was found to be much better for bivalent ions specially Ba^{2+}.

Homogeneous membranes of this compound also could not be prepared. Consequently both the binders i.e., polystyrene and araldite were tried, but desirable membranes could only be prepared with polystyrene. Minimum amount of binder, which will provide stable membranes, temperature and pressure used in the press was decided after a great deal of experimentation. Intimately mixed powder of doped-mixed oxide and polystyrene (20 percent by weight) was heated at 90°C and 6000 to 6500 psi pressure as described in chapter 2. Membranes thus obtained were quite stable and generated stable and reproducible potentials. Observations recorded with membranes having different amounts of polystyrene are given in Table 3.10. Obviously the best one is obtained with 20% polystyrene.

Membranes were equilibrated with 0.1 M $BaCl_2$ solution for 48 hours. Again the equilibration time and concentration of equilibrating solution was obtained by trial when noiseless and reproducible potentials are recorded by the membrane. Equilibrated membranes were thoroughly washed with distilled water and used as sensors. Potentials observed with 10^{-1} to 10^{-5}M Ba^{2+} solutions using 10^{-1} M $BaCl_2$ as reference are recorded in Fig.3.4. The plot is linear in the range 10^{-1} to 10^{-4} M concentration and the slope of the plot is 46.2 mV per decade of concentration which is far away from the Nernstian value (29.58 mV per decade of

concentration), A non-Nernstian behaviour is no deterrant for the membrane to be used as a sensor for Ba^{2+}.

As per IUPAC recommendations on ion selective electrodes, the practical limit of detection may be taken as the concentration corresponding to the point of intersection of the two extrapolated lines of potential-concentration plot. Thus the lowest limit of detection up to which this sensor can measure is $7x10^{-5}$ M concentration of Ba^{2+}.

Different concentrations of reference solution were also tried but best results could only be obtained with 10^{-1}M $BaCl_2$ solution. The response time of the membrane electrode is 30 seconds over the entire working concentration range of the sensor and a repeated monitoring of potentials show a standard deviation of 0.4 mV in the functional concentration range. One single membrane electrode was used for Ba^{2+} measurement for three months at a stretch and no drift in potential was observed. After a prolonged use the electrode was regenerated by equilibrating with 0.1M $BaCl_2$ solution for 36 h. In comparison to fresh equilibration, smaller time is required for regeneration during use. If the membrane, in use, fails to give a proper response even after regeneration, it should be discarded and replaced by a new one.

Potential change in the electrode with varying hydrogen ion concentration is depicted in Fig.3.5. It is evident that the Ce(IV) doped membrane of Ti-Fe mixed oxide can be used in pH range 5 to 9. A sharp change in potential at low pH may be attributed to the interference of hydrogen ions.

The electrode system has also been tested in partially non-aqueous solvents. Membrane sensor can tolerate and works well in solvents having 25 percent non-aqueous content or less. Potential VS concentration plots in 25% methanolic, ethanolic and acetonic solutions are shown in Fig.3.6. These curves run parallel to the calibration plots except that their slopes are slightly

smaller. At higher non-aqueous content, stable potentials are not obtained with the membrane sensor.

Selectivity of this electrode for barium ions over other cations has been assessed in terms of selectivity coefficients $K^{pot}_{A,B}$. These have been obtained by fixed interference method for several other cations (Table 3.11). Most of the monovalent, bivalent and trivalent cations do not interfere with the working of the electrode assembly except Li^+, NH_4^+ and Cu^{2+} ions. If these ions are present in solution at 10^{-3} M concentration there are 20 to 30 percent chances of their interference. At concentration lesser than 10^{-3}M, these cations may also not interfere. Anions like NO_3^- and CH_3COO^- also do not cause any interference in the working of the electrode assembly.

It has been possible to use this membrane as indicator electrode in the titration of barium ions, the titration plot of 10^{-3} M $BaCl_2$ (30 ml) with 10^{-2} M K_2SO_4 is shown in Fig. 3.7. A fall in potential is observed as Ba^{2+} are removed from the system. After the end point potentials stay almost constant (Fig.3.7). A sharp end point with perfect stoichiometry is a significant feature of this titration.

TABLE 3.10

S.No	Amount of polystyrene (Percent by weight)	Detection limit(M)	Slope mV/decade of concentration
1	15.0	$1x10^{-4}$	42.0
2.	20.0	$7x10^{-5}$	46.2
3.	25.0	$8x10^{-5}$	52.4
4.	30.0	$2x10^{-4}$	34.5

TABLE 3. 11

Selectivity coefficient ($K^{Pot}_{A,B}$) values of doped-mixed hydrous oxide membrane at 10^{-3} M concentration of interfering ions :

Ions	$(K^{Pot}_{A,B})$
Na^+	$0.46x10^{-1}$
K^+	$0.21x10^{-1}$
NH_4^+	$2.2x10^{-1}$
Rb^+	$0.32x10^{-1}$
Cs^+	$0.34x10^{-1}$
Li^+	$2.8x10^{-1}$
Sr^{2+}	$1.6x10^{-2}$
Ca^{2+}	$2.7x10^{-2}$
Cd^{2+}	$2.2x10^{-2}$
Cu^{2+}	$3.2x10^{-1}$
Mg^{2+}	$3.5x10^{-2}$
Mn^{2+}	$3.7x10^{-2}$
Hg^{2+}	$2.5x10^{-2}$
Fe^{3+}	$8.6x10^{-3}$
Cr^{3+}	$8.9x10^{-3}$
La^{3+}	$7.5x10^{-3}$

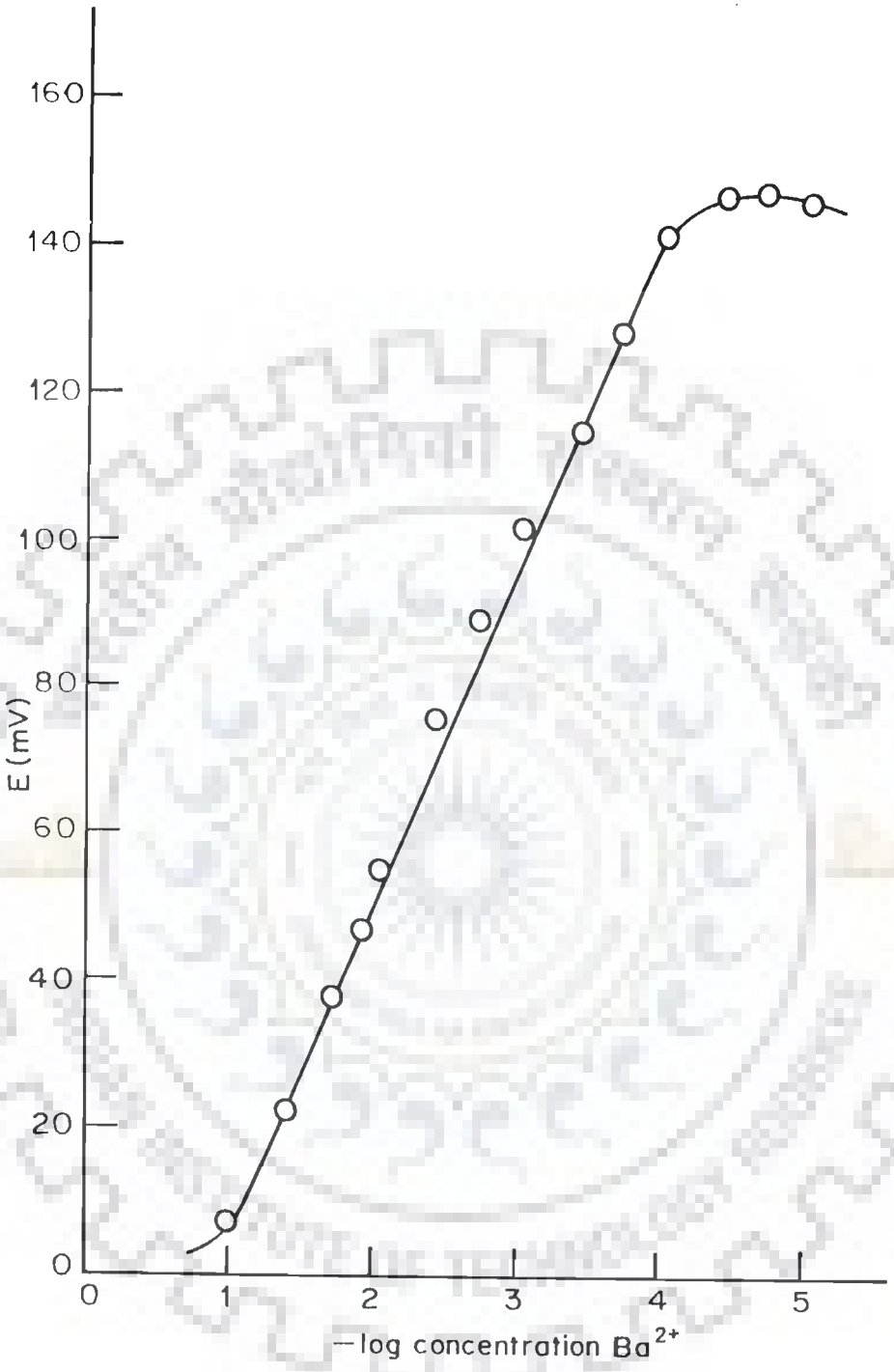


Fig. 3.4 Potential versus $[Ba^{2+}]$ plot.

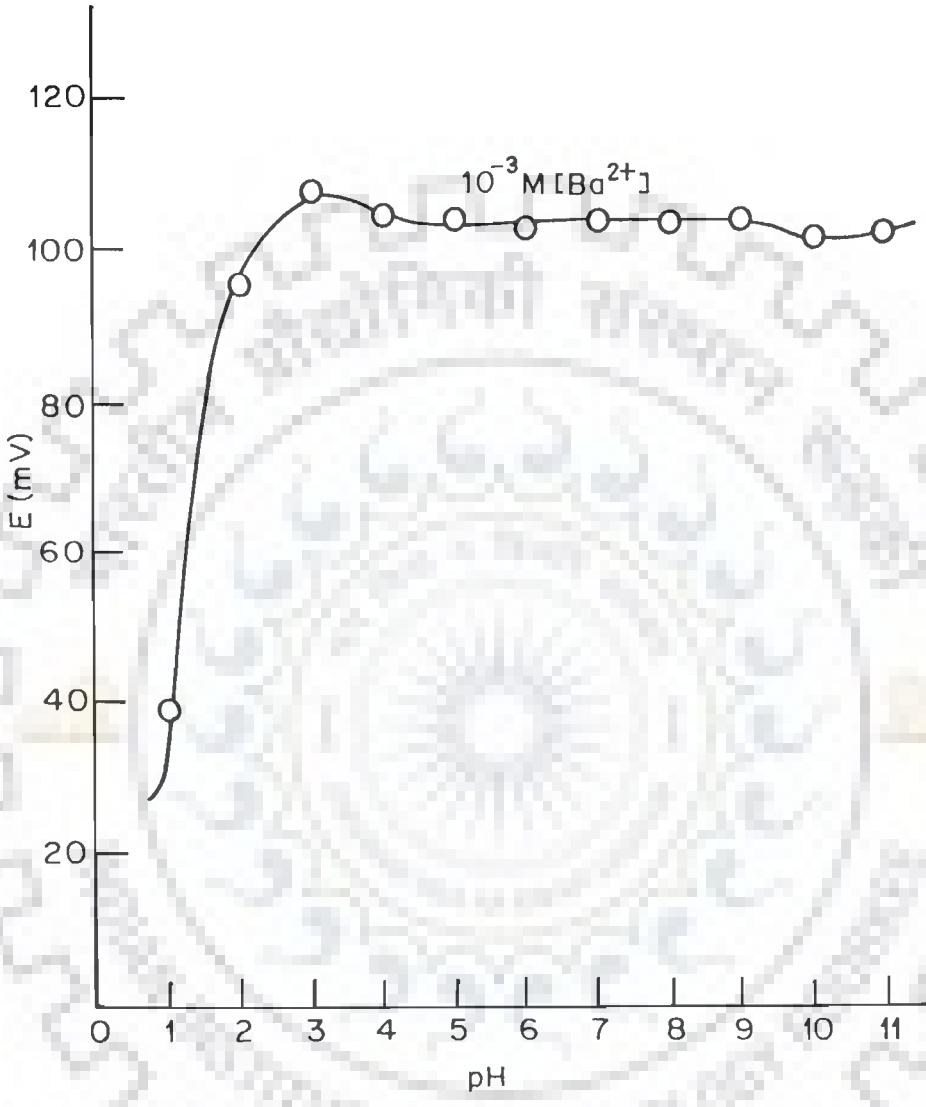


Fig. 3.5 Variation of membrane potential with pH.

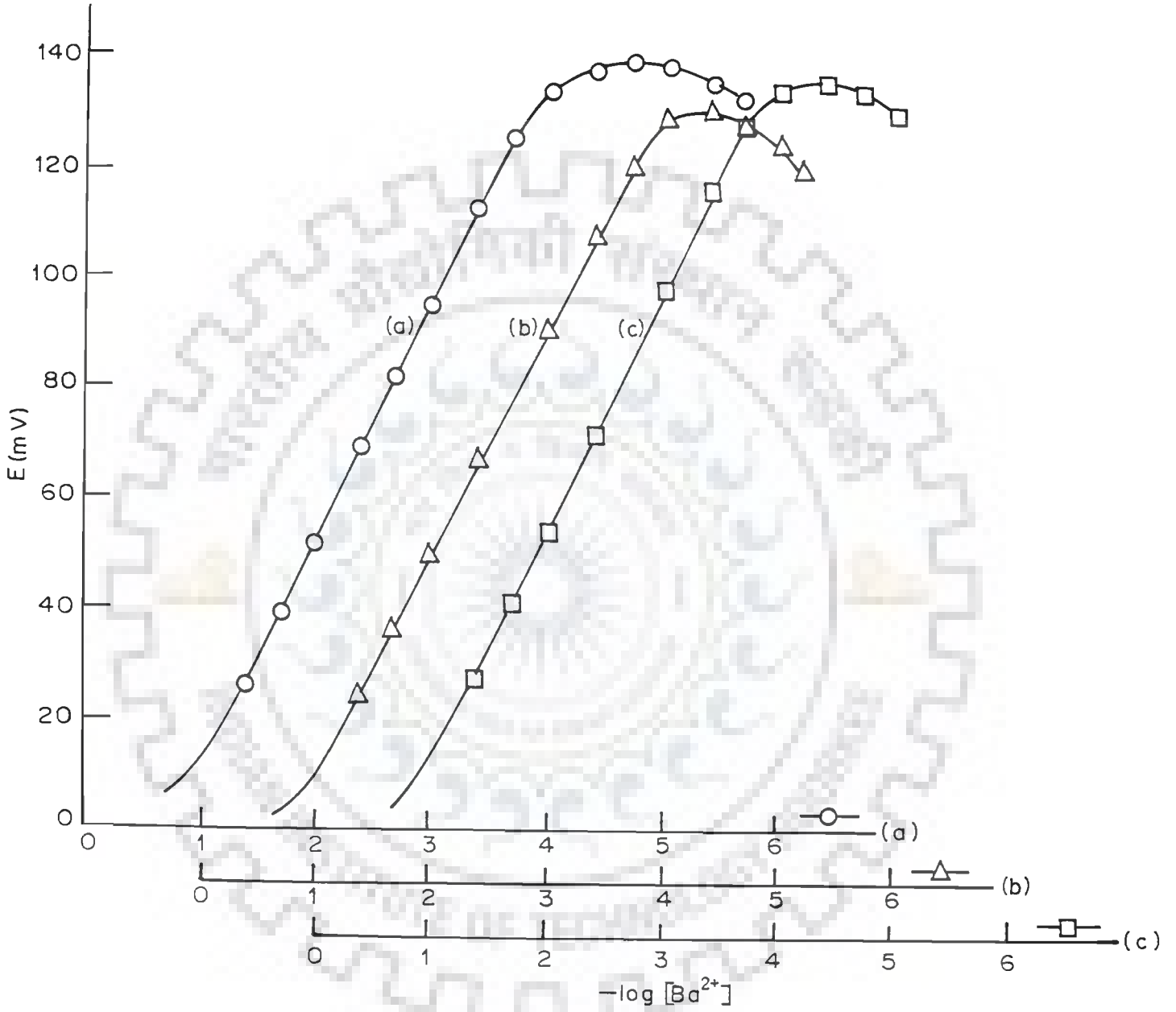


Fig. 3.6 Potential versus $[Ba^{2+}]$ plots in 25% (a) methanolic, (b) ethanolic & (c) acetonic solutions.

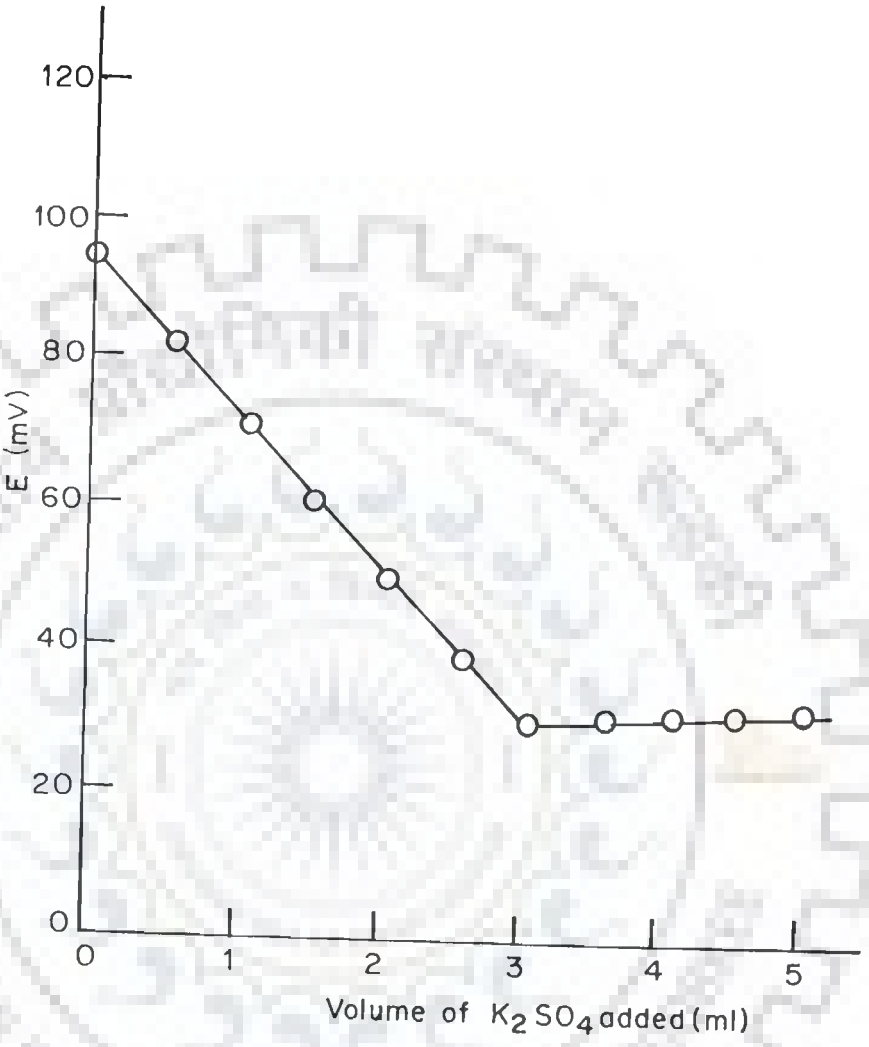


Fig. 3.7 Titration plot of 30ml of 10^{-3} M $BaCl_2$ with 10^{-2} M K_2SO_4.

