Fabrication of Electrochemical Sensors for Pharmaceutical Analysis

THESIS

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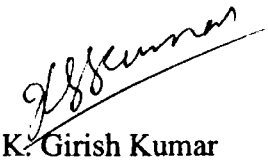
Certificate

Certified that the present work entitled "**Fabrication of Electrochemical Sensors for Pharmaceutical Analysis**", submitted by Ms. Sareena John, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry to Cochin University of Science and Technology, is an authentic and bonafide record of the original research work carried out by her under my supervision at the Department of Applied Chemistry. Further, the results embodied in this thesis, in full or in part, have not been submitted previously for the award of any other degree.



K. Girish Kumar
(Supervising Guide)

Declaration

I hereby declare that the work presented in this thesis entitled "**Fabrication of Electrochemical Sensors for Pharmaceutical Analysis**" is based on the original work carried out by me under the guidance of Dr. K. Girish Kumar, Reader in Analytical Chemistry, Department of Applied Chemistry, Cochin University of Science and Technology and has not been included in any other thesis submitted previously for the award of any degree.

Sareena John

Kochi – 22

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Preface

A reliable and specific assay is of great importance for characterization of disposition, tolerance and safety of a drug. Recent years have seen an upsurge of interest in the application of potentiometric sensors in the field of medicinal analysis. This provides fast, accurate, reproducible and selective determination of various species. Chemical sensors have changed the way we think about analytical chemistry and clinical testing procedures. The applications of potentiometeric sensors are manifold. It has been especially useful in pharmaceutical analysis.

As part of the present investigations eighteen sensors have been fabricated for the drugs mebendazole, pefloxacin, ambroxol, sildenafil citrate, dextromethorphan and tetracycline.

The thesis is divided into nine chapters. A brief account of the different chapters is given below.

Chapter 1 gives a detailed description about the electroanalytical techniques in use. It gives a brief description about the history of the development of the potentiometric sensors. Various types of potentiometric sensors are described in detail. The chapter also gives an account on the potentiometric sensors fabricated for different drugs.

Chapter 2 discusses in detail the synthesis of each of the ion associations used in the fabrication of the different sensors. It also describes the general method of fabrication of the two types of sensors. The chapter also discusses the general procedure for the analysis of the pharmaceutical formulations and real samples employed in the studies.

Chapter 3 presents the fabrication of PVC membrane sensor for mebendazole (MBZ) based on the ion associations of the drug with molybdophosphoric acid (MPA), silicotungstic acid (STA) and phosphotungstic acid (PTA) as ionophores. The sensors exhibited stable, fast Nernstian response over a wide concentration range. The developed sensors have also been used for the determination of the drug in pharmaceutical preparations and also for the determination of the drug in urine samples.

Chapter 4 illustrates the fabrication and electrochemical response characteristics of the sensors of pefloxacin (PEF). The sensors are based on the ion association of the drug with the ion pairing reagents silicotungstic acid (STA) and molybdophosphoric acid (MPA). The sensor matrix composition was optimized and the response studied. The analytical applications of the developed sensors were also investigated.

Chapter 5 focusses on the fabrication of carbon paste electrodes for ambroxol (AMB) based on the ion association of the drug with molybdophosphoric acid (MPA) and phosphotungstic acid (PTA). The electrochemical response characteristics were studied in detail. The developed sensors were successfully applied for the determination of the drug in tablets and for its recovery from urine samples.

Chapter 6 deals with the study of the response characteristics of sensors developed for sildenafil citrate (SIL). Optimization of the response characteristics the sensors developed is dealt with in detail. The analytical applications of the developed sensors are also given in detail.

Chapter 7 is devoted to the detailed description about the sensors developed for dextromethorphan (DEX) based on the ion association of the drug with two ion-pairing reagents such as sodium tetraphenyl borate (NaTPB) and

phosphotungstic acid (PTA). The various response parameters of the developed sensors are discussed in detail.

Chapter 8 presents a detailed account of the two types of sensors developed for tetracycline (TCE). The ion association of the drug with sodium tetraphenyl borate (NaTPB) has been employed for the fabrication of both the PVC membrane sensor and carbon paste electrode. The electrochemical response characteristics are given in detail. The developed sensors were applied for the determination of the drug in pharmaceutical formulations and also for the determination of the drug in urine samples.

Chapter 9 gives the summary and the conclusions of the work carried out.

References are given under separate head as the last part of the thesis.

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Chapter 1

Introduction

Analytical chemistry deals with methods for determining the chemical composition of the samples of matter. A qualitative method yields information about the atomic or molecular species or the functional group that exist in the sample; a quantitative method, in contrast, provides numerical information as to the relative amount of one or more of these components. Chemical analysis is the resolution of a chemical compound into its proximate or ultimate parts; the determination of its elements or the foreign substances it may contain[1]. This definition outlines the broad scope of analytical chemistry. There is an escalating need and desire for us to monitor all aspects of our environment in real time and it has been brought about by our increasing concern with pollution, our health and safety. There is always a desire to determine contaminants and analytes at lower and lower levels and one could say that the aim of all modern analytical chemistry is to lower the detection limits and to improve the accuracy and precision of those methods. There are a number of methods available for the determination of chemical composition of the various species such as titrimetry, absorption/emission spectroscopy, thermal methods etc. Electroanalytical method is yet another technique developed for trace level analysis. Of the different electroanalytical techniques, development of sensors is a promising field and getting wider attention now a days. Sensors can be categorized into

two general groups. They are physical sensors, which are sensitive to such physical responses as temperature, pressure, magnetic field and force and these do not have a chemical interface. Then there are the chemical sensors which rely on a particular chemical reaction for their response[2].

Chemical analysis has especially become important in industrial processes, hospitals, geological surveys etc; appropriate choice of the method of chemical analysis is very important. Important factors which must be taken into account when selecting an appropriate method for analysis include, the nature of information sought, size of sample available, proportion of constituent to be determined and purpose for which analytical data is required. Chemical analysis may be proximate analysis, partial analysis, trace constituent analysis and complete analysis with respect to the information which is furnished. On the basis of sample size, analytical methods are classified as macro, meso, micro, submicro and ultramicro[3].

1.1 Common techniques

The main techniques employed in quantitative analysis are based upon quantitative performance of suitable chemical reactions, appropriate electrical measurements and measurement of certain optical properties. In some cases a combination of optical or electrical measurements and quantitative chemical reaction may be used.

The quantitative execution of chemical reactions is the basis of traditional or classical methods of chemical analysis: gravimetry, titrimetry and volumetry. In gravimetry the substance being determined is converted into an insoluble precipitate which is collected and weighed; in the special case of electrogravimetry, electrolysis is carried out and the material

deposited on one of the electrodes is weighed. Volumetry and titrimetry involves measuring the volume of gas or solution involved in a chemical reaction[4]. The need for trace level analysis led to the development of chromatography, spectrophotometry and electroanalysis. Chromatography is a separation process employed for the separation of mixtures of substances. It is widely used for the identification of components of mixtures. It is often possible to make quantitative determination particularly when using gas chromatography and high performance liquid chromatography. In spectrophotometric analysis, a source of radiation is used that extends to the ultraviolet region of the spectrum. The fundamental law that governs spectrophotometry is the Beer's law. Atomic absorption spectroscopy (AAS), atomic fluorescence spectroscopy (AFS), flame emission spectroscopy (FES) and inductively coupled plasma (ICP) make use of absorption/emission spectroscopy[5]. Electroanalytical technique has become relevant due to its lower detection limits. Electroanalysis is often compared with atomic absorption spectroscopy (AAS) or its modern version, inductively coupled plasma (ICP). Unlike AAS and ICP, the electrochemical approach when applied to solution samples, will give a rapid answer without digestion, as to the labile fraction of a given element in a particular oxidation state and the experiment can be performed on-site in the field.

Electroanalysis can be defined as the application of electrochemistry to solve real life problems[6]. The principal criterion of an electroanalytical technique is that the species which is desired to be measured should react directly (or indirectly through coupled reaction) at, or be adsorbed onto the electrode. Electroanalytical measurements can only be carried out in situations in which the medium between the two electrodes making up the

electrical circuits be sufficiently conducting[7]. Electroanalytical measurements offer a number of important potential benefits[8]:

(i) selectivity and specificity

(ii) selectivity resulting from choice of material

(iii) high sensitivity and low detection limits

(iv) possibility of giving results in real time or close to real time

(v) application as miniaturized sensors in situations where other sensors may not be usable.

1.2 Types of electroanalysis

There are essentially three types of electro analytical measurements that can be performed:

(i) *Conductimetry*: The concentration of charge is obtained through measurement of solution resistance. The method is therefore not species selective. It is useful in situations where it is necessary to ascertain whether the total ion concentration is below a certain permissible maximum level or for use as an on-line detector after separation of a mixture of ions by ion chromatography.

(ii) *Potentiometry*: It is the procedure of using a single measurement of electrode potential to determine the concentration of an ionic species in solution. The electrode whose potential is dependent upon the concentration of the ion to be determined is termed as the indicator electrode and the case where the ion to be determined is directly involved in the reaction, it is an electrode of the first kind. When the concentration of the ion to be

determined is not directly concerned in the electrode reaction, it is an electrode of the second kind. The measurement is made at effectively zero current. The current paths between the electrodes can be highly resistive. By judicious choice of electrode material, the selectivity of the response to one particular ion can be increased, in some cases with very minimal interference in the measured potential from other ions. Such electrodes are known as ion selective electrodes. Detection limits of the order of 100 nanomolecules per litre of the total concentration of the ion present in a particular oxidation state can be achieved. It is possible to measure 100 picomolar differences in concentration.

(iii) *Amperometry and Voltammerty*: In amperometry, a fixed potential is applied to the electrode, which causes the species to be determined to react and a current to pass. If this potential is conveniently chosen, then the magnitude of current is directly proportional to the concentration. Detection limits in the micromolar region can be obtained.

Voltammetry is concerned with the study of current-voltage-time relationships during electrolysis carried out in a cell. The technique commonly involves studying the influence of changes in applied voltage on the current flowing in the cell, but in some circumstances, the variation of current with time may be investigated. Using this technique several species which react at different applied potentials can be determined almost simultaneously in the same experiment without the need of any previous separation step. Very low detection limits of down to

picomolar concentrations can be reached using state-of-the-art instrumentation and preconcentration of the analyte on the electrode surface.

1.3 Electrochemical sensors

An overview of development of analytical chemistry demonstrates that electrochemical sensors represent the most rapidly growing class of chemical sensors. A chemical sensor can be defined as a device that provides continuous information about its environment. Ideally, a chemical sensor provides a certain type of response directly related to the quantity of a specific chemical species. All chemical sensors consist of a transducer, which transforms the response into a detectable signal on modern instrumentation and a chemically selective layer, which isolates the response of the analyte from its immediate environment. They can be classified according to the property to be determined as: electrical, optical, mass and thermal sensors and they are designed to detect an analyte in the gaseous, liquid or solid state[9].

Compared to optical, mass and thermal sensors, electrochemical sensors are especially attractive because of their remarkable detectability, experimental simplicity and low cost[10]. They have a leading position among the presently available sensors that have reached the commercial stage and which have found a vast range of applications in the fields of clinical, industrial, environmental and agricultural analyses.

Potentiometric sensors are a type of electrochemical sensors. Potentiometric sensors have found the most widespread practical applicability since the early 1930s due to their simplicity, familiarity and

cost. There are three types of potentiometric devices: ion selective electrodes (ISEs), coated wire electrodes (CWEs) and field effect transistors (FETs).

1.4 ISEs

ISEs are classified as potentiometric sensors since some selective chemistry takes place at the surface of the electrode producing an interfacial potential. Species recognition is achieved with a potentiometric chemical sensor through a chemical equilibrium reaction at the sensor surface. Thus the surface must contain a component which will react chemically and reversibly with the analyte. This is achieved by using ion selective membranes which make up the sensor surface.

Most analytical sensors are electrodes of the 2^{nd} kind. As with all electrodes that are not metal - metal ion electrode of the 1^{st} kind [M/M+], speed of response and reversibility is of critical importance for accuracy and reproducibility of measurements. In fact the issue of reversibility and consideration of all electrochemical systems as equilibrium processes was one of the major contributions of Nernst. The Nernst equation describes that a change in potential of an electrochemical system is linear to change in the ion activity (in logarithmic units) of the selected analyte ion.

The development of ion selective electrodes during the last 20 years has quickly been followed by many applications in addition to those in inorganic analysis. The field of applications was broadened by the introduction of liquid ion - exchanger membranes, membranes containing electroneutral macrocyclic compounds, enzyme electrodes and gas sensors[11-14]. Such new electrode materials facilitated the development of

potentiometric sensors for most of the important inorganic ions and several types of organic compounds, many of which are of ionic character[15].

1.5 Classification

There are 4 categories of membranes used in potentiometric chemical sensors.

1) *Glass membranes*: These are selective for ions such as H^+, Na^+ and NH_4^+. Glass membranes have a very high electrical resistance in the M Ω range; however they must conduct ionic charge to some extent in order to be able to make measurements with them. When a glass membrane is put in water, a charge separation process occurs across the glass / H_2O interface giving rise to an electrical potential difference; the magnitude of which depends on the position of the equilibrium which in turn depends on the number of hydrogen ions in the aqueous solution originally.

2) *Sparingly soluble inorganic salt membrane*: This type consists of a section of a single crystal of an inorganic salt such as LaF_3 or a pressed powdered disc of an inorganic salt or mixtures of salts such as $Ag_2S/AgCl$. Such membranes are selective for ions such as F^-, S^{2-} and Cl^-. Three types of sensor membranes employing sparingly soluble inorganic salts are known. They are

    a) Single crystal membranes.

    b) Pressed powder membranes.

    c) Membranes where the powdered salt is held together by an inert binder. (usually a polymer.)

3) *Polymer immobilized ionophore membranes*: In these an ion-selective complexing agent or an ion exchanger is immobilized in a plastic matrix such as poly (vinyl chloride).

Ion selective electrodes are classified according to the physical state of the substances forming the electrode membrane, or possibly according to the nature of the substances affecting ion exchange in the membrane[16-18].

(i) Ion selective electrodes with solid membranes: The membrane can either be homogeneous (a single crystal, a crystalline substance or a glass which is considered to be a solid with regard to the immobility of the anionic groups) or heterogeneous where a crystalline substance is built into a matrix made from a suitable polymer.

(ii) Ion selective electrodes with liquid membranes: In this case the electrode membrane is represented by a water immiscible liquid, in which a dissolved substance capable of exchanging the ion in the solution for which the electrode is selective. This substance is either an associate of this ion with an oppositely charged ion, soluble in the membrane or it is a complex of the ion for which the electrode is selective.

Selective sensors have been used for analytical determination of a wide variety of ions since the 1900s.

4) *Gel-immobilized and chemically bonded enzyme membranes*: These membranes use the highly specific reactions catalysed by enzymes. The enzyme is incorporated into a matrix or bonded onto to a solid substrate surface.

1.6 Potentiometric sensors

Potentiometric chemical sensors make use of the development of an electrical potential at the surface of a solid material when it is placed in a solution containing ions which can exchange with the surface. The magnitude of the potential is related to the number of ions in solution. There occurs a charge separation across the interface which gives rise to an electrical potential difference. In potentiometry, it is said that the measurement of cell potential is made under a zero current condition.

Ion-selective electrodes (ISEs) are the chemical sensors with longest history and probably the most frequent routine application. James Ross and Martin Frant of Orion Research are the founding fathers of ISEs. The calcium and fluoride ISEs they developed in the mid 1960s were the big bang that started a new era in potentiometric analysis[19].

The common glass[20-24] electrode for pH measurement is an example of a potentiometric sensor and has been known for more than 80 years, well before the development of the so called new breed of ion selective electrodes such as the fluorides in 1960s. The membrane in a pH electrode is essentially a sodium silicate glass made by fusing a mixture of Al_2O_3, Na_2O and SiO_2. Increasing the amount of Al_2O_3 in the glass leads to an increasing response to other monovalent cations such as Na^+, K^+ and Li^+. The selectivity[25] of glass electrodes to alkali metal ions was systematically studied by Eisenman *et al.* In all cases however, the glass membrane also responds to pH.

Liquid membrane containing a dissolved ion exchanger was first used by Sollner and Shean[26,27]. In 1961, the first ISE with precipitate containing heterogeneous membranes were prepared by Pungor and Hallos-Rokosinyi. Compact ion exchange membranes were obtained by Frant and Ross[28].

Concepts from medicine and physiology also spurred the development of ISEs. In 1964, Cyril Moore and Besston C Pressman observed that neutral macro cyclic antibiotics induce ion permeation in mitochondria, leading to the development of neutral carrier electrodes. Wilhem Simon an eminent organic chemist, used extracts of poisonous mushrooms containing the dipsipetide valinomycin dissolved in a liquid ion exchanger membrane. Although the response was slow, an electrode that measured the K^+ in the presence of a 5000 fold excess of Na^+ was soon developed and patented. His studies on the structure selectivity relationships of many synthetic ionophores, plasticizers and additives allowed him to fabricate ISEs[29].

In 1966, it was discovered that a slice of a single crystal of lanthanum fluoride attached to the end of an electrode barrel could be used to sense the fluoride ion in aqueous solution[30]. In 1967, a liquid membrane ion selective electrode was produced for the first time, which provided the means for direct determination of the activity of calcium ions in solution. This was of great importance in the biological and chemical sciences because of the importance of calcium in biological fluids[31].The most significant advance in liquid membrane electrodes, other than the original discovery occurred in 1970 when it was shown that organic liquid of the liquid membrane ion selective electrode could be immobilized into poly (vinyl chloride) to produce a polymer film with sensing properties for calcium, as good as, if not better than, the liquid membrane itself. The use of PVC to make sensor membranes originated from the laboratory of Prof. J.D.R. Thomas[32].

In an effort to miniaturize the sensor and to avoid using internal filling solution, the coated wire electrode was developed in 1971. The response of coated wire electrode is similar to that of classical ISE, with

regard to detectability and range of concentration. The great advantage is that the design eliminates the need for an internal reference electrode, resulting in the benefits during miniaturization. This is particularly useful for the in vivo and in vitro biomedical and clinical monitoring of different kind of analytes[33-36]. Pungor and his co-workers developed an iodide ion selective electrode by incorporating finely dispersed silver iodide into a silicone rubber monomer and then carrying out polymerization[37-39]. An enzyme ISE for amygdalin has also been proposed[40]. The electrode contains a cyanide solid state electrode coated with an acryl-amide gel containing β-glucosidase[41,42]. Ruzika *et al* introduced liquid state electrode based on carbon in 1970[43]. In 1973, Mesaric and Dahmen developed sensors using spectral grade graphite powder, nujol and metal salts of low solubility in a plastic body[44]. In 1980, Heineman *et al*, first described the use of polymer film chemically modified carbon paste electrode[45].

Carbon paste electrodes (CPEs) belong to a group of heterogenous carbon electrodes[46-47]. CPEs are represented by carbon paste, ie, a mixture prepared from graphite powder and a suitable liquid binder packed into a suitably designed electrode body[48]. Due to numerous advantages, properties and characteristics, these electrodes are widely used for potentiometry, voltammetry, amperometry and coulometry. Adams, the inventor of CPEs[49] and his research group were the first to publish an extensive study on carbon pastes comprising numerous test measurements[50,51]. Their investigations have been primarily focused on the characterization of CPEs with respect to their applicability in anodic and cathodic voltammetry. A study of Farsang[52] can be regarded as a pioneering attempt to optimize the carbon paste composition via the chemical structure of the binder by observing the

behaviour of several CPEs prepared from silicone oils with different molecular weight. Lindquist[53] carried out a systematical comparison of the properties of carbon pastes when investigating mainly the effect of liquid binders with respect to their content in the paste mixture.

Various online monitoring systems have benefited from the inherent specificity, wide scope, dynamic behaviour and simplicity of potentiometric sensors[54]. They have become widely used as detectors in high speed automated flow analyzers such as air segmented[55,56] and flow injection systems[57]. In addition, the coupling of modern ion chromatography with potentiometric detection has been with significant success[58]. Miniaturization of ISE has also permitted their use as on-column detectors for capillary electrophoresis[59].

Rohwedder *et al*[60] and Fatibello and co-workers[61-64] have shown the use of coated graphite epoxy ISE for determination of cations using ion pair formation with tricaprylmethylammonium cation in a PVC matrix. Rover *et al* have described the construction of tubular ISE useful for the determination of saccharin[65]. The construction and application of ISEs applied for the determination of pharmaceutical compounds such as acetyl salicylic acid and vitamin B6 have also been described[66].

1.7 Performance factors

Some critical issues that will arise with all ion selective sensors are detection limits, linear measurement range and selectivity over interfering ions. In addition, the operational pH, temperature and pressure limits of the sensor greatly determine its use in real world industrial and laboratory

applications. Another very important criterion for utility of any given sensor is the expected life time under constant use[67].

1.7.1 Slope (Response) of the electrode

The slope also called the response of the electrode is the main characteristic of the potentiometric sensors. The value of the slope is given by Nernst: 59.16/z mVdecade^{-1} of concentration, where z is the charge of the ion that has to be determined. The value can be deducted from Nernst equation. Nernstian response implies ideal sensitivity. The slope is dependent upon the stability of the compound formed at membrane solution interface[68].

1.7.2 Linear concentration range

The linear concentration range represents the range of concentration of a substance (or ion) over which sensitivity of the electrode is constant within a specified variation usually ±5%. The reproducibility of the linear range is connected with the working conditions of the electrode such as pH, composition of the solution, history and pre conditioning of the electrode and temperature[69].

1.7.3 pH range

The pH plays a very important role in the response of the potentiometric sensors. It can influence the formation of protonated and unprotonated species of the same substance, it can favour the redox processes at the electrode or the electrode can become pH selective under certain conditions[70].

1.7.4 Response time

IUPAC defined the response time as the time which elapses between the instant when the electrodes are brought into contact with a sample solution and the first instant at which the slope of the working electrode becomes equal to a limiting value selected on the basis of the experimental conditions and/or requirements concerning accuracy[71]. For ISE the response time depends on concentration as well as on the stability of the compound formed between the ion that has to be determined and the ligand at the membrane solution interface.

1.7.5 Selectivity

Selectivity is one of the basic characteristics of the electrochemical sensors. It depends on the composition of the membrane, ratio between the activities of the main and interfering ion in solution, complexity of the matrix sample that is analyzed, current applied and pH of the solution. The selectivity of an ion pair based sensor depends on the physico-chemical characteristics of the ion exchange process at the sensor – sample solution interface and the mobility of the respective ions in the sensor[72].

1.7.6 Shelf life or Life time

Life time may be defined as the storage or operational time for the sensitivity of the sensor to decrease by a factor of 10% to 50%, within the concentration range[73]. The lifetime of a sensor refers to the period of time during which the sensor can be used for the determination of the analyte and it is determined by the stability of the selective material.

1.8 A brief review on potentiometric sensors for drugs

Quality assurance plays a central role in determining the safety and efficiency of medicines. Highly specific and sensitive analytical techniques hold the key to the design, development, standardization and quality control of medicinal products. Modern physical methods of analysis are extremely sensitive, providing precise and detailed information from small samples of material. They are for the most part rapidly applied and in general are readily amenable for automation[74].

ISEs have found many successful applications in pharmaceutical analysis[75-79] mainly because of their low cost, ease of use and maintenance and the simplicity and the speed of assay procedures. It is usually possible to develop procedures for the determination of drugs in pharmaceutical preparations that need only a pre-dilution step with a suitable buffer (eg; injection preparations) or dissolution of tablets in the measuring solvent. Turbidity due to tablet matrix is not usually a problem so that even the filtration step can be avoided.

The vast majority of solvent polymeric ISEs for organic ions that have been reported so far are ionophore free ion-exchanger electrodes[80, 81] but a considerable number of ionophore based ISEs for organic analytes have also been described[82]. Cationic organic analytes that have been measured with ionophore based ISEs are 1-phenyl ethyl amine, ephedrine, norephedrine, amphetamine, dopamine, amino acid amides, benzyl amine, mexiletine, local anesthetics (procaine, prilocaine, lidocaine, bupivacaine, lignocaine), imipramine, desipramine, trimipramine diquat and paraquat (herbicides), guanidine, metformin, phenformin, creatinine, protamine and the condensates of a Girard reagent with glucose or other aldehydes. Anionic

analytes that were detected with ionophore based ISEs are acetate[83], nucleotides, heparin salicylate, phthalate, maleate and a number of other carboxylates.

While a number of these ISEs were used to measure analytes in relatively uncomplicated samples such as drug capsules and tablets, a few of them were also tested for use in more complex matrices. Examples are the measurement of acetate in vinegar[84], salicylate in urine[85,86] and blood serum[87,88], phenyl pyruvate in urine[89], benzoate in blood serum[90], glucose in human blood upon reaction of the analyte with a Girard reagent[91] and protamine and heparin in blood samples[92].

Magda *et al*[93] were successful in developing two new potentiometric methods for the determination of famotidine. The famotidine selective membrane sensor was based on the use of the ion association formed between famotidine and tetraphenyl borate. The sensor exhibited a linear response in the range $10^{-3} - 10^{-5}$ M.

Amodiaquine polymeric membrane sensors were developed by Kauffmann *et al.* The sensing components were composed of the ion association formed between the drug and sodium tetraphenyl borate or tetrakis (4-chlorophenyl) borate. The sensors gave a near Nernstian response over the pH range 3.7 and 5.5. The sensors were successfully applied for the determination of amodiaquine in pharmaceutical dosage forms[94].

A conventional polymer membrane, graphite coated and carbon paste electrode for triprolidine was prepared by S.I.M. Zayed. The sensors incorporated triprolidine-sodium tetraphenyl borate ion pair as the electro active material. It exhibited a fairly wide pH range of 4.70 - 8.75. The sensors showed very good selectivity for triprolidine[95].

A mexiletine selective membrane electrode based on the crown ether 4', 4", (5') -di-tert-butyldicyclohexano-18-crown-6 showing the highest sensitivity and a detection limit of 30 μM has been reported[96]. The sensor was successfully employed for the determination of mexiletine in saliva.

Khalil *et al* were successful in fabricating a membrane sensor for phenothiazine. The electroactive materials were either phenothiazine drug - tetraphenyl borate or phenothiazine drug - naphthalene sulphonate ion pairs. The electrodes exhibited useful analytical characteristics for the direct or indirect determination of phenothiazine drugs in pure form or in pharmaceutical preparations[97].

The ion pair complexes formed between fluphenazine hydrochloride and nortriptyline hydrochloride with sodium tetraphenyl borate or tetrakis (4-chlorophenyl) borate were used for the fabrication of the sensors for these drugs. These sensors gave Nernstian slopes over the concentration range 10^{-2} -10^{-5} M. These sensors were used for the determination of the corresponding drugs in pharmaceutical dosage forms and in presence of their degradates[98].

Liquid membrane ion selective electrodes with the ion association complexes of novocaine with tetraphenyl borate or dipicrylamines were proposed for use in the determination of novocaine by Cosofret *et al*. The developed sensors gave a linear response in the range 10^{-1} - 10^{-5} M[99].

The scopolamine sensor developed by G.A.E. Mostafa was based on the ion association of the drug with phosphotungstic acid. The sensor gave a stable near Nernstian response for 10^{-2} - 10^{-6} M scopolamine over the pH range 3-7. The direct determination of scopolamine in some formulations

gave results that compare favourably with those obtained by the USP method[100].

Two novel potentiometric PVC membrane sensors responsive to pyridoxine hydrochloride were reported. These sensors have the ion associations of the drug formed with molybdophosphoric acid or tungstophosphoric acid as the electroactive material. The developed sensors had a lower detection limit of 4×10^{-5} M and a fast response time of nearly 35 - 45s and was selective to pyridoxine over a number of interfering ions. The determination of pyridoxine in some pharmaceutical preparations using the proposed sensors gave satisfactory results comparable with BP method[101].

Rizk *et al* developed polyurethane sensors for thiopental on solid graphite support. The electroactive materials of thiopental with Cu (II) and Co (II) bathophenanthroline were dispersed in a polyurethane matrix. The sensors showed a fast response time, low detection limit and a long life time. The sensors were used for the direct potentiometry of thiopental in pharmaceutical formulation and human serum[102].

A ketoconazole membrane sensor was developed based on an ion association of ketoconazole with sodium tetraphenyl borate. The sensor gave a Nernstian slope within the concentration range 10^{-3} – 10^{-6} M. The developed sensor was used to evaluate the equilibrium constants of α and β cyclodextrin ketoconazole complexes in addition to its use in the determination of ketoconazole in pharmaceutical preparations and biological fluids[103].

Wen and co-workers reported a pethidine selective membrane sensor based on the ion association of the drug with silicotungstic acid. The

electrode had a detection limit as low as 9.91×10^{-7} mol/dm^3. The sensor could be used for the determination of pethidine in tablets and injections[104].

Enein and his co-workers used the ion association formed with silicotungstic acid for the determination of propranolol in pharmaceutical formulations. It had a short conditioning time of three hours and a fast response time[105].

A potentiometric sensor immobilized in a graphite matrix for the determination of diclofenac was reported by Pezza and his group. Studies on the determination of diclofenac in pharmaceutical formulations, especially tablet dosage formulations and injectable ampoules were carried out to illustrate the feasibility of the proposed sensor[106].

S. S. M. Hassan and his group explored the use of 5,10,15,20-tetraphenylporphyrinato indium (III) as ionophore in fabrication of a sensor for ibuprofen in a PVC and polyurethane matrix. The sensors were found to be useful for the quantification and quality control assessment of ibuprofen in pharmaceutical preparations[107].

Enein and his group studied the response of a PVC membrane sensor for methacycline based on methacycline - tetraphenyl borate as the electroactive material. The membrane could be used for the determination of methacycline in tablets and the results agreed well with pharmacopoeia method[108].

A new oxymetazoline ion selective PVC membrane electrode based on oxymetazoline - phosphotungstate ion association as the ionophore was reported. It had a fairly wide pH range of 1.0 - 9.4. The electrode was used for the determination of oxymetazoline in nasal drops[109].

Shamsipur's group developed a clotrimazole selective membrane sensor based on clotrimazole - phosphomolybdate ion pair complex. The electrode gave a Nernstian response and displayed a good selectivity for clotrimazole. The membrane sensor was successfully applied to the determination of clotrimazole in tablets and creams[110].

Ozsoz and his group published their results of polymeric membrane sensors for antidepressant nefazodone based on its ion pair complex with phosphotungstate, tetraphenyl borate, silicotungstate and reinckate. The best results were obtained with nefazodone - phosphotungstate and the sensor was used for the determination of the drug in pure solutions[111].

A potentiometric sensor immobilized in a graphite matrix for the determination of p-aminobenzoate in pharmaceutical formulations has been reported. It had a greater lifetime of over six months[112].

A clotrimazole - triiodide ion pair was used for the fabrication of triiodide selective sensor. The sensor gave a super Nernstian response. It was however used as an indicator electrode in the potentiometric titration of triiodide ions and indirect potentiometric determination of clotrimazole in pharmaceutical preparations[113].

G.A.E. Mostafa reported a metoclopramide selective membrane sensor incorporating metoclopramide - tetraiodomercurate as the ionophore. The membrane sensor showed a stable near Nernstian response. The determination of metoclopramide in tablets, injection and syrup using the sensor gave very good response[114].

The group of Salem developed solid contact ion selective electrodes for bromazepam, clonazepam and 1, 4-benzodizepines. The electrodes were

based on PVC membranes doped with the drug - phosphotungstic acid ion pair complexes as electroactive materials. The electrodes were applied for the determination of the drugs in pharmaceutical preprations[115].

Construction and characterization of potentiometric membrane sensors for quantification of diclofenac and warfarin drugs have been described by S. S. M. Hassan *et al.* The membrane sensors incorporated iron (II) phthalocyanin as a molecular recognition reagent. The sensor was applied to the determination of these drugs in dosage forms[116].

Shamsipur and co-workers reported a diclofenac selective membrane sensor having diclofenac - hexadecyl pyridinium bromide as the ionophore. The sensor was applied for the determination of diclofenac in tablets and also for its recovery from blood serum and urine samples[117].

Moghimi *et al* reported a potentiometric sensor immobilized in a graphite matrix for the determination of picrate ion. The electrode was successfully applied to the potentiometric determination of picrate ions and indirect determination of some pharmaceuticals such as quinidine, through precipitation reaction with quinidines[118].

Ghoreshi and his group fabricated both conventional and coated graphite type electrodes for the determination of naphazoline based on naphazoline - tetraphenyl borate ion pair. Both the sensors gave Nernstian slopes and were used for the determination of naphazoline in pure state and in pharmaceutical preparations[119].

Pedreno *et al* discussed several plasticized membranes for the determination of some multi drug resistance reversers. PVC membranes were doped with tetrabutyl ammonium tetraphenyl borate. The proposed sensor

was applied for the determination of chlorpromazine, clomipramine, imipramine, desipramine and verapamil in pharmaceutical preparations[120].

Ibrahim and his group discussed a carbon paste electrode for dicyclomine hydrochloride. The electrode was based on a mixture of two ion exchangers namely dicyclominium phosphomolybdate and dicyclominium tetraphenyl borate as the electroactive material. The sensors were applied for the determination of dicyclomine hydrochloride in tablets and biological fluids[121].

Badawy *et al* fabricated a hydralazine ion selective PVC membrane electrode based on hydralazinium tetraphenyl borate as the electroactive material. The electrode was successfully applied for the determination of hydralazine in pure form and in pharmaceutical preparations[122].

The PVC membrane sensor for diphenhydramine reported by Badawy and his group used diphenhydramine - tetraphenyl borate ion pair. Diphenhydramine in pure solutions and in anti histamine syrups could be determined using the proposed sensor[123].

Cosofret *et al* reported a membrane sensor for amantadine which was successfully used for the determination of the drug in pharmaceutical formulations[124].

Vire *et al* conducted a comparative study of three polymeric membrane electrodes for tizanidine. The electrodes gave Nernstian response and were applied for the determination of tizanidine in tablet form[125].

Montenegro and his group fabricated quinidine ion selective electrode without inner reference solution based on quinidine tetrakis (4-

chlorophenyl) borate as the ion exchanger. Quinidine in pharmaceutical preparations could be determined using the proposed sensor[126].

The group of Hampp reported ion selective PVC membrane sensor for muscle relaxants pancuronium, tubocurarino, gallamine and succinyl choline based on two different counter ions dipicrylaminate and tetraphenyl borate[127].

Enein and his co workers explored the response characteristics of an amiodarone selective membrane sensor based on amiodarone – dipicryl amine ion pair complex. It exhibited a detection limit of 4×10^{-9} M. Though ephedrine and polyvinylprolidone interfered, it was found to be useful for the determination of amiodarone in dosage forms such as tablets and ampoules[128].

Khalil and Aliem successfully estimated the benazepril hydrochloride content in pure form and in pharmaceutical preparations. They employed a coated wire benazepril selective electrode based on the incorporation of benazepril - tetraphenyl borate ion pair in a PVC coated membrane. It had a wide usable pH range of 2.5 – 9.2[129].

The group of S.S.M. Hassan explored the response characteristics of PVC membrane sensors for some β-blockers such as atenolol, bisoprolol, metoprolol, propranolol and timolol. The electroactive materials for these sensors were the ion association complexes of the respective β-blockers with phosphotungstic acid. Validation of the method according to the quality assurance standards showed the suitability of the proposed sensors for use in the quality control assessment of these drugs. The determination of these β-

blockers in some pharmaceutical preparations was also possible using the proposed sensors[130].

Kharitonov was successful in fabricating an ion selective membrane electrode based on tridecylmethyl ammonium chloride with ethylenediaminetetraacetate anion selective to bismuth (III). These electrodes were useful for direct potentiometric monitoring of bismuth (III) in stomach antiacids. The sensor exhibited a very good selectivity for [Bi (EDTA)] over a variety of complex metal ions with EDTA[131].

M. B. Saleh *et al* explored the possibility of using [4-(4'-nitrobenzyl)-1-phenyl-3,5-pyrazolidinedion] as an ionophore for the fabrication of PVC membrane sensor for the determination of cerium (III) ions. It had a fast response time of < 10 s. The proposed sensor was used successfully as an indicator electrode in potentiometric titration of phosphate, oxalate in aqueous media and carbonate, fluoride acetylsalicylate in some drugs[132].

