Development of Electrochemical Sensors for the Determination of Certain Pharmaceuticals

Thesis

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Certificate

Certified that the present work entitled "**Development of Electrochemical Sensors for the Determination of Certain Pharmaceuticals**", submitted by Ms. Pearl Augustine, 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 "**Development of Electrochemical Sensors for the Determination of Certain Pharmaceuticals**" is based on the original work carried out by me under the guidance of Dr. K. Girish Kumar, Head, 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.

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Pearl Augustine

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Preface

Electrochemical techniques are powerful and versatile analytical techniques that offer high sensitivity, accuracy, and precision as well as a large linear dynamic range, with relatively low-cost instrumentation. Electrochemistry is a well established and fast growing area with a number of possible applications in the pharmaceutical field. The improvement of quality of life has stimulated considerable research in drug design, bioavailability and safety. Thus, in order to achieve these targets, highly sensitive and specific methods of analysis are necessary. The society of today demands safe and cost effective manufacturing of a variety of high quality products with a minimum of negative effects on the environment. Electrochemical techniques are well suited for the determination of drugs in various samples, that is, raw material, pharmaceutical dosage forms even those involving a complex matrix such as syrups, tablets, creams, suppositories, or ointments or else in biological fluids. The principal advantage of the modern electrochemical methods is that the excipients do not interfere, and generally the separation and extraction procedure is not necessary.

Potentiometric sensors are an important class of electrochemical sensors in which the analytical information is obtained by converting the recognition process into a potential signal, which is proportional (in a logarithmic fashion) to the concentration (activity) of species generated or consumed in the recognition event. Such devices rely on the use of ion selective electrodes for obtaining the potential signal. The inherent selectivity of these devices is attributed to highly selective interactions between the membrane material and the target ion. Potentiometric sensors are very

attractive for field operations because of their high selectivity, simplicity and low cost.

The thesis presents the development, electrochemical characterization and analytical application studies of sixteen electrochemical sensors developed for six drugs viz., Trimethoprim, Ketoconazole, Lamivudine, Domperidone, Nimesulide and Lomefloxacin. Two different types potentiometric sensors have been developed in the study. These include both PVC membrane potentiometric sensor and carbon paste sensor.

Thus a total of 16 sensors have been developed. The thesis is divided into nine chapters.

A brief idea of the chapters is given below.

Chapter 1 gives a general introduction on the various electroanalytical techniques and their application. The chapter gives an idea of the different types of chemical sensors and discusses in detail about electrochemical sensors. It also gives a brief review of the important potentiometric sensors developed for different drugs.

Chapter 2 gives a brief sketch of the materials and methods used in the investigations. The general method for the synthesis of different ion associations and also the methods used for the fabrication of the two types of sensors are described in the chapter. It also gives an idea of the general procedure for the analysis of drug content in pharmaceutical formulations and also in real samples like urine. The instruments used in the present study are also discussed.

Chapter 3 describes the fabrication of two carbon paste sensors for the quantitative determination of Trimethoprim (TMP) .The sensors incorporate the ion association of the drug with molybdophosphoric acid (MPA) and phosphotungstic acid (PTA) as electroactive materials. The analytical applications of the developed sensors in the determination of the drug in pharmaceutical formulations and real sample like urine was also clearly investigated.

Chapter 4 deals with the development of two novel electrochemical sensors for the determination of the drug Ketoconazole (KET) based on KET-MPA (molybdophosphoric acid) ion pair as the electroactive material. The electrochemical response characteristics are described in detail and the application study of the developed sensors in the determination of the drug in pharmaceuticals and urine samples have also been dealt with in detail.

Chapter 5 deals with the development of sensors for the drug Lamivudine (LAM) based on the ion pair complexes of the drug with molybdophosphoric acid (MPA) and phosphotungstic acid (PTA). The response parameters of the newly developed sensors as well as their analytical applications have been discussed clearly in this chapter.

Chapter 6 presents the fabrication and response behaviour of the sensors developed for the drug Domperidone (DOM) based on the ion association complex DOM-PTA (phosphotungstic acid). The analytical applications of the developed sensors in the determination of pharmaceutical formulations and real samples have also been discussed in this chapter.

Chapter 7 deals with the development of sensors for the drug Nimesulide (NIM) based on the ion pair complexes of the drug with molybdophosphoric acid (MPA) and silicotungstic acid (STA). Optimization of membrane and carbon paste composition, response characteristics and analytical applications are dealt with in detail in this chapter.

Chapter 8 discusses the development and performance characteristics of membrane sensors for the drug Lomefloxacin (LOM) based on the ionophores LOM-STA and LOM-MPA. The application studies of the developed sensors in the determination of the drug in pharmaceutical formulations and urine samples are also explained in the chapter.

Chapter 9 presents the summary and important conclusions of the work done.

References are given as a separate section at the end of the thesis.

Contents

Chapter 1

INTRODUCTION

Analytical Chemistry is the study of the chemical composition of natural and artificial materials. It is a sub discipline of chemistry that has the broad mission of understanding the chemical composition of all matter and developing the tools to elucidate such compositions[1]. This differs from other sub disciplines of chemistry in that it is not intended to understand the physical basis for the observed chemistry as with physical chemistry and it is not intended to control or direct chemistry as is often the case in organic chemistry and it is not necessarily intended to provide engineering tactics as are often used in material science. Analytical chemistry generally does not attempt to use chemistry or understand its basis; however, these are common outgrowths of analytical chemistry research. It has a significant overlap with other branches of chemistry, especially those that are focused on a certain broad class of chemicals, such as organic chemistry, inorganic chemistry or biochemistry, as opposed to a particular way of understanding chemistry, such as theoretical chemistry. Analytical chemistry is particularly concerned with the questions of "what chemicals are present, what are their characteristics and in what quantities are they present?" These questions are often involved in questions that are more dynamic such as what chemical reaction an enzyme catalyzes or how fast it does it, or even more dynamic such as what is the transition state of the reaction. Although analytical chemistry addresses these types of questions, it stops after they are answered. The next logical steps of understanding what it

means, how it fits into a larger system, how can this result be generalized into theory or how it can be used are not analytical chemistry. Since analytical chemistry is based on firm experimental evidence and limits itself to some fairly simple questions to the general public it is most closely associated with hard numbers such as how much lead is in drinking water.

Chemical Analysis may be defined as the application of a process or a series of processes in order to identify and/or quantify a substance, the components of a solution or mixture or the determination of structures of chemical compounds. Chemical analysis generally consists of a chain of procedures to quantify and / or identify one or several components in a sample of matter. The needs for improved analytical methods are increasing, especially for compounds with known or possible effects on human health due to increasing number of environmental pollutants, drugs and their metabolites, and additives used in the food industry.

With increasing demands for pure water, better food control and cleaner atmospheres, the analytical chemist has a greater and greater role to play within modern society. From the study of raw materials such as crude oil and minerals to the finest quality scents and perfumes, the analytical chemist is called upon to play a part in determining composition, purity and quality[2]. Manufacturing industries rely upon both quantitative and qualitative chemical analysis to ensure their raw meet certain specifications and to check the quality of final product. These needs place high demands on the analytical methods employed, which must be efficient, accurate and predominantly automated. Recent advances in instrumentation and the range of detectors available enable analytical scientists to measure and identify target analytes at lower and lower concentrations. Thus the scope of analytical chemistry is

very broad and embraces a wide range of manual, chemical and instrumental techniques and procedures. The objective and purpose of the analysis has to be sensibly assessed before selecting an appropriate procedure.

1.1 Different Methods of Analytical Techniques

A qualitative method in analytical chemistry yields information about the identity of atomic or molecular species or the functional groups in the sample. Whereas a quantitative method in contrast provides numerical information as to the relative amount of one or more of these constituents[3]. The main techniques employed in quantitative analysis are based on

(i) The quantitative performance of suitable chemical reactions and either measuring the amount of reagent needed to complete the reaction product obtained.

(ii) Appropriate electrical measurements

(iii) The measurement of certain spectroscopic properties

(iv) The characteristic movement of a substance through a defined medium under controlled conditions.

The quantitative execution of chemical reactions is the basis of the traditional or classical methods of chemical analysis: gravimetry, titrimetry and volumetry. In gravimetric analysis the substance being determined is converted into an insoluble precipitate which is collected and weighed. In electrogravimetry, electrolysis is carried out and the material deposited on one of the electrodes is weighed. Some common techniques record a parameter as a function of temperature or time. Thermogravimetry records the change in weight, differential thermal analysis record the difference in temperature between the test substance and an inert reference material. Differential

scanning calorimetry records the energy needed to establish a zero temperature difference between a test substance and a reference material.

The titrimetric analysis is carried out by determining the volume of a solution of accurately known concentration which is required to react quantitatively with a measured volume of a solution of the substance to be determined. Volumetry measures the volume of a gas evolved or absorbed in a chemical reaction.

Spectroscopic methods of analysis depend on measuring the amount of radiant energy of a particular wavelength absorbed by the sample, or measuring the amount of radiant energy of a particular wavelength emitted by the sample. Atomic absorption spectroscopy (AAS), atomic fluorescence spectroscopy (AFS), flame emission spectroscopy (FES) and inductively coupled plasma (ICP) make use of absorption/emission spectroscopy[4]. Chromatography encompasses a diverse and important group of methods that permit the scientist to separate closely related components of complex mixtures when many of these separations are impossible by other means[5].

Electroanalytical chemistry encompasses a group of quantitative analytical methods that are based upon the electrical properties of a solution of the analyte when it is made a part of an electrochemical cell[6]. These techniques are capable of producing exceptionally low detection limits and a wealth of characterization information describing electrochemically addressable systems. Electrochemical techniques are powerful and versatile analytical techniques that offer high sensitivity, accuracy, and precision as well as a large linear dynamic range. Electroanalytical measurements offer a number of important benefits [7]:

(a) selectivity and specificity

(b) selectivity resulting from the choice of electrode material

(c) high sensitivity and low detection limit

(d) possibility of furnishing results in real time or close to real time

(e) application as miniaturized sensors where other sensors may not be useful

Electrochemical measurements are two-dimensional, with the potential being related to qualitative properties (with thermodynamic or kinetic control) and the current related to quantitative properties (controlled either by mass transport process or reaction rates). Thus, compounds can be selectively detected by electrochemical methods. The principal criterion for electroanalytical measurements 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 circuit is sufficiently conducting.

1.2 Classification of Electroanalytical Techniques

Electroanalytical Techniques can be in general classified into three types. They are:

(1) Conductimetry

(2) Potentiometry

(3) Amperometry and voltammetry

1.2.1 Conductimetry:

Here the concentration of charge is obtained through measurement of solution resistance. This is therefore not species selective. It is useful when the total ion concentration is below a certain permissible maximum level or for use as an on-line detector after separation of mixture of ions by ion chromatography. Conductimetry measures the conductance of a solution, using inert electrodes, alternating current, and an electrical null circuit , thereby ensures no net current flow and no electrolysis. The concentration of ions in the solution is estimated from the conductance [8].

1.2.2 Potentiometry:

In potentiometry the measuring set up always consists of two electrodes: the measuring electrode, also known as the indicator electrode, and the reference electrode. Both electrodes are half-cells. When placed in a solution together they produce a certain potential. The equilibrium potential of an indicator electrode is measured against a selected reference electrode using a high impedance voltmeter, ie. effectively at zero current. Thus the current path between the two electrodes can be highly resistive. Potential-determining transitions always occur at the phase boundaries, e.g. between the solution and the electrode surface. 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 are of the order of 100 nanomoles per litre of the total concentration of the ion present in a particular oxidation state, although down to 10 picomolar differences in concentration can be measured.

1.2.3 Amperometry and Voltammetry:

In amperometry, a fixed potential is applied to the electrode, which causes the species to be determined to react and a current to pass. Depending on the potential that is applied, the magnitude of the current is directly proportional to the concentration. In amperometric titrations, the titrant undergoes reaction at the indicator electrode to produce a current which is proportional to the concentration of the electroactive substance. Detection limits in the micromolar region can be obtained.

The common characteristic of all voltammetric techniques is that they involve the application of a potential (E) to an electrode and the monitoring of the resulting current (i) flowing through the electrochemical cell. In many cases the applied potential is varied or the current is monitored over a period of time (t). Thus, all voltammetric techniques can be described as some function of E, i, and t. They are considered active techniques (as opposed to passive techniques such as potentiometry) because the applied potential forces a change in the concentration of an electroactive species at the electrode surface by electrochemically reducing or oxidizing it. The analytical advantages of the various voltammetric techniques include excellent sensitivity with a very large useful linear concentration range for both inorganic and organic species (10^{-12} to 10^{-1} M).

1.3 Sensors

A sensor can be defined as something which senses a particular analyte or a substance. It is a device which measures a physical quantity and converts it into a signal which can be read by an observer or by an instrument. Sensors are designed to detect and respond to an analyte in the gaseous, liquid or solid

state[9]. Sensors can be broadly classified into physical sensor and chemical sensor.

Physical sensors are sensitive to such physical responses as temperature, pressure, magnetic field, force and these do not have a chemical interface. Chemical sensors rely on a particular chemical reaction for their response.

A chemical sensor is a device which responds to a particular analyte in a selective way through a chemical reaction and can be used for the qualitative or quantitative determination of the analyte[10]. A useful definition for a chemical sensor is a small device that as the result of a chemical interaction or process between the analyte and the sensor device, transforms chemical or biochemical information of a quantitative or qualitative type into an analytically useful signal. The role of the chemical sensor is to provide information about the chemical state of the process and one can say that the chemical sensor is the "eye" of the process control system. Chemical sensors can also provide essential information about the chemical state of our environment. There are two parts to a chemical sensor - a region where selective chemistry takes place and the transducer.

1.4 Types of Chemical Sensors

Chemical sensors are categorized into the following groups depending on the transducer type

(1) Electrochemical

(2) Optical

(3) Mass sensitive

(4) Heat sensitive

1.4.1 Electrochemical Sensors

These include potentiometric sensors (ion selective electrodes, ion selective field effect transistors) and voltammetric / amperometric sensors including solid electrolyte gas sensors. Electrochemical sensors can be applied for solid, liquid, or gaseous analytes with the latter two most common[11].

1.4.2 Optical Sensors

In optical sensors there is a spectroscopic measurement associated with the chemical reaction. Optical sensors are often referred to as 'optodes' and the use of optical fibres is a common feature. Absorbance, reflectance, and luminescence measurements are used in the different types of optical sensors.

1.4.3 Mass Sensitive Sensors

These make use of the piezoelectric effect and include devices such as the surface acoustic wave sensor and are particularly useful as gas sensors. They rely on a change in mass on the surface of an oscillating crystal which shifts the frequency of oscillation. The extent of the frequency shift is a measure of the amount of material adsorbed on the surface.

1.4.4 Heat Sensitive Sensors

The heat of a chemical reaction involving the analyte is monitored with a transducer such as a thermistor or a platinum thermometer. They are often called calorimetric sensors.

Compared to optical, mass and thermal sensors, electrochemical sensors are especially attractive because of their remarkable detectability, experimental simplicity and low cost. They have a leading position among the presently available sensors that have reached the commercial stage and

which have found a vast range of important applications in the fields of clinical, industrial, environmental and agricultural analyses[12].

1.5 Potentiometric Sensors

Potentiometric sensors come under the class of electrochemical sensors. They 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 the solution. The charge separation formed across the interface gives rise to an electrical potential difference. In potentiometric sensors, a local equilibrium is established at the sensor interface, where either the electrode or membrane potential is measured, and information about the composition of a sample is obtained from the potential difference between two electrodes. Potentiometric sensors have found the most widespread practical applicability since the early 1930s, due to their simplicity, familiarity and cost. There are three basic types of potentiometric sensors or devices: ion selective electrodes (ISEs), coated wire electrodes (CWEs) and ion selective field effect transistors (ISFETs).

1.5.1 Ion Selective Electrodes (ISEs)

The ion selective electrode is an indicator electrode capable of selectively measuring the activity of a particular ionic species. In the classic configuration, such electrodes are mainly membrane-based devices, consisting of permselective ion-conducting materials, which separate the sample from the inside of the electrode. One electrode is the working electrode whose potential is determined by its environment. The second electrode is a reference electrode whose potential is fixed by a solution containing the ion of interest at a constant activity. Since the potential of the reference electrode is constant, the value of the

potential difference (cell potential) can be related to the concentration of the dissolved ion. It is related to processes taking place at the membrane interface[13,14]. 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. In contrast to metal electrodes, an ISE does not measure a redox potential. If the ion to be measured is contained in the sample solution then this ion can penetrate the membrane. This alters the electrochemical properties of the membrane and causes a change in potential. One hundred percent selectivity for exactly one type of ion is only possible on rare occasions. Most ion-selective electrodes have only a particular sensitivity for a special type of ion, but also often react with ions with similar chemical properties or a similar structure.

1.5.2 Coated-Wire Electrodes (CWEs)

CWEs were first introduced in the mid of 1970's by Freiser[15,16]. In the classical CWE design, a conductor is directly coated with an appropriate ion-selective polymer membrane usually poly (vinyl chloride), poly (vinyl benzyl chloride) or poly (acrylic acid) to form an electrode system that is sensitive to electrolyte concentrations. The CWE response[17] 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 benefits during miniaturization, for example. This is particularly useful for the in vitro and in vivo biomedical and clinical monitoring of different kind of analytes.

1.5.3 Ion Selective Field Effect Transistors (ISFETs)

Ion selective field effect transistors work as an extension of CWE. ISFET incorporate the ion sensing membrane directly on the gate area of a field effect transistor (FET). The FET is a solid state device that exhibits high input impedance and low output impedance and therefore is capable of monitoring charge buildup on the ion sensing membrane. The construction is based on the technology used to fabricate microelectronic chips[18-19], and the great contribution is that it is possible to prepare small multisensor systems with multiple gates, for sensing several ions simultaneously, while their small size permits the in vivo determination of analytes.

There are generally four categories of membranes of ion selective electrode potentiometric sensors. These are:

1.5.1.1 *Glass Membrane*

The most widely used glass electrode is the pH electrode, which has been used for several decades. 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. Its success is attributed to a series of undisputed advantages, such as simplicity, rapidity, non destructiveness, low cost, applicability to a wide concentration range and, particularly, to its extremely high selectivity for hydrogen ions. Nevertheless, measurements of pH can also be performed using other types of potentiometric sensors. Application of glass electrodes for other monovalent cations, including sodium, lithium[20,21], ammonium and potassium sensors based on new glass compositions, have also been reported[22].

1.5.1.2 *Sparingly Soluble Inorganic Salt Membranes*

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

(i) Single crystal membranes.

(ii) Pressed powder membranes.

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

1.5.1.3 *Polymer-immobilized Ionophore Membranes*

In these, an ion-selective complexing agent or ion-exchanger is immobilized in a plastic matrix such as poly (vinyl chloride).

1.5.1.4 *Gel-immobilized and Chemically Bonded Enzyme Membranes*

These membranes use the highly specific reactions catalyzed by enzymes. The enzyme is incorporated into a matrix or bonded onto a solid substrate surface.

According to the nature of the substances affecting ion exchange in the membrane[23-25], ion selective electrodes can also be classified as (a) ion selective electrodes with solid membranes and (b) ion selective electrodes with liquid membranes.

In ion selective electrodes with solid membranes, the membrane can be either 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.

In the second case, the electrode membrane is represented by a water immiscible liquid, in which is dissolved a 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.

1.6 Potentiometric Ion Selective Electrodes

Ion selective sensors including ion selective membrane electrodes have been becoming one of the effective and powerful means for analytical scientists in the determination of drug substances and are playing an important role in pharmaceutical analysis[26-28] due to offering advantages of simplicity, rapidity and accuracy over more established pharmaceutical analysis methods. Moreover the interest in developing small sensing devices for biomedical use is growing rapidly[29]. The key problem associated with development of small or miniaturized ion selective sensors used for in vivo assay of drugs and for the determination of drug in a flow system is how to eliminate an internal reference electrode together with the corresponding inner filing electrolyte in the conventional polymeric membrane ion-selective electrodes. The response of most ion-selective electrodes (ISEs) has been described on the basis of the Nicolskii-Eisenman selectivity formalism[30-32].

The different independent achievements in the mid-1960s marked the starting point of modern potentiometry [33]. In 1967, Ross described the first membrane electrode based on a liquid ion exchanger [34]. James Ross and Martin Frant of Orion Research are the founding fathers of ISEs. Bloch and

co-workers introduced the first ionophore-based solvent polymeric membrane based on PVC [35], a matrix still widely used today. At about the same time, Stefanac and Simon discovered that antibiotics inducing selective ion transport through biological membranes also generate a selective potentiometric response in liquid membranes [36].

Liquid membrane electrode ISE, based on water immiscible liquid substances impregnated in a polymeric membrane, are widely used for direct potentiometric measurements of several polyvalent cations as well as certain anions. The polymeric membrane is used to separate the test solution from the inner compartment containing a solution of the target ion. The membrane-active recognition can be by a liquid ion exchanger[37] or by a neutral macrocyclic compound[38] having molecule–sized dimensions containing cavities to surround the target ions. The construction of a hydrogen ion selective potentiometric electrode based on a tridodecylamine ionophore dispersed in a poly (vinylchloride) membrane[39], or poly(1-aminoanthracene) films[40] has been described. 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[41-42]. Rodwedder *et al*[43] and Fatibello and co-workers[44-47] have shown the use of coated graphite epoxy ion selective electrodes for determination of cations using ion-pair formation with tricaprylmethylammonium cation in a PVC matrix. Using a similar system with incorporation of saccharinate anion and toluidine, Rover *et al*[48] have described the construction of a tubular ion selective electrode useful for determination of saccharin. Alfaya *et al*[49] described a more sensitive system for saccharin determination using a thin film of silsesquioxane 3-n-propylpyridinium chloride polymer coated on a

graphite rod. The successful use of thin film electrodes modified, by nickel(II) hexacyanoferrate, for potassium determination has been described by Stradiotto and co-workers[50].

1.7 Solid State Ion Selective Electrodes

Solid electrodes began to be used in electroanalysis about forty years ago[51]. Solid-state electrodes are miniaturized version of an electrochemical sensor. Solid state membrane electrodes are preferred over liquid membrane electrodes because they can easily be used in all kinds of media which are suitable for environmental analysis, food analysis, clinical analysis as well as for in vivo analysis.

Carbon materials in the form of graphite, glassy carbon, carbon fibres etc are important solid state electrodes for several reasons. This is because carbon has rich surface chemistry, which can be explored to influence reactivity. Also the adsorption on carbon surfaces can be used to enhance analytical utility. Adams, the inventor of CPEs[52], and his research group were the first who published an extensive study on carbon pastes comprising numerous test measurements[53,54]. Carbon paste electrodes (CPEs) belong to a special group of solid state ion selective electrodes. 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[55]. The biggest disadvantage of CPEs, which limits their applicability in practical analysis, is that the success in working with carbon-paste based electrodes depends on the experience of the user[56].

Mixtures containing merely two main components ie, carbon powder and the liquid binder are commonly classified as unmodified carbon pastes.

The modified graphite paste electrodes represent a class of electrodes with high reliability of the construction and with high potential in accommodation of molecules of different sizes. The base of modified carbon paste is usually a mixture of powered graphite and non electrolytic binder and modifying agent. The composition of modified carbon paste will influence the response characteristics of the designed electrode. Carbon paste based biosensors contain enzyme (or its carrier) together with appropriate mediator[57]. The main adavantages of CPEs is that it does not require any internal solution or internal reference electrode. Also the surface of the electrode can be renewed easily.

Thus it is possible to conclude that potentiometric sensors have been important since 1930, when the commercialization of a glass electrode resulted in the foundation of one of the most successful analytical instrument companies (Beckman Instruments). History also shows that, since 1960, when ion-selective electrodes revolutionized the approach to the difficult analysis of inorganic ions, up to now, the growth of patents for different formulations of glass, for different membrane types and for diverse shapes and sizes of electrodes testify to interest in the area. Therefore, there are many commercially available ion-sensing potentiometric devices. These systems tend to be low in cost, simple to use, easily automated for rapid sampling, with low interferences from the matrix, and can be applied to small volumes. These advantages make potentiometric sensors an ideal choice for both clinical and industrial measurements where speed, simplicity, and accuracy are essential.

1.8 Performance Factors of a Potentiometric Ion Selective Electrode

1.8.1 Slope of the Electrode

The slope, S (also called response of the electrode), is the main characteristic of the potentiometric electrodes. The ideal value of the slope is

given by Nernst: 59.16/z mV/decade of concentration, where z is the charge of the ion that has to be determined. This value can be computed from the Nernst equation.

$E = E^0 \pm (RT/zF) \log a$,

where E is the potential of the electrode, E^0 is the standard electrode potential, R = 8.31 J/mol K, F = 96500 C, T = 298 K, and a is the activity of the ion. From this equation, the slope of the potentiometric electrode is given by: S = RT/zF

Nernstian response implies ideal sensitivity, but not necessarily ideal selectivity since interfering ions may also give Nernstian response when present as the sole potential determining species. The slope is dependent on the stability of the compound formed at the membrane-solution interface[58]. The slope depends on some parameters which characterize the matrix such as polarity of the plasticizer, oil or solvent. The slope of the potentiometric electrodes can be improved by choosing a suitable selector that forms a compound with higher stability or by changing the composition of the matrix[59].

1.8.2 Limit of Detection

The value of the limit of detection can be deducted from the calibration graph as the concentration defined by the intersection of the extrapolated two linear regions of the curve. Experimental conditions such as composition of the test solution, history of the electrode and stirring rate should be recorded[60].

1.8.3 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[61].

1.8.4 Influence of pH

The pH can influence the formation of protonated and unprotonated species of the same substance. The pH plays a very important role in the response of the potentiometric sensors. Special care must be accorded to the buffering of solutions, because a small difference in pH may cause a significant change in the potential, and that will result in an error in the measurement.

1.8.5 Response Time

IUPAC defined the response time as the time which elapses between the instant when the electrodes of the potentiometric cells are brought into contact with the sample solution (or at which the activity of the ion of interest in solution is changed) 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 the accuracy[62]. The response time is influenced by the membrane-solution interface processes. The response time increases with decreasing the concentration of the molecule that has to be assayed.

1.8.6 Selectivity

Selectivity is one of the basic characteristics of the electrochemical sensors. It depends on the composition of the membrane (active sites as well as matrix), ratio between the activities of the main ion and interfering ion in the solution, complexity of the matrix of the sample that is analysed, current applied, and the pH of the solution. This property of electrochemical sensors

restricts their utilization for the assay of an ion from a complex matrix. IUPAC defined the interfering substance as any substance, other than the ion being determined, whose presence in the sample solution affects the measured emf of a cell. The degree of selectivity of an electrochemical sensor is given by the value of potentiometric selectivity coefficient.

1.8.7 Life Time or Shelf Life

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 [63]. 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.

1.9 Electroanalytical Techniques for Drugs

Electrochemical techniques are well suited for the determination of drugs in various samples, that is, raw material, pharmaceutical dosage forms even those involving a complex matrix such as syrups, tablets, creams, suppositories, or ointments or else in biological fluids. The principal advantage of the modern electrochemical methods is that the excipients do not interfere, and generally the separation and extraction procedure is not necessary. Thus, sample preparation usually consists of dissolving out the active ingredient from the pharmaceutical dosage form with a suitable solvent and performing direct analysis on an aliquot portion of this solution. In addition to the analytical aspect, electrochemistry allows the establishment of the electrochemical behaviour of a given drug through mechanistic studies. This is of particular interest knowledge of the drug. Electrochemical techniques are most suitable to investigate the redox properties of a new drug; this can give insights into its metabolic fate.

Progress in the pharmaceutical industry results in the creation of a great number of drugs including substances of various structures and compositions, differing in their pharmacological action and therapeutic properties. This situation poses a problem of controlling not only the quality of drugs but the content of drugs (and their metabolites) in various media as well, including biological liquids of the human and animal organism, food products, waste waters of pharmaceutical plants, etc. Applications of potentiometry techniques in pharmaceutical analysis and clinical chemistry have been reviewed by Vytras and Granzhan et al [64]. There is a constant increase in the number of electrodes capable of selectively identifying various drugs. The potentiometric determination of drugs can be based on either direct potentiometry or potentiometric titration. The detection is performed with the aid of transducers, sensitive to either inorganic or organic ions, including biosensors. However, the present study is mainly concentrated on the potentiometric methods of drug analysis using ISEs.

The methods for determining drug cations are mostly based on ISE involving associates between the cations and large ions such as tetraphenyl borate (TPB)[65,66], tris (octylhydroxy) benzenesulfonate (TOBS)[67], molybdophosphate (MPA)[68,69], and diphenylhydroxyacetate (DHAA)[70]. The anions of drugs are determined using electroactive substances with counterions represented by cations of tertiary ammonium bases. For example, benzyldimethylcetylammonium is used for determining antibiotics of the penicillin group[71,72] and tetraoctylammonium (TOA) is employed for the analysis of 5,5-diethylbarbiturates[73]. In the initial stage of development of drug ionometry, the investigations had a purely applied character. At present, most works are devoted to establishing the factors providing desired modification of the

properties of ion-selective membranes. In this context, interesting results were reported on the effects of the ion association[74] and the character of a plasticizing solvent[75] on the selective properties of counter electrodes for the organic cations of drugs.

If the ion to be determined forms more stable associates than does the interfering ion, the potentiometric selectivity was increased by introducing a considerable amount of a well dissociated ion exchanger salt with a highly hydrophobic cation into the membrane [76]. The counterions in the electroactive substances are mostly represented by heteropolycomplexes between cations of the drugs analyzed and anions of molybdophosphoric ($PMo_{12}O_{40}^{3-}$), molybdosilicic ($SiMo_{12}O_{40}^{4-}$), and molybdotungstogallic ($GaMo_2W_{10}O_{40}^{5-}$) acids. Study of the electrode characteristics depending on the nature of the central atom of a heteropolyanion showed that an increase in the negative charge of the heteropolyanion leads to decreasing sensitivity and selectivity of the electrodes and increasing potential drift. Optimum electrode characteristics were observed for the electrodes based on molybdophosphoric acid. The determination of drugs by the method of direct potentiometry with ISE offers a rapid and simple procedure satisfying all requirements of pharmaceutical analyses.

1.10 A Brief Review on Potentiometric Sensors for Drugs

The control of drug quality is a branch of analytical chemistry that has a wide impact on public health. So the development of reliable, quick and accurate methods for the determination of the active ingredients is welcomed. In recent years, ion selective membrane electrodes have been used more and more in drug quality control, but no pharmacopoeias yet introduced their use for assays, though this will probably be done in the next few years. An

important advantage of ion selective membrane electrodes is that they can in principle, be designed for any ionic species, even for organic ions with complicated structures and high molecular weight[77-79]. Ion selective electrodes, including ion selective membrane electrodes have been becoming one of the effective and powerful means for analytical scientists in the determination of drug substance and are playing an increasing role in pharmaceutical analysis[80-82], due to offering advantages of simplicity, rapidity and accuracy over more established pharmaceutical analysis methods. Most of the ISEs sensitive to medically important ionic compounds such as sulpha drugs[83], vitamins[84] and alkaloids[85] belong to the class of ion-pair based liquid membrane electrodes.

A liquid membrane electrode based on an amiodarone-dipicrylamine ion-pair complex was developed by Aboul-Enien *et al.* The electrode exhibited a Nernstian slope of 57.3 mV/decade with a detection limit of 4×10^{-9} M. The electrode was used for the determination of amiodarone in pharmaceutical dosage forms such as tablets[86].

S.S.M.Hassan and his group reported four novel potentiometric sensors for the selective determination of cinnarizine. These sensors are based on the use of the ion-association complexes of the cinnarizinium cation with tetraphenylborate, flavianate, reineckate and 12-molybdatophosphate counter anions as ion exchange sites in a plasticized PVC matrix. These sensors proved useful for determining cinnarizine in various dosage forms, monitoring tablet dissolution rates and testing tablet uniformity[87].

Hassan Y. Aboul-Enein and his co-workers reported the construction and the performance characteristics of an ion-selective PVC membrane electrode for flurbiprofen. The sensor exhibited a near-Nernstian response in

the concentration range 1×10^{-2} - 7×10^{-5}M. The active component flurbiprofen in pharmaceutical preparations were determined using the proposed electrode[88].

A mexiletine-sensitive membrane electrode based on crown ether and ion exchanger was described by T. Katsu *et al.* The electrode exhibited high selectivity against many inorganic cations. The sensor was used to determine mexiletine content in saliva[89].

M. A. Ahmed and M.M. Elbeshlawy proposed five novel polyvinyl chloride matrix membrane sensors for the selective determination of sulbutamine. These sensors were based on molybdate, tetraphenylborate, reineckate, phosphotungstate and phosphomolybdate, as possible ion-pairing agents. These sensors display rapid near Nernstian stable response over a relatively wide concentration range of sulbutamine, with calibration slopes 28 - 32.6mV/decade over a reasonable pH range 2 - 6. The proposed sensors proved to have a good selectivity for salbutamine over some inorganic and organic cations. The five potentiometric sensors were applied successfully in the determination of the drug in a pharmaceutical preparation (arcalion-200) using both direct potentiometry and potentiometric titration[90].