3.5 ELECTROANALYTICAL PERFORMANCE OF THORIUM TELLURITE MEMBRANE (ESTIMATION OF Cr^{6+})

Most of the inorganic ion exchangers are found to be cation selective. Except some hydrous oxides under specified physical conditions, no other inorganic gel had been reported to possess anion exchange characteristics till late eighties. During the course of investigations (in this lab.) on the sol-gel behaviour of some compounds it was possible to prepare a positively charged gel of zirconium molybdate and some tellurites (3.27). Thorium and stannic tellurite were found to possess promising anion exchange characteristics and their membranes were tried for the estimation of anions.

Thorium tellurite membrane shows much better selectivity for chromate and dichromate anions, although sulphate and phosphate occupy a superior position in the selectivity sequence of this compound. Probably the presence of binder in membrane plays a significant role in its electroanalytical selectivity.

Homogeneous membranes of this compound were not stable. As such polystyrene supported membranes were prepared. The minimum amount of polystyrene, required to cast stable membranes was determined by trial. Chemically stable membranes, having small response time and capable of generating reproducible potentials were prepared by heating a mixture of tellurite and polystyrene (20% by wt.) at 100°C under a pressure of 7000 to 7500 psi. Table 3.12 shows the data obtained with membranes having different amount of polystyrene. Slope close to Nernstian value and best working concentration range is obtained with 20 per cent polystyrene by weight.

Membranes were equilibrated with 0.1 M K_2CrO_4 or $K_2Cr_2O_7$ solution for 48 hours. A solution concentration of 0.1M and 48 hours time for equilibration is sufficient so that membranes develop noiseless and reproducible potentials. These equilibrated membranes were thoroughly washed with distilled water and used for

the estimation of Cr^{6+} ions. Membrane potentials were observed (using cell given in chapter 2) with varying concentration of potassium dichromate or chromate. A reference solution of Cr^{6+} of 10^{-1} M concentration was used. Varying concentrations of reference solution (10^{-2}M and $5x10^{-1}$ M) were also tried (data not included in the text). An increase in response time, a more divergent slope and smaller working concentration range were observed in both case.

The plot of electrode potential vs concentration (Fig. 3.8) is perfectly linear in the concentration range 10^{-4} to 10^{-1}M which exhibits the utility of membrane electrode for the estimation of chromium as chromate or dichromate. As per IUPAC recommendations the practical limit of detection is $5x10^{-5}$ M(Fig. 3.8). The slope of the plot is 22 mV per decade of concentration not much away from the Nernstian value (29 mV per decade of concentration). The wide concentration range over which this membrane can be used (10^{-1} to $5x10^{-5}$M) makes it practically useful in the continuous analysis of waste effluent in which the constituents may show major variations in concentration.

The response time of the membrane electrode as measured at various concentrations of salt solution is 20 seconds and the potential remains stable for more then 5 minutes. Beyond this time a very slow drift is observed. If properly stored in water and cross contamination avoided, one membrane can be easily used for four months.

The precision of the electrode system was estimated in the early phase of this work and potentials generated by the membrane at a particular concentration were repeatedly monitored and the standard deviation was ± 0.5 mV in the higher concentration range and ± 2mV in the lower concentration range.

The pH dependence of membrane electrode is shown in terms of potential Vs pH plots of CrO_4^{2-} and $Cr_2O_7^{2-}$ ions at $1x10^{-3}$ M concentration (Fig. 3.9). The

working pH range of the assembly is 3 to 6 if Cr^{6+} is taken as dichromate and 8 to 11 if Cr^{6+} is taken as chromate.

The response of membrane sensor has also been observed in partially non-aqueous solvents. A linear potential vs concentration plot with almost the same slope (as that of calibration plot) is observed in 32 per cent (v/v) methanol-water, ethanol-water and acetone-water solvents (Fig. 3.10). A drift in potential is observed in solution containing a higher percentage of alcohol and the response time rises to about 2 minutes.

The performance of the electrode system has been assessed in presence of other interfering ions and its selectivity for chromate/dichromate over other anions has been determined in terms of selectivity coefficient values obtained by the fixed interference method.

Selectivity coefficient values for a number of anions, when present with chromate or dichromate, at an interference level of 10^{-3}M are given in Table 3.13. All the ions, except Cl^-, Br^-, NO_3^- , have very low selectivity coefficient values and do not interfere with the working of this membrane sensor. Cl^-, Br^- and NO_3^- ions would interfere only when present in larger concentrations in comparison to primary ion.

The electrode functions well even in the presence of other cations (other than K^+) (all experiments were conducted with potassium chromate/dichromate). Na^+, Li^+, Zn^{2+} etc. do not interfere at all . Lead, silver and barium interfere at levels at which these decrease the chromium concentration by precipitation.

The proposed membrane sensor was tried for chromium estimation in industrial effluents. Effluents, in addition to chromium may also contain some washings or anionic detergent. Thus, it was necessary to know the effect of anionic detergent on the working of the proposed electrode assembly. Membrane potentials were therefore recorded in the presence of Cr^{6+} in the concentration

range 10^{-2} to 10^{-5} M with and without the background concentration of 10^{-5} M sodium dodecylsulphate. The observations recorded in Table 3.14 show that the small addition of anionic surfactant causes a little shift in membrane potential to negative side. The shift in potential is definitely more when the concentrations of Cr^{6+} as well as the interfering ion are comparable.

The effect is attributed to initial monolayer formation at the membrane solution interface, followed by extraction into the membrane phase itself. It is possible to overcome this interference by treating the membrane with the surfactant solution (10^{-5}M concentration) for one hour. The treatment conditions the membrane and it becomes immune to the disturbing effect of anionic detergent. The conditioned membrane was thoroughly washed with distilled water and membrane potentials were recorded in Cr^{6+} solution (10^{-2} to 10^{-5}M concentration) containing 10^{-5}M sodium dodecyl sulphate (Table 3.14). The conditioned membrane does not sense any disturbance due to the presence of anionic surfactant except that there is a little shift of potentials to negative side and the slope of the plot also changes slightly in comparison to the original value recorded in calibration plot (Fig. 3.8). The shifting of potentials, in no way, affects the use of the membrane electrode and the same can be safely employed even in the presence of surfactant anions. The treated membrane can tolerate the presence of anionic detergent due to the desensitization of the sensing element and similar desensitization of electrode has also been reported earlier by Llenado (3.30).

The membrane sensor has also been used for the titration of chromate ions. Fig. 3.11. depicts the titration of chromate ions with barium acetate. A neat plot and an end point with perfect stoichiometry are characteristic features of this titration. A significant fall in potential is observed with the depletion of chromate ions and beyond the end point potentials stay almost constant (Fig. 3.11).

TABLE 3.12

S.No.	Amount of polystyrene (percent by wt.)	Detection limit (M)	Slope mV/decade of concentration
1	15.0	$8x10^{-5}$	19.5
2	20.0	$5x10^{-5}$	22.0
3	25.0	$1x10^{-4}$	36.4
4	30.0	$2.x10^{-4}$	39.5

TABLE 3.13

Selectivity coefficient values ($K^{pot}_{A,B}$) of various interfering ions at 10^{-3} M concentration for thorium tellurite membrane with CrO_4^{2-} and $Cr_2O_7^{2-}$ as primary ions

Interfering ion	$K^{Pot}_{A,B}$	
	CrO_4^{2-}	$Cr_2O_7^{2-}$
SCN^-	0.20×10^{-1}	0.20×10^{-1}
VO_3^-	0.32×10^{-1}	0.32×10^{-1}
Cl^-	1.20×10^{-1}	1.24×10^{-1}
Br^-	1.12×10^{-1}	1.22×10^{-1}
NO_3^-	1.50×10^{-1}	1.55×10^{-1}
SO_3^{2-}	0.35×10^{-2}	0.54×10^{-2}
SO_4^{2-}	0.12×10^{-2}	0.14×10^{-2}
MoO_4^{2-}	0.24×10^{-2}	0.26×10^{-2}
$S_2O_3^{2-}$	0.31×10^{-2}	0.34×10^{-2}
PO_4^{3-}	0.50×10^{-2}	0.51×10^{-2}
$Fe(CN)_6^{4-}$	0.23×10^{-2}	0.43×10^{-2}
WO_4^{2-}	0.30×10^{-2}	0.32×10^{-2}

TABLE 3.14

Potentials of thorium tellurite membrane (untreated and treated) observed with and without the presence of 10^{-5}M sodium dodecyl sulphate (SDS) in potassium chromate solutions of 10^{-2}- 10^{-5}M concentrations

Concentration of Cr^{6+} ion (M)	Potentials (mV)			
	Membrane untreated		Membrane treated	
	without SDS	with SDS (10^{-5}M)	without SDS	with SDS (10^{-5}M)
10^{-2}	-24.2	-29.2	-33.2	-33.4
10^{-3}	-47.1	-52.2	-58.5	-58.7
10^{-4}	-69.2	-73.4	-82.4	-82.5
10^{-5}	-71.3	-78.2	-84.2	-84.8

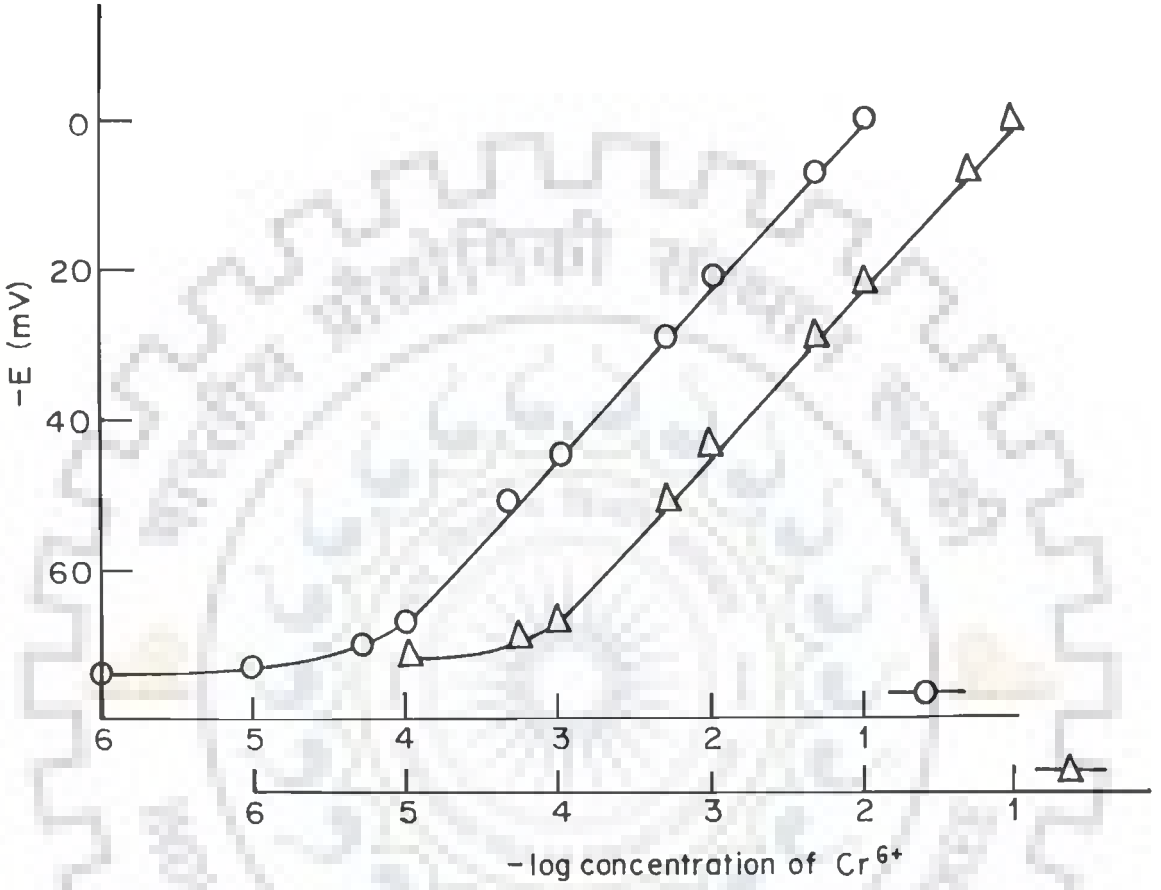


Fig. 3.8 Plot of cell potential versus -log concentration of chromium ions

—○— Chromate ions

—△— Dichromate ions

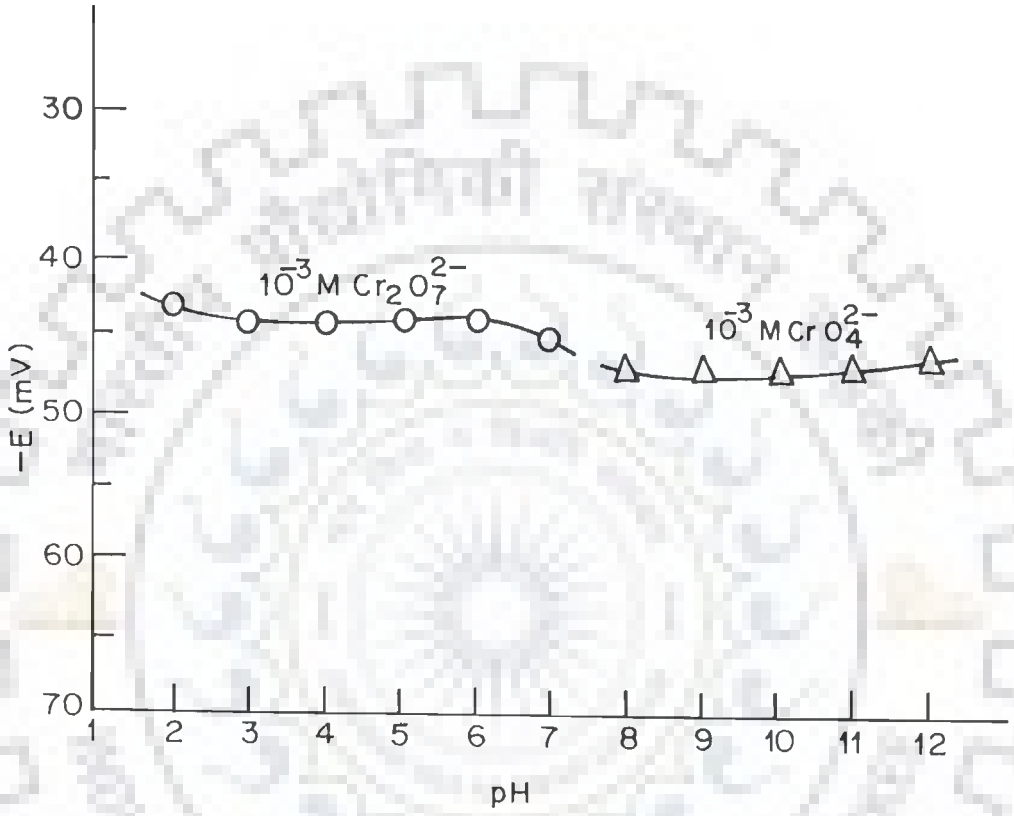


Fig. 3.9 Variation of membrane potential with pH.

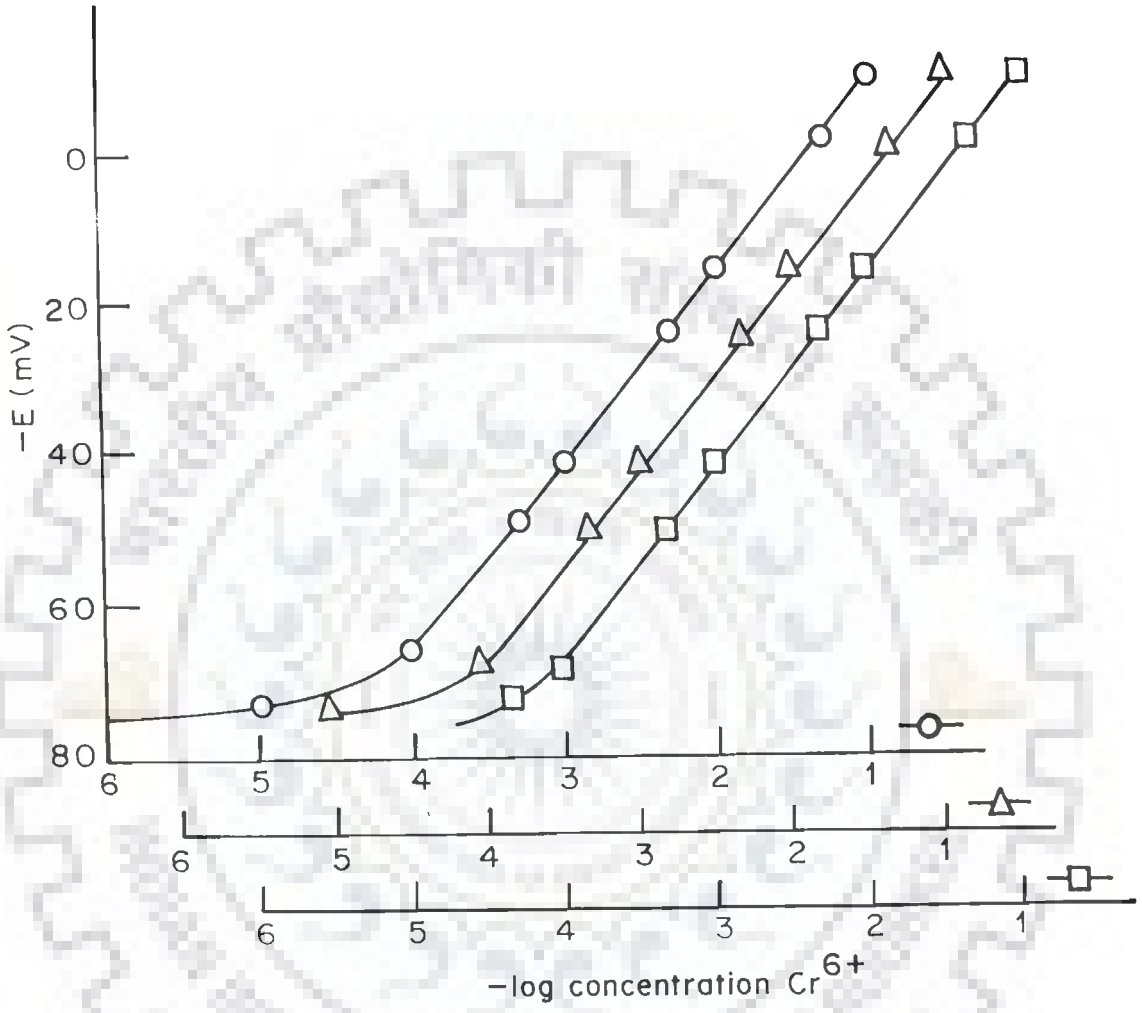


Fig. 3.10 Potential versus $[Cr^{6+}]$ plots in 32% —○— methanolic, —△— ethanolic and —□— acetonic solutions.