A PVC membrane sensor selective to cimetidine with cimetidine - phospotungstate ion pair complex as the ionophore has been reported. It gave a Nernstian slope and could be successfully applied to the determination of cimetidine in tablets and for its recovery from urine sample[133].

The construction and performance characteristics of four novel PVC membrane sensors responsive to cinnarizinium cation have been reported. These sensors were based on the use of ion association complexes of cinnarizinium cation with tetraphenyl borate, flavinate, reineckate and molybdophosphate counter anions as ion exchange sites in a plasticized PVC matrix. The sensors proved useful in determining cinnarizine in various dosage forms, in monitoring tablet dissolution rates and in testing tablet uniformity[134].

In an attempt to detect illicit drugs and stimulants using ISEs, the group of Watanabe, fabricated a cocaine selective membrane electrode using sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate as ion exchanger. They used tetrakis(2-ethylhexyl)pyromellitate as solvent mediator to suppress the response to lipophilic quarternary ammonium ions and strengthened the response to cocaine. The electrode was applied for the determination of cocaine in a drug mixture containing cocaine and morphine which is widely used to suppress pain in cancer patients[135].

Drozd and Hopkala published their results of polymeric membrane electrodes for cyproheptadine hydrochloride. They are based on the use of cyproheptadine-tetrakis(4-chlorophenyl) borate and cyproheptadine-dipicrylamine as electroactive compound. The electrode was successfully applied for the determination of cyproheptadine in bulk substance and tablets[136].

A potentiometric sensor immobilized in a graphite matrix has been reported for the determination of diclofenac. The electrode gave a Nernstian slope and was used in the determination of diclofenac in pharmaceutical preparations[137].

Katsu and Mori discussed a disopyramide sensitive membrane electrode for determining free disopyramide levels in blood serum. The sensor incorporated sodium tetrakis[3,5-bis(2-methoxyhexafluro-2-propyl)phenyl]borate as the ion exchanger. None of the similar antiarrhythmic drugs except bretylium interfered[138].

An internal solid contact sensor for the determination of doxycycline hydrochloride was developed based on a conducting polypyrrole film

immobilized on a glassy carbon electrode surface coated by a plasticized PVC membrane. The ion pair of the drug with tetraphenyl borate was used as the electroactive material. The sensor was successfully applied for the determination of the drug in pharmaceutical formulations[139].

The group of S. S. M. Hassan explored the response characteristics of PVC matrix membrane sensors for fluorouracil. The sensors incorporate ion association complexes of flurouracil with bathophenanthroline nickel (II), bathophenanthroline iron (II) and phenanthroline iron (II) as electroactive materials. The sensors were used for the direct determination of fluorouracil in pharmaceutical preparations. They were also used to follow the stability of the drug in the presence of its degradates namely formaldehyde, fluoroacetate and urea[140].

A flurbiprofen sensor based on tricaprylmethyl ammonium chloride has been reported. The sensor was applied for the determination of dissolution profile of flurbiprofen[141].

Shehata *et al* constructed four glutathione selective electrodes with different techniques and in different polymeric matrices. The developed sensors were used in the determination of glutathione in pharmaceutical preparations as well as for its recovery from plasma[142].

Alizadeh *et al* developed an ion selective membrane electrode for ketamine hydrochloride which had ionic end groups as ion exchanger sites. The electrode gave a perfect Nernstian slope. In addition to its use in the determination of the drug from pharmaceutical preparations, it was also used to study the interaction of bovine serum albumin with ketamine[143].

Sadeghi and his group constructed a potentiometric sensor based on a molecularly imprinted polymer for the recognition and determination of levamisole hydrochloride. It had a longer life span of four months and was used for the determination of levamisole in pure and tablet forms[144].

Hassan and his co-workers developed potentiometric, spectrofluorimetric and spectrophotometric methods for metformin determination. The ion association for the drug was obtained with reinckate and tungstosilicate[145].

Stefan employed lauryl sulphate as the counter ion for the construction of ion selective electrodes for moclobemide and disopyramide[146].

Katsu *et al* fabricated a PVC membrane sensor responsive to a stimulant phentermine. They employed sodium tetrakis[3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl]borate as the ion exchanger. This electrode was applied to determine phentermine in ion exchange complexes containing this stimulant[147].

Khalil *et al* constructed a plasticized membrane electrode selective to prazosinium cation based on prazosinium phosphotungstate ion association. The sensor was found to be useful in determining the prazosin content in pure solution and in pharmaceutical preparations[148].

The prenalterol selective membrane sensor reported by the group of Khalil was based on an ion association of the drug with sodium tetraphenyl borate. The determination of prenalterol in pure form and in pharmaceutical formulations was achieved by the developed sensor[149].

The piroxicam and tenoxicam selective membrane sensor fabricated by Khalil and his group proved to be useful in the determination of the active ingredient in their respective pharmaceutical formulations. The electroactive materials were based on the ion pair complexes of the drug with aliquot 336S cation[150].

Shoukry and co workers developed PVC membrane sensors for the determination of reproterol hydrochloride based on ion associates of the drug with phosphotungstic acid, phosphomolybdic acid and a mixture of both; which proved to be useful for the determination of the drug in its pharmaceutical preparations[151].

The five different potentiometric sensors developed for sulbutiamine by Ahmed *et al* were useful for the determination of the drug in micro gram quantities. The ion pairs were based on phosphotungstate, molybdate, tetraphenyl borate, reinckate and phosphomolybdate[152].

Wang and his co workers immobilized heptakis(2,6-di-O-isobutyl)-β-cyclodextrin in a PVC matrix to fabricate a sensor selective to tetracycline[153].

Theophylline sensor reported by Shamsipur's group was based on 2,6-bis(phenyl)-4-(phenyl)-3-H-thiopyran ionophore. The membrane sensor was successfully applied to the determination of theophylline in tablets[154].

Solid state valproate ion selective sensor based on conducting polypyrrole films was fabricated for the determination of valproate in pharmaceutical preparations. It had a long life span of 4 months[155].

Shahawi reported ion selective membrane electrodes for sildenafil citrate. The sensors were based on the formation of the complex ion associates of sildenafil citrate with tetraphenyl borate or phosphomolybdic

acid. The proposed sensors were tested for the analysis of sildenafil citrate in pure form, pharmaceutical preparations and blood serum[156].

The group of Sanchez Pedreno in 2007 reported the construction of a PVC membrane sensor for tiapride. The sensor incorporates an ion association of the drug with tetraphenyl borate. The sensor exhibited a fast, stable, Nernstian response over a wide concentration range $1 \times 10^{-5} - 1 \times 10^{-2}$ M with a slope of 57.2 mV/decade. The developed sensor was applied for the determination of tiapride in human urine and iontophoresis solution[157].

Zholt Khormosh and his co-workers in 2007 reported a diclofenac selective membrane sensor for the drug based on the ion associate of the drug with rhodamine B. The potentiometric response characteristics of the sensor based on the ion association in different plasticizers were extensively studied. The sensor exhibited a linear response within the diclofenac concentration range $1 \times 10^{-5} - 5 \times 10^{-2}$ M and a slope of 60 mV/decade[158].

H. Y. A. Enein *et al* were successful in developing a pethidine selective PVC membrane sensor based on the ion association of the drug with phosphomolybdate. It exhibited a near Nernstian response of 55.24 mV/decade over the concentration range $1 \times 10^{-5} - 1 \times 10^{-2}$ M. The useful pH range has been observed to be 2 - 7. The developed sensor has been found to be useful for the determination of the drug in pure form, pharmaceutical formulations and in biological fluids[159].

1.9 Scope of the present investigations

The quality of a drug is determined after establishing its authenticity by testing its purity and quality of the pure substance in the drug and its

formulations. A number of methods including physical, chemical, physico-chemical and biological ones are employed for determining the quality of the drugs. Among the physico-chemical methods, the electrochemical is the most widely used one. In continuation to the development of spectrophotometric[160-164] and electrochemical methods[165-168] for the determination of drugs in our laboratories, the present investigations involve the preparation of potentiometric sensors for drugs such as mebendazole, pefloxacin, ambroxol, sildenafil citrate, dextromethorphan and tetracycline. For all the sensors fabricated, the various analytical parameters studied include linear response range, calibration slope, detection limit, effect of pH, shelf life and selectivity. The developed sensors have been applied for the determination of the drugs in pharmaceutical formulations and in real samples.

Chapter 2

Materials and Methods

This chapter discusses in detail the synthesis of each of the ion associations used in the fabrication of the different sensors. It also describes the general method of fabrication of the two types of sensors viz; the PVC membrane sensor and the carbon paste electrode (CPE). Details about the general reagents and the instruments used in the investigations are also discussed in this chapter. The chapter also discusses the general procedure for the analysis of the pharmaceutical formulations and real samples employed in the studies.

2.1 Reagents

All reagents used were of analytical grade and distilled water was used throughout the studies. High molecular weight PVC, perchloric acid and all the metal salts were obtained from Merck, Germany and were used as received. The ion pairing reagents such as silicotungstic acid (STA), phosphotungstic acid (PTA), molybdophosphoric acid (MPA) and sodium tetraphenyl borate (NaTPB) were obtained from sd fine chem. India. The plasticizers Bis(2-ethyl hexyl) phthalate (BEP), Bis(2-ethyl hexyl) sebacate (BES), Di-n-butyl phthalate (DBP), Di-butyl sebacate (DBS) and Bis(2-ethyl hexyl) adipate (BEA) were all products of Lancaster UK and were used without any further purification. High purity graphite was purchased from Sigma Aldrich Corporation, USA and was used as received. Tetrahydrofuran

(THF), 2, 2-diphenyl-1-picrylhydrazyl, 5,5-diethylbarbituric acid, ethyl acetate, acids and indicators were products of sd fine chem. India. Pure drugs viz, tetracycline, dextromethorphan, mebendazole, ambroxol, sildenafil citrate and pefloxacin were obtained as gift samples. Pharmaceutical formulations containing the drugs were purchased from local drug stores.

2.2 Synthesis of the ion association complexes

The ion association complexes (ionophores) for each drug were prepared using the respective ion pairing reagents. The different ion pairing reagents employed were silicotungstic acid (STA), phosphotungstic acid (PTA), molybdophosphoric acid (MPA) and sodium tetraphenyl borate (NaTPB).

2.2.1 Mebendazole-STA ion association

0.29 g of mebendazole (MBZ) was dissolved in very dil. HNO_3 and made upto 100 mL with distilled water. 75 mL of the 10^{-2} M MBZ solution thus obtained was mixed with 25 mL 10^{-2} M STA solution. The resulting solution was stirred well for 10 minutes. The white coloured precipitate obtained was filtered and washed several times. The precipitate was dried at room temperature and stored in a desiccator.

2.2.2 Mebendazole-PTA ion association

10^{-2} M MBZ solution was prepared by dissolving 0.29 g of pure drug in very dil. HNO_3 and made upto 100 mL with distilled water. MBZ-PTA ion association was prepared by mixing 75 mL of this drug solution with 25 mL of 10^{-2} M PTA solution and stirred well for 10 minutes. The yellow coloured precipitate thus formed was filtered, washed, dried at room temperature and stored in a desiccator.

2.2.3 Mebendazole-MPA ion association

MBZ-MPA ion association was prepared by mixing 75 mL 10^{-2} M MBZ solution and 25 mL 10^{-2} M MPA. The solution was stirred well for 10 minutes. The resulting yellow coloured precipitate was filtered and washed repeatedly. The ion association was dried at room temperature and stored.

2.2.4 Pefloxacin-STA ion association

0.33 g of pure pefloxacin (PEF) was dissolved in distilled water and made upto 100 ml to get 10^{-2} M PEF solution. 75 mL of this solution was mixed with 25 mL of 10^{-2} M STA and stirred well. The yellow coloured precipitate was filtered and washed. It was dried at room temperature and stored.

2.2.5 Pefloxacin-MPA ion association

A 10^{-2} M PEF solution was prepared in 100 mL by dissolving 0.33 g of the pure drug in distilled water.75 mL of this solution was mixed with 25 mL 10^{-2} M MPA and stirred for 10 minutes. The brown coloured precipitate obtained was filtered, washed several times and dried at room temperature.

2.2.6 Ambroxol-MPA ion association

0.41 g of pure ambroxol (AMB) was dissolved in dil. methanol and made upto 100 mL to get a 10^{-2} M AMB solution. 75 mL of this solution was mixed with 25 mL, 10^{-2} M of MPA and the brown coloured precipitate obtained was washed several times. The precipitate was dried at room temperature and stored in a desiccator.

2.2.7 Ambroxol-PTA ion association

75 mL 10^{-2} M ambroxol (AMB) solution was mixed with 25 mL 10^{-2} M PTA solution. The solution was allowed to stand for a few minutes and

the faint yellow coloured precipitate formed was filtered and washed. The precipitate dried at room temperature was stored in a desiccator.

2.2.8 Sildenafil citrate-PTA ion association

0.47 g of sildenafil citrate (SIL) was dissolved in dilute methanol and made upto 100 mL to prepare a 10^{-2} M SIL solution. 75 mL of this solution was mixed with 25 mL 10^{-2} M PTA and stirred well for 15 minutes. It was allowed to stand for a few more minutes and the resulting yellow coloured precipitate was washed repeatedly. The dried precipitate was stored in a desiccator.

2.2.9 Sildenafil citrate-STA ion association

The SIL – STA ion association was prepared by mixing 10^{-2} M (75 mL) SIL with 10^{-2} M (25 mL) STA. The solution was stirred well for 15 minutes and allowed to stand. The brown coloured precipitate formed was washed repeatedly, dried at room temperature and was stored in a desiccator.

2.2.10 Dextromethorphan-NaTPB ion association

10^{-2} M solution of dextromethorphan (DEX) was prepared in 100 mL by dissolving 0.37 g of the drug in distilled water. 100 mL of 10^{-2} M solution of NaTPB was prepared. The ion association of DEX with NaTPB was obtained by mixing 50 mL each of these prepared solutions and stirred well. The resulting white precipitate was filtered, washed with distilled water and dried at room temperature. The dried precipitate is stored in a desiccator for future use.

2.2.11 Dextromethorphan-PTA ion association

The DEX-PTA ion association was prepared by mixing solutions of DEX and the ion-pairing reagent PTA. 10^{-2} M solution of dextromethorphan (DEX) was prepared in 100 mL by dissolving 0.37 g of the drug in distilled

water and 10^{-2} M solution of PTA was also prepared. The DEX-PTA ion association was prepared by mixing 75 mL of 10^{-2} M DEX with 25 mL of 10^{-2} M PTA. The mud coloured precipitate obtained was filtered, washed repeatedly, dried at room temperature and stored in a desiccator.

2.2.12 Tetracycline – NaTPB ion association

The ion association for tetracycline (TCE) was based on the ion-pairing reagent NaTPB. A 10^{-2} M solution of the drug (100 mL) was prepared by dissolving 0.44 g TCE in water. Similarly 10^{-2} M (100 mL) solution of NaTPB was also prepared. The ion association was prepared by mixing 10^{-2} M (50 mL) solution of the drug with 10^{-2} M (50 mL) of NaTPB. This was then stirred well. The resulting yellow coloured precipitate was filtered, washed several times with distilled water and dried at room temperature. The ion association thus obtained is stored in a desiccator.

2.3 Preparation of the drug solutions

A 10^{-1} M stock solution in suitable solvents was prepared for each of the drugs. The dilution series for the analysis was obtained by the serial dilution of 10^{-1} M stock solution.

2.4 Preparation of the buffer solutions

The buffer solutions were used to adjust the pH of the test solutions. The different buffer solutions were freshly prepared according the Robinson table[169].

2.4.1 pH 1.0

To 50 mL 0.2 M potassium chloride solution, 134.0 mL of 0.2 M HCl solution was added to give the buffer having pH 1.0.

2.4.2 pH 2.0

To 50 mL 0.2 M potassium chloride solution, 13.0 mL of 0.2 M HCl solution was added to give the buffer having pH 2.0.

2.4.3 pH 3.0

To 100 mL 0.1 M potassium hydrogen phthalate solution, 44.6 mL of 0.1 M HCl solution was added to give the buffer having pH 3.0.

2.4.4 pH 4.0

To 100 mL 0.1 M potassium hydrogen phthalate solution, 0.2 mL of 0.1 M HCl solution was added to give the buffer having pH 4.0.

2.4.5 pH 5.0

To 100 mL 0.1 M potassium hydrogen phthalate solution, 45.2 mL of 0.1 M NaOH solution was added to give the buffer having pH 5.0.

2.4.6 pH 6.0

To 100 mL 0.1 M potassium dihydrogen phosphate, 11.2 mL of 0.1 M NaOH solution was added to give the buffer having pH 6.0.

2.4.7 pH 7.0

To 100 mL 0.1 M potassium dihydrogen phosphate, 58.2 mL of 0.1 M NaOH solution was added to give the buffer having pH 7.0.

2.4.8 pH 8.0

To 100 mL 0.025 M borax, 41.0 mL of 0.1 M HCl solution was added to give the buffer having pH 8.0.

2.4.9 pH 9.0

To 100 mL 0.025 M borax, 9.2 mL of 0.1 M HCl solution was added to give the buffer having pH 9.0.

2.4.10 pH 10.0

To 100 mL 0.05 M sodium bicarbonate, 21.4 mL of 0.1 M NaOH solution was added to give the buffer having pH 10.0.

2.4.11 pH 11.0

To 100 mL 0.05 M sodium bicarbonate, 45.4 mL of 0.1 M NaOH solution was added to give the buffer having pH 11.0.

2.4.12 pH 12.0

To 50 mL 0.2 M potassium chloride, 12.0 mL of 0.2 M NaOH solution was added to give the buffer having pH 12.0.

2.5 Analysis of the pharmaceutical formulations

2.5.1 Tablets for Tetracycline (Resteclin and Tetracycline)

The contents of ten capsules of each of Resteclin (NPIL - India) and Tetracycline (Dabur - India) were accurately weighed and powdered well in a mortar. About 250 mg of the powder was accurately weighed and dissolved in minimum amount of distilled water and filtered into a 100 mL volumetric flask. This was made up to the mark and shaken well. 10 mL of this solution was transferred into a 100 mL titrimetric flask and made up to the volume. The pH of the solution was adjusted to 6.0. 20 mL of this solution was transferred into a beaker and electrochemical studies were conducted.

2.5.2 Syrup for Dextromethorphan (TUSQ-DX)

10 mL of the syrup (Tusq-DX – Blue Cross - India) was transferred into a 100 mL volumetric flask. It was dissolved in minimum amount of distilled water by shaking well for 10 minutes. The pH of the solution was maintained at 5.0 using appropriate buffer solution. The solution was then

quantitatively diluted. 20 mL of this solution was taken in a beaker and electrochemical studies were carried out.

2.5.3 Tablet for Mebendazole (Mebex)

Ten tablets of Mebex (Cipla - India) were accurately weighed and finely powdered. The mass equivalent to the mass of one tablet was dissolved in dil. HNO_3 and filtered into a 100 mL volumetric flask. This was made upto the mark and shaken well. 10 mL of this solution was transferred to a 50 mL volumetric flask. The pH of the solution was adjusted to 6.0 by adding buffer solution and the solution was quantitatively diluted. 20 mL of this solution was transferred to a beaker and electrochemical studies were performed.

2.5.4 Tablet for Ambroxol (Ambrolite)

Ten tablets of Ambroxol (Ambrolite -Tablets India) were weighed, crushed and finely powdered. The mass equivalent to the mass of one tablet was accurately weighed and dissolved in dil. methanol. It was then made up to the mark in a 100 mL volumetric flask. The pH of the solution was maintained at 6.0. 20 mL of this solution was transferred to a beaker and the electrochemical response was investigated.

2.5.5 Tablet for Sildenafil citrate (Silagra)

The weight of ten tablets of Sildenafil citrate (Silagra – Cipla - India) was accurately determined and powdered well. The quantity of powder equivalent to the mass of one tablet was dissolved in minimum volume of distilled water and shaken well for 15 minutes. This was made up in a 100 mL titrimetric flask after adjusting the pH to 5.0. 10 mL of this solution was transferred to a 50 mL volumetric flask and made up. 20 mL of this solution was taken to carry out the electrochemical studies.

2.5.6 Tablet for Pefloxacin (Pelox)

Ten tablets of Pelox (Wockhardt - India) were weighed and finely powdered. The mass equivalent to the mass of one tablet was dissolved in distilled water and made up in a 100 mL titrimetric flask. 10 mL of this solution was transferred into a 100 mL titrimetric flask and quantitatively diluted. The pH of the solution was maintained at 5.0. 20 mL of this solution was taken in a beaker and electrochemical studies were performed.

2.6 Standard methods

2.6.1 Tetracycline[170]

The contents of ten capsules were accurately weighed and finely powdered. The mass equivalent to the mass of one capsule was accurately weighed and dissolved in minimum quantity of distilled water. 2 mL of 0.2 M calcium chloride and 2 mL of 0.5 M 5,5-diethylbarbituric acid were added and the undecomposed drug was extracted with three 10 mL portions of ethyl acetate. The organic phase was filtered through anhydrous sodium sulphate and the ethyl acetate was evaporated off. The residue was dissolved in methanol and made upto 100 mL. 1 mL of this solution was transferred to a 25 mL standard flask and 2 mL buffer solution (pH-6) and 2 mL of 2,2-diphenyl-1-picrylhydrazyl was added and mixed well. This solution was heated at 60 °C for 12 minutes. It was cooled and completed to the volume. The absorbance of the solution was measured at 520 nm against reagent blank. The amount of drug was calculated from the calibration graph.

2.6.2 Dextromethorphan[171]

20 mL of the syrup was dissolved in 20 mL ethanol, made up to 100 mL in a volumetric flask and titrated with 0.1 M sodium hydroxide determining the end point potentiometrically.

2.6.3 Mebendazole[172]

0.1124 g of Mebex was dissolved in 1.5 mL of anhydrous formic acid and 20 mL of anhydrous acetic acid was added. The solution was made up in a 100 mL volumetric flask. 10 mL of this solution was transferred to 100 mL titrimetric flask and made up. 20 mL of this solution was titrated with 0.1 M perchloric acid, determining the end point potentiometrically. 1 mL of 0.1 M perchloric acid is equivalent to 29.53 mg of MEB.

2.6.4 Ambroxol[172]

The weight equivalent to the weight of five tablets was dissolved in 70 mL of alcohol and 5 mL of 0.01 M hydrochloric acid was added. It was made up to the volume in a 100 mL titrimetric flask. Potentiometric titration was carried out using 0.1 M sodium hydroxide.

2.6.5 Sildenafil citrate[173]

0.1225 g of the tablet was dissolved in distilled water and taken in a separating flask. To this solution 2 mL of bromocresol green and 3 mL of buffer (pH 2) was added followed by 5 mL of chloroform. This was shaken well and kept aside for 1 minute. After the organic layer was transferred to a beaker, aqueous phase was again extracted with 5 ml of the same solvent. The successive organic layers were mixed and dried over anhydrous sodium sulphate and transferred to a 50 mL volumetric flask. The absorbance of the solution was measured at 415nm against a reagent blank.

2.6.6 Pefloxacin[172]

0.2864 g of pefloxacin was dissolved in 15 mL of anhydrous acetic acid and 75 mL of acetic anhydride was added.10 mL of this solution was transferred to a 100 mL volumetric flask and made up. It was then titrated with 0.1 M perchloric acid and the end point was determined potentiometrically.

2.7 Analysis of urine sample

The developed sensors were used for the determination of the corresponding drugs in urine samples. Standard addition method was employed for this purpose. 20 mL of the urine sample containing the drug was taken in a beaker and the electrochemical studies were carried out. It was then spiked with 2 mL of known concentration of the drug solution and again the potential value was determined. The potential readings were noted and the amount of the drug present in the urine sample was calculated[174].

2.8 Fabrication of the sensors

The design of the electrochemical sensors plays a crucial role in determining the electrochemical response characteristics of the sensors. Two types of sensors were fabricated for the studies.

2.8.1 Fabrication of the PVC membrane sensor

The PVC membrane electrodes belong to the class of liquid membrane electrodes. PVC based sensor membranes contain a reagent dissolved in a suitable solvent, which selectively binds with the ion of interest, the ionophore. The general method of fabrication of the PVC membrane sensor was according to a procedure reported by Cragg's and

Moody[175]. The active membrane ingredients were varied to arrive at an optimum composition. Ionophore, plasticizer and PVC were taken in suitable percentage-weight ratios. The % w/w of these components vary approximately as 1-5 : 60-65 : 30-35 for ionophore, plasticizer and PVC respectively. The preliminary step involves dissolving the ionophore in THF followed by the plasticizer and the PVC in specific ratios. The resulting mixture was then poured into glass rings struck on a glass plate. It was then covered with a filter paper and left to dry allowing the slow evaporation of the solvent. Small disc shaped membranes thus obtained were cut out and glued to one end of a glass tube using Araldite and M-seal. This was then left to dry and the prepared membrane sensor was finally conditioned by dipping it in the drug solution of suitable concentration for 24 hrs. The internal filling solution consisted of a mixture of 1×10^{-3} M drug and 1×10^{-1} M NaCl solution.

2.8.2 Fabrication of the carbon paste sensor

The carbon paste electrodes (CPEs) belong to a special group of heterogeneous carbon electrodes. CPEs are represented by carbon paste, ie; a mixture prepared from graphite and ionophore with a suitable liquid binder packed into a suitably designed electrode body. One of the most significant advantages of the CPEs is that it does not require an internal filling solution. The ion association and high purity graphite were thoroughly mixed in a mortar with acetone in suitable proportions. It was homogenized and left at room temperature to evaporate off acetone. Then weighed amount of plasticizer was added to this carbon paste. This paste was then packed to one end of the Teflon holder in which electrical contact was made with a copper rod through the centre of the electrode. The electrode surface was polished

using a filter paper to produce reproducible working surface. The sensors were then conditioned by dipping it in suitable concentrations of the drug solutions.

2.9 Selectivity studies

If the full scope of a chemical sensor is to be fully realized, their selectivity in presence of various interferents demands proper and reliable assessment. The selectivity of the developed sensors for the drug in presence of various foreign ions has been determined using the Fixed Interference Method (FIM). The value of the selectivity coefficient $K_{A,B}^{pot}$ determines the extent of selectivity of the drug in presence of foreign ions. The potential of the cell comprising the sensor and the reference electrode was measured for solutions of constant activity of the interfering ion, a_B, and varying activity of the primary ion. The potentials obtained versus the logarithm of the activity of the primary ion were plotted and the intersection of the extrapolated linear portions of this graph indicates the value of a_A. The $K_{A,B}^{pot}$ value is calculated using the following equation.

$$K_{A,B}^{pot} = a_A / (a_B)^{z_A / z_B}$$

z_A and z_B are charge numbers of the primary ion, A and of the interfering ion, B. The selectivity coefficient indicates the extent to which a foreign ion interferes with the response of an electrode to its primary ion.

2.10 Potential measurement and calibration.

The potential measurements were carried out at 25±1 °C on a Metrohm 781 ion meter. A saturated calomel electrode was used in conjunction with the developed sensors. The cell assembly for the potentiometric measurements can be represented as follows:

For PVC membrane sensor,

Internal reference electrode | internal filling solution (1×10^{-3} M drug solution + 1×10^{-1} M NaCl solution) | PVC membrane | test solution | external reference electrode.

For carbon paste electrode (CPE),

Reference electrode | test solution | Graphite electrode.

The electrochemical response of the developed sensors was investigated by measuring the potential response of the test solutions. The solutions were stirred well and the stable potential readings were recorded.

2.11 Instruments used

The CHN analysis was done on a CHN analyzer, Elementar Vario EL III at Sophisticated Test and Instrumentation Centre (STIC), Kochi. Spectrophotometric measurements were carried out on a UV3500 Labomed Inc. spectrophotometer. All the potential measurements were carried out on a Metrohm 781 ion meter.

Chapter 3

Development of Sensors for Mebendazole

This chapter illustrates the fabrication of PVC membrane sensor for mebendazole (MBZ) based on the ion associations of the drug with molybdophosphoric acid (MPA), silicotungstic acid (STA) and phosphotungstic acid (PTA) as ionophores. The electrochemical response characteristics of the developed sensors were studied in detail. The utility of the developed sensors were investigated in the determination of the drug in pharmaceutical formulations and also for the determination of the drug from urine samples.

Mebendazole (MBZ), a derivative of benzimidazole (5-benzoyl-2-benzimidazolylcarbamic acid methyl ester) ($C_{16}H_{13}N_3O_2$) (Figure 3.1) is a synthetic broad spectrum anthelminthic drug producing high cure rates in single or mixed infestations by ascaris, thread worms, pin worm, common round worm, hook worms and whip worms[176,177]. MBZ causes slow immobilization and death of the worms by selectively and irreversibly blocking uptake of glucose and other nutrients from susceptible adult intestine where helminths dwell. It is a spindle poison that induces chromosome nondisjunction. Studies also show that MBZ is effective in the treatment of cancer and other angiogenesis-dependent diseases. MBZ has no effect on normal endothelial cell growth but directly target tumor cells *in vivo*[178].

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MBZ causes degenerative alterations in the tegument and intestinal cells of the worm by binding to the colchicine-sensitive site of the tubulin, thus inhibiting its polymerisation or assembly into microtubules. The loss of cytoplasmic microtubules leads to impaired uptake of glucose by the larval and adult stages of the susceptible parasites, and depletes their glycogen stores. Degenerative stages in the endoplasmic reticulum, the mitochondria of the geminal layer and the subsequent release of lysosomes result in decreased production of adenosine triphosphate, which is the energy required for the helminth. Due to diminished energy production, the parasite is immobilised and it eventually dies.

Much of the early work on the benzimidazoles as a class was undertaken by Janssen Pharmaceutical in Belgium under the direction of Dr Paul Janssen. Benzimidazoles was originally developed as plant fungicides and later as veterinary anthelminthics. The first benzimidazole to be developed and licensed for human use was thiabendazole in 1962. Although thiabendazole was very effective, it was also moderately toxic which led to the discovery of better and safer compounds such as benzimidazole carbamates. The first benzimidazole carbamate to make it into humans was mebendazole (MBZ) (1975) followed by flubendazole (both Janssen products)[179].

Several analytical techniques available in the literature for the quantitative determination of MBZ include spectrophotometry[180], high performance liquid chromatography[181-186], liquid chromatography[187], thin layer chromatography[188] and cathodic stripping voltammetry[189]. Most of these methods are complicated and need sophisticated instruments.

This chapter presents the results of development of three PVC membrane sensors for MBZ based on three different ionophores and their use in the determination of MBZ in pharmaceutical formulations.

3.1 Preparation of the ion associations

The sensors for MBZ determination were based on three different ion associations. These ion associations were prepared using the ion pairing reagents molybdophosphoric acid (MPA), silicotungstic acid (STA) and phosphotungstic acid (PTA). The detailed procedure for the preparation of these ion associations has been discussed in Section 2.2 of Chapter 2. The ion associations were prepared by mixing the drug and the respective ion pairing agents in the ratio 3:1 (drug: ion pairing agent). The ion associations obtained were dried and stored in desiccator. The structure of the ion associations was confirmed by elemental analysis. The composition of all three complexes have been found to be 3:1 (drug : ion pairing reagent).

MBZ-STA ion association

Found (%) – C – 20.07, H – 1.57, N – 4.25

Calculated (%) – C –19.96, H – 1.45, N – 4.19

MBZ-PTA ion association

Found (%) – C – 15.25, H – 1.44, N – 3.27

Calculated (%) – C – 15.31, H – 1.12, N – 3.35

MBZ-MPA ion association

Found (%) – C – 22.03, H – 2.04, N – 4.74

Calculated (%) – C – 21.86, H –1.97, N – 4.65

3.2 Fabrication of PVC membrane sensor

The general method for fabrication of the PVC membrane sensor has been discussed in Section 2.8.1 in Chapter 2. The fabrication of the sensor membrane involves dissolving the ion association, PVC and plasticizer in THF. The resulting mixture was poured into glass rings on a glass plate. The disc shaped membrane obtained after slow evaporation of the solvent was stuck onto one end of a glass tube. The glass tube was filled with a solution consisting of 1×10^{-3} M MBZ and 1×10^{-1} M NaCl. The developed sensors were conditioned by dipping them in 1×10^{-3} M MBZ for 24 hours.

In preparing the PVC membrane sensors for each of the ionophore, various composition ratios of the ionophore, PVC and plasticizer were tried, so as to obtain an optimum composition for the membrane which gave a Nernstian response.

3.3 Potential measurement and calibration

Potentials were measured at 25±1 °C on a Metrohm 781 ion meter. A saturated calomel reference electrode was used in conjunction with the developed sensor.

The cell assembly for potentiometric measurements can be represented as follows:

Internal reference electrode | internal filling solution (1×10^{-3} M drug solution + 1×10^{-1} M NaCl solution) | PVC membrane | test solution | external reference electrode.

The performance of the developed sensors were investigated by measuring the potential in MBZ solutions prepared in the concentration range 1.0×10^{-1} – 1.0×10^{-6} M. The solutions were stirred and the stable potential reading was taken.

3.4 Optimization of membrane composition

The optimization of PVC membrane composition is very important because the sensitivity and selectivity of the PVC membrane sensor is highly influenced by the amount of the ionophore, nature of the plasticizer and the plasticizer/PVC ratio. In order to study the effect of varying the membrane composition on the potential response of the developed sensors, a set of 20 different compositions for the PVC membrane sensors were prepared for each ion association. The five different plasticizers used were bis(2-ethyl hexyl) phthalate (BEP), Bis(2-ethyl hexyl) sebacate (BES), Di-n- butyl phthalate (DBP), Di-butyl sebacate (DBS), Bis(2-ethyl hexyl) adipate (BEA).

MBZ-STA ion association was used for the fabrication of PVC membrane sensor for mebendazole. All the active ingredients of the membrane matrix were changed so as to obtain an optimum composition for the membrane. The nature as well as the amount of the plasticizer and the amount of the ionophore was varied. The optimum amount of ionophore has been found to be 1.0% (w/w). For the sensor fabricated using MBZ - STA ion pair, the plasticizer that gave the near Nernstian slope of 55.8 mV/decade is BEP. Table 3.1 clearly shows how the slopes of the sensors vary on changing the composition of the membrane. The best membrane composition for this ion association has been found to be 1.0:35.0:64.0 % (w/w) (ion-

association: PVC: plasticizer). This sensor (S_{M5}) exhibited a linear response over the range 1.0×10^{-6} - 5.0×10^{-2} M of MBZ with a lower detection limit of 6.3×10^{-7} M and response time of 30 s. The calibration plot for S_{M5} is given as Figure 3.2.

The dielectric constant of the plasticized poly (vinyl chloride) (PVC) is an important factor to be considered in liquid membranes. In the ion - selective electrodes the dielectric constant of the PVC is a function of the nature of the plasticizer as well as its proportion in the polymeric matrix[190, 191].

It is generally expected that the values of the dielectric constants of the liquid membranes are similar to that of pure liquid plasticizer. This is due to the fact that most liquid membranes used as sensors contain between 60 to 70% by weight of a plasticizer. The latter can be explained by considering that the pK_a of the plasticized PVC is above its glass transition temperature (Tg) at room temperature. The variation observed in sensor response using different plasticizers may be attributed to different carrier mechanisms[192].

Membrane sensors incorporating MBZ - PTA ion association as the electroactive species were also fabricated. The sensors were constructed by varying the membrane components such as the plasticizer, amount of ionophore and plasticizer /PVC ratio. The amount of the ionophore was found to influence the sensitivity of the sensors. The optimum amount of ionophore has been found to be 1.2% (w/w). As is well known, the property of the plasticizer influences the response of the sensor. The best response was found to be for the sensor fabricated using DBP. A 62.8% (w/w) of plasticizer was found to give the near Nernstian slope of 52.6 mV/decade. Table 3.2 illustrates the variation in slopes of the sensors with changing

membrane composition. Thus a membrane sensor (P_2) incorporating 1.2 : 36.0 : 62.8 % (w/w) (ion association : PVC : plasticizer) of the active membrane ingredients gave best response in terms of slope (52.6 mV/decade) working concentration range ($1.0 \times 10^{-6} - 7.0 \times 10^{-2}$ M) and a lower detection limit (3.6×10^{-7} M). The response time was observed to be 45s. Figure 3.3 depicts the calibration plot of P_2.