The construction and evaluation of tripelennamine conventionally shaped ion selective electrodes and tubular detectors for the determination of this compound in pharmaceutical formulations are described. The electrodes showed a linear response in a concentration range of about 4×10^{-5}M - 1×10^{-1}M and a slope near the theoretical value. The electrodes were applied to

different pharmaceutical formulations such as creams, gels and syrups and good results were obtained[91].

Kiyoyuki *et al* were successful in developing a cocaine selective membrane electrode with the use of sodium tetrakis [3,5-bis (trifluoromethyl) phenyl]borate as an ion-exchanger. The lower limit of detection was 4×10^{-5}M cocaine. Interference by other drugs (morphine and codeine) and a stimulant (methamphetamine) was negligible. This electrode was successfully applied for the determination of cocaine in a drug mixture containing cocaine and morphine, which is widely used to suppress acute pain in cancer patients[92].

The construction and electrochemical response characteristics of poly (vinyl chloride) matrix membrane sensors fluorouracil are described by Hassan *et al*. The membranes incorporate ion association complexes of fluorouracil anion with bathophenanthroline- nickel (II), bathophenanthroline -iron (II) and phenanthroline-iron (II) as electroactive materials. These sensors exhibited fast response time, low determination limit (1×10^{-5}M), good stability (4 - 8 weeks) and reasonable selectivity. The sensors were used for direct potentiometry and potentiometric titration for the determination of fluorouracil in some pharmaceutical preparations[93].

R. I. Stefan reported the construction and performance characteristics of two ion-selective membrane electrodes based on ion pair complexes between lauryl sulphate and moclobemide (I) and disopyramide. The electrodes presented a wide linear range and also showed high selectivity to moclobemide and disopyramide in the presence of other foreign ions[94].

S. Khalil1 and N. Borham reported the construction and performance characteristics of ion selective membrane electrodes for phenothiazine drugs based on their ion pair complexes with tetraphenylborate and dinonylnaphthalenesulfonate in a poly (vinyl chloride) matrix. The electrodes showed a near Nernstian response over various ranges depending on the nature of the phenothiazine drug. The reported sensors were used to determine the phenothiazine drugs in pharmaceutical preparations with satisfactory results[95].

A novel ion selective electrode was reported for the determination of ranitidine by Hassen *et al.* The potentiometric technique was based on direct measurements of the drug cation with novel PVC matrix membrane sensors incorporating ranitidine-reineckate, tungstophosphate and tungstosilicate ion association complexes as electroactive compounds with 2-nitrophenyl ether as plasticizing solvent mediator. The sensors were used for the determination of ranitidine in a variety of pharmaceutical dosage forms[96].

Salem *et al* reported two novel, simple, low cost and sensitive ion-selective electrodes for the determination of some 1,4-benzodiazepines in their pharmaceutical preparations as well as in biological fluids. Sodium tetraphenyl borate and phosphotungstic acid were used for the formation of ion pairs with the drugs. The electrodes were used for determining trace amounts of bromazepam, clonazepam and diazepam in their pharmaceutical preparations as well as in biological fluids[97].

Naader Alizadeh and Rasoul Mehdipour developed an ion selective membrane electrode for the drug ketamine hydrochloride using a modified PVC membrane which has ionic end groups as ion exchanger sites and was

plasticized with ortho nitrophenyloctyl ether (o-NPOE) as plasticizer. This drug electrode showed excellent Nernstian responses (59 mV per decade) in the concentration range 1×10^{-5}- 1×10^{-2} M with a detection limit of 5×10^{-6} M. The electrode was applied for determination of ketamine hydrochloride in pharmaceutical preparations using direct potentiometry[98].

Newly developed solid contact ion selective electrodes have been proposed for determining diazepam, bromazepam and clonazepam 1,4-benzodiazepines in pure forms and in pharmaceutical preparations. The electrodes are based on polyvinyl chloride membranes doped with drug-tetraphenyl borate (TPB) or drug-phosphotungstic acid (PTA) ion. Successful application of developed electrodes for drugs determinations in pharmaceutical preparations was obtained[99].

Takashi Katsu and Yuki Mori fabricated a disopyramide-sensitive membrane electrode. Sodium tetrakis [3,5-bis (2-methoxyhexafluoro-2-propyl) phenyl]borate was incorporated as the ion exchanger and 2-fluoro-2′-nitrodiphenyl ether was used as the solvent mediator in a poly(vinyl chloride) membrane matrix[100].

Hisham E. Abdellatef and coworkers were successful in reporting the construction of a plasticized poly (vinyl chloride) matrix type famotidine ion selective membrane electrode and its use in the potentiometric determination of famotidine in pharmaceutical preparations. It is based on the use of the ion associate species, formed by famotidine cation and tetraphenyl borate (TPB) counter ion. The electrode exhibited a linear response for 1×10^{-3} - 1×10^{-5} M of famotidine solutions over the pH range 1 - 5[101].

A PVC membrane electrode was reported for the stimulant phenetermine by Takashi Katsu and his group using the ion exchanger sodium tetrakis [3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl] borate. The electrode showed high selectivity to the stimulant in presence of other analogous compounds. This electrode was applied to the determination of phenetermine in ion exchange resin complexes containing this stimulant[102].

The construction and general performance characteristics of potentiometric plastic membrane sensors for piroxicam and tenoxicam drug-anions are described by Khalil and group. The electroactive materials are based on ion pair complexes with aliquot 336S cation. The selectivity of the electrodes to a number of organic and non organic anions is reported. The electrodes exhibit useful analytical characteristics for the determination of the active ingredients in their respective pharmaceutical tablet formulations without any prior separation with good reproducibility[103].

Hassan *et al* reported the construction and characterization of five poly (vinyl chloride) matrix membrane sensors responsive to some β -blockers (atenolol, bisoprolol, metoprolol, propranolol and timolol). The sensors are based on the use of the ion association complexes of the β -blocker cations with tungstophosphate anion as electroactive materials. The performance characteristics of these sensors, evaluated according to IUPAC recommendations, reveal fast, stable and near Nernstian response for 1×10^{-2} - 2×10^{-7} M of different β-blockers over the pH range 2 - 9. The sensors were used for direct potentiometry of β-blockers in some pharmaceutical preparations[104].

An internal solid contact sensor was developed for the determination of doxycycline hydrochloride by Sun *et al.* This was based on a conducting polypyrrole (PPy) film immobilized on a glassy carbon electrode surface casted by a plasticized polyvinyl chloride membrane containing an ion pair compound of doxycycline hydrochloride with tetraphenylborate (TPB) and dibutylphthalate (DBP) as plasticizer. The detection limit obtained was 4.0×10^{-6} M. The sensor was successfully applied to determination of doxycycline hydrochloride in pharmaceutical formulation[105].

S. Khalil and group were successful in reporting an ion selective electrode for prazosin based on prazosinium - phosphotungstate ion associate. The electrode exhibited a linear response with an approximate Nernstian slope over the range of 2.7×10^{-6}- 1×10^{-2} M. The working pH of the electrode ranged from 1.5 to 6.4 and exhibited very good selectivity for the prazosin with respect to a large number of inorganic cations and organic substances of biological importance[106].

A coated-wire benazepril-selective electrode based on incorporation of the benazepril - tetraphenylborate ion pair in a poly (vinylchloride) coating membrane was constructed by S. Khalil and S. Abd El-Aliem. The influences of membrane composition, temperature, pH of the test solution, and foreign ions on the electrode performance were investigated. The electrode was successfully used for potentiometric determination of benazepril hydrochloride both in pure solutions and in pharmaceutical preparations[107].

Shamsipur *et al* developed a new cimetidine ion selective electrode, characterized and used in pharmaceutical analysis as well as its recovery

from urine samples. The electrode incorporated PVC membrane with cimetidine-phosphotungstate ion pair complex. The electrode exhibited a Nernstian response for cimetidine in the concentration range 1.0×10^{-5} - 1.0×10^{-2} M with a slope of 58 mVper decade. The limit of detection was 5.0×10^{-6} M[108].

The construction and electrochemical response characteristics of four poly (vinyl chloride), membrane sensors for determination of fluphenazine hydrochloride and nortriptyline hydrochloride are described by El-Ragehy *et al.* The method is based on the formation of the ion pair complexes between the two drugs cations and sodium tetraphenylborate (NaTPB) or tetrakis (4-chlorophenyl) borate (KtpClPB). The proposed sensors were used for the direct potentiometric determination of fluphenazine and nortriptyline hydrochlorides in their pure forms and pharmaceutical dosage forms[109].

Hassan *et al* reported the fabrication of metformin ion selective electrodes based on metformin – reineckate and metformin - tungstosilicate ion pairs as electroactive species with dioctylphthalate and o-nitrophenyloctylether as plasticizers, respectively. These sensors give rapid Nernstian response for 1×10^{-1} - 1×10^{-5} M metformin in the pH range 5-11[110].

S. Khalil and M.A. EL-Ries reported a new prenalterol ion selective PVC membrane electrode based on the ion pair complex of prenalterol with sodium tetraphenylborate and its performance characteristics were studied. The electrode exhibited a linear response with a good Nernstian slope over a relatively wide range of concentration. The electrode showed very good selectivity for the drug with respect to a large number of inorganic and organic cations. The standard addition method and potentiometric titration

were used to determine prenalterol in pure solutions and in pharmaceutical formulations[111].

Reproterol hydrochloride selective PVC membranes based on ion associates of reproterolium-phosphotungstate, reproterolium-phosphomolybdate or a mixture of both were prepared by Shoukry and group. The electrodes displayed a linear response over the concentration range of 6.3×10^{-6} - 1.0×10^{-1} mol dm^{-3} of reproterol. The electrodes showed good selectivity to the reproterolium ion with respect to many inorganic cations, sugars and amino acids[112].

Two novel potentiometric membranes sensors responsive to the drug ibuprofen have been developed by Hassan and co-workers. The sensors showed a near Nernstian response of -53 and -55 mV/decade over a wide concentration range. Both the sensors were used for the quantification and quality control assessment of ibuprofen in pharmaceutical preparations[113].

Gamal Abdel El-Hafeez Mostafa reported a novel PVC membrane electrode for the determination of scopolamine ion based on the formation of an ion association complex of scopolamine with phosphotungstate counter anion as an electroactive material dispersed in a PVC matrix. The sensor showed a fast stable near Nernstian response over a wide concentration range within a pH range of 3 - 7[114].

Ribeiro and coworkers developed a simple, precise, rapid and low cost potentiometric method for captopril determination in pure form and in pharmaceutical preparations. No interferences were observed in the presence of common components of the tablets as lactose, microcrystalline cellulose, croscarmellose sodium, starch and magnesium stearate. The analytical results

obtained by applying the proposed method compared very favourably with those obtained by the United States Pharmacopoeia Standard procedure[115].

A plastic membrane electrode for the determination of methacycline hydrochloride was fabricated by Hassan *et al* based on the use of methacycline-tetraphenylborate as the electroactive substance, and dioctylphtalate as the plasticizing agent. The electrode was successfully applied to determination of methacycline hydrochloride in tablet by direct potentiometric method. The result obtained with the electrode was in good agreement with the value obtained by using the official method[116].

Zhi-Hua-Liu and co-workers reported a novel PVC membrane electrode for the drug pethidine with dibutyl phthalate as plasticizer based on pethidine-silicotungstate as the electroactive material. The membrane electrode was highly stable and had a longer life time compared to the earlier reported electrode. The electrode was successfully applied to determination of pethidine hydrochloride in tablet and injections[117].

H.Y. Aboul-Enein and Xian Xiang Sun developed novel ion selective PVC membrane electrode for determination of propranolol. Silicotungstic acid was used as the counter ion and diisononyl phthalate was used as the plasticizer. The electrode exhibited excellent potential response properties, showing a Nernstian response in the concentration of 3.0×10^{-6} - 2.6×10^{-2} M with the slope of 54.7 mV per decade. The electrode was successfully used for the analysis of propranolol in pharmaceutical formulation[118].

Khalil Farhadi and Ramin Maleki reported a triiodide ion selective electrode based on clotrimazole-triodide ion as the membrane carrier. The electrode showed a linear range within 10^{-3} to 10^{-6}M with a slope of

-68.9 mV/decade and detection limit of 5×10^{-6}M. The electrode was used for the indirect potentiometric determination of clotrimazole in pharmaceutical preparations[119].

G.A.E. Mostafa discussed the construction and characteristic performance of a metoclopramide - polyvinyl chloride membrane sensor. The sensor was based on the use of metoclopromide- tetraiodomercurate ion pair as electroactive material in PVC matrix in presence of dioctylphthalate as solvent mediator. The membrane sensor showed a stable, near Nernstian response over the concentration range 1×10^{-2} - 6×10^{-5}M of metoclopromide in the pH range 3 - 7 with cationic slope of 53.0 mV/decade. The determination of metoclopromide in tablets, injection, and syrup gave results that compare favorably with those obtained by the British pharmacopoeia method[120].

The construction and performance characteristics of ion selective membrane electrodes for a newly found phenylpiperazine antidepressant, Nefazodone, based on its ion pair complexes with phosphotungstate, tetraphenylborate, tungstosilicate and reineckate in a poly (vinyl chloride) matrix were described by Erdem *et al.* The selectivity of these electrodes to Nefazodone in the presence of a number of sugar molecules, cations and drugs was reported. The potentiometric standard addition method was used to determine the drug in pure solution[121].

G. A. E. Mostafa described the construction and general performance characteristics of two novel potentiometric PVC membrane sensors responsive to the pyridoxine hydrochloride known as vitamin B6.These sensors are based on the use of the ion association complexes of the

pyridoxine cation with molybdophosphate and tungstophosphate counter anions as ion pairs in a plasticized PVC matrix. The electrodes showed a stable, near Nernstian response for 6×10^{-5} - 1×10^{-2}M vitamin B6 over the pH range 2 - 4 with a cationic slope of 54.0 and 54.5 mV per concentration decade for pyridoxine-molybdophosphate and pyridoxine-tungstophosphate, respectively[122].

Kauffmann *et al* described the construction and electrochemical response characteristics of two types of poly (vinyl chloride) membrane sensors for the determination of amodiaquine hydrochloride. The sensing membrane comprised an ion pair formed between the cationic drug and sodium tetraphenyl borate or potassium tetrakis(4-chlorophenyl) borate in a plasticized PVC matrix. Eight PVC membrane ionselective electrodes were fabricated and studied. The sensors displayed a fast, stable and near Nernstian response over a relative wide amodiaquine concentration range (3.2×10^{-6} to 2.0×10^{-2}M), with slopes comprised between 28.5 and 31.4 $mVdec^{-1}$ in a pH range between 3.7 and 5.5[123].

Mojtaba Shamsipur and group reported the preparation and characterization of a novel diclofenac ion selective electrode. The electrode exhibited a Nernstian slope for diclofenac in the concentration range 1.0×10^{-5} to 1.0×10^{-2}M with a limit of detection of 4.0×10^{-6}M. The electrode displayed a good selectivity for diclofenac with respect to a number of common inorganic and organic species. The membrane sensor was successfully applied to the determination of diclofenac in its tablets as well as for its recovery from blood serum and urine samples[124].

Four glutathione selective electrodes were reported by Shehata *et al* with different ion exchangers in different polymeric matrices. The proposed sensors were successfully used for the determination of glutathione in capsules and also in plasma[125].

Khadiga M. Kelani reported the construction and electrochemical response characteristics of 4 polymeric membrane sensors for potentiometric determination of zolpidem hemitartrate. A linear response was obtained over the concentration range of 1×10^{-5} - 1×10^{-2}M with a cationic slope of 29 mV per concentration decade. The 4 proposed sensors were also applied successfully to the determination of the drug in tablets and in biological fluids[126].

Joanna Drozd and Hanna Hopkala described sensitive and reasonably selective poly (vinyl chloride) membrane electrodes for cyproheptadine. They are based on the use of cyproheptadine-tetrakis (4-chlorophenyl)borate and cyproheptadine-dipicrylamine as a novel electroactive compounds. The electrodes were used in the potentiometric determination of cyproheptadine in bulk substance and tablets. The results were in good agreement with those obtained by UV spectrophotometric method[127].

Pezza and group reported the characteristics, performance and application of a potentiometric sensor immobilized in graphite matrix for diclofenac ion. This electrode responds to diclofenac over the range 5.0×10^{-5} to 1.0×10^{-2} mol l^{-1} at pH 6.5 - 9.0 and a detection limit of 3.2×10^{-5} mol l^{-1}. The proposed sensor displayed good selectivity for diclofenac in the presence of several substances, especially concerning carboxylate and inorganic

anions. It was used to determine diclofenac in pharmaceutical preparations by means of the standard addition method[128].

Moghimi *et al* reported a potentiometric sensor for the selective determination of picrate ion immobilized in a graphite matrix. The electrode showed easy construction, fast response time (about 25 s), low cost, and excellent response stability (lifetime > 6 months, in continuous use). The proposed sensor showed high selectivity towards picrate ion over many hydrophilic and lipophilic anions. 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 picrates[129].

The construction and performance characteristics of a potentiometric sensor for p-amino benzoate ion immobilized in graphite matrix were reported by Pezza *et al.* The proposed sensor displayed good selectivity for p-aminobenzoate in the presence of several substances, especially, concerning carboxylate and inorganic anions. It was used to determine p-aminobenzoate in pharmaceutical formulations by means of the standard additions method[130].

El-Shahawi *et al* described the construction and performance characteristics of ion selective membrane electrodes for sildenafil citrate. The proposed sensors were based on the formation of the complex ion associates of the drug with sodium tetraphenylborate and phosphomolybdic acid as ionophores in poly vinyl chloride membrane. The sensors showed a linear and stable potential response with near Nernstian slope of 55.5 and 53.5 mV per decade over a wide range of concentration 10^{-2} to 10^{-5} M sildenafil with

good reproducibility. The selectivity coefficients indicated good selectivity for the drug over a large number of nitrogenous compounds and some inorganic cations. The proposed sensors were tested for the analysis of sildenafil citrate in pure form, pharmaceutical preparations and blood serum[131].

Hassan *et al* reported the construction and characterization of potentiometric membrane sensors for quantification of diclofenac. The membranes of the sensors incorporate iron (II) - phthalocyanine as a molecular recognition reagent, dibutylsebaccate solvent mediator, tridodecylmethylammonium chloride as membrane additive in poly(vinyl chloride) matrix. The sensors displayed a wide linear range and pH range of 5.5 - 9.0[132].

New PVC membrane electrodes selective for the determination of hyoscyamine ion (Hy^+) based on hyoscyamine tetraphenylborate (Hy-TPB) or hyoscyamine phosphotungstate (Hy-PT) ion exchangers as electroactive materials were described by Badawy and group. The electrodes gave near Nernstian slopes of 56.5 and 57.8 mV/decade for Hy-TPB and Hy-PT respectively.The electrodes have been applied to the potentiometric determination of hyoscyamine in pure solution and in pharmaceutical preparations under batch and FIA conditions and as end point indicator electrode for the determination of hyoscyanine using potentiometric titration[133].

Y.M. Issa and S.I.M Zayed reported a new oxymetazoline ion selective electrode based on the ion associate of oxymetazoline with phosphotungstic acid. The electrode exhibited a linear response with a calibration slope of

57.16 mV/decade. The proposed sensor showed high selectivity for oxymetazoline ion with respect to a large number of inorganic cations and compounds[134].

Rizk *et al* developed polyurethane sensors for thiopental on solid graphite support. The sensors were based on the electroactive materials of thiopental with Cu (II) and Co (II) bathophenanthroline 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[135].

A novel potentiometric PVC membrane sensor for determination of diclofenac in pharmaceutical preparations has been developed by Hassan and group. The sensor is based on the use of the 2,4,6-tri(2-pyridyl)-s-triazine iron(II) diclofenac complex as an electroactive material in a plasticized PVC membrane matrix. No significant interferences were caused by inorganic and organic anions and various drug excipients and diluents[136].

Ibrahim *et al* reported 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 proposed sensor exhibited high selectivity for dicyclomine ion with respect to a large number of inorganic cation, sugars, aminoacids and organic compounds[137].

Ghoreishi and group reported new naphazoline ion selective membrane electrodes of both conventional and coated graphite types prepared based on the ion-pair of naphazoline tetraphenylborate. The conventional type

electrode was fully characterized in terms of membrane composition, life span, pH, ionic strength and temperature[138].

Wang *et al* developed a potentiometric PVC membrane sensor for determination of the drug tetracycline. The response time of the sensor was less than 2.0 min. In addition to high reproducibility and reversibility, the sensor also exhibited good selectivity over some common pharmaceutical species and some common organic and inorganic compounds[139].

A novel theophylline potentiometric sensor was prepared, characterized and used in pharmaceutical analysis by Mousavi *et al.* The sensor was based on a PVC membrane containing dibutyl phthalate as plasticizer, 2,6-bis(phenyl)-4(phenyl)3H-thiopyran as ionophore and oleic acid as additive. A linear response in the range of 1.0×10^{-6} to 1.0×10^{-2} M of theophylline was established[140].

A novel plastic membrane electrode for the determination of diclofenac anion was described by Hassan *et al.* The sensing membrane of the electrode consisted of diclofenac-nickel(II) bathophenanthroline as an ion-exchanger site in a poly(vinyl chloride) matrix plasticized with o-nitrophenyl phenyl ether. In borate buffer solutions of pH 8 - 12, the electrode exhibited a fast, stable and linear response for 1×10^{-2} - 5×10^{-5} M diclofenac solutions[141].

Mojtaba Shamsipur and Fahimeh Jalali proposed a novel clotrimazole ion selective electrode. The electrode incorporated PVC membrane with clotrimazole-phosphomolybdate ion pair complex. The electrode exhibited a Nernstian response for clotrimazole in the concentration range 1.38×10^{-5} - 1.0×10^{-3}M [142].

Chlordiazepoxide Hydrochloride electrodes were prepared by Issa *et al* using the ion associates of the drug with phosphomolybdic acid and phosphotungstic acid. The electrodes exhibited a mean slope of 59.4 and 60.8 mV/decades of chlordiazepoxide hydrochloride. The electrodes could be used within the concentration range 10^{-2} to 10^{-6}M[143].

Wassil *et al* reported the construction of PVC matrix type β-blockers (sotalol, carvedilol, and betaxolol) ion selective electrodes and their use for direct potentiometry of their respective species. The proposed sensors are based on the complex ion associates of β-blockers with tungstophosphate and ammonium reineckate ionophores in polyvinyl chloride membrane with dioctylphthalate plasticizer. The four electrodes showed stable potential response with near Nernstian slope within a range of concentration $1.0 \times 10^{-}$ - $1.0 \times 10^{-}$ M β-blockers[144].

A new triiodide ion selective PVC membrane sensor based on diprotonated ketoconazole-triiodide, as ion pair, was proposed Khalil Farhadi and group. The electrode has a linear dynamic range between 2.0×10^{-3} and 7.0×10^{-6} M with a detection limit of 3.0×10^{-6} M. The potentiometric response is independent of the pH of the solution in the pH range 2 - 8.5. The proposed triiodide membrane sensor was used in development of a new, simple, fast, inexpensive and precise method for the determination of Ketoconazole in formulations[145].

Fuglein *et al* reported ion selective electrodes for the determination of the antibiotic drug chlortetracycline. The proposed electrode was based on chlortetracycline - tungstosilicate ion pair. The proposed sensor

exhibited high selectivity for chlortetracycline in presence of other interfering ions[146].

Xian Xiang Sun and Hassan Y. Aboul-enein reported an internal solid contact sensor for the determination of methacycline hydrochloride based on the use of conducting poly (pyrrole) as solid contact material and methacycline - phosphotungstate as the ion exchanger and dibutyl phthalate as the plasticizer. The determination of methacycline hydrochloride in tablets were carried out by direct potentiometry[147].

Two potentiometric sensors responsive to sildenafil citrate drug were described, characterized, compared and used for drug assessment by Hassan *et al.* The sensors are based on the use of the ion association complexes of cation with tungstophosphate and reineckate anions as electroactive materials in plasticized poly(vinyl chloride) membranes. The sensors display good selectivity for the drug over many nitrogeneous compounds, some inorganic cations and excipients and diluents commonly used in drug formulations. Validation of the assay methods with both sensors by measuring the lower detection limit, range, accuracy, precision and repeatability reveals good performance characteristics confirming applicability for continuous determination of sildenafil in pharmaceutical formulations and in spiked human serum[148].

Mojtaba Shamsipur and Fahimeh Jalali developed a novel atenolol ion selective electrode characterized, and used in pharmaceutical analysis. The electrode incorporates PVC membrane with atenolol tetrakis (p-chlorophenyl) borate ion pair complex. The electrode exhibited a Nernstian response for atenolol in the concentration range 3.0×10^{-5} to 8.0×10^{-2} M. The limit of detection was 1.0×10^{-5}M[149].

Sergey V. Kharitonov reported the construction and electrochemical response characteristics of poly (vinyl chloride) matrix ion selective electrodes for papaverine hydrochloride. The membranes incorporate ion association complexes of papaverine with tetraphenylborate, picrate, tetraiodomercurate, Reinecke salt and heteropoly compounds of Keggin structure-molybdophosphoric acid, tungstophosphoric acid, molybdosiliconic acid, and tungstosiliconic acid as electroactive materials. These electrodes showed linear response for papaverine hydrochloride over the range from 1×10^{-5} up to 5×10^{-2} mol/l with cationic slopes from 42 up to 58 mVper concentration decade[150].

A new polymeric membrane electrode has been developed by M. Rachidi and J. Elharti for the determination of azithromycin. The electrode was constructed by incorporating the azithromycin-tetraiodomercurate ion pair complex into a polyvinyl chloride matrix plasticized by nitrobenzene. This sensor exhibited good linear response over the concentration range 1.0×10^{-2} - 7.0×10^{-6}M[151].

Four poly (vinyl chloride) membrane sensors for the determination of hyoscine butylbromide were described and characterized by Saharty *et al.* The sensors were based on the use of the ion association complexes of hyoscine cation with ammonium reineckate counter anions as ion exchange sites in the PVC matrix. The membranes incorporate ion association complexes of hyoscine with dibutylsebaccate, dioctylphthalate, nitrophenyl octyl ether and β-cyclodextrin The sensors were used for determination of hyoscine butylbromide in laboratory prepared mixtures, pharmaceutical formulations in combination with ketoprofen and in plasma[152].

Sadeghi *et al* reported a potentiometric sensor based on a molecularly imprinted polymer for recognition and determination of levamisole hydrochloride. The proposed sensor was highly responsive to levamisole in the presence of other similar structures like thiabendazole, (2-methylthio) benzothiazole or (2-amino) benzothiazole. The potentiometric selectivity coefficients of the proposed sensor were evaluated and it exhibited good selectivity to levamisole with respect to the electrode based on a non-imprinted polymer or the plasticized PVC membrane electrode based on levamisole hydrochloride-tetraphenyl borate[153].

Jalali *et al* reported a novel gabapentin ion selective electrode based on the ion association of the drug with phosphomolybdic acid. The electrode displayed a good selectivity for gabapentin with respect to a number of pharmaceuticals that may be taken with gabapentin simultaneously. The sensor can be used in a pH range of 1.8 - 3.2. The membrane sensor was successfully applied to the determination of gabapentin in its tablets as well as its recovery from blood serum samples[154].

Mojtaba Shamsipur and Fahimeh Jalali reported a PVC membrane sensor for the determination of ketoconazole in pharmaceutical preparations and biological samples. The membrane used in this electrode was made from liquid plasticized PVC and was based on a water insoluble ketoconazole-tetraphenyl borate ion pair as the ion exchanger[155].

V.D. Vaze and A.K. Srivastava developed a simple, rapid and sensitive sensor for the assay of pyridoxine hydrochloride. The method was based upon the use of calix-8-arene as a neutral carrier in the presence of phosphotungstic

acid as an ion extruder and di isooctyl phthalate as plasticizer. The sensor was found to have a short response time of 20 s to pyridoxine concentration[156].

M.N. Abbas and A.A. Radwan reported a novel potentiometric lipoate-selective sensor based on mercuric lipoate ion pair as a membrane carrier The electrode was prepared by coating the membrane solution containing PVC, plasticizer, and carrier on the surface of graphite electrode. Fast and stable response, good reproducibility, long term stability, applicability over a pH range of 8.0 - 9.5 is demonstrated. The sensor has a response time of $\leq$12 s and can be used for at least 6 weeks without any considerable divergence in its potential response. The proposed sensor has been applied for the direct and flow injection potentiometric determination of lipoic acid in pharmaceutical preparations and urine; and has been also utilized as an indicator electrode for the potentiometric titration of lipoic acid[157].

Ipratropium ion selective electrode has been constructed from poly (vinyl chloride) matrix membrane containing Ipratropium-tetraphenylborate as the electroactive component using 2-nitrophenyloctylether as plasticizer. The electrode exhibits near Nernstian response to Ipratropium bromide over the concentration range 1×10^{-5} to 1×10^{-2} M and detection limit 5.1×10^{-6}M. The electrode was successfully used as indicator electrode in the potentiometric titration of Ipratropium bromide versus sodium tetraphenylborate and in the determination of the drug in pharamaceutical formulations and spiked urine samples applying batch and flow injection techniques, with satisfactory results[158].

Shoukry *et al* developed new chlorpromazinium plastic membrane electrodes of the conventional type based on incorporation of

chlorpromazinium-reineckate ion pair, chlorpromazinium-phosphotungstate or chlorpromazinium-phosphomolybdate ion associate into poly (vinyl chloride) membrane. The electrodes exhibited calibration graph slopes of 49.83, 52.87, and 61.30 mV/decade over life spans of 1, 5, and 3 days, respectively. All electrodes proved to be selective for chlorpromazine and have been applied to the assay of a pharmaceutical preparation[159].

M. Arvand *et al* constructed an ion selective membrane electrode for the drug atenolol, based on incorporation of the atenolol phosphotungstate ion associate in a PVC coating membrane with acetophenone as plasticizer. The influence of the membrane composition, temperature, conditioning time of the electrode, pH of the test solution, and foreign ions on the electrode performance were investigated. The drug electrode showed Nernstain responses in the concentration range 5×10^{-7} - 1×10^{-2} M and was found to be very selective, precise and usable within the pH range 3 - 6[160].

Ali A. Ensafi and Ali R. Allafchian reported a new PVC membrane sensor for amiloride. The sensor was based on amiloride-sodium tetraphenyl phthalate ion pair as an electroactive material. The sensor was highly selective for amiloride over a large number of similar compounds. The sensor showing a fast response time of 6 s and was used over a period of 2 months with a good reproducibility. The sensor was successfully applied to determination of amiloride in pharmaceutical samples with satisfactory results[161].

A PVC membrane sensor for diclofenac was developed by Maleki *et al* based on its ion pair complex with silver. The electrode gave a Nernstian response for diclofenac anions over a wide linear range from 5.2×10^{-5} to

1.1 × 10^{-2} M at 25 ± 1 °C. The electrode was successfully used for determination of diclofenac in pharmaceuticals and also in potentiometric study of interaction of diclofenac with bovine serum albumin[162].

Nesrin K. Ramadan and Hala E. Zaazaa developed five poly (vinyl chloride) matrix membrane electrodes responsive to the β-blockers atenolol, bisoprolol fumarate, timolol maleate, and levobunolol HCl. Ammonium reineckate anion and tungstophosphate anion were used to form the ion association with the drugs. The method was successively applied for the determination of β-blockers in their pharmaceutical formulations[163].

Alizadeh *et al* reported a solid state valproate ion selective sensor based on conducting polypyrrole films. The sensors showed a quasi Nernstian behavior over 4×10^{-5} - 4×10^{-2} M with a detection limit of 1×10^{-5}M in an aqueous solution. The response time of the electrode was about 20 s and the electrode could be used at least for 4 months without any divergence. The drug sensor was applied for determination of valproate ions in pharmaceutical preparations by using titration potentiometry[164].

Rachidi *et al* developed a cetirizine selective membrane electrode based on the ion association of the drug with sodium tetraphenyl borate anion. The developed electrode showed a good Nernstian response in the concentration range of 6.3×10^{-7} -1.0×10^{-2}M with a slope of 58.2 mV per decade and a lower quantification limit of 3.16×10^{-7} M. Cetirizine selective electrode was successfully applied for the determination of cetirizine in pharmaceutical preparations using direct potentiometric method with high accuracy[165].

Kulapina *et al* reported the construction of ion selective electrodes with plasticized membranes based on ion pairs of gentamycin and kanamycin with

tetraphenylborate and Acid Chrome Black Special. The developed electrodes were used for the rapid potentiometric determination of gentamycin and kanamycin in biological fluids and pharmaceuticals[166].

Sayed S. Badway and group developed potentiometric membrane electrodes for the determination of Ranitidine Hydrochloride. These electrodes were based on the ion association of the drug formed with sodium tetraphenyl borate and phosphotungstate anions. The sensors showed fast and stable responses .The sensors were applied to the determination of the drug in pharmaceutical preparations[167].