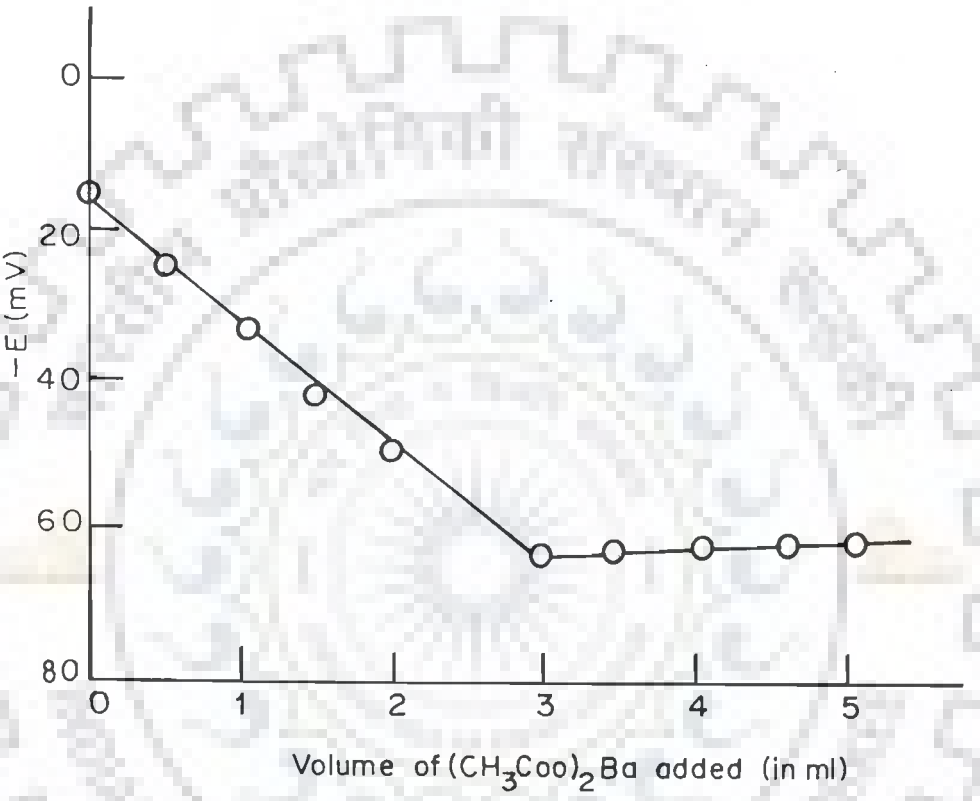


Fig. 3.11 Titration plot of CrO_4^{2-} (30ml of 10^{-2} M) with 10^{-1} M $(CH_3Coo)_2Ba$.

3.6 ELECTROANALYTICAL PERFORMANCE OF STANNIC TELLURITE MEMBRANE (ESTIMATION OF SO_4^{2-})

Stannic tellurite was prepared by the addition of stannic chloride to potassium tellurite at pH ~ 1. This compound also exhibits promising anion exchange properties. As such the membranes of this compound have also been tried for the estimation of anions of analytical interest.

The compound shows maximum affinity to sulphate ions and fortunately the membrane also exhibits the same trend. Stable membranes were prepared by embedding the compound with araldite binder (30 per cent by weight). Methodology used for preparing araldite based membrane is detailed in chapter 2. Optimum quantity of binder material, necessary for the fabrication of stable membranes, useful for electroanalytical work, was decided after preliminary investigations (Table 3.15). Thirty percent binder based membranes exhibit a wide working concentration range and almost Nernstian slope (30 mV per decade of concentration).

The concentration of solution and the duration of equilibration was also decided after a good deal of laboratory work and the membranes equilibrated with 0.1M K_2SO_4 solution for 48 hours were used for subsequent investigations. Membranes equilibrated, in this way generated noiseless, stable potentials.

Potentials observed with the membrane, when interposed between solutions of potassium sulphate (concentration range 10^{-1} - 10^{-6} M) and a reference solution of 10^{-1} M concentration, are recorded in Fig. 3.12. For this particular membrane the readings were also taken at constant ionic strength and recorded in the same figure. Although the plot is apparently linear upto 10^{-4}M, SO_4^{2-} concentration with a slope of 30 mV per decade of concentration the lower limit of detection is 7.5×10^{-5} M as per IUPAC norms (3.31). Slopes of this order are generally observed and are reported for solid state membrane sensors (3.32). The plot obtained at

constant ionic strength is in absolute correspondence with the one obtained without adjusting the ionic strength (Fig. 3.12).

The response time of the membrane is 30 seconds in concentrated SO_4^{2-} solution and 40 seconds in dilute solutions. Potentials remain constant for 3 to 4 minutes without any drift.

For determining the reproducibility of the membrane five replicates at different concentrations were observed and the standard deviation was 0.4 mV. One membrane was used for four months and no divergence was recorded. After this period it was again equilibrated with 0.1M K_2SO_4 for 24 hours and could be used for two months more. Thereafter it was discarded and replaced by a fresh membrane.

Different concentrations of reference solution were also tried but the best results were obtained with 0.1M K_2SO_4 solution.

Thus a low response time and a wide functional concentration range over which this sensor can be used to measure sulphate ions, makes it useful in the continuous analysis of waste effluents in which SO_4^{2-} ion constituent may show major variations in concentration.

Changes in membrane potential with pH at 10^{-3} and 10^{-4} M concentration of SO_4^{2-} are depicted in Fig. 3.13. Potentials stay constant between pH 7-10 which is the working pH range of this electrode assembly.

Potentials recorded in partially non-aqueous solvents show the applicability of the membrane in solutions having a maximum of 25 per cent non-aqueous content (Fig. 3.14). The response time of the electrode rises to one minute and a potential drift is also recorded in solutions having a higher non-aqueous content (data not included in the dissertation).

The performance of the electrode has also been assessed in presence of other ions and its selectivity for SO_4^{2-} over other anions has been determined by fixed

interference method (3.33). Selectivity coefficient values for various interfering ions are given in Table 3.16. It is observed that the polyvalent ions do not interfere at all in the working of this membrane sensor. Some monovalent ions like Cl^-, Br^- and NO_3^- may cause some interference. Apart from this the electrode functions well even in the presence of cations like Na^+, K^+, Zn^{2+}, Cd^{2+}, Mn^{2+}, Mg^{2+}. Alkaline earth metal ions interfere only when these are present in significant amount. Ag^+ and Pb^{2+} affect the electrode assembly at all concentrations.

This membrane has also been used as an end point indicator in the titration involving SO_4^{2-}. The titration of potassium sulphate (20 ml, 10^{-3}M) was performed with $BaCl_2$ solution ($5x10^{-3}$M) and the same has been shown in Fig. 3.15. The end point is sharp and stoichiometric. Fall in potential with the depletion of sulphate ions and a very small variation beyond the end point are the normal features of such titrations (Fig. 3.15).

This membrane sensor has also been tried for the monitoring of SO_4^{2-} in waste water. Since the samples of waste water are likely to be contaminated with anionic detergents also (released after some washings etc.) it is desirable to see the applicability of the electrode system in solutions of SO_4^{2-} mixed with an anionic detergent sodium dodecylsulphate (SDS). Potentials observed with SO_4^{2-} solutions at three different concentrations with and without the presence of 10^{-4} M SDS are recorded in Table 3.17. A perusal of the data shows the shifting of potentials to more negative side in the presence of SDS and the effect is naturally more pronounced when the concentration of SO_4^{2-} and SDS is same.

The disturbance caused by the anionic detergent on the working of the proposed electrode system can be removed by treating the membrane with $5x10^{-4}$M SDS solution for four hours. The concentration of solution and the duration of treatment has again been decided by several trials when the membrane develops

perfect immunity to the presence of surfactant anions. This technique of developing immunity has been successful only with few membranes. The chemical nature of membrane matrix is probably responsible for this discriminative behaviour. Potentials recorded with conditioned membrane in contact with SO_4^{2-} in the presence and absence of SDS are also given in Table 3.17. An almost perfect matching of potentials in presence and absence of SDS (Table 3.17) very well reflect the applicability of treated membrane for the estimation of sulphate ions even in presence of anionic detergent.

TABLE 3.15

S.NO.	Amount of Araldite (per cent by weight)	Detection limit (M)	Slope mV/decade of concentration
1.	20.0		unstable membranes
2.	25.0	1.0×10^{-4}	22.5
3.	30.0	7.5×10^{-5}	30.0
4.	35.0	8.5×10^{-6}	24.6

TABLE 3.16

Selectivity coefficient ($K^{Pot}_{A.B}$) values of interfering anions (10^{-3}M) for stannic tellurite membrane sensor

Anions	$K^{pot}_{A.B}$ values
Cl^-	11.4×10^{-2}
Br^-	10.2×10^{-2}
NO_3^-	7.6×10^{-2}
SCN^-	4.6×10^{-2}
VO_3^-	3.8×10^{-2}
SO_3^{2-}	2.5×10^{-2}
$S_2O_3^{2-}$	2.8×10^{-2}
MoO_4^{2-}	1.4×10^{-2}
WO_4^{2-}	2.2×10^{-3}
CrO_4^{2-}	1.5×10^{-3}
PO_4^{3-}	3.8×10^{-3}
$Fe(CN)_6^{4-}$	4.5×10^{-3}

TABLE 17

Potentials observed with potassium sulphate and potassium sulphate plus sodium dodecyl sulphate (SDS)solutions with treated and untreated stannic tellurite membrane

Concentration of SO_4^{2-} ion (M)	Potentials (mV)			
	Membrane untreated		Membrane treated	
	without SDS	with SDS(10^{-4}M)	wihtout SDS	with SDS(10^{-4}M)
10^{-2}	-30.0	-32.8	-30.2	-30.2
10^{-3}	-60.7	-64.3	-61.2	-61.5
10^{-4}	-90.2	-93.6	-90.6	-90.8

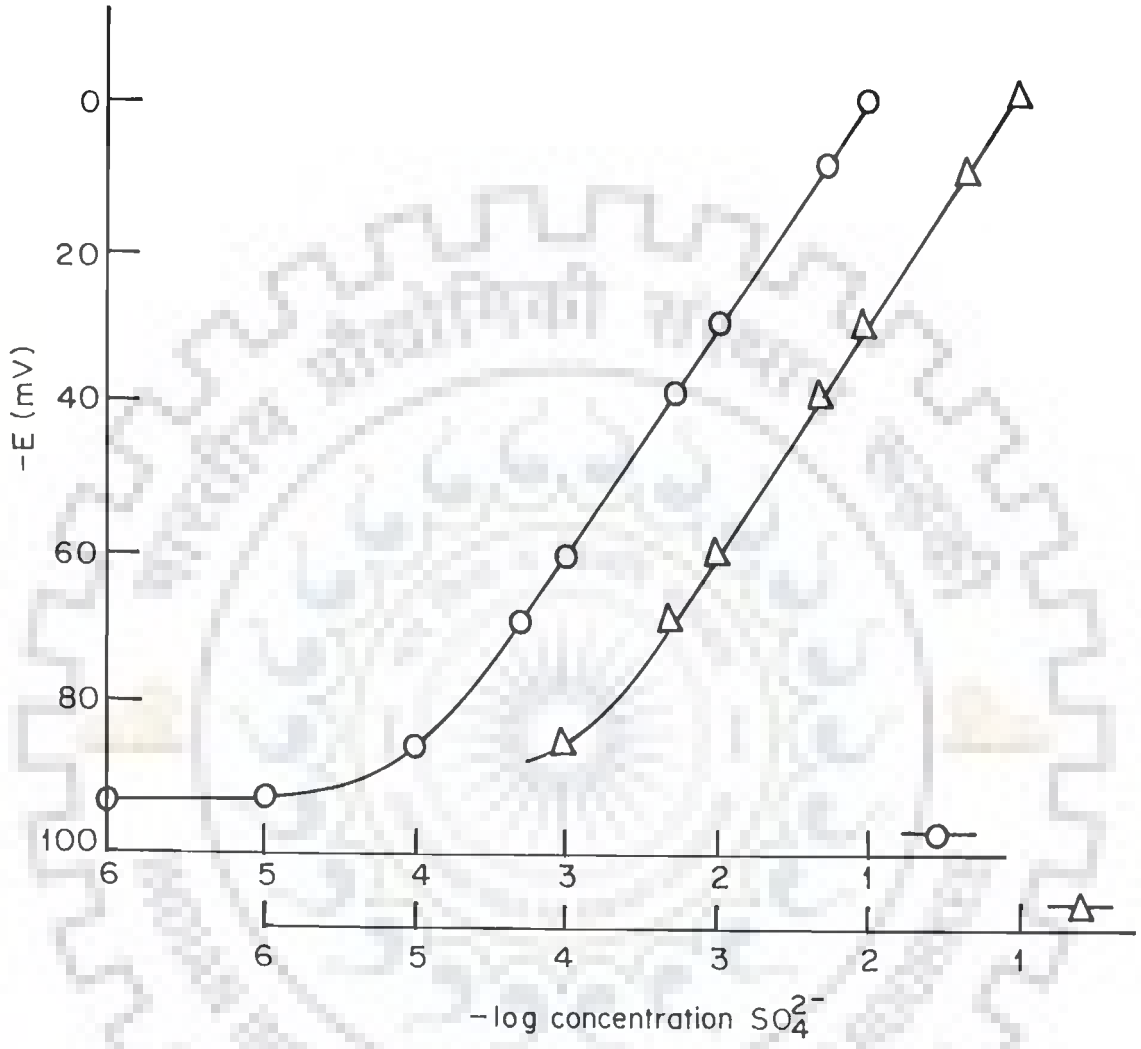


Fig. 3.12 Plot of cell potential versus -log concentration of Sulphate ions.

—○— Sulphate ions alone

—△— At constant ionic strength

(Sulphate + Sodium per chlorate)

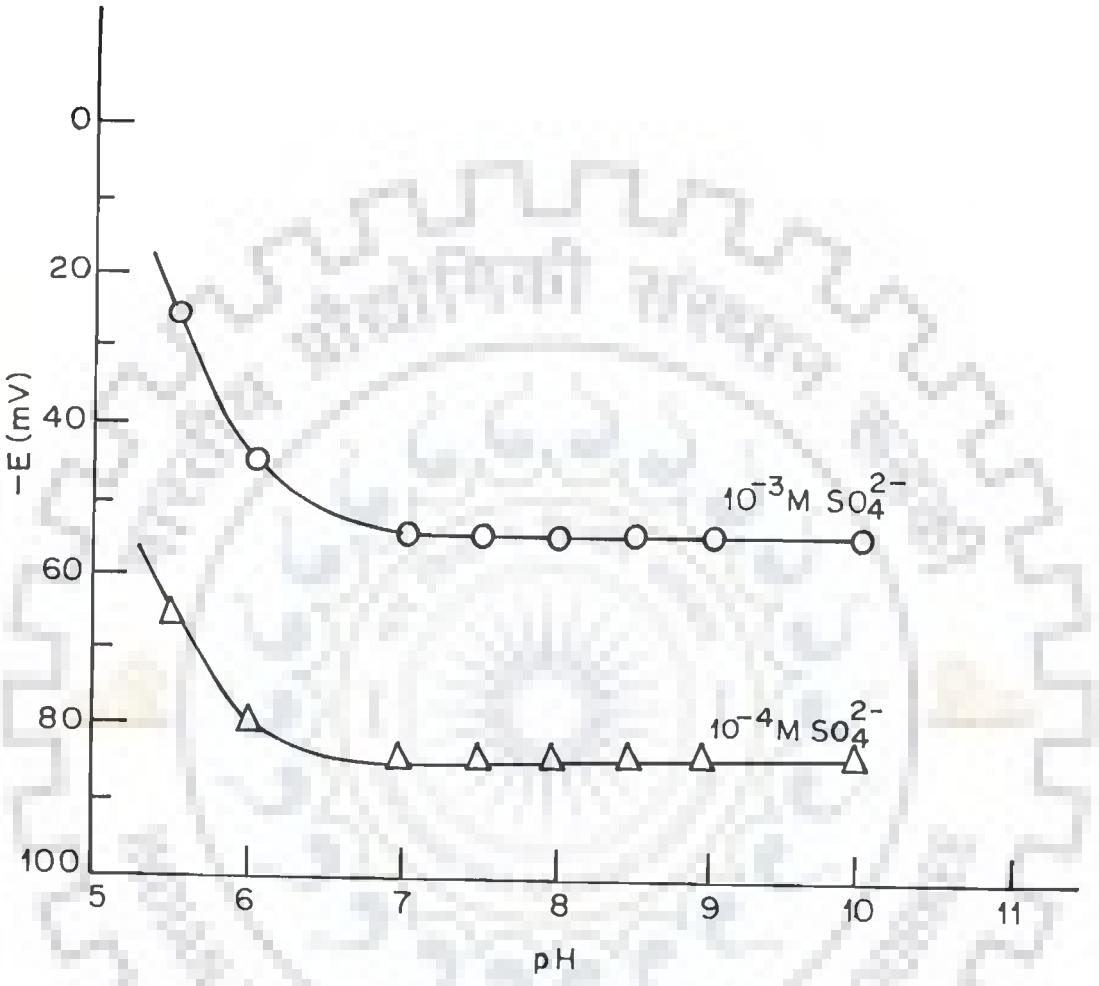


Fig. 3.13 Variation of membrane potential with pH.