The optimum amount of ionophore for the construction of the sensor incorporating MBZ-MPA ion association as the electro active material has been found to be 1.6% (w/w). The nature of the plasticizer was varied and the optimum amount was found to be 67.2% (w/w). From Table 3.3 it is clear that the sensor (M_7) is the best among all the sensors fabricated for this ion association and BEP is the most suitable plasticizer. The composition ratio of the sensor (M_7) is 1.6 : 31.2 : 67.2 (ion association : PVC : plasticizer) giving a slope of 57.4 mV/decade (Figure 3.4) working concentration range of $1.0 \times 10^{-6} - 8.9 \times 10^{-3}$ M and a lower detection limit of 4.8×10^{-7} M. The sensor exhibited a good response time of < 35 s.

The deviation from the Nernstian value may in some cases be due to the saturation of the membrane surface or failure to attain membrane equilibrium, due to less diffusivity of the active ingredients of the membranes.

The response characteristics of S_{M5}, P_2 and M_7 are summarized in Table 3.4

3.5 Effect of concentration of the internal filling solution

The influence of concentration of internal filling solution on the potential response of the developed mebendazole selective membrane sensors was studied. In order to study this effect, the MBZ concentration was changed from 1.0×10^{-3} M to 1.0×10^{-5} M. It has been observed that changing the concentration of the internal solution did not cause any significant change in the response of the sensor. Hence 1.0×10^{-3} M MBZ solution was used as the internal filling solution for the developed sensors.

3.6 Effect of pH

The effect of pH of the test solution on the potential response of all the sensors S_{M5}, P_2, M_7 was examined for two fixed concentrations of MBZ viz; 1.0×10^{-3} M and 1.0×10^{-4} M in the pH range 1-11 (Figures 3.5, 3.6, 3.7). The pH of the solution was adjusted using buffer. For the sensor S_{M5}, there is no change in the potential response within the pH range 4 - 7. The potential corresponding to the pH range 5 - 7 remained constant for the sensor P_2 whereas for the sensor M_7, the pH range where the potential remained unchanged is 4 - 7. At higher pH (pH>7) the solution turned turbid due to the decomposition of the drug and at lower pH the observed increase in potential may be due to the dissociation of the complex.

3.7 Potentiometric selectivity

The most important characteristic of any ion selective sensor is its response to the primary ion in the presence of other ions present in the solution, which is expressed in terms of the potentiometric selectivity coefficient ($K_{A,B}^{pot}$). The extent of interference of other ions and species on

the sensors S_{M5}, P_2 and M_7 were studied by Fixed Interference Method (FIM). The calculated selectivity coefficients are summarized in Table 3.5. The results reveal that the developed sensors are selective to MBZ in presence of the foreign ions tested. The values of the selectivity coefficients given in the table reveal that the developed sensors S_{M5}, P_2 and M_7 show very good selectivity to mebendazole in the presence of ions such as of Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, citric acid, lactose, urea, ascorbic acid and glycine. There is no interference from the tablet excipients such as starch and talc. Since there is no interference from these ions and urea the developed sensors can be applied for the determination of mebendazole in urine sample.

3.8 Shelf Life or Life Time

The stability and life time of the developed sensors were monitored. Detectable loss of performance characteristics has not been found in the sensor S_{M5} upto 6 weeks. The sensor P_2 could be used for 4 weeks, whereas M_7 could be used for 7 weeks without any significant deviation from the optimized response characteristics. After this, the calibration slope and linear range of response gradually decreased for all the three sensors probably due to leaching of the ionophore from the sensor.

3.9 Analytical applications

The developed sensors S_{M5}, P_2 and M_7 were employed for the assay of MBZ content in tablets (Mebex - Cipla - India). The detailed procedure is given in Section 2.5.3 in Chapter 2. Ten tablets were weighed, finely powdered and thoroughly mixed. An accurately weighed portion of the powder equivalent to weight of one tablet was dissolved in dil. HNO_3 and

filtered into a volumetric flask. 20 mL of the drug solution was transferred to a beaker and potentials were recorded. The results (Table 3.6) were compared with those obtained by the European Pharmacopoeia procedure[172]. The results show that there is a satisfactory agreement between the MBZ content determined by the proposed method and the standard method. The developed sensors could be used for the determination of MBZ in tablets with high accuracy and precision.

S_{M5}, P_2 and M_7 were employed for the determination of mebendazole in spiked urine samples. The results given in Table 3.7 clearly reveal that the sensors could be used for the determination of MBZ in spiked urine samples with high accuracy and precision. The % recovery of mebendazole using the sensors S_{M5}, P_2 and M_7 were found to be 99.3, 98.7 and 100.7 respectively.

The ion associations of the drug with the ion pairing reagents molybdophosphoric acid (MPA), silicotungstic acid (STA) and phosphotungstic acid (PTA) were employed for the fabrication of PVC membrane sensors for MBZ. M_7 was found to give a near Nernstian slope of 57.4 mV/decade, within a concentration range of $1.0 \times 10^{-6} - 8.9 \times 10^{-3}$ M. The detection limit has been as low as 4.8×10^{-7} M. S_{M5} and P_2 also gave a Nernstian response of 55.8 and 52.6 mV/decade respectively. The working concentration ranges for S_{M5} and P_2 are $1.0 \times 10^{-6} - 5.0 \times 10^{-2}$ M and $1.0 \times 10^{-6} - 7.0 \times 10^{-2}$ M. The lower detection limit for S_{M5} was found to be 6.3×10^{-7} M. The working pH range for both S_{M5} and M_7 was observed to be 4-7, whereas for P_2 the pH range was found to be 5-7. The shelf life for the sensors S_{M5}, P_2 and M_7 are 6, 4 and 7 weeks respectively.

Table 3.1

Optimization of membrane ingredients for PVC membrane sensor based on MBZ-STA ion association

	Composition % (w/w)			Slope
Membrane	Ionophore	PVC	Plasticizer	mV/decade
S_{M1}	1.0	35.0	64.0, DBP	49.8
S_{M2}	1.2	36.0	62.8, DBP	47.6
S_{M3}	1.6	31.2	67.2, DBP	32.4
S_{M4}	2.0	29.1	68.9, DBP	30.1
S_{M5}	**1.0**	**35.0**	**64.0, BEP**	**55.8**
S_{M6}	1.2	36.0	62.8, BEP	47.0
S_{M7}	1.6	31.2	67.2, BEP	62.3
S_{M8}	2.0	29.1	68.9, BEP	69.4
S_{M9}	1.0	35.0	64.0, DBS	50.1
S_{M10}	1.2	36.0	62.8, DBS	61.5
S_{M11}	1.6	31.2	67.2, DBS	63.5
S_{M12}	2.0	29.1	68.9, DBS	65.4
S_{M13}	1.0	35.0	64.0, BES	72.1
S_{M14}	1.2	36.0	62.8, BES	70.0
S_{M15}	1.6	31.2	67.2, BES	70.9
S_{M16}	2.0	29.1	68.9, BES	69.1
S_{M17}	1.0	35.0	64.0, BEA	62.4
S_{M18}	1.2	36.0	62.8, BEA	64.3
S_{M19}	1.6	31.2	67.2, BEA	32.2
S_{M20}	2.0	29.1	68.9, BEA	39.9

Table 3.2

Optimization of membrane ingredients for PVC membrane sensor based on MBZ-PTA ion association

	Composition % (w/w)			Slope
Membrane	Ionophore	PVC	Plasticizer	mV/decade
P_1	1.0	35.0	64.0, DBP	49.1
P_2	**1.2**	**36.0**	**62.8, DBP**	**52.6**
P_3	1.6	31.2	67.2, DBP	63.2
P_4	2.0	29.1	68.9, DBP	65.0
P_5	1.0	35.0	64.0, BEP	71.9
P_6	1.2	36.0	62.8, BEP	65.5
P_7	1.6	31.2	67.2, BEP	73.8
P_8	2.0	29.1	68.9, BEP	75.3
P_9	1.0	35.0	64.0, DBS	31.6
P_{10}	1.2	36.0	62.8, DBS	45.7
P_{11}	1.6	31.2	67.2, DBS	49.6
P_{12}	2.0	29.1	68.9, DBS	69.3
P_{13}	1.0	35.0	64.0, BES	38.1
P_{14}	1.2	36.0	62.8, BES	40.6
P_{15}	1.6	31.2	67.2, BES	49.8
P_{16}	2.0	29.1	68.9, BES	36.4
P_{17}	1.0	35.0	64.0, BEA	50.3
P_{18}	1.2	36.0	62.8, BEA	49.4
P_{19}	1.6	31.2	67.2, BEA	45.2
P_{20}	2.0	29.1	68.9, BEA	49.0

Table 3.3

Optimization of membrane ingredients for PVC membrane sensor based on MBZ-MPA ion association

	Composition % (w/w)			Slope
Membrane	Ionophore	PVC	Plasticizer	mV/decade
M_1	1.0	35.0	64.0, DBP	36.0
M_2	1.2	36.0	62.8, DBP	39.4
M_3	1.6	31.2	67.2, DBP	42.6
M_4	2.0	29.1	68.9, DBP	40.1
M_5	1.0	35.0	64.0, BEP	49.1
M_6	1.2	36.0	62.8, BEP	45.0
M_7	**1.6**	**31.2**	**67.2, BEP**	**57.4**
M_8	2.0	29.1	68.9, BEP	61.5
M_9	1.0	35.0	64.0, DBS	42.3
M_{10}	1.2	36.0	62.8, DBS	48.6
M_{11}	1.6	31.2	67.2, DBS	47.6
M_{12}	2.0	29.1	68.9, DBS	45.9
M_{13}	1.0	35.0	64.0, BES	42.3
M_{14}	1.2	36.0	62.8, BES	44.9
M_{15}	1.6	31.2	67.2, BES	45.1
M_{16}	2.0	29.1	68.9, BES	47.3
M_{17}	1.0	35.0	64.0, BEA	32.6
M_{18}	1.2	36.0	62.8, BEA	34.8
M_{19}	1.6	31.2	67.2, BEA	33.1
M_{20}	2.0	29.1	68.9, BEA	30.9

Table 3.4

Response characteristics of the sensors S_{M5}, P_2 and M_7

Parameter	Response Characteristics		
	S_{M5}	P_2	M_7
Slope (mV decade^{-1})	55.8	52.6	57.4
Working concentration range (M)	$1.0\times10^{-6} - 5.0\times10^{-2}$	$1.0\times10^{-6} - 7.0\times10^{-2}$	$1.0\times10^{-6} - 8.9\times10^{-3}$
Detection limit (M)	6.3×10^{-7}	3.6×10^{-7}	4.8×10^{-7}
pH range	4-7	5-7	4-7
Shelf life	6 weeks	4 weeks	7 weeks
Response time(s)	< 30	< 45	< 35

Table 3.5

Selectivity coefficients for the sensors S_{M5}, P_2 and M_7 using fixed interference method.

Interfering ion (X)	$K_{A,B}^{pot}$		
	S_{M5}	P_2	M_7
Na^+	5.4×10^{-3}	6.2×10^{-3}	4.8×10^{-3}
K^+	1.8×10^{-3}	1.3×10^{-4}	2.2×10^{-4}
Ca^{2+}	1.3×10^{-4}	8.1×10^{-3}	7.9×10^{-3}
Co^{2+}	2.9×10^{-3}	6.4×10^{-3}	3.3×10^{-3}
Mg^{2+}	3.4×10^{-3}	3.5×10^{-3}	4.7×10^{-3}
Zn^{2+}	4.8×10^{-2}	2.0×10^{-3}	3.9×10^{-3}
Citric acid	1.8×10^{-4}	9.5×10^{-3}	8.0×10^{-3}
Lactose	7.5×10^{-4}	6.9×10^{-4}	7.1×10^{-4}
Urea	6.0×10^{-3}	5.1×10^{-3}	4.3×10^{-3}
Ascorbic acid	5.8×10^{-4}	9.2×10^{-3}	1.2×10^{-4}
Starch	3.6×10^{-4}	3.3×10^{-4}	9.7×10^{-3}
Glycine	8.1×10^{-3}	6.8×10^{-3}	4.9×10^{-3}
Talc	4.7×10^{-3}	7.2×10^{-3}	1.1×10^{-4}

Table 3.6

Determination of MBZ in pharmaceutical formulations

Sample	Declared Amount (mg/tablet)	Method adopted	Found * (mg/tablet)	SD*	CV*
MEBEX (Cipla - India)	100	S_{M5}	98	2.05	2.09
		P_2	98	1.88	1.91
		M_7	99	1.90	1.91
		Standard Method	97	2.31	2.38

*Average of six replicates

Table 3.7

Determination of MBZ in urine sample using the developed sensors

Drug added (M)	Sensor	Drug found* (M)	Recovery %
1.50×10^{-4}	S_{M5}	1.49×10^{-4}	99.3
	P_2	1.48×10^{-4}	98.7
	M_7	1.51×10^{-4}	100.7

*Average of six replicates

Figure 3.1

Structure of the drug - Mebendazole

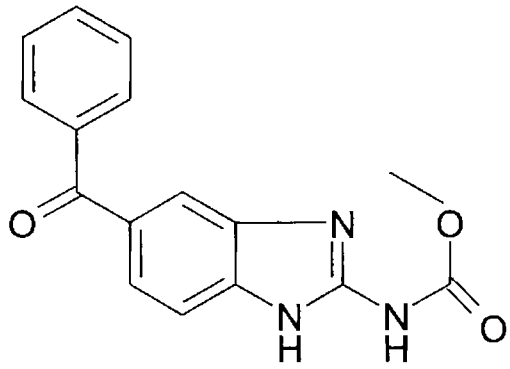


Figure 3.2

Calibration graph for MBZ selective PVC membrane sensor based on MBZ-STA ion association (S_{M5})

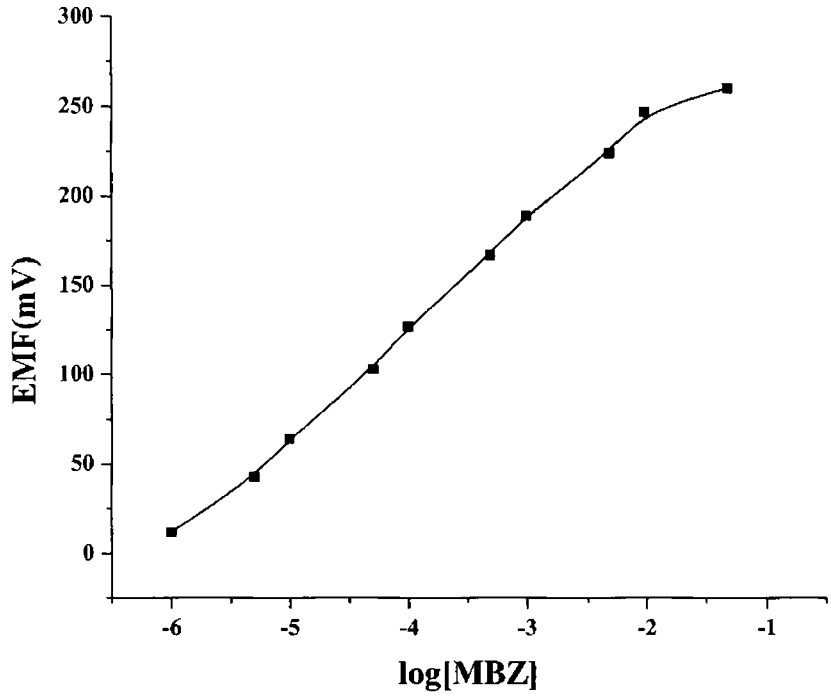


Figure 3.3

Calibration graph for MBZ selective PVC membrane sensor based on MBZ-PTA ion association (P_2)

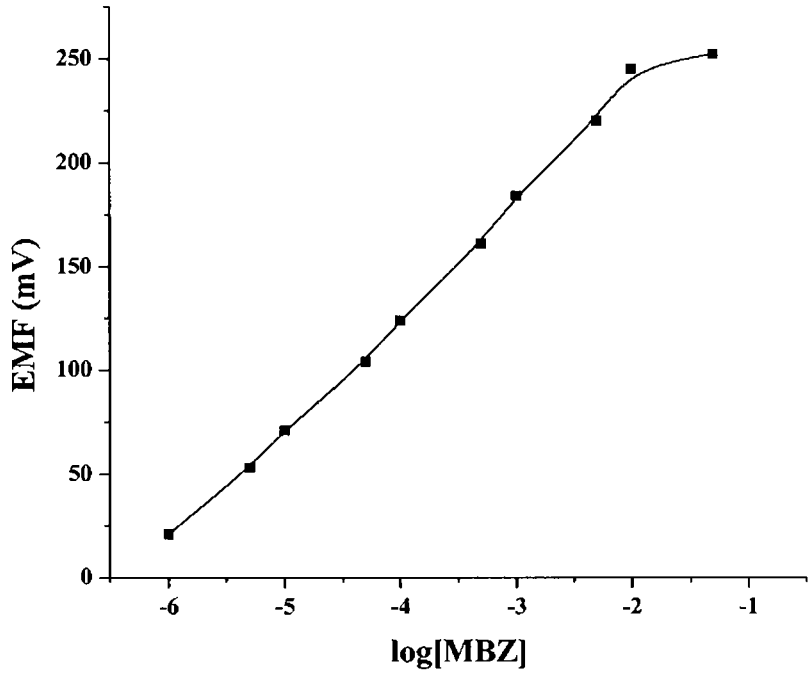


Figure 3.4

Calibration graph for MBZ selective PVC membrane sensor based on MBZ-MPA ion association (M_7)

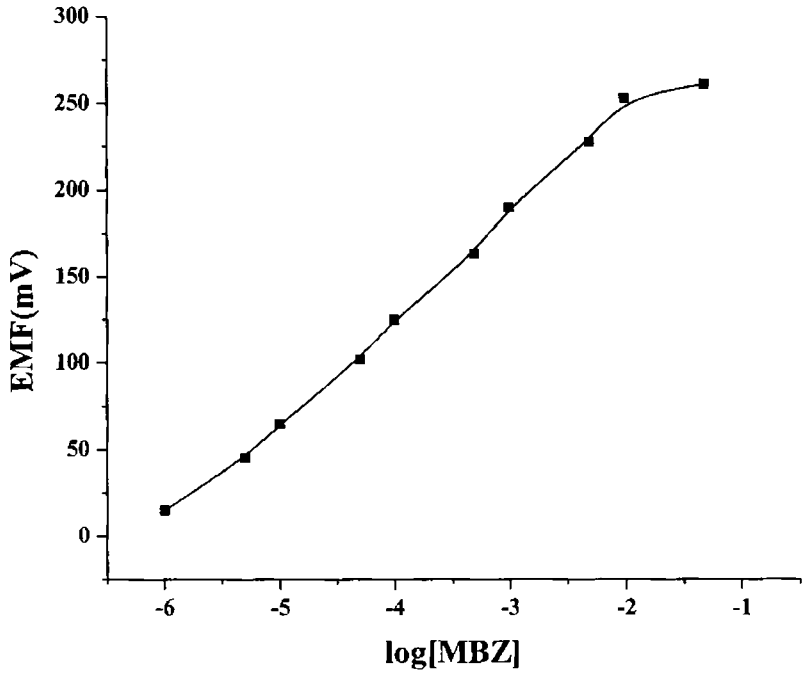


Figure 3.5

Effect of pH on the cell potential of the MBZ membrane sensor S_{M5}
1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

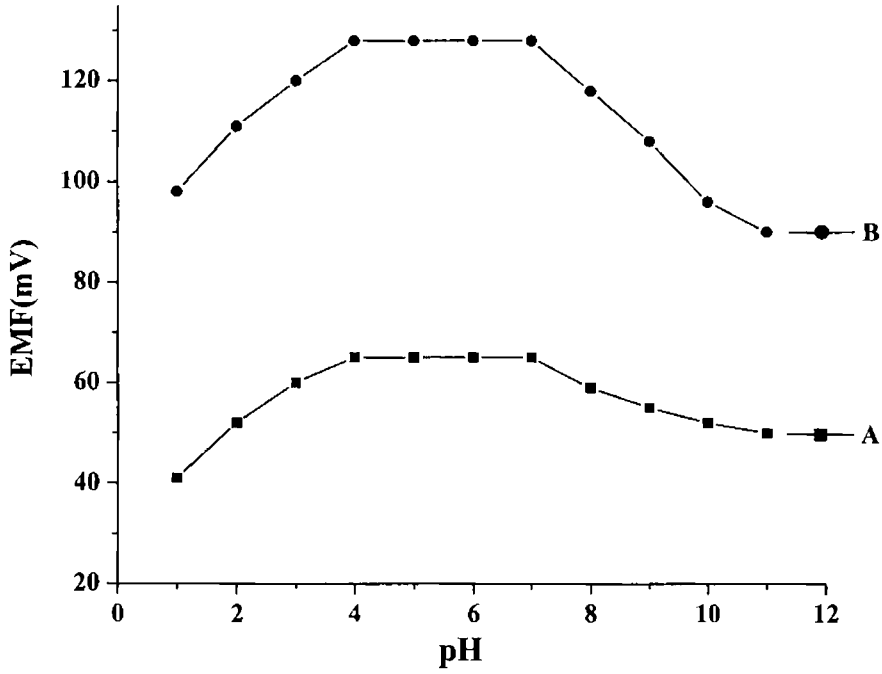


Figure 3.6

Effect of pH on the cell potential of the MBZ membrane sensor P_2 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

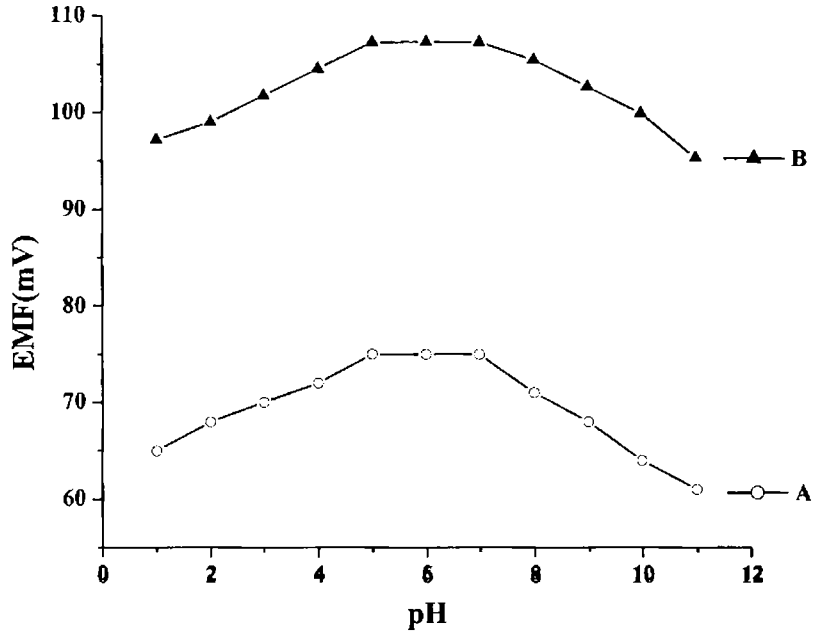
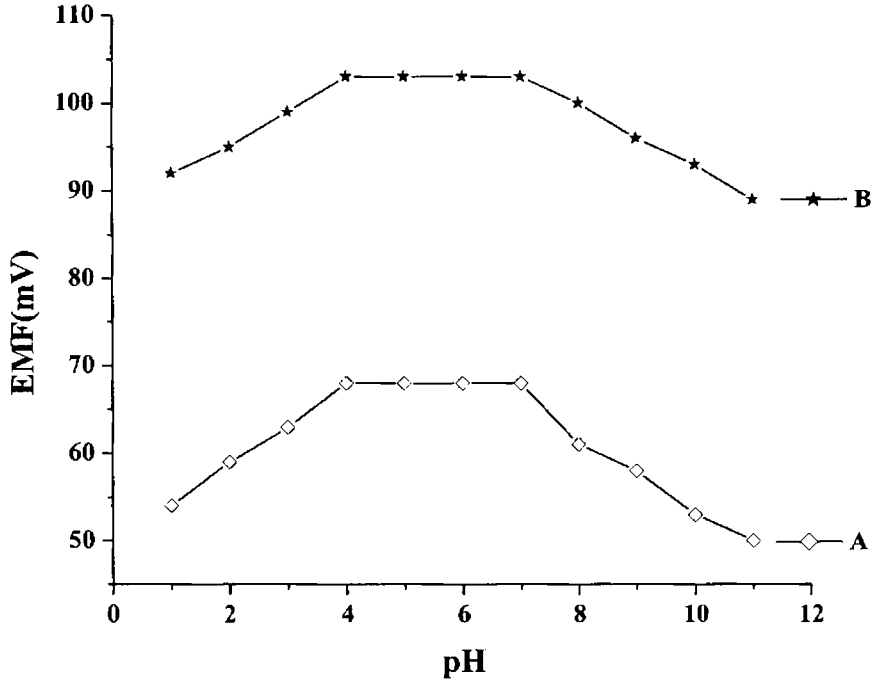


Figure 3.7

Effect of pH on the cell potential of the MBZ membrane sensor M_7 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)



Chapter 4

Development of Sensors for Pefloxacin

The details about the fabrication and electrochemical response characteristics of the sensors of pefloxacin (PEF) are discussed in this chapter. Both PVC membrane sensors and carbon paste electrodes were fabricated for PEF. The sensors are based on the ion association of the drug with the ion pairing reagents silicotungstic acid (STA) and molybdophosphoric acid (MPA). The sensor matrix composition was optimized and the response studied. The analytical applications of the developed sensors were also investigated.

The 1962 synthesis of nalidixic acid by Lesher and Froelich started a new line of research into chemotherapeutic agents: quinolones[193]. Today quinolones are a widely used family of antibacterial agents in clinical practice. At first, they were indicated only for the treatment of infections of urinary tract, but later their major contribution to the treatment of several systemic pathologies were confirmed, arousing great interest in the field of chemotherapy[194]. This growing interest was reflected in three different chronological stages, based on the structural modifications. These structural modifications brought about a broadening of the antibacterial spectrum and possess superior pharmacokinetic characteristics, such as good bioavailability when taken orally, greater tissue penetration and a long half

life. Pefloxacin (PEF) belongs to the third generation quinolones whose development started with synthesis by Koga *et al* in 1980[195]. Pefloxacin is a fluoroquinolone antibiotic. Chemically pefloxacin is 1-ethyl-6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid (Figure 4.1). It possesses excellent activity against gram-negative aerobic bacteria such as *E. coli* and *Neisseria gonorrhoea* as well as gram positive bacteria including *S. pneumoniae* and *Staphylococcus aureus.* They also possess effective activity against shigella, salmonella, campylobacter, gonococcal organisms, multidrug resistant pseudomonas and enterobacter. Pefloxacin also demonstrates favorable cellular penetration characteristics yielding high tissue/serum ratios. This has obvious implications for the treatment of infections caused by intracellular pathogens[196]. With respect to the pharmacokinetic profile of quinolones, the serum protein binding capacity of pefloxacin is in the range 20 – 30 %[197,198].

The bactericidal action of pefloxacin results from interference with the activity of the bacterial enzymes DNA gyrase and topoisomerase IV, which are needed for the transcription and replication of DNA. DNA gyrase appears to be the primary quinolone target in gram negative bacteria. Topoisomerase IV appears to be the preferential target in gram positive organisms. Interference with DNA gyrase and topoisomerase results in strand breakage of the bacterial chromosome, supercoiling and resealing. As a result, DNA replication and transcription is inhibited

A number of methods for the quantitative determination of PEF are known. These include HPLC[199,200], spectrophotometry[201-203], capillary electrophoresis[204], voltammetry[205,206], UV spectrophotometry[207,208] and derivative spectrometry[209]. Most of these methods, however, utilize

expensive instrumentation, suffer from lack of selectivity, involve careful control of reaction conditions, derivatization reactions or require time consuming pretreatment steps which affect their usefulness for routine analysis.

This chapter deals with the fabrication and electrochemical studies of both PVC membrane electrode and carbon paste electrode for PEF based on its ion association with STA and MPA. These sensors were applied for the determination of PEF in pharmaceutical formulations.

4.1 Preparation of the ion associations

The ion associations of PEF with STA and MPA were used for the fabrication of sensors for the drug. The detailed procedure for the preparation of ion associations is discussed in Section 2.2 in Chapter 2. The ion associations were prepared by mixing equimolar solutions of the drug and the ion pairing agent. The mixtures were stirred for 10 minutes and precipitates were filtered, washed and dried at room temperature. Elemental analysis revealed the formation of 3:1 (drug : ion pair reagent) complex for both PEF- STA and PEF-MPA ion associations.

PEF-STA ion association

Found (%) – C – 21.98, H – 3.12, N – 4.45

Calculated (%) – C –22.01, H – 3.20, N – 4.40

PEF-MPA ion association

Found (%) – C – 18.52, H – 2.39, N – 3.58

Calculated (%) – C –18.46, H – 2.36, N – 3.60

4.2 Fabrication of the sensors

The sensors fabricated for PEF included the PVC membrane electrode and the carbon paste electrode. These sensors incorporated the PEF-MPA and PEF-STA ion association as the electro active materials.

4.2.1 Fabrication of the PVC membrane sensor

PVC membrane sensor is one of the conventional forms of electrochemical sensors. The steps involved in the fabrication of PVC membrane sensor are discussed in detail in Chapter 2 (Section 2.8.1). The fabrication of the sensor involves dissolving the mixture of ionophore, PVC and plasticizer in 5-7 mL THF. The cocktail was then poured onto glass rings on a glass plate. It was covered with a filter paper allowing the slow evaporation of the solvent. The small disc shaped membranes thus obtained were cut out and glued to one end of glass tube using M-seal. This was allowed to dry. The sensor thus prepared was conditioned by soaking in 1×10^{-3} M PEF solution for 24 hours. The internal filling solution was 1×10^{-3} M PEF and 1×10^{-1} M NaCl.

The optimum composition for the PVC membrane sensor fabricated based on PEF-STA ion association has been found to be 1.2:36.0:62.8 (ionophore : PVC : plasticizer) and that for PEF-MPA based sensor to be 2.0:30.1:67.9 (ionophore : PVC : plasticizer) in terms of slope, concentration range and detection limit.

4.2.2 Fabrication of carbon paste sensor

Carbon paste sensors possess the advantage that it requires no internal filling solution. Its fabrication is reliable, fast, easy and simple.

High purity graphite and ionophore in varying proportions were thoroughly mixed in acetone. This mixture was allowed to stand overnight

enabling the slow evaporation of acetone. This mixture was then made into a paste with weighed amount of plasticizer. Five different plasticizers were tried to find out the most suitable one that would give best slope, wide concentration range and fairly good detection limit. The paste thus obtained was packed to the open end of a Teflon holder in which electrical contact was made with a copper rod through the centre of the holder. One of the merits of this type of sensor is that the working surface can be renewed by rubbing the surface on a filter paper.

4.3 Potential measurement and calibration

Potentials were measured at 25±1 °C on a Metrohm 781 ion meter. A saturated calomel reference electrode was used in conjunction with the developed sensor.

The cell assembly for potentiometric measurements can be represented as follows:

For PVC membrane sensor,

Internal reference electrode | internal filling solution (1×10^{-3} M drug solution + 1×10^{-1} M NaCl solution) | PVC membrane | test solution | external reference electrode.

For carbon paste sensor,

Reference electrode | test solution | Graphite electrode.

The performance of the developed sensors were investigated by measuring the potential in PEF solutions prepared in the concentration range

$1.0 \times 10^{-1} - 1.0 \times 10^{-6}$ M. The solutions were stirred and the stable potential reading was taken.

4.4 Optimization of the sensor matrix composition.

The sensitivity and selectivity of membrane sensors is largely influenced by amount of ionophore, PVC and amount/nature of plasticizer. So these factors are carefully varied to arrive at an optimum composition for the membrane. The PVC acts as a regular support matrix for the membrane but its use creates a need for a plasticizer[210]. A plasticizer on the other hand is a substance which increases the flexibility, softness and distensibility or workability of a polymer[211]. The presence of a plasticizer may affect the response characteristics of the sensor such as sensitivity and response time[212].

The ion association of the drug with STA was employed for the fabrication of PVC membrane sensor for PEF. The amount of the active ingredients of the membrane was varied so as to obtain the membrane composition which could give best response characteristics. The amount of the ionophore is one of the most important parameters which influence the response of the sensor. Table 4.1 clearly shows how the slopes change as the membrane composition is varied. 1.2% (w/w) was found to be the optimum amount of ionophore for the sensor based on PEF-STA ion association as ionophore. A further increase in the ionophore amount resulted in a diminished response, which may be attributed to the saturation of membrane. Addition of plasticizers improves the nature of the PVC membrane and thus gives a better response. Among the 5 different plasticizers viz, bis(2-ethyl hexyl) phthalate (BEP), bis(2-ethyl hexyl) sebacate (BES), di-n- butyl

phthalate (DBP), di-butyl sebacate (DBS), bis(2-ethyl hexyl) adipate (BEA) used, BEP was found to be the most effective plasticizing agent in the preparation of the PVC membrane whereas others gave a non Nernstian response. The plasticizer content was restricted to 62.8% (w/w). The addition of plasticizer improves the mobility of the ionophore in the membrane matrix. Thus for the PVC membrane sensor incorporating PEF-STA ion association as the electro active material, the optimum composition of membrane matrix has been obtained to be 1.2:36.0:62.8 % (w/w) (ionophore : PVC : plasticizer). The sensor (P_{PS5}) with this composition has been found to give a Nernstian slope of 58.8 mV/decade. The calibration graph for P_{PS5} is given as Figure 4.2. The sensor P_{PS5} with a slope of 58.8 mV/decade was used for further studies. P_{PS5} has got a wide linear range of 1.0×10^{-5} -1.2×10^{-2} M with a lower detection limit of 1.5×10^{-6} M. The response time of P_{PS5} has been found to be < 30 s.

The response of the carbon paste sensor too depends on the composition of the sensor matrix. Table 4.2 illustrates how the slopes of the sensors change with changing composition of the sensor matrix. 2.8% (w/w) has been found to be the optimum amount of ionophore for the carbon paste sensor incorporating PEF-STA ion association as the electro active material. The most suitable plasticizer in this case was BEP. The concentration of the plasticizer in the sensor matrix greatly influences the response of the sensors fabricated. The sensor P_{GS7} incorporating 2.8% (w/w) of ionophore, 61.2% (w/w) graphite and 36.0% (w/w) of plasticizer has been observed to give a Nernstian slope of 57.5 mV/decade. A deviation from this composition resulted in a deviation from the Nernstian behaviour. The calibration graph for P_{GS7} is given as Figure 4.3. The sensor, P_{GS7} was used for further studies

as it was best in terms of slope, concentration range and detection limit. The sensor exhibited a detection limit of 1.2×10^{-6} M and a working concentration range of $1.0 \times 10^{-5} - 5.0 \times 10^{-3}$ M. The response time of the sensor has been found to be < 35 s.

PEF-MPA ion association was also employed for the fabrication of both PVC membrane sensor and carbon paste electrode. While fabricating the PVC membrane sensor for PEF-MPA, the membrane composition was varied (Table 4.3). The optimum amount of ionophore has been found to be 2.0% (w/w). It has been observed that varying the ionophore content in the membrane would affect the sensitivity of the sensors. On increasing the amount of ionophore, co extraction of the ions from the sample solution will occur which may cause a deviation from Nernstian behavior. Another important constituent of the sensor matrix which influences the sensitivity of the electrode is the plasticizer. BEP has been observed to be the best plasticizer in terms of slope and concentration range. The plasticizer amount that gave a Nernstian behaviour has been observed to be 67.9% (w/w). The free energy of interaction of the ions in solution with the ionophore is highly influenced by the concentration of the plasticizer used and hence the response characteristics are changed on changing the amount of the plasticizer[213-215]. The factors viz, amount of ionophore and nature / amount of plasticizer were taken into consideration while fabricating the PVC membrane sensor for PEF based on PEF-MPA ion association. The sensor P_{PM8} with an optimum composition 2.0:30.1:67.9 % (w/w) (ionophore : PVC : plasticizer) was fabricated and used for further studies. It is evident from the table that sensor P_{PM8} has got a near Nernstian slope of 58.6 mV/decade. P_{PM8} has got a wide concentration range of 1.9×10^{-5} - 8.3×10^{-3} M and a

fairly good detection limit of 1.6×10^{-6} M. The response time has been observed to be < 25 s. The calibration graph of P_{PM8} is given as Figure 4.4.