S. V. Kharitonov reported the construction and characterization of potentiometric ion selective electrodes based on different lipophilic derivatives of tetraphenylborate for the drug drotaverine hydrochloride. The electrodes were applied to the determination of drotaverine hydrochloride in different pharmaceutical forms by potentiometry and potentiometric precipitation titration[168].

Ghani *et al* reported new plastic membrane electrodes for dothiepin hydrochloride based on dothiepin phosphotungstate, dothiepin phosphomolybdate and a mixture of both. The selectivity of the electrodes towards many inorganic cations, sugars, and amino acids was also tested. The electrodes were applied to the potentiometric determination of the dothiepinium ion in its pure state and pharmaceutical preparations in batch and flow injection conditions[169].

The construction and electrochemical response characteristics of a poly (vinyl chloride) membrane selective electrode for the determination of tiapride was described by Pedreno *et al*. The sensing membrane comprised an

ion pair formed between the protonated drug and tetraphenylborate in a plasticized PVC matrix. The electrode has been applied to the determination of tiapride in human urine and iontophoresis solution[170].

Mostafa *et al* reported the construction and characterization of two novel potentiometric membrane sensors for the drug acebutolol. The sensors were based on the use of ion association complexes of the drug formed with sodium tetraphenyl borate and phosphomolybdate anions in a PVC matrix. The sensors were applied to the determination of the drug in pharmaceutical preparations[171].

1.11 Scope of the Present Investigation

Ion selective sensors have been becoming one of the effective and powerful means for analytical scientists in the determination of drug substances and are playing an increasing role in pharmaceutical analysis. Many methods have been employed in our laboratory for the quantitative analysis of drugs in pure form as well as in dosage forms[172-179]. Apart from all the other methods developed, ISE's are cost effective, easy to prepare and can be rapidly manipulated. Thus it was aimed to develop potentiometric ion selective sensors for six drugs namely, Trimethoprim (TMP), Ketoconazole (KET), Lamivudine (LAM), Domperidon (DOM), Nimesulide (NIM) and Lomefloxacin (LOM). The sensors fabricated include both PVC membrane sensor as well as carbon paste sensor. A total of sixteen different sensors were developed. The response parameters of all the sensors have been studied and the sensors were applied to the determination of the drugs in pharmaceutical formulations and also in real samples like urine.

Chapter 2

MATERIALS AND METHODS

A brief sketch of the materials and methods used in the investigations is presented in this chapter. The general method for the synthesis of the ten ion association complexes and also the fabrication of the two types of sensors, viz., PVC membrane sensor and Carbon paste sensor are described in this chapter. Details about the general reagents and the instruments used in the investigations are also discussed in this chapter. It also covers the general procedure for the analysis of drug content in pharmaceutical formulations and also in real samples like urine.

2.1 Reagents

The reagents and solvents used were of analytical grade and were procured from local vendors. Distilled water was used through out the studies. The metal salts, high molecular weight PVC and perchloric acid were obtained from Merck, Germany and were used as received. The ion exchanger reagents such as molybdophosphoric acid (MPA), phosphotungstic acid (PTA) and silicotungstic acid (STA) were obtained from sd fine chem. India. High purity graphite was purchased from Sigma Aldrich Corporation, USA. This was used as received. Tetrahydrofuran (THF), orthophenanthroline, ammonium vanadate and other common reagents 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 purchased from Lancaster UK and were used as such without any further purification. Pure drugs such as Trimethoprim (TMP), Ketoconazole (KET), Lamivudine (LAM), Domperidone (DOM), Nimesulide (NIM) and Lomefloxacin (LOM) were obtained as gift samples. Pharmaceutical formulations containing the drugs were purchased from local drug stores.

2.2 Instruments used

All the potential measurements were carried out on a Metrohm 781 ion meter. 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. The SEM analysis was done on JOEL 6390 LV at STIC, Kochi.

2.3 Synthesis of the Ion Association Complexes

The ion association complexes for each drug were prepared by mixing the drug with its respective ion pairing reagents. The ion pairing reagents used were molybdophosphoric acid (MPA), phosphotungstic acid (PTA) and silicotungstic acid (STA).

2.3.1 Trimethoprim – MPA Ion Association (TMP-MPA)

The ion association TMP-MPA was prepared by mixing 75 mL of 10^{-2} M TMP with 25 mL 10^{-2} M solution of MPA. 0.2903 g of TMP was dissolved in methanol in a 100 mL volumetric flask and made upto the mark. Similarly 0.5644 g of MPA was dissolved in distilled water in a 25 mL volumetric flask.75 mL of the drug solution was mixed with 25 mL of MPA solution with thorough stirring to obtain a dull yellow precipitate. The

obtained precipitate was filtered, washed several times with distilled water and dried at room temperature. It was then stored in a desiccator.

2.3.2 Trimethoprim – PTA Ion Association (TMP-PTA)

TMP-PTA ion association was prepared by mixing 75 mL 10^{-2}M solution of TMP with 25 mL 10^{-2}M PTA solution. 0.2903 g of TMP was dissolved in methanol and made upto volume in a 100 mL standard flask. 25 mL 10^{-2}M solution of PTA was prepared by dissolving 0.7200 g PTA in distilled water in 25 mL volumetric flask. 75 mL of the drug solution was mixed with 25 mL of PTA solution with thorough stirring. A dull white precipitate was obtained. It was filtered, washed with distilled water and dried at room temperature. The dried precipitate was stored in a desiccator for future use.

2.3.3 Ketoconazole – MPA Ion Association (KET-MPA)

0.5314 g of KET was dissolved in very dilute HCl and the solution was made up to volume using distilled water in a 100 mL volumetric flask. 25 mL 10^{-2}M solution of MPA was also prepared. 75 mL of the drug solution was mixed with 25 mL of 10^{-2} M MPA solution. The solution was stirred vigorously. A light brown precipitate was formed. This was then filtered, washed with distilled water and dried. The dried precipitate was then stored in a desiccator for future use.

2.3.4 Lamivudine – MPA Ion Association (LAM-MPA)

A 10^{-2}M solution of LAM was prepared by dissolving 0.2293 g of LAM in distilled water. The solution was then made upto 100 mL in a volumetric flask. 25 mL 10^{-2}M MPA solution was also prepared in distilled water. LAM-MPA ion association was prepared by mixing the 75 mL LAM

solution with 25 mL MPA solution. The resulting solution was stirred well for 10 minutes. A light brown precipitate was formed. This was then filtered, washed with distilled water and dried at room temperature. The dried precipitate was stored in a desiccator.

2.3.5 Lamivudine – PTA Ion Association (LAM-PTA)

0.2293 g of LAM was dissolved in distilled water in a 100 mL volumetric flask and made upto volume. 25 mL 10^{-2}M PTA solution was prepared by dissolving 0.7200 g of PTA in distilled water in a 25 mL volumetric flask. 75 mL of LAM solution was mixed with 25 mL of PTA solution to obtain a flesh coloured precipitate. The solution was thoroughly stirred while mixing. The precipitate thus formed was filtered, washed, dried at room temperature and stored in a desciccator.

2.3.6 Domperidone – PTA Ion Association (DOM-PTA)

0.1065 g of DOM was dissolved in methanol in a 25 mL volumetric flask. The solution was made upto 25 mL using distilled water. A 10^{-2}M solution of PTA was also prepared in a 25 mL standard flask using distilled water. The two solutions were mixed together. The solution was stirred vigorously while mixing. A light green precipitate was formed which was then filtered and washed repeatedly. The ion association was dried at room temperature and stored.

2.3.7 Nimesulide – MPA Ion Association (NIM-MPA)

0.3083 g of NIM was dissolved in methanol in a 100 mL volumetric flask and made upto the mark using methanol to obtain a 10^{-2}M solution. 25 mL 10^{-2}M solution of MPA was also prepared. 45 mL of the freshly prepared NIM solution was mixed with 15 mL of MPA solution. Upon mixing, the

solution was stirred continuously. The yellow coloured precipitate formed was filtered and washed. The precipitate was dried at room temperature and was stored in a desiccator.

2.3.8 Nimesulide – STA Ion Association (NIM-STA)

A 10^{-2}M solution of NIM was prepared in 100 mL volumetric flask. 25 mL of 10^{-2}M STA solution was prepared by dissolving 0.7196 g of STA in distilled water and the solution was then made upto to the mark in a volumetric flask. 75 mL of NIM solution was mixed with 25 mL of STA solution with thorough stirring. A white coloured precipitate was formed. The solution was allowed to stand for a few minutes and the precipitate formed was filtered and washed. The precipitate was dried at room temperature and was stored in a desiccator.

2.3.9 Lomefloxacin – STA Ion Association (LOM-STA)

0.3878 g of LOM was dissolved in 2 drops of con. HNO_3 and made upto 100 mL in a volumetric flask using distilled water. 25 mL of 10^{-2}M STA solution was also prepared by dissolving 0.7196 g of STA in distilled water. 75 mL of the 10^{-2} M LOM solution thus obtained was mixed with 25 mL 10^{-2} M STA solution. A light brown precipitate was obtained. It was filtered, washed and dried at room temperature and then stored in a desiccator.

2.3.10 Lomefloxacin – MPA Ion Association (LOM-MPA)

75 mL of 10^{-2}M LAM solution was mixed with 25 mL 10^{-2}M MPA solution. The solution was stirred well. A light yellow coloured precipitate was formed. The obtained precipitate was filtered, washed several times with distilled water and dried at room temperature. The dried precipitate was stored in a desiccator.

2.4 Fabrication of the Sensors using the Prepared Ionophores

Ionophore is the most important part of an ion selective sensor. Two different types of sensors were fabricated for the above drugs using the synthesized ionophores. A brief description of the stages involved in the fabrication of the two types of sensors is given in the next section.

2.4.1 Fabrication of the PVC Membrane Sensor

The general method for the fabrication of PVC membrane sensor was first reported by Cragg's and Moody[180]. The main components of a PVC membrane sensor are ionophore, PVC and plasticizer. The PVC membrane electrodes belong to the class of liquid membrane electrodes. PVC based sensor membranes all contain a reagent, the ionophore, dissolved in a suitable solvent, which selectively binds with the ion of interest. The main requirements for the organic liquid membrane is that it should be immiscible with water, have low volatility, interact reversibly with the ion of interest and exhibit some degree of charge conduction, which presumably occurs by the transport of ions by reagent molecules. For the preparation of the membrane, the ionophore, plasticizer and PVC were taken in the appropriate percentage-weight ratios and dissolved in THF. The solution was poured into glass rings struck onto 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 formed were cut out and glued to one end of a hollow Pyrex glass tube using Araldite and M-seal. The Pyrex glass tube was filled with the internal filling solution which consisted of a mixture of 1.0×10^{-3}M drug and 1.0×10^{-1}M NaCl solution. The membrane was conditioned by dipping it in a 1.0×10^{-3}M drug solution. Figure 2.1 represents the stages involved in the fabrication of a PVC membrane sensor.

2.4.2 Fabrication of the Carbon Paste Sensor

The carbon paste electrodes belong to the group of solid state ion selective electrodes. High purity graphite and the ionophore (referred to as the modifier) were mixed thoroughly in the appropriate percentage - weight ratios, using a mortar and pestle to give a homogeneous mixture. To this mixture a weighed amount of plasticizer was added and the paste was thoroughly mixed. The mixing is repeated several times in order to obtain a mixture as homogeneous as one can get. This paste was then packed to one end of the Teflon holder in which electrical contact was made with a copper rod that runs through the centre of the electrode holder. Its filling is made in small portions when each of them being pressed intimately before adding the next one. Appropriate packing and a smooth surface was achieved by pressing the surface of the sensor against a smooth filter paper. The prepared sensor is then left unused for a certain time (1 - 2 hrs) to allow their final homogenization to proceed. The carbon paste sensor was then conditioned by dipping it in drug solutions of suitable concentrations. The most significant advantage of a carbon paste sensor is that it does not require an internal filling solution. Also the electrode surface could be polished using a filter paper to produce reproducible working surface. Figure 2.2 represents the stages involved in the fabrication of a carbon paste sensor.

2.5 Preparation of the Drug Solutions

A 10^{-1}M stock solution was prepared for each of the drugs in suitable solvents. The stock solution was diluted to get the required concentration.

2.5.1 Trimethoprim Solution

2.9030 g of TMP was dissolved in methanol in a 100 mL volumetric flask and made upto the mark.

2.5.2 Ketoconazole Solution

5.3140 g of KET was dissolved in very dilute HCl and the solution was made up to volume using distilled water in a 100 mL volumetric flask.

2.5.3 Lamivudine Solution

2.2926 g of LAM was dissolved in distilled water in a 100 mL volumetric flask and made upto volume.

2.5.4 Domperidone Solution

4.2590 g of DOM was dissolved in methanol in a 100 mL volumetric flask. The solution was made upto 100 mL using distilled water.

2.5.5 Nimesulide Solution

3.0830 g of NIM was dissolved in methanol in a 100 mL volumetric flask and made upto the mark using methanol.

2.5.6 Lomefloxacin Solution

3.8780 g of LOM was dissolved in 2 drops of con. HNO_3 and made upto 100 mL using distilled water.

2.6 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 to the Robinson table[181].

2.6.1 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.

2.6.2 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.

2.6.3 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.

2.6.4 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.

2.6.5 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.

2.6.6 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.

2.6.7 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.

2.6.8 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.

2.6.9 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.

2.6.10 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.

2.6.11 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.

2.6.12 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.

2.7 Potential Measurement and Calibration

The potential measurements were carried out at 25±1 °C on a Metrohm 781 ion meter. 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 | carbon paste electrode.

A saturated calomel electrode was used in conjunction with the developed sensors as the reference electrode. The performances of the sensors were investigated by measuring the emf values between different concentrations of the respective drug solutions. The solutions were stirred

well and the stable potential readings were recorded. The potentials were plotted as a function of logarithm of concentration of the drug taken.

2.8 Selectivity Study of a Developed Sensor

Selectivity is one of the basic characteristics of an electrochemical sensor. In a potentiometric sensor the cell potential is mainly influenced by the ion of interest (primary ion), but there will also be a contribution from other ions which can interact with the sensor membrane. Selectivity represents to what extend the electrode is selective to a specified analyte ion. The selectivity of a developed sensor for a particular drug in presence of the various interfering species is determined using the Fixed Interference Method (FIM) and the selectivity coefficient values are evaluated. In this method, the potential of a cell comprising an ion selective sensor and a reference electrode is measured with solutions of constant activity of the interfering ion, a_B, and varying activity of the primary ion, a_A. The potential values obtained are plotted versus the logarithm of the activity of the primary ion. The intersection of the extrapolation of the linear portions of the curve will indicate the value of a_A which is used to calculate $K_{A,B}^{pot}$ from the following equation[182].

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

where z_A and z_B are charge numbers of the primary ion, A and of the interfering ion, B. The selectivity coefficients define the ability of an ion selective electrode to distinguish a particular ion from other interfering ions. The selectivity coefficient indicates the extent to which a foreign ion interferes with the response of an electrode to its primary ion. A value of selectivity coefficient that is close to 1 suggests that the sensor is almost

equally selective to the interfering ion as to the primary ion. A value greater than 1 says that the sensor prefers the interfering ion and that it would strongly interfere with the primary ion response.

2.9 Preparation and Analysis of Pharmaceutical Formulations

2.9.1 Trimethoprim Formulation - Aubril

Ten tablets (Aubril - Novartis Pharma, India) were accurately weighed and finely powdered. The mass equivalent to the mass of one tablet was dissolved in methanol and filtered into a 100 mL volumetric flask. The filtrate was washed with methanol several times and was made upto the mark and shaken well. 10 mL of this solution was transferred to a 100 mL volumetric flask. The pH of the solution was adjusted to 5.0 by adding buffer solution and the solution was quantitatively diluted. 15 mL of this solution was transferred to a beaker and electrochemical studies were performed.

2.9.2 Ketoconazole Formulations - Ketovate and Ketozole

Ten tablets of each type (Ketovate, Bal Pharma, India and Ketozole, Rexcel, India) were accurately weighed and finely powdered. The mass equivalent to the mass of one tablet was dissolved in distilled water after adding one drop of con. HCl. This was then decanted and filtered into a 100 mL volumetric flask. The solution was made upto the mark and shaken well. 10 mL of this solution was transferred to a 100 mL volumetric flask. The pH of the solution was adjusted to 6.0 by adding buffer solution and the solution was quantitatively diluted. 15 mL of this solution was transferred to a beaker and electrochemical studies were conducted.

2.9.3 Lamivudine Formulation - Lamivir

Ten tablets (Lamivir - Cipla, India) were accurately weighed and finely powdered. The mass equivalent to the mass of one tablet was dissolved in distilled water in a beaker and filtered into a 100 mL volumetric flask. The beaker was washed several times with distilled water and the contents were filtered into the flask. The solution was made upto the mark and shaken well. 10 mL of this solution was transferred to a 100 mL volumetric flask. The pH of the solution was maintained at 6.0 by adding buffer solution and the solution was quantitatively diluted. 15 mL of this solution was transferred to a beaker and electrochemical studies were performed.

2.9.4 Domperidone Formulations - Vomihtop and Domitil

Ten tablets of Domperidone (Vomihtop Cipla, India and Domitil, Bal Pharma, India) were weighed, crushed and finely powdered. The mass equivalent to the mass of one tablet was taken, dissolved in dilute methanol and filtered into a 100 mL volumetric flask. It was then made up to the mark. 10 mL of this solution was transferred to a 100 mL volumetric flask and after adjusting the pH to 5.0 with appropriate buffer, the solution was made up to the mark. 15 mL of this solution was transferred to a beaker and the potential response was conducted.

2.9.5 Nimesulide Formulation - Nimulase

The mass of ten tablets of Nimesulide (Nimulase, Kniss Laboratories PVT.Ltd, India) were taken and then powdered well. The mass of powder equivalent to the mass of one tablet was taken and dissolved in methanol. The solution was filtered to a 100 mL volumetric flask. The filtrate was washed with methanol several times and the solution was made upto the mark.10 mL of this solution was transferred to a 100 mL volumetric flask. The pH of the

solution was adjusted to 6.0 by adding buffer solution and the solution was quantitatively diluted. 15 mL of this solution was transferred to a beaker and electrochemical studies were carried out.

2.9.6 Lomefloxacin Formulations - Lomedon and Lomegen

Ten tablets of Lomefloxacin (Lomedon, Indon, India and Lomegen Genix, India) were weighed and finely powdered. The mass equivalent to the mass of one tablet was dissolved in dil.HNO_3.The solution was decanted several times, filtered and then transferred completely to a 100 mL volumetric flask and made upto volume. 10 mL of this solution was transferred to a 100 mL volumetric flask. The pH of the solution was maintained at 8.0 by adding buffer solution and the solution was made up to the mark. 15 mL of this solution was taken in a beaker and electrochemical studies were performed.

2.10 Analysis of Urine Sample

The developed sensors were applied to the determination of the corresponding drug content in urine samples. Standard addition technique was used to evaluate the drug content. 20 mL of the urine sample containing the drug was taken in a beaker and the potential reading was taken. It was then spiked with 2 mL of known concentration of the drug solution and the potential value was determined again. The difference in the potential readings between the spiked sample and the unspiked samples were noted. From the difference, the amount of drug present in the original unspiked sample was calculated[183].

2.11 Standard Methods

2.11.1 Trimethoprim[184]

0.25 g of of Aubril tablet was dissolved in 50 mL of acetic acid. 20 mL of this solution was taken in a beaker. It was then titrated with 0.1M

perchloric acid determining the end point potentiometrically. Each mL of 0.1M perchloric acid is equivalent to 29.03 mg of TMP.

2.11.2 Ketoconazole [185]

0.200 g of the tablets was dissolved in 70 mL of a mixture of 1 volume of anhydrous acetic acid and 7 volumes of methyl ethyl ketone. 10 mL of this solution was transferred to 100 mL titrimetric flask and quantitatively diluted. 20 mL of this solution was titrated with 0.1 M perchloric acid, determining the end point potentiometrically.

2.11.3 Lamivudine[186]

Ten tablets of Lamivir were accurately weighed and finely powdered. The mass equivalent to the mass of one tablet was accurately weighed and dissolved in distilled water and made upto volume in a 100 mL volumetric flask.10 mL of this solution was transferred to a 100mL volumetric flask and quantitatively diluted. A known volume of the drug was taken and a measured excess of n - bromosuccinimide (NBS) in HCl - acetate buffer was added. After 5 min, a known amount of iron(II) is added to the mixture which reduces the unreacted NBS. The residual iron(III) is complexed with orthophenanthroline. The absorbance is measured at 510 nm.The amount of NBS reacted corresponds stoichiometrically to the amount of LAM.

2.11.4 Domperidone[185]

0.300 g of the tablets were dissolved in 50 mL of a mixture of 1 volume of anhydrous acetic acid and 7 volumes of methyl ethyl ketone. 10 mL of this solution was transferred to a 100 mL volumetric flask and quantitatively diluted. 20 mL of this solution was taken in a beaker. Potentiometric titration was carried out using 0.1M perchloric acid. The

point of inflexion was taken as the end point.1 mL of 0.1M perchloric acid is equivalent to 42.59 mg of DOM.

2.11.5 Nimesulide[185]

0.2500 g of the tablet was dissolved in 3 mL of anhydrous formic acid and 40 mL of anhydrous acetic acid was added. It was then made up to volume in a 50 mL volumetric flask. 10 mL of this solution was transferred to a 100 mL volumetric flask and the solution was made up to the mark. 20 mL of the solution was then titrated with 0.1 M perchloric acid and the end point was determined potentiometrically.

2.11.6 Lomefloxacin[187]

Ten tablets were accurately weighed and finely powdered. An accurately weighed amount equivalent to weight of one tablet was transferred to 100 mL volumetric flask and diluted to the mark with appropriate solvent. 10 mL of this solution was transferred to a 100 mL volumetric flask and quantitatively diluted. To different aliquots of solution containing 0.2-1.0 mg of the drug, 3 mL of 5% w/v ammonium vanadate was added in a 10 mL volumetric flask followed by 2 mL of concentrated sulphuric acid. The mixture was mixed well and boiled gently for 20 min. in water bath, then cooled and diluted to volume with bidistilled water. The absorbance was measured at 766 nm against blank. The amount of drug was calculated from the calibration graph.

Figure 2.1 Stages involved in the fabrication of a PVC membrane sensor.

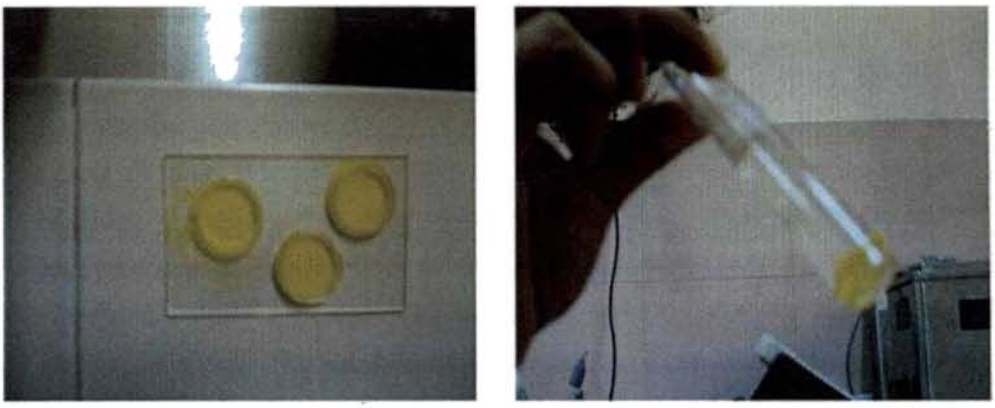
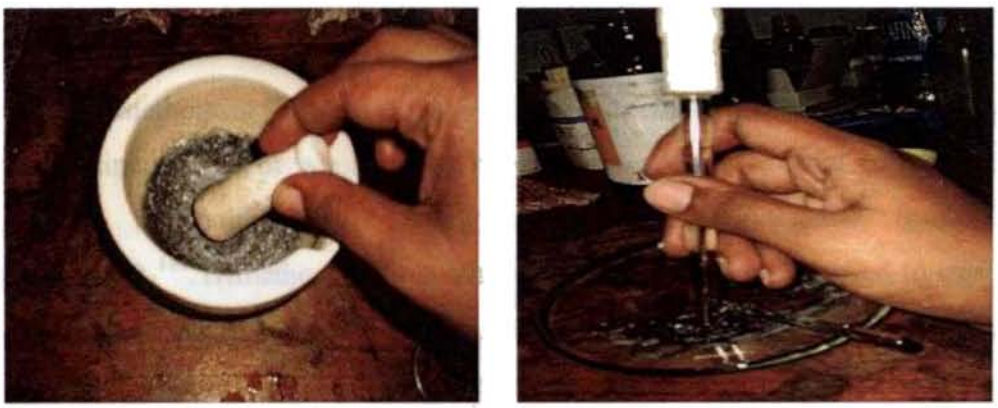


Figure 2.2 Stages involved in the fabrication of a carbon paste sensor.



Chapter 3

SENSORS FOR THE DETERMINATION OF TRIMETHOPRIM

The fabrication of two carbon paste sensors for the quantitative determination of Trimethoprim (TMP) are discussed in detail in this chapter. The sensors incorporate the ion association complex of the drug with molybdophosphoric acid (MPA) and phosphotungstic acid (PTA) as electroactive materials. The different electrochemical response characteristics of the sensors have been studied in detail. The analytical applications of the developed sensors in the determination of the drug in pharmaceutical formulations and real sample like urine was also clearly investigated.

3.1 Introduction

Trimethoprim (Figure3.1), is 5- [(3,4,5-trimethoxyphenyl)methyl] -2,4-pyrimidinediamine. It is a white to light yellow odourless, bitter compound with a molecular weight of 290.32, and the molecular formula $C_{14}H_{18}N_4O_3$[188].Trimethoprim, a synthetic antiinfective agent, is used to treat and prevent urinary tract infections, diarrhoea, and, when combined with sulfamethoxazole or dapsone, for prevention and treatment of Pneumocystis carinii infections. TMP interferes with the production of tetrahydrofolic acid by inhibiting the enzyme responsible for making tetrahydrofolic acid from dihydrofolic acid. TMP inhibits the bacterial enzyme more than the

corresponding human enzyme. Therefore, TMP has less effect on the production of tetrahydrofolic acid by humans. Trimethoprim binds to bacterial dihydrofolate reductase, subsequently interfering with the uptake of p-aminobenzoic acid (PABA) into folic acid. As folic acid is a coenzyme responsible for the transport of one-carbon fragments from one molecule to another, it is an essential component of bacterial development. Sulfonamides inhibit bacterial dihydrofolate synthetase, the enzyme immediately preceding dihydrofolate reductase, and therefore act synergistically with trimethoprim. Trimethoprim is widely used in conjunction with sulpha drugs, most commonly sulphamethoxazole, because the antimicrobial activity is greater than when the sulpha drug is used alone.

TMP is effective against a wide variety of bacteria. TMP was first approved by the FDA in combination with sulphamethoxazole in 1973.It was approved as a stand-alone drug in 1980.

The importance of this drug has thus prompted the development of many methods for its determination. Several analytical methods have been reported for quantitative determination of TMP. These include visible spectrofluorometry[189], ultraviolet (UV) spectrophotometry[190], non aqueous titrimetry[191,192], high performance liquid chromatography[193,194] (HPLC) and stripping voltammetric and polarographic techniques[195]. The titrimetric method suffers from lack of selectivity and interference from other basic substances. The other methods are time consuming and also require expensive and sophisticated instruments. Potentiometric membrane sensors have been reported for the determination of TMP[188,196]. But there are no reports of carbon paste potentiometric sensors for the selective determination of this drug.

Carbon paste sensors have been successfully applied as potentiometric sensors for the determination of various species [197]. These sensors are based on the ion-exchange mechanism of the active component incorporated into the carbon paste matrix. These sensors possess advantages of ease of preparation, ease of regeneration and very stable response in addition to very low ohmic resistance [198,199] which is probably due to the formation of a very thin film of the pasting liquid coated on to small particles of carbon powder [200,201].They have found direct application in a variety of analytical situations such as amperometry[202-204] and voltammerty[205,206] in addition to potentiometry[207,208].

This chapter presents the detailed results of development and analytical applications of two carbon paste sensors for the determination of the drug TMP. The ion associations of the drug with molybdophosphoric acid (MPA) and phosphotungstic acid (PTA) were prepared. The TMP selective carbon paste sensors are based on the ion associations of the drug with molybdophosphoric acid and phosphotungstic acid. The developed sensors exhibit useful analytical characteristics for the direct or indirect determination of the drug either in the pure form or in pharmaceutical preparations and real samples.

3.2 Synthesis of the Ion Associations

Ionophore is the most important part of an ion selective sensor. It is the electroactive ingredient which is responsible for the selective recognition of the ion in the developed sensor. The present study involves the synthesis of two different ion associations for the drug TMP based on the ion pairing reagents phosphotungstic acid (PTA) and molybdophosphoric acid (MPA). These ion associations were used for the fabrication of carbon paste sensors

for the determination of TMP. The ion associations of the drug with molybdophosphoric acid and phosphotungstic acid was prepared by mixing equimolar solutions of the drug and the ion pairing reagents in the ratio 3:1. The mixtures obtained in both cases were shaken well for about 10 min. The precipitates thus produced were filtered through a Whatman filter paper and washed thoroughly using distilled water. The obtained precipitates were dried at room temperature and stored in a desiccator.

The compositions of both ion association complexes were confirmed by the elemental analysis to be 3:1 (TMP: MPA and TMP: PTA). The elemental analysis data obtained for the ion associations is as follows:

TMP-MPA ion association

Found (%) – C – 16.23, H – 1.74, N – 5.39

Calculated (%) – C – 16.11, H – 1.82, N – 5.36

TMP-PTA ion association

Found (%) – C – 13.26, H – 1.37, N – 4.51

Calculated (%) – C – 13.43, H – 1.44, N – 4.47

3.3 Fabrication of the Carbon Paste Sensor

Two carbon paste sensors have been fabricated for the drug TMP using the ion associations TMP – MPA and TMP – PTA. Weighed amount of the ion associations and high purity graphite were mixed together. To this mixture a weighed amount of the plasticizer was added and the paste was again mixed thoroughly. This paste was then packed into the open end of a Teflon holder (12 cm length) with a hole at one end. Electrical contact is made with a copper rod that runs through the centre of the electrode.

Appropriate packing and a smooth surface was achieved by rubbing the surface of the sensor against a filter paper. Renewal of the electrode surface is one of the basic advantages of a carbon paste sensor. Even if the drug gets adsorbed onto the electrode surface, a fresh surface can be easily regenerated by removing a small amount of the paste from the tip of the electrode. Excess paste is scraped off and the new surface is polished once again by pressing against a filter paper. Both the sensors were conditioned by dipping them in 1.0×10^{-3}M TMP solution for 12 hrs. The sensors then generate stable potentials when placed in contact with TMP solutions.

3.4 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 | carbon paste electrode.

A 1.0×10^{-1}M solution of the drug was prepared in methanol in a 100 mL volumetric flask. The dilution series were prepared by serial dilution of the stock solution with methanol in 50 mL volumetric flasks. The CPE's were immersed in each of the different solutions and performances of the electrodes were investigated by measuring the emf values between 1.0×10^{-2}M and 1.0×10^{-7}M concentrations of the respective TMP solutions. The solutions were stirred and the stable potential readings were taken. The resultant calibration graph was used for subsequent determination of unknown TMP.

For the carbon paste electrode there is no need for an internal filling solution. This is one of the significant advantages of the carbon paste sensor.

3.5 Performance Characteristics of the Developed Sensors

The functional potential of any ion selective electrode depends on many factors including potential activity responses, selectivity in the presence of various interferents, pH range, detection limit, range of linear response, response time, and operative life or shelf life. Each of these parameters are discussed in the next section.

3.5.1 Optimization of the Carbon Paste Composition

Optimization of the carbon paste composition is done by varying the amount of ionophore, graphite powder and also the amount of plasticizer used. The sensitivity and linearity for a given carbon paste sensor depends significantly on the amount of modifier in the paste composition. It is also reported that the response characteristics of a carbon paste electrode is largely affected by the nature of the plasticizer used[209-211]. Thus in the present work, the influence of the plasticizer type and also their concentration on the performance characteristics of the sensors were studied. The five different plasticizers used to study their effect on the electrochemical response of the developed sensors 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).

In the case of the sensor incorporating TMP-MPA ion association, after varying the composition of the sensor matrix, it was found that the sensor containing 1.4% ion pair exhibited the best result in terms of slope (Table 3.1). The optimized composition of ingredients in the case of this

sensor (T_{M8}) may be represented as 1.4:40.0:58.6 (ionophore: graphite: plasticizer). Of the five different plasticizers used, BEP gave a near Nernstian linear plot over the concentration range studied. From Table 3.1 it is clear that in the case of the other solvent mediators, the slopes of the potentiometric response are much different from the expected Nernstian value of 59.5 mV/decade. The use of BEP gave a near Nernstian slope of 54.5 mV/decade (Figure 3.2).