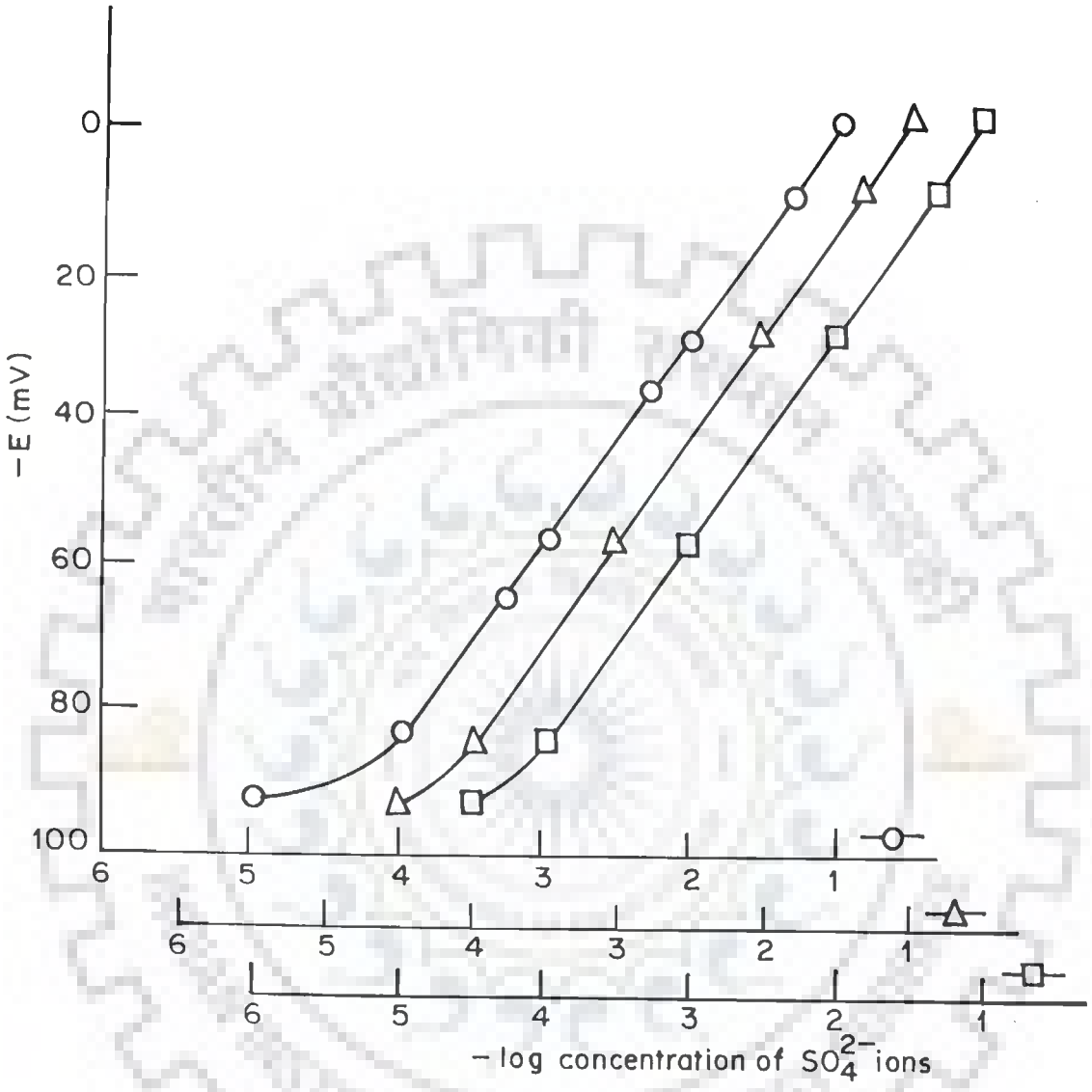


Fig. 3.14 Plot of cell potential versus -log concentration of sulphate ions in 25% methanolic (—○—), ethanolic (—△—) and acetonic(—□—) solutions.

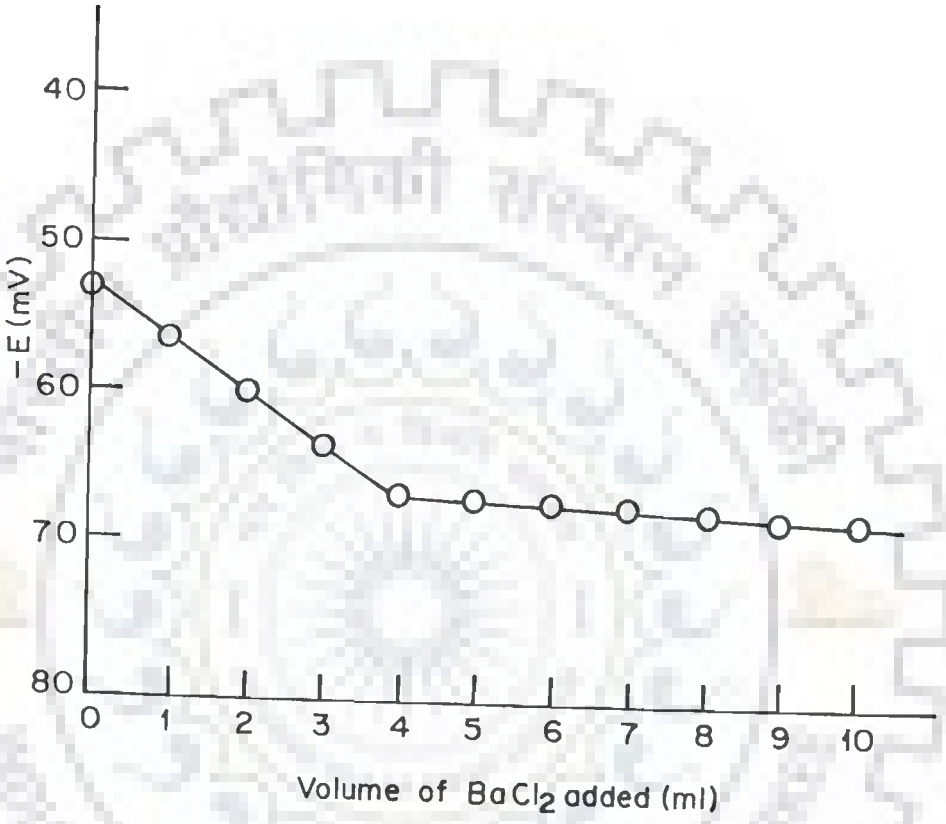


Fig. 3.15 .Titration curve of 20 ml, 10^{-3} M K_2SO_4 with 5×10^{-3} M $BaCl_2$ solution.

3.7 APPLICATION OF TELLURITE MEMBRANE SENSORS

(a) The utility of thorium tellurite membrane electrode has been observed for the analysis of actual wastes obtained from an electroplating and tannery plant. The details for the same are given in Tables 3.18 and 3.19. The samples of waste water were obtained from Modinagar and Moradabad (U.P.India). Official analytical data was also provided by the Analytical Section of the same industry. The samples were not subjected to any treatment except that the suspended particles were removed. Potentiometric evaluation of chromium was performed by the membrane electrode, under investigation. The results obtained were quite promising and the electrode system can be successfully used for the estimation of Cr^{6+} as CrO_4^{2-} /$Cr_2O_7^{2-}$ even in the presence of a significant amount of other ions. Measurements in more heterogeneous environment reveal that the system works well even in the presence of smaller amounts of protein. Larger amount of these substances however corrode the membrane surface.

It is worth mentioning, that estimation of Cr^{6+} by spectrophotometry, is possible in much wider range but the sensitivity and interference by large number of ions deter the use of this technique. The proposed method is applicable in comparatively narrow range of concentration (2.60 to 5200 mg l^{-1}) but is free of interference by various ions of concern in spectrophotometry. Besides this the electrode can be easily set up and the assembly is quite economical and useful for trend analysis.

Utility of stannic tellurite membrane sensor has also been observed for the estimation of SO_4^{2-} in actual wastes obtained from tannery and paper and pulp industry. Samples were collected from neighbouring towns (Meerut and Saharanpur) and the analytical data was also obtained from them. Samples were centrifuged and used as such. In the case of tannery waste the samples were treated with aluminium chloride, to mask the chloride ions, and sodium perchlorate was added

before measurements were made using the proposed membrane electrode. The analytical data obtained from the industry and the results obtained with the membrane electrode are given in Tables 3.20 and 3.21. Electrode performance is quite satisfactory and the sensor can be successfully employed for monitoring sulphate ions in wastewater.

Although sulphate estimation is possible by numerous other methods, but the range of concentration in which these methods can be used is quite high. Very few membrane sensors, so far, have been reported, in literature, for SO_4^{2-} , but most of these get disturbed by the presence of almost all the monovalent anions.

TABLE 3.18

Analysis of plating waste (concentration in ppm) using thorium tellurite membrane electrode

	pH	Cu	Fe	Cr^{6+}	CN^-	Cr^{6+} observed
A*	4.0	35	8.0	555	1.2	552
B*	3.6	58	1.2	620	0.2	617

* Analytical data obtained from the industry.

TABLE 3.19

Analysis of Tannery waste (concn. in ppm) using thorium tellurite membrane sensor

	pH	Permanganate value mg O_2L^{-1}	BOD mg O_2L^{-1}	Total nitrogen mg L^{-1}	Cl^- mgL^{-1}	S^{2-} mg L^{-1}	Cr^{6+} mg L^{-1}	Cr^{6+} observed
A*	8.5	232	235	177	2140	9.0	2250	2246
B*	9.0	515	476	370	2582	360	3226	3220

*Analytical data obtained from the industry.

TABLE 3.20

Analysis of paper and pulp waste using stannic tellurite membrane electrode

Ingredients	Sample 1		Sample 2	
	Data obtained from industry (ppm)	Estimated SO_4^{2-} (ppm)	Data obtained from industry (ppm)	Estimated SO_4^{2-} (ppm)
Soda	150	-	190	-
SO_4^{2-}	135	130	165	161
SO_3^{2-}	123	-	150	-

TABLE 3.21

Analysis of Tannery Waste using stannic tellurite membrane electrode

Ingredients	Sample 1		Sample 2	
	Data obtained from industry (ppm)	Estimated SO_4^{2-} (ppm)	Data obtained from industry (ppm)	Estimated SO_4^{2-} (ppm)
NaCl	384	-	524	-
Lime	672	-	760	-
SO_4^{2-}	175	169	250	232

3.8 REFERENCES

3.1. Coetzee, C.J., "Inorganic ion exchangers as ion sensors", Ion Selective Electrode Rev., 3, 105 (1981).

3.2. Frant, M.S. and Ross, J.W., "Lanthanum flouride electrode for flouride ions", Science., 154, 1553(1966).

3.3. Bailey, P.L., "Analysis with Ion selective electrodes" Heyden(1982).

3.4. Fagg, A.G., Pathan, A.S. & Burns, D.T., " A solid membrane sensor for potassium ions", Anal. Lett., 7,539(1974).

3.5. Rock, P.A., Eyrich, T.L. and Styer, S., J. Electrochem. Soc., 124, 531(1978).

3.6. D' Olieslager, and Heerman, L., " Estimation of Potassium and cesium by ferrocyanide membrane sensor", J. Electrochem. Soc., 126, 347(1979).

3.7. Vlasselaer, S. and D' Olieslager, "Membrane sensors for alkali metal ions(I), J.Inorg, Nucl. Chem., 38, 327(1976)

3.8. Vlasselaer, S. and D" Olieslager, "Membrane sensors for alkali metal ions(II). J. Radioanal. Chem., 35, 211(1977).

3.9. Malik, W.U., Srivastava, S.K., Razdan, P and Kumar S., "Tungstoarsenates as ion selective electrodes for cesium and thallium ions", J. Electroanal. Chem., 72, 111 (1976).

3.10. Jain, A.K., Srivastava, S.K., Singh R.P. and Agrawal, S., " Studies with inorganaic membranes - A membrane selective to strontium ion", J. App. Chem. & Biotech., 27, 680(1977).

3.11. Srivastava S.K., Jain A.K., Agrawal, S., and Singh, R.P., "Measurement of the activity and CMC of cationic surfactants using an inorganic ion exchange membrane electrode", J. Electroanal. Chem., 90, 291(1978).

3.12. Srivastava, S.K., Jain, A.K., Agrawal, S. and Singh, R.P., "Studies with inorganic ion exchange membranes", Talanta, 25, 157(1978).

3.13. Srivastava, S.K., Sahgal, V. and Vardhan, H., " A solid inorganic gel membrane for mercury", Sensors and Actuators(B)., 13, 391(1993).

3.14. Dorren, L. and Cheng, K.L., " Anion Selective membrane sensors based on insoluble salts", Talanta, 37, 901(1990).

3.15. Purdy, W.C., "Studies with carbon supported zinc electrode", Talanta, 37, 307(1990).

3.16. Beg, M.N and Arshad, M., "Use of nickel phosphate membrane as ion sensor with special reference to phosphate ion", Indian J. Chem., 27A, 460(1988).

3.17. Guilbault, G.G. and Brignac, Jr. P.J., "Studies of various inorganic phosphate salt membranes impregnated in silicone rubber, as electrodes, Anal. Chem., 41, 1136(1969).

3.18. Ihn, G.S., Nash C.F. and Buck, R.P., " Mono hydrogen phosphate - sensing electrode formulations", Anal. Chim. Acta., 121, 101(1980).

3.19. Midgley, D., " Solid State electrodes for phosphate ions", Talanta., 26, 261 (1979).

3.20. Ovozamsky and Riemsdijk, W.H.V., " The behaviour of silver phosphate as phosphate sensitive electrode", Anal. Chem. Acta., 85, 41(1976).

3.21. Tang, T.C. and Huang, H.J., Anal. Chim., 67, 2299(1995). "Flow injection analysis of sulphate ion",

3.22. Janata, J. and Bezegh, A., "Chemical Sensors", Anal. Chem., 60, 62R(1988).

3.23. Janata J., "Chemical Sensors", Anal. Chem., 62, 33R(1990)

3.24. Janata, J., " Chemical Sensors", Anal. Chem., 64, 196R(1992).

3.25. Janata, J., Josowicz, M. and De Vaney, D.M., "Chemical Sensors", Anal. Chem., 66, 207R(1994).

3.26. Srivastava, S.K. Saraswat, I.P. and Shardanand, "Selective separation of rubidium on titanium(IV) ferricyanide gel columns". J. Anal. Sci., (Japan) 2, 343(1986).

3.27. Srivastava, S.K., Singh, A.K. and Khanna, R., "Anion exchange properties of some tellurites", Indian J. Chem., 24, 254(1988).

3.28. Evmiridis, N.P., Demertzis, A.G. and Vlessidis, M.A., "Potassium ion estimation by synthetic zeolite membranes", Anal. Chem., 145, 340(1991).

3.29. Engel, D. and Grabner, E.W., "Membrane sensor for K^+ " Ber, Bunsenges, Phys. Chem., 89, 982(1985).,

3.30. Hartmann, M., Grabner, E.W. and Bergveld, P., " Ion slective electrodes for potassium" Anal. Chim. Acta., 242, 249(1991).

3.31. Llenado, R.A., "Potentio, response of a calcium selective membrane electrode ---" Anal. Chem., 47, 2243 (1975).

3.31. IUPAC recommendations for publishing manuscripts on ion selective electrodes. Ion Selective Electrode Reviews., 1, 139(1979).

3.32. Jasinski, R., Trachtenberg, I. and Andrychuk, D., Potentiometric measurement of copper in sea water., Anal. Chem., 46, 364(1974).

3.33. Malik, W.U., Srivastava, S.K.and Bansal, A., "Potentiometric estimation of molybdate with a solid membrane electrode", Anal. Chem., 54, 1399(1982).

Chapter - 4
Biosensors

4.1 INTRODUCTION

Biosensor is a device incorporating a biological sensing element either intimately connected to or integrated within a transducer. Generally the aim is to produce a digital electronic signal which is proportional to the concentration of specific chemical or a set of chemicals. The apparently alien marriage of two contrasting disciplines combines the specificity and sensitivity of biological systems with the computing power of microprocessor. This emerging technology crosses many traditional academic delineations and offers a powerful new tool which has radically altered our attitude to analytical science.

Biosensors were first described in 1962 in a symposium at New York Academy of Science (4.1). Significant characteristics of biosensors over other analytical techniques include simple operation, optimal sample frequency, extension to new substances, adapted sensitivity and improved specificity.

Efforts made to fabricate biosensors for glucose and phenol are described in this chapter.

4.2 BASIC PRINCIPLES OF BIOSENSORS

Biosensors are constructed by holding a biocatalytic substance near the tip of an ion or gas selective electrode. The electrode responds to diffusion of specific substrate for biochemical reaction into a by product, which the electrode can sense. The analysis is complete when the electrode response reaches a steady state potential. In biosensor the enzyme or microorganism is physically trapped or held at the sensing electrode surface. This process is called immobilization. There have been other methods of attachment which rely on chemical binding of enzyme / microorganism to the membrane surface (4.2). Some of these are :

(a) Adsorption

Adsorption is a very simple and quick technique. Typically a cell suspension is recirculated through a column containing the adsorbent. After attachment of cells the column is washed to remove free cells.

(b) Entrapment

The entrapment technique in polymeric matrices is most commonly applied and is a versatile technique in cases where cells are physically confined in the pores of a carrier. The pores must be small enough to prevent cell leakage, but sufficiently large to allow diffusion of substrate and product.

(c) Covalent bonding

In this bonding of a catalyst on a carrier is possible via reactive groups on the matrix or through the reactive bonding which links the biocatalyst to the carrier.

(d) Crosslinking

The simplest way of achieving the cell seggregation in the form of large particles with high cell density is the cell to cell crosslinking by addition of crosslinking chemicals. Chemicals mainly used are low molecular weight bi or multi function reagents like glutartaldehyde.

(e) Encapsulation

In this technique the whole cells are physically confined inside a porous capsule and remain in contact with each other.

4.3 TYPES OF BIOSENSORS

Biosensors are categorized into five classes on the basis of their applications :

(a) Potentiometric biosensors

In this type a signal is obtained by a non-enzymatic biochemical reaction. Analytical solution of steady state response of enzyme pH sensors has been derived and tested on penicillin and urea sensors (4.3).