Carbon paste sensor was also fabricated for PEF based on PEF-MPA ion association. The carbon paste sensors are easy to prepare, easy to regenerate and they give stable response. The response characteristics of a CPE depend on factors such as amount of ionophore and nature / amount of plasticizer. Various proportions of ionophore were tried while fabricating the carbon paste sensor. The amount of ionophore in the sensor was varied from 2.0 – 3.0 % (w/w). 2.5% (w/w) of ionophore has been found to be the optimum amount. A deviation from this amount resulted in a deviation from Nernstian behavior. A higher quantity of ionophore gave a non Nernstian behavior which may be attributed to the saturation of the sensor matrix. When the amount of ionophore is low, the rate of diffusion of the ions from aqueous phase to the sensor matrix phase is slow and insufficient. Hence the deviation in potential response of the sensor is observed. Another important factor that affects the response of the carbon paste sensor is the plasticizer. Five different plasticizers viz; bis(2-ethyl hexyl) phthalate (BEP), bis(2-ethyl hexyl) sebacate (BES), di-n- butyl phthalate (DBP), di-butyl sebacate (DBS) and bis(2-ethyl hexyl) adipate (BEA) were employed for the fabrication of the carbon paste sensor to study the effect of plasticizer on the response of the sensors. It has been observed that both the nature and the amount of the plasticizer in the sensor matrix influence the response of the carbon paste sensor. From Table 4.4 it is clear that 46.5% (w/w) is the optimum amount of BEP, the most effective plasticizer. At low amount of plasticizer, all the active sites of the ionophore are not able to interact properly with the drug ions in solution, hence the extent of free energy change of interaction is also

smaller but when the plasticizer is in optimum quantity all the active sites of the ionophore interact with the drug ions and hence the observed response. The sensor (P_{GM6}) with the composition 2.5 : 51.0 : 46.5 (ionophore : graphite : plasticizer) gave a slope of 57.2 mV/decade (Figure 4.5). The working concentration range for P_{GM6} has been found to be 5.0×10^{-5} - 1.2×10^{-2} M. The lower detection limit is 1.9×10^{-6} M. The response time of P_{GM6} has been found to be < 30 s. Thus P_{GM6} has been found to be the best carbon paste sensor for PEF based on PEF-MPA ion association in terms of slope, concentration range and detection limit.

The average response time is the time required for the electrode to reach a stable potential within ± 1 mV of the final equilibrium value after the successive immersion of the sensors in different PEF solution each having a 10 - fold difference in concentration. The response time of all the 4 sensors, P_{PS5}, P_{GS7}, P_{PM8} and P_{GM6} were examined by taking measurements for PEF solutions with a 10 - fold difference in concentration in the sequence high to low concentration and vice versa. The response time may be influenced by the fast interactions of the primary ions in the aqueous phase with the ionophore. This may also depend on the rate of exchange reaction between the ionophore in the sensor matrix and primary ions in solution which in turn depend on the sensor composition. The response time for the sensors P_{PS5}, P_{GS7}, P_{PM8} and P_{GM6} were found to be < 30 s, < 35 s, < 25 s and < 30 s respectively.

The response characteristics of the sensors are summarized in Table 4.5.

4.5 Effect of concentration of internal filling solution

The effect of concentration of the internal filling solution on the response characteristics of the PVC membrane sensors was critically investigated. To study this effect the concentration of the internal filling solution was changed from 1.0×10^{-4} M to 1.0×10^{-2} M for the sensors P_{PS5} and P_{PM8}. The calibration plots hence obtained was studied and it has been found that the slopes remain unchanged. So there is no effect of the concentration of the internal filling solution on the response of the sensors. Hence for further studies, the concentration of the internal filling solution for the sensors P_{PS5} and P_{PM8} were fixed at 1.0×10^{-3} M. There is no need for an internal filling solution for carbon paste sensors P_{GS7} and P_{GM6}, which is one of the advantages of carbon paste electrodes.

4.6 Effect of pH

The effect of pH of the test solution on the potential of the developed sensors was investigated. The effect was studied for two different concentrations viz; 1×10^{-3} M and 1×10^{-4} M solutions of PEF. The pH of the solution was varied using buffer solutions. For the sensors, P_{PS5} and P_{GS7}, based on PEF-STA ion association, the pH versus EMF profile gave a linear plot in the range 4 -7. The potential remained constant in the pH range 4 – 7. This has been illustrated in Figure 4.6 and 4.7. This has been found to be the useful pH range for both the PVC membrane sensor and carbon paste electrode for PEF based on PEF – STA ion association.

The potential of the sensors based on PEF-MPA ion association has been found to remain constant in the pH range 5 – 7 as is evident from Figure 4.8 and 4.9. So the pH range 5 – 7 has been taken to be the useful pH

for the sensors P_{PM8} and P_{GM6}. In all these cases, the measurements were hindered in the alkaline media owing to the formation of a precipitate in the test solutions. The variations in concentration of the PEF solution did not affect the useful pH range of all the four sensors developed.

4.7 Potentiometric selectivity

The selectivity is the most important characteristic of a sensor as it determines the extent of the utility of the sensor in real sample measurement. The potential response of the developed PEF sensors P_{PS5}, P_{GS7}, P_{PM8} and P_{GM6} was investigated in presence of foreign ions by using the Fixed Interference Method (FIM). The selectivity coefficient values ($K_{A,B}^{pot}$) are shown in Table 4.6. The selectivity coefficient values indicate that the developed sensors are highly selective to PEF over a number of ions tested. The selectivity coefficient values of the ions Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, citric acid, lactose, urea, ascorbic acid and glycine show that the developed sensors are selective to the drug. Even the tablet excipients such as starch and talc were not found to interfere with the species of interest.

4.8 Shelf life or Life time

The stability or shelf life of all the four sensors developed for PEF was tested over a period of 10 weeks. The operative lifetime of the sensor P_{PS5} was found to be 5 weeks. The shelf life of both the sensors based on PEF-MPA ion associations P_{PM8} and P_{GM6} was found to be 5 weeks. P_{GS7} was found to have an operative lifetime of 4 weeks. During this period the sensors showed no deviation from its optimized response characteristics. All the sensors were kept immersed in 1.0×10^{-3} M pefloxacin solution when not

in use. As explained earlier the working surface of the carbon paste sensors can be renewed.

4.9 Analytical applications

The utility of the developed sensors P_{PS5}, P_{GS7}, P_{PM8} and P_{GM6} were investigated in the determination of PEF in tablets (Pelox Wockhardt - India). As explained in Section 2.5.6 of Chapter 2, electrochemical studies have been carried out. The results are given in Table 4.7. The results were compared with those obtained by the European Pharmacopoeia procedure[172]. The results given in the table shows that there is good agreement between the values obtained by the standard method and the proposed method. The developed sensors P_{PS5}, P_{GS7}, P_{PM8} and P_{GM6} could be used for the determination of pefloxacin in tablets.

The developed sensors could be used for the determination of pefloxacin in spiked urine sample. The results are consolidated in Table 4.8. The average % recovery of pefloxacin using the sensors P_{PS5}, P_{GS7}, P_{PM8} and P_{GM6} has been found to be 98.0, 99.3, 101.3 and 100.7 respectively. The sensors are selective to the drug and it does not interfere with the constituents of urine. Hence it is of great practical utility. Thus the developed sensors can be used for the analysis of real samples.

All the four sensors fabricated for PEF based on the ion associations of the drug with silicotungstic acid (STA) and molybdophosphoric acid (MPA) has been found to give the Nernstain slopes. The working concentration range of P_{PS5} has been observed to be 1.0×10^{-5} - 1.2×10^{-2} M with a lower detection limit of 1.5×10^{-6} M. The sensors P_{GS7}, P_{PM8} and P_{GM6} exhibited Nernstain slopes within the concentration range of 1.0×10^{-5} - 5.0

$\times 10^{-3}$, 1.9×10^{-5} - 8.3×10^{-3} and 5.0×10^{-5} -1.2×10^{-2} M respectively. A working pH range of 4-7 was observed for P_{PS5} and P_{GS7} and 5-7 for P_{PM8} and P_{GM6}. The shelf life for all the three sensors except P_{GS7} has been found to be 5 weeks. A response time of less than 30 s was obtained for P_{PS5} and P_{GM6}. P_{GS7} and P_{PM8} gave response times of less than 35 s and 25 s respectively.

Table 4.1

Optimization of membrane ingredients for PVC membrane sensor for PEF based on PEF-STA ion association

	Composition % (w/w)			Slope
Membrane	Ionophore	PVC	Plasticizer	mV/decade
P_{PS1}	1.2	36.0	62.8, DBP	36.7
P_{PS2}	1.6	35.0	63.4, DBP	38.9
P_{PS3}	1.8	36.2	62.0, DBP	35.2
P_{PS4}	2.0	30.1	67.9, DBP	32.0
P_{PS5}	**1.2**	**36.0**	**62.8, BEP**	**58.8**
P_{PS6}	1.6	35.0	63.4, BEP	62.3
P_{PS7}	1.8	36.2	62.0, BEP	65.1
P_{PS8}	2.0	30.1	67.9, BEP	69.4
P_{PS9}	1.2	36.0	62.8, DBS	32.4
P_{PS10}	1.6	35.0	63.4, DBS	33.9
P_{PS11}	1.8	36.2	62.0, DBS	36.7
P_{PS12}	2.0	30.1	67.9, DBS	35.0
P_{PS13}	1.2	36.0	62.8, BES	69.1
P_{PS14}	1.6	35.0	63.4, BES	68.4
P_{PS15}	1.8	36.2	62.0, BES	71.3
P_{PS16}	2.0	30.1	67.9, BES	72.2
P_{PS17}	1.2	36.0	62.8, BEA	65.1
P_{PS18}	1.6	35.0	63.4, BEA	66.9
P_{PS19}	1.8	36.2	62.0, BEA	70.5
P_{PS20}	2.0	30.1	67.9, BEA	68.4

Table 4.2

Optimization of composition of the carbon paste sensor for PEF based on PEF-STA ion association

	Composition % (w/w)			Slope
Sensor	Ionophore	Graphite	Plasticizer	mV/decade
P_{GS1}	2.0	34.5	63.5, DBP	39.2
P_{GS2}	2.5	51.0	46.5, DBP	35.4
P_{GS3}	2.8	61.2	36.0, DBP	39.4
P_{GS4}	3.0	49.7	47.3, DBP	36.1
P_{GS5}	2.0	34.5	63.5, BEP	48.5
P_{GS6}	2.5	51.0	46.5, BEP	51.3
P_{GS7}	**2.8**	**61.2**	**36.0, BEP**	**57.5**
P_{GS8}	3.0	49.7	47.3, BEP	49.9
P_{GS9}	2.0	34.5	63.5, DBS	32.0
P_{GS10}	2.5	51.0	46.5, DBS	34.2
P_{GS11}	2.8	61.2	36.0, DBS	38.8
P_{GS12}	3.0	49.7	47.3, DBS	35.1
P_{GS13}	2.0	34.5	63.5, BES	36.8
P_{GS14}	2.5	51.0	46.5, BES	39.1
P_{GS15}	2.8	61.2	36.0, BES	35.2
P_{GS16}	3.0	49.7	47.3, BES	33.9
P_{GS17}	2.0	34.5	63.5, BEA	65.4
P_{GS18}	2.5	51.0	46.5, BEA	69.3
P_{GS19}	2.8	61.2	36.0, BEA	72.6
P_{GS20}	3.0	49.7	47.3, BEA	73.9

Table 4.3

Optimization of membrane ingredients for PVC membrane sensor for PEF based on PEF-MPA ion association

	Composition % (w/w)			Slope
Membrane	Ionophore	PVC	Plasticizer	mV/decade
P_{PM1}	1.2	36.0	62.8, DBP	44.3
P_{PM2}	1.6	35.0	63.4, DBP	46.7
P_{PM3}	1.8	36.2	62.0, DBP	43.2
P_{PM4}	2.0	30.1	67.9, DBP	40.0
P_{PM5}	1.2	36.0	62.8, BEP	45.9
P_{PM6}	1.6	35.0	63.4, BEP	46.8
P_{PM7}	1.8	36.2	62.0, BEP	50.2
P_{PM8}	**2.0**	**30.1**	**67.9, BEP**	**58.6**
P_{PM9}	1.2	36.0	62.8, DBS	34.8
P_{PM10}	1.6	35.0	63.4, DBS	37.1
P_{PM11}	1.8	36.2	62.0, DBS	39.1
P_{PM12}	2.0	30.1	67.9, DBS	38.2
P_{PM13}	1.2	36.0	62.8, BES	41.2
P_{PM14}	1.6	35.0	63.4, BES	38.6
P_{PM15}	1.8	36.2	62.0, BES	35.2
P_{PM16}	2.0	30.1	67.9, BES	34.7
P_{PM17}	1.2	36.0	62.8, BEA	65.6
P_{PM18}	1.6	35.0	63.4, BEA	63.8
P_{PM19}	1.8	36.2	62.0, BEA	68.1
P_{PM20}	2.0	30.1	67.9, BEA	70.0

Table 4.4

Optimization of composition of the carbon paste sensor for PEF based on PEF-MPA ion association

	Composition % (w/w)			Slope
Sensor	Ionophore	Graphite	Plasticizer	mV/decade
P_{GM1}	2.0	34.5	63.5, DBP	47.1
P_{GM2}	2.5	51.0	46.5, DBP	48.2
P_{GM3}	2.8	61.2	36.0, DBP	45.5
P_{GM4}	3.0	49.7	47.3, DBP	42.9
P_{GM5}	2.0	34.5	63.5, BEP	50.3
P_{GM6}	**2.5**	**51.0**	**46.5, BEP**	**57.2**
P_{GM7}	2.8	61.2	36.0, BEP	49.4
P_{GM8}	3.0	49.7	47.3, BEP	48.2
P_{GM9}	2.0	34.5	63.5, DBS	34.1
P_{GM10}	2.5	51.0	46.5, DBS	35.6
P_{GM11}	2.8	61.2	36.0, DBS	37.8
P_{GM12}	3.0	49.7	47.3, DBS	36.2
P_{GM13}	2.0	34.5	63.5, BES	65.4
P_{GM14}	2.5	51.0	46.5, BES	67.8
P_{GM15}	2.8	61.2	36.0, BES	69.2
P_{GM16}	3.0	49.7	47.3, BES	73.1
P_{GM17}	2.0	34.5	63.5, BEA	36.7
P_{GM18}	2.5	51.0	46.5, BEA	39.1
P_{GM19}	2.8	61.2	36.0, BEA	45.8
P_{GM20}	3.0	49.7	47.3, BEA	40.1

Table 4.5

Response characteristics of the sensors P_{PS5}, P_{GS7}, P_{PM8} and P_{GM6}

Parameter	Response Characteristics			
	P_{PS5}	P_{GS7}	P_{PM8}	P_{GM6}
Slope (mV/decade)	58.8	57.5	58.6	57.2
Working concentration range (M)	1.0×10^{-5} - 1.2×10^{-2}	1.0×10^{-5} - 5.0×10^{-3}	1.9×10^{-5} - 8.3×10^{-3}	5.0×10^{-5} - 1.2×10^{-2}
Detection limit (M)	1.5×10^{-6}	1.2×10^{-6}	1.6×10^{-6}	1.9×10^{-6}
pH range	4-7	4-7	5-7	5-7
Shelf life	5weeks	4 weeks	5weeks	5weeks
Response time(s)	< 30	< 35	< 25	< 30

Table 4.6

Selectivity coefficients for the sensors P_{PS5}, P_{GS7}, P_{PM8} and P_{GM6} using fixed interference method.

Interfering ion (X)	$K^{pot}_{A,B}$			
	P_{PS5}	P_{GS7}	P_{PM8}	P_{GM6}
Na^+	1.5×10^{-4}	8.8×10^{-3}	9.4×10^{-3}	8.1×10^{-3}
K^+	9.1×10^{-3}	7.9×10^{-3}	7.2×10^{-3}	6.9×10^{-3}
Ca^{2+}	3.2×10^{-3}	2.8×10^{-3}	4.0×10^{-3}	2.9×10^{-3}
Co^{2+}	6.5×10^{-3}	7.0×10^{-3}	7.1×10^{-3}	6.8×10^{-3}
Mg^{2+}	1.0×10^{-4}	1.1×10^{-4}	9.2×10^{-3}	9.6×10^{-3}
Zn^{2+}	4.9×10^{-3}	5.4×10^{-3}	5.1×10^{-3}	5.8×10^{-3}
Citric acid	5.2×10^{-3}	6.1×10^{-3}	5.5×10^{-3}	6.0×10^{-3}
Lactose	6.9×10^{-3}	7.3×10^{-3}	1.1×10^{-4}	8.7×10^{-3}
Urea	7.1×10^{-3}	8.8×10^{-3}	1.4×10^{-4}	2.0×10^{-4}
Ascorbic acid	6.3×10^{-3}	5.9×10^{-3}	6.1×10^{-3}	5.1×10^{-3}
Starch	1.3×10^{-4}	9.9×10^{-3}	8.6×10^{-3}	1.6×10^{-4}
Glycine	5.1×10^{-3}	4.8×10^{-3}	6.0×10^{-3}	7.2×10^{-3}
Talc	2.8×10^{-4}	9.1×10^{-3}	8.8×10^{-3}	1.8×10^{-4}

Table 4.7

Determination of PEF in pharmaceutical formulations

Sample	Declared Amount (mg/tablet)	Method adopted	Found * (mg/tablet)	SD*	CV*
Pelox (Wockhardt - India)	200	P_{PS5}	198	1.41	0.71
		P_{GS7}	198	1.94	0.97
		P_{PM8}	200	2.48	1.24
		P_{GM6}	198	2.52	1.27
		Standard Method	199	1.67	0.83

*Average of six replicates

Table 4.8

Determination of PEF in urine sample using the developed sensors

Drug added (M)	Sensor	Drug found* (M)	Recovery %
1.50×10^{-4}	P_{PS5}	1.47×10^{-4}	98.0
	P_{GS7}	1.49×10^{-4}	99.3
	P_{PM8}	1.52×10^{-4}	101.3
	P_{GM6}	1.51×10^{-4}	100.7

*Average of six replicates

Figure 4.1

Structure of the drug - pefloxacin

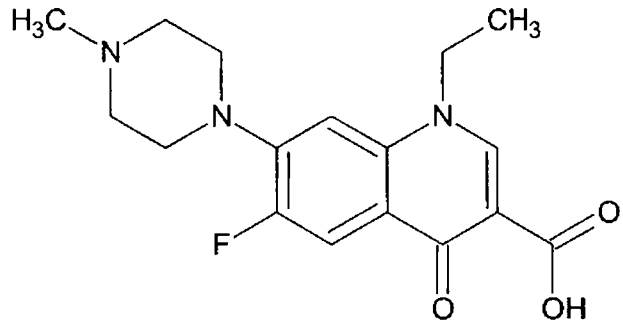


Figure 4.2

Calibration graph for PEF selective PVC membrane sensor based on PEF-STA ion association (P_{PS5})

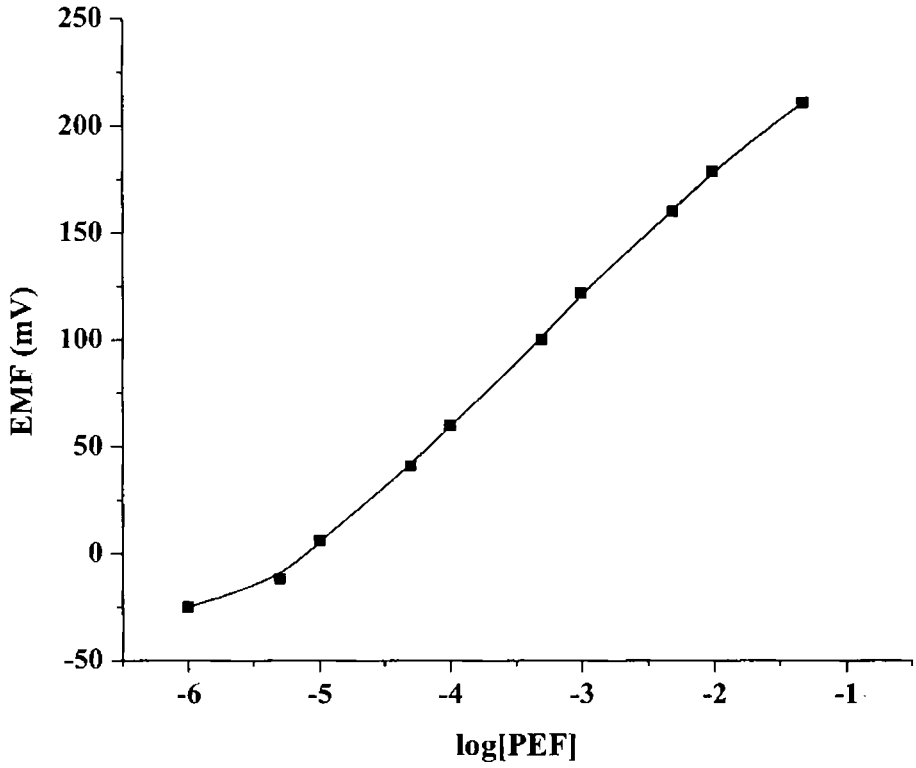


Figure 4.3

Calibration graph for PEF selective carbon paste sensor based on PEF-STA ion association (P_{GS7})

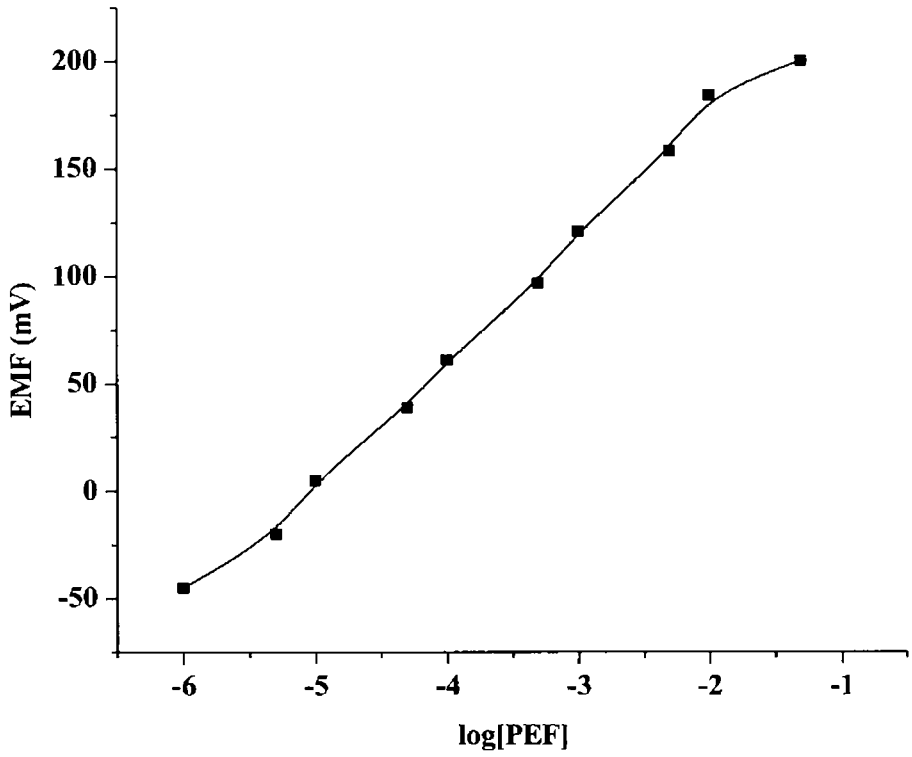


Figure 4.4

Calibration graph for PEF selective PVC membrane sensor based on PEF-MPA ion association (P_{PM8})

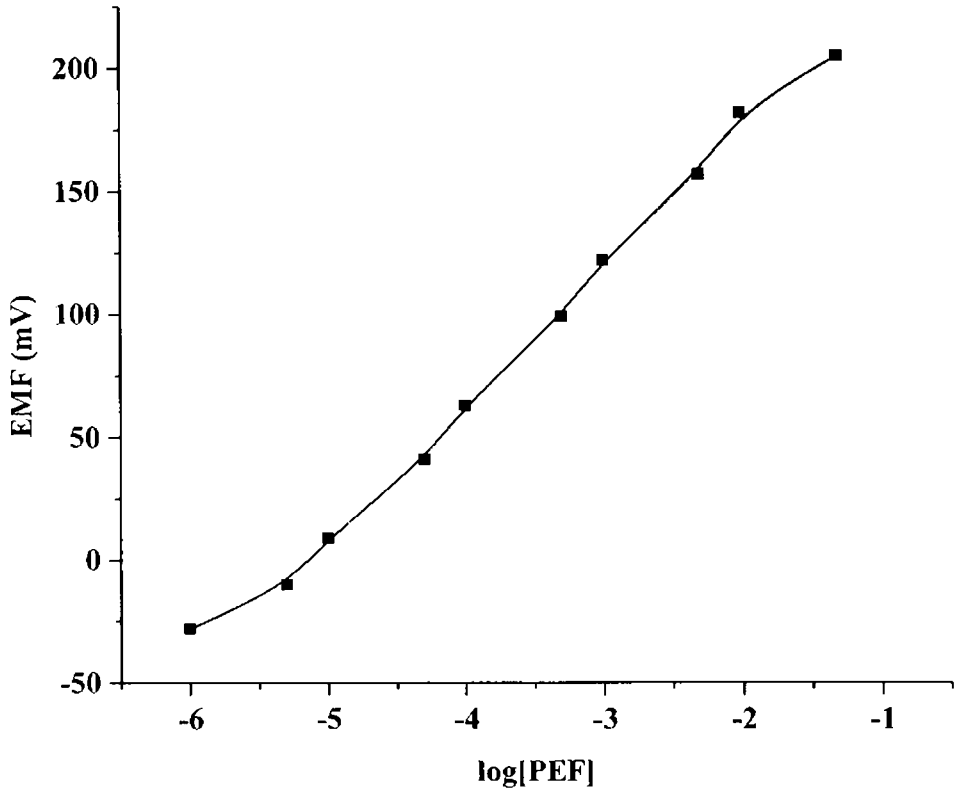


Figure 4.5

Calibration graph for PEF selective carbon paste sensor based on PEF-MPA ion association (P_{GM6})

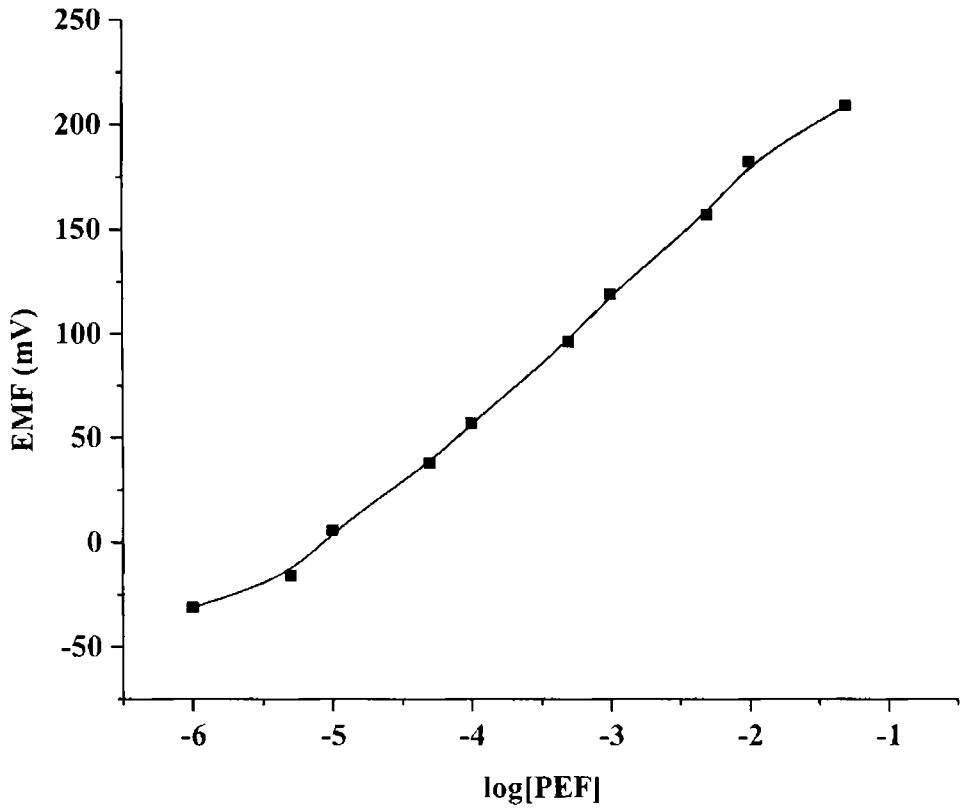


Figure 4.6

Effect of pH on the cell potential of the PEF membrane sensor based on PEF-STA ion association (P_{PS5})

1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

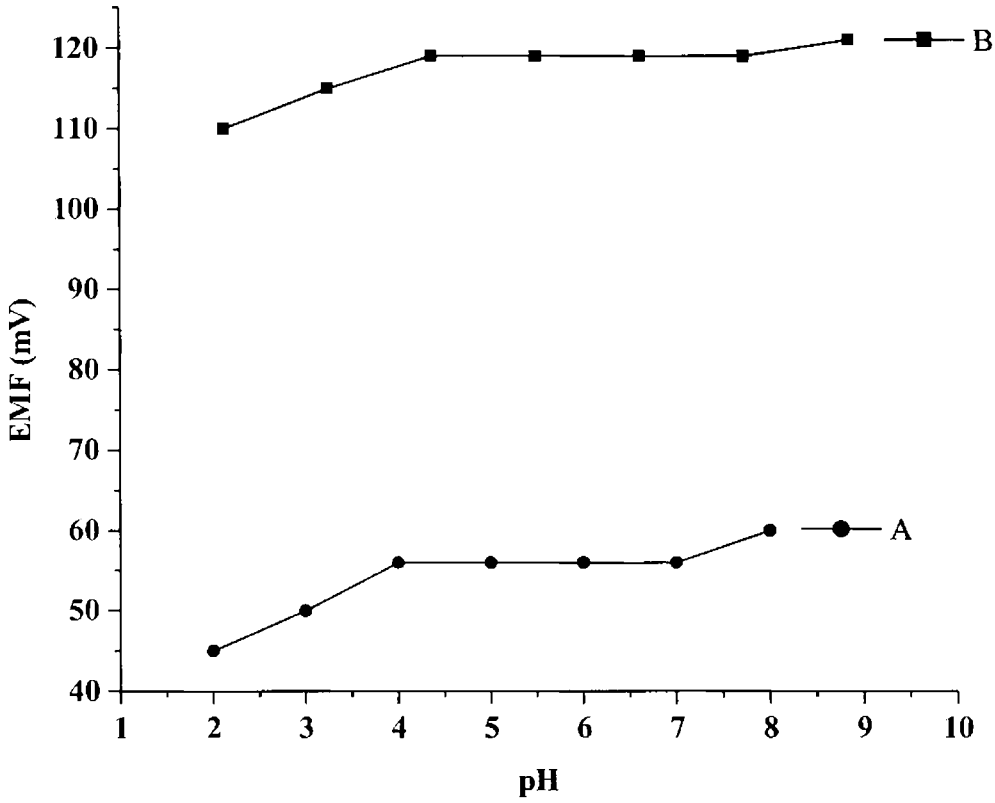


Figure 4.7

Effect of pH on the cell potential of the PEF selective carbon paste sensor based on PEF-STA ion association (P_{GS7})

1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

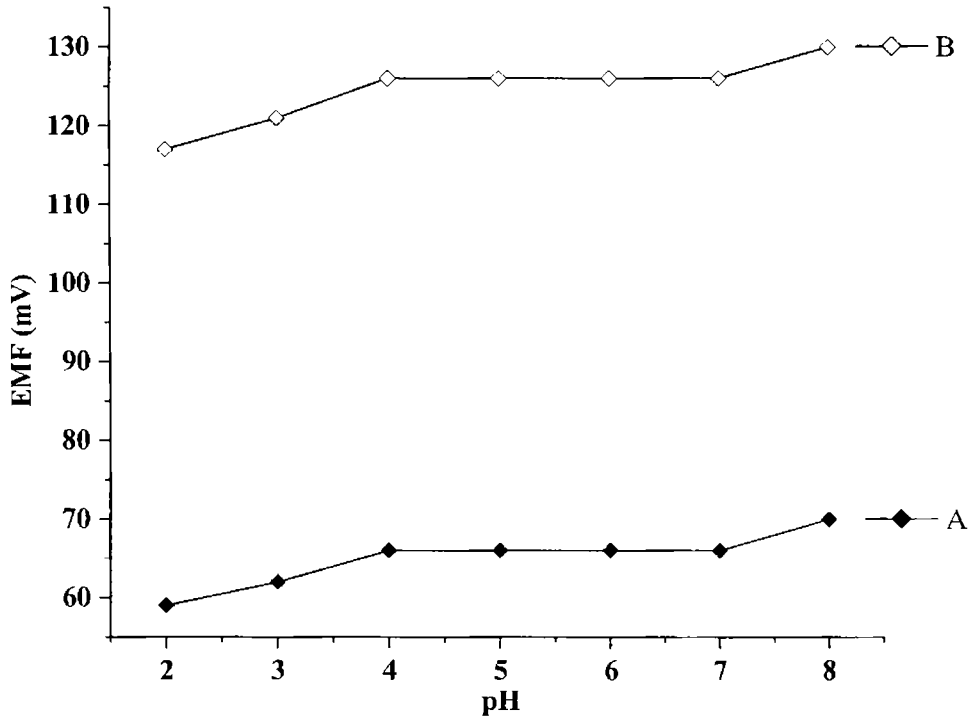


Figure 4.8

Effect of pH on the cell potential of the PEF membrane sensor based on PEF-MPA ion association (P_{PM8})

1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

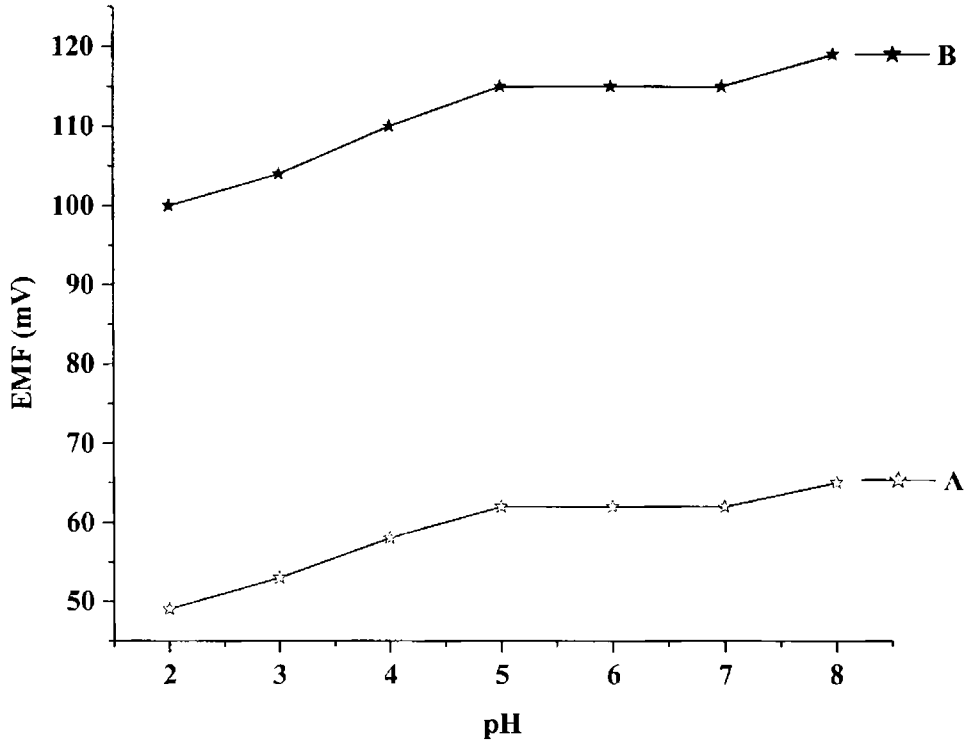
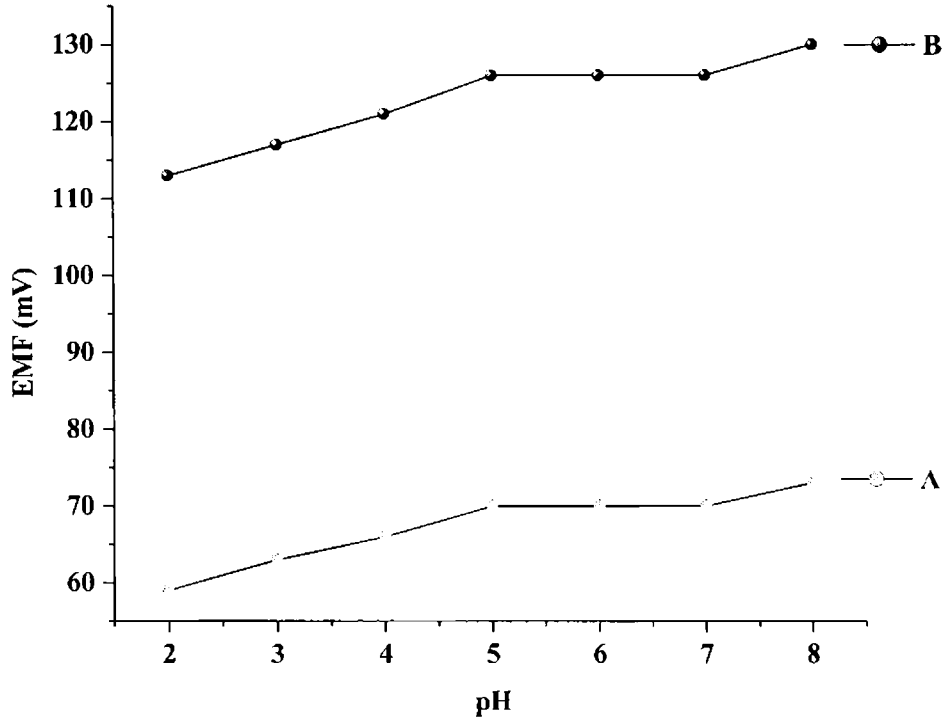


Figure 4.9

Effect of pH on the cell potential of the PEF selective carbon paste sensor based on PEF-MPA ion association (P_{GM6})

1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)



Chapter 5

Development of Sensors for Ambroxol

This chapter deals with the fabrication of carbon paste electrodes for ambroxol (AMB) based on the ion association of the drug with molybdophosphoric acid (MPA) and phosphotungstic acid (PTA). The response characteristics of the developed sensors were studied in detail. Various parameters that illustrate the response of the sensors were also investigated in detail. The analytical application of the sensors in the determination of the drug in tablets was investigated.