The response characteristics of the sensor based on TMP-PTA ion association as the electro active material was also studied. The performance of the sensor depends on the composition of the sensor matrix as the carbon paste sensors are based on the ion exchange mechanism of the active component incorporated into the carbon paste matrix. The sensor matrix composition was varied to find the most suitable composition. The results are given in Table 3.2.The ionophore content was varied and it was found that the best response was obtained with the sensor having 1.2 % (w/w) of the ionophore (T_{P7}). The effect of all the five different plasticizers on the response was also studied as in the above case. For both the sensors, BEP has been found to be the best plasticizer in terms of slope. This may be due to its high polarity and less lipophilicity. The studies revealed that the best composition for the sensor matrix was 1.2:37.2:61.6%(w/w) (ionophore: graphite: plasticizer). The sensor (T_{P7}) exhibited a near Nernstian slope of 57.2 mV/decade (Figure 3.3).

The two sensors, T_{M8} and T_{P7} were chosen for further electrochemical studies. The response characteristics of both the sensors, T_{M8} and T_{P7} are consolidated in Table 3.3.

3.5.2 Working Concentration Range, Slope and Response Time

The working concentration range for the sensors T_{M8} and T_{P7} was found to be $1.0 \times 10^{-2} - 1.0 \times 10^{-5}$ M and $1.0 \times 10^{-2} - 5.0 \times 10^{-5}$ M respectively. The slopes calculated from the calibration graph were found to be 54.5 mV/decade and 57.2 mV/decade for the sensors T_{M8} and T_{P7} respectively showing near Nernstian behaviour. Deviations from linear behaviour of Nernstian equations are observed at low and high drug concentrations. At regions of very low concentrations the sensor becomes insensitive. The detection limit was calculated from the graph by the intersection of the two extrapolated linear segments of the calibration plot and was found to be 2.95×10^{-6} M and 1.47×10^{-5} M for the sensors T_{M8} and T_{P7} respectively.

The average time required for the TMP sensor to reach a stable potential within ±1 mV of the final equilibrium value after successive immersion in a series of TMP solutions, each having a 10 - fold difference in concentration was measured. Stable responses were achieved within 30 - 40 s for TMP concentrations of 1.0×10^{-2} - 1.0×10^{-7}M. The response time of the sensor T_{M8} was found to be <40 s and a response time of <30 s was shown by T_{P7} sensor.

3.5.3 Effect of pH

The influence of pH on the potential response of TMP sensors was investigated by recording the emf at two different concentrations (viz., 1.0×10^{-3} M and 1.0×10^{-4} M) of TMP solutions at different pH values. Figures 3.4 and 3.5 clearly depict the effect of pH of the test solution on the potential response of the developed sensors T_{M8} and T_{P7}. The pH of the solution was varied from 1-12 using buffer. For T_{M8} the potential remained constant in the pH range 2 - 6 whereas in the case of T_{P7} the potential remained unchanged

in the range 3 - 6. At pH>7, trimethoprim tends to precipitate, causing the potential to decrease. The potential decreases at pH <2 in the case of T_{M8} sensor and but it tends to decrease at pH<3 for the T_{P7} sensor.

3.5.4 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 solution. Selectivity represents to what extend the electrode is selective to the specified analyte ion. It is usually expressed in terms of the potentiometric selectivity coefficient. The selectivity coefficients for different foreign ions using the developed sensors were determined by the Fixed Interference Method (FIM). The selectivity coefficient values were determined graphically using the expression $K_{A,B}^{pot} = a_A / (a_B)^{z_A / z_B}$. The influence of some common cations and organic species on the response of the developed TMP selective sensors have been studied .The resultant selectivity coefficient values are summarized in Table 3.4. The very small selectivity coefficient values obtained indicate that none of the examined ions were found to interfere with the determination of the primary ion. The values of the selectivity coefficients given in the table reveal that the developed sensors show very good selectivity to TMP in the presence of ions such as of NH_4^+, Na^+, K^+, Mg^{2+}, Co^{2+}, Ca^{2+} etc. A close look on the table reveals that the commonly used tablet excepients and additives in the preparation of pharmaceutical formulations such as starch, lactose and talc did not interfere with the determinations. As TMP is usually available as a combination drug with sulphonamides, the interference of sulphamethoxazole and sulphadiazine have also been studied. A very low selectivity coefficient values of the

combination drugs sulphamethoxazole and sulphadiazine indicated that the sensors could be used for the selective determination of TMP.

3.5.5 Shelf Life or Life Time

The life time of the sensors were investigated by performing the calibration periodically with standard solutions of the drug and calculating the response slopes. The period through which the electrode retains the Nernstian response is known as the shelf life or life span. It was found that the life time of an ion selective electrode is fundamentally affected by the loss of one or more of its components with the consequent loss of its response [212-215]. It was found that the sensor T_{M8} could be used continuously for about two weeks without considerable decrease in its slope values. The sensor T_{P7} showed a lifetime of about three weeks.

3.6 Analytical Applications

The sensors T_{M8} and T_{P7} developed for TMP were employed for its determination in tablet form. The utility of the developed sensors in the determination TMP in real sample like urine was also studied.

3.6.1 Determination of TMP in Pharmaceutical Formulations (Tablets)

The developed sensors T_{M8} and T_{P7} were applied to the determination of TMP in pharmaceutical formulation commercialized as Aubril (Novartis Pharma, India). As the active components did not interfere with the determinations, the combination drug of TMP and sulphadiazine (Aubril) was used for the analysis. The TMP content was determined by the proposed ion selective sensors using the calibration method. A detailed procedure for the determination is given in section 2.9.1 of Chapter 2. The results summarized in Table 3.5 explains that the precision and accuracy of the method is very

high. The results obtained were compared with those obtained by the standard method reported in US Pharmacopoeia[184]. It has been found that there is satisfactory agreement between the TMP content determined by the proposed sensors and the official method.

3.6.2 Recovery of TMP from Urine Sample

The sensors proved useful for the determination of TMP content in biological samples such as urine using the standard addition method. The results are in good agreement and are within an acceptable range of error. The accuracy of the proposed method was confirmed by carrying out recovery experiments by standard addition technique by adding a known amount of standard to the pre- analyzed sample. The results are illustrated in Table 3.6. The % recoveries of TMP obtained using the sensors T_{M8} and T_{P7} were found to be 98.5 and 99.0 respectively.

3.7 Conclusion

Two carbon paste sensors, viz. T_{M8} and T_{P7} were developed for the selective determination of trimethoprim. The sensors exhibited near Nernstian slopes of 54.5 and 57.2 mV/decade for T_{M8} and T_{P7} respectively. The linear range of T_{M8} has been found to be $1.0 \times 10^{-2} - 1.0 \times 10^{-5}$M and that for T_{P7} to be $1.0 \times 10^{-2} - 5.0 \times 10^{-5}$M. A lower detection limit of 2.95×10^{-6}M was obtained for T_{M8} and 1.47×10^{-5}M was obtained for T_{P7}.The effect of pH on the potential response indicated that a pH range of 2-6 was the optimum pH range in the case of T_{M8} and 3-6 was the optimum pH range for the sensor T_{P7}.The response time of the sensor T_{M8} was found to be less than 40 s and its shelf life was about 2 weeks. In the case of T_{P7} the response time was less than 30 s. It had a lifetime of about 3 weeks. The sensor T_{P7} was best in terms of slope, fast response time and also long shelf life, whereas T_{M8} was

superior to T_{P7} in having a much lower detection limit and a wider pH range. Both the fabricated sensors were successfully applied to the determination of trimethoprim in pharmaceutical preparation. A combination drug of TMP and sulphadiazine (Aubril) was used for the application studies. The developed sensors gave very close values to the declared amount of 90mg per tablet clearly indicating that the combination drug sulphadiazine did not interfere with the determination of TMP. The sensors were also applied to the determination of the drug in real samples like urine. The analytical method proposed proved to be a simple, rapid and accurate method.

Table 3.1 Optimization of composition of carbon paste sensor using TMP – MPA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	Graphite	Plasticizer	
T_{M1}	1.2	37.2	61.6, BEA	64.2
T_{M2}	1.4	40.0	58.6, BEA	66.8
T_{M3}	1.6	35.0	63.4, BEA	48.2
T_{M4}	1.2	37.2	61.6, DBS	67.8
T_{M5}	1.4	40.0	58.6, DBS	39.4
T_{M6}	1.6	35.0	63.4, DBS	67.1
T_{M7}	1.2	37.2	61.6, BEP	52.4
T_{M8}	***1.4***	***40.0***	***58.6, BEP***	***54.5***
T_{M9}	1.6	35.0	63.4, BEP	62.6
T_{M10}	1.2	37.2	61.6, BES	48.7
T_{M11}	1.4	40.0	58.6, BES	68.4
T_{M12}	1.6	35.0	63.4, BES	44.6
T_{M13}	1.2	37.2	61.6, DBP	50.8
T_{M14}	1.4	40.0	58.6, DBP	64.6
T_{M15}	1.6	35.0	63.4, DBP	51.7

Table 3.2 Optimization of composition of carbon paste sensor using TMP – PTA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	Graphite	Plasticizer	
T_{P1}	1.2	37.2	61.6, BEA	49.7
T_{P2}	1.4	40.0	58.6, BEA	70.1
T_{P3}	1.6	35.0	63.4, BEA	64.3
T_{P4}	1.2	37.2	61.6, DBS	64.6
T_{P5}	1.4	40.0	58.6, DBS	44.6
T_{P6}	1.6	35.0	63.4, DBS	66.1
T_{P7}	***1.2***	***37.2***	***61.6, BEP***	***57.2***
T_{P8}	1.4	40.0	58.6, BEP	61.7
T_{P9}	1.6	35.0	63.4, BEP	49.5
T_{P10}	1.2	37.2	61.6, BES	65.1
T_{P11}	1.4	40.0	58.6, BES	62.4
T_{P12}	1.6	35.0	63.4, BES	51.4
T_{P13}	1.2	37.2	61.6, DBP	50.4
T_{P14}	1.4	40.0	58.6, DBP	49.9
T_{P15}	1.6	35.0	63.4, DBP	67.3

Table 3.3 Response characteristics of the developed sensors T_{M8} and T_{P7}

Parameter	Carbon paste sensor T_{M8}	Carbon paste sensor T_{P7}
Slope (mV per decade)	54.5	57.2
Linear range (M)	$1.0 \times 10^{-2} - 1.0 \times 10^{-5}$	$1.0 \times 10^{-2} - 5.0 \times 10^{-5}$
pH range	2 - 6	3 - 6
Detection limit (M)	2.95×10^{-6}	1.47×10^{-5}
Response time (s)	< 40	< 30
Shelf life	2 weeks	3 weeks

Table 3.4 Selectivity coefficient values of various interfering species, K^{pot}

Interfering Species	$K^{pot}_{A,B}$ Carbon paste sensor T_{M8}	Carbon paste sensor T_{P7}
NH_4^+	3.7×10^{-2}	1.5×10^{-2}
K^+	2.6×10^{-2}	4.5×10^{-2}
Na^+	6.5×10^{-2}	3.3×10^{-2}
Mg^{2+}	3.4×10^{-3}	4.1×10^{-2}
Co^{2+}	3.8×10^{-3}	5.3×10^{-3}
Ca^{2+}	4.4×10^{-3}	3.9×10^{-3}
Ni^{2+}	9.2×10^{-4}	7.4×10^{-4}
Zn^{2+}	5.1×10^{-4}	4.9×10^{-3}
Urea	6.2×10^{-3}	7.8×10^{-3}
Sulphamethoxazole	4.7×10^{-2}	5.2×10^{-2}
Sulphadiazine	3.3×10^{-2}	4.1×10^{-2}
Ascorbic acid	1.3×10^{-2}	3.6×10^{-2}
Starch	2.5×10^{-2}	3.7×10^{-2}
Talc	5.3×10^{-2}	4.6×10^{-2}
Glycine	2.9×10^{-3}	4.3×10^{-3}
Lactose	1.7×10^{-2}	3.2×10^{-2}

Table 3.5 Determination of TMP in pharmaceutical formulation

Sample	Declared Amt (mg/tablet)	Method adopted	Found * (mg/tablet)	SD	CV
Aubril (Novartis Pharma, India)	Trimethoprim = 90	T_{M8}	88	0.95	1.08
		T_{P7}	89	0.92	1.03
	Sulphadiazine = 410	Standard Method	89	0.93	1.04

* Average of six replicates.

Table 3.6 Determination of TMP in urine sample using the developed sensors

Drug taken (M)	Sensor	Drug found* (M)	Recovery %
2.00×10^{-4}	T_{M8}	1.97×10^{-4}	98.5
	T_{P7}	1.98×10^{-4}	99.0

* Average of six replicates.

Figure 3.1 Structure of Trimethoprim

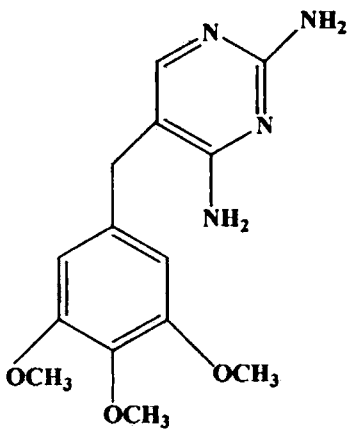


Figure 3.2 Calibration graph for TMP selective carbon paste sensor based on TMP - MPA ion association (T_{M8})

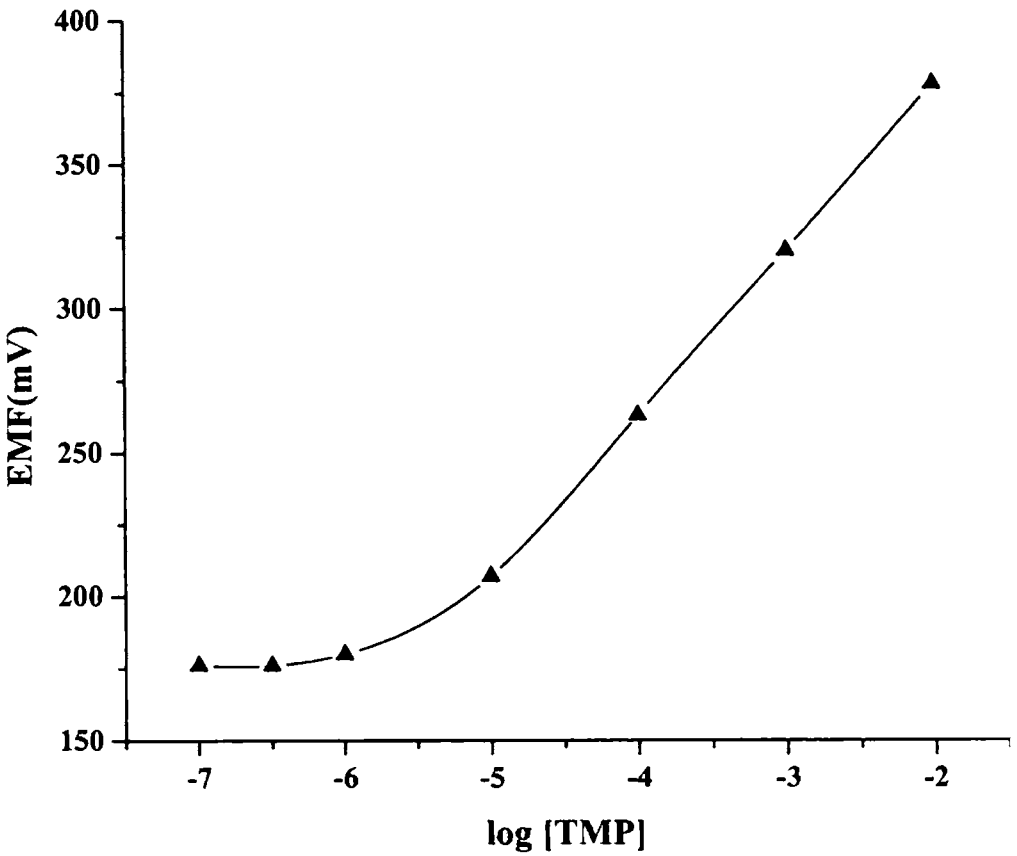


Figure 3.3 Calibration graph for TMP selective carbon paste sensor based on TMP - PTA ion association (T_{P7})

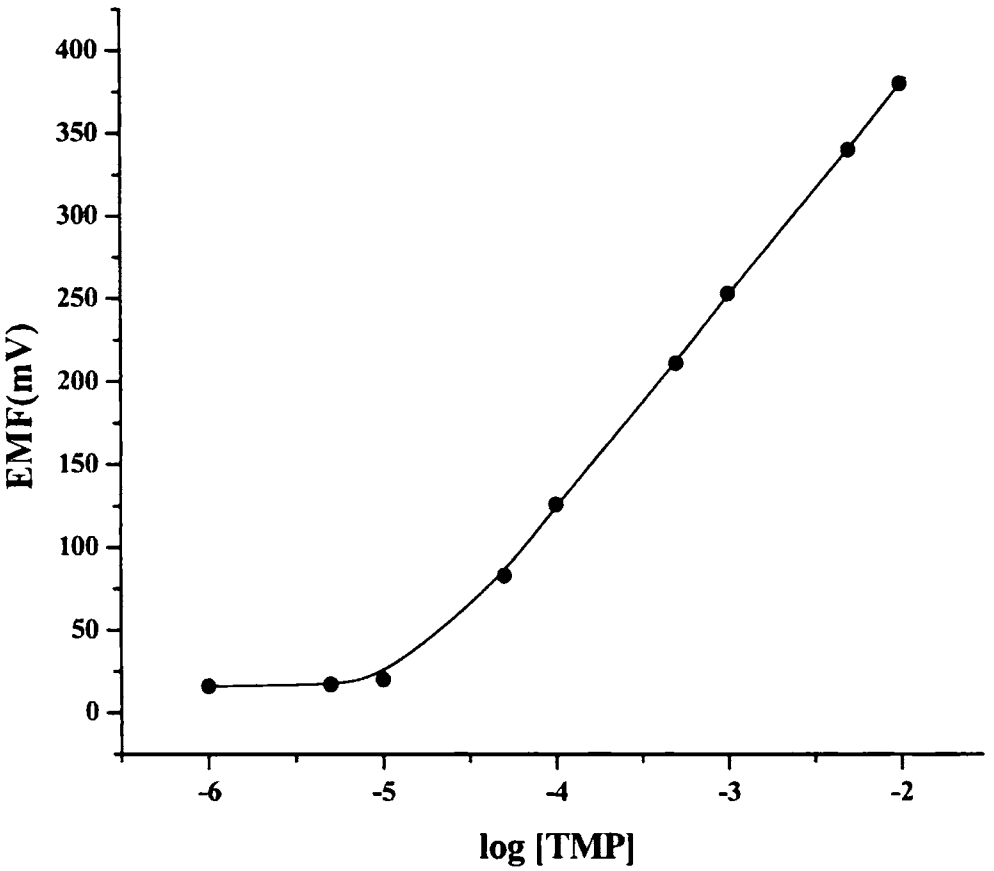


Figure 3.4 Effect of pH on the cell potential of TMP selective carbon paste sensor T_{M8} at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

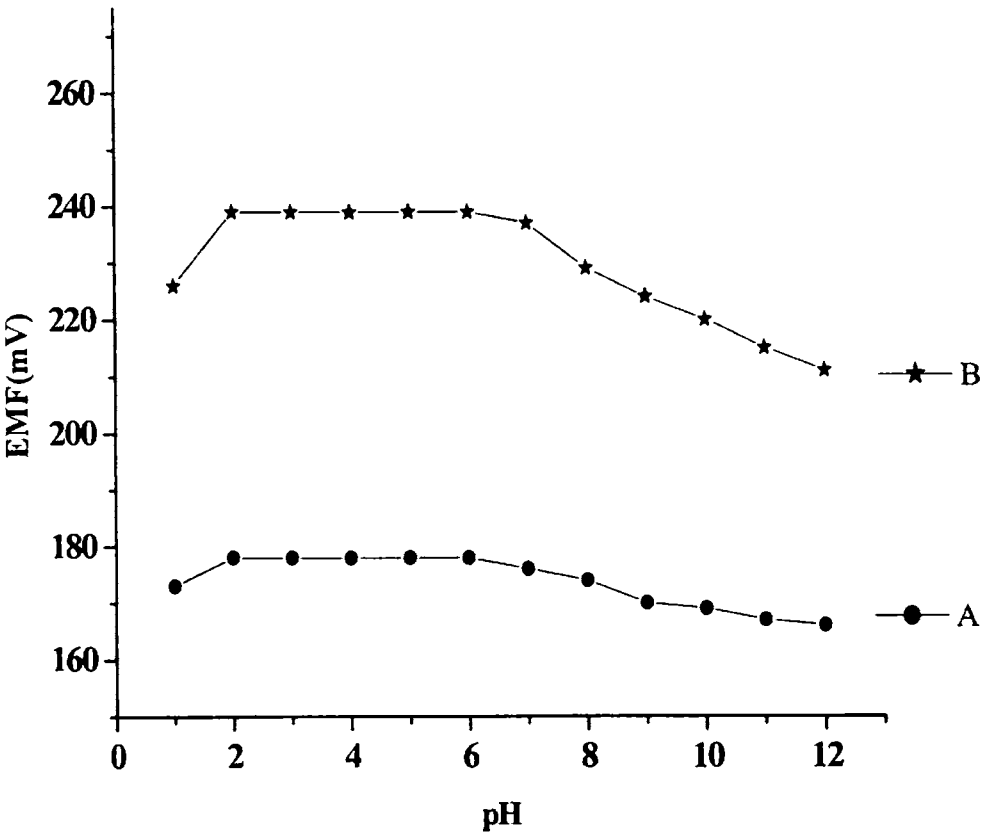
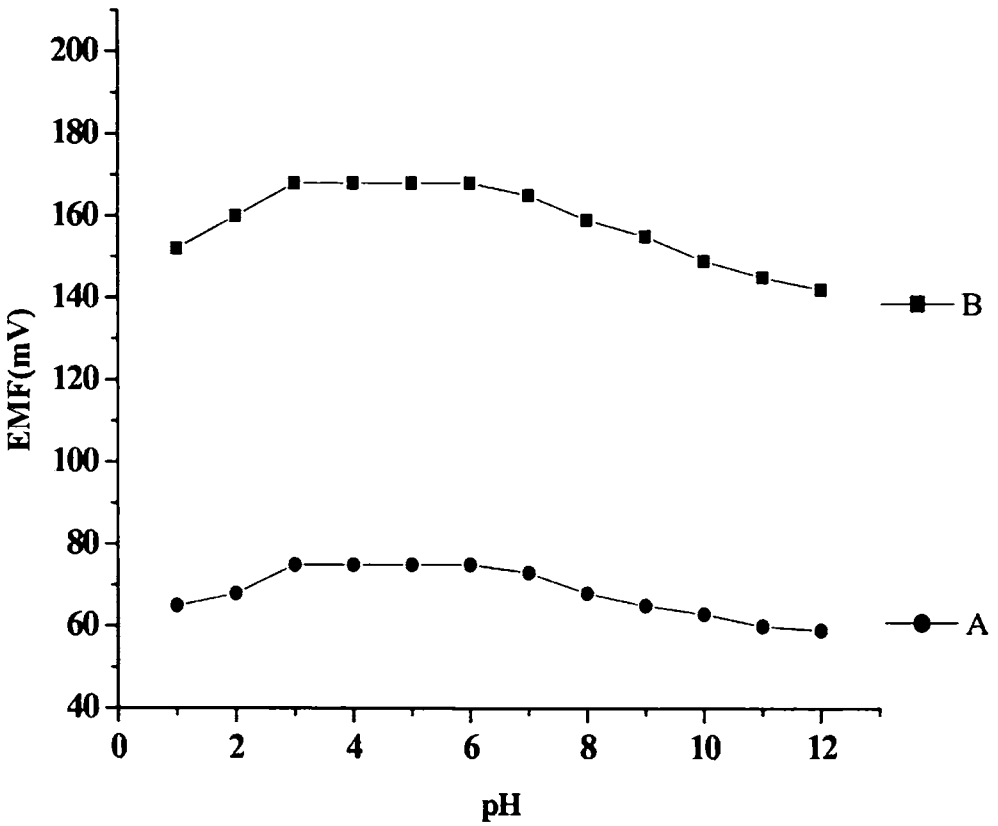


Figure 3.5 Effect of pH on the cell potential of the TMP selective carbon paste sensor T_{P7} at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)



Chapter 4

SENSORS FOR THE DETERMINATION OF KETOCONAZOLE

This chapter deals with the development of two novel electrochemical sensors for the determination of the drug Ketoconazole (KET) based on KET-MPA (molybdophosphoric acid) ion pair as the electroactive material. The optimization studies have been carried out and response characteristics of the developed sensors have been studied in detail. The developed sensors were successfully applied to the determination of the drug in pharmaceutical formulations and also its recovery from biological fluids like urine sample has been discussed.

4.1 Introduction

Ketoconazole (Figure 4.1), [(±)-*cis*-1-acetyl-4-(4-{[2-(2,4-dichlorophenyl) -2- (1*H*-imidazol-1-ylmethyl)-1,3-dioxolan- 4-yl] methoxy} phenyl) piperazine], is an orally active antimycotic agent from the class of imidazole derivatives. It is active against Cryptococcus neoformans and Pseudallescheria boydi [216]. It is used to treat a wide variety of dermal and systematic Mycoses and has the advantage over other imidazole derivatives of producing adequate sustained blood levels following oral administration [217,218]. Moreover, it has been found that ketoconazole may cause changing in cytochrome P-450 dependent monooxygenase activities[219,220] as well as in epoxide hydrolase[221]. Thus, the

determination of ketoconazole in biological specimens and dosage forms has been the subject of considerable interest.

Ketoconazole is the only member of the imidazole derivatives currently used for the treatment of systemic infections. The continually increasing number of patients suffering from mycoses due to decreased immunity (AIDS, organ transplantations, *etc.*) has led to a rather frequent application of pharmacologically active agents from this class [216].

Ketoconazole is used as a broad-spectrum antifungal agent for the treatment or prevention of fungal infections especially against thrush, gastrointestinal infections, and infections of the skin, nail, and scalp. It is also topically used in the formulation of cosmetic creams and in shampoos as an anti-dandruff agent.

Due to the vital importance of ketoconazole determination in pharmaceutical preparations and in biological fluids, several analytical methods have been developed for quantitative determination of ketoconazole. These include visible spectrophotometry [222], ultraviolet (UV) spectrophotometry [223-233], spectrofluorimetry[234], thin-layer chromatography[235], supercritical fluid chromatography with UV detection[236], capillary electrophoresis with diode array detection[237], high performance liquid chromatography (HPLC) using different detection modes such as UV [238-240], diode array [241] and electrochemical detection[242,243] and stripping voltammetric and polarographic techniques [244]. However, most of these methods require expensive and sophisticated instruments and are time- consuming. Hence a simple and sensitive method for the analysis of ketoconazole is developed here.

This chapter describes the fabrication of potentiometric sensors for the determination of KET in pure form and in dosage forms. The reported sensors

include a PVC membrane sensor and a carbon paste sensor prepared by incorporating the ion association complex of ketoconazole with molybdophosphoric acid. Performance characteristics of the two sensors were also studied. The sensors were successfully applied for the determination of KET in pure solutions and pharmaceutical preparations and the results obtained are in good agreement with that obtained by the official method. The application of the developed sensors to biological fluids such as urine sample was also conducted.

4.2 Synthesis of the Ion Association

The KET – MPA ion association was prepared by mixing 75 mL 10^{-2}M KET with 25 mL 10^{-2}M MPA solutions. The mixture was then shaken well for 10 min and the produced precipitate was filtered through a Whatman filter paper, washed thoroughly with distilled water, dried at room temperature and stored in a desiccator. The composition of the ion association was confirmed by elemental analysis to be 3:1 (KET: ion pairing reagent). The elemental analysis data obtained for the ion association is as follows:

KET- MPA ion association

Found (%): – C – 24.34, H – 2.21, N – 4.31

Calculated (%): – C –24.29, H – 2.18, N – 4.36

4.3 Fabrication of KET Membrane Sensor

The membrane electrode was constructed according to the Craggs procedure. The membrane composition was studied by varying the weight percentages of the ion pair, PVC and plasticizer, until the optimum composition that exhibits the best performance was obtained. The PVC membrane was prepared by dissolving the required amount of the ion pair, plasticizer and PVC in 5-7 mL of THF. The mixture was then poured into glass rings struck onto a

glass plate and allowed to stand overnight. After the slow evaporation of solvent the sensing membrane is formed. Small portions of the membrane were cut and glued to one end of a glass tube. The electrode body was filled with an inner filling solution containing NaCl (1.0×10^{-1}M) and KET (1.0×10^{-3}M). The finished electrode was conditioned in KET solution (1.0×10^{-3}M) for 24 hrs. The electrode was washed with distilled water before measurement.

4.4 Fabrication of KET Carbon paste Sensor

For the preparation of the carbon paste sensor, weighed amount of the ion pair (KET-MPA) was mixed with spectroscopic graphite powder. Plasticizer was added to the mixture and it was again thoroughly mixed until it was uniformly wetted. A teflon holder with a hole at one end for the carbon paste filling served as the electrode body. Electrical contact was made with a copper rod that runs through the center of the electrode holder. The electrode surface was polished using a filter paper to produce reproducible working surface. The sensor was conditioned by soaking in a 1.0×10^{-3} M KET solution.

4.5 Potential Measurement and Calibration

All emf measurements were carried out using the following cell assembly. A saturated calomel electrode (SCE) was used as the external as well as the internal reference electrode. The electrochemical cell assembly may be represented as,

For membrane sensor:

Saturated calomel electrode | internal filling solution (1×10^{-1} M NaCl solution + 1×10^{-3}M drug solution) | PVC membrane | test solution | saturated calomel electrode.

For carbon paste sensor:

Reference electrode | test solution | Graphite electrode.

A Metrohm 781 ion meter was used for potential measurements. All emf measurements were carried out at 25 ± 1^0C.

Standard solutions of the analyte were prepared by serial dilution of stock solution. The emf of the cell was directly measured by the developed sensors using the previously mentioned cell assembly. Calibration graph was obtained by plotting EMF (mV) versus log [KET]. The calibration graph was used for subsequent determination of unknown KET concentrations.

4.6 Performance Characteristics of the Developed Sensors

The performance characteristics of a developed sensor are described in terms of its linear range, detection limit, slope, response time and shelf life. These factors are discussed in detail in the next section.

4.6.1 Optimization Studies of the Two Types of Sensors

The nature of the plasticizer has a marked influence on the response slope, linear domain and also on the selectivity of PVC membrane electrodes. Optimizations of the membrane composition are presented in Table 4.1. Five different plasticizers viz. BEP, BES, DBS, BEA, DBP were employed to study their effect on the electrochemical behaviour of the membrane. Of the five different plasticizers used, DBP was found to give a near Nernstian response. Hence, the sensor with DBP was selected for further studies. Different ratios of membrane composition were employed to evaluate their effects on the response characteristics of membrane sensor. The results revealed that the best composition was ion association: PVC: plasticizer (DBP) as 2.2:40.2:57.6 wt% (Kp_5). The sensor gave a linear response behaviour within the concentration

range $1.0 \times 10^{-2} - 5.0 \times 10^{-5}$M solution of KET with a slope of 57.8 mV/decade and a lower detection limit of 7.94×10^{-5} M (Figure 4.2).

The surface morphology of the developed membrane was analyzed using SEM. This technique allows the study of membrane surface characteristics, such as morphological homogeneity and chemical composition. Homogeneity of the membrane may affect the response characteristics of a sensor. The SEM image of the membrane of Kp_5 sensor is shown in Figure 4.3.The image obtained shows a homogenous membrane with the absence of solid particles or cumulus.

The response characteristics of the carbon paste sensors are largely affected by the nature of the plasticizer used. All the five plasticizers which were tried for membrane sensors have been tried in the case of carbon paste sensors also. Optimization of the carbon paste composition are consolidated in Table 4.2.The use of BEP resulted in a near Nernstian linear plot over the concentration range 1×10^{-2} - 1×10^{-5}M (Figure 4.4). In the case of other plasticizers used, the slopes of the potentiometric response are much different from the expected Nernstian value. The results show that the sensor made of 2.4% KET-MPA ion pair (Kc_3) exhibits the best performance (slope 55.2 mV/ decade and detection limit of 2.45×10^{-6}M). In all subsequent studies, the sensor made of 2.4% KET-MPA ion pair was used in case of carbon paste electrode. The response characteristics of the two types of sensors under investigation are summarized in Table 4.3.

4.6.2 Effect of Concentration of Internal Filling Solution

The influence of the concentration of the internal filling solution on the potential response of the KET selective membrane sensor was studied. The

KET concentration was changed from 1.0×10^{-4} to 1.0×10^{-2} M and the EMF vs. log [KET] plot was obtained. It was found that the variation in concentration of the internal solution did not cause any significant difference in the potential. Hence a 1.0×10^{-3}M KET solution was fixed as the internal filling solution in the case of the membrane sensor. For the carbon paste electrode there is no need for an internal filling solution. This is one of the significant advantages of the carbon paste sensor.