(b) Amperometric biosensors

Amperometric or current measuring sensors monitor faradaic current that arise when electrons are exchanged between a biological system and an electrode held at an appropriate potential (4.4).

(c) Optical biosensors

An optical biosensor is a small device which together with its measuring instrument uses optical principles quantitatively or activities of interest in biology, into electrical signals. These electrical signals may be compensated, calibrated and interpreted, by using electronic signal processing in the instrument into a format suitable for display or for driving an actuator (4.5).

(d) Semiconductor biosensors

The device is based on the use of a catalytic metal as a gate of a field effect structure where the hydrogen sensitive Pd-gate metal oxide semiconductor (MOS) structure is the prototypical device (4.6)

(e) Immunosensors

These are based on the selective reaction between an antigen and the corresponding antibody that ensures specificity of the immunosensor in biological fluid analysis (4.7).

4.4 APPLICATIONS OF BIOSENSORS

The industrial applications of biosensors in the field such as the production of pharmaceuticals, food manufacturing, waste water treatment and energy production are on increase. Biosensor plays a very important role in monitoring raw materials, cell population and product formation in fermentation process. By the use of biosensors, samples can be measured over a wide concentration range without pretreatment and do not need to be optically clear. Microbial sensors are suitable for 'on line' control of biochemical processes.

Different varieties of glucose oxidase electrodes and equipment have been successfully used for years in health institutions for the measurement of sugar concentration in blood and urine (4.8,4.9).

4.5 PREPARATION OF GLUCOSE BIOSENSOR

General

Glucose is extensively used in most foodstuffs medicines, breweries etc. as a sweetener and it is very important to develop a fast, simple and reproducible method for its estimation. The operational characteristics of biosensor should be as follows:

(i) The substrate must be transported to the surface of electrode.

(ii) The substrate must diffuse through the membrane to the active site.

(iii) Reaction occurs at the active site.

(iv) The product formed in the enzymatic reaction is transported through the membrane to the surface of the electrode.

(v) Product is measured at the electrode surface.

Literature review

The first working electrode was reported by Updike and Hickes (4.10) using glucose oxidase immobilized in a gel over a polarographic oxygen electrode to measure the concentration of glucose in biological solution and tissues. These were both voltammetric or amperometric probes, i.e., the current produced upon the application of a constant applied voltage was measured. Hikuma et. al., described (4.11) a microbial sensor consisting of immobilized whole living cells of *Brevibacterium lactofermentum* and an oxygen electrode was constructed for the continuous determination of total assimilable sugars (glucose, fructose and sucrose)in a fermentation broth. Bacteria were immobilized in a strip of nylon net (1cm x 1 cm, 20 mesh) and attached to an electrode. The response time was 10 min using a steady state determination and 1 min by the pulse method. Sensitivity of the microbial sensor to glucose, fructose and sucrose existed in a ratio of 1.00: 10.80: 0.92.

Karube et al., constructed (4.12) a microbial sensor having immobilized whole cells of *pseudomonas fluorescens* and an O_2 electrode was developed for the determination of glucose. The sensor responded slightly to fructose, mannose and saccharose, but no response was observed in the case of amino acids. A linear relationship was observed between the current and the concentration of glucose between 20 mgl^{-1} by steady state determination and the minimum detectable concentration of glucose was 2 mgl^{-1}. The current was reproducible to within ± 6% when a sample solution containing 10 mgl^{-1} of glucose was employed. No decrease in current output was observed over a two week period and upto 150 assays.

Demura and Asakura prepared (4.13) a glucose sensor by immobilizing glucose oxidase (GOD) in *Bomby x mori* silk fibroin membrane by physical treatment i.e. stretching without any chemical reagents. Glucose sensor prepared with this GOD-immobilised fibrioin membrane was developed and the capabilities such as response time, calibration curve, and repeating usage were determined. The response time was 8.5 seconds and the standard error of repeating usage was around 0.9% in the pH range of 5-8.

Abdulla described (4.14) enzyme electrodes based on fluoride and iodine-selective coated wire electrode for the determination of glucose. The electrodes consisted of a homogeneous polyvinyl chloride membrane containing both the electroactive species and immobilized glucose oxidase. Two enzyme electrodes based on iodine were investigated. The first of these utilized an ammonium molybdate catalyst and had a life time of 4 days. The life time of the second electrode, for which peroxidase was also immobilized in the electrode membrane was six days. The enzyme electrode based on fluoride also had a life time of six days.

Palleschi et al. suggested (4.15) that lactase and glucose were measured in whole blood of athletes running on a treadmill by using two extracorporreal electrochemical biosensors. The lactase sensor was fixed to an endocrine artificial pancreas which had been used in previous extra carporreal experiments. The lactase sensor gave a signal which resulted in a well defined curve that allowed the evaluation of the aerobic as well as the anaerobic threshold. The results obtained with the glucose sensor supported the theory that muscle anaerobic glycolysis is dependent on muscle glycogen rather than on blood glucose.

Alva et al. prepared (4.16) a glucose sensor by spreading a solution of glucose oxydase (50μl) in 0.5 M phosphate buffer containing 17.5% bovine serum

albumin mixed with glutaraldehyde solution (20μl) to a Pt strip on both sides. The Pt strip was used as the working electrode with a Pt mesh counter electrode and a SCE for the potentiometric determination of glucose. The calibration graph was rectilinear from 0.1 to 5 mM.

Cardosi and Birch described (4.17) the manufacturing of disposable strip type electrode for glucose measurement. Glucose oxidase was immobilized onto a platinized Vulcan XC-72 carbon black particle by screen printing following activation of the carbon surface with N-hydroxy succinamide. The sensor construction allowed coupling of the biocatalytic reaction with the electrocatalytic oxidation of the liberated peroxide. Calibration graphs were rectilinear from 20 μM to around 8 mM of glucose concentration.

Hoa et al., suggested (4.18) a biosensor for glucose based on the use of a polyaniline conducting polymer containing glucose oxidase. A polyaniline film was deposited on two platinum discs by cycling the potential from -0.2 to 0.8V VS SCE in 0.1M anilene solution in 0.1M H_2SO_4. A second glucose oxidase polyanilene film was deposited on the first layer by cycling between -0.2 and + 1.2V VS SCE. Sensors were immersed in sample solution for 20 second before glucose determination. Response was rectilinear upto 10 mM of glucose concentration.

Snejdarkova et al., reported (4.19) the preparation of a glucose sensitive electrode containing essentially a streptoviridin glucose oxidase complex bonded to a biotin modified phospholipid bilayer and coated on to a stainless-steel wire (0.3 mm diam), precoated with a polyoxyphenylase film (5 μ mol). In operation enzymically generated H_2O_2 was monitored at + 670 mV vs a SCE as reference. Measurement of glucose upto 50 mmol were possible in 0.1M - Tris - HCl- 0.1M-KCl (1:1) at pH 7.0. The calibration graph was rectilinear upto 7 m mol of glucose. The practicability of measurement of glucose in blood and urine was explored.

Karyakin et.al., (4.20) have recently reported a first generation amperometric glucose biosensor based on a Prussian blue modified electrode. This was developed by immobilization of glucose oxidase with a Nafian layer on Prussian blue modified electrode. The biosensor response exhibited a linear dependence for analyte concentration in the range 10^{-6} to $5x10^{-3}$M.

In the present study a new biosensor for specific determination of glucose was developed. The construction of the biosensor was based on the idea that glucose will be utilized by the microorganism in its metabolic pathway and the production of hydrogen ion in these reactions will be directly proportional to the concentration of glucose present in the solution. The biosensor was developed by coupling a pH electrode with the membrane of immobilized microorganism to measure the decrease in pH.

Lactobacillus species, during preliminary investigations, was found to be capable of degrading glucose and in the process lactic acid is produced. Extending the same investigations it was found that Lactobacillus bulgaricus was capable of utilizing glucose in a very short duration of time and there was a significant change in hydrogen ion concentration of the solution due to the production of lactic acid. Consequently efforts were made to develop a biosensor for glucose by using this particular microorganism and a glass electrtode was taken as a transducer to observe the decrease in pH.

4.6 MATERIALS AND METHODS

a) Cultivation of Micro-organism

The cultures of bacterial strain lactobacillus bulgaricus were obtained from the National Chemical Laboratory, Pune, India. The strains were checked for their purity according to Bergeys manual of systematic bacteriology (4.21).

Bacterial strain was cultivated in the following medium:

Glucose	20 gl^{-1}
Peptone	6 gl^{-1}
Sodium acetate	6 gl^{-1}
Yeast extract	5 gl^{-1}
Salt A	5 ml
Salt B	5 ml

Salt A contains K_2HPO_4 (100gl^{-1}) KH_2PO_4 (100 gl^{-1}) and salt B contains $MgSO_4$ (40 gl^{-1}), NaCl (2 gl^{-1}), $MnSO_4$ (2 gl^{-1}) and $FeSO_4$ (2 gl^{-1}). pH of the medium was kept 7.6 before autoclaving with phosphate buffer. Bacterial strains were maintained on the slants of nutrient agar medium at 4-8^{0}C and transferred periodically.

For the cultivation of micro-organism, bacteria were grown in 500 ml conical flask containing 150 ml of the above medium. Medium was inoculated with strain and incubated on a rotary shaker at 30^{0}C for 72 hours. The bacteria were collected by centrifugation on a R24 REMI research centrifuge at 1000 g for 15 minutes and washed with sterile water. These bacterial cells were used for the preparation of membrane after immobilization.

4.7 PREPARATION OF MEMBRANE

The entrapment technique was used to immobilize the cells of Lactobacillus Bulgaricus for the preparation of membrane. For immobilization, agar plates were prepared having 2% of agar and 0.2M $CaCl_2$. Two grams of cells (dry weight) were taken and mixed with 2% of sodium alginate. This was poured (a thickness of

0.5 mm) on the agar plates and kept at room temperature for over night to make the membrane ready.

4.8 ELECTRODE PREPARATION

The membrane of immobilized cells (1x1 cm) was coated over a pH electrode with an O-ring and covered with nylon net to protect the membrane. The electrode response was measured with a pH meter.

4.9 MEASUREMENT OF ELECTRODE RESPONSE

A standard glucose solution was prepared with double distilled water and the biosensor was washed with 0.9% saline water for 3 minutes after each analysis. The volume of glucose solution taken for analysis was 20 ml. The signal of electrode was detected after every 2 minutes for 30 minutes with a biosensor connected with the pH meter. When the steady-state method was used in measuring glucose concentration, the signal of electrode was detected until no further change in digital signal was observed and the response value at steady state was correlated with glucose concentration. A steady state was reached in about 3 minutes after the sensor was placed in the glucose solution. Effect of sample pH, temperature, specificity and stability of biosensor was also studied and all the measurements were carried out at 31^0C.

4.10 ANALYSIS BY HPLC

Glucose concentrations were also confirmed by HPLC (Water Assoc. Milford, MA). The column used was Sugar-PAC I and column temperature was maintained at 90^0C. Water containing 50 mgl^{-1} Ca-EDTA was used as mobile phase, the flow rate was 0.6 ml min^{-1}. Column eluant was detected by refractive index (R-401, Water Assoc.).

4.11 RESULTS AND DISCUSSION

It was found that Lactobacillus species are capable of utilising glucose and in the process of its degradation lactic acid is produced in a very short duration of time and a significant decrease in hydrogen ion concentration takes place.

increase

(a) Preparation of Calibration Curve

Typical response curve at different concentrations of glucose were observed in order to obtain the calibration curve. The digital values were detected (initially after shorter durations) for 30 minutes using a biosensor connceted to pH meter. These curves are shown in (Fig.4.1). The digital signal from the biosensor decreases more rapidly with an increase in the concentration of glucose. It can be observed that one assay can be completed within a span of three minutes. A calibration curve (Fig. 4.2) is obtained with the help of the plots depicted in Fig.4.1. The calibration plot (Fig. 4.2) is quite satisfactory, being linear upto 2500 mgl^{-1} concentration of glucose. Beyond that no significant change is observed in pH. Reproducibility of the biosensor was observed by monitoring a solution of glucose (concn. 1500 m $mol.l^{-1}$) 15 times in a series and the divergence in various readings was ± 12 m $mol.l^{-1}$.

b. Effect of sample pH

Since the biosensor detects a change in hydrogen ion concentration, the effect of sample pH on its response has been investigated and shown in Fig. 4.3. It is apparent that the electrode response is linear (Fig. 4.3) in pH range 5.0 to 7.0 which can be taken as the functional pH range of the glucose sensor. An increase in hydrogen ion concentration is observed beyond this concentration. For optimum working of the electrode a pH range of 5 to 7 is quite necessary.

(c) Effect of temperature

The effect of temperature on biosensor response is depicted in Fig.4.4 The response of electrode has been observed from 15 to 50^0C. It is observed that the optimum temperature for the working of electrode is 31^0C. As such subsequent measurements were conducted at 31^0C.

(d) Specificity of the biosensor

The interference of various substances such as sucrose and other mono and disaccharides is a serious problem in measuring glucose concentration in sample solution. In order to evaluate the specificity of the biosensor, the effect of these substances on the sensor response was investigated. Since the interferents are present simultaneously with the primary determinand, investigations were planned to observe the effect of the presence of sucrose, fructose, lactose, cellubiose, maltose, sorbose and galactose on glucose estimation using this electrode system. It is observed (Table 4.1) that sucrose, fructose and lactose cause interference. The top horizental column of Table 4.1 depicts the concentration of various interferents while the electrode response (pH), with interferent and glucose both present in solution, is recorded in the vertical columns. If these three sugars sucrose, fructose and lactose are present in solution alongwith glucose the biosensor would show divergence. These three sugars also, may not cause any disturbance if the same are present at concentrations equal to or less than 1500 mgl^{-1}. Beyond this limit the presence of sugars would cause a drift in the response of the biosensor. Other sugars e.g., cellubiose, maltose etc. do not interfere at all.

(e) Stability of biosensor

Fig.4.5 shows the stability of glucose biosensor under operational

conditions. Durability of a biosensor is characterized by its storage and operational stability. When stored at 4^0C in 50 m mol $CaCl_2$ solution the biosensor does maintain hundred percent activity for more than ten days. The operational stability of the biosensor was examined by measuring the response of the electrode as a function of time under optimized conditions (Fig.4.5). The biosensor seems to remain stable for eight days under operational conditions and slight divergence is recorded after this time (Fig.4.5).

Practically, all glucose biosensors are stored referigerated and immersed in an appropriate buffer, their life time is known to be 4-5 days. The purpose of this procedure is to minimize microbial metabolism.

A response time of 3 minutes, sufficient working pH range, tolerance of other sugars and stability of eight days makes it specially useful for analysing glucose in food stuffs.

TABLE 4.1

Response of glucose biosensor in presence of different interferents

Substrate	Concentration (mg/l)					
	1000	1500	2000	2500	3000	3500
	(Electrode response, (pH)					
Sucrose	6.2	6.1	5.5	5.1	4.8	4.6
Fructose	6.2	6.1	5.7	5.2	4.9	4.7
Lactose	6.2	6.1	5.6	5.3	4.9	4.7
Cellubiose	6.2	6.2	6.2	6.2	6.2	6.2
Maltose	6.2	6.2	6.2	6.2	6.2	6.2
Sarbose	6.2	6.2	6.2	6.2	6.2	6.2
Galactose	6.2	6.2	6.2	6.2	6.2	6.2