Ambroxol HCl (AMB), trans-4-(2-amino-3, 5-dibromobenzylamino) cyclohexanol hydrochloride, (Figure 5.1) a pharmacologically active metabolite of bromohexine, is a compound with potent mucolytic activity, for which it is used as an expectorant and bronchosecretolytic in therapeutics. Ambroxol is administered as hydrochloride salt in daily doses of 30±120 mg using mostly oral formulations like tablets and syrups[216]. Ambroxol also seems to have additional antioxidant and anti-inflammatory properties[217-219]. AMB inhibits the release of inflammatory mediators from human leucocytes and mast cells[220]. It is used in the treatment of chronic bronchitis and neonatal respiratory distress syndrome[221]. The pharmacological effects of AMB have been reported as mucoregulation on gland cells and enhanced production of surfactant[222]. AMB is used in the treatment of acute and chronic disorders, characterized by the production of

thick or excess mucus. It works to decrease mucus viscosity by altering its structure.

It has also been reported that instillation of ambroxol into the eye inhibited corneal reflex[223]. A recent study has shown that AMB suppressed the pain associated with sore throat[224].

Several analytical methods for the determination of ambroxol are described in literature, including high performance liquid chromatography[225-234], UV-visible spectrophotometry[235], flow injection analysis[236,237], gas chromatography[238,239], liquid chromatography[240], capillary zone electrophoresis[241-243], colorimetry[244] and voltammetry[245]. But some of these reported methods require time consuming sample preparation or expensive instrumentation.

As part of the present investigations, two different ion associations for AMB were synthesized based on molybdophosphoric acid (MPA) and phosphotungstic acid (PTA). These ion associations were used as electro active component in the fabrication of carbon paste sensors for the determination of AMB.

5.1 Preparation of the ion associations

The ion associations of the drug based on MPA and PTA have been employed for the fabrication of the carbon paste sensor. The ion associations were prepared as described in Section 2.2 of Chapter 2. The ion associations were prepared by mixing equimolar solutions of the drug and the ion pairing agent in the ratio 3:1. The resulting precipitate was filtered, washed and dried. The ion association thus obtained is stored in a descicator. The structures of the ion associations have been confirmed by elemental analysis.

AMB-MPA ion association

Found (%) – C – 15.76, H – 2.10, N – 2.77

Calculated (%) – C –15.89, H – 2.25, N – 2.38

AMB-PTA ion association

Found (%) – C – 12.27, H – 1.64, N – 2.13

Calculated (%) – C – 12.10, H – 1.80, N – 1.99

5.2 Fabrication of the carbon paste sensor

Carbon paste sensors have been successfully applied as potentiometric sensors for the determination of various species[246]. These sensors possess advantages of ease of preparation, ease of regeneration and very stable response in addition to very low ohmic resistance[247,248] which is probably due to the formation of a very thin film of the pasting liquid coated on to small particles of carbon powder[249,250].

Carbon paste sensors were prepared by mixing weighed amount of the ion associations and high purity graphite with acetone. It was homogenized and left at room temperature to evaporate acetone and the impregnated carbon powder was added to weighed amount of plasticizer. This paste was then packed to one end of the Teflon holder in which electrical contact was made with a copper rod through the centre of the electrode holder. The electrode surface was polished using a filter paper to produce reproducible working surface. The sensor was conditioned by dipping it in a 1×10^{-3} M AMB solution for 10 hrs.

The composition of the sensor matrix was optimized by varying the amount of ionophore and the plasticizer. The best composition ratio was obtained to be 2.5:51.0:46.5 (ionophore : graphite : plasticizer) for the sensor

based on AMB - MPA ion association. The sensor fabricated for AMB – PTA ion association gave a near Nernstian slope when the composition was 3.0:49.7:47.3 (ionophore : graphite : plasticizer). In either case, the most suitable plasticizer was found to be bis(2-ethyl hexyl) phthalate (BEP).

5.3 Potential measurement and calibration

Potentials were measured at 25±1 °C on a Metrohm 781 ion meter. A saturated calomel reference electrode was used in conjunction with the developed sensor.

The cell assembly for potentiometric measurements can be represented as follows:

Reference electrode | test solution | Graphite electrode.

The performance of the developed sensors were investigated by measuring the potential in AMB solutions prepared in the concentration range 1.0×10^{-1} – 1.0×10^{-6} M. The solutions were stirred and the stable potential reading was taken.

5.4 Optimization of the composition of the sensors

The carbon paste sensors are based on the ion exchange mechanism of the active component incorporated into the carbon paste matrix. The sensitivity and linearity of a carbon paste sensor depends significantly on the amount of ionophore and nature of the plasticizer used[251-253].

The performance of the sensor depends on the composition of the sensor matrix. Thus the amount of the ionophore and the nature / amount of the plasticizer influences the electrochemical response characteristics of the developed sensor. To arrive at an optimum sensor matrix composition, amount of the active ingredients were varied. Sensors incorporating AMB – MPA and AMB – PTA ion associations as ionophore were prepared. The five different plasticizers used were bis(2-ethyl hexyl) phthalate (BEP), Bis(2-ethyl hexyl) sebacate (BES), Di-n- butyl phthalate (DBP), Di-butyl sebacate (DBS) and Bis(2-ethyl hexyl) adipate (BEA).

A set of 20 different sensors were fabricated by varying the sensor matrix components. 2.5% has been found to be the optimum amount of the ionophore for AMB-MPA based sensor. The change in the amount of ionophore from this value changes the ratio of the ionic sites to the ionophore in the sensor matrix, which affects the electrode response from Nernstian to sub Nernstian. Table 5.1 gives an idea about the slopes of the different sensors developed for AMB – MPA ion association by varying the sensor matrix components. For the sensor incorporating AMB – MPA ion association as ionophore, the optimum composition has been found to be 51.0:2.5:46.5 (graphite: ionophore: plasticizer). This sensor (A_{M6}) gave a near Nernstian slope of 56.7 mV/decade. The slopes are sub-Nernstian with sensors fabricated using all other plasticizers except BEP.

It is clear from Table 5.2 that a carbon paste sensor (A_{P8}) modified with 3.0% (w/w) of AMB-PTA 49.7% (w/w) of graphite and 47.3% (w/w) of plasticizer exhibited a slope of 58.6 mV/decade. It exhibited a wide linear range of $7.4 \times 10^{-6} - 1.0 \times 10^{-2}$ M. On decreasing the amount of ionophore the slopes decreased which can be attributed to the decrease in conductance

of the sensor material. These two sensors, A_{M6} and A_{P8} were chosen for further electrochemical studies. In both of these sensors, BEP has been found to be the best plasticizer in terms of slope and working concentration range, where as in the case of other plasticizers, slopes were much different from the expected Nernstian value. This may be due to the variation in the free energy of interaction of the electroactive ions and the ionophore in the sensor matrix. BEP has been observed to be the best plasticizer which can be explained to its high polarity and less lipophilicity. The sensor (A_{M6}) incorporating 2.5% (w/w) ionophore with a slope of 56.7 mV/decade has got a working concentration range $5.8 \times 10^{-6} - 1.0 \times 10^{-2}$ M. The lower detection limit, as obtained by the intersection of the two extrapolated segments of the calibration curve, of this sensor has been found to be 8.8×10^{-7} M. The response time of A_{M6} is < 30 s. From Table 5.2, it is evident that the sensor A_{P8} with the composition 3.0:49.7:47.3 (ionophore: graphite: plasticizer) has a slope of 58.6 mV/decade and its working concentration range is $7.4 \times 10^{-6} - 1.0 \times 10^{-2}$ M. The lower detection limit of A_{P8} is 7.8×10^{-7} M with a response time of < 25 s.

The response characteristics of both the sensors, A_{M6} and A_{P8} are consolidated in Table 5.3.

The calibration graph, which is a plot of EMF versus log [AMB] for the sensors A_{M6} and A_{P8} are shown as Figure 5.2 and Figure 5.3.

5.5 Effect of pH

The effect of pH of the test solution on the potential response of the sensors were studied at two different concentrations of the test solution viz; 1.0×10^{-3} M and 1.0×10^{-4} M. The pH of the solution was varied from 1-12

and the change in potentials were recorded. The pH of the solution was adjusted using buffer. For A_{M6} the potential remained constant in the pH range 4 – 8 whereas in the case of A_{P8} the potential remained unchanged in the range 5 – 8. After this, the potential decreases as the free base precipitates in the test solution and consequently the concentration of unprotonated species is gradually increased. Figures 5.4 and 5.5 clearly depict the effect of pH of the test solution on the potential response of the developed sensors A_{M6} and A_{P8}.

5.6 Potentiometric selectivity

If the full scope of the developed ion selective sensors is to be usefully realized, then their selectivity for the primary ion in the presence of foreign ions demands proper and reliable assessment. The potentiometric selectivity coefficients ($K_{A,B}^{pot}$) which describes the preference of the sensor for the foreign ions relative to primary ion, were determined by the fixed interference method (FIM). The potentiometric selectivity coefficient values for the sensors A_{M6} and A_{P8} have been calculated to find out how efficient is the sensors in the determination of AMB. The resultant selectivity coefficient values are summarized in Table 5.4. None of the examined ions such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, citric acid, lactose, urea, ascorbic acid and glycine were found to interfere as shown by the small values of $K_{A,B}^{pot}$. Even the tablet excipients such as starch and talc were found to be non - interfering. It has been observed that there was no interference from constituents of urine and hence the sensors could be used for the determination of the drug in urine samples. The values indicate that the developed sensors are highly selective to AMB.

5.7 Shelf life or Life time

Long time stability of the electrode potential is an important parameter in practical applications of ion selective electrodes. Large potential drift is a major drawback. Potential stability of the developed sensors (A_{M6} and A_{P8}) was monitored continuously over a period of 10 weeks by measuring their potentials in $1.0 \times 10^{-6} - 1.0 \times 10^{-2}$ M standard drug solutions each day. The slope of each sensor was calculated and compared with the slope of the original calibration plot. The sensor A_{M6} showed a decrease in potential response after a period of 5 weeks and the A_{P8} was found to be fully operational up to 4 weeks, thereafter a decrease in the stability of the sensors was observed. The decrease in the electrode stability may be attributed to the leaching of the ionophore from the sensor matrix.

5.8 Analytical applications

The carbon paste sensors A_{M6} and A_{P8} developed for ambroxol were employed for the determination of AMB in tablets. The AMB content in the tablets (Ambrolite -Tablets India) was determined using the sensors. The detailed procedure for the determination is given in Chapter 2. As discussed in Section 2.5.4 of Chapter 2 the electrochemical studies were carried out. The result obtained was compared with that obtained by the standard method[172]. The results are summarized in Table 5.5. The results reveal that the method is effective in the determination of the drug in pharmaceutical formulations.

The utility of the developed sensors in the determination of real samples was also studied. A_{M6} and A_{P8} were employed for the determination of ambroxol in spiked urine samples. The results illustrated in Table 5.6

indicate that these sensors could be used for the determination of the drug in urine sample with good accuracy and precision. The obtained % recovery of AMB using the developed sensors A_{M6} and A_{P8} are 99.0 and 99.5 respectively.

Carbon paste electrodes were fabricated for AMB based on the ion associations of the drug with molybdophosphoric acid (MPA) and phosphotungstic acid (PTA). Both the sensors exhibited Nernstain slopes of 56.7 (A_{M6}) and 58.6 mV/decade (A_{P8}). The working concentration range of A_{M6} has been observed to be $5.8 \times 10^{-6} - 1.0 \times 10^{-2}$ M and that for A_{P8} to be $7.4 \times 10^{-6} - 1.0 \times 10^{-2}$ M. A lower detection limit of 8.8×10^{-7} M was achieved for A_{M6}, and 7.8×10^{-7} M for A_{P8}. The working pH range for A_{M6} has been found to be 4 – 8 whereas for A_{P8} it was observed to be 5 – 8. The senor A_{M6} having a shelf life of 5 weeks gave a response time of < 30 s. A_{P8}, having a shelf life of 4 weeks exhibited a response time of < 25 s.

Table 5.1

Optimization of composition of the carbon paste sensor based on AMB-MPA ion association

	Composition % (w/w)			Slope
Sensor	Ionophore	Graphite	Plasticizer	mV/decade
A_{M1}	2.0	34.5	63.5, DBP	51.2
A_{M2}	2.5	51.0	46.5, DBP	50.8
A_{M3}	2.8	61.2	36.0, DBP	48.6
A_{M4}	3.0	49.7	47.3, DBP	45.0
A_{M5}	2.0	34.5	63.5, BEP	50.0
A_{M6}	**2.5**	**51.0**	**46.5, BEP**	**56.7**
A_{M7}	2.8	61.2	36.0, BEP	62.8
A_{M8}	3.0	49.7	47.3, BEP	67.7
A_{M9}	2.0	34.5	63.5, DBS	48.5
A_{M10}	2.5	51.0	46.5, DBS	42.3
A_{M11}	2.8	61.2	36.0, DBS	43.9
A_{M12}	3.0	49.7	47.3, DBS	40.8
A_{M13}	2.0	34.5	63.5, BES	41.7
A_{M14}	2.5	51.0	46.5, BES	45.6
A_{M15}	2.8	61.2	36.0, BES	39.9
A_{M16}	3.0	49.7	47.3, BES	44.2
A_{M17}	2.0	34.5	63.5, BEA	49.1
A_{M18}	2.5	51.0	46.5, BEA	46.8
A_{M19}	2.8	61.2	36.0, BEA	47.0
A_{M20}	3.0	49.7	47.3, BEA	44.4

Table 5.2

Optimization of composition of the carbon paste sensor based on AMB-PTA ion association

	Composition % (w/w)			Slope
Sensor	Ionophore	Graphite	Plasticizer	mV/decade
A_{P1}	2.0	34.5	63.5, DBP	52.3
A_{P2}	2.5	51.0	46.5, DBP	50.9
A_{P3}	2.8	61.2	36.0, DBP	51.4
A_{P4}	3.0	49.7	47.3, DBP	49.2
A_{P5}	2.0	34.5	63.5, BEP	49.1
A_{P6}	2.5	51.0	46.5, BEP	49.3
A_{P7}	2.8	61.2	36.0, BEP	50.5
A_{P8}	**3.0**	**49.7**	**47.3, BEP**	**58.6**
A_{P9}	2.0	34.5	63.5, DBS	61.4
A_{P10}	2.5	51.0	46.5, DBS	68.9
A_{P11}	2.8	61.2	36.0, DBS	65.1
A_{P12}	3.0	49.7	47.3, DBS	64.7
A_{P13}	2.0	34.5	63.5, BES	62.0
A_{P14}	2.5	51.0	46.5, BES	64.1
A_{P15}	2.8	61.2	36.0, BES	65.9
A_{P16}	3.0	49.7	47.3, BES	64.8
A_{P17}	2.0	34.5	63.5, BEA	63.7
A_{P18}	2.5	51.0	46.5, BEA	69.0
A_{P19}	2.8	61.2	36.0, BEA	70.6
A_{P20}	3.0	49.7	47.3, BEA	71.5

Table 5.3

Response characteristics of the sensors A_{M6} and A_{P8}

Parameter	Response Characteristics	
	A_{M6}	A_{P8}
Slope (mV decade^{-1})	56.7	58.6
Working concentration range (M)	$5.8\times10^{-6} - 1.0\times10^{-2}$	$7.4\times10^{-6} - 1.0\times10^{-2}$
Detection limit (M)	8.8×10^{-7}	7.8×10^{-7}
pH range	4 - 8	5 - 8
Shelf life	5 weeks	4 weeks
Response time(s)	< 30	< 25

Table 5.4

Selectivity coefficients for the sensors A_{M6} and A_{P8} using fixed interference method.

Interfering ion (X)	$K^{pot}_{A,B}$	
	A_{M6}	A_{P8}
Na^+	2.8×10^{-4}	9.8×10^{-3}
K^+	3.4×10^{-3}	4.7×10^{-3}
Ca^{2+}	9.1×10^{-3}	8.2×10^{-3}
Co^{2+}	7.2×10^{-3}	6.9×10^{-3}
Mg^{2+}	6.5×10^{-3}	5.4×10^{-3}
Zn^{2+}	1.9×10^{-4}	1.1×10^{-4}
Citric acid	8.1×10^{-3}	6.9×10^{-3}
Lactose	2.1×10^{-4}	3.4×10^{-4}
Urea	3.2×10^{-4}	2.9×10^{-4}
Ascorbic acid	8.8×10^{-3}	7.1×10^{-3}
Starch	2.1×10^{-4}	1.4×10^{-4}
Glycine	5.8×10^{-3}	6.7×10^{-3}
Talc	6.4×10^{-3}	3.3×10^{-4}

Table 5.5

Determination of AMB in pharmaceutical formulations

Sample	Declared Amount (mg/tablet)	Method adopted	Found * (mg/tablet)	SD*	CV*
Ambrolite (Tablets India)	30	A_{M6}	27	2.01	7.44
		A_{P8}	29	2.39	8.24
		Standard Method	28	1.70	3.57

*Average of six replicates

Table 5.6

Determination of AMB in urine sample using the developed sensors

Drug added (M)	Sensor	Drug found* (M)	Recovery %
2.00×10^{-4}	A_{M6}	1.98×10^{-4}	99.0
	A_{P8}	1.99×10^{-4}	99.5

*Average of six replicates

Figure 5.1

Structure of the drug -ambrxol

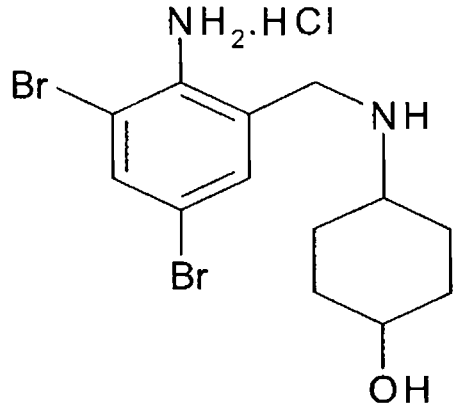


Figure 5.2

Calibration graph for AMB selective carbon paste sensor based on AMB-MPA ion association (A_{M6})

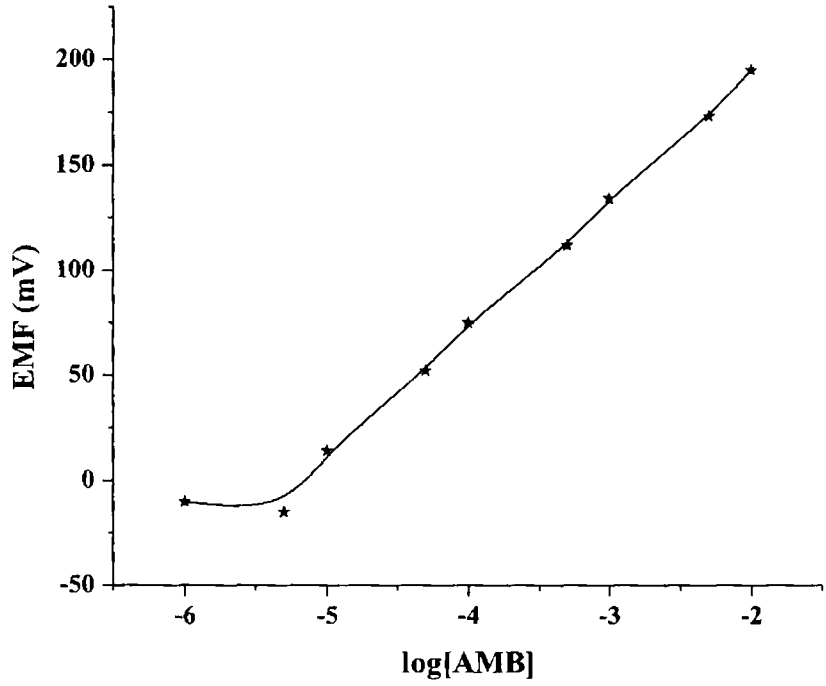


Figure 5.3

Calibration graph for AMB selective carbon paste sensor based on AMB-PTA ion association (A_{P8})

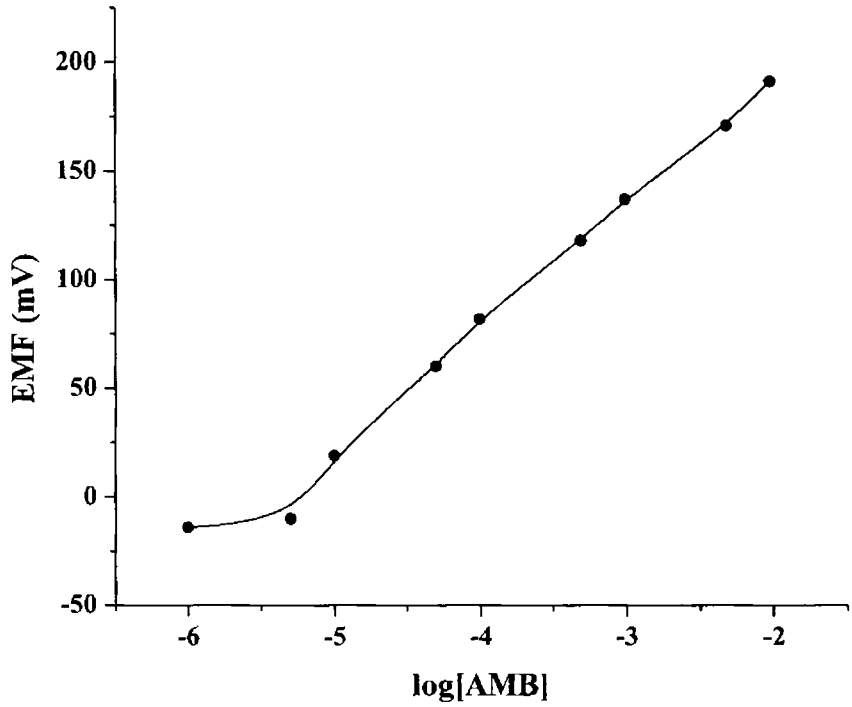


Figure 5.4

Effect of pH on the cell potential of the AMB selective carbon paste sensor A_{M6}

1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

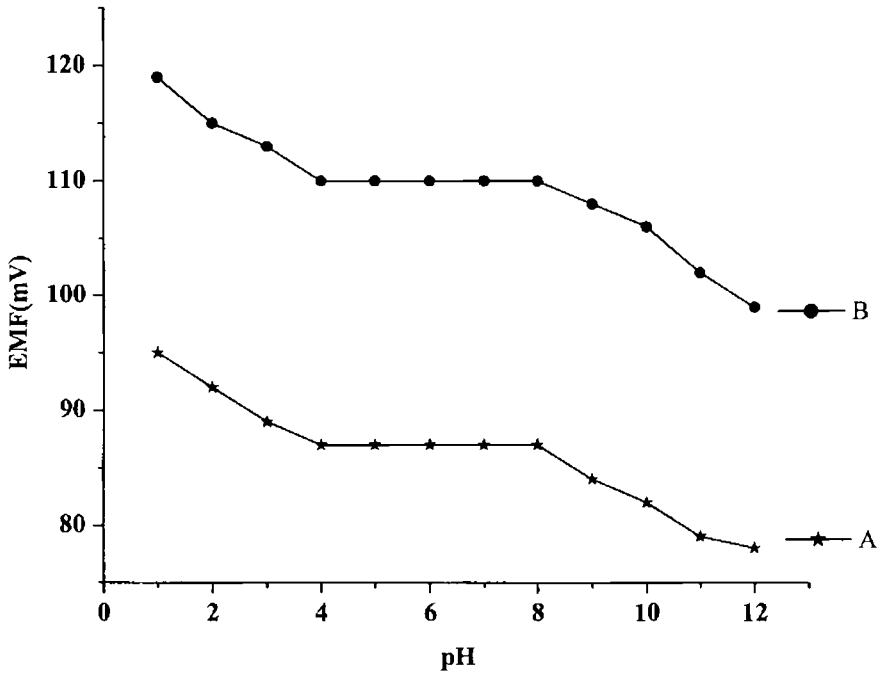
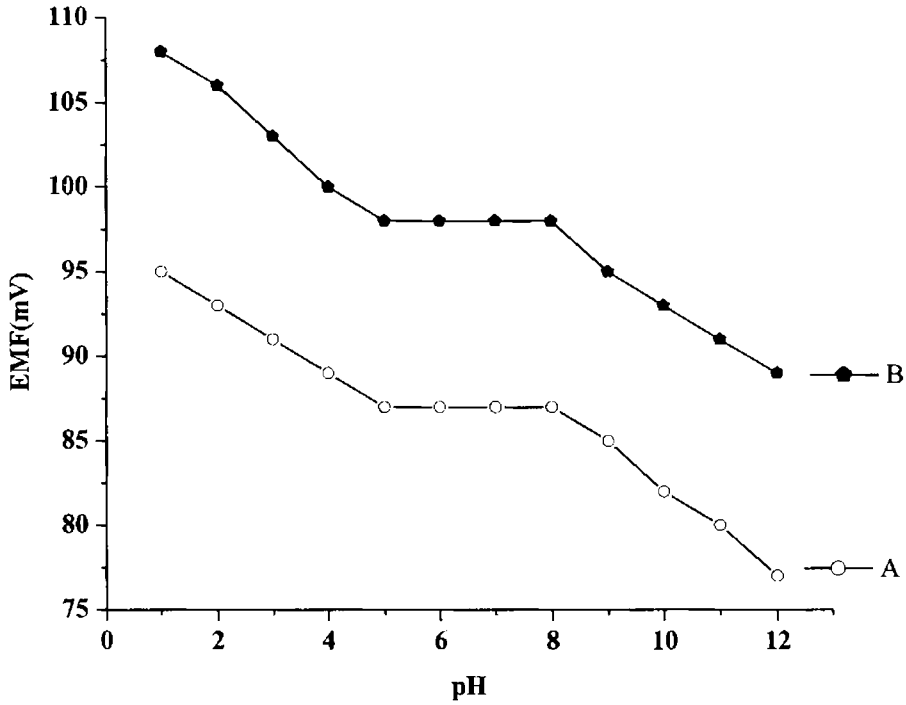


Figure 5.5

Effect of pH on the cell potential of the AMB selective carbon paste sensor A_{P8}

1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)



Chapter 6

Development of Sensors for Sildenafil citrate

This chapter gives details about the response characteristics of sensors developed for sildenafil citrate (SIL). Silicotungstic acid (STA) and phosphotungstic acid (PTA) were used as ion pairing reagents for the preparation of the ion associations. SIL-STA ion association was used for the fabrication of both the PVC membrane sensor and the carbon paste sensor, while a carbon paste sensor was fabricated based on SIL-PTA ion association. The developed sensors worked over a fairly wide concentration range. The developed sensors were found to be useful in the determination of SIL in pharmaceutical formulations.

Sildenafil citrate (SIL) is known chemically as: 1- [4 – ethoxy - 3-(6,7 – dihydro -1-methyl-7-oxo-3-propyl-1H-pyrazolo-[4,3-d] pyirimidin-5-yl) phenyl sulphonyl]-4-methylpiperazine citrate (Figure 6.1). This drug is a potent and selective inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 (PDES5)[254,255]. SIL is highly effective for the treatment of erectile dysfunction and the mode of action of SIL involves the release of nitric oxide (NO) in the corpus cavernosum. The produced NO activates the enzyme guanylate cyclase, which results in increased levels of cGMP, producing smooth muscle relaxation of the penile in the corpus cavernosum and therefore having the potential to improve penile erectile function by allowing inflow of blood [256]. The molecular structure of

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sildenafil is similar to that of cGMP and acts as a competitive binding agent of PDE5 in the corpus cavernosum resulting in more cGMP and better erections. Sildenafil citrate is metabolized by hepatic enzymes and excreted by both the liver and kidneys.

Sildenafil citrate was synthesized by a group of pharmaceutical chemists working at Pfizer's Sandwich, Kent research facility. It was initially studied for use in hypertension and angina pectoris. Phase I clinical trials under the direction of Ian Osterloh suggested that the drug had little effect on angina, but that it could induce marked penile erections[257, 258]. So the studies were stopped till 1992. Later Pfizer decided to market it for erectile dysfunction rather than for angina. The drug was finally patented in 1996 [259]. Pfizer's worldwide patent on sildenafil citrate will expire in 2011-2013. The UK patent of Pfizer on PDE5 inhibitors as treatment of impotence was invalidated in 2000 because of obviousness.

A reliable and specific assay is of great importance for characterization of a drug's disposition, tolerance and safety. Spectrophotometry[260], resonance Rayleigh-scattering[261], chromatography[262-265] and electroanalytical techniques[266-269] are among the most common methods employed for the quantification of sildenafil citrate. But most of these methods are expensive, suffer from lack of selectivity and require careful control of conditions and considerable time for routine control analysis[270, 271].

Recent years have seen an upsurge of interest in the application of ion sensors in the field of medicinal analysis. This provides fast, accurate, reproducible and selective determination of various species[272,273]. This chapter presents the fabrication of ion selective sensors for SIL based on

their ion associations with silicotungstic acid (STA) and phosphotungstic acid (PTA). SIL-STA ion association was used for the fabrication of both the conventional PVC membrane sensor and the carbon paste sensor, while only carbon paste sensor was fabricated based on SIL-PTA ion association. The electrochemical response characteristics of these electrodes were investigated.

6.1 Preparation of the ion associations

Ion associations of SIL with STA and PTA were prepared for the fabrication of sensors selective to the drug. The procedure for the preparation of these ion associations has been discussed in detail in Section 2.2 of Chapter 2. The solutions of the drug (10^{-2} M, 75 mL) and the respective ion pairing agents (10^{-2} M, 25 mL) are mixed together. The obtained precipitates are washed well, dried and stored in desiccator. The composition of the ion associations have been found to be 3:1 (drug : ion pairing reagent). The structure of the ion associations was confirmed by elemental analysis.

SIL-PTA ion association

Found (%) – C – 10.51, H – 1.58, N – 3.24

Calculated (%) – C – 10.50, H – 1.63, N –3.28

SIL-STA ion association

Found (%) – C – 13.59, H – 2.35, N – 4.26

Calculated (%) – C –12.88, H –2.36, N –4.11

6.2 Fabrication of the sensors

6.2.1 Fabrication of PVC membrane sensor

The fabrication of the PVC membrane sensor was in accordance with a procedure reported by Cragg and Moody. The sensing component ie; the ionophore, PVC and plasticizer was thoroughly mixed. The resulting cocktail was dissolved in 5-7 mL of THF. The aliquot was then poured onto glass rings on a glass plate. It was left to stand overnight allowing the slow evaporation of the solvent. The disc shaped membranes obtained were cut out and glued to one end of a glass tube. The sensor thus prepared was conditioned by soaking in 1×10^{-3} M SIL for 24 hrs. The internal filling solution was a mixture of 1×10^{-3} M SIL and 1×10^{-1} M NaCl.

The selectivity and sensitivity of the PVC membrane sensors are largely influenced by the ratio of the PVC, plasticizer and ionophore. So while fabricating PVC membrane sensor for SIL based on SIL-STA ion association, all these factors were considered and best membrane composition was obtained to be 1.8:41.0:57.2 (ionophore : PVC : plasticizer).

6.2.2 Fabrication of the carbon paste sensor

Carbon paste sensor forms a very important class of chemical sensors. It is one of the most reliable methods, the construction being simple and fast.

The ionophore was mixed with graphite in acetone. It was left overnight allowing the slow evaporation of acetone. This was then made into a paste using a suitable plasticizer. The resulting paste was packed to the open end of a Teflon holder. A copper rod through the centre of the electrode

body provides the electrical contact. The proportion of the ionophore, graphite and plasticizer was varied to give an optimum composition of the carbon paste sensor.

6.3 Potential measurement and calibration

Potentials were measured at 25±1 °C on a Metrohm 781 pH/ion meter. A saturated calomel reference electrode was used in conjunction with the developed sensor.

The cell assembly for potentiometric measurements can be represented as follows:

For PVC membrane sensor,

Internal reference electrode | internal filling solution (1×10^{-3} M drug solution + 1×10^{-1} M NaCl solution) | PVC membrane | test solution | external reference electrode.

For carbon paste sensor,

Reference electrode | test solution | Graphite electrode.

The performance of the developed sensors were investigated by measuring the potential in SIL solutions prepared in the concentration range 1.0×10^{-1} – 1.0×10^{-6} M. The solutions were stirred and the stable potential reading was taken.

6.4 Optimization of composition of the sensors

The type and properties of the polymer matrices used for the fabrication of the electrodes significantly influences the response characteristics of the electrode. The addition of plasticizer influences the selectivity, sensitivity and working range of the electrodes due to the variation in free energy of interaction of the electroactive ions and ionophore in polymer matrices. It also depends on the amount of ionophore and plasticizer used in its fabrication. Hence the response parameters for the developed sensors were optimized as a function of amount of ionophore and amount/nature of plasticizer.

SIL-STA ion association was employed for the fabrication of PVC membrane sensor. A number of membrane compositions were investigated and their response characteristics studied. A total 20 membrane sensors were prepared by varying the plasticizer and also the amount of the ionophore. In order to obtain a Nernstian response, the electrode must contain sufficient amount of ionophore. 1.8% (w/w) of ionophore has been found to be the optimum amount. As the amount of ionophore was increased a deviation from Nernstian behaviour occurred. The reason for this behaviour may be that, the high amount of ionophore may induce strong interactions between polymeric chains and ionophore, preventing mobility of the segments[274].

The plasticizers too play a vital role in the fabrication of the sensor. The addition of the plasticizer helps to control the various equilibria between ionophore ions in the membranes phase. It makes the chain flexible and improves the mobility of the ionophore. A set of five different plasticizers viz; Bis(2-ethyl hexyl) phthalate (BEP), Bis(2-ethyl hexyl) sebacate (BES), Di-n- butyl phthalate (DBP), Di-butyl sebacate (DBS) and Bis(2-ethyl hexyl)

adipate (BEA) were tried to study the effect of plasticizer on the response behaviour of the PVC membrane sensor. Of the five different plasticizers studied, BEP has been found to be the most suitable one in terms of slope and concentration range. This may be due to the greater polarity of the BEP which make it suitable for the SIL selective membrane electrode. It affects the rate of exchange reaction between the ionophore in the membrane phase and primary ions of the solution. Table 6.1 illustrates how the slopes of the sensors vary with varying sensor matrix composition. Considering all the parameters that contribute to the response of the SIL-STA based PVC membrane electrode, an optimum composition for the membrane was arrived at, which was found to be 1.8:41.0:57.2 (ionophore:PVC:plasticizer). The PVC membrane sensor with the composition 1.8:41.0:57.2 % (w/w) gave a Nernstian slope of 58.6 mV/decade. The sensor S_{S7} exhibited a linear working range of $5.0 \times 10^{-5} - 1.0 \times 10^{-2}$ M and a response time of < 35 s. The lower detection limit of S_{S7} has been observed to be 3.1×10^{-6} M. The calibration graph of S_{S7} is given as Figure 6.2.