4.6.3 Effect of pH

The effect of pH of the test solution on the electrode potential was investigated by following the variation in potential with change in pH. Figures 4.5 and 4.6 clearly depict the effect of pH on the potential response of the two sensors. Two different concentrations of the test solution namely 1.0×10^{-4}M and 1.0×10^{-3}M KET were used to study the effect of pH. The pH was adjusted using different buffer solutions. From the pH studies, it was found that there was no change in the potential response for both the electrodes within the pH range 3 - 6 and hence this was chosen as the working pH range of the sensors.

4.6.4 Potentiometric Selectivity

Selectivity is one of the basic characteristics of an electrochemical sensor. The response of any ion-selective sensor to the primary ion in the presence of other ions present in the solution is expressed in terms of the potentiometric selectivity coefficient. It gives a basic source of information on the interferences in the ion selective electrode response. The selectivity of an ion pair based membrane electrode depends on the physico-chemical characteristics of the ion exchange process at the membrane–sample solution interface, on the mobility of the respective ions in the membrane, and on the hydrophobic interactions between the primary ion and the organic membrane

[245]. The interference of various substances on the selectivity of the developed sensors has been examined using the fixed interference method[182]. The potentiometric selectivity coefficients values were determined and the resulting selectivity coefficients are summarized in Table 4.4. The results reveal that there are no significant interferences from all of the tested substances namely urea, ascorbic acid, glycine and cations such as NH_4^+, K^+, Na^+, Mg^{2+}, Co^{2+}, Ni^{2+}, $Ca^{2+}$and Zn^{2+}. Also the tablet execepients such as talc, lactose etc have no interfering action with the determination of KET as indicated by the very low selectivity coefficient values. Hence the sensors can be selectively used for the determination of KET.

4.6.5 Response Time and Life Time of the Sensors

The average response time is the time required for the sensor to reach a stable potential within a value of ±1 mV of the final equilibrium value. The response time for the sensor Kp_5 was less than 30 s and that of the Kc_3 sensor was less than 45 s. The lifetime of the electrode was investigated by measuring the potentials in standard drug solutions each day. The response slope of the sensors was calculated each time. A near Nernstian slope was obtained for a period of 4 weeks in the case of membrane sensor Kp_5 and 2 weeks for the carbon paste sensor Kc_3.

4.7 Analytical Applications

The application of the developed sensors was conducted by determining the KET content in pharamaceutical formulation such as tablets. The sensors were also applied for the determination of KET in real sample like urine.

4.7.1 Determination of KET in Pharmaceutical Formulations (Tablets)

The developed sensors were successfully applied for the determination of KET in commercially available pharmaceutical formulations such as Ketovate (Bal Pharma) and Ketozole (Rexcel). The detailed procedure for the determination is given in section 2.9.2 of Chapter 2. The results obtained are summarized in Table 4.5. The SD and CV values obtained indicate that the determination of the drug using the developed sensors is highly precise and accurate. The results were compared with those obtained by the standard method (potentiometric titration)[185] reported in European Pharmacopoeia. The results show that there is a satisfactory agreement between the KET content determined by the proposed method and the standard method.

4.7.2 Recovery of KET from Urine Sample

The newly developed sensors were applied to the determination of the drug in biological fluids. The sensors were applied to the recovery of KET from urine samples. The KET content of the solution was then determined by the proposed sensors using the standard addition method, the detailed procedure of which is given in Chapter 2.The results of the determination are summarized in Table 4.6. The results show that the proposed sensors can detect the investigated drug in urine samples with high accuracy and high % recovery without pretreatment procedures of the sample. High percentage recovery values of 99.7 and 100.3 were obtained using Kp_5 and Kc_3.

4.8 Conlcusion

Potentiometric sensors were developed for the selective determination of the drug ketoconazole. The developed sensors include both membrane sensor and a carbon paste sensor. The linear range of the membrane sensor

was obtained between 1×10^{-2} and 5×10^{-5}M whereas in the case of the carbon paste sensor it was between 1×10^{-2} and 1×10^{-5}M. Both sensors had an optimum pH range of 3 - 6. A much lower detection limit was obtained for the carbon paste sensor. The slope, response time and shelf life of the membrane sensor was found to be far superior when compared to the carbon paste sensor. Moreover, the use of the newly developed sensors for the determination of ketoconazole in pharmaceutical formulations and real samples at less cost, with accuracy and without the need for any special treatment of the samples are all advantageous over other reported techniques.

Table 4.1 Optimization of composition of PVC membrane sensor using KET-MPA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	PVC	Plasticizer	
Kp_1	2.0	33.0	65.0, BEP	53.7
Kp_2	2.2	40.2	57.6, BEP	62.8
Kp_3	2.4	42.0	55.6, BEP	73.6
Kp_4	2.0	33.0	65.0, DBP	55.6
Kp_5	***2.2***	***40.2***	***57.6, DBP***	***57.8***
Kp_6	2.4	42.0	55.6,DBP	52.2
Kp_7	2.0	33.0	65.0, DBS	37.2
Kp_8	2.2	40.2	57.6, DBS	52.9
Kp_9	2.4	42.0	55.6, DBS	56.9
Kp_{10}	2.0	33.0	65.0, BES	50.5
Kp_{11}	2.2	40.2	57.6, BES	65.9
Kp_{12}	2.4	42.0	55.6, BES	73.2
Kp_{13}	2.0	33.0	65.0, BEA	67.9
Kp_{14}	2.2	40.2	57.6, BEA	49.8
Kp_{15}	2.4	42.0	55.6, BEA	46.5

Table 4.2 Optimization of composition of carbon paste sensor using KET - MPA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	Graphite	Plasticizer	
Kc_1	2.0	33.0	65.0, BEP	50.5
Kc_2	2.2	40.2	57.6, BEP	48.6
Kc_3	***2.4***	***42.0***	***55.6, BEP***	***55.2***
Kc_4	2.0	33.0	65.0, DBP	46.7
Kc_5	2.2	40.2	57.6, DBP	39.7
Kc_6	2.4	42.0	55.6,DBP	52.2
Kc_7	2.0	33.0	65.0, DBS	52.9
Kc_8	2.2	40.2	57.6, DBS	37.2
Kc_9	2.4	42.0	55.6, DBS	49.8
Kc_{10}	2.0	33.0	65.0, BES	73.2
Kc_{11}	2.2	40.2	57.6, BES	65.9
Kc_{12}	2.4	42.0	55.6, BES	63.7
Kc_{13}	2.0	33.0	65.0, BEA	50.4
Kc_{14}	2.2	40.2	57.6, BEA	67.5
Kc_{15}	2.4	42.0	55.6, BEA	71.2

Table 4.3 Response characteristics of the developed sensors Kp_5 and Kc_3

Parameter	PVC Membrane sensor Kp_5	Carbon paste sensor Kc_3
Slope (mV per decade)	57.8	55.2
Linear range (M)	$1 \times 10^{-2} - 5 \times 10^{-5}$	$1 \times 10^{-2} - 1 \times 10^{-5}$
pH range	3-6	3-6
Detection limit (M)	7.94×10^{-5}	2.45×10^{-6}
Response time (s)	< 30	< 45
Shelf life	4 weeks	2 weeks

Table 4.4 Selectivity coefficient values of various interfering species, K^{pot}

Interfering species	$K^{pot}_{A,B}$	
	PVC membrane sensor Kp_5	**Carbon paste sensor** Kc_3
NH_4^+	4.6×10^{-3}	2.7×10^{-2}
K^+	3.2×10^{-2}	3.1×10^{-3}
Na^+	6.1×10^{-2}	4.3×10^{-4}
Mg^{2+}	1.7×10^{-3}	5.1×10^{-2}
Co^{2+}	4.2×10^{-3}	2.8×10^{-3}
Ca^{2+}	5.1×10^{-3}	5.7×10^{-3}
Ni^{2+}	4.7×10^{-4}	3.5×10^{-4}
Zn^{2+}	5.9×10^{-4}	3.8×10^{-3}
Urea	2.8×10^{-3}	5.1×10^{-3}
Starch	3.6×10^{-3}	4.5×10^{-3}
Talc	2.8×10^{-2}	3.9×10^{-2}
Ascorbic acid	1.6×10^{-3}	4.8×10^{-3}
Glycine	3.4×10^{-3}	2.5×10^{-3}
Lactose	2.2×10^{-3}	3.6×10^{-3}

Table 4.5 Determination of KET in pharmaceutical formulations

Sample	Declared Amt (mg/tablet)	Method adopted	Found * (mg/tablet)	SD	CV
Ketovate	200	**Kp_5**	196	0.91	0.46
(Bal Pharma, India)		**Kc_3**	195	0.89	0.46
		Standard Method	198	0.95	0.48
Ketozole	200	**Kp_5**	198	0.95	0.48
(Rexcel, India)		**Kc_3**	197	0.91	0.46
		Standard Method	198	0.98	0.49

* Average of six replicates.

Table 4.6 Determination of KET in urine sample using the developed sensors

Drug taken (M)	Sensor	Drug found* (M)	Recovery %
3.00×10^{-4}	**Kp_5**	2.99×10^{-4}	99.7
	Kc_3	3.01×10^{-4}	100.3

* Average of six replicates.

Figure 4.1 Structure of Ketoconazole

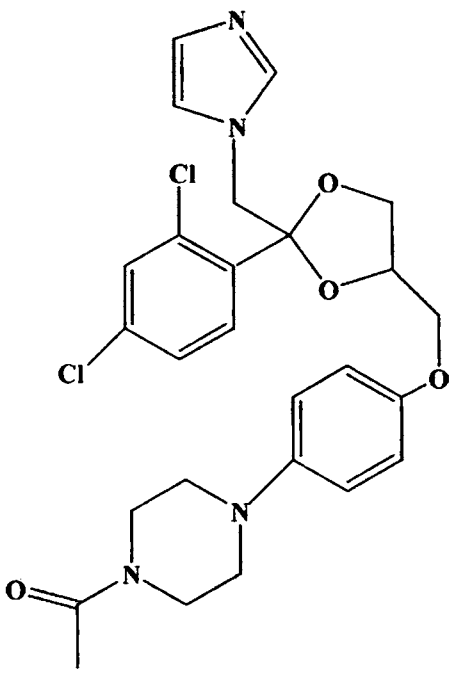


Figure 4.2 Calibration graph for KET selective PVC membrane sensor based on KET - MPA ion association (Kp_5)

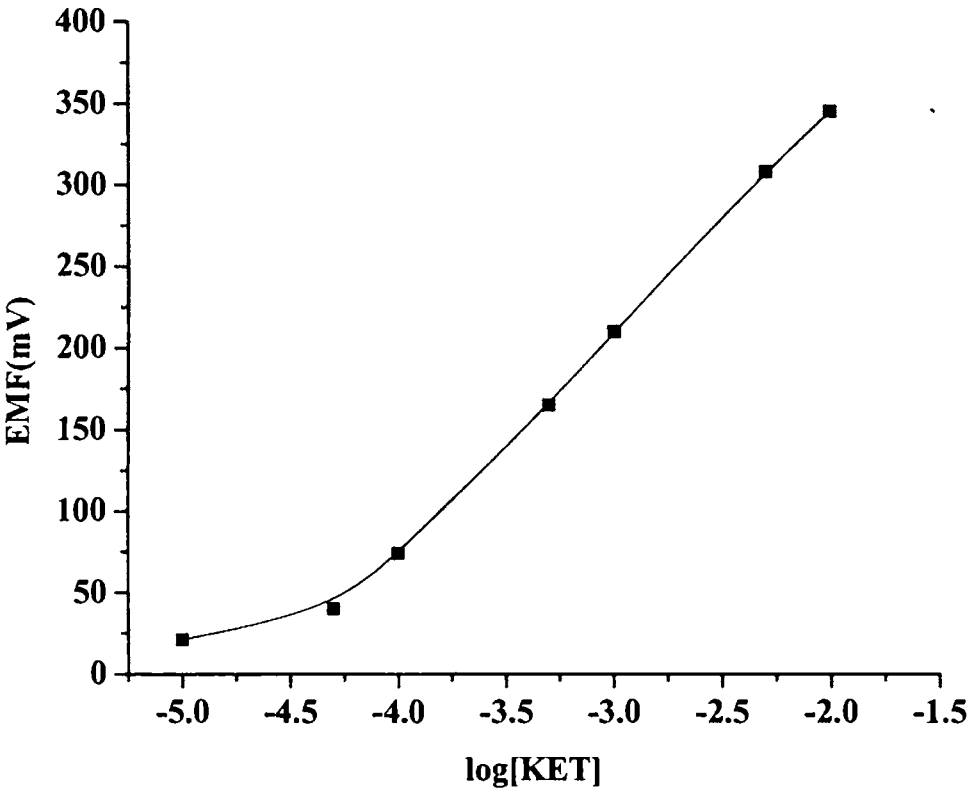


Figure 4.3 SEM image of the polymeric membrane of $\mathbf{Kp_5}$ sensor

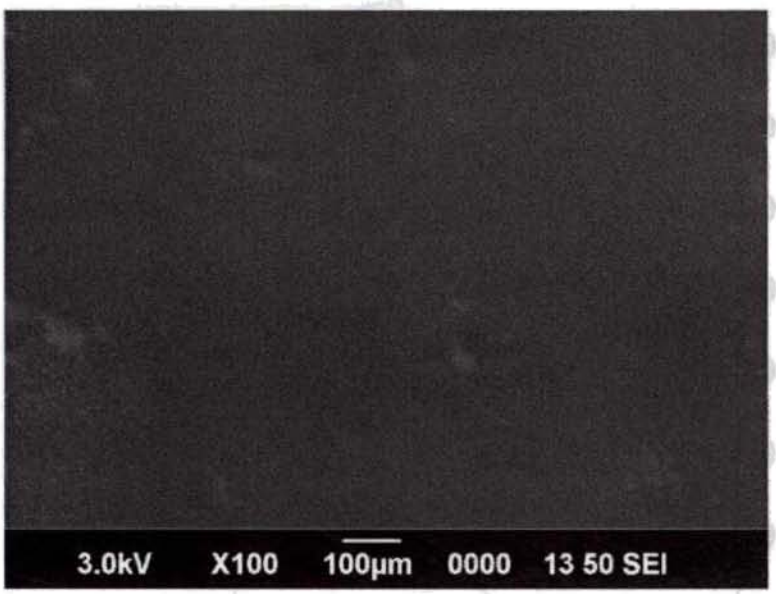


Figure 4.4 Calibration graph for KET selective carbon paste sensor based on KET - MPA ion association (Kc_3)

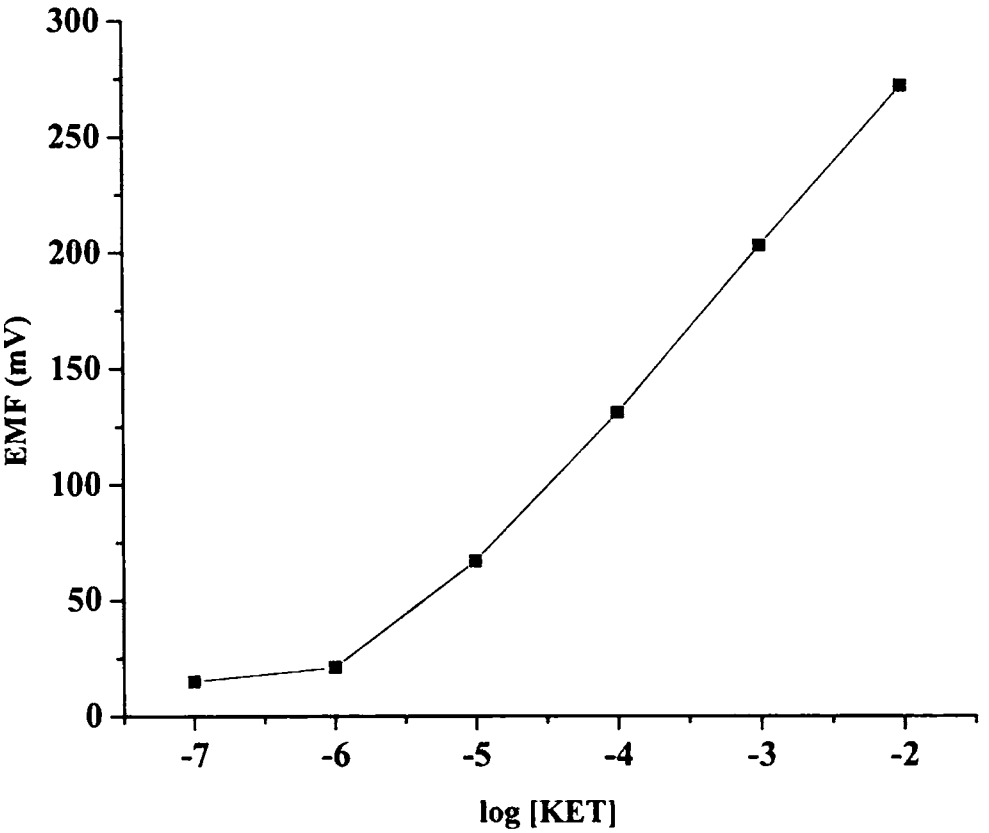


Figure 4.5 Effect of pH on the cell potential of the KET selective PVC membrane sensor **Kp_5** at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

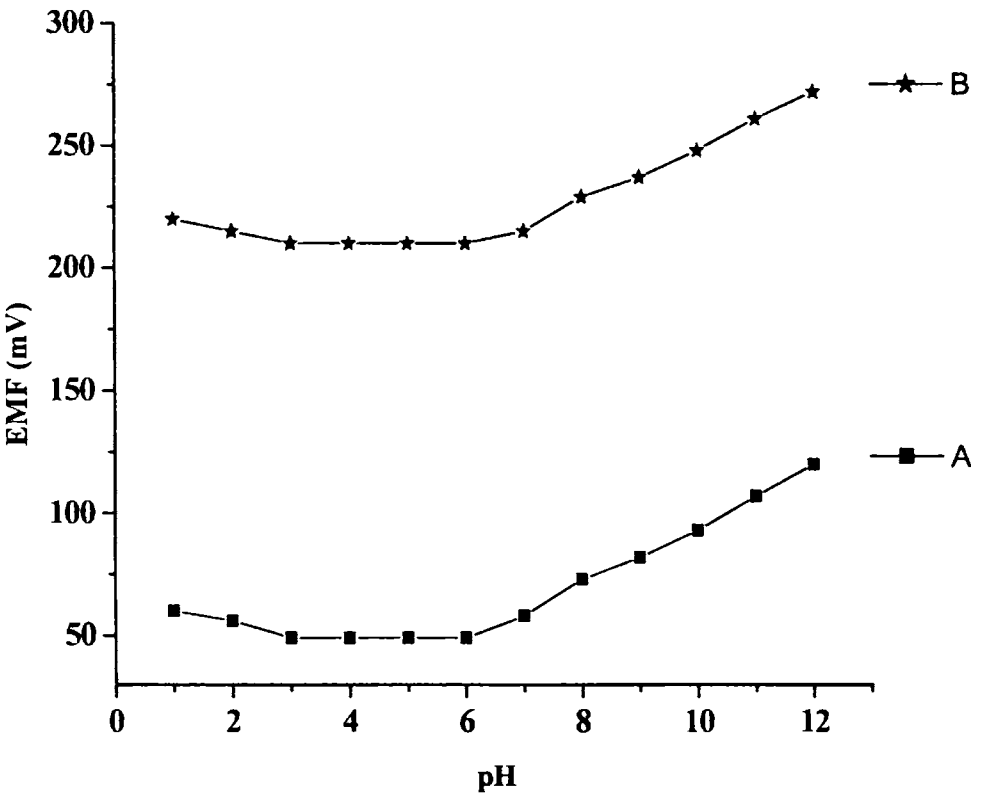
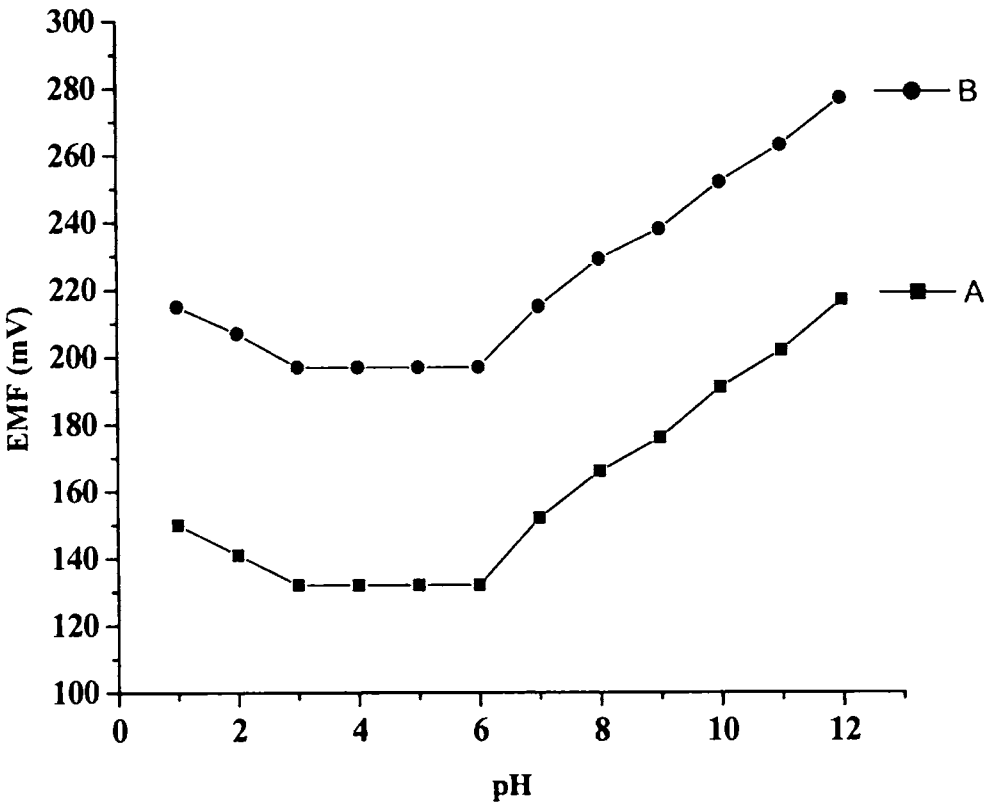


Figure 4.6 Effect of pH on the cell potential of the KET selective carbon paste sensor **Kc$_3$** at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)



Chapter 5

SENSORS FOR THE DETERMINATION OF LAMIVUDINE

The construction and performance characteristics of four different types of sensors for the drug Lamivudine (LAM) have been discussed clearly in the chapter. The four different types of sensors include two carbon paste sensors and also two polymeric membrane sensors. Both sensors incorporated the ion association of the drug with molybdophosphoric acid (MPA) and phosphotungstic acid (PTA) as electroactive materials. Along with their response characteristics, the present study has covered the applicability of the newly developed sensors in various fields such as pharmaceutical formulations and real samples like urine.

5.1 Introduction

Lamivudine (Figure 5.1) belongs to the class of dideoxynucleoside reverse transcriptase inhibitors, and is a potent in vitro and in vivo inhibitor of human immunodeficiency virus (HIV), the causative agent of the acquired immunodeficiency syndrome (AIDS)[246-249]. Furthermore, lamivudine shows activity against hepatitis B virus[250-252]. The pharmacokinetic profile of lamivudine in HIV infected patients has not been investigated thoroughly yet.

Human immunodeficiency virus type-1 (HIV-1), the agent that causes acquired immunodeficiency affected syndrome (AIDS), is found in blood, spleen, lymph nodes, brain, saliva, cervicovaginal secretions and semen of infected male patients[253-255]. Synthetic nucleoside analogs are commonly used to treat HIV-1 infection. Human intracellular kinases phosphorylate these synthetic compounds to form triphosphorylated analogs, which are inserted into the viral DNA. This insertion blocks further elongation of viral DNA. For this reason, compounds in this class of drugs are commonly called nucleoside analog chain terminators. Lamivudine and zidovudine are two commonly used drugs of this class[256]. The use of zidovudine plus lamivudine combination therapy causes a decrease of HIV-1 in blood plasma.

Lamivudine is 2',3'-dideoxy-3'-thiacytidine and as mentioned earlier it is a potent reverse transcriptase inhibitor of the class nucleoside analog reverse transcriptase inhibitor (NARTI). It is also called 3TC. It is an analogue of cytidine.Lamivudine has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV. In combination with other antiretroviral agents, lamivudine can be effective in suppressing viral replication. The drug is also effective in suppressing the Hepatitis B virus. It improves the seroconversion of e-antigen positive hepatitis B and also improves histology staging of the liver. Long term use of lamivudine unfortunately leads to emergence of a resistant hepatitis B virus mutant. Despite this, lamivudine is still used widely as it is well tolerated. 3TC has also been shown to be much less toxic towards human bone marrow cells as compared to nucleoside analogs currently used in AIDS therapy[257]and its high selectivity was also observed in liver cells, a critical target site for anti-hepatitis drugs[258]. Recent clinical trials evaluating the anti-HIV and anti-

HBV effects of 3TC have confirmed the in vitro findings with an excellent safety profile without major dose-limiting toxicity at doses ranging from 0.5 to 20 mg/kg per day [259,260].

Lamivudine was invented by Bernard Belleau and Nghe Nguyen-Ba at the Montreal-based IAF BioChem International, Inc. laboratories in 1989. The drug was later licensed to the British pharmaceutical company Glaxo for a 14 percent royalty.Lamivudine was approved by the Food and Drug Administration (FDA) on Nov 17, 1995 for use with Zidovudine (AZT) and again in 2002 as a once-a-day dosed medication. The fifth antiretroviral drug in the market, it was the last NARTI for three years while the approval process switched to protease inhibitors. Its patent will expire in the United States on 2016-05-18.

A high-performance liquid chromatographic (HPLC) assay with UV detection for the quantitation of this compound in human urine has been described by Morris et al[261]. An HPLC assay for the determination of lamivudine in human serum has been described by Harker et al[262]. Furthermore, an HPLC methodology for the quantitative determination of lamivudine in perfusion solutions from isolated perfused rat kidney studies was described by Hsyu and Lloyd[263]. A radioimmuno assay for the quantitation of lamivudine was described, which may be of use for the determination of intracellular phosphorylated lamivudine[264]. High Throughput Analysis of lamivudine in pharmaceutical preparations using monolithic silica HPLC column was described by Hassan et al[265]. A rapid HPLC method with UV detection for the analysis of lamivudine in commercial pharmaceutical dosage forms (tablets and oral solutions) and human serum was developed by Sibel and Bengi[266]. Also few spectrophotometric[267,268] and titrimetric techniques[269] have also been reported for

the determination of lamivudine. Numerous analytical methods such as high performance liquid chromatography (HPLC)–UV assays [270-272], HPLC with tandem mass spectrometry[273] and immunoassay[274-276] have been reported for the simultaneous determination of lamivudine and zidovudine.

Most of the methods reported are time consuming and also require expensive and sophisticated instruments. The HPLC method requires a costly gradient elution system and more time to equilibrate the column from one analytical run to another. But up to now there are no reports of potentiometric sensors for the selective determination of this drug. Also no other electrochemical determination has been reported for this drug till now. Hence, a simple, selective, eco-friendly and cost effective technique is required for their routine analysis in dosage form as well as in biological fluids. This is for the first time that potentiometric sensors are developed for the determination of Lamivudine. As part of the present investigations, two types of such sensors have been developed. Further, the application of this method extended to the analysis of the drug in pharmaceutical formulations and real sample like urine.

Instrumental methods have one disadvantage; it needs rather complicated and laborious sample preparations prior to determination. Indeed, methods for the preparation of the electrodes that are selective towards LAM using ion selective electrode do not appear in the literature. The development and analytical applications of two PVC membrane sensors and two carbon paste sensors for the determination of the drug LAM has been described in this chapter. Two sensors each were prepared using the ion associations of the drug with molybdophosphoric acid (MPA) and phosphotungstic acid (PTA). The electrochemical response characteristics of

each of the four different sensors were evaluated. The membrane sensors were successfully applied to the determination of LAM in its tablets as well as its recovery from urine samples.

5.2 Synthesis of the Ion Associations

The ion associations prepared for the fabrication of sensors for LAM are based on the ion exchange reagents molybdophosphoric acid (MPA) and phosphotungstic acid (PTA). The general procedure for the synthesis of these ion associations has been discussed in Sections 2.3.4 and 2.3.5 of Chapter 2.The ion pair LAM - MPA was prepared by mixing 75 mL of 10^{-2}M LAM with 25 mL of 10^{-2}M MPA solution. The ion association LAM - PTA was prepared by mixing 75 mL of 10^{-2}M LAM with 25 mL of 10^{-2}M PTA solution. The mixtures obtained in both cases were shaken well for about 10 min. The resulting water insoluble precipitates were filtered through a Whatman filter paper, washed using distilled water and dried at room temperature. The precipitates were then stored in a desiccator.

The elemental analysis of both the ion associations were carried out. The elemental analysis confirmed the composition of the LAM – MPA complex to be 3:1 (LAM: MPA). The elemental analysis data obtained for the ion association is as follows:

LAM-MPA ion association

Found (%) – C - 9.84, H - 1.22, N - 4.21

Calculated (%) – C - 9.77, H - 1.12, N - 4.27

The composition of LAM-PTA ion association was also confirmed by elemental analysis to be 3:1 (LAM: PTA). The elemental analysis data obtained for the ion association is as follows:

LAM-PTA ion association

Found (%) – C - 8.11, H - 0.98, N - 3.55

Calculated (%) – C - 8.07, H - 0.92, N - 3.53

5.3 Fabrication of LAM Membrane Sensor

Two membrane sensors were fabricated for LAM using the ion pairs LAM-MPA and LAM-PTA. They were constructed according to Craggs procedure as described in Chapter 2. The ion-pair, PVC and plasticizer were taken in specified percentage-weight ratios and dissolved in THF. The solution was poured into glass rings struck onto a glass plate. It was then allowed to stand overnight for slow evaporation of the solvent. Small disc shaped membranes were cut out and glued to one end of a hollow Pyrex glass tube using Araldite. The electrode body was filled with an inner filling solution. The internal solution consisted of NaCl (1.0×10^{-1}M) and LAM (1.0×10^{-3}M). The finished electrode was conditioned in 1.0×10^{-3}M LAM solution for 12 hrs. The electrode was washed with distilled water before measurement.

5.4 Fabrication of LAM Carbon paste Sensor

The carbon paste sensors were prepared by thoroughly mixing high purity graphite powder and ionophore in the specified percentage-weight ratios using a mortar and pestle. The homogeneous mixture obtained was made into a paste using a suitable plasticizer. This paste was then packed into the open end of a Teflon holder for which a copper rod in the centre provides the electrical contact. A smooth surface for the electrode was obtained by pressing the surface against a filter paper. The two carbon paste sensors were conditioned by soaking in a 1.0×10^{-3} M LAM solution for 12 hrs.

5.5 Potential Measurement and Calibration

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

For membrane sensor:

Saturated calomel electrode | internal filling solution (1×10^{-1} M NaCl solution + 1×10^{-3}M drug solution) | PVC membrane | test solution | saturated calomel electrode

For carbon paste sensor:

Reference electrode | test solution | carbon paste electrode.

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.

Standard solutions of LAM were prepared by serial dilution of the stock solution (1.0×10^{-1}M). The calibration of the sensors were carried out by measuring the potential of 1.0×10^{-7}- 1.0×10^{-2}M drug solutions starting from low to high concentrations. The potential readings were recorded after stabilization and were plotted as a function of log [LAM]. The calibration graph was used for subsequent determination of unknown LAM concentrations.

5.6 Performance Characteristics of the Developed Sensors

Any potentiometric ion selective sensor can be evaluated based on its response characteristics. The response characteristics of a newly developed sensor depend on various parameters.

5.6.1 Optimization of the Membrane Composition

The composition of the matrix (eg: the nature of components and the ratio of the components of the matrix and electroactive material) influences the reliability of the electrode response through membrane equilibrium. Electroactive material has the primary role in the response characteristics. The durability and mechanical strength properties of PVC based membranes can be improved by selecting the best components and ratio between the components in the design of the sensors[277]. Hence it is necessary to optimize the membrane composition in order to get the best response for the developed sensors. As a result the composition of the electroactive component was varied between 1.0 and 1.4 mg. The choice of the plasticizer also proved to be very important for the electrode response. The plasticizer and PVC along with the ionophore represent the matrix of the membrane. The parameters connected with the plasticizer that affect the response of the electrode are the polarity of the plasticizer [278] and the ratio of PVC and plasticizer [279,280]. Accordingly five different types of plasticizers were chosen for the study which involves 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).Also a total of fifteen different sensors were prepared for both the ion associations by varying the ratios of ionophore, plasticizer and PVC.

Table 5.1 shows the optimization of membrane composition of PVC membrane sensor based on LAM-MPA as the ionophore. In the case of this sensor, out of three different compositions studied (1.0, 1.2 and 1.4 mg), best results were obtained with 1.4 mg ionophore composition. The optimized composition of the corresponding sensor (LP_{M9}) was ion association: PVC: plasticizer as 1.4: 32.6: 66.0 wt%. All the three compositions were tried for each

of the five plasticizers. The best plasticizer in terms of slope was found to be BEP. Hence the sensor LP_{M9} with BEP as plasticizer, which gave a Nernstian slope of 58.8 mV/decade (Figure. 5.2) was chosen for further studies.