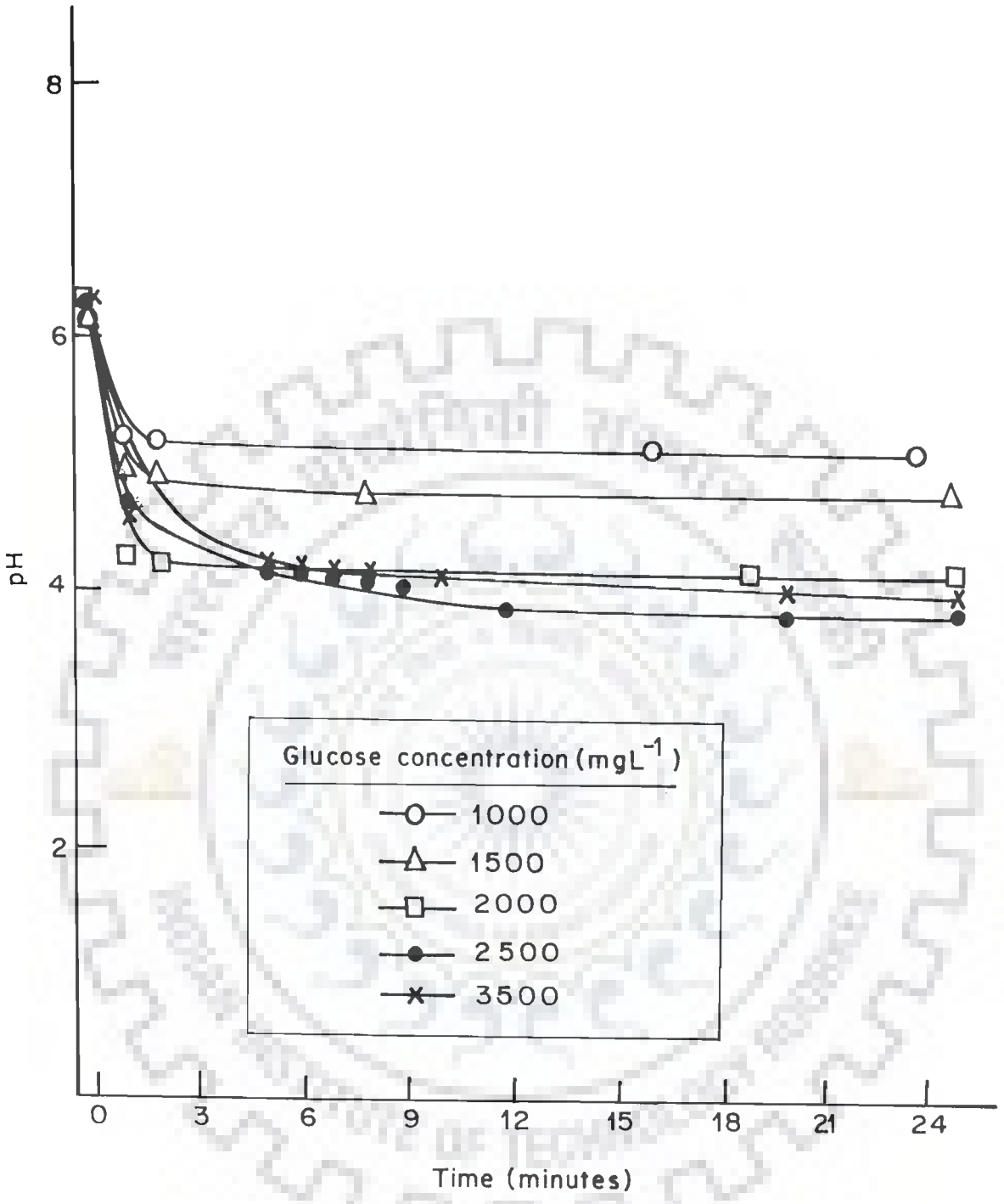


Fig. 4.1 Effect of glucose concentration and optimization of response time

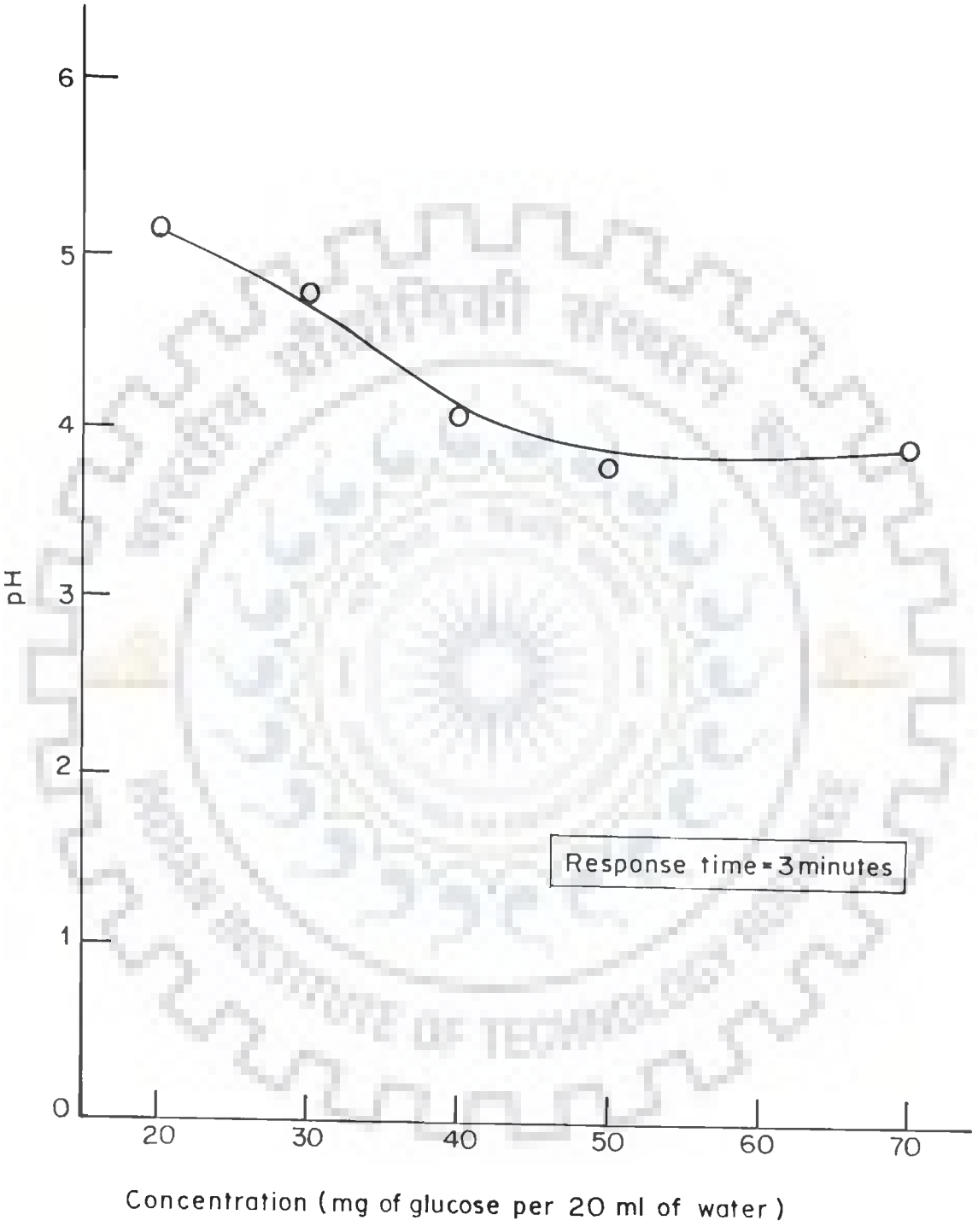


Fig. 4.2 Calibration curve of glucose

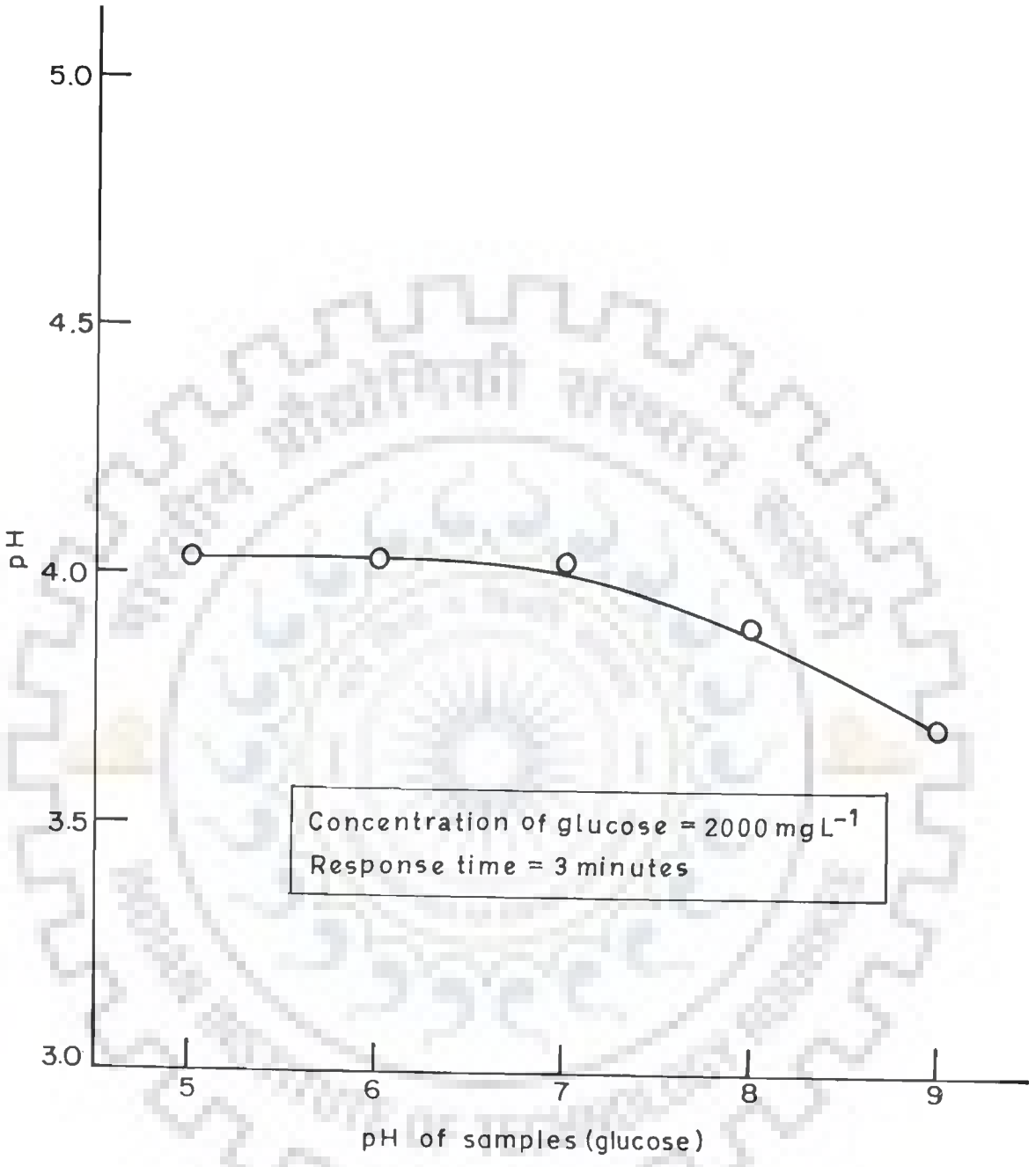


Fig. 4.3 Effect of sample pH on electrode response

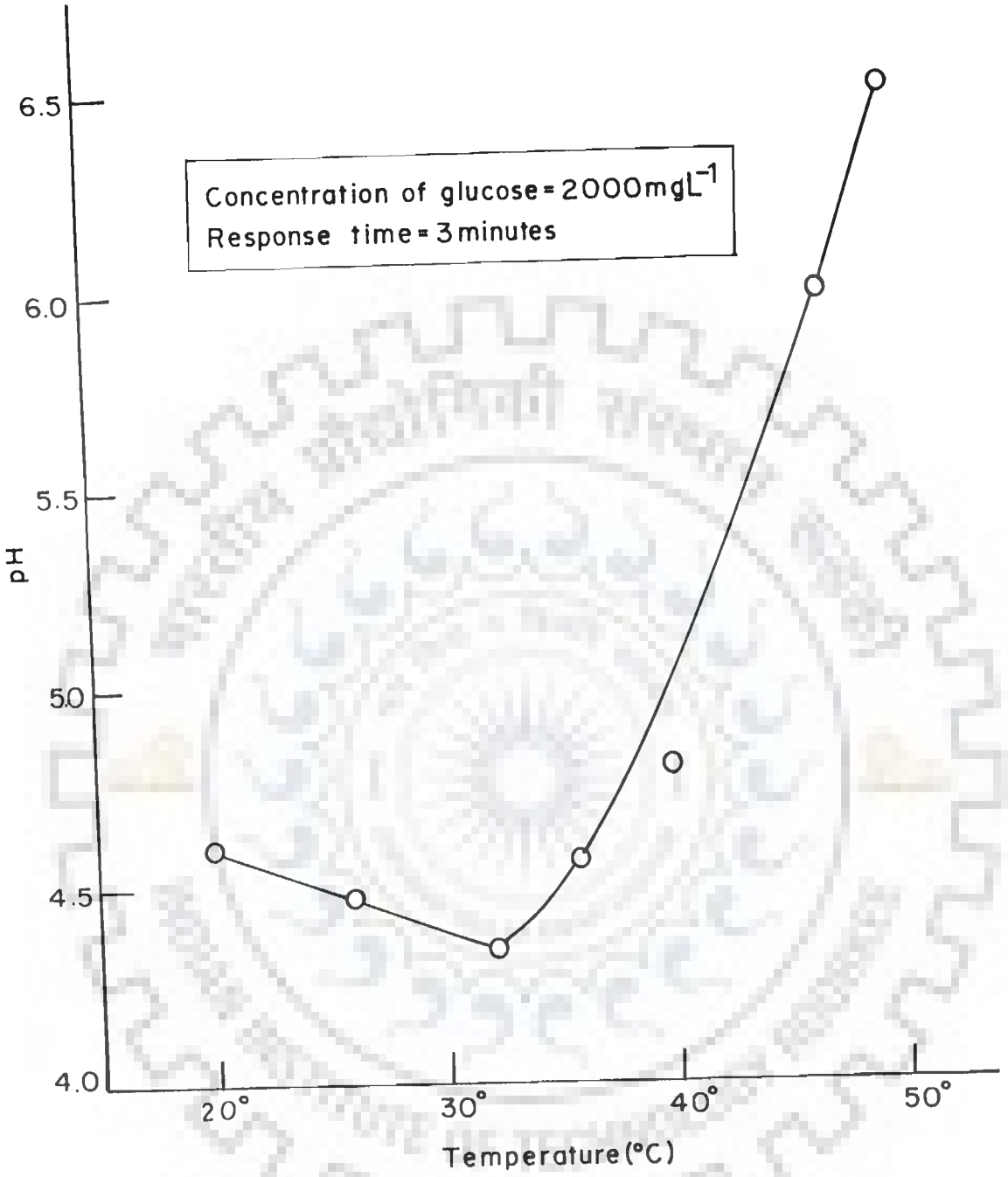


Fig. 4.4 Effect of temperature on electrode response

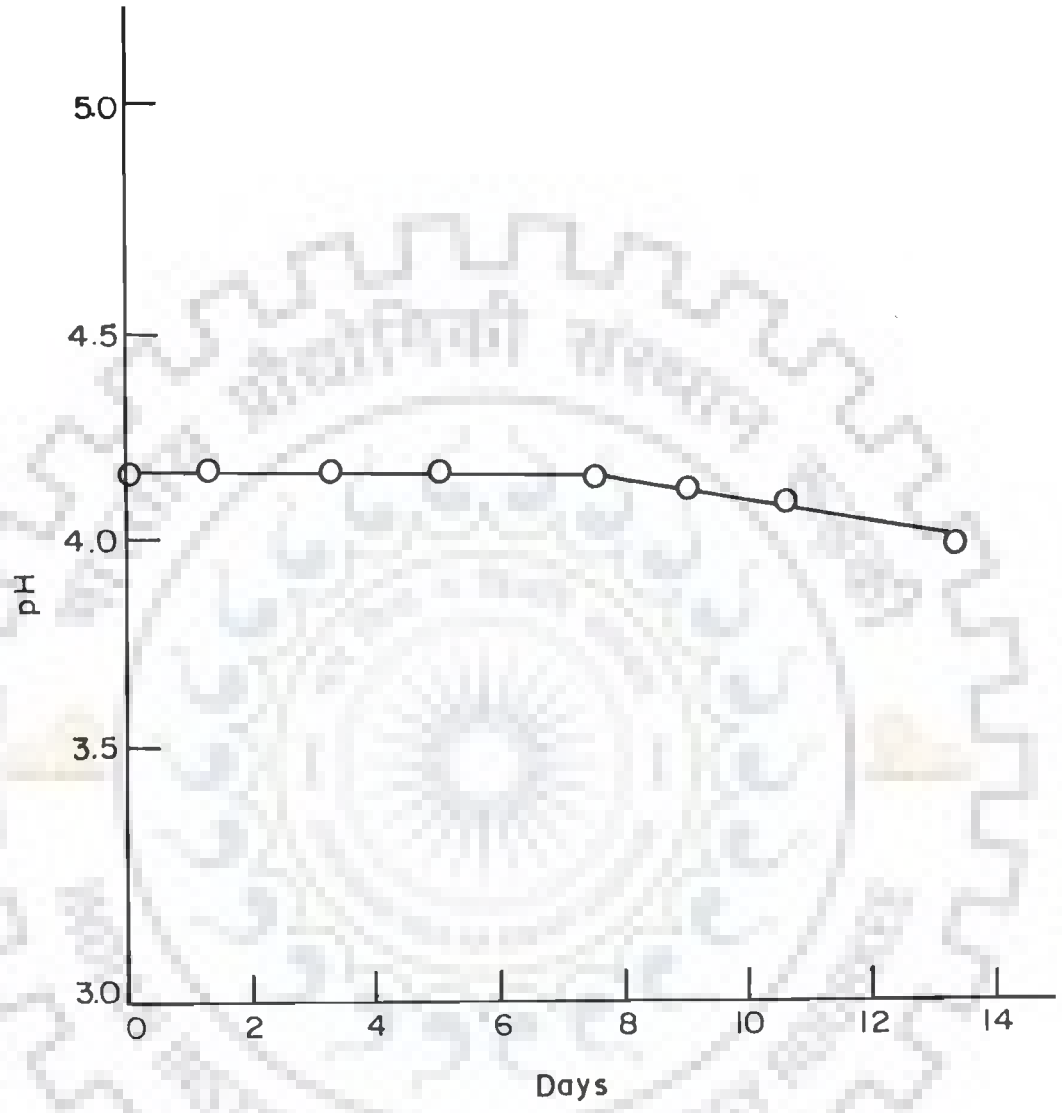


Fig. 4.5 Stability of glucose biosensor under operational conditions

4.12 PREPARATION OF PHENOL BIOSENSOR

Considerable attention has been given to reliable quantification of phenols in complex environmental, food, pharmaceutical and industrial matrices.

Phenolic compounds are present in nature as polymers such as tannins, anthocyanins and flavolans i.e., substances responsible for the organoleptic properties and colours of many flowers and fruits. Although some phenols are used in medicine and as detergents and disinfectants, these are generally very toxic and damage urinogenital organs, liver and kidneys. Pollution from phenols may be due to industrial wastes from pulp mills, distilleries and refineries. As such a fast and easy method of analytical determination of phenol is of great importnace.

Various methods e.g., Spectrophotometry, fluorimetry, gas chromatography and HPLC have been used for this purpose but for environmental pruposes and with waste samples the techniques should be rapid, inexpensive and capable of being used 'in situ' and biosensors are quite suitable in this respect. Efforts made to develop a phenol biosensor and its details are mentioned in subsequent paragraphs.

4.13 LITERATURE REVIEW

Mizutani et. al., (4.22) reported an enzyme electrode with a chemically amplified response for hydroquinone, norepinephrine and other dihydroxyphenols such as catechol, dopamine, epinephrine etc. It was constructed from a glassy carbon electrode and a layer containing immobilized glucose oxidase. Dihydroxyphenol molecules were consumed at the electrode surface, but were regenerated by the glucose oxidase reaction in the layer. The consumption/ regeneration cycle for dihydroxyphenols resulted in an electrode response

amplified by 2-120 times and accordingly, in an increased sensitivity. The detection limit of hydroquinone, catechol or dopamine was as low as one μ mol.

Tillyer and Gobin described (4.23) a catechol enzyme electrode in which a clark-type oxygen electrode was coupled to immobilise polyphenol oxidase in albumin cross-linked with glutaralhedyde on a dialysis membrane. Electrode calibration, response time, pH response, stability, detection limit and selectivity were evaluated and the feasibility of using the sensor for the measurement of catecholamines in the urine of patients with neural crest tumors was assessed.

Ghindilis et al., proposed (4.24) a new method of amperometric determination of phenolic compounds using an enzyme electrode constructed by the combination of oxygen electrode and immobilized laccase. The time needed for analysis in the flow injection mode was below 100 seconds. A column with immobilized enzyme could be used for as many as 500 determinations of phenolic compounds without any decrease in enzyme activity. The practical validity of the method was demonstrated by tannin analysis in tea of different brands.

Mazzei et. al. (4.25) suggested the quantitative determination of catechol and other biologically active polyphenols by means of plant tissue electrode built up by a whole tissue containing a gas-selective enzyme (polyphenol oxidase, PPO). This type of electode was found to be more stable than those prepared by using purified PPO enzyme.