Carbon paste electrodes (CPEs) were also fabricated based on SIL-STA and SIL-PTA ion associations. In CPEs also the sensor matrix ingredients viz; graphite, plasticizer and ionophore were varied (Table 6.2). These were found to influence the electrochemical response characteristics of the carbon paste sensors. The amount of ionophore in the optimized carbon paste sensor for SIL based on SIL – STA ion association has been found to be 2.5% (w/w). When the amount of the ionophore in the sensor matrix was increased, a sub Nernstian response was obtained. The plasticizer was also changed; all the five plasticizers were tried. The amount of the plasticizer was also varied. Table 6.2 shows the variation in slope with

changing matrix composition. For the sensor based on SIL-STA ion association, good results were obtained with BEP plasticized sensor with the composition 2.5:51.0:46.5 % (w/w) (ionophore : graphite : plasticizer). The sensor (S_{GS6}) gave a near Nernstian response of 56.3 mV/decade. The working concentration of the sensor S_{GS6} has been found to be 1.2×10^{-5} – 5.0×10^{-3} M with a lower detection limit of 1.5×10^{-6} M. S_{GS6} had a response time of < 30 s. Figure 6.3 illustrates the calibration plot of S_{GS6}.

The carbon paste sensor incorporating SIL-PTA ion association as ionophore was fabricated and tested for its electrochemical response. The composition of the sensor matrix was varied to arrive at an optimum composition. One of the most important factors that determine the response of the sensor is the amount of the ionophore in the sensor matrix. The amount of the ionophore was changed from 2.0 to3.0 % (w/w). 2.0% (w/w) of ionophore, in the sensor matrix gave a near Nernstian response of 57.7 mV/decade as is evident from Table 6.3. Out of the five different plasticizers examined, BEP was found to give the best response in terms of slope and concentration range. The optimized carbon paste sensor (S_{GP5}) for SIL incorporating SIL-PTA ion association has the composition, 2.0:34.5:63.5 % (w/w) (ionophore : graphite : plasticizer). The best plasticizer in the case of sensor S_{GP5} has been obtained to be BEP, which can again be related to its high polarity. The calibration graph of S_{GP5} is given in Figure 6.4. The lower detection limit of S_{GP5} is 1.2×10^{-6} M. The working concentration range is 1.0×10^{-5} – 1.0×10^{-2} M. The sensor exhibited a response time of < 30 s.

The response characteristics of all three sensors, S_{S7}, S_{GS6} and S_{GP5} are summarized in Table 6.4.

6.5 Effect of concentration of internal filling solution

The effect of concentration of the internal filling solution on the response characteristics of the PVC membrane sensor for SIL based on SIL – STA ion association, S_{S7} has been investigated. The concentration of the internal filling solution was varied from 1.0×10^{-4} – 1.0×10^{-2} M SIL. The calibration plot obtained showed no significant deviation in its response with changing concentration of the internal filling solution. Hence 1.0×10^{-3} M SIL has been used as the internal filling solution for all the studies.

6.6 Effect of pH

The dependence of the potential response of the three sensors S_{S7}, S_{GS6} and S_{GP5} over various pH was critically investigated. The two different concentrations for SIL that was studied are 1×10^{-3} and 1×10^{-4}. The pH was varied from 2-12 using buffer solution. The data revealed a linear potential profile versus pH in the pH range 4-6 for all the 3 sensors S_{S7}, S_{GS6} and S_{GP5}. All the three sensors showed a similar trend in pH profile. An increase in potential was observed at pH < 4 which may be due to the interference of H^+ ions[275-277]. In SIL solutions of pH < 4 more than one positive site can be identified at the available nitrogen centres in the drug molecule which subsequently increase the potentiometric response. At pH > 6 the potential readings for the sensors decreases sharply due to precipitation or hydrolysis of SIL. The gradual increase in unprotonated species may also account for such behavior[278]. The pH dependence of the three sensors is plotted as Figures 6.5, 6.6 and 6.7.

6.7 Potentiometric selectivity

Selectivity is one of the most important characteristics of ion selective electrodes that is expressed by the potentiometric selectivity coefficients and describes the preference of the sensor for an interfering ion relative to the primary ion. The potentiometric selectivity coefficients for the sensors S_{S7}, S_{GS6} and S_{GP5} were determined by the Fixed Interference Method (FIM). The value of the selectivity coefficient $K_{A,B}^{pot}$, determines the selectivity of the sensor. The selectivity coefficient values for all the three sensors are summarized in Table 6.5. The results given in the table shows that all the three sensors S_{S7}, S_{GS6} and S_{GP5} exhibit good selectivity for SIL even in the presence of the different foreign ions tested viz; Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, citric acid, lactose, urea, ascorbic acid and glycine including the tablet excipients such as talc and starch. None of the ions tested was found to interfere with any of the three sensors developed.

6.8 Shelf life or Life time

The average life time or the shelf life of the developed sensors was studied. As already explained the working surface of the carbon paste electrode can be renewed. The potential measurements were recorded every day over a period of time to determine the shelf life of the sensors. The sensors were all kept in 1.0×10^{-3} M sildenafil citrate solution when not in use. The operative life time of the SIL - selective PVC membrane sensor incorporating SIL-STA ion association S_{S7} has been observed to be 4weeks. The operative lifetime of carbon paste sensor S_{GS6} using SIL-STA ion association, has been found to be 5 weeks. S_{GP5} had a shelf life of 3 weeks.

During this period the sensors showed no appreciable deviation from their optimized response characteristics.

6.9 Analytical applications

The sensors developed for SIL such as S_{S7}, S_{GS6} and S_{GP5} were employed for the determination of sildenafil citrate in tablets (Silagra - Cipla - India). The detailed procedure for the determination of the SIL content in tablets is given in Section 2.5.5 of Chapter 2. As discussed in Chapter 2 electrochemical studies were carried out for the tablet. The determination of SIL in tablets was carried out by the proposed method and compared with the results obtained with the spectroscopic method[173]. Table 6.6 illustrates the results obtained using both the methods. There is good agreement between the values obtained by both the methods. From the table it is clear that the developed sensors S_{S7}, S_{GS6} and S_{GP5}, could be used for the determination of SIL in tablets with high accuracy and precision.

The developed sensors were employed for the determination of sildenafil citrate in urine samples. The detailed procedure is given in Chapter 2. The results (Table 6.7) show that the developed sensors can detect the drug content in the spiked urine sample with high accuracy and precision. The % recovery of the drug using the sensor S_{S7} has been found to be 103.8 whereas for the sensors S_{GS6} and S_{GP5} it has been found to be 99.2 and 100.8 respectively.

All three sensors fabricated for SIL viz; S_{S7}, S_{GS6} and S_{GP5} gave near Nernstian slopes of 58.6, 56.3 and 57.7 mV/decade respectively. A detection limit of 3.1×10^{-6} M was achieved for S_{S7} having a working concentration range of $5.0 \times 10^{-5} - 1.0 \times 10^{-2}$ M. S_{GS6} and S_{GP5} exhibited Nernstian

behaviour within the range $1.2 \times 10^{-5} - 5.0 \times 10^{-3}$ M and $1.0 \times 10^{-5} - 1.0 \times 10^{-2}$M. The lower detection limits for the sensors S_{GS6} and S_{GP5} has been found to be 1.5×10^{-6} M and 1.2×10^{-6} M. The pH range for all the sensors was observed to be 4 – 6. The response time for S_{GS6} and S_{GP5} has been observed to be < 30 s while for S_{S7} it was < 35 s. The shelf life for S_{S7}, S_{GS6} and S_{GP5} has been found to be 4, 5 and 3 weeks respectively.

Table 6.1

Optimization of membrane ingredients for PVC membrane sensor for SIL based on SIL-STA ion association

	Composition % (w/w)			Slope
Membrane	Ionophore	PVC	Plasticizer	mV/decade
S_{S1}	1.0	33.5	65.5, DBP	30.1
S_{S2}	1.2	46.0	52.8, DBP	35.4
S_{S3}	1.8	41.0	57.2, DBP	42.0
S_{S4}	2.0	40.8	57.2, DBP	32.2
S_{S5}	1.0	33.5	65.5, BEP	49.1
S_{S6}	1.2	46.0	52.8, BEP	52.1
S_{S7}	**1.8**	**41.0**	**57.2, BEP**	**58.6**
S_{S8}	2.0	40.8	57.2, BEP	50.6
S_{S9}	1.0	33.5	65.5, DBS	44.2
S_{S10}	1.2	46.0	52.8, DBS	33.6
S_{S11}	1.8	41.0	57.2, DBS	64.2
S_{S12}	2.0	40.8	57.2, DBS	66.1
S_{S13}	1.0	33.5	65.5, BES	63.5
S_{S14}	1.2	46.0	52.8, BES	72.9
S_{S15}	1.8	41.0	57.2, BES	46.3
S_{S16}	2.0	40.8	57.2, BES	48.0
S_{S17}	1.0	33.5	65.5, BEA	73.4
S_{S18}	1.2	46.0	52.8, BEA	70.6
S_{S19}	1.8	41.0	57.2, BEA	76.4
S_{S20}	2.0	40.8	57.2, BEA	49.6

Table 6.2

Optimization of composition of the carbon paste sensor for SIL based on SIL-STA ion association

	Composition % (w/w)			Slope
Sensor	Ionophore	Graphite	Plasticizer	mV/decade
S_{GS1}	2.0	34.5	63.5, DBP	65.8
S_{GS2}	2.5	51.0	46.5, DBP	68.1
S_{GS3}	2.8	61.2	36.0, DBP	72.0
S_{GS4}	3.0	49.7	47.3, DBP	74.5
S_{GS5}	2.0	34.5	63.5, BEP	49.2
S_{GS6}	**2.5**	**51.0**	**46.5, BEP**	**56.3**
S_{GS7}	2.8	61.2	36.0, BEP	48.2
S_{GS8}	3.0	49.7	47.3, BEP	46.9
S_{GS9}	2.0	34.5	63.5, DBS	38.4
S_{GS10}	2.5	51.0	46.5, DBS	40.6
S_{GS11}	2.8	61.2	36.0, DBS	47.5
S_{GS12}	3.0	49.7	47.3, DBS	49.9
S_{GS13}	2.0	34.5	63.5, BES	70.3
S_{GS14}	2.5	51.0	46.5, BES	73.6
S_{GS15}	2.8	61.2	36.0, BES	45.8
S_{GS16}	3.0	49.7	47.3, BES	36.8
S_{GS17}	2.0	34.5	63.5, BEA	40.2
S_{GS18}	2.5	51.0	46.5, BEA	43.9
S_{GS19}	2.8	61.2	36.0, BEA	45.9
S_{GS20}	3.0	49.7	47.3, BEA	49.0

Table 6.3

Optimization of composition of the carbon paste sensor for SIL based on SIL-PTA ion association

	Composition % (w/w)			Slope
Sensor	Ionophore	Graphite	Plasticizer	mV/decade
S_{GP1}	2.0	34.5	63.5, DBP	61.5
S_{GP2}	2.5	51.0	46.5, DBP	62.3
S_{GP3}	2.8	61.2	36.0, DBP	65.9
S_{GP4}	3.0	49.7	47.3, DBP	70.2
S_{GP5}	**2.0**	**34.5**	**63.5, BEP**	**57.7**
S_{GP6}	2.5	51.0	46.5, BEP	54.0
S_{GP7}	2.8	61.2	36.0, BEP	49.6
S_{GP8}	3.0	49.7	47.3, BEP	45.1
S_{GP9}	2.0	34.5	63.5, DBS	63.1
S_{GP10}	2.5	51.0	46.5, DBS	69.2
S_{GP11}	2.8	61.2	36.0, DBS	76.4
S_{GP12}	3.0	49.7	47.3, DBS	77.0
S_{GP13}	2.0	34.5	63.5, BES	32.5
S_{GP14}	2.5	51.0	46.5, BES	36.0
S_{GP15}	2.8	61.2	36.0, BES	39.2
S_{GP16}	3.0	49.7	47.3, BES	40.1
S_{GP17}	2.0	34.5	63.5, BEA	40.6
S_{GP18}	2.5	51.0	46.5, BEA	43.3
S_{GP19}	2.8	61.2	36.0, BEA	32.1
S_{GP20}	3.0	49.7	47.3, BEA	69.1

Table 6.4

Response characteristics of the sensors S_{S7}, S_{GS6} and S_{GP5}

Parameter	Response Characteristics		
	S_{S7}	S_{GS6}	S_{GP5}
Slope (mV decade^{-1})	58.6	56.3	57.7
Working concentration range (M)	5.0×10^{-5} – 1.0×10^{-2}	1.2×10^{-5} – 5.0×10^{-3}	1.0×10^{-5} – 1.0×10^{-2}
Detection limit (M)	3.1×10^{-6}	1.5×10^{-6}	1.2×10^{-6}
pH range	4-6	4-6	4-6
Shelf life	4weeks	5weeks	3weeks
Response time(s)	< 35	< 30	< 30

Table 6.5

Selectivity coefficients for the sensors S_{S7}, S_{GS6} and S_{GP5} using fixed interference method.

Interfering ion (X)	$K^{pot}_{A,B}$		
	S_{S7}	S_{GS6}	S_{GP5}
Na^+	3.5×10^{-3}	6.2×10^{-3}	5.8×10^{-3}
K^+	2.8×10^{-4}	4.2×10^{-3}	4.8×10^{-3}
Ca^{2+}	1.9×10^{-4}	9.0×10^{-3}	8.6×10^{-3}
Co^{2+}	8.6×10^{-3}	1.1×10^{-4}	9.7×10^{-3}
Mg^{2+}	5.3×10^{-3}	6.3×10^{-3}	6.2×10^{-3}
Zn^{2+}	6.0×10^{-3}	2.8×10^{-4}	7.1×10^{-3}
Citric acid	5.7×10^{-3}	7.2×10^{-3}	5.9×10^{-3}
Lactose	3.1×10^{-3}	6.6×10^{-3}	1.0×10^{-4}
Urea	6.7×10^{-3}	1.9×10^{-4}	8.2×10^{-3}
Ascorbic acid	4.2×10^{-3}	5.4×10^{-3}	1.2×10^{-4}
Starch	2.2×10^{-4}	6.1×10^{-3}	8.6×10^{-3}
Glycine	8.4×10^{-3}	5.1×10^{-3}	1.1×10^{-4}
Talc	1.9×10^{-4}	7.4×10^{-3}	9.8×10^{-3}

Table 6.6

Determination of SIL in pharmaceutical formulations

Sample	Declared Amount (mg/tablet)	Method adopted	Found * (mg/tablet)	SD*	CV*
Silagra (Cipla - India)	100	S_{S7}	99	2.00	2.02
		S_{GS6}	99	1.73	1.74
		S_{GP5}	98	2.00	2.04
		Standard Method	99	1.41	1.42

*Average of six replicates

Table 6.7

Determination of SIL in urine sample using the developed sensors

Drug added (M)	Sensor	Drug found* (M)	Recovery %
1.20×10^{-4}	S_{S7}	1.21×10^{-4}	100.8
	S_{GS6}	1.19×10^{-4}	99.2
	S_{GP5}	1.21×10^{-4}	100.8

*Average of six replicates

Figure 6.1

Structure of the drug – Sildenafil citrate

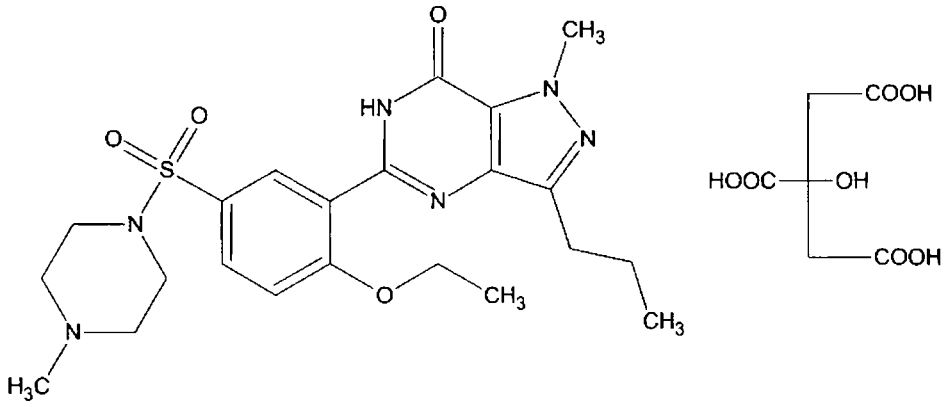


Figure 6.2

Calibration graph for SIL selective PVC membrane sensor based on SIL-STA ion association (S_{S7})

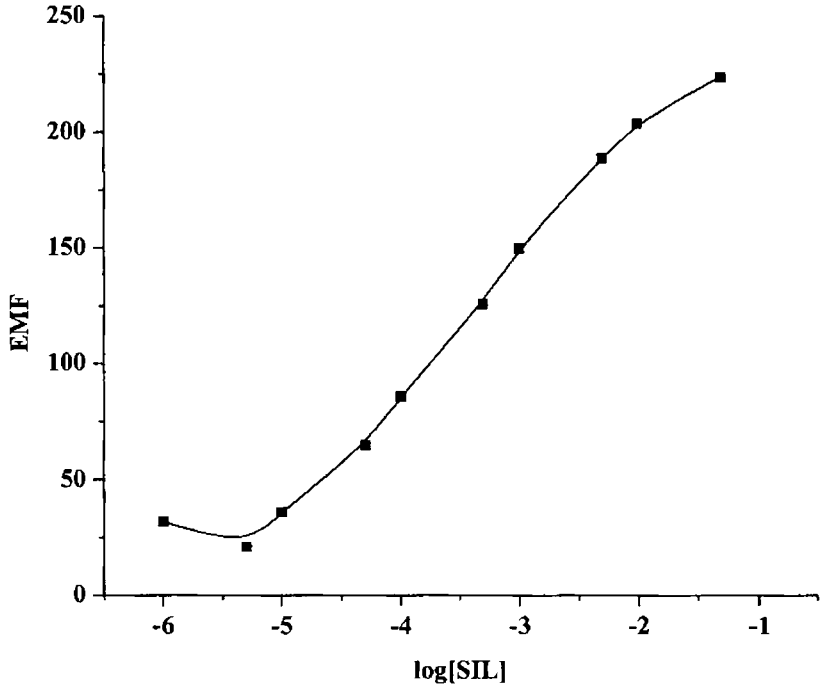


Figure 6.3

Calibration graph for SIL selective carbon paste sensor based on SIL-STA ion association (S_{GS6})

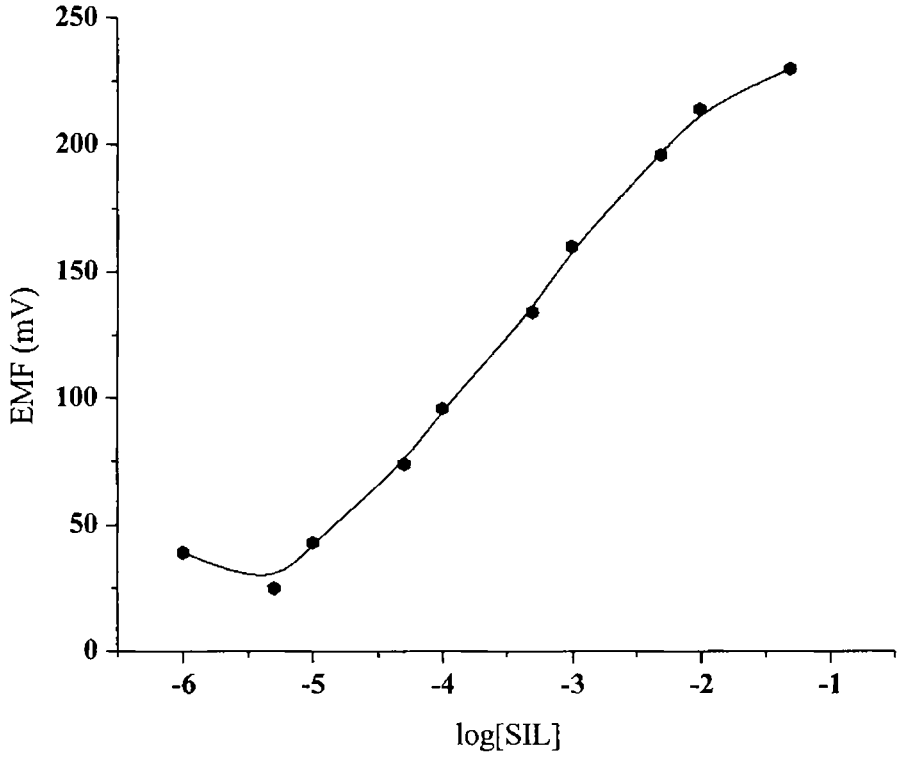


Figure 6.4

Calibration graph for SIL selective PVC membrane sensor based on SIL-PTA ion association (S_{GP5})

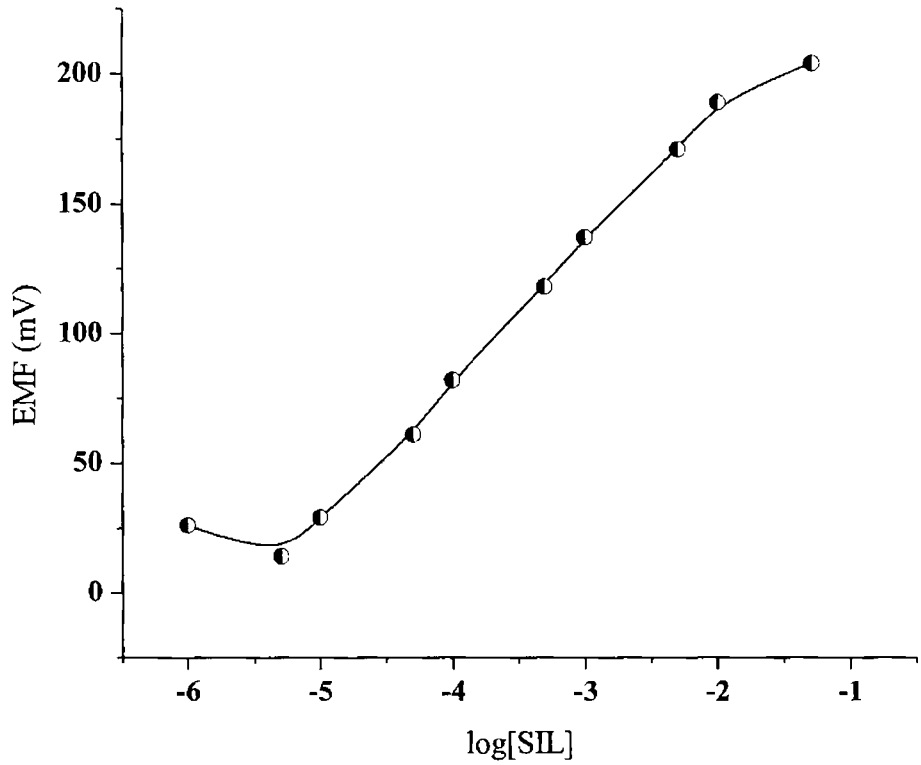


Figure 6.5

Effect of pH on the cell potential of the SIL selective PVC membrane sensor based on SIL-STA ion association S_{S7}

1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

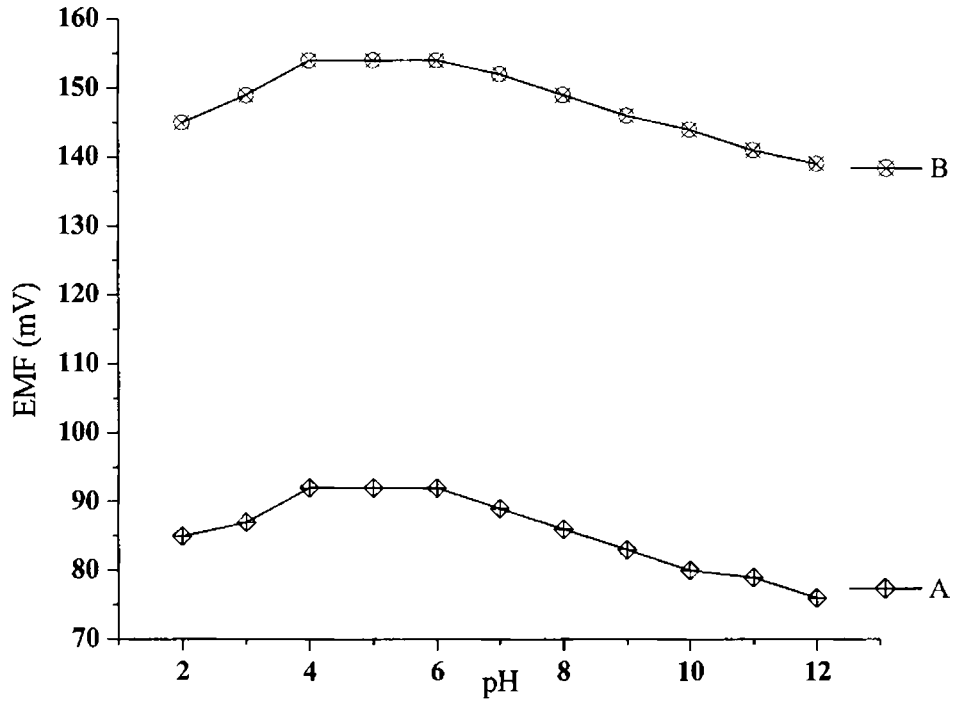


Figure 6.6

Effect of pH on the cell potential of the SIL selective carbon paste sensor based on SIL-STA ion association S_{GS6}

1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

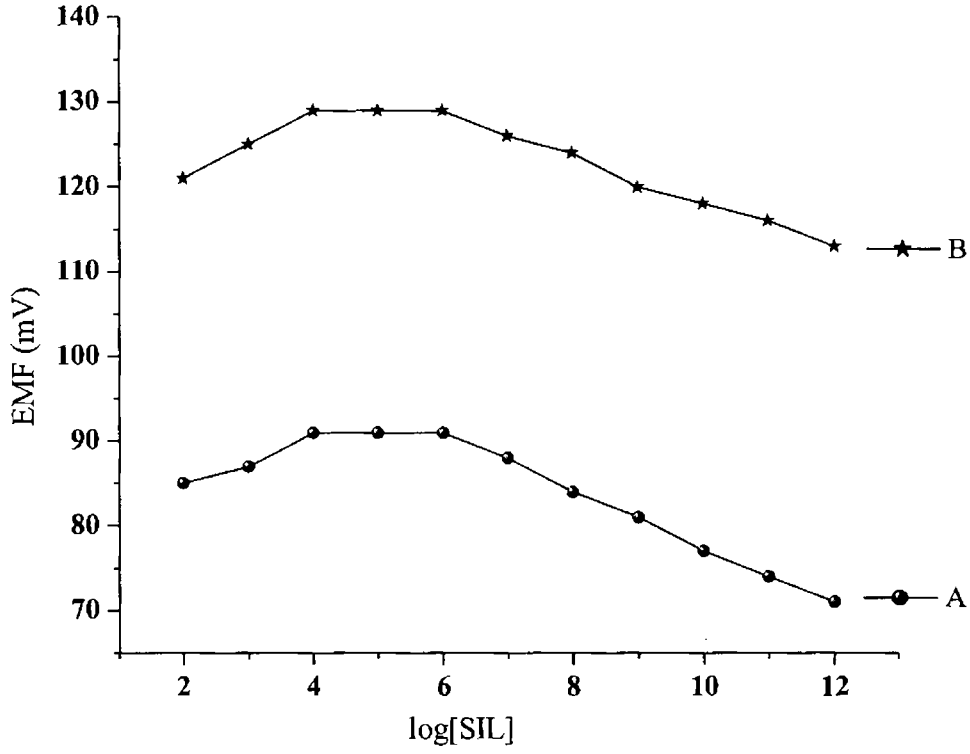
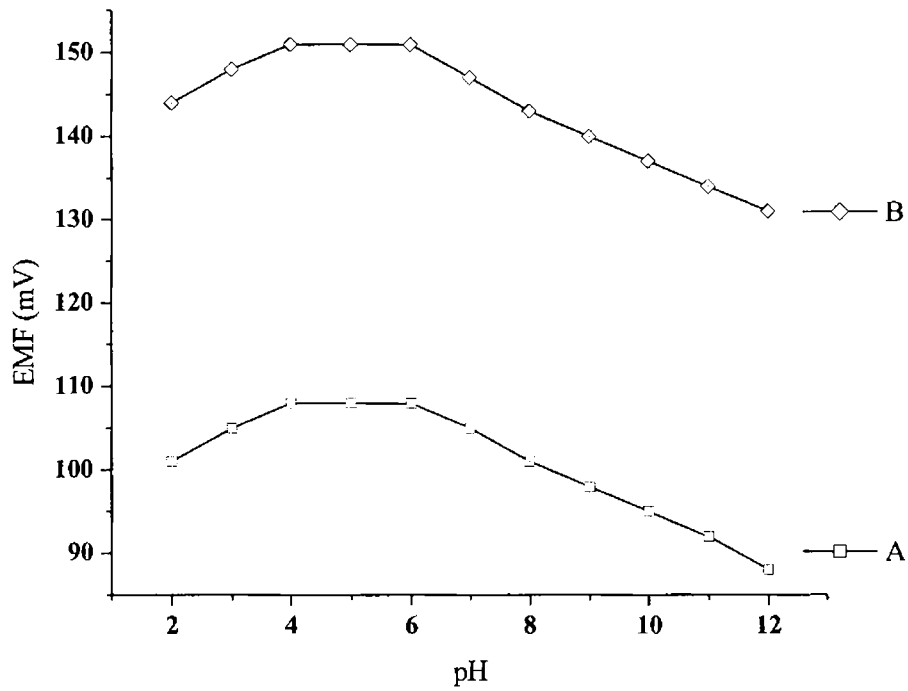


Figure 6.7

Effect of pH on the cell potential of the SIL selective carbon paste sensor based on SIL-PTA ion association S_{GP5}



Chapter 7

Development of Sensors for Dextromethorphan

This chapter discusses in detail the response characteristics of two types of potentiometric sensors fabricated for dextromethorphan (DEX) based on two different ionophores. These ionophores are based on the ion association of the drug with two ion-pairing reagents, sodium tetraphenyl borate (NaTPB) and phosphotungstic acid (PTA). Both PVC membrane sensors and carbon paste electrodes (CPEs) were fabricated using the ion associations prepared. Various response parameters of the developed sensors are discussed in detail. The developed sensors were applied for the determination of the drug in pharmaceutical formulations. The sensors were also found to be useful in the determination of the drug in urine sample.

Dextromethorphan (DEX) is a synthetic morphine analog, similar to levorphanol. It has no traditional opiate-like activity, and is not a substitute for codeine as an analgesic[279-281]. DEX is a centrally acting antitussive, which is comparable to codeine on a mg basis for cough suppression but lacking analgesic or addictive properties. Although it is chemically related to morphine, it has no analgesic or addictive properties. Its antitussive activity is equal to that of codeine. Dextromethorphan has little to no dissociative/hallucinogenic effect at medically approved dosages, which range from about 5–60 milligrams. People who study the specific effects of psychotropic substances classify DEX as a dissociative drug, a major

subclass of hallucinogenic drugs, along with ketamine and phencyclidine. Biochemically, DEX's psychological effects have been attributed largely to dextrorphan (DX), a chemical by-product that is produced when DEX metabolizes within the body. It generally does not produce withdrawal symptoms characteristic of physically addictive substances, but psychological addiction has been reported by some users.

Dextromethorphan (DEX) suppresses the cough reflex by a direct effect on the cough centre in the medulla of the brain. DEX shows high affinity binding to several regions of the brain, including the medullary cough center. The drug acts centrally to elevate the threshold for coughing. DEX is rapidly absorbed from the gastrointestinal tract, and exerts its activity within 15 to 60 minutes of ingestion. The duration of action after oral administration is approximately three to eight hours.

DEX was first patented with U.S. Patent 2,676,177, and was approved for over-the-counter purchase as an antitussive in 1958. During the 1960s and 1970s, DEX became available in an over-the-counter tablet form by the brand name Romilar. It was put on the shelves in hopes of cutting down on codeine cough remedies. In 1973, Romilar was taken off the shelves after a burst in sales due to common recreational use. It was then replaced by cough syrup, in an attempt to cut down on recreational usage.

Chemically, DEX is [(+)-cis-1,3,4,9,10,10a-hexahydro-6-methoxy-11-methyl- 2H-10,4a-iminoethanophenanthrene], and is also known as 3-methoxy-17-methyl-(9alpha,13alpha,14alpha)-morphinan hydrobromide (Figure 7.1).

The different analytical techniques available in the literature for the quantitative determination of DEX include capillary electrophoresis[282], gas

chromatography[283], liquid-liquid extraction and LC with fluorescence detection[284], reversed phase LC[285], first derivative spectrophotometry[286], high performance liquid chromatography[287-289] and LC-MS[290]. Most of these methods require expensive and sophisticated instruments and are time-consuming. Hence it is worthwhile to develop a simple and sensitive method for the analysis of this drug.

The present chapter deals with the fabrication of two types of sensors for the determination of DEX incorporating the ion associations of the drug with phosphotungstic acid (PTA) and sodium tetraphenyl borate (NaTPB).

7.1 Preparation of the ion associations

The sensors for DEX are based on the ion associations of the drug with PTA and NaTPB. As discussed in Section 2.2 of Chapter 2, the DEX-PTA ion pair was prepared by mixing the solutions of DEX (10^{-2} M, 75 mL) with PTA (10^{-2} M, 25 mL) and the DEX-NaTPB ion pair was obtained by mixing equimolar solutions of both DEX and NaTPB (10^{-2} M, 50 mL). The precipitate thus obtained was filtered, washed several times with distilled water and dried at room temperature. The precipitate was stored in a desiccator. The structure of the ion associations was confirmed by elemental analysis. The composition of the DEX – PTA ion association is 3:1 and 1:1 for DEX-NaTPB ion association (drug : ion pairing agent).

DEX- PTA

Found (%) – C – 17.45, H – 2.09, N – 1.04

Calculated (%) – C – 17.24, H – 2.00, N – 1.12

DEX – NaTPB

Found (%) – C – 84.14, H –8.17, N – 2.28

Calculated (%) – C – 84.32, H – 8.19, N – 2.35

7.2 Fabrication of the sensors

Two types of sensors, one based on the conventional PVC membrane and the other carbon paste electrode were prepared and studied for their electrochemical performances. These sensors incorporated the DEX – NaTPB and DEX - PTA ion associations as electroactive materials.

7.2.1 Fabrication of PVC membrane sensor

The general method for the fabrication of the PVC matrix membrane sensor was according to the Cragg's procedure as discussed in Section 2.8 in Chapter 2. The preliminary step in the fabrication of the PVC membrane sensor involves dissolving ionophore, PVC and plasticizer in THF. The sensing membrane was prepared by pouring the PVC cocktail into glass rings struck on a glass plate. The membrane thus obtained was glued to one end of a glass tube. The glass tube was filled with 1×10^{-3} M DEX and 1×10^{-1} M NaCl solutions. The prepared sensor was then left for conditioning for 24 hours.

The composition of the membrane was optimized by varying the % w/w of the different membrane constituents viz; PVC, ionophore and plasticizer. Only appropriate selection of each of these constituents finally guarantees for a highly selective and sensitive electrode that exhibits a stable and reproducible potential response[291]. For the DEX-PTA based membrane sensor the best performance was obtained with BEP as the plasticizer and the composition of the membrane was found to be 1.8:62.0:36.2 (ionophore :

plasticizer : PVC) (% w/w). The composition ratio of the membrane that gave the best response in terms of slope, concentration range and response time for the DEX-NaTPB based membrane sensor was found to be 1.6:63.4:35.0 (ionophore :plasticizer : PVC) (% w/w). In this case the plasticizing agent used was DBP.