Table 5.2 shows the optimization of membrane composition of LAM-PTA based PVC membrane sensor. The optimized composition in the case of LAM-PTA based sensor was 1.0: 42.4: 56.6 wt% (LP_{P7}). This sensor gave a near Nernstian slope of 55.1 mV/decade (Figure. 5.3). Of the five plasticizers used, BEP gave the best result in terms of the slope. Thus in the case of both membrane sensors BEP was found to be the best plasticizer. This may be due to high polarity of this plasticizer.

5.6.2 Optimization of Carbon Paste Composition

As in the case of optimization of membrane ingredients of a membrane sensor, the optimization of carbon paste composition was conducted for a carbon paste sensor. The percentage ratios of the ionophore, graphite and plasticizer were varied. Three different ionophore compositions were tried and a total of fifteen carbon paste sensors were prepared. All the five plasticizers were tried in the case of carbon paste sensors also. As the ionophore content was varied an irregular pattern of slope was observed which might be due to the decrease in conductance of the sensor material.

The optimization of carbon paste composition of the sensor incorporating LAM-MPA ionophore is given in Table 5.3 The optimized composition in the case of this carbon paste sensor was 2.2: 40.2: 57.6 wt% (LC_{M5}) (ionophore: graphite: plasticizer). The calibration plot of LC_{M5} is presented in Figure 5.4. This sensor gave a near Nernstian slope of 52.5 mV/decade. The plasticizer which gave the best result in the case of this sensor was DBP.

The response characteristics of the carbon paste sensor containing LAM-PTA as the ionophore was also studied by varying the ionophore percentage from 2.0 to 2.4.The results are summarized in Table 5.4. The sensor LC_{P4} was found to give the best response. The optimized composition in the case of LC_{P4} was 2.0:33.0:65.0 (ionophore: graphite: plasticizer). A deviation from Nernstian behaviour was observed for most of the sensors when the ionophore content was changed from 2.0 to 2.4. The concentration of the plasticizer in the sensor matrix greatly influences the response of the sensors fabricated. DBP has been found to be the best plasticizer in terms of slope in the case of both carbon paste sensors and slopes were much different from the expected Nernstian value in the case of the other plasticizers. The sensor LC_{P4} gave a near Nernstian slope of 55.7 mV/decade. Figure 5.5 shows the calibration plot of LC_{P4}.

5.6.3 Effect of Concentration of Internal Filling Solution

The influence of concentration of internal filling solution on the potential response of the LAM-selective membrane sensors were studied in a 1.0×10^{-2} to 1.0×10^{-4}M concentration range. The results showed that a variation of the concentration of internal solution does not cause any significant difference in the potential response of the electrodes. A 1.0×10^{-3}M concentration of internal solution was quite appropriate for smooth functioning of the sensor systems. A carbon paste sensor requires no internal filling solution. This is one of the significant advantages of the carbon paste sensor.

5.6.4 Working Concentration Range, Slope and Response Time

The linear range in the case of the membrane sensor LP_{M9} was 1.0×10^{-1} - 2.69×10^{-5} M. The slope calculated from the calibration graph was 58.8 mV/decade. 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 1.62×10^{-5}M. The response time for this sensor was found to be less than 35 sec. In the case of the membrane sensor LP_{P7} the linear range was found to be 1.0×10^{-1} - 1.0×10^{-6}M. The calibration plot gave a near Nernstian slope of 55.1 mV/decade with a lower detection limit of 7.94×10^{-7}M for this sensor. The response time for the sensor was less than 20 s. In the case of the two membrane sensors LP_{M9} and LP_{P7}, the best sensor in terms of linear range, detection limit and response time was LP_{P7}, ie, the sensor incorporating LAM-PTA as the ionophore.

The carbon paste sensor LC_{M5} gave linear range between 1.0×10^{-2} - 1.0×10^{-6}M. The calibration plot gave a near Nernstian slope of 52.5 mV/decade with a lower detection limit of 7.40×10^{-7}M for this sensor. The dynamic response time of the sensors were tested by measuring the time required to achieve a steady state potential within ±1 mV of the final equilibrium value. The response time for this sensor was less than 30 s. The working concentration range for the sensor LC_{P4} was found to be 1.0×10^{-1} - 1.0×10^{-5}M. The detection limit was calculated from the graph as 6.76×10^{-6}M. The slope calculated from the calibration graph was found to be 55.7 mV/decade showing near Nernstian behaviour. The response time of this sensor was found to be less than 35 s. The carbon paste sensor LC_{M5} based on LAM-MPA ion association was superior to LC_{P4} in terms of wide linear range, lower detection limit and a faster response time.

The response characteristics of all the four sensors, LP_{M9}, LP_{P7}, LC_{M5} and LC_{P4} are consolidated in Table 5.5.

The SEM images were taken inorder to investigate the surface morphology of the optimized membranes. SEM is a strong and reliable tool for studying in detail the influence of surface morphology of the membranes and the reproducibility of the preparation conditions. The SEM images (Figures 5.6 and 5.7) give an idea of the surface structure of the membranes LP_{M9} and LP_{P7}. The homogeneity of the membranes may also affect the sensitivity and response characteristics of the sensors. A much less homogeneous surface is found to be obtained for membrane LP_{P7} when compared to LP_{M9}. This may be the reason for a deviation of the slope of LP_{P7} from the Nernstian value.

5.6.5 Effect of pH

The pH effect of the test solutions on the electrode potentials were studied. The pH profiles for all the four sensors are clearly depicted in Figures 5.8, 5.9, 5.10 and 5.11. For each of the four sensors, the pH study was conducted at two different concentrations, viz., 1.0×10^{-3} M and 1.0×10^{-4} M. For each pH value, the potential was recorded and thus the potential - pH curves for two LAM concentrations were constructed for the four sensors. The pH of the solution was varied from 1-12 using buffer. For the sensors based on the ion association LAM-MPA, the potential remained constant in the pH range 4 – 8. Whereas in the case of sensors incorporating LAM-PTA ion association ie, LP_{P7} and LC_{P4} the potential remained unchanged in the range 5 – 8. The figures show that at pH < 4 there may be an interference of hydronium ion or a gradual increase of protonated species and dependence of e.m.f. on the pH of the solution. At higher pH values (pH > 8), free base precipitates in the test solution and consequently, the concentration of unprotonated species gradually increased. As a result, lower e.m.f. readings were recorded.

5.6.6 Potentiometric Selectivity

The selectivity of the ion pair based membrane electrodes depend on the selectivity of the ion exchange process at the membrane-test solution interface and the mobilities of the respective ions within the membrane[281]. Fixed Interference Method (FIM) was used to determine the selectivity coefficients for different foreign ions using the developed sensors. The influence of some inorganic cations, organic species and some pharmaceutical excepients on the electrode response was investigated. Table 5.6 lists the selectivity coefficient values of various interfering species tested. From the table it is clear that very low selectivity coefficient values have been obtained for the ions such as NH_4^+, K^+, Na^+, Mg^{2+}, Co^{2+}, Ni^{2+}, $Ca^{2+}$and $Zn^{2+}$and also for pharmaceutical excepients like lactose, talc etc. The selectivity coefficients obtained by this method showed that the proposed sensors were highly selective towards LAM.

5.6.7 Shelf Life or Life Time

Plotting the calibration curve periodically with standard solutions and calculating the response slopes investigated the life time of the electrodes. The polarity of the plasticizer is found to influence the life time of a sensor. ie, as the polarity of a plasticizer increases the life time of the sensor decreases. This is due to the higher migration rate of the ionophore to the aqueous phase by exudation[282]. It was indicated that the conventional membrane electrodes could be used continuously for about three weeks and the carbon paste sensors could be used without major variation in slope values for about four weeks. The linear range and slope of all the developed sensors were lost after this period. This may be due to the leaching of the ionophore from the matrix.

5.7 Analytical Applications

Analytical application studies were conducted using all the four fabricated sensors. The sensors were employed for the determination of the drug content in its tablet form. The application of the developed sensors in the determination of LAM in real sample like urine was also studied.

5.7.1 Determination of LAM in Pharmaceutical Formulations (Tablets)

The proposed sensors proved to be useful for the determination of LAM content of a pharmaceutical preparation by using direct reading of the potential. It was used to analyze the LAM content of a tablet Lamivir (Cipla - India) containing 150 mg of the drug, as declared by the company. The detailed procedure for the determination is given in section 2.9.3 of Chapter 2. The results obtained are summarized in Table 5.7. The results obtained from the measurements were found to be in satisfactory agreement with the declared amount as well as with the results obtained by the standard spectrophotometric method[188]. The SD values obtained for the determination are found to be 0.96, 0.95, 0.97 and 0.98 which shows that the method developed is highly precise. The close agreement of the found values with the declared amount is indicative of non-interference of the other ingredients and excepients that are present in the formulation.

5.7.2 Recovery of LAM from Urine Sample

The developed sensors were applied for the determination of the LAM in spiked urine samples. The detailed procedure has been given in Chapter 2.The percentage recoveries of the drug using the sensors LP_{M9}, LC_{M5}, LP_{P7}, and LC_{P4} has been found to be 97.0, 103.0, 99.0, and 98.0 respectively. The results are given in Table 5.8.The results show that the sensors are highly selective to the drug.

5.8 Conclusion

A total of four sensors were fabricated for the drug Lamivudine. These include two polymeric membrane sensors based on the ion associations LAM-MPA and LAM-PTA and also two carbon paste sensors based on the same ionophores. The slopes of the sensors LP_{M9}, LC_{M5}, LP_{P7}, and LC_{P4} were found to be 58.8, 52.5, 55.1 and 55.7 mV/decade respectively. The PVC membrane sensors LP_{M9}, LP_{P7} had a linear of 1.0×10^{-1} - 2.69×10^{-5} M and 1.0×10^{-1} - 1.0×10^{-6}M respectively. The optimum pH range in the case of LP_{M9} and LC_{M5} was 4 - 8 and that for the sensors based on LAM-PTA ionophore was 5 - 8. The lower detection limit for the membrane sensors was found to be 1.62×10^{-5}M and 7.94×10^{-7} respectively. The carbon paste sensors LC_{M5} and LC_{P4} showed a linear range 1.0×10^{-2} - 1.0×10^{-6}M and 1.0×10^{-1} - 1.0×10^{-5}M with a lower detection limit of 7.40×10^{-7}M and 6.76×10^{-6}M. The response time for the membrane sensors LP_{M9} and LP_{P7}were found to be less than 35 s and less than 20 s respectively. The response time of LC_{M5} and LC_{P4} were less than 30 and 35 s respectively. The membrane electrodes had a shelf life of three weeks whereas the carbon paste sensors had a shelf life of four weeks.

Performance characteristics of these sensors reveal low detection limit, high sensitivity, good selectivity, fast response, long life span and applicability for accurate determination of the drug in dosage forms as well as in real samples. Hence the developed sensors were applied to the determination of LAM in pharmaceutical formulation and biological fluids like urine. In general the LAM sensors described in this chapter are sufficiently simple and specific for quantitative determination of the drug. Moreover, they are cost effective and can be used for the direct determination of LAM in complex matrix without the need for prior separation.

Table 5.1 Optimization of composition of PVC membrane sensor using LAM – MPA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	PVC	Plasticizer	
LP_{M1}	1.0	42.4	56.6, DBP	50.1
LP_{M2}	1.2	50.2	48.6, DBP	42.8
LP_{M3}	1.4	32.6	66.0, DBP	63.8
LP_{M4}	1.0	42.4	56.6, BES	39.9
LP_{M5}	1.2	50.2	48.6, BES	48.7
LP_{M6}	1.4	32.6	66.0, BES	50.7
LP_{M7}	1.0	42.4	56.6, BEP	49.7
LP_{M8}	1.2	50.2	48.6, BEP	52.7
LP_{M9}	***1.4***	***32.6***	***66.0, BEP***	***58.8***
LP_{M10}	1.0	42.4	56.6, DBS	68.7
LP_{M11}	1.2	50.2	48.6, DBS	45.2
LP_{M12}	1.4	32.6	66.0, DBS	47.5
LP_{M13}	1.0	42.4	56.6, BEA	37.4
LP_{M14}	1.2	50.2	48.6, BEA	67.6
LP_{M15}	1.4	32.6	66.0, BEA	54.3

Table 5.2 Optimization of composition of PVC membrane sensor using LAM – PTA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	PVC	Plasticizer	
LP_{P1}	1.0	42.4	56.6, DBP	53.4
LP_{P2}	1.2	50.2	48.6, DBP	35.6
LP_{P3}	1.4	32.6	66.0, DBP	62.4
LP_{P4}	1.0	42.4	56.6, BES	46.3
LP_{P5}	1.2	50.2	48.6, BES	49.5
LP_{P6}	1.4	32.6	66.0, BES	65.2
LP_{P7}	***1.0***	***42.4***	***56.6, BEP***	***55.1***
LP_{P8}	1.2	50.2	48.6, BEP	53.5
LP_{P9}	1.4	32.6	66.0, BEP	60.2
LP_{P10}	1.0	42.4	56.6, DBS	38.7
LP_{P11}	1.2	50.2	48.6, DBS	44.6
LP_{P12}	1.4	32.6	66.0, DBS	63.1
LP_{P13}	1.0	42.4	56.6, BEA	50.8
LP_{P14}	1.2	50.2	48.6, BEA	49.8
LP_{P15}	1.4	32.6	66.0, BEA	45.6

Table 5.3 Optimization of composition of carbon paste sensor using LAM – MPA ion association

Sensor	Composition % (w/w)			Slope
	Ion pair	Graphite	Plasticizer	mV/decade
LC_{M1}	2.0	33.0	65.0, BEP	48.2
LC_{M2}	2.2	40.2	57.6, BEP	50.9
LC_{M3}	2.4	42.0	55.6, BEP	65.6
LC_{M4}	2.0	33.0	65.0, DBP	46.7
LC_{M5}	***2.2***	***40.2***	***57.6, DBP***	***52.5***
LC_{M6}	2.4	42.0	55.6,DBP	43.1
LC_{M7}	2.0	33.0	65.0, DBS	49.7
LC_{M8}	2.2	40.2	57.6, DBS	65.8
LC_{M9}	2.4	42.0	55.6, DBS	38.9
LC_{M10}	2.0	33.0	65.0, BES	39.5
LC_{M11}	2.2	40.2	57.6, BES	45.2
LC_{M12}	2.4	42.0	55.6, BES	67.7
LC_{M13}	2.0	33.0	65.0, BEA	48.7
LC_{M14}	2.2	40.2	57.6, BEA	42.9
LC_{M15}	2.4	42.0	55.6, BEA	51.2

Table 5.4 Optimization of composition of carbon paste sensor using LAM – PTA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	Graphite	Plasticizer	
LC_{P1}	2.0	33.0	65.0, BEP	48.7
LC_{P2}	2.2	40.2	57.6, BEP	52.5
LC_{P3}	2.4	42.0	55.6, BEP	50.8
LC_{P4}	***2.0***	***33.0***	***65.0, DBP***	***55.7***
LC_{P5}	2.2	40.2	57.6, DBP	50.6
LC_{P6}	2.4	42.0	55.6,DBP	63.1
LC_{P7}	2.0	33.0	65.0, DBS	47.8
LC_{P8}	2.2	40.2	57.6, DBS	53.1
LC_{P9}	2.4	42.0	55.6, DBS	44.4
LC_{P10}	2.0	33.0	65.0, BES	42.1
LC_{P11}	2.2	40.2	57.6, BES	37.3
LC_{P12}	2.4	42.0	55.6, BES	47.4
LC_{P13}	2.0	33.0	65.0, BEA	47.8
LC_{P14}	2.2	40.2	57.6, BEA	64.9
LC_{P15}	2.4	42.0	55.6, BEA	52.3

Table 5.5 Response characteristics of the developed sensors LP_{M9}, LC_{M5}, LP_{P7} and LC_{P4}

Parameter	Response Characteristics			
	LP_{M9}	**LC_{M5}**	**LP_{P7}**	**LC_{P4}**
Slope (mV per decade)	58.8	52.5	55.1	55.7
Linear range (M)	1.0×10^{-1} - 2.69×10^{-5}	1.0×10^{-2} - 1.0×10^{-6}	1.0×10^{-1} - 1.0×10^{-6}	1.0×10^{-1} - 1.0×10^{-5}
pH range	4-8	4-8	5-8	5-8
Detection limit (M)	1.62×10^{-5}	7.40×10^{-7}	7.94×10^{-7}	6.76×10^{-6}
Response time (s)	< 35	< 30	< 20	< 35
Shelf life	3 weeks	4 weeks	3 weeks	4 weeks

Table 5.6 Selectivity coefficient values of various interfering species, K^{pot}

Interfering Species	$K^{pot}_{A,B}$			
	LP_{M9}	**LC_{M5}**	**LP_{P7}**	**LC_{P4}**
NH_4^+	3.1×10^{-3}	4.2×10^{-3}	3.4×10^{-3}	4.6×10^{-3}
K^+	4.5×10^{-3}	6.4×10^{-3}	5.6×10^{-3}	4.9×10^{-3}
Na^+	5.7×10^{-3}	6.9×10^{-3}	5.3×10^{-3}	7.1×10^{-3}
Mg^{2+}	8.1×10^{-3}	7.4×10^{-3}	8.3×10^{-3}	7.8×10^{-3}
Co^{2+}	1.7×10^{-2}	2.5×10^{-2}	3.2×10^{-2}	2.7×10^{-3}
Ca^{2+}	7.9×10^{-3}	8.6×10^{-3}	7.4×10^{-3}	6.7×10^{-3}
Ni^{2+}	6.5×10^{-2}	7.3×10^{-2}	6.9×10^{-2}	7.5×10^{-2}
Zn^{2+}	3.4×10^{-3}	4.5×10^{-3}	3.7×10^{-4}	5.3×10^{-3}
Ascorbic acid	2.7×10^{-2}	4.1×10^{-2}	2.8×10^{-2}	1.9×10^{-3}
Glycine	7.9×10^{-3}	6.6×10^{-3}	7.1×10^{-3}	8.4×10^{-3}
Lactose	3.6×10^{-3}	4.9×10^{-3}	4.2×10^{-3}	3.9×10^{-3}
Urea	5.6×10^{-4}	6.8×10^{-3}	5.7×10^{-4}	9.3×10^{-3}
Starch	4.3×10^{-3}	3.9×10^{-3}	4.8×10^{-3}	5.1×10^{-3}
Talc	8.6×10^{-4}	4.5×10^{-3}	5.2×10^{-3}	5.8×10^{-3}

Table 5.7 Determination of LAM in pharmaceutical formulation

Sample	Declared Amount (mg/tablet)	Method adopted	Found * (mg/tablet)	SD*	CV*
Lamivir (Cipla - India)	150	LP_{M9}	149	0.96	0.64
		LC_{M5}	148	0.95	0.64
		LP_{P7}	149	0.97	0.65
		LC_{P4}	149	0.98	0.66
		Standard Method	149	0.91	0.61

*Average of six replicates

Table 5.8 Determination of LAM in urine sample using the developed sensors

Drug added (M)	Sensor	Drug found* (M)	Recovery %
1.00×10^{-4}	LP_{M9}	0.97×10^{-4}	97.0
	LC_{M5}	1.03×10^{-4}	103.0
	LP_{P7}	0.99×10^{-4}	99.0
	LC_{P4}	0.98×10^{-4}	98.0

*Average of six replicates

Figure 5.1 Structure of Lamivudine

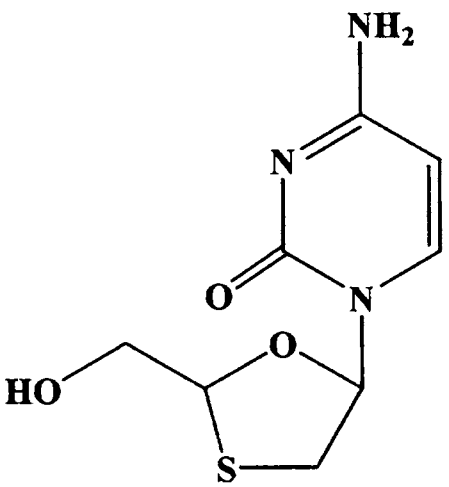


Figure 5.2 Calibration graph for LAM selective PVC membrane sensor based on LAM - MPA ion association (LP_{M9})

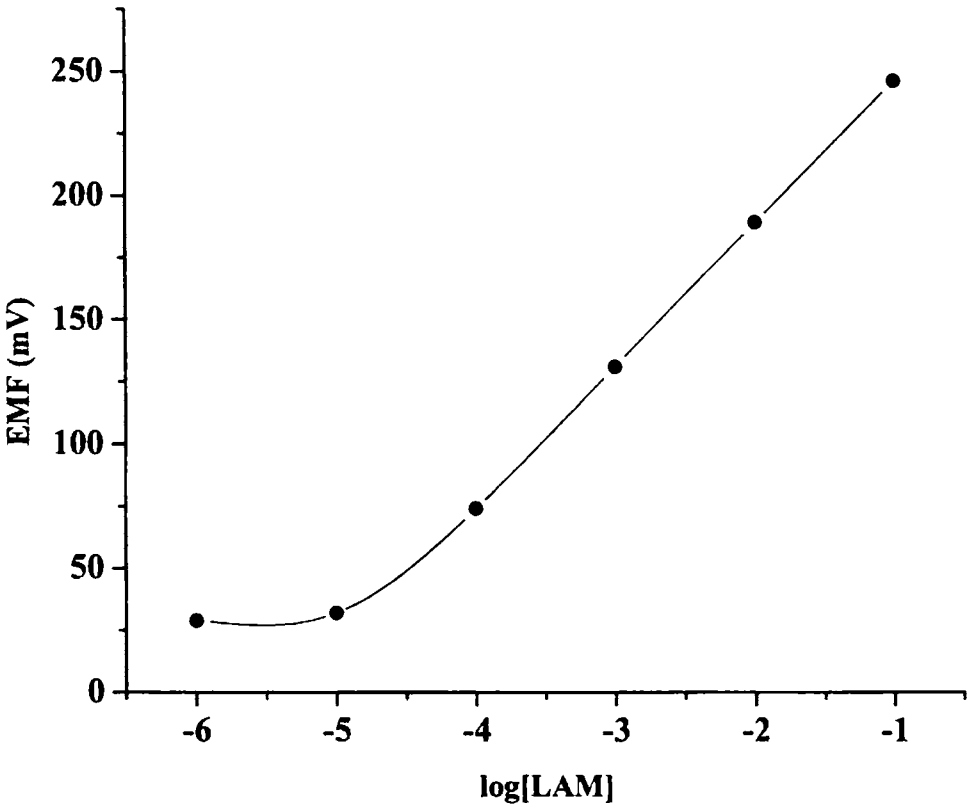


Figure 5.3 Calibration graph for LAM selective PVC membrane sensor based on LAM - PTA ion association (LP_{P7})

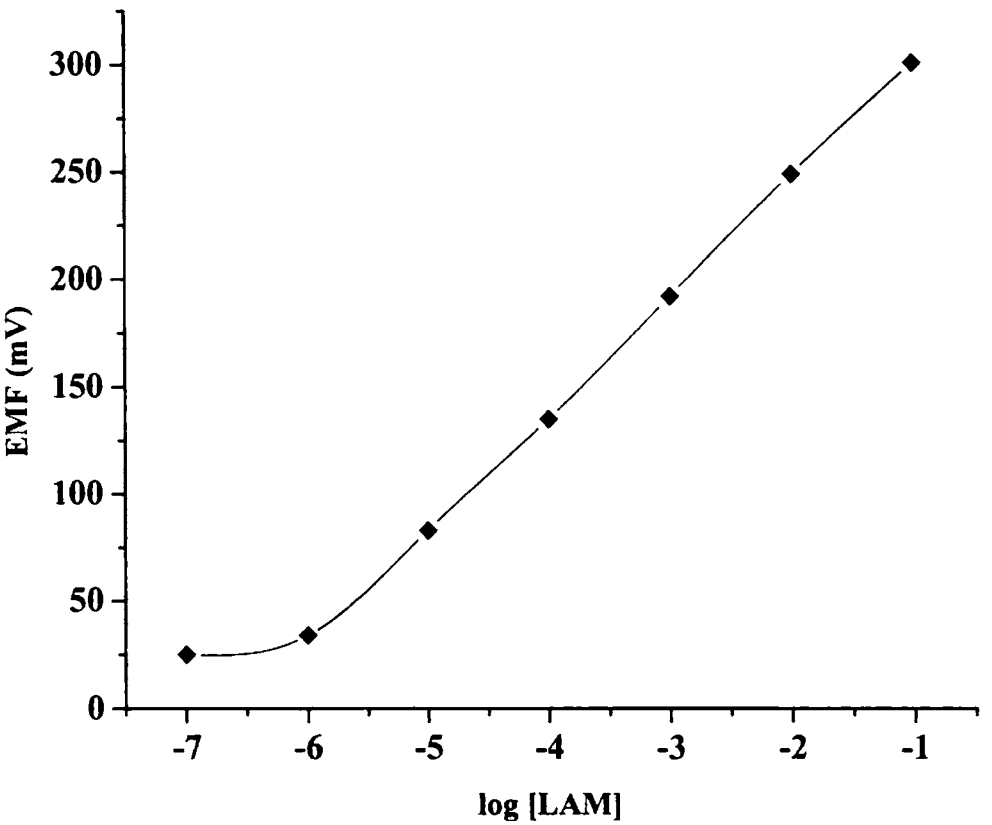


Figure 5.4 Calibration graph for LAM selective carbon paste sensor based on LAM - MPA ion association (LC_{M5})

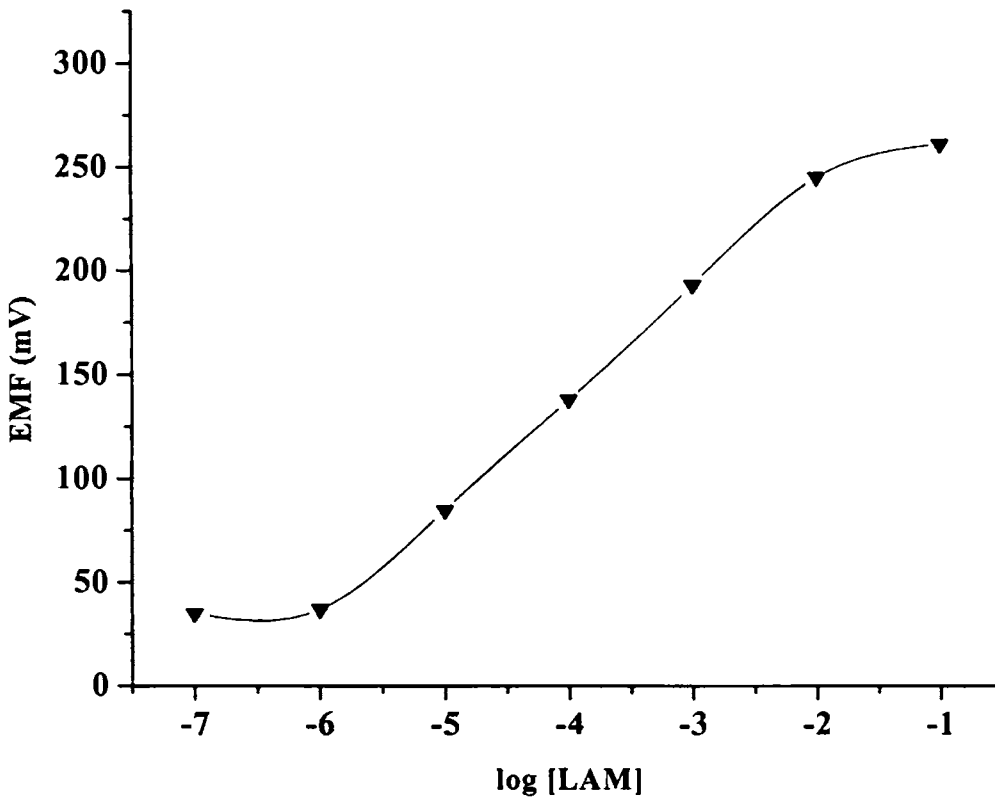


Figure 5.5 Calibration graph for LAM selective carbon paste sensor based on LAM - PTA ion association (LC_{P4})

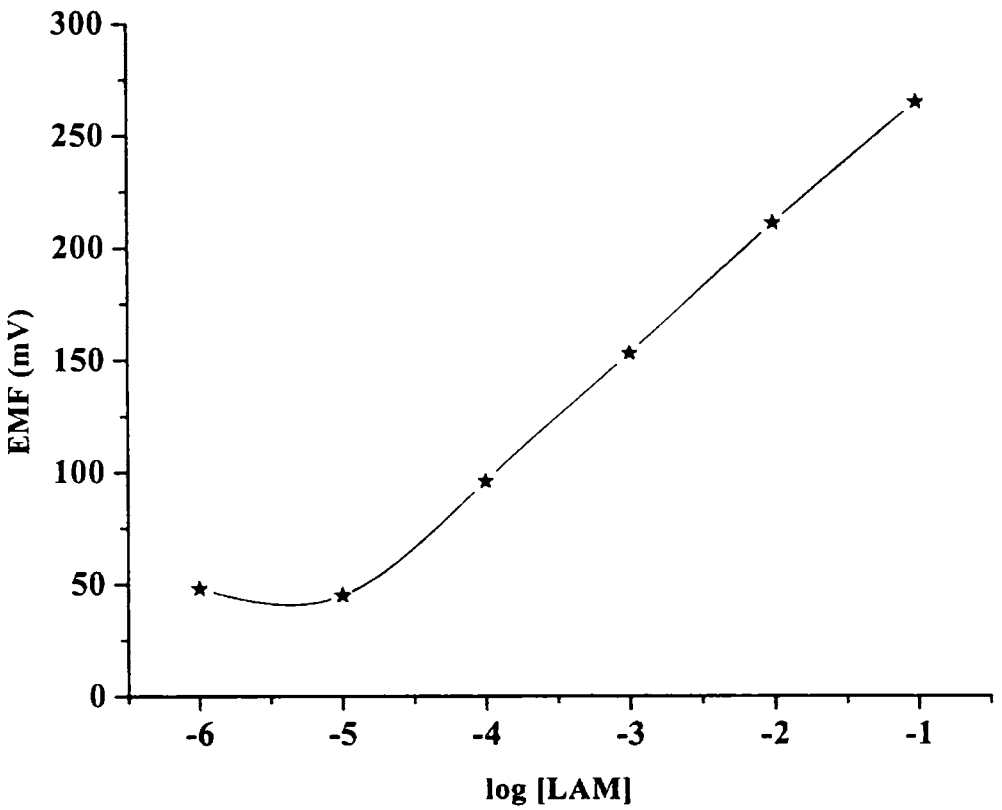


Figure 5.6 SEM image of the polymeric membrane of LP_{M9} sensor

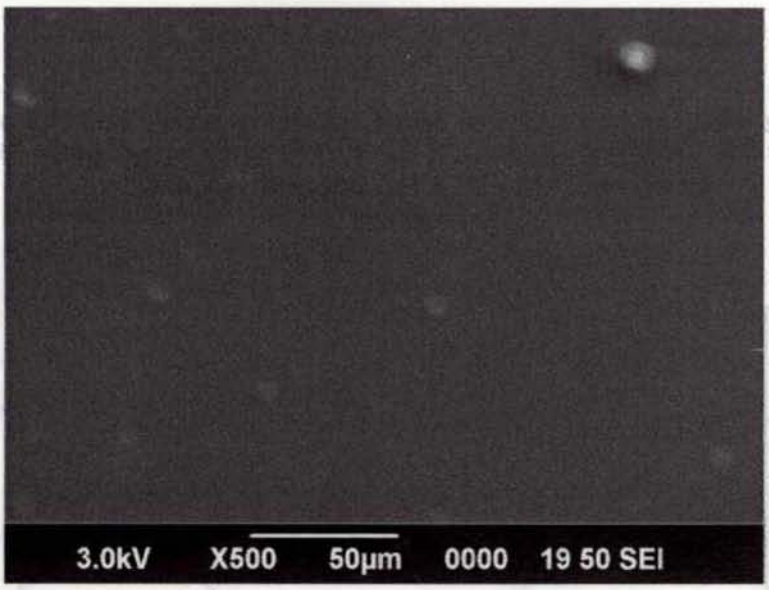


Figure 5.7 SEM image of the polymeric membrane of LP_{P7} sensor

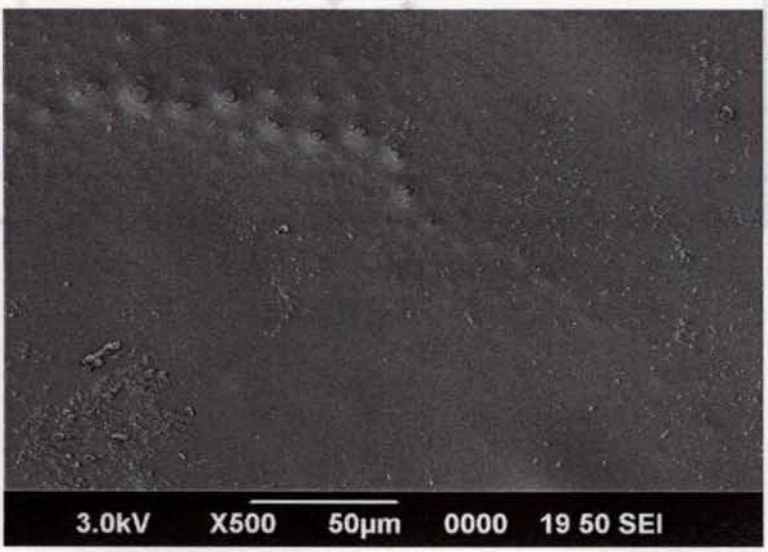


Figure 5.8 Effect of pH on the cell potential of LAM selective PVC membrane sensor (LP_{M9}) at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