Campanella et.al., performed (4.26) research, using tyrosinase enzyme to develop a suitable probe sensitive to phenols, working in non-aqueous media. For this purpose a clark O_2 electrode as indicating sensor, alongwith tyrosinase enzyme immobilized in a dialysis membrane and n-hexane as non-aqueous solvent, were used. A comparison of the response between the sensor, when working in n-hexane, and in aqueous buffer, was also reported.

Tan and Chen fabricated (4.27) a polyphenol oxidase sensor by covering a dissolved oxygen probe with a polycarbonate membrane on which fine apple powder, which was thoroughly washed and vacuum dried,was immobilized. The sensor showed good response towards dopamine and other phenolic related compounds. The pretreated apple powder, used in the present sensor, showed preferential sensitivity to dopamine. Major interfering solutes include, L-cysteine, tyrosine, catechols and L-histidine.

Kotte et.al., (4.28) have very recently reported a methyl phenazonium modified enzyme sensor based on polymer thick films for the detection and estimation of phenols. A methyl phenazonium-zeolite modified enzyme sensor based on a planar screen printed two electrode arrangements has been developed for phenols. It shows marked sensitivity to eight phenolic compounds usually present in industrial waste water.

During the course of investigations to identify a suitable bacterial strain for the development of phenol biosensor, it was observed that *pseudomonas cruciviae* is able to degrade phenol in the presence of oxygen. The decrease in oxygen is found to be proportional to phenol concentration.

4.14 MATERIALS AND METHODS

(a) Cultivation of Microorganism

The cultures of bacterial strain *Pseudomonas Cruciviae* (NCIM 2004) were collected from National Chemical Laboratory, Pune, India. Stock cultures were maintained in nutrient agar and checked for their purity according to Bergey's mannual of systematic bacteriology (4.21). Bacterial strains were maintained at 4-8^{0}C and transferred periodically.

For cultivation of microorganism, initially bacteria were grown in basal medium having the following composition :

$(NH_4)_2SO_4$	—	0.26%
K_2HPO_4	—	0.1%
KH_2PO_4	—	0.01%
$MgSO_4$	—	0.02%
$CaCl_2$	—	0.001%
$FeSO_4$	—	0.0001%
Yeast extract	—	0.01%
Glucose	—	0.2%

The pH of the medium was adjusted at 7.4 with phosphate buffer before autoclaving. 150 ml of the inoculated medium was inoculated in 500 ml erlenmeyer flask on a rotary shaker (135 rpm) at 34^{0}C. After 48 hours, cells were collected by centrifugation at 1000g for 15 minutes on R-24 REMI Research centrifuge and washed throughly with sterilized distilled water under sterile conditions. These bacterial cells were used for the preparation of membrane after immobilization.

(b) Preparation of membrane

Membrane was prepared according to the procedure described by Kierstan and Bucke (4.29). Approximately, 20g of wet cells were suspended in 64 ml of 0.9% saline solution and inoculated at 4^{0}C for 24 hours before immobilization. To this cold solution were added 12.0 g of acrylamide, 0.64 of bis-acrylamide, 8.0 ml of 5% TEMED and 8.0 ml of 2.5% ammonium per sulphate. The reaction mixture was shaken well to dissolve these solids and poured into 2 or 3 glass petri plates to give a thickness of less than 0.5 mm and covered. The gel was allowed to polymerize at 10^{0}C and washed with phosphate buffer (pH 7.4). The fine gel was finally separated.

(C) Electrode preparation

Membrane having immobilized cells (1x1 cm) was attached to the surface of gas permeable membrane on the oxygen electrode using an '0'-ring. This membrane was covered with a dialysis membrane as shown in Fig.4.6 for the protection of inner membrane from toxic materials. When not in use, the electrode was stored in phosphate buffer at room temperature. All measurements were carried out at room temperature (25°C) using standards or samples diluted with phosphate buffer (pH 7.2). Solutions were equilibrated with air, stirred during measurements and steady state readings were obtained.

(d) Measurement of electrode response

Electrode was washed with 0.9% saline water for three minutes after each analysis. The volume of phenol solution taken during measurement was 20ml for each analysis. The signal at electrode was detected after a certain period of time on a digital D.O.meter. At steady state the value of DO was recorded and correlated with phenol concentration. Effect of phenol concentration, pH, specificity and stability on the working of the concerned biosensor was also studied.

4.15 RESULTS AND DISCUSSION

It was observed that the strain pseudomonas cruciviae has phenol oxidase enzyme which is able to degrade phenol in the presence of oxygen. As such oxygen is utilized in this process and decrease in oxygen can be measured by DO meter and correlated with phenol concentration.

(a) Preparation of calibration curve

The electrode was calibrated with solutions of phenol in double distilled

water in the concentration range of 0.25-4 millimols l^{-1}. All solutions were prepared a fresh and five assays at each concentration were performed. Almost hundred percent sensor response was recorded and steady state was achieved in four minutes in each case, which may be taken as the response time of the biosensor (Fig. 4.7).

The calibration plot, which correlates the phenol concentration with the DO values is shown in Fig.4.8. Plots are not linear beyond 4 milli-mol concentration of phenol (data not recorded here). When a sample of phenol concentration 2 millimol l^{-1} was monitored 15 times in series a divergence in values by 0.002 millimol l^{-1} was observed.

The effect of varying pH on electrode response was observed in the pH range 4 to 10.0. The same is recorded in Fig.4.9. A perusal of the data reveals maximum activity in the range 5.5 to 8.0 pH. This may be taken as the working pH range of the biosensor since the electrode response is linear in this range.

(c) Specificity of the biosensor

In industrial waste like refinery wastewater many types of phenols and its derivatives are present. The expected range of these compunds vary from 0.05 millimol to 50.0 millimol l^{-1}. These compunds may cause interference during measurement of phenol by the biosensor. The response of the concerned biosensor to different phenolic compunds is shown in Table 4.2. It can be observed that a positive response is shown by m-cresol, chlorophenols and catechol while O-cresol, p-cresol, amino and nitrophenol and resorcinol do not cause any interference with the electrode system i.e. the change in D.O.was found to be negligible.

It is quite well known that phenol oxidase present in bacteria responds to other phenols also. This enzyme catalyses a number of phenolic compounds and

besides phenol it is relatively specific to phenol, m-cresol., chlorophenol etc. which may show unwanted interference. Reaction mechanism of probable catalytic activity of the enzyme phenol oxidase is shown in Fig. 4.10.

(d) Stability of the biosensor

The stability of the biosensor under oprational conditions is recorded in Fig. 4.11. A perusal of the data indicates that deterioration in response starts after one week, followed by a rapid decline over two weeks, probably due to the inactivation of enzyme by polymerization of o-quinone in aqueous solution (4.30). The electrode was stored at 4^{0}C under non-operatinal conditions to overcome the relatively short biosensor life.

(e) Response of the electrode system in wastewater

The electrode response was also observed in a synthetic wastewater sample spiked with a variety of phenols in the range of concentration 0.25-4.0 milli mol l^{-1}. The data is recorded in Fig.4.8. Although various phenolic derivatives were present in wastewater, the response of the electrode was found to be parallel to that of pure phenolic solution. This parallel response of phenol sensor in water containing mixture of phenols suggests its efficacy for the estimation of phenol in wastewater (with due consideration of the interferents).

TABLE 4.2

Response of Phenol sensor to other derivatives

Phenolic Derivatives	Concn.(milli mol l^{-1})					
	0.25	0.5	1.0	2.0	3.0	4.0
	Electrode response (D.O, mg/l)					
o-cresol	7.8	7.8	7.8	7.8	7.8	7.8
p-cresol	7.8	7.8	7.8	7.8	7.8	7.8
m-cresol	6.6	5.9	5.2	4.6	4.1	3.8
Resorcinol	7.8	7.8	7.7	7.7	7.7	7.7
o-chlorophenol	6.9	6.3	5.6	5.1	4.7	4.2
Pentacholoro Phenol	7.1	6.5	6.1	5.7	5.1	4.8
4-amino Phenol	7.7	7.7	7.7	7.7	7.7	7.7
2,4,6.tri amino phenol	7.8	7.8	7.7	7.7	7.7	7.7
p-nitro phenol	7.7	7.7	7.7	7.7	7.7	7.7
o-nitro phenol	7.7	7.7	7.7	7.7	7.7	7.7
Catechol	6.7	6.0	5.3	4.7	4.2	3.9