7.2.2 Fabrication of the carbon paste sensor

The carbon paste sensors offer very attractive properties for electrochemical investigation of various inorganic and organic species. Carbon pate sensors possess advantages of ease of preparation, ease of regeneration and very stable response in addition to the very low ohmic resistance [247, 248] which is probably due to the formation of a very thin film of pasting liquid coated onto small particles of powder[251, 252].

The carbon paste was prepared by mixing graphite and the ionophore in varying proportions. This was then packed into the open end of a Teflon holder. The copper rod in the centre provides the electrical contact. The working surface was polished using a filter paper.

7.3 Potential measurement and calibration

Potentials were measured at 25±1 °C on a Metrohm 781 ion meter. A saturated calomel reference electrode was used in conjunction with the developed sensor. The cell assembly for potentiometric measurements can be represented as follows:

For PVC membrane sensor,

Internal reference electrode | internal filling solution (1×10^{-3} M drug solution + 1×10^{-1} M NaCl solution) | PVC membrane | test solution | external reference electrode.

For carbon paste sensor,

Reference electrode | test solution | Graphite electrode.

The performance of the developed sensor was investigated by measuring the potential in DEX solutions prepared in the concentration range 1.0×10^{-1} – 1.0×10^{-6} M. The solutions were stirred and the stable potential reading was taken.

7.4 Optimization of sensor matrix composition

The response of a sensor is largely dependent on the nature of the plasticizer used, the amount of the ionophore, plasticizer and PVC. Different PVC membrane sensors for DEX based on DEX-NaTPB ion association were fabricated by changing the amount of the membrane constituents such as ionophore, plasticizer and also by varying the nature of the plasticizers. The various plasticizers that were employed for the fabrication of the membrane sensors include Bis(2-ethyl hexyl) phthalate (BEP), Bis(2-ethyl hexyl) sebacate (BES), Di-n- butyl phthalate (DBP), Di-butyl sebacate (DBS) and Bis(2-ethyl hexyl) adipate (BEA). Out of the 20 different PVC membrane sensors fabricated based on DEX- NaTPB ion association, the one

with DBP as the plasticizer gave the best slope. The optimum amount of DBP has been found to be 63.4% (w/w). The major component of the membrane in an ion selective membrane sensor is the plasticizer, which ensures the mobility of the free and complexed ionophore, set the dielectric constant and provide suitable mechanical property for the membrane. As a result, the plasticizer would highly influence the selectivity, measuring range and detection limit of the sensor [292-295]. In order to obtain Nernstian response, the sensor must have sufficient amount of the ionophore, otherwise the electrode will not respond in the Nernstian way. 1.6% (w/w) of ionophore has been observed to give a Nernstian slope of 57.7 mV/decade. The sensor with sufficient amount of ionophore has been observed to show improved sensitivity. On further increasing the amount of ionophore, a change in the ratio of ionic sites to the ionophore in the membrane phase occurs which affects the electrode response from Nernstian to non-Nernstian behaviour. Figure 7.2 illustrates the calibration plot of the optimized membrane sensor for DEX (D_{N2}) based on DEX-NaTPB ion association as the electroactive material. The results consolidated in Table 7.1 shows that the optimum composition of the PVC membrane sensor D_{N2} is 1.6: 35.0:63.4 % (w/w) (ionophore : PVC : plasticizer). The working concentration range of D_{N2} has been found to be 1.0×10^{-5} - 1.2×10^{-2} M with a lower detection limit of 5.1 $\times 10^{-6}$ M. The response time of the optimized sensor D_{N2} has been found to be < 30 s.

The DEX-PTA ion association was also used for the fabrication of the PVC membrane sensor for DEX. Table 7.2 illustrates how the slope of the sensors vary on changing the membrane composition. The amount of ionophore in the membrane matrix is one of the most important factors

which influence the response of the PVC membrane sensor. The optimum amount of ionophore in the PVC membrane sensor for DEX based on DEX-PTA ion association has been found to be 1.8% (w/w). A deviation from this amount resulted in a non Nernstian response. A higher amount of ionophore leads to saturation of membrane matrix. If the ionophore content in the sensor matrix is less than 1.8% w/w, a deviation from Nernstian response is observed which may be attributed to the decreased mobility of the ionophore across the polymer membrane. Plasticizers play a key role in the behaviour of sensors. The plasticizers should have certain properties and characteristics such as high lipophilicity, high molecular weight, low vapour pressure etc. The amount of plasticizer in addition to the nature of the plasticizer influences the response of the sensor. The PVC membrane sensor shows a substantial variation in potential response at different amounts of plasticizer because the water uptake capacity of the PVC membranes changes with the amount of the plasticizer[296,297]. Plasticizers help to impart such properties like flexibility and smoothness to the polymer membrane. Of the various different plasticizers used BEP has been found to give the best response (Table 7.2). The optimum amount of BEP in the sensor matrix has been obtained to be 62.0% (w/w). At a higher concentration of plasticizer, the free energy of interactions of DEX ions in solution with ionophore in polymer matrix is highly influenced, hence the response characteristics of the electrode is changed. The optimum composition of the PVC matrix membrane sensor (D_{P7}) for DEX, incorporating DEX-PTA ion association is 1.8:36.2:62.0 % (w/w) (ionophore : PVC : plasticizer) which gave a Nernstian slope of 56.4 mV/decade. D_{P7} has a working concentration range of $1.1 \times 10^{-5} - 5.0 \times 10^{-2}$ M and a lower detection limit of 6.2×10^{-6} M. The

response time of D_{P7} is < 35 s. The calibration plot of D_{P7} is given in Figure 7.3.

The ion associations of DEX with NaTPB and PTA were also employed for the fabrication of carbon paste sensors for DEX. Carbon paste electrodes have the advantage that it does not require an internal filling solution and its surface can be renewed. Here again the sensor matrix composition was varied to arrive at an optimum composition. In fabricating the CPE for DEX, based on DEX-NaTPB as ion association, the amount of the ionophore was varied. As is evident from Table 7.3 when the ionophore content in the sensor matrix is higher a non-Nernstian response is obtained. So 2.0% (w/w) of DEX-NaTPB ion association was found to be the optimum amount. As already reported the nature / amount of plasticizers also determine the response characteristics of the carbon paste electrode. It influences the mobility of the ionophore in the polymer matrix. The different plasticizers that were employed in the study include Bis(2-ethyl hexyl) phthalate (BEP), Bis(2-ethyl hexyl) sebacate (BES), Di-n- butyl phthalate (DBP), Di-butyl sebacate (DBS) and Bis(2-ethyl hexyl) adipate (BEA). Of these five plasticizers used, BEA has been observed to give the best response in terms of slope. The optimum amount of BEA was 63.5% (w/w). The calibration graph of D_{GN17} is given as Figure 7.4.This sensor of DEX D_{GN17} has the optimum composition 2.0:34.5:63.5 % (w/w) (ionophore : graphite : plasticizer). The slope of D_{GN17} has been found to be 60.3 mV/decade with a working concentration range of 1.5×10^{-5} - 5.0×10^{-2} M and a lower detection limit of 1.1×10^{-6} M. The response time of D_{GN17} is < 30 s.

DEX-PTA ion association was employed in the fabrication of CPE for DEX. The concentration of the active ingredients of the sensor was

varied to find out the most suitable composition for the sensor. The ionophore mobility and the equilibrium thus established influence the response of the sensor. So an adequate amount of ionophore should be present in the matrix. The ionophore content was varied and it has been concluded that 2.8% (w/w) of ionophore in the sensor gave a Nernstian response of 57.0 mV/decade (Figure 7.5). In addition to the ionophore content, the next important factor which influences the sensor's response is the nature / amount of the plasticizer. The best plasticizer for the DEX sensor incorporating DEX-PTA ion association has been found to be BEA, the optimum amount being 36.0% (w/w). This may be due to the good solubility and fairly good distribution ratio of the ion association. A higher quantity of plasticizer resulted in a deviation in its response. The sensor D_{GP19} used for further studies has the optimized composition 2.8:61.2:36.0 % (w/w) (ionophore : graphite : plasticizer) (Table 7.4). The response time for D_{GP19} is < 25 s. The working concentration range of D_{GP19} is 1.0×10^{-5} - 1.0×10^{-2} M with a detection limit as low as 1.9×10^{-6} M.

The response characteristics of D_{P7}, D_{N2}, D_{GN17} and D_{GP19} are summarized in Table 7.5.

7.5 Effect of concentration of internal filling solution

The influence of concentration of the internal filling solution on the response characteristics of the PVC membrane sensors, D_{P7} and D_{N2} selective to DEX were studied. The concentration of the internal filling was changed from 1.0×10^{-4} M to 1.0×10^{-2} M. The calibration plots were obtained in each case. It has been observed that there is no change in the response of the sensors D_{P7} and D_{N2} when the concentration of the internal filling solution

was changed. Hence the concentration of the internal filling solution was fixed at 1.0×10^{-3} M for all the studies.

7.6 Effect of pH

The effect of pH of the test solution on the response characteristics of the developed sensors was studied. The pH of the solution was changed from 2 - 12 using buffer solution. The effect of pH was studied at two fixed concentrations of the test solutions. The pH profile of all the four sensors D_{N2}, D_{P7}, D_{GN17} and D_{GP19} are given in Figures 7.6 - 7.9. For all the sensors except D_{P7}, the pH profile gave a linear plot in the pH range 5 - 8. In the case of D_{P7}, the potential remained constant in the pH range 4 - 8. The potential showed an increasing trend till pH 4 and remained constant till 8 and finally it started to decrease probably due to precipitation. In the sensors D_{N2}, D_{GN17} and D_{GP19}, the potential increased up to pH 5 and finally it decreased beyond pH 8 after remaining constant in the range 5 - 8.

7.7 Potentiometric selectivity

The selectivity of the ion pair associate based sensors depend on the selectivity of the ion exchange process at the sensor - test solution interface and the mobilities of the respective ions within the membrane[298]. The extent of interference of various species was studied by the Fixed Interference Method (FIM). The selectivity coefficient values for all the four sensors D_{P7}, D_{N2}, D_{GN17} and D_{GP19} are given in Table 7.6. The developed sensors were found to be particularly selective to the drug in presence of ions such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, citric acid, lactose, urea, ascorbic acid and glycine. None of the examined ions were found to interfere as shown by the

small values of $K_{A,B}^{pot}$. The values indicate that the developed DEX selective sensors are highly selective to DEX over a number of ions tested.

7.8 Shelf life or Life time

The operative lifetime of the developed sensors D_{P7}, D_{N2}, D_{GN17} and D_{GP19} were studied. The life time of the sensor D_{P7} was found to be 4 weeks whereas the shelf life of the other three sensors was found to be 3 weeks. During this time the sensors showed no deviation from the optimized response characteristics. The working surface of the CPEs can however be renewed by squeezing out small amount of the paste and rubbing off the excess. The working surface can be polished using a filter paper. This can be conditioned and used. When not in use the sensors are dipped in 1×10^{-3} M DEX solution.

7.9 Analytical applications

The practical utility of the developed sensors D_{P7}, D_{N2}, D_{GN17} and D_{GP19} were examined. The sensors were applied for the determination of dextromethorphan content in syrup (Tusq-DX - Blue Cross - India). The detailed procedure is given in Section 2.5.2 in Chapter 2. The results consolidated in Table 7.7 clearly show that the developed sensors can be successfully applied for the determination of DEX in pharmaceutical formulations such as syrup. The results given in the table shows that there is a satisfactory agreement between the DEX content determined by the proposed method and the standard method[171]. The examined syrup contained chlorpheniramine maleate (2mg) and phenylephrine hydrochloride (5mg),

but from the studies conducted it has been observed that there was no interference from these also. The developed sensors were highly selective to the drug even in the presence of these excipients.

The sensors were also used for the determination of DEX in urine sample. The average % recovery of DEX using the developed sensors D_{P7}, D_{N2}, D_{GN17} and D_{GP19} has been found to be 98.7, 98.0, 100.7, and 97.3 respectively. The results are illustrated in Table 7.8. The % recovery values indicate that the sensors are selective to DEX even in presence of constituents that make up urine.

Phosphotungstic acid (PTA) and sodium tetraphenyl borate (NaTPB) were employed as the ion pairing reagents for the fabrication of PVC membrane sensor and carbon paste electrode for DEX. D_{N2} exhibited a Nernstian slope of 57.7 mV/decade within the concentration range 1.0×10^{-5} - 1.2×10^{-2} M. D_{N2} having a lower detection limit of 5.1×10^{-6} M had a response time of < 30 s. The lower detection limit of D_{P7} was observed to be 6.2×10^{-6} M and it exhibited a working concentration range of 1.1×10^{-5} – 5.0×10^{-2} M. A response time of < 35 s was observed for D_{P7}. The sensors D_{GN17} and D_{GP19} gave slopes of 60.3 and 57.0 mV/decade over the concentration ranges 1.5×10^{-5} - 5.0×10^{-2} M and 1.0×10^{-5} - 1.0×10^{-2} M. The lower detection limits for D_{GN17} and D_{GP19} has been 1.1×10^{-6} M and 1.9×10^{-6} M respectively. The shelf life for D_{N2}, D_{GN17} and D_{GP19} was observed to be 3 weeks whereas for D_{P7} it was observed to be 4 weeks. The working pH range has been found to be 5-8 for all the four sensors except D_{P7}, which had a pH range of 4-8. A response time of < 30 s and < 25 s was observed for D_{GN17} and D_{GP19}.

Table 7.1

Optimization of membrane ingredients for PVC membrane sensor for DEX based on DEX-NaTPB ion association

Membrane	Composition % (w/w)			Slope
	Ionophore	PVC	Plasticizer	mV/decade
D_{N1}	1.2	36.0	62.8, DBP	52.1
D_{N2}	**1.6**	**35.0**	**63.4, DBP**	**57.7**
D_{N3}	1.8	36.2	62.0, DBP	49.1
D_{N4}	2.0	30.1	67.9, DBP	47.2
D_{N5}	1.2	36.0	62.8, BEP	68.8
D_{N6}	1.6	35.0	63.4, BEP	67.5
D_{N7}	1.8	36.2	62.0, BEP	66.4
D_{N8}	2.0	30.1	67.9, BEP	65.8
D_{N9}	1.2	36.0	62.8, DBS	69.2
D_{N10}	1.6	35.0	63.4, DBS	70.6
D_{N11}	1.8	36.2	62.0, DBS	71.4
D_{N12}	2.0	30.1	67.9, DBS	72.0
D_{N13}	1.2	36.0	62.8, BES	65.8
D_{N14}	1.6	35.0	63.4, BES	69.3
D_{N15}	1.8	36.2	62.0, BES	70.8
D_{N16}	2.0	30.1	67.9, BES	72.9
D_{N17}	1.2	36.0	62.8, BEA	32.5
D_{N18}	1.6	35.0	63.4, BEA	41.7
D_{N19}	1.8	36.2	62.0, BEA	49.6
D_{N20}	2.0	30.1	67.9, BEA	40.8

Table 7.2

Optimization of membrane ingredients for PVC membrane sensor for DEX based on DEX-PTA ion association

	Composition % (w/w)			Slope
Membrane	Ionophore	PVC	Plasticizer	mV/decade
D_{P1}	1.2	36.0	62.8, DBP	48.7
D_{P2}	1.6	35.0	63.4, DBP	49.0
D_{P3}	1.8	36.2	62.0, DBP	63.4
D_{P4}	2.0	30.1	67.9, DBP	45.3
D_{P5}	1.2	36.0	62.8, BEP	50.1
D_{P6}	1.6	35.0	63.4, BEP	53.9
D_{P7}	**1.8**	**36.2**	**62.0, BEP**	**56.4**
D_{P8}	2.0	30.1	67.9, BEP	64.1
D_{P9}	1.2	36.0	62.8, DBS	63.3
D_{P10}	1.6	35.0	63.4, DBS	69.1
D_{P11}	1.8	36.2	62.0, DBS	72.6
D_{P12}	2.0	30.1	67.9, DBS	68.7
D_{P13}	1.2	36.0	62.8, BES	74.2
D_{P14}	1.6	35.0	63.4, BES	70.0
D_{P15}	1.8	36.2	62.0, BES	69.4
D_{P16}	2.0	30.1	67.9, BES	71.3
D_{P17}	1.2	36.0	62.8, BEA	42.3
D_{P18}	1.6	35.0	63.4, BEA	51.0
D_{P19}	1.8	36.2	62.0, BEA	49.4
D_{P20}	2.0	30.1	67.9, BEA	48.6

Table 7.3

Optimization of composition of the carbon paste sensor for DEX based on DEX- NaTPB ion association

Sensor	Composition % (w/w)			Slope
	Ionophore	Graphite	Plasticizer	mV/decade
D_{GN1}	2.0	34.5	63.5, DBP	45.8
D_{GN2}	2.5	51.0	46.5, DBP	47.1
D_{GN3}	2.8	61.2	36.0, DBP	49.3
D_{GN4}	3.0	49.7	47.3, DBP	32.9
D_{GN5}	2.0	34.5	63.5, BEP	72.0
D_{GN6}	2.5	51.0	46.5, BEP	75.6
D_{GN7}	2.8	61.2	36.0, BEP	76.3
D_{GN8}	3.0	49.7	47.3, BEP	77.4
D_{GN9}	2.0	34.5	63.5, DBS	69.4
D_{GN10}	2.5	51.0	46.5, DBS	70.2
D_{GN11}	2.8	61.2	36.0, DBS	73.3
D_{GN12}	3.0	49.7	47.3, DBS	74.9
D_{GN13}	2.0	34.5	63.5, BES	34.9
D_{GN14}	2.5	51.0	46.5, BES	35.2
D_{GN15}	2.8	61.2	36.0, BES	49.0
D_{GN16}	3.0	49.7	47.3, BES	36.5
D_{GN17}	**2.0**	**34.5**	**63.5, BEA**	**60.3**
D_{GN18}	2.5	51.0	46.5, BEA	50.4
D_{GN19}	2.8	61.2	36.0, BEA	63.1
D_{GP20}	3.0	49.7	47.3, BEA	65.2

Table 7.4

Optimization of composition of the carbon paste sensor for DEX based on DEX-PTA ion association

	Composition % (w/w)			Slope
Sensor	Ionophore	Graphite	Plasticizer	mV/decade
D_{GP1}	2.0	34.5	63.5, DBP	44.4
D_{GP2}	2.5	51.0	46.5, DBP	49.1
D_{GP3}	2.8	61.2	36.0, DBP	50.0
D_{GP4}	3.0	49.7	47.3, DBP	47.1
D_{GP5}	2.0	34.5	63.5, BEP	41.6
D_{GP6}	2.5	51.0	46.5, BEP	43.9
D_{GP7}	2.8	61.2	36.0, BEP	46.8
D_{GP8}	3.0	49.7	47.3, BEP	49.5
D_{GP9}	2.0	34.5	63.5, DBS	69.0
D_{GP10}	2.5	51.0	46.5, DBS	73.8
D_{GP11}	2.8	61.2	36.0, DBS	74.0
D_{GP12}	3.0	49.7	47.3, DBS	76.9
D_{GP13}	2.0	34.5	63.5, BES	42.3
D_{GP14}	2.5	51.0	46.5, BES	46.8
D_{GP15}	2.8	61.2	36.0, BES	47.9
D_{GP16}	3.0	49.7	47.3, BES	49.2
D_{GP17}	2.0	34.5	63.5, BEA	48.9
D_{GP18}	2.5	51.0	46.5, BEA	50.1
D_{GP19}	**2.8**	**61.2**	**36.0, BEA**	**57.0**
D_{GP20}	3.0	49.7	47.3, BEA	63.1

Table 7.5

Response characteristics of the sensors D_{P7}, D_{N2}, D_{GN17} and D_{GP19}

Parameter	Response Characteristics			
	D_{N2}	D_{P7}	D_{GN17}	D_{GP19}
Slope (mV decade^{-1})	57.7	56.4	60.3	57.0
Working concentration range (M)	1.0×10^{-5} - 1.2×10^{-2}	1.1×10^{-5} - 5.0×10^{-2}	1.5×10^{-5} - 5.0×10^{-2}	1.0×10^{-5} - 1.0×10^{-2}
Detection limit (M)	5.1×10^{-6}	6.2×10^{-6}	1.1×10^{-6}	1.9×10^{-6}
pH range	5-8	4-8	5-8	5-8
Shelf life	3 weeks	4 weeks	3weeks	3weeks
Response time(s)	< 30	< 35	< 30	< 25

Table 7.6

Selectivity coefficients for the sensors D_{P7}, D_{N2} D_{GN17} and D_{GP19} using fixed interference method.

Interfering ion (X)	$K_{A,B}^{pot}$			
	D_{N2}	D_{P7}	D_{GN17}	D_{GP19}
Na^+	1.1×10^{-4}	3.2×10^{-3}	6.3×10^{-3}	2.4×10^{-3}
K^+	5.5×10^{-3}	4.8×10^{-3}	1.9×10^{-4}	9.1×10^{-3}
Ca^{2+}	6.2×10^{-3}	3.9×10^{-3}	3.9×10^{-3}	1.6×10^{-4}
Co^{2+}	2.0×10^{-4}	7.4×10^{-3}	8.8×10^{-3}	3.5×10^{-3}
Mg^{2+}	6.4×10^{-3}	8.1×10^{-3}	4.9×10^{-3}	5.8×10^{-3}
Zn^{2+}	4.8×10^{-3}	6.6×10^{-3}	1.1×10^{-4}	5.2×10^{-3}
Citric acid	6.9×10^{-3}	1.6×10^{-4}	5.6×10^{-3}	6.0×10^{-3}
Lactose	7.5×10^{-3}	5.2×10^{-3}	7.0×10^{-3}	6.9×10^{-3}
Urea	4.8×10^{-3}	2.7×10^{-3}	5.9×10^{-3}	4.4×10^{-3}
Ascorbic acid	7.6×10^{-3}	8.6×10^{-3}	7.5×10^{-3}	7.0×10^{-3}
Starch	5.0×10^{-3}	7.3×10^{-3}	6.6×10^{-3}	1.0×10^{-4}
Talc	1.7×10^{-4}	9.4×10^{-3}	7.5×10^{-3}	6.2×10^{-3}

Table 7.7

Determination of DEX in pharmaceutical formulations

Sample	Declared Amount (mg/mL)	Method adopted	Found * (mg/mL)	SD*	CV*
TUSQ-DX (Blue Cross - India)	3	D_{N2}	3	0.13	4.32
		D_{P7}	3	0.11	3.66
		D_{GN17}	3	0.14	4.67
		D_{GP19}	3	0.13	4.33
		Standard Method	3	0.10	3.33

*Average of six replicates

Table 7.8

Determination of DEX in urine sample using the developed sensors

Drug added (M)	Sensor	Drug found* (M)	Recovery %
1.50×10^{-4}	D_{N2}	1.48×10^{-4}	98.7
	D_{P7}	1.47×10^{-4}	98.0
	D_{GN17}	1.51×10^{-4}	100.7
	D_{GP19}	1.46×10^{-4}	97.3

*Average of six replicates

Figure 7.1

Structure of the drug – Dextromethorphan

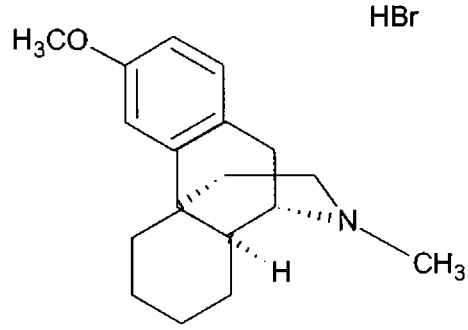


Figure 7.2

Calibration graph for DEX selective PVC membrane sensor based on DEX-NaTPB ion association (D_{N2})

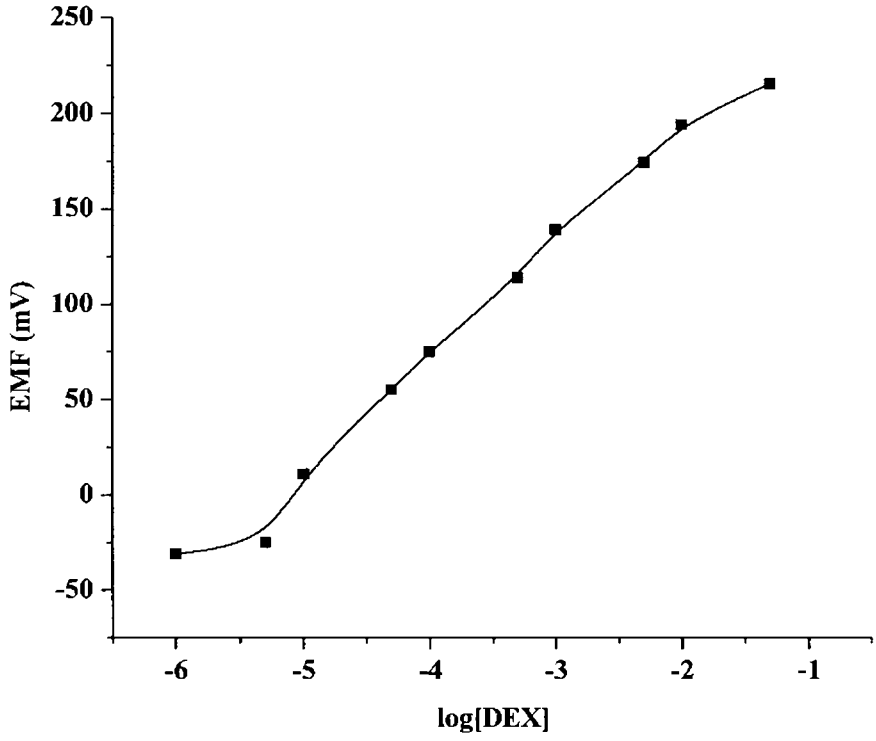


Figure 7.3

Calibration graph for DEX selective PVC membrane sensor based on DEX-PTA ion association (D_{P7})

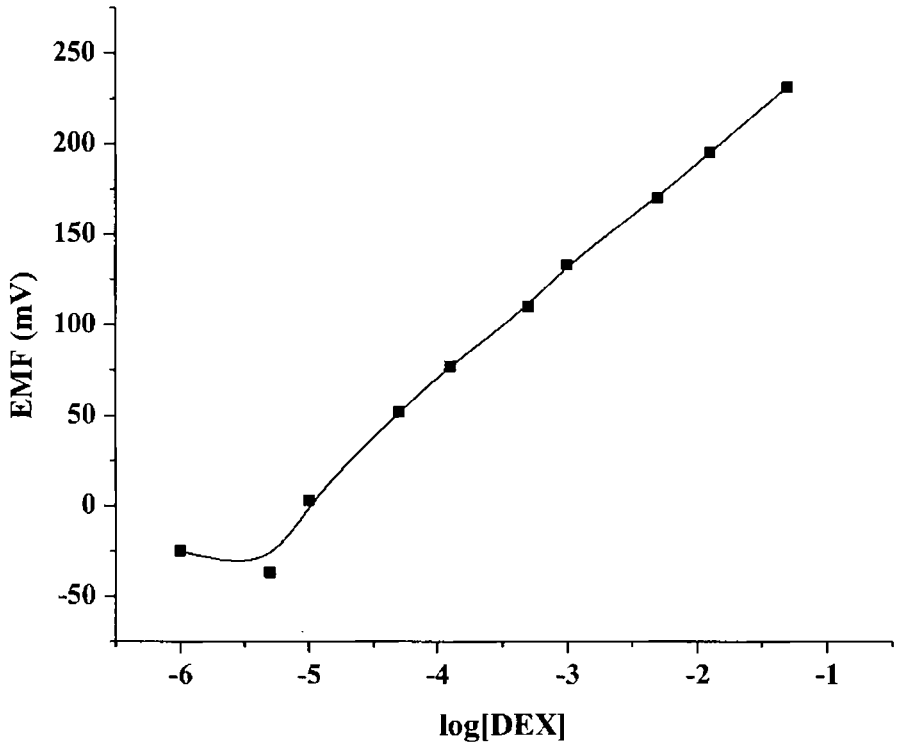


Figure 7.4

Calibration graph for DEX selective carbon paste sensor based on DEX-NaTPB ion association (D_{GN17})

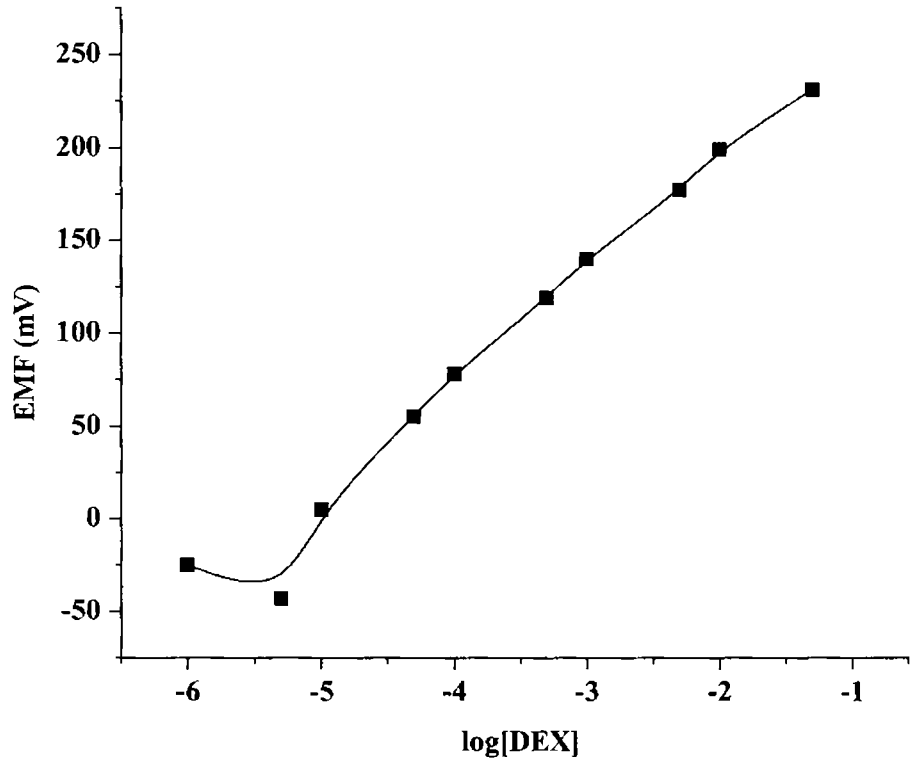


Figure 7.5

Calibration graph for DEX selective carbon paste sensor based on DEX-PTA ion association (D_{GP19})

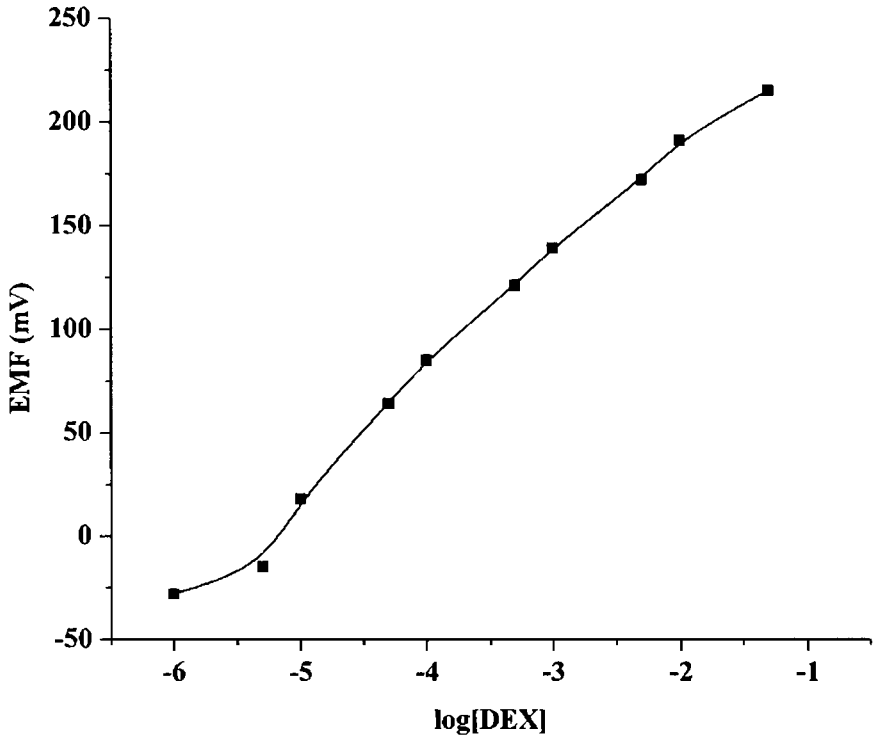


Figure 7.6

Effect of pH on the cell potential of the DEX membrane sensor based on DEX-NaTPB ion association (D_{N2})

1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

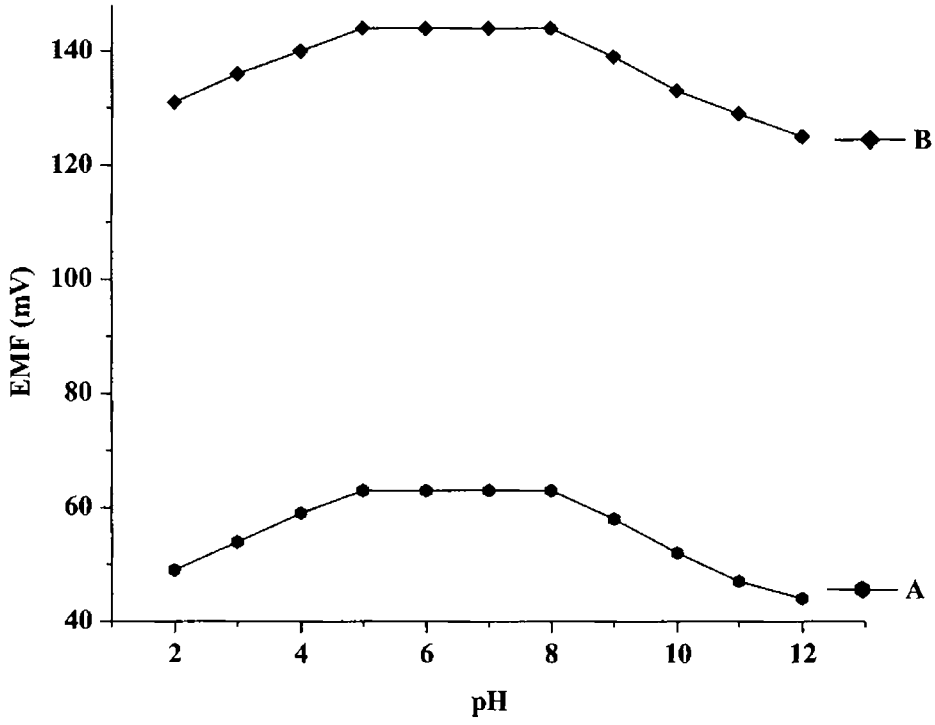


Figure 7.7

Effect of pH on the cell potential of the DEX membrane sensor based on DEX-PTA ion association (D_{P7})

1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

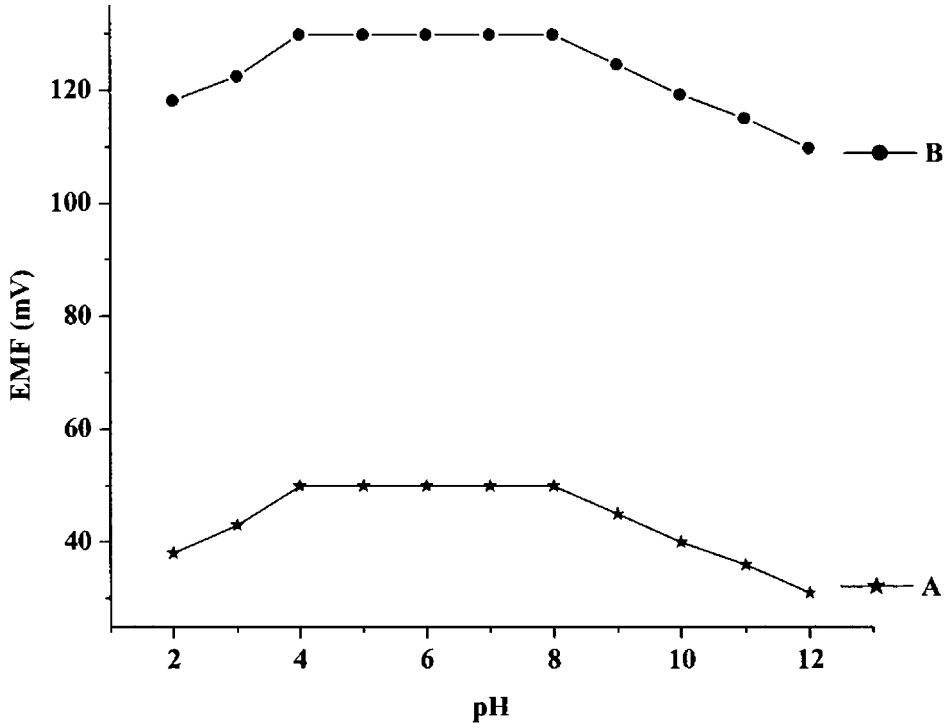


Figure 7.8

Effect of pH on the cell potential of the DEX selective carbon paste sensor based on DEX-NaTPB ion association (D_{GN17})