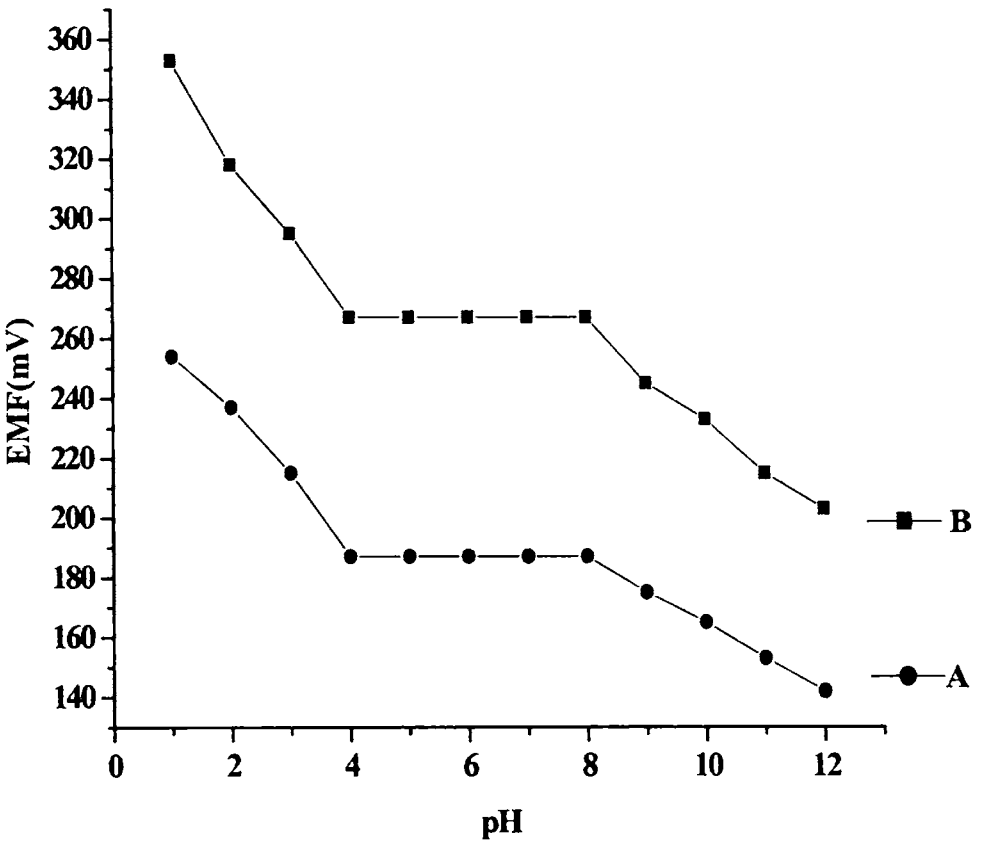


Figure 5.9 Effect of pH on the cell potential of LAM selective carbon paste sensor (LC_{M5}) at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

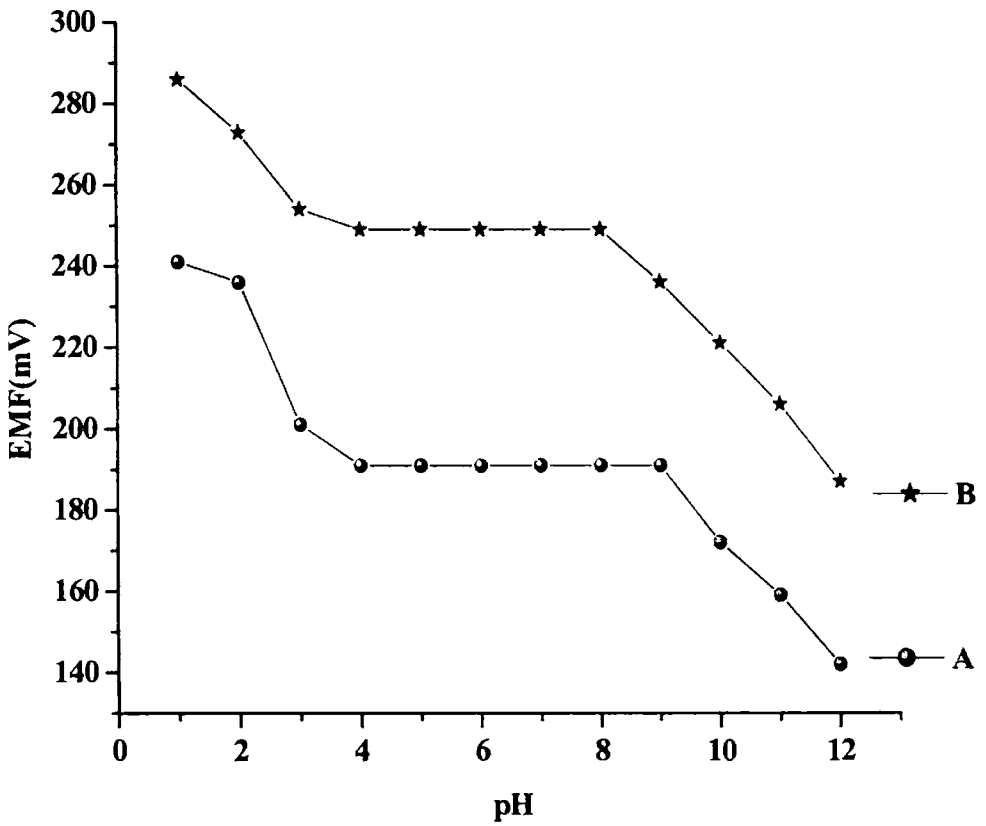


Figure 5.10 Effect of pH on the cell potential of the LAM selective PVC membrane sensor ($\mathbf{LP_{P7}}$) at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

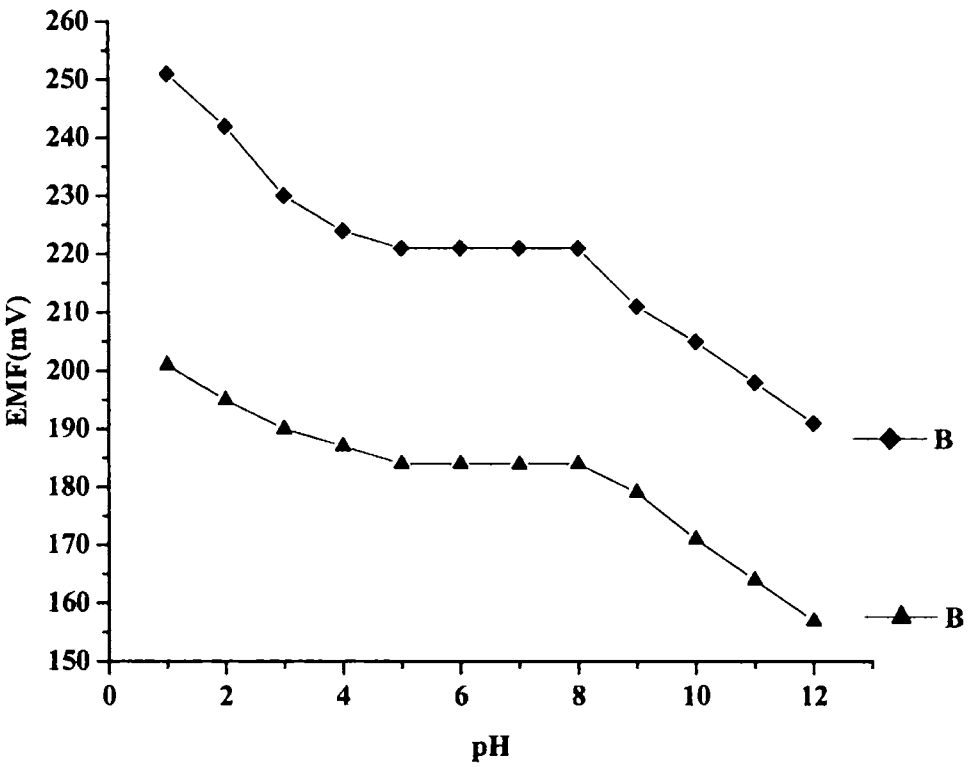
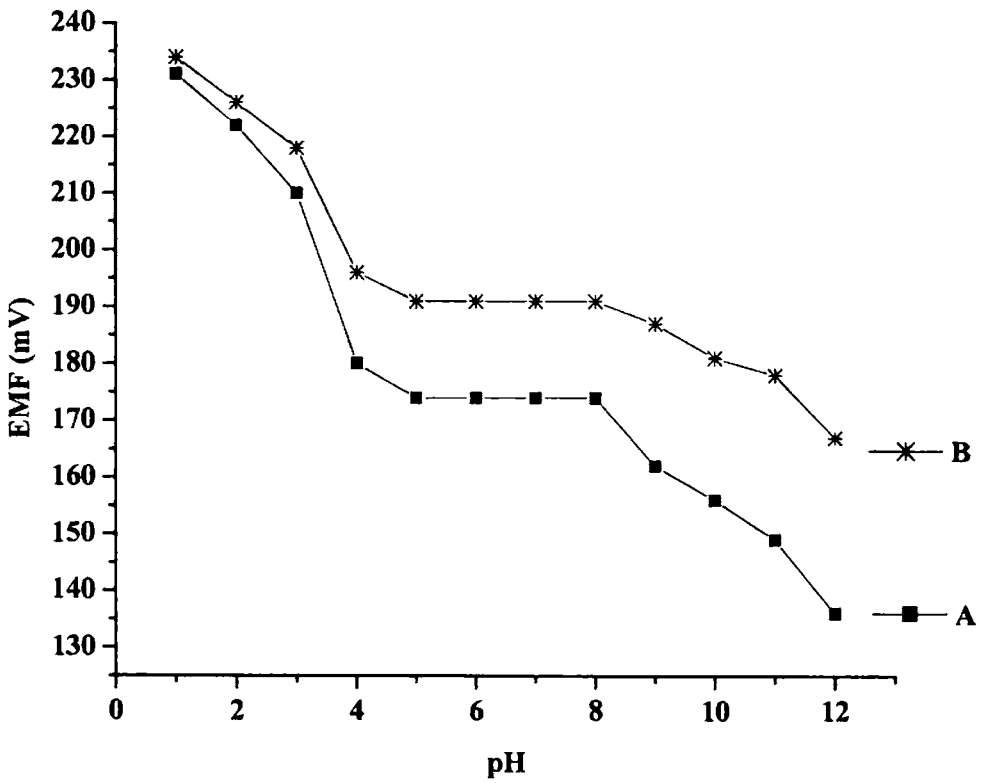


Figure 5.11 Effect of pH on the cell potential of the LAM selective carbon paste sensor (LC_{P4}) at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)



Chapter 6

SENSORS FOR THE DETERMINATION OF DOMPERIDONE

This chapter deals with the fabrication of two novel electrochemical sensors for the selective determination of Domperidone (DOM). The two sensors were fabricated based on DOM – PTA (phosphotungstic acid) ion pair as the electroactive material .The sensors include a PVC membrane sensor and a carbon paste sensor. The response characteristics of the developed sensors have been studied. The analytical application of the developed sensors in the determination of the drug in pharmaceutical formulations such as tablets was investigated. The sensors were also applied for the determination of DOM in real samples like urine by the standard addition method.

6.1 Introduction

Domperidone, 5-chloro - 1- [1- [3- (2-oxo-1,3- dihydrobenzoimidazo l-1- yl) propyl]-4-piperidyl]-1,3-dihydrobenzoimidazol-2-one, is a white or almost white powder (Figure 6.1). It is a dopamine antagonist used as an antiemetic for the short term treatment of nausea and vomiting of various etiologies[283]. It may also be used in those patients who experience peripheral effects with levodopa. Domperidone is indicated for treating symptoms associated with upper gastrointestinal motility disorders caused by chronic and sub acute gastritis. It is a gastrointestinal emptying adjunct, a peristaltic

stimulant, and also exhibits antiemetic properties. Domperidone helps the stomach to empty more quickly in people where this is a problem. It helps to reduce reflux (stomach acid coming back up) and the sensation of fullness. Domperidone is also used to prevent stomach problems associated with the use of certain medications used to treat Parkinson's disease.

Domperidone is a drug that has a side effect of increasing milk production, probably by increasing prolactin production by the pituitary gland. Prolactin is the hormone that stimulates the cells in the mother's breast to produce milk. Domperidone increases prolactin secretion indirectly, by interfering with the action of dopamine, whose action is to decrease the secretion of prolactin by the pituitary gland. There are several studies that show that it works to increase milk production and that it is safe. It has been used for several years, in small infants who spit up and lose weight, but was replaced until a few years ago by cisapride (cisapride has since been taken off the market because it can cause serious cardiac problems). Domperidone is not in the same family of medication as cisapride. Another related, but older medication, metoclopramide, is also known to increase milk production, but it has frequent side effects which have made its use for many nursing mothers unacceptable (fatigue, irritability, depression). Domperidone has many fewer side effects because it does not enter the brain tissue in significant amounts (does not pass the blood-brain barrier).

In June of 2004, the Federal Drug Administration (FDA) in the US put out a warning against using domperidone because of possible cardiac side effects. This unfortunate step was taken without considering the fact that the cardiac side effects occurred only when the drug was taken intravenously by otherwise very sick patients. Incidentally, the Federal Drug Administration

has no authority outside the US, and even in the US, compounding pharmacies, which are not regulated by the FDA, are continuing to provide patients with domperidone. Domperidone is excreted in breast milk, and no studies on its effects on breast feeding infants have been reported in the literature. Individual incidents of problems with the drug include cardiac arrest and arrhythmia, complications with other medications, as well as complications with improper intravenous use.

Metabolism and excretion in dog, rat and man has been studied with high performance liquid chromatography (HPLC) as the dominant analytical technique[291]. The pharmacokinetics and bioavailability in man have been studied with domperidone levels measured by a radioimmunoassay (RIA) method using antibodies raised in rabbits against domperidone [284]. The effect of domperidone on prolactin levels was studied with the plasma levels of domperidone measured by an HPLC technique[285]. Several analytical methods have been reported for quantitative determination of domperidone including spectrophotometry[286-288], high performance liquid chromatography[289-395], anodic differential pulse voltammetry[396], and titrimetry[397]. However, 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.

Potentiometric sensors have the advantages of low cost, easy of use and maintenance, and also simplicity and speed of assay procedure. The reliability of the analytical information make them very attractive for the assay of pharmaceutical products. Suitable ISEs for drugs have enough selectivity towards the drugs over pharmaceutical excipients and they can be useful in the quantitative analysis of the drugs in pharmaceutical preparations

without prior separations. In particular, ISEs are very useful in the case of drugs which are unstable during prior separation[398-300] .The high selectivity of these electrodes impart a great advantage over other techniques. Modern ion selective electrodes based on material transport accross a specific membrane are now widely used in the determination of trace amounts of analytes.The material transport includes both neutral and charged complex species or simple ions.

This chapter describes the construction, electrochemical evaluation, and pharmaceutical applications of two novel potentiometric sensors for domperidone. These sensors incorporate the ion association complex of the drug domperidone (DOM) with phosphotungstic acid (PTA). In the present work, a polymeric membrane sensor and a carbon paste sensor were constructed for DOM using the same ion pair DOM-PTA and the performance characteristics were studied. The sensors were successfully applied for the determination of DOM in pure solutions, pharmaceutical preparations and real sample like urine and the results obtained are in good agreement with that obtained by the official method.

6.2 Synthesis of the Ion association

Ionophore is the electroactive component of a sensor. The ionophore in the case of DOM sensor was based on the ion pairing reagent phosphotungstic acid. The DOM – PTA ion association was prepared by mixing 25 mL 10^{-2}M DOM with 25 mL 10^{-2}M PTA solutions. The mixture was then shaken well for 10 min and the produced precipitate was filtered through a Whatman filter paper, washed thoroughly with distilled water, dried at room temperature and stored in a desiccator. The procedure for the preparation of the ion association has been discussed in detail in Sec 2.3.6 of

Chapter 2. The composition of the ion association was confirmed by elemental analysis to be 1:1 (DOM: ion pairing reagent). The elemental analysis data obtained for the ion association is as follows:

DOM - PTA ion association

Found (%) – C - 7.85, H - 0.89, N - 2.23

Calculated (%) – C -7.98, H - 0.82, N - 2.12

6.3 Fabrication of DOM Membrane Sensor

The membrane electrode was constructed according to the Craggs procedure which has been discussed in detail in Chapter 2. The PVC membrane was prepared by dissolving the required amount of the ion pair, plasticizer and PVC in 5-7 mL of THF. The mixture was then poured into glass rings struck onto a glass plate. It was allowed to stand overnight for slow evaporation of solvent and formation of the sensing membrane. Small portions of the membrane was cut out and glued to one end of a glass tube. The electrode body was filled with an inner filling solution containing NaCl (1.0×10^{-1}M) and DOM (1.0×10^{-3}M). The finished electrode was conditioned in DOM solution (1.0×10^{-3}M) for 24 hrs. The electrode was washed with distilled water before measurement.

6.4 Fabrication of DOM Carbon Paste Sensor

The carbon paste sensor was fabricated as detailed under Section 2.4.2 in Chapter 2. Weighed amount of the ion associations and high purity graphite were mixed together. To the homogenized mixture, a weighed amount of the plasticizer was added. The carbon paste thus obtained was then filled to one end of a teflon holder with a hole at one end for the carbon paste filling. Electrical contact is made with a copper rod that runs through the

centre of the electrode. Appropriate packing and a smooth surface was achieved by polishing the surface of the sensor against a filter paper. The electrode surface can be easily regenerated by removing a small amount of the paste from the tip of the electrode. The sensor was conditioned by soaking it in a 1.0×10^{-3}M solution of DOM solution.

6.5 Potential Measurement and Calibration

All emf measurements were carried out using the following cell assembly. A saturated calomel electrode (SCE) was used as the external as well as the internal reference electrode. The electrochemical cell assembly may be represented as:

For membrane sensor:

Saturated calomel electrode | internal filling solution (1×10^{-1} M NaCl solution + 1×10^{-3}M drug solution) | PVC membrane | test solution | saturated calomel electrode.

For carbon paste sensor:

Reference electrode | test solution | carbon paste electrode.

A Metrohm 781 ion meter was used for potential measurements. All emf measurements were carried out at 25 ± 1^{0}C.

A 1.0×10^{-1}M solution of the drug was prepared in methanol in a 100 mL volumetric flask. The dilution series were prepared by serial dilution of the stock solution with distilled water in 50 mL volumetric flasks. The fabricated sensors were immersed in each of the different concentrations and performances of the electrodes were investigated by measuring the emf values between different concentrations of the respective DOM solutions.

The solutions were stirred and the stable potential readings were taken. The resultant calibration graph was used for subsequent determination of unknown DOM.

6.6 Performance Characteristics of the Developed Sensors

The functional parameters of any sensor are expressed in terms of its slope, detection limit, linear range, selectivity, response time and life span. A detailed study of the response characteristics of the two fabricated sensors are given in the next section.

6.6.1 Optimization Studies of the Two Types of Sensors

The optimization of composition of the two types sensors were carried out by varying the nature and also the amount of the plasticizer and ionophore used. This is because the sensitivity of the electrode is dependent on amount of the ion exchanger and also on the nature and amount of the plasticizer used. The addition of plasticizer influences selectivity, sensitivity, and the working range of the electrodes due to variation in free energy of interaction of electroactive ions and ionophore in polymer matrices.

In the present study, five different plasticizers were employed to study their effect on the electrochemical behaviour of the membrane. 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). The membrane composition was studied by varying the percentages of the ion pair, PVC and plasticizer. The membrane compositions were varied until the optimum composition that exhibits the best performances was obtained. The potential responses of a set of 15 different sensors constructed with different plasticizers and different

composition ratios are given in Table 6.1. The results revealed that the best composition was ion association: PVC: plasticizer (BEP) as 1.2: 50.2: 48.6 wt%. The slopes of the sensors varied from the Nernstian values depending on the use of plasticizer, the proportion of the plasticizer with PVC and of the electroactive compound. The PVC acts as a regular support matrix for the membrane but its use creates a need for a plasticizer[301]. In the present investigation, BEP was found to be the optimum available plasticizer for the PVC membrane sensor. It plasticizes the membrane, dissolves the ion association complexes and adjusts both the membranes permittivity and mobility of the ion-exchanger sites to give highest possible selectivity and sensitivity [302,303]. The calibration plot of the sensor D_{P8} is shown in Figure 6.2.The sensor D_{P8} gave a linear response behaviour within the concentration range 1.0×10^{-1} - 1.0×10^{-5}M of DOM with a slope of 56.5 mV/decade and a lower detection limit of 7.36×10^{-5}M.

SEM analysis was conducted inorder to study the surface morphology of the developed D_{P8} membrane. Figure 6.3 gives the SEM image for D_{P8} membrane. The extend of homogeneity of a membrane surface is clearly visible in the SEM image. A homogeneous membrane is found to exhibit good response characteristics such as high sensitivity and long shelf life. The deviation of the slope of the sensor D_{P8} from the Nernstian value may be due to its irregular or less uniform surface.

The composition of the carbon paste ingredients were also varied inorder to get the best response. A set of 15 carbon paste sensors were fabricated with varying compositions of ionophore to graphite ratio with different solvent mediators and the results obtained are consolidated in Table 6.2. The optimized composition in the case of this sensor (D_{C5}) may be

represented as 2.2: 40.2: 57.6 (ionophore: graphite: plasticizer). All the five plasticizers which were tried for membrane sensors have been tried in the case of carbon paste sensors also. The use of DBP resulted in a Nernstian linear plot over the concentration range 1.0×10^{-1} - 3.55×10^{-6} M (Figure 6.4). The results show that the sensor made of 2.2% DOM-PTA ion pair exhibits the best performance (slope 57.8 mV/dec, detection limit 1.0×10^{-6}M). In all subsequent studies, the sensor made of 2.2% DOM - PTA ion pair was used in case of carbon paste electrode.

The response characteristics of the two types of sensors under investigation are summarized in Table 6.3.

6.6.2 Effect of Concentration of Internal Filling Solution

The influence of the concentration of the internal filling solution on the potential response of the DOM selective membrane sensor was studied. The proposed membrane electrode was examined with different concentrations of the internal solution from 1.0×10^{-2} to 1.0×10^{-4} M of DOM. The variation in concentration did not cause any effect on the functioning of the membrane sensor. A 1.0×10^{-3} M concentration was fixed as the internal solution for smooth functioning of the membrane electrode system.

6.6.3 Effect of pH

The pH dependence of the developed sensors was examined for two fixed concentrations (1.0×10^{-3} M and 1.0×10^{-4} M). The pH range was varied between 1 and 12 using different buffer solutions. The effect of pH of the test solution on the electrode potential was investigated by following the variation in potential with change in pH. Figures 6.5 and 6.6 clearly depict that the potentials remained constant in the pH range 4 - 6 for both the

electrodes. Hence this was chosen as the working pH range of the sensors. The potential decrease at pH less than 4 may be due to the gradual increase in the protonated species. At pH greater than 6, the solution turned turbid due to the decomposition of the drug and potential values were observed to decrease. The variations in concentration of DOM did not affect the useful pH range of the two sensors developed.

6.6.4 Selectivity Studies

The preference of a sensor towards a particular analyte in presence of other foreign species is determined from the selectivity coefficient values. The interference of various substances on the selectivity of the developed sensors has been examined using the fixed interference method. Potential response of the developed sensors were tested in the presence of some inorganic cations and some organic compounds. The potential values were used for the determination of the concentration of primary ion, with the help of which the selectivity coefficient values are determined and resulting selectivity coefficients are summarized in Table 6.4. The values of the selectivity coefficients given in the table reveal that the developed sensors show very good selectivity to DOM in the presence of ions such as NH_4^+, K^+, Na^+, Mg^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Zn^{2+}, lactose, urea, ascorbic acid and glycine. There is no interference from the tablet excipients such as starch and talc and hence the sensors can be selectively used for the determination of DOM in pharamaceutical formulations.

6.6.5 Response Time and Life Time of the Sensors

Response time of the DOM-PTA membrane sensor was less than 25 s and that in the case of carbon paste sensor was less than 20 s. The average response time is the time required for the sensor to reach a stable potential

within ±1 mV of the final equilibrium value. The life time of an electrode is limited by the diffusion of the membrane components from the membrane to the aqueous solution[304-307]. The amount of these components lost differs from one electrode to the other depending on its conception. The life time of the electrode was investigated by measuring the potentials in standard drug solutions each day. The response slope of the sensors was calculated each time. A Nernstian slope was obtained for a period of 3 weeks in the case of membrane sensor and 2 weeks for carbon paste sensor. During this period, the sensors showed no significant deviation in the optimized response characteristics. Both the sensors were kept immersed in 1.0×10^{-3} M DOM solution when not in use.

6.7 Analytical Applications

The response characteristics of the DOM ion selective sensors suggest that these sensors could be used for the determination of DOM in pharmaceutical preparations and in real samples. With a view to proving their analytical usefulness, direct potentiometric determinations were performed. These have been discussed in detail in the next section.

6.7.1 Determination of DOM in Pharmaceutical Formulations (Tablets)

The developed sensors D_{P8} and D_{C5} were applied for the determination of DOM in commercially available pharmaceutical formulations such as Vomihtop (Cipla, India) and Domitil (Bal Pharma, India). The detailed procedure for the determination is explained in Sec 2.9.4 of Chapter 2. The results were compared with those obtained by the standard European Pharmacopoeia method[185]. The results are illustrated in Table 6.5. The data given in the table clearly indicate a satisfactory agreement between the DOM content determined by the proposed sensors and by the reported method.

6.7.2 Recovery of DOM from Urine Sample

The developed sensors were applied for the determination of the drug from urine samples. The DOM content of the solution was then determined by the proposed sensors using the standard addition method, the detailed procedure of which is given in Chapter 2.The results of the determination are summarized in Table 6.6.The results show that the proposed sensors can detect the investigated drug in spiked urine samples with high accuracy and high % recovery without pretreatment procedures of the sample. The average % recoveries of the drug using the sensors D_{P8} and D_{C5} has been found to be 99.0 and 99.3 respectively.

6.8 Conclusion

Domperidone selective sensors have been developed using the ionophore DOM-PTA. A PVC membrane sensor as well as a carbon paste sensor has been developed for the drug with the same ionophore. The developed sensors are found to have good characteristics in terms of slope, concentration range, detection limit, response time and pH range. The carbon paste sensor was found to be superior to the PVC membrane sensor in terms of linear range, detection limit and also fast response time. It had a linear range of 1×10^{-1} - 3.55×10^{-6}, detection limit of 1.0×10^{-6} and a fast response time of less than 20 s. The sensors are cost effective, easy to prepare and to use. The sensors are also found to be highly selective over a number of other ions. Further, the developed sensors can be used for the determination of DOM content in pharmaceutical formulations and also in urine samples. The results are highly satisfactory and agree with the determination of DOM content in the same samples by the standard reported method.

Table 6.1 Optimization of composition of PVC membrane sensor using DOM - PTA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	PVC	Plasticizer	
D_{P1}	1.0	42.4	56.6, DBP	50.8
D_{P2}	1.2	50.2	48.6, DBP	49.3
D_{P3}	1.4	32.6	66.0, DBP	52.4
D_{P4}	1.0	42.4	56.6, BES	48.5
D_{P5}	1.2	50.2	48.6, BES	49.2
D_{P6}	1.4	32.6	66.0, BES	43.1
D_{P7}	1.0	42.4	56.6, BEP	52.3
D_{P8}	***1.2***	***50.2***	***48.6, BEP***	***56.5***
D_{P9}	1.4	32.6	66.0, BEP	49.2
D_{P10}	1.0	42.4	56.6, DBS	48.3
D_{P11}	1.2	50.2	48.6, DBS	50.9
D_{P12}	1.4	32.6	66.0, DBS	49.8
D_{P13}	1.0	42.4	56.6, BEA	47.6
D_{P14}	1.2	50.2	48.6, BEA	47.8
D_{P15}	1.4	32.6	66.0, BEA	51.8

Table 6.2 Optimization of composition of carbon paste sensor using DOM - PTA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	Graphite	Plasticizer	
D_{C1}	2.0	33.0	65.0, BEP	48.9
D_{C2}	2.2	40.2	57.6, BEP	52.4
D_{C3}	2.4	42.0	55.6, BEP	51.6
D_{C4}	2.0	33.0	65.0, DBP	50.4
D_{C5}	***2.2***	***40.2***	***57.6, DBP***	***57.8***
D_{C6}	2.4	42.0	55.6,DBP	51.4
D_{C7}	2.0	33.0	65.0, DBS	49.5
D_{C8}	2.2	40.2	57.6, DBS	43.6
D_{C9}	2.4	42.0	55.6, DBS	45.2
D_{C10}	2.0	33.0	65.0, BES	39.8
D_{C11}	2.2	40.2	57.6, BES	48.3
D_{C12}	2.4	42.0	55.6, BES	49.6
D_{C13}	2.0	33.0	65.0, BEA	50.7
D_{C14}	2.2	40.2	57.6, BEA	49.3
D_{C15}	2.4	42.0	55.6, BEA	49.2

Table 6.3 Response characteristics of the developed sensors D_{P8} and Dc_5

Parameter	PVC Membrane sensor D_{P8}	Carbon paste sensor Dc_5
Slope (mV per decade)	56.5	57.8
Linear range (M)	1.0×10^{-1} - 1.0×10^{-5}	1.0×10^{-1} - 3.55×10^{-6}
pH range	4 - 6	4 - 6
Detection limit (M)	7.36×10^{-5}	1.00×10^{-6}
Response time (s)	< 25	< 20
Shelf life	3 weeks	2 weeks

Table 6.4 Selectivity coefficient values of various interfering species, K^{pot}

Interfering species	K^{pot} PVC membrane Sensor D_{P8}	Carbon paste Sensor Dc_5
NH_4^+	3.8×10^{-4}	4.7×10^{-4}
K^+	3.4×10^{-3}	2.9×10^{-3}
Na^+	5.6×10^{-3}	7.4×10^{-3}
Mg^{2+}	7.8×10^{-2}	6.2×10^{-2}
Co^{2+}	5.7×10^{-2}	8.3×10^{-2}
Ca^{2+}	3.7×10^{-3}	4.1×10^{-3}
Ni^{2+}	6.1×10^{-3}	4.9×10^{-3}
Zn^{2+}	7.3×10^{-3}	8.7×10^{-3}
Urea	5.6×10^{-3}	6.7×10^{-3}
Ascorbic acid	3.4×10^{-2}	2.1×10^{-2}
Glycine	8.4×10^{-3}	9.4×10^{-3}
Starch	4.3×10^{-2}	3.9×10^{-2}
Lactose	4.3×10^{-3}	5.2×10^{-3}

Table 6.5 Determination of DOM in pharmaceutical formulations

Sample	Declared Amt (mg/tablet)	Method adopted	Found * (mg/tablet)	SD	CV
Vomihtop (Cipla, India)	10	D_{P8}	8.71	0.29	3.33
		Dc_5	9.23	0.22	2.38
		Standard Method	9.34	0.21	2.25
Domitil (Bal Pharma, India)	10	D_{P8}	9.14	0.28	3.06
		Dc_5	8.33	0.25	3.00
		Standard Method	9.63	0.19	1.97

* Average of six replicates.

Table 6.6 Determination of DOM in urine sample using the developed sensors

Drug taken (M)	Sensor	Drug found* (M)	Recovery %
3.00×10^{-3}	D_{P8}	2.97×10^{-3}	99.0
	Dc_5	2.98×10^{-3}	99.3

* Average of six replicates.

Figure 6.1 Structure of Domperidone

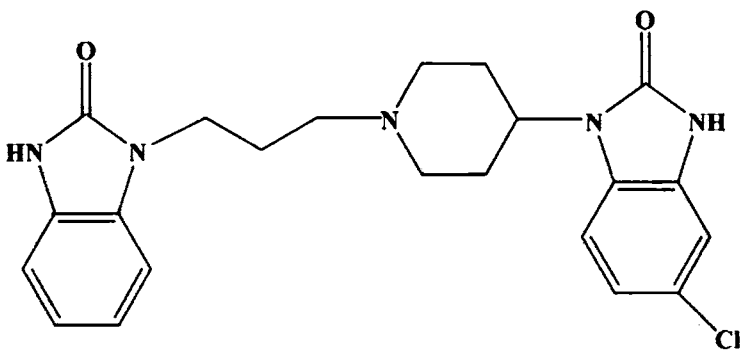


Figure 6.2 Calibration graph for DOM selective PVC membrane sensor based on DOM - PTA ion association (D_{P8})

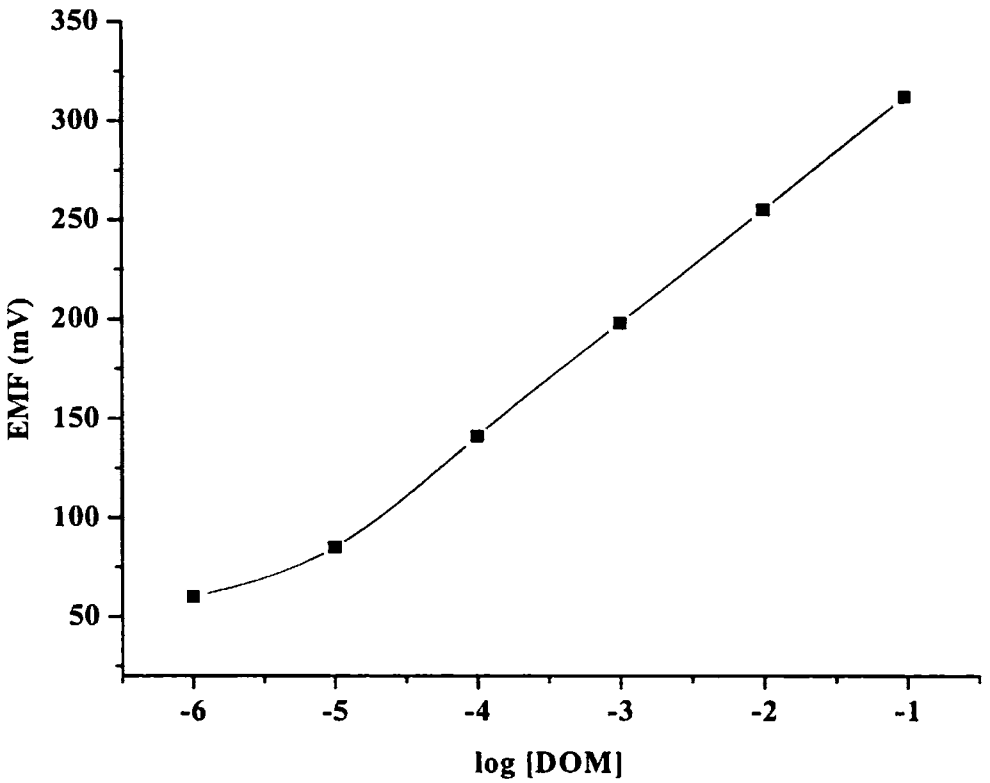


Figure 6.3 SEM image of the polymeric membrane of $\mathbf{D_{P8}}$ sensor

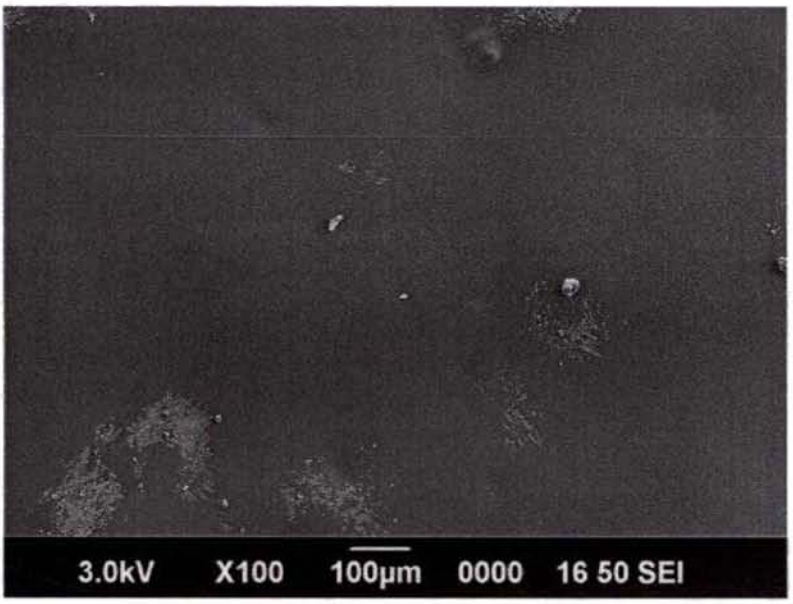


Figure 6.4 Calibration graph for DOM selective carbon paste sensor based on DOM - PTA ion association ($\mathbf{Dc_5}$)

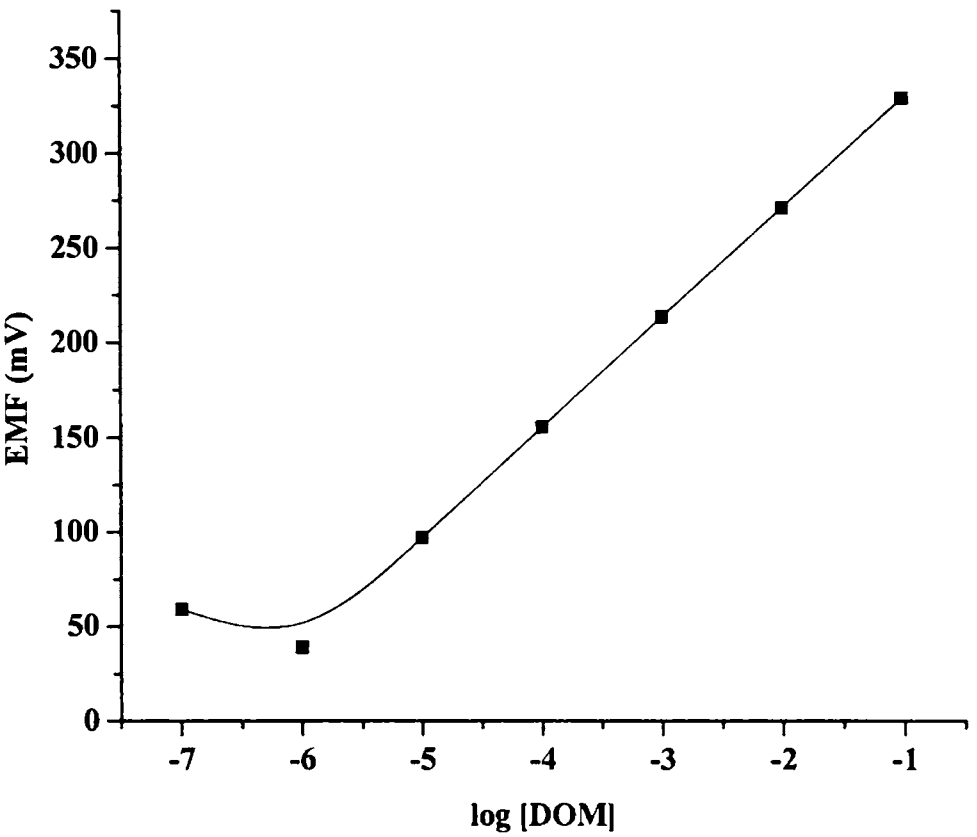


Figure 6.5 Effect of pH on the cell potential of the DOM selective PVC membrane sensor $\mathbf{D_{P8}}$ at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

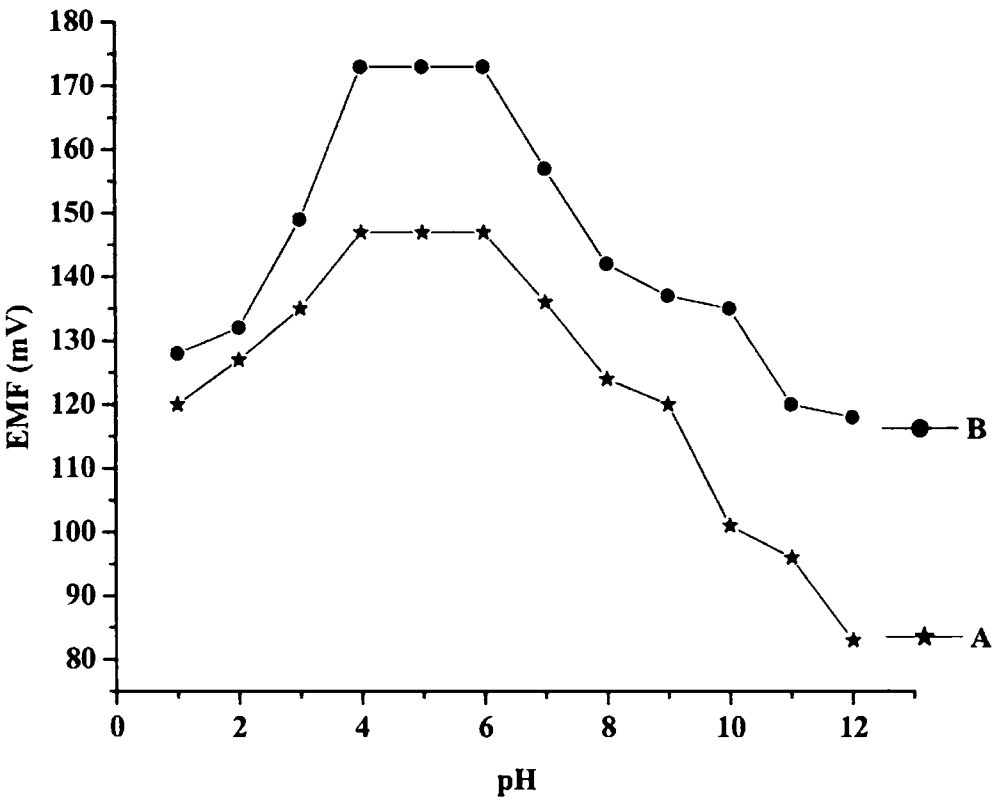
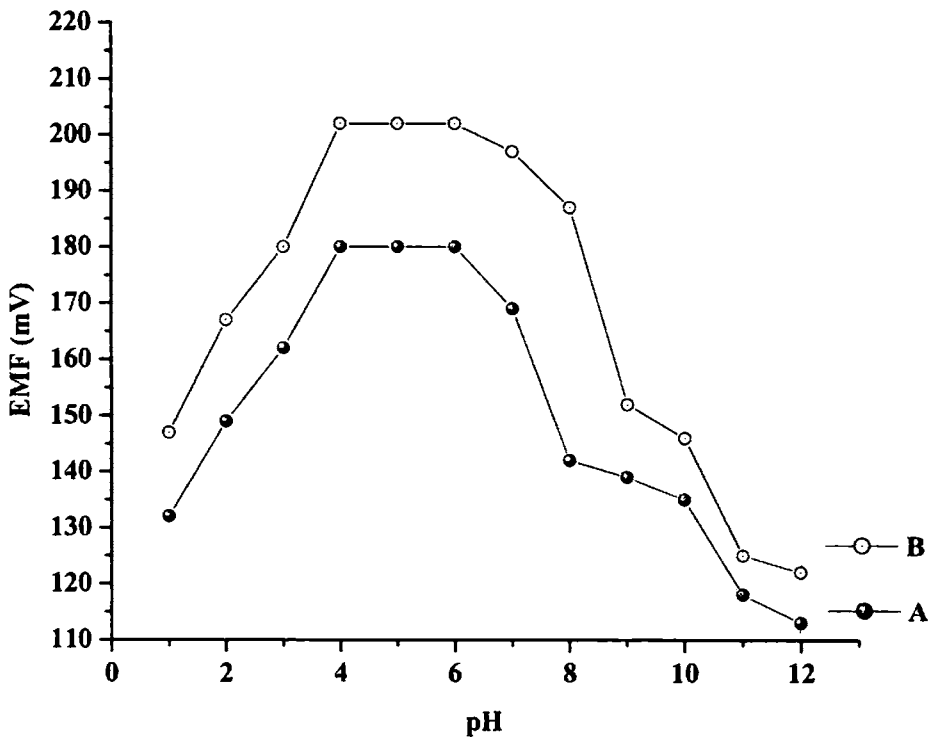


Figure 6.6 Effect of pH on the cell potential of the DOM selective carbon paste sensor $\mathbf{Dc_5}$ at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)



Chapter 7

SENSORS FOR THE DETERMINATION OF NIMESULIDE

This chapter describes the fabrication and response characteristics of four types of sensors for the drug Nimesulide (NIM). The sensors are based on the ion pairs of the drug with molybdophosphoric acid (MPA) and silicotungstic acid (STA). Both PVC membrane sensors and carbon paste sensors were fabricated using the ion associations prepared. Different response parameters of the developed sensors have been discussed in detail. The analytical applications of the developed sensors have been illustrated. The developed sensors were applied for the determination of the drug in pharmaceutical formulation and also in urine sample.

7.1 Introduction

Nimesulide, *N*-(4-nitro-2-phenoxiphenyl) methane sulfonamide ($C_{13}H_{12}N_2O_2S$), is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic activity[308] (Figure 7.1). This NSAID has been shown to be effective and well tolerated in adult patients with a variety of inflammatory and painful conditions. Nimesulide is one of the newest NSAIDs which acts principally through the selective inhibition of cyclooxygenase 2 or COX-2. Nimesulide seems to cause less severe gastrointestinal side effects than do other NSAIDs and aspirin.

Safety of nimesulide has been a matter of public concern worldwide for some years because of its serious side effects and decreasing use in several countries. In India, marketing approval for the drug was granted in 1994 for painful inflammatory febrile disorders but it is being promoted as first line antipyretic therapy. Nimesulide obtained government approval in India in 1994 to be used for 'painful inflammatory febrile disorders'. How an NSAID category drug primarily meant for musculo-skeletal disorders, became a first-line for fever, that too in children remains a mystery. It is true that all NSAIDs due to their inherent properties can be used to bring down fever. But this does not mean that an NSAID should be allowed to be a first-line fever therapy.

Several analytical methods have been reported for the quantitative determination of NIM. Several HPLC methods have been reported for the determination of NIM in biological fluids[309-315]. Other methods include spectrophotometry[316-318], capillary zone electrophoresis[319]and HPLC with UV[320] or a glassy carbon electrode[321] as a detector. For biological fluid samples, a thin layer chromatographic method was used for the determination of nimesulide in plasma[322]. Electrochemical detection of analyte is a very elegant method in analytical chemistry. Electrochemical sensors satisfy many of the requirements for such tasks particularly owing to their inherent specificity, speed of response, sensitivity and simplicity of preparation. Up to date, there are only a few available electrochemical methods for the determination of nimesulide in the literature including polarography[323], differential pulse polarography[324,325] and adsorptive stripping voltammetry with mercury electrodes[326]. Catarino et al proposed an amperometric method with a glassy carbon electrode for the determination of nimesulide[327,328].

Most of these methods involve several time consuming manipulation steps and require sophisticated instruments. Hence it is worthwhile to develop a simple and sensitive method for the analysis of this drug.

There has been a continuing increase in the number of PVC membrane sensors that have been prepared for a variety of substances. These sensors can be prepared by incorporating the ion exchanger within the plasticized membrane and can be used as very useful tool for clinical, chemical and environmental analysis. Ion selective electrodes have found useful applications that are simple, economical, applicable over a wide range of concentration and offer sufficient selectivity to the drug in presence of pharmaceutical excipients.

This chapter describes the preparation, electrochemical evaluation and possible applications of 4 novel potentiometric sensors for nimesulide based on the use of nimesulide - molybdophosphoric acid (NIM-MPA) ion pair complex and nimesulide - silicotungstic acid (NIM-STA) ion pair complex as electroactive materials. A PVC membrane sensor as well as a carbon paste sensor was developed by each of the two ionophores. The sensors showed high selectivity to the drug with good performance characteristics.

7.2 Synthesis of the Ion Associations

Ionophore is the key component of any sensor which imparts the selectivity that enables the sensor to respond selectively to a particular analyte, thus avoiding interferences from other substances. NIM – MPA ion association was prepared by mixing an aliquot of 45 mL of 10^{-2}M nimesulide with 15 mLof 10^{-2} M MPA. The ion association NIM - STA was prepared by mixing 75 mL of 10^{-2}M NIM with 25 mL of 10^{-2}M STA solution. The

precipitates obtained were shaken well for about 30 min. The resulting water insoluble precipitates were filtered through a Whatman filter paper, washed using distilled water and dried at room temperature. The precipitates were then stored in a desiccator.

The elemental analysis confirmed the compositions of NIM-MPA and NIM-STA complexes to be 3:1 (NIM: ion pairing reagent). The elemental analysis data obtained for the ion associations is as follows:

NIM-MPA ion association

Found (%) – C - 14.56, H - 1.17, N - 2.54

Calculated (%) – C - 14.71, H - 1.13, N - 2.64

NIM-STA ion association

Found (%) – C - 12.45, H - 1.01, N - 2.18

Calculated (%) – C - 12.31, H - 0.94, N - 2.21

7.3 Fabrication of NIM Membrane Sensor

The electrode was constructed according to the Craggs procedure. The key to constructing such an electrode is to produce a sensitive and selective membrane that responds to a particular drug. Such a membrane is usually prepared by incorporating an appropriate ion exchanger and solvent mediator into a poly (vinyl chloride) membrane matrix. Four different membrane compositions were studied by varying the percentages of ion association, PVC and the plasticizer. The ion association, plasticizer and PVC was mixed in 5-7 mL of THF and poured into glass rings stuck onto a glass plate. The glass plate was covered with a filter paper and left to stand overnight to allow slow evaporation of the solvent. The membrane thus obtained was removed

and glued to one end of a hollow Pyrex glass tube using Araldite. The membrane was conditioned by dipping it in a 1.0×10^{-3} M NIM solution for 12 hours. The electrode was washed with distilled water before measurement. The electrode was kept dipped in conditioning solution when not in use for a long time.

7.4 Fabrication of NIM Carbon Paste Sensor

The carbon paste sensors were prepared by thoroughly mixing high purity graphite powder and ionophore in the specified percentage-weight ratios using a mortar and pestle. The homogeneous mixture obtained, was made into a paste using a suitable plasticizer. This paste was then packed into the open end of a Teflon holder for which a copper rod in the centre provides the electrical contact. A smooth surface for the electrode was obtained by pressing the surface against a filter paper. The sensors were conditioned by soaking in a 1×10^{-3} M NIM solution for 24 hours.

7.5 Potential Measurement and Calibration

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

For membrane sensor:

Saturated calomel electrode | internal filling solution (1×10^{-1} M NaCl solution + 1×10^{-3}M drug solution) | PVC membrane | test solution | saturated calomel electrode.

For carbon paste sensor:

Reference electrode | test solution | carbon paste electrode.

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.

Standard solutions (1×10^{-7} - 1×10^{-2} M) were prepared by serial dilution of a 1×10^{-1} M NIM solution. The electrodes were placed into well stirred standard solutions in the order 1×10^{-7} - 1×10^{-2} M and stable potentials were recorded. The resulting calibration graph was used for subsequent determination of unknown NIM concentration.

7.6 Performance Characteristics of the Developed Sensors

Several parameters were investigated in order to evaluate the performance of the nimesulide ion selective electrodes based on ionophores NIM-MPA and NIM-STA in terms of membrane/carbon paste composition, calibration curve slopes, reproducibility, linear range, limit of detection, response time, selectivity and shelf life. The next section discusses in detail each of these parameters.

7.6.1 Optimization of the Membrane Composition

It is known that the potentiometric sensitivity and linearity for a given ion selective electrode depend significantly on its membrane composition[329,330]. In PVC membrane sensors it is essential to use external plasticizers (membrane solvents) that can reduce the glass transition temperature of the polymer to below room temperature, increase the elasticity of the polymeric membrane and aid in providing mechanical stability. Furthermore, plasticizers also provide a lipophilic environment within the membrane for improving the solubility of electroactive species [331]. The nature of plasticizers also influence the mobility of ionophore molecules[332,333]. A

total of 20 different PVC membrane sensors were prepared for both the ionophores. Since the type of plasticizer used for the membrane construction also plays a very important role in the response of the electrode, five different types of plasticizers were chosen for the study. These 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).

Generally, use of plasticizers improve certain characteristics of the membranes, and in some cases, the slopes get affected adversely. The potentiometric response characteristics of the NIM sensor based on the use of NIM - MPA ion pair as the electroactive material in a PVC matrix were examined (Table7.1). Several different membrane compositions that contained 1%, 1.2%, 1.8% and 2% of ion pair were investigated. The results revealed that the best composition of the membrane was ion association: PVC: plasticizer as 2: 40.8: 57.2 (NP_{M8}). It was found that the use of BEP gave a near Nernstian slope.

An increase in the amount of plasticizer improves to large extent the adhesive properties of the membrane but, on the other hand, aids in the deterioration of the membrane depending on the properties of both the ion exchanger and the matrix. Table 7.2 presents the optimization of composition of PVC membrane sensor based on NIM-STA ion association. In the case of this sensor incorporating NIM-STA as ion association, best results were obtained with 1.2 mg ionophore composition. The optimized composition of the corresponding sensor (NP_{S6}) was ion association: PVC: plasticizer as 1.2: 46.0: 52.8 wt%. In the case of this sensor also, the best plasticizer in terms of

slope was found to be BEP. This sensor gave a slope of 57.12 mV/decade. Hence the sensor NP_{S6} was chosen for further studies.

7.6.2 Optimization of the Carbon Paste Composition

The choice of carbon paste components, their quality and mutual ratio in the mixture as well as the way of preparation of carbon pastes and their optimal homogenization - all these aspects determine the resulting behaviour of a CPE. As in the case of optimization of membrane composition of a membrane sensor, the optimization of carbon paste composition was conducted for a carbon paste sensor. The percentage ratios of the ionophore, graphite and plasticizer were varied. Several compositions for the electrodes were investigated in which the plasticizer and ion pair percentages ranged from 65 to 55% and from 2 to 2.4% respectively. For each composition, the electrodes were repeatedly prepared five times using five different solvent mediators. All the five plasticizers were tried in the case of carbon paste sensors also. As the ionophore content was varied an irregular pattern of slope was observed which might be due to the decrease in conductance of the sensor material.

A study of the influence of solvent mediators on the potentiometric response characteristics of the NIM selective carbon paste sensor based on NIM - MPA ion pair complex was conducted and the results are summarized in Table 7.3. As seen, among the five different plasticizers used, the use of 57.6% BEP in the presence of 2.2% ionophore (NC_{M2}) gave the best sensitivity (with a Nernstian slope of 57.14 mV/decade). The optimized composition in the case of NIM - MPA based carbon paste sensor was 2.2: 40.2: 57.6 wt% (ionophore: graphite: plasticizer).

The response characteristics of the carbon paste sensor containing NIM - STA as the ionophore was also studied by varying the ionophore percentages from 2.0 to 2.4 (Table7.4). The optimized composition was found to be 2.0:33.0:65.0 (ionophore: graphite: plasticizer). The concentration of the plasticizer in the sensor matrix greatly influences the response of the sensors fabricated. Of the five plasticizers used, BEP gave the best result in terms of the slope. Thus in the case of all the four fabricated sensors, BEP was found to be the best plasticizer. This may be due to the high polarity of this plasticizer[334].

7.6.3 Effect of Concentration of Internal Filling Solution

Ionophore based membranes are prone to suffer from electrolyte coextraction at the inner membrane side; as a result, the composition of the internal solution influences both the achievable lower detection limit and the shape of the response function[335-338]. The influence of concentration of internal solution on the potential response of the NIM selective membrane sensors were studied in a 1.0×10^{-2} to 1.0×10^{-4}M concentration range. The obtained results showed that the variation in concentration of the internal solution did not produce any significant difference in the potential response. A 1.0×10^{-3}M concentration of drug solution was chosen as internal solution in all subsequent studies.

7.6.4 Working Concentration Range, Slope and Response Time

The calibration plot of the PVC membrane sensor based on NIM - MPA ion pair is presented in Figure 7.2. The membrane sensor NP_{M8} gave a linear range 1.0×10^{-2} - 1.0×10^{-6}M with a slope of 55.6 mV/decade (Figure 7.2). The lower detection limit of this sensor was 6.7×10^{-6}M. Figure 7.3 shows the calibration plot for the PVC membrane sensor based on NIM - STA ionophore. The linear range in the case of the membrane sensor based

on NIM - STA was 1.0×10^{-1} - 3.63×10^{-6}.M. The slope calculated from the calibration graph was 57.12 mV/decade (NP_{S6}). 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 8.91×10^{-7}M. The response time for NP_{M8} was found to be less than 45 s. In the case of the membrane sensor NP_{S6}, the response time was less than 35 s.

Figures 7.4 and 7.5 show the calibration plots for the carbon paste sensors NC_{M2} and NC_{S1}.The carbon paste sensor based on NIM - MPA ion association (NC_{M2}) gave a linear range between 1.0×10^{-2} - 2.04×10^{-5}M. The calibration plot gave a near Nernstian slope of 57.14 mV/decade with a lower detection limit of 8.32×10^{-6}M for this sensor (Figure 7.4). The working concentration range for NIM-STA based carbon paste sensor NC_{S1} was found to be 1.0×10^{-1} - 1.26×10^{-5}M. The lower detection limit calculated from the graph gave a value of 3.38×10^{-6}M. The slope calculated from the calibration graph was found to be 54.34 mV/decade (Figure 7.5).

The response time for the sensor NC_{M2} was less than 30. The sensor NC_{S1} gave a fast response time of less than 25 s. The response characteristics of all the four sensors, NP_{M8}, NP_{S6}, NC_{M2} and NC_{S1} are consolidated in Table 7.5.

The surface structures of the membranes were studied using SEM images. The SEM images of the membranes NP_{M8} and NP_{S6} are presented in Figures 7.6 and 7.7.The high resolution electron micrographs demonstrate a much homogeneous and uniform surface morphology for NP_{S6} compared to NP_{M8}.This may account for the better response characteristics of NP_{S6} when compared to NP_{M8}. A much large deviation of the slope from Nernstian value

is seen for NP_{M8}.The membrane sensor NP_{S6} is superior to NP_{M8} in having a much lower detection limit and fast response time.

7.6.5 Effect of pH

The effect of pH of the test solution on the potential response of the sensors was investigated by following the variation in potential with change in pH. Two different concentrations of the test solution viz., 1.0×10^{-4}M and 1.0×10^{-3}M NIM were used to study the effect of pH. The pH was adjusted using different buffer solutions. From the Figures 7.8 and 7.9, it was observed that, for the sensors NP_{M8} and NC_{M2} there is no change in the potential response within the pH range 5 - 8 and hence this was chosen as the working pH range of the sensors. Figures 7.10 and 7.11 depict the effect of pH of the test solution on the potential response of the sensors based on NIM-STA ion association. For the sensors based on the ion association NIM-STA, the potential remained constant in the pH range 5 - 7. At higher pH 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 interference of hydrogen ions.

7.6.6 Potentiometric Selectivity

Selectivity of a membrane sensor is its response to primary ion in presence of foreign ions. This is the most important characteristic of a sensor, which determines the extent to which a sensor can be employed in the analysis of real samples. This is measured in terms of potentiometric selectivity coefficients. The selectivity coefficients were determined using the fixed interference method. Selectivity coefficient data for various ions are presented in Table 7.6. A value of selectivity coefficient <1 indicates that the electrode is selective to the primary ion over the interfering ion. The influence of some inorganic cations, organic species and some pharmaceutical excepients on the electrode response was

investigated. Table 7.6 shows that the selectivity coefficient values for all the ions studied are smaller than 1. The developed sensors were selective to the drug in presence of various ions like NH_4^+, K^+, Na^+, Mg^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, $Zn^{2+}$and some pharmaceutical excepients such as starch, lactose, talc etc. Hence, these sensors could be used for the selective determination of the drug NIM.

7.6.7 Shelf Life or Life Time

Sensor life span was examined by repeated monitoring of the slope of the drug calibration curve periodically. It was observed that the investigated electrodes exhibited good stability in terms of slope in the linear domain of concentration and the membrane electrodes NP_{M8} and NP_{S6} could be used continuously for about 4 weeks and 2weeks respectively without considerable change in its slope value. The two carbon paste sensors showed a shelf life of 3 weeks. During this time the sensors showed no deviation from the optimized response characteristics. The working surface of the carbon paste sensors could be renewed. All of the developed sensors were stored in conditioning solutions when not in use.

7.7 Analytical Applications

Analytical application studies were conducted using all the four fabricated sensors. The sensors were employed for the determination of the drug content in its tablet form and also for the determination of NIM content in real sample like urine.

7.7.1 Determination of NIM in Pharmaceutical Formulations (Tablets)

The developed sensors were successfully applied for the determination of NIM in commercially available pharmaceutical formulation such as Nimulase (Kniss Laboratories PVT Ltd, India). The detailed procedure for

the determination is given in section 2.9.5 of Chapter 2. The results obtained are summarized in Table 7.7. The values obtained clearly shows that the sensors are highly selective to the drug NIM and that, common tablet excepients such as talc, lactose, starch etc did not interfere with the determination as is evident from the selectivity studies. The results were compared with those obtained by the standard potentiometric titration method reported in European Pharmacopoeia[185]. It has been found that there is satisfactory agreement between the NIM content determined by the proposed sensors and the official method.

7.7.2 Recovery of NIM from Urine Sample

The developed sensors were employed for the determination of NIM content in urine samples. The detailed procedure is given in Chapter 2. The results are given in Table 7.8.The results indicate that the developed sensors can detect the drug content in the spiked urine sample with high accuracy and precision. The percentage recoveries of the drug using the sensors NP_{M8}, NC_{M2}, NP_{S6} and NC_{S1} were found to be respectively 99.0, 99.0, 98.5 and 99.5.

7.8 Conclusion

Two PVC membrane sensors and two carbon paste sensors were fabricated for the drug NIM. The sensors were developed using the ion associations formed by molybdophosphoric acid and silicotungstic acid with the drug. The membrane sensors NP_{M8} and NP_{S6} gave slope values of 55.60 and 57.12 mV/decade respectively. The optimum pH range in the case of these sensors was 5 - 8 and 5 - 7 respectively. The response time for the membrane sensors NP_{M8} and NP_{S6} were found to be less than 45 s and less than 35 s respectively. Also their shelf lives were obtained as 4 weeks and 2 weeks respectively. The carbon paste sensors NC_{M2} and NC_{S1} showed a

linear range 1.0×10^{-2} - 2.04×10^{-5}M and 1.0×10^{-1} - 1.26×10^{-5}M with a lower detection limit of 8.32×10^{-6}M and 3.38×10^{-6}M. The sensors NC_{M2} and NC_{S1} gave slope values of 57.14 and 54.34 mV/decade respectively. The response time of these sensors were less than 30 and 25 s respectively. Both NC_{M2} and NC_{S1} had a shelf life of 3 weeks.

The developed sensors were found to have good characteristics in terms of slope, concentration range, detection limit, response time, pH range and shelf life. The sensors showed high selectivity to NIM drug in the presence of other foreign species. The membrane sensor NP_{S6} was superior to NP_{M8} in terms of slope, lower detection limit and fast response time. In the case of the two carbon paste sensors, NC_{S1} gave a lower detection limit and fast response when compared to NC_{M2}. Further, the developed sensors could be used in the determination of NIM in pharmaceutical formulations and also in body fluids like urine.

Table 7.1 Optimization of composition of PVC membrane sensor using NIM - MPA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	PVC	Plasticizer	
NP_{M1}	1.0	49.5	49.5,DBP	47.9
NP_{M2}	1.2	46.0	52.8, DBP	62.3
NP_{M3}	1.8	63.5	34.7, DBP	48.2
NP_{M4}	2.0	40.8	57.2, DBP	52.3
NP_{M5}	1.0	49.5	49.5,BEP	37.2
NP_{M6}	1.2	46.0	52.8, BEP	62.5
NP_{M7}	1.8	63.5	34.7, BEP	73.6
NP_{M8}	***2.0***	***40.8***	***57.2, BEP***	***55.6***
NP_{M9}	1.0	49.5	49.5,BES	39.7
NP_{M10}	1.2	46.0	52.8, BES	67.8
NP_{M11}	1.8	63.5	34.7, BES	49.4
NP_{M12}	2.0	40.8	57.2, BES	45.7
NP_{M13}	1.0	49.5	49.5,DBS	50.7
NP_{M14}	1.2	46.0	52.8, DBS	47.1
NP_{M15}	1.8	63.5	34.7, DBS	40.3
NP_{M16}	2.0	40.8	57.2, DBS	64.5
NP_{M17}	1.0	49.5	49.5,BEA	52.4
NP_{M18}	1.2	46.0	52.8, BEA	48.3
NP_{M19}	1.8	63.5	34.7, BEA	49.5
NP_{M20}	2.0	40.8	57.2, BEA	68.1

Table 7.2 Optimization of composition of PVC membrane sensor using NIM - STA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	PVC	Plasticizer	
NP_{S1}	1.0	49.5	49.5,DBP	50.8
NP_{S2}	1.2	46.0	52.8, DBP	48.7
NP_{S3}	1.8	63.5	34.7, DBP	49.1
NP_{S4}	2.0	40.8	57.2, DBP	63.5
NP_{S5}	1.0	49.5	49.5,BEP	49.9
NP_