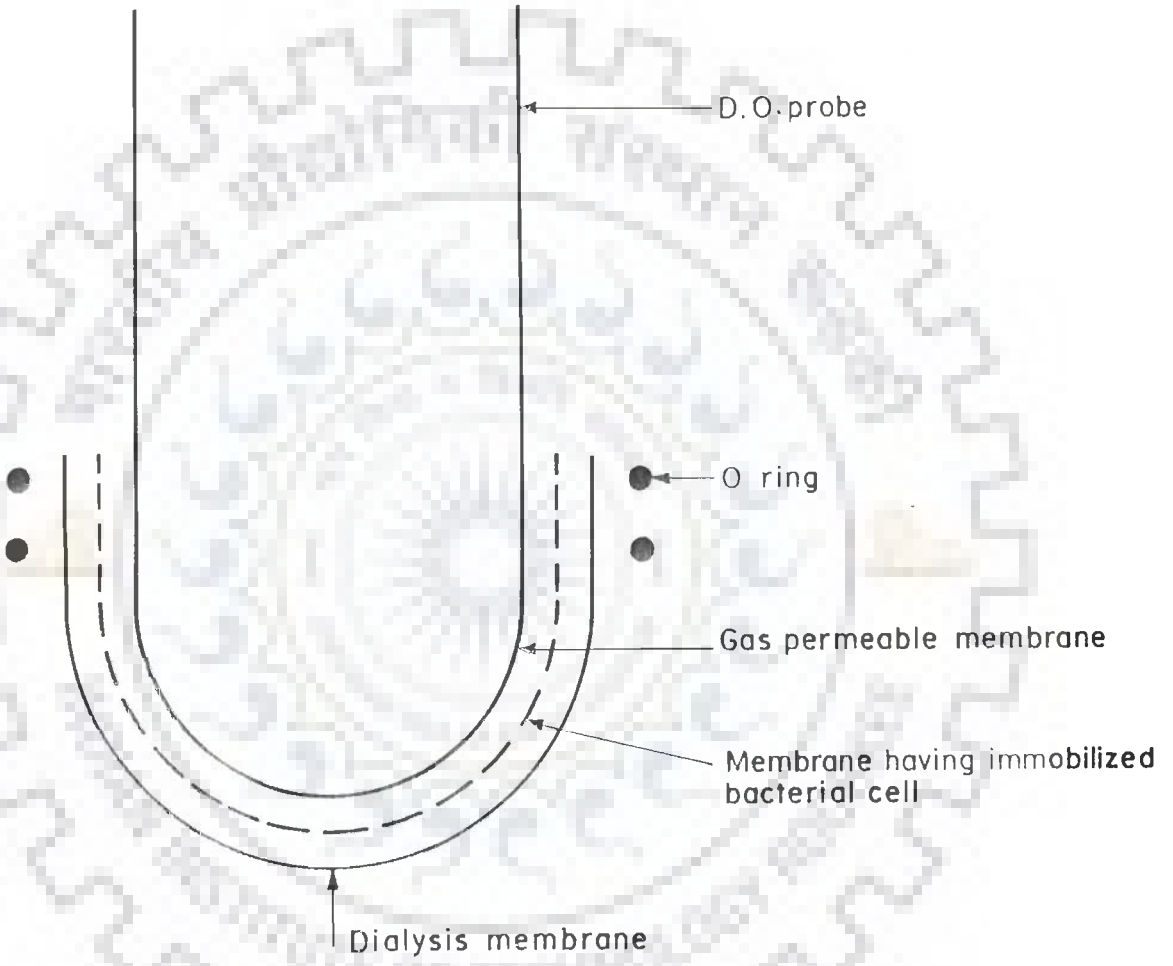


Fig. 4.6 Schematic diagram of phenol biosensor

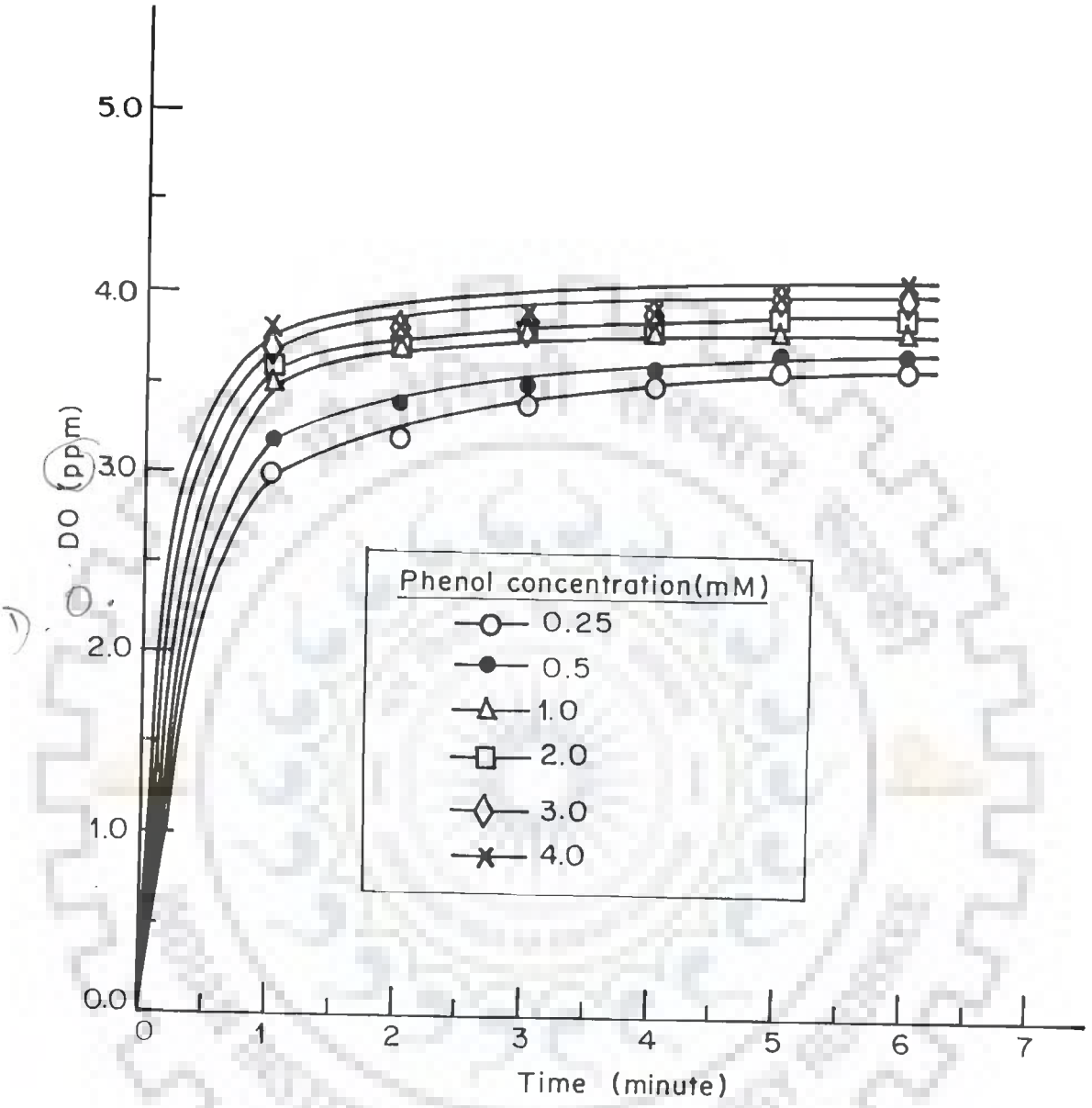


Fig. 4.7 Effect of phenol concentration and optimization of response time

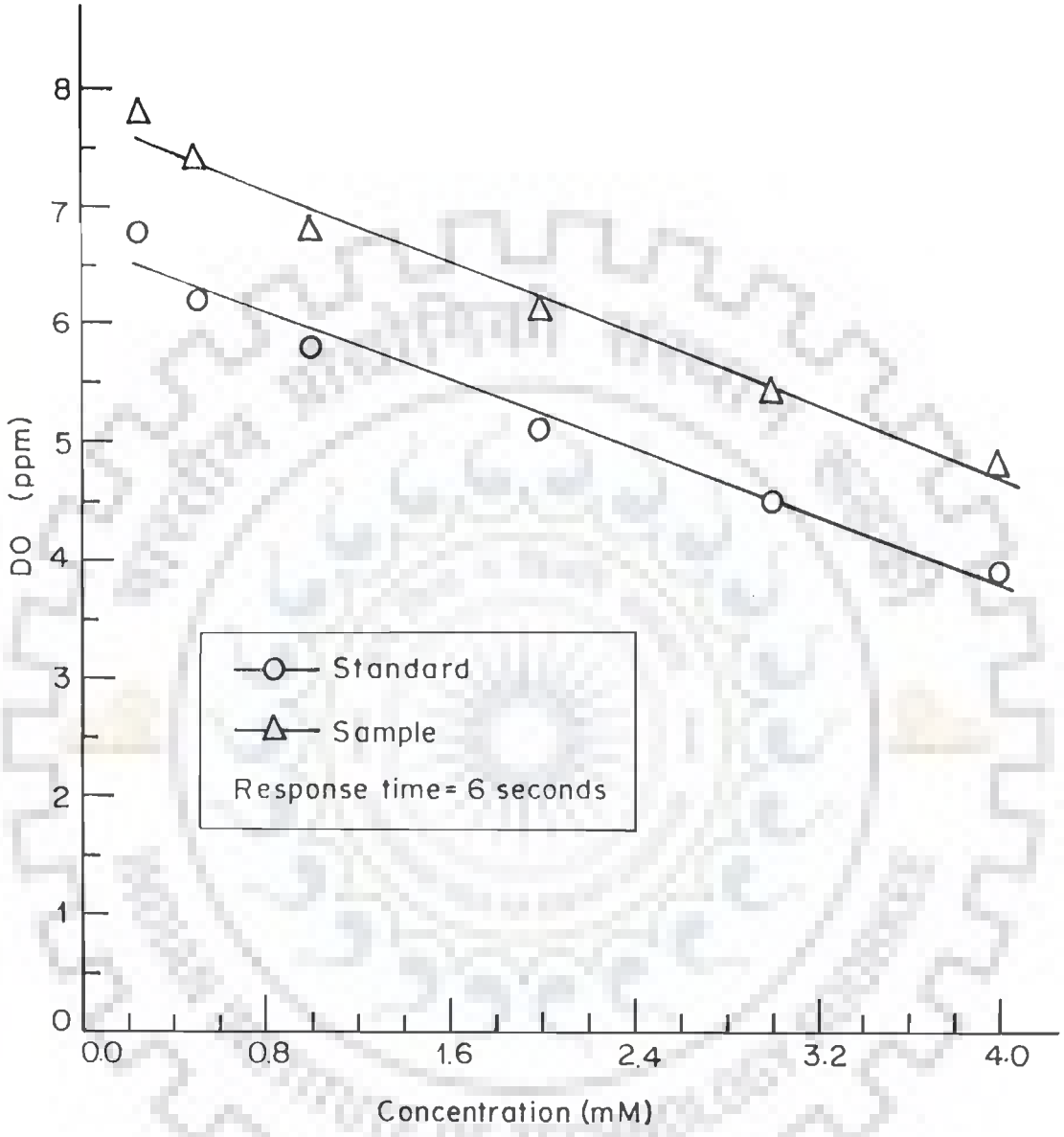


Fig. 4.8 . Calibration curve of standard and an unknown sample of phenol

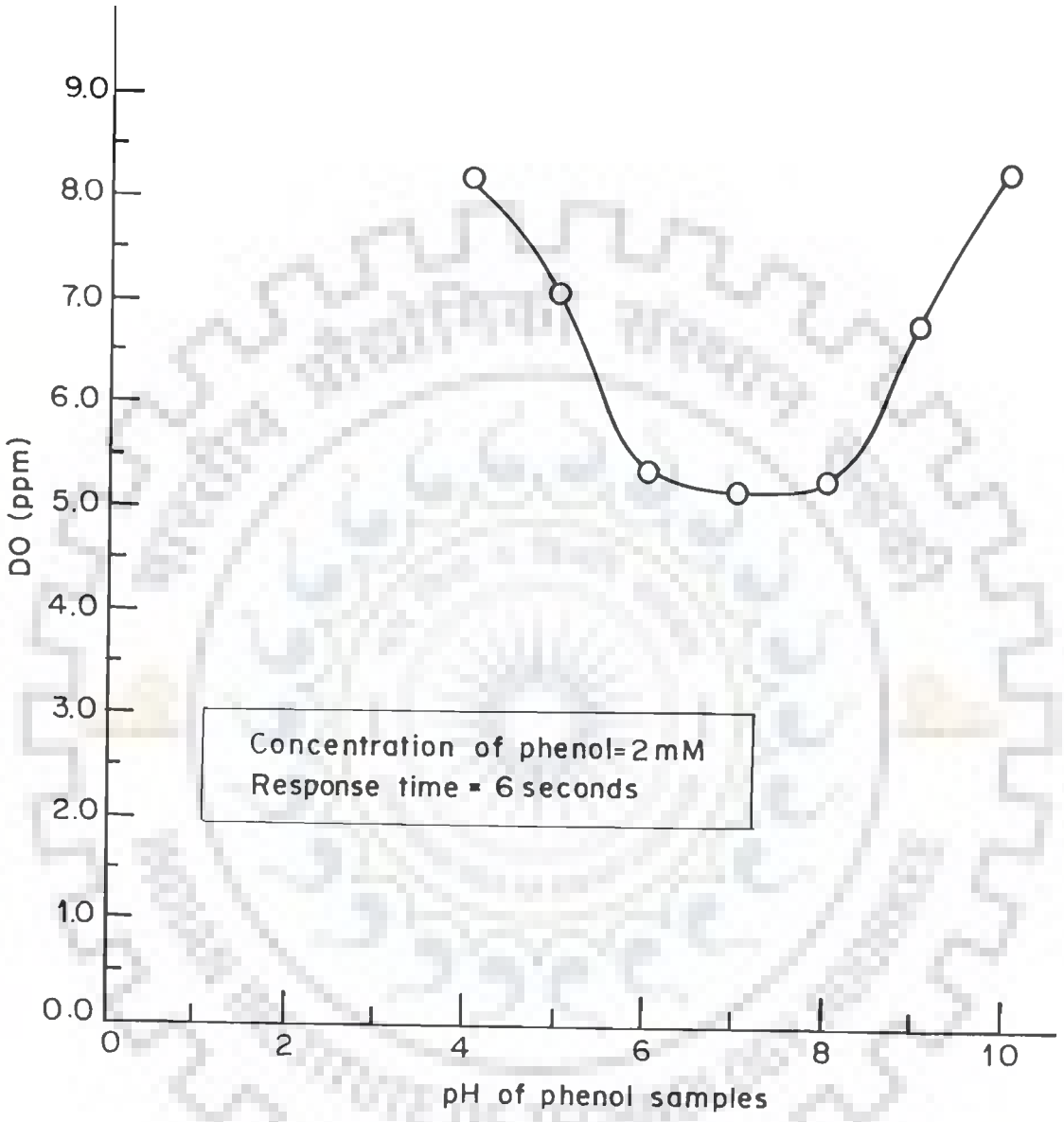


Fig. 4.9 Effect of sample pH on electrode response

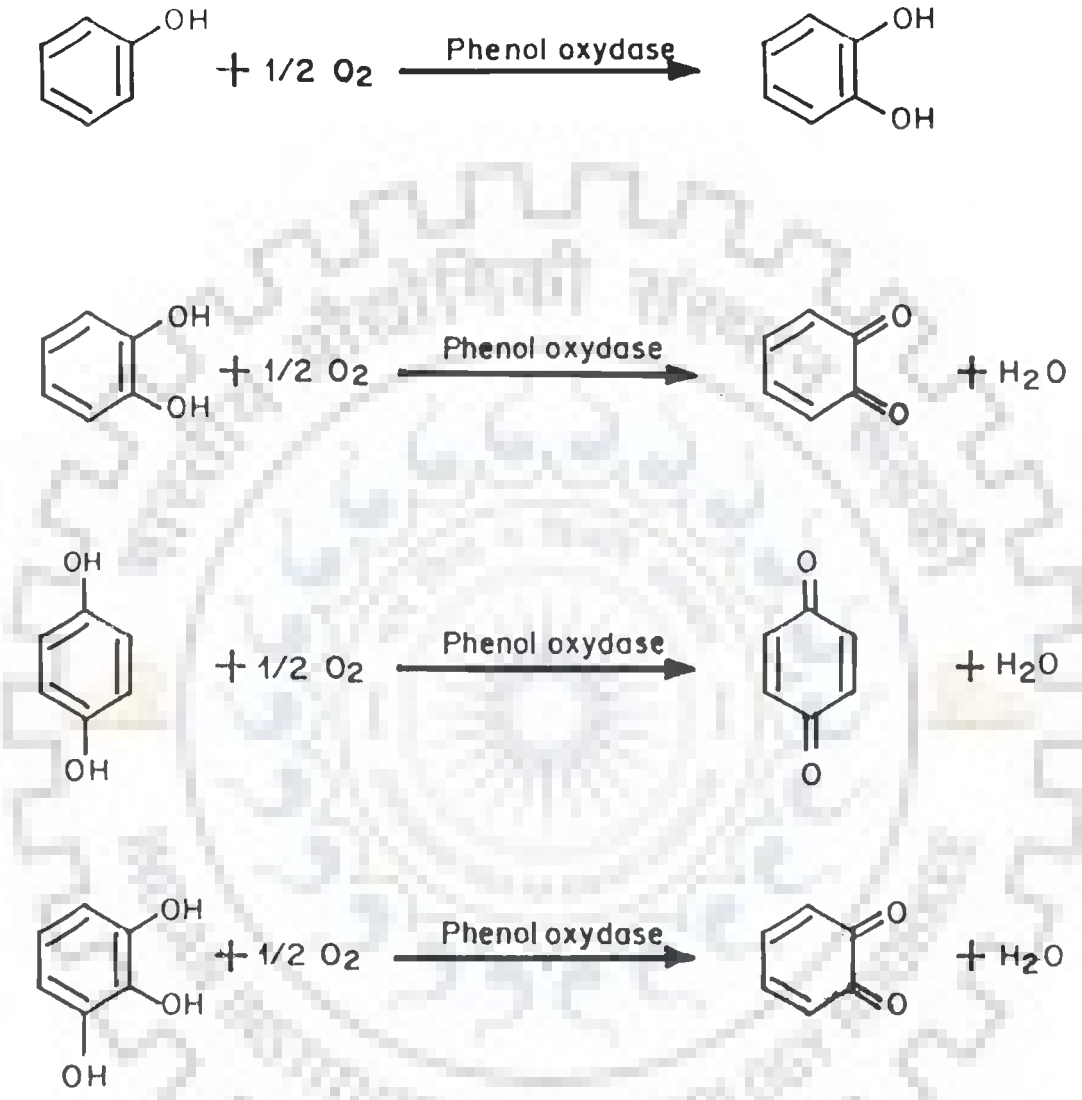


Fig. 4.10 Reactions of phenol oxydase

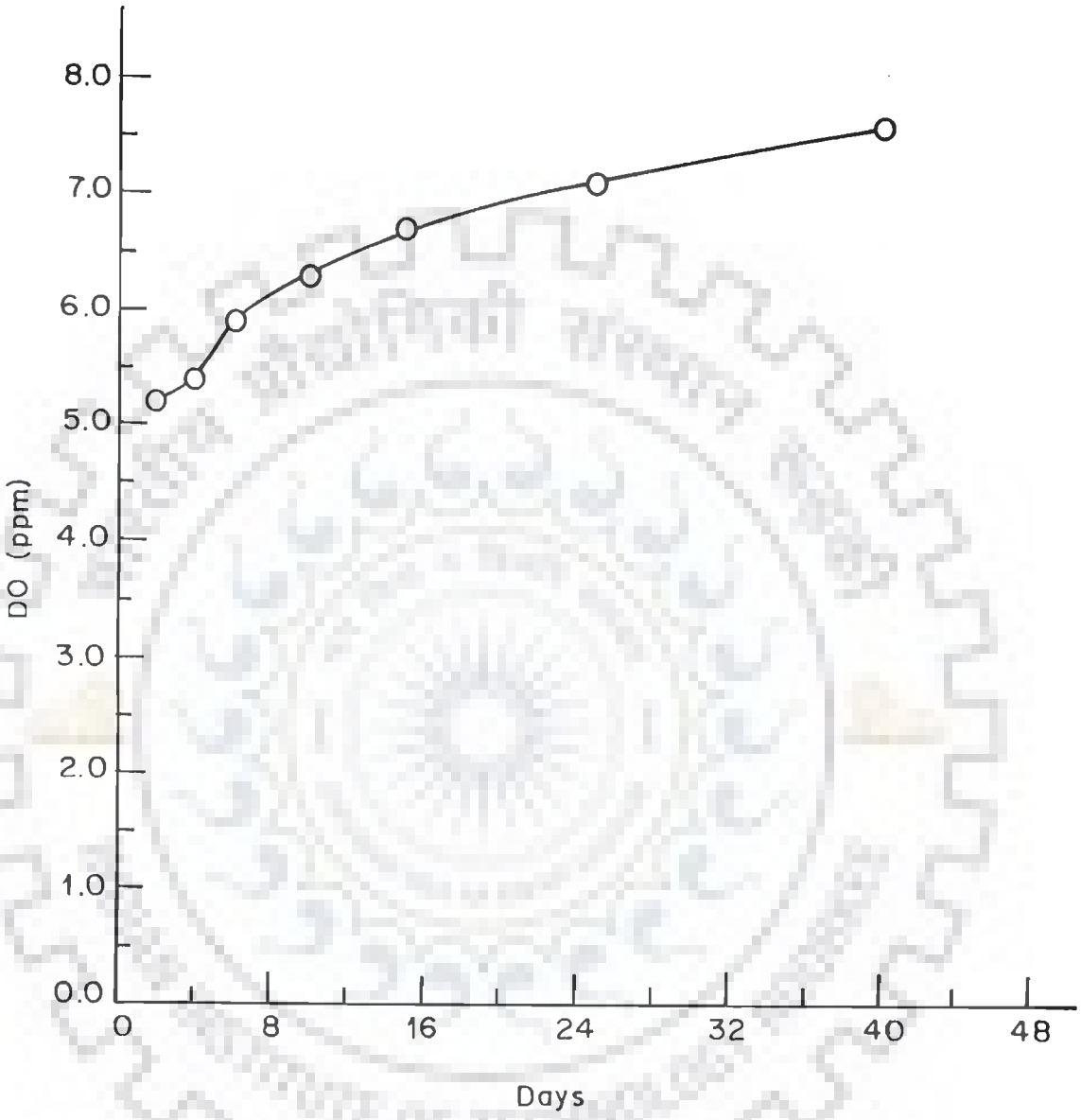


Fig. 4.11 Stability of phenol biosensor under operational conditions

4.16 REFERENCES

4.1. Clark, L.C. Jr. and Lyons, C., "Electrode System for Continuous Monitoring in Cordiovascular Surgery", Ann. N.Y. Acd. Sci., **102**, 29-45 (1962).

4.2. Robert, L.S., "Ion Selective Electrode", Anal Chem., **162**, 21 (29R) (1990).

4.3. Janata, J., "Chemical Sensors" Anal. Chem., **62**, 33R (1990).

4.4. Albeny, W.J. and Bartlet, F.S.R., "Amperometric Enzyme Electrodes", Trans Royal Society, London, **316**, 106(1987).

4.5. Badley, R.A., Shanks, I.A. and Drake, L., "Optical Biosensors for Immunoassy Philosphical Trans. Royal Society, London, **316**, 143 (1987).

4.6. Lundstrom, L. and Spetz, A., "Semiconductor Biosensor", Philosphical Trans Royal Society, London, **316**, 47 (1987).

4.7. Aizawa, M., "Immunosensors" Philosophical Trans Royal Society, London", **316**, 121(1987)

4.8. Schimid, R.D., "Biosensors, Application in Medicine, Environmental Protection and Process Control", VCH Publication, USA, 273 (1989).

4.9. Janchen, M. and Scheller, F., "Biosensors, Application in Medicine, Environmental Protection and Process Control" VCH Publication, USA, 17(1989).

4.10. Updike, S.J. and Hicks, G.P., Nature **214**, 986(1971).

4.11. Hikuma, M., Kubo, T., Obana, H., Yasuda, T., Karube, I., Suzuki, S. and Yasuda, T., "Amperometric determination of Total Assimilable Sugars in Fermentation Broths by use of Immobilized whole Cells", Enzyme Microb Tech., **2**, 234(1980).

4.12. Karube, I., Mastunaga, T., Mitsuda S., and Suzuki, S., "Glucose Sensor using Immobilized whole cells of Pseudomonas Fluorecens", J. Appl. Microbiol and Biotechnol, **7**, 343 (1979).

4.13. Demura, M. and Askura, T., "Immobilization glucose oxidase with Bombyx Mori Silk Fibroin by Stretching treatment and its application to Glucose Sensor", Biotechnology and Bioengineering, **33**, 598(1988).

4.14 Abdulla, H.M., Greenway, G.M. and Platt, A.E., "Biosensing with coated wire electrode", Analyst, 114, 785(1989).

4.15. Palleschi, G., Mascini, M., Bernadi, L.and Zeppine, P., "Lactose and Glucose Electrochemical Biosensors for the Evaluation of the Aerobic and Anaerobic Threshold in Runners", Med. & Biol. Engineering, 28, 25(1990).

4.16. Alva, S., Gupta, S.S., Phaaker, R.S. and Govip, G.,"Glucose oxidase Immobilized Electrode for Potentiometric Estimation of Glucose", Biosensors and Bioelectronics, **6**, 63(1991).

4.17. Cardosi, M.F.and Birch, S.N., "Screen printed Glucose Electrodes based on Platenized Carbon Particles and Glucose oxidase", Anal. Chem. Acta, **276**, 69(1993).

4.18. Hoa, B.T., Suresh Kumar, T.N., Srinivasa, R.S., Lal, R. and Contractor, A.Q., "Anal Chem", 64, 2645(1994).

4.19. Snejdarkova, M., Rohat, M. and Otto, M., "Design of a Glucose Minisensor Based on Glucose Oxydase complex coupling with Self-assembled phospholipid Membrane on solid Support", Anal. Chem., 65, 668(1993).

4.20. Karyakin, A.A., Gitelmacher, O.V. and Karyakina, E.E., "Prussian Blue Based first Generation Biosensor for Glucose", Anal. Chem., 67(14), 2419(1995).

4.21. Bachanan, R.E.and Gibbons, N.E., "Bergeys Mannual Systematic Bacteriology, vol.1, Williams and Williams, Baltimore, 1984.

4.22. Mizutani, F., Yabuki., S.and Asai, M., "Highly Sensitive Measurement of Hydroquinone with an Enzyme Electrode", Biosensors and Bioelectronics", **6(4)**, 305(1991).

4.23. Tillyes, C.R. and Gobin, P.T., "The development of a catechol enzyme electrode and its possible use for the diagnosis and monitoring of Renal Crest Tumors", Biosensors and Bioelectronics; **6**, 569(1991).

4.24. Ghindilis, A.L., Gavvilova, V.P.and Yavopolo, V.A.I.," Laccase-based biosensor for the determination of Polyphenols : Determination of Catechols in Tea ", Biosensors and Bioelectronics, **7**, 122(1992).

4.25. Mazzei, F., Botre, F., Lanzi, M., Loventi, G.and Porcelli, F., "Plant Metabolism as an Analytical Tool :Some Applications of Plant Tissue Electrode for the Selective Determination of Catecholamines", Sensors and Actuators (B), **7** 427(1992).

4.26. Campanella, L., Sammarline, M.P. and Tomassetti, N., "New Enzyme Sensor for Phenol Determination in Non-aqueous and Aqueous medium", Sensors and Actuators B., 7, 383(1992).

4.27. Tan, T.C. and Chen, V., "Sensing Characteristics of an Immobilized apple powder enzymic sensor ", Sensors and Actuators - B, **17**, 101(1994).

4.28. Kotte, H., Grunding, B., Vorlop, K., and Stottmeiste, U., " A Methylphenazonium-zeolite modified Enzyme Sensor Based on Polymer Thick Films for Phenols", Anal. Chem., **67(1)**, 65-70(1995).

4.29. Kierstan, M.P.J. and Bucke, C., " Biotechnology and Bioengg., **19**, 387(1977).

4.30. Hall, G.F., Best, O.J. and Turner, A.P.F., "The determination of p-Cresol in Chloroform with an Enzyme Electrode", Anal. Chim. Acta, **213,** 113(1988).