1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

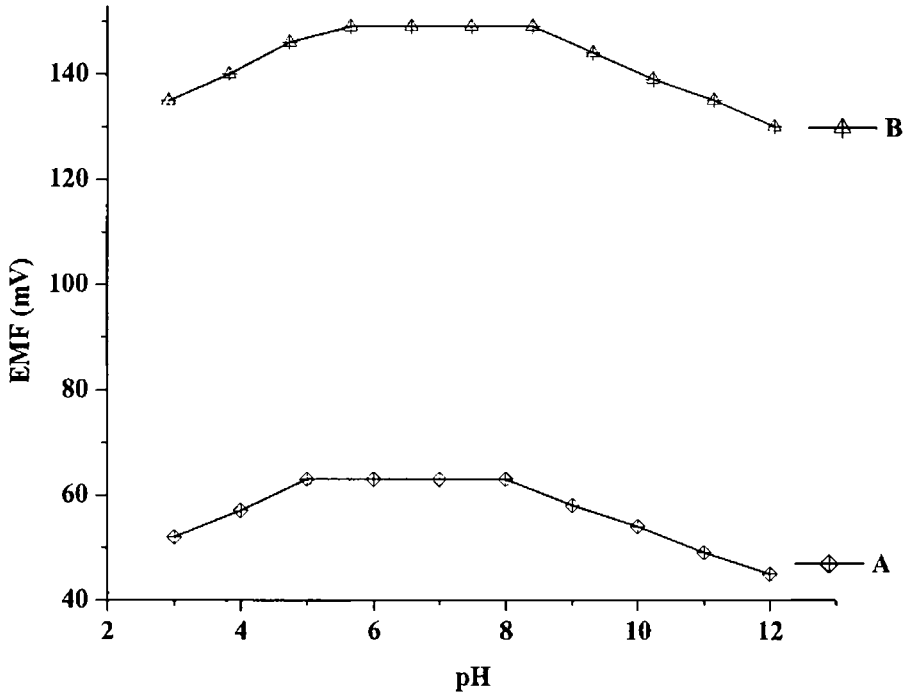
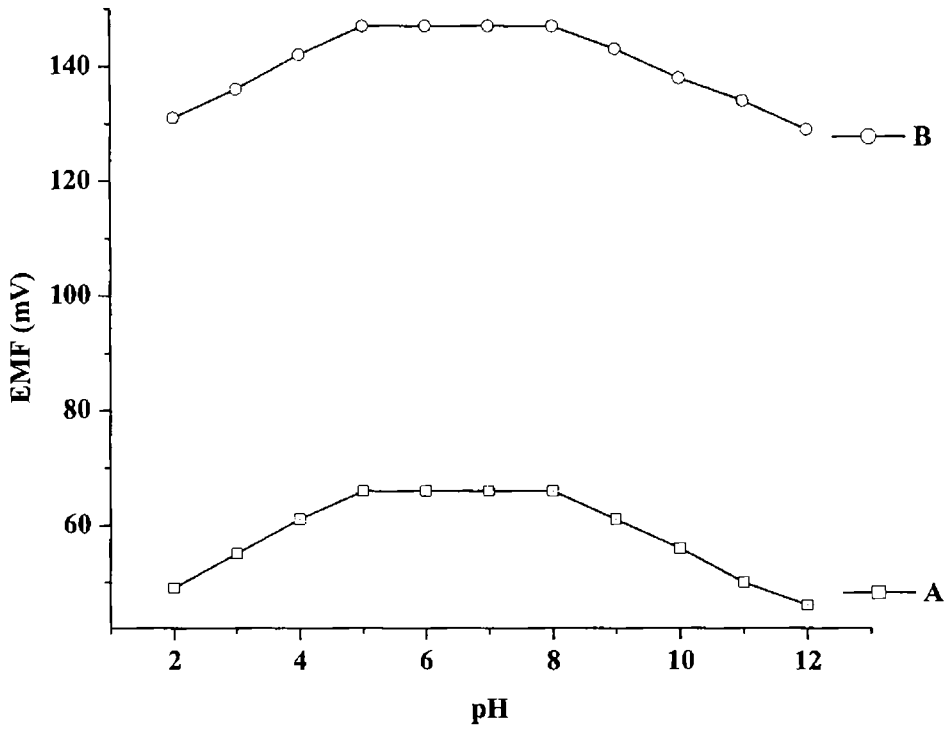


Figure 7.9

Effect of pH on the cell potential of the DEX selective carbon paste sensor based on DEX-PTA ion association (D_{GP19})

1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)



Chapter 8

Development of Sensors for Tetracycline

This chapter is devoted to the complete discussion on the development of two types of sensors for Tetracycline (TCE). The ion association of the drug with sodium tetraphenyl borate (NaTPB) has been employed for the fabrication of both the PVC membrane sensor and carbon paste electrode. The electrochemical response of the sensors is described in detail. Various response parameters such as effect of pH, response time, shelf life and selectivity studies were examined. The developed sensors were applied for the determination of TCE in pharmaceutical formulations and also in urine samples.

Tetracycline (TCE), as an antibiotic and bacterial drug is widely used owing to the broad spectrum of its antibacterial activity against nearly all gram positive and gram negative bacteria. It is effective against a wide variety of bacteria including *Haemophilus influenzae, Streptococcus pneumoniae, Mycoplasma pneumoniae, Chlamydia psittaci, Chlamydia trachomatis, Neisseria gonorrhoeae* and many others. TCE is often an alternative drug for people who are allergic to penicillin. Moreover TCEs could be applied to the diagnosis and therapeutics of carcinomas. It is used in combination with other medications to treat *Helicobacter pylori*, the bacteria associated with ulcers of stomach and duodenum. They also have non antibiotic properties, such as anti inflammatory effects, inhibition of

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metalloproteinases, reduce new blood vessel formation and reduce programmed cell death. Therefore the study of analytical methods for the determination of TCEs has been a noticeable project in the field of drug analysis[299-301].

The first drug of the TCE family chlortetracycline was introduced in 1948, by Dr. Benjamin Duggar a scientist employed by Lederle laboratories, who derived the substance from a golden coloured, fungus like, soil dwelling bacterium named *Streptomyces aureofaciens*. Oxytetracycline was discovered shortly afterwards by A.C. Finlay et al, it came from a similar soil bacterium named *Streptomyces rimosus*. Robert Burns Woodward determined the structure of oxytetracycline enabling Lloyd. H. Conover to successfully prepare TCE as a synthetic product. In June 2005, tigecycline, the first member of a new subgroup of TCEs named glycylcyclines was introduced to treat infections which were resistant to other antimicrobics.

TCE was the first therapeutically superior drug to be made by chemical alteration of an antibiotic produced by microbial metabolism.

Tetracyclines inhibit a lot of enzyme reactions essential for the vital processes of bacterial cells. The most sensitive biochemical reaction that is inhibited is the synthesis of proteins. Tetracycline works by binding specifically to the 30S ribosome of the bacteria, preventing attachment of the aminoacyl tRNA to the RNA-ribosome complex. It simultaneously inhibits other steps of the protein biosynthesis.

Tetracycline can also alter the cytoplasmic membrane and this in turn causes leakage of nucleotides and other compounds out of the cell. This does not directly kill the bacteria but instead inhibit it[302].

Chemically, Tetracycline hydrochloride is: (2-(amino-hydroxy-methylidene)-4-dimethylamino-6,10,11,12a-tetrahydroxy-6-methyl-4,4a,5,5a-tetrahydrotetracene-1,3,12-trione monochloride (Figure 8.1).

Many reactions and various techniques have been developed for the quantitative determination of TCE. Spectrophotometry based on the oxidation of TCE with vanadate[303] and chelation of TCE with metal ions[304], fluorimetry[305] and derivative fluorimetry[306] have been proposed. These methods require cumbersome pre treatment of the sample solution and limited selectivity. HPLC[307] and potentiometry[308] with ion-selective electrodes have been applied for the selective determination of TCE and show some remarkable advantages over other methods in the area of analysis[309].

In the present work, a plastic membrane electrode and a carbon paste electrode for TCE was developed based on a sodium tetraphenyl borate (NaTPB) ion association and the performance characteristics were studied. The developed sensors were also used for the determination of TCE in pharmaceutical preparations.

8.1 Preparation of the ion association

The sensor for the determination of TCE is based on an ion association of the drug with sodium tetraphenyl borate. The general procedure for the preparation of the ion association is given in Section 2.2 of Chapter 2. The ion association of TCE with NaTPB was prepared by mixing equimolar solutions of TCE and NaTPB. A 10^{-2} M solution of TCE and NaTPB was prepared and mixed together. The precipitate thus obtained was filtered, washed several times with distilled water and dried at room

temperature. The precipitate was stored in a desiccator. The structure of the ion association has been confirmed by elemental analysis.

Found (%) – C – 68.61, H – 4.61, N – 3.36

Calculated (%) – C – 69.01, H – 4.56, N – 3.40

8.2 Fabrication of the sensors

Two types of sensors, one based on the conventional PVC membrane and the other carbon paste electrode were prepared and studied for their electrochemical performances. These sensors incorporated the TCE - NaTPB ion association as the electroactive material.

8.2.1 Fabrication of PVC membrane sensor

The design of electrochemical sensors plays a very important role in their behaviour. As discussed in Chapter 2, Cragg's procedure[175] was followed in the preparation of PVC matrix membrane sensor. The first step of sensor fabrication consists of dissolving PVC, plasticizer and the sensing compound in THF. The resulting mixture was then poured in to glass rings stuck on a glass plate. Small disc shaped membranes were cut out and glued on to one end of a glass tube, using M-Seal and Araldite. It was left to dry and finally the prepared sensor was dipped in 1×10^{-3} M TCE solution. The glass tube was filled with solution consisting of 1×10^{-3} M TCE and 1×10^{-1} M NaCl. The prepared sensor was then left for conditioning for 24 hours.

Various ratios of the ionophore, PVC and plasticizer were taken to prepare the membrane as the composition of the matrix influences the reliability of the response of the electrode through membrane equilibrium. In the case of PVC membrane sensor, the composition ratio for the membrane that gave the best response in terms of slope, concentration range, response

time and detection limit was found to be 1.6:57.2:41:2 (ionophore : plasticizer : PVC) % (w/w) where the plasticizer used was DBP.

8.2.2 Fabrication of the carbon paste sensor

The modified carbon paste electrode represents a separate class of electrodes with high reliability of construction and with high potential in accommodation of molecules of different sizes. Their design is one of the most reliable and furthermore the construction is fast and easy[310].

Graphite and the ion association complex were thoroughly mixed in varying (% w/w) proportions. Acetone was added to this mixture and left to stand overnight for the acetone to evaporate completely. It was made into a paste using dibutyl phthalate. The paste was then packed into the open end of Teflon holder in which electrical contact was made with a copper rod through the centre of the electrode holder. The electrode surface was polished using a filter paper to produce reproducible working surface. The finished electrode was conditioned by dipping it overnight in 1×10^{-3} M drug solution.

8.3 Potential measurement and calibration

Potentials were measured at 25±1 °C on a Metrohm 781 ion meter. A saturated calomel reference electrode was used in conjunction with the developed sensor.

The cell assembly for potentiometric measurements can be represented as follows:

For PVC membrane sensor,

Internal reference electrode | internal filling solution (1×10^{-3} M drug solution + 1×10^{-1} M NaCl solution) | PVC membrane | test solution | external reference electrode.

For carbon paste sensor,

Reference electrode | test solution | Graphite electrode.

The performance of the developed sensor was investigated by measuring the potential in TCE solutions prepared in the concentration range 1.0×10^{-1} – 1.0×10^{-6} M. The solutions were stirred and the stable potential reading was taken.

8.4 Optimization of sensor matrix composition

The reliability of the sensor design is influenced by the matrix composition, electroactive material distribution in the membrane and the reliability of response characteristics[311].

Electroactive material has the primary role in sensor. The choice of the plasticizer has also been proved to be very important for the electrode response[312]. The PVC and plasticizer represent the matrix of the membrane which in turn forms the medium for the electroactive material.

A number of membrane compositions were investigated by varying the plasticizer and also the ratio of PVC, plasticizer and ionophore. A total of 25 membrane sensors were fabricated. Of the five different plasticizers viz; bis(2-ethyl hexyl) phthalate (BEP), Bis(2-ethyl hexyl) sebacate (BES), Di-n-butyl phthalate (DBP), Di-butyl sebacate (DBS) and Bis(2-ethyl hexyl) adipate (BEA) tested, DBP resulted in best slope (near Nernstian) (Table

8.1). The main purpose of the plasticizer is to lower the glass transition temperature of PVC and produce homogeneous and flexible films with good mechanical stability. It acts as a fluidizer allowing homogeneous dissolution and diffusional mobility of the ion association inside the membrane[313]. It has been found that sensor fabricated with DBP gave a near Nernstian slope of 52.2 mV/decade. The influence may be due to the polarity of the plasticizer which is estimated from the interaction of the charged species with a continuum of given dielectric constant[314, 292]. The other sensors gave either super Nernstian or sub Nernstian slope (Table 8.1).

The potentiometric response of the sensor towards TCE is found to be dependent on the concentration of the ionophore used. Hence different compositions of the ionophore were also tried inorder to evaluate their influence on the response characteristics of the membrane sensor. The results show that the electrode (S_3) made with 1.6% w/w ionophore exhibits the best performance characteristics in terms of slope (52.2 mV/decade), usable concentration range (1.0×10^{-5} - 2.0×10^{-3} M) and detection limit (6.7×10^{-6} M) (Figure 8.2).

The detection limit was calculated from the graph by the intersection of the two extrapolated linear segments of the calibration plot. Thus the sensor (S_3) with an optimum membrane composition of 1.6 : 57.2 : 41:2 % w/w of ionophore : plasticizer : PVC was chosen for further studies. Thus sensor (S_3) is found to be the best among the different PVC membrane sensors fabricated.

The weight ratio of graphite, ionophore and the plasticizer was varied to arrive at an optimum composition for CPE. In case of CPE (Table 8.2) the results showed that the sensor made of 3.0% ionophore exhibits the best

performance. The calibration plot of G_4 (Figure 8.3) gives a slope of 58.3 mV/decade. The working concentration range for G_4 is 2.9×10^{-5} - 2.0×10^{-3} M. The sensor gave a detection limit of 8.4×10^{-6} M and a good response time of < 40 s.

It has been found that the sensor G_4 with the composition ratio 3.0 : 51.8 : 45.2 (ionophore : graphite : DBP) % w/w was best in terms of slope, detection limit, concentration range, response time and this sensor was used for further studies.

The response characteristics of the sensors S_3 and G_4 are summarized in Table 8.3.

8.5 Effect of concentration of internal filling solution

The potential response of the proposed PVC membrane sensor was examined at different concentrations of the internal filling solution. The concentration of the internal solution of TCE in the sensor was changed from 1.0×10^{-3} M to 1.0×10^{-4} M and the potential response of the developed sensor was measured. It was found that variation of concentration of internal filling solution does not cause any significant difference in potential response of the sensor. A 1.0×10^{-3} M concentration of internal solution was quite appropriate for the proper functioning of the sensor.

8.6 Effect of pH

The influence of pH on the potential response of the sensors at two different concentrations, 1.0×10^{-3} M and 1.0×10^{-4} M were tested in the pH range 1 - 10 for S_3 (Figure 8.4) and for G_4 (Figure 8.5). The pH of the

solutions were adjusted using buffer solutions. As seen from the figures, the potential remains constant from pH 2 - 3 and 6 - 7 in either case. It is known that TCEs are unstable due to either forming the isomeric compounds containing inner ether at basic solution or undergo dehydration at acidic solution. So the pH range 6 - 7 has been used as the working pH range of both the sensors.

8.7 Potentiometric selectivity

Selectivity is one of the basic characteristics of the electrochemical sensors. The selectivity of an ion pair based sensor depends on the physico-chemical characteristics of the ion exchange process at the sensor – sample solution interface and the mobility of the respective ions in the sensor

The selectivity of the sensors is related to the free energy of transfer of the TCE drug cation between aqueous and organic phases. The degree of selectivity of the electrochemical sensors is given by the potentiometric selectivity coefficients ($K_{A,B}^{pot}$). The response of the sensors towards different substances has been checked and the selectivity coefficient values ($K_{A,B}^{pot}$) was used to evaluate the degree of interference. The potentiometric selectivity coefficients of the sensors S_3 and G_4 were determined by the Fixed Interference Method (FIM)[315,316]. The selectivity coefficient values are given in Table 8.4. The selectivity coefficient values indicate that the developed sensor is selective towards TCE over a number of foreign substances as none of the tested substances were found to interfere. It has been found that there was no interference from the ions tested such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, citric acid, lactose, urea, ascorbic acid and

glycine. The sensors were found to be selective to the drug in the presence of a number of tablet excipients such as starch and talc.

8.8 Shelf life or Life time

The practical shelf life of the developed sensors was also investigated. The practical life of the sensor S_3 was found to be 6 weeks while that of G_4 was 4 weeks.

8.9 Analytical applications

The proposed membrane sensors were employed for the assay of TCE content in tablets (Resteclin - NPIL - India and Tetracycline - Dabur - India). The detailed procedure is given in Section 2.5.1 of Chapter 2. Assay of TCE in pharmaceutical formulations using the proposed method was carried out and compared with the spectrophotometric method[170]. The results are summarized in Table 8.5. The data given in the table clearly indicate a satisfactory agreement between the TCE content determined by the proposed sensors and by the reported method.

The developed sensors were applied for the determination of the drug from urine samples. The average % recovery of the drug using the sensors S_3 and G_4 has been found to be 99.3 and 98.7 respectively. The results are given in Table 8.6. The results show that the sensors are highly selective to the drug.

The ionophore employed for the fabrication of the PVC membrane sensor and carbon paste electrode for TCE was the ion association of the drug with sodium tetraphenyl borate (NaTPB). S_3 gave a slope of 52.2

mV/decade over a concentration range of $1.0 \times 10^{-5} - 2.0 \times 10^{-2}$ M and a lower detection limit of 6.7×10^{-6} M. The working concentration range of G_4 having a slope of 58.3 mV/decade has been found to be $2.9 \times 10^{-5} - 2.0 \times 10^{-3}$ M. The lower detection limit of 8.4×10^{-6} M was achieved for G_4. S_3 and G_4 have been found to work over a pH range of 6-7. The shelf life for S_3 having a response time of < 30 s has been found to be 6weeks whereas for G_4 it was found to be 4 weeks. The response time of G_4 has been observed to be < 40 s.

Table 8.1

Optimization of membrane ingredients for PVC membrane sensor

	Composition % (w/w)			Slope
Membrane	Ionophore	PVC	Plasticizer	mV/decade
S_1	1.0	33.5	65.5, DBP	49.1
S_2	1.2	46.0	52.8, DBP	38.1
S_3	**1.6**	**41.2**	**57.2, DBP**	**52.2**
S_4	1.8	63.5	34.7, DBP	68.9
S_5	2.0	40.8	57.2, DBP	71.0
S_6	1.0	33.5	65.5, BEP	50.4
S_7	1.2	46.0	52.8, BEP	39.0
S_8	1.6	41.2	57.2, BEP	42.2
S_9	1.8	63.5	34.7, BEP	67.4
S_{10}	2.0	40.8	57.2, BEP	43.8
S_{11}	1.0	33.5	65.5, DBS	74.1
S_{12}	1.2	46.0	52.8, DBS	72.3
S_{13}	1.6	41.2	57.2, DBS	63.5
S_{14}	1.8	63.5	34.7, DBS	69.3
S_{15}	2.0	40.8	57.2, DBS	74.9
S_{16}	1.0	33.5	65.5, BES	48.6
S_{17}	1.2	46.0	52.8, BES	69.4
S_{18}	1.6	41.2	57.2, BES	65.0
S_{19}	1.8	63.5	34.7, BES	49.9
S_{20}	2.0	40.8	57.2, BES	68.1
S_{21}	1.0	33.5	65.5, BEA	37.9
S_{22}	1.2	46.0	52.8, BEA	73.4
S_{23}	1.6	41.2	57.2, BEA	47.6
S_{24}	1.8	63.5	34.7, BEA	65.8
S_{25}	2.0	40.8	57.2, BEA	48.6

Table 8.2

Optimization of composition of carbon paste sensor

	Composition % (w/w)			Slope
Sensor	Ionophore	Graphite	Plasticizer	mV/decade
G_1	2.0	47.9	50.1, DBP	50.5
G_2	2.5	48.0	49.5, DBP	70.0
G_3	2.8	49.2	48.0, DBP	73.2
G_4	**3.0**	**51.8**	**45.2, DBP**	**58.3**
G_5	2.0	47.9	50.1,BEP	**49.8**
G_6	2.5	48.0	49.5, BEP	46.6
G_7	2.8	49.2	48.0, BEP	39.0
G_8	3.0	51.8	45.2, BEP	35.2
G_9	2.0	47.9	50.1, DBS	63.9
G_{10}	2.5	48.0	49.5, DBS	68.6
G_{11}	2.8	49.2	48.0, DBS	69.0
G_{12}	3.0	51.8	45.2, DBS	70.1
G_{13}	2.0	47.9	50.1, BES	69.9
G_{14}	2.5	48.0	49.5, BES	72.6
G_{15}	2.8	49.2	48.0, BES	75.8
G_{16}	3.0	51.8	45.2, BES	77.4
G_{17}	2.0	47.9	50.1, BEA	49.2
G_{18}	2.5	48.0	49.5, BEA	47.6
G_{19}	2.8	49.2	48.0, BEA	39.0
G_{20}	**3.0**	**51.8**	45.2, BEA	36.1

Table 8.3

Response characteristics of the sensors S_3 and G_4

Parameter	Response Characteristics	
	S_3	G_4
Slope (mV/decade)	52.2	58.3
Working concentration range (M)	$1.0\times10^{-5} - 2.0\times10^{-2}$	$2.9\times10^{-5} - 2.0\times10^{-3}$
Detection limit (M)	6.7×10^{-6}	8.4×10^{-6}
pH range	6-7	6-7
Shelf life	6 weeks	4 weeks
Response time(s)	< 30	< 40

Table 8.4

Selectivity coefficients for the sensors S_3 and G_4 using fixed interference method.

Interfering ion (X)	$K_{A,B}^{pot}$	
	S_3	G_4
Na^+	2.9×10^{-2}	1.5×10^{-2}
K^+	3.6×10^{-2}	2.8×10^{-2}
Ca^{2+}	2.0×10^{-3}	2.7×10^{-3}
Co^{2+}	1.3×10^{-2}	4.6×10^{-3}
Zn^{2+}	7.7×10^{-3}	6.4×10^{-3}
Mg^{2+}	1.2×10^{-2}	3.7×10^{-2}
Glycine	2.3×10^{-3}	8.4×10^{-2}
Talc	8.1×10^{-3}	7.2×10^{-3}
Citric acid	1.1×10^{-4}	9.7×10^{-3}
Lactose	3.1×10^{-4}	1.5×10^{-4}
Urea	7.2×10^{-3}	8.1×10^{-3}
Ascorbic acid	5.6×10^{-3}	7.0×10^{-3}
Starch	4.9×10^{-3}	6.6×10^{-3}

Table 8.5

Determination of TCE in pharmaceutical formulations

Sample	Declared amount (mg/tablet)	Method adopted	Found * (mg/tablet)	SD*	CV*
Tetracycline (Dabur- India)	250	PVC membrane sensor	249	0.22	0.08
		Carbon paste sensor	245	0.53	0.22
		Standard Method	248	0.62	0.25
Resteclin (NPIL- India)	250	PVC membrane sensor	247	0.26	0.10
		Carbon paste sensor	248	0.26	0.15
		Standard Method	248	0.38	0.03

*Average of six replicates

Table 8.6

Determination of TCE in urine sample using the developed sensors

Drug added (M)	Sensor	Drug found* (M)	Recovery %
1.50×10^{-4}	S_3	1.49×10^{-4}	99.3
	G_4	1.48×10^{-4}	98.7

*Average of six replicates

Figure 8.1

Structure of the - Tetracycline

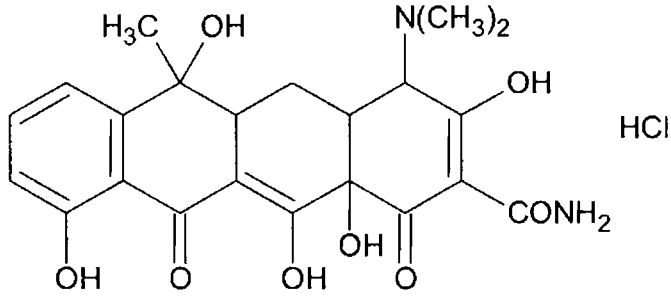


Figure 8.2

Calibration graph for TCE- selective PVC membrane sensor (S_3)

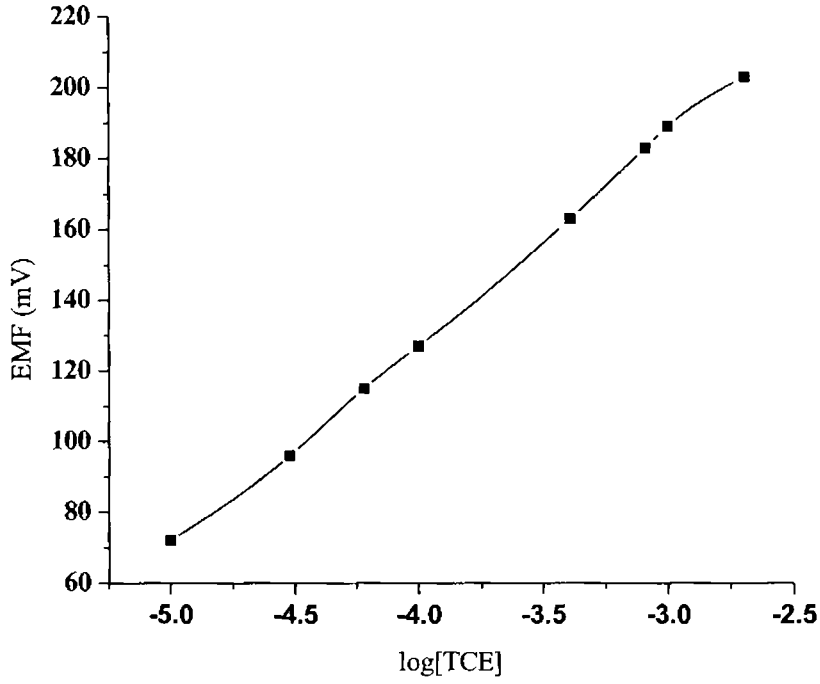


Figure 8.3

Calibration graph for TCE- selective carbon paste sensor (G_4)

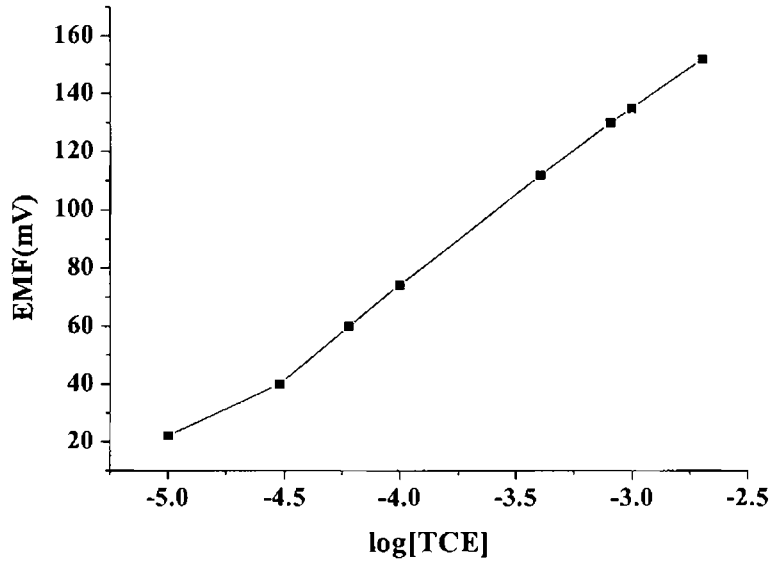


Figure 8.4

Effect of pH on the cell potential of the TCE membrane sensor (S_3) 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

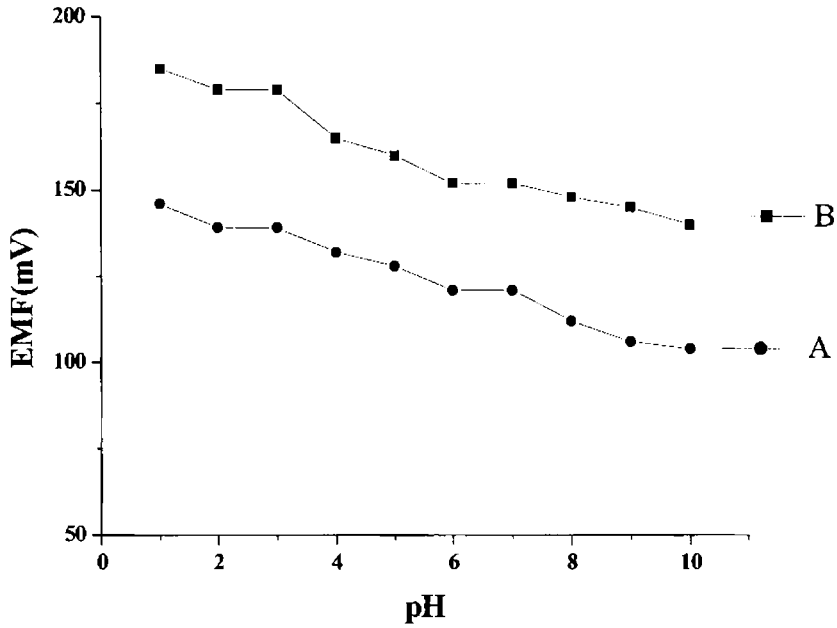
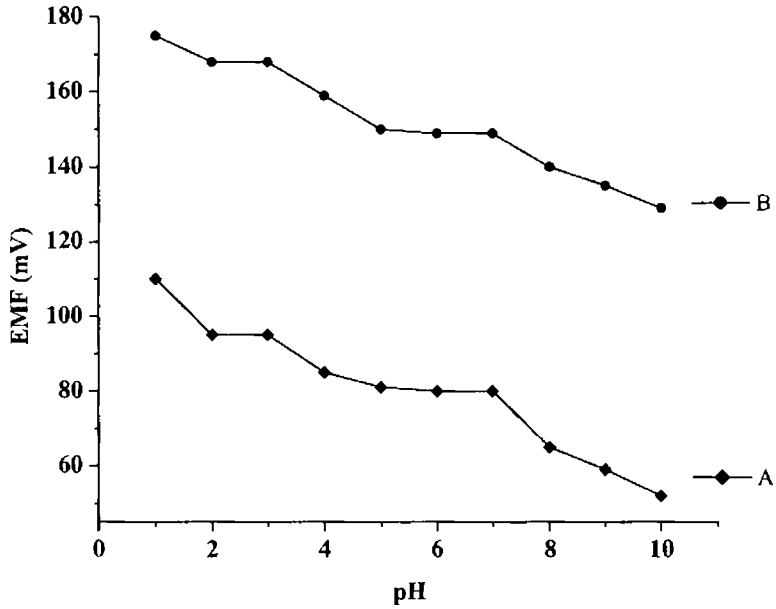


Figure 8.5

Effect of pH on the cell potential of the TCE carbon paste sensor (G_4) 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)



Chapter 9

Conclusions

This chapter discusses in brief the important results obtained as part of the investigations that was carried out. A brief outline of the various sensors fabricated for the different drugs are given in this chapter.

The present study was aimed at the fabrication of potentiometric sensors for the drugs mebendazole, pefloxacin, ambroxol, sildenafil citrate, dextromethorphan and tetracycline.

The main objectives of the work are:

(1) Synthesis of the ion association

(2) Characterization of the ion association using elemental analysis

(3) Fabrication of the different types of sensors: - PVC membrane sensors and Carbon paste electrodes (CPEs)

(4) Investigation of the response of the developed sensors towards the corresponding drugs

(5) Optimization of the sensor matrix composition

(6) Study on the response characteristics of the developed sensors such as effect of pH, shelf life, response time, effect of concentration of the internal filling solution and selectivity.

(7) Analytical applications of the developed sensors

A total of 18 sensors have been developed for the determination of the drugs mebendazole, pefloxacin, ambroxol, sildenafil citrate, dextromethorphan and tetracycline. The major step in the fabrication of the sensor was the preparation of the ion association. Two types of sensors viz; PVC membrane sensor and carbon paste electrode (CPE) were fabricated. The general method of fabrication of the PVC membrane sensor was in accordance with a procedure reported by Cragg's and Moody. The response characteristics of the different sensors fabricated were studied. Various response parameters such as response time, lifetime, selectivity and the effect of pH were studied. The sensor matrix composition was optimized by varying the ratio of the PVC/graphite, plasticizer and the ionophore. The developed sensors were also employed for the determination of the drugs in pharmaceutical formulations and also for the recovery of the drug from urine samples. The selectivity studies reveal that the developed sensors are highly selective to the drug even in presence of foreign ions.

The eighteen sensors developed for the drugs are:

Mebendazole – PVC membrane sensor on the ion associations of the drug with PTA, STA and MPA (3 nos)

Pefloxacin - PVC membrane sensor and CPE based on the ion associations of the drug with STA and MPA (4 nos)

Ambroxol - CPE based on the ion associations of the drug with PTA and MPA (2 nos)

Sildenafil citrate - PVC membrane sensor and CPE based on the ion associations of the drug with PTA and STA (3 nos)

Dextromethorphan - PVC membrane sensor and CPE based on the ion associations of the drug with PTA and NaTPB (4 nos)
Tetracycline - PVC membrane sensor and CPE based on the ion association of the drug with NaTPB (2 nos)

Potentiometric chemical sensors, an important class of electrochemical sensors are widely used in pharmaceutical analysis because of its inherent advantages. Attempts are being made to miniaturize sensors. The field of potentiometric sensors, as a mature technology, has experienced important changes in the past few years. The principal developments in this area focus on reducing the detection limit to true trace levels and there are important advances in the areas of materials and active components design. Clearly the results of the present investigations are highly promising and fruitful.

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Papers presented

1. Fabrication of a Novel Potentiometric Sensor for the Determination of Terazosin (National seminar on Emerging Trends and New Vistas in Chemistry, Calicut University, Kerala, 2005)
2. Differential Pulse Voltammetric Determination of Sparfloxacin in Pure Form and in Dosage Forms (National seminar on Emerging Trends and New Vistas in Chemistry, Calicut University, Kerala, 2005)
3. A novel Manganese selective plasticized membrane sensor using a Schiff base as ionophore (National seminar on Emerging Trends and New Vistas in Chemistry, Calicut University, Kerala, 2005)
4. Fabrication of a nickel ion sensor (National Seminar on Frontiers in Chemistry, Cochin University of Science and Technology, Kochi, India, 2006)
5. Fabrication of a novel tetracycline membrane sensor and its application to pharmaceutical analysis (National Seminar on Frontiers in Chemistry, Cochin University of Science and Technology, Kochi, India, 2006)
6. Fabrication of a PVC membrane sensor for the determination of nimesulide in pharmaceutical formulation (National Seminar on Frontiers in Chemistry, Cochin University of Science and Technology, Kochi, India, 2006)
7. PVC matrix membrane sensors for the determination of Dextromethorphan (International Conference on Materials for the Millennium-2007, Cochin University of Science and Technology, Kochi, India, 2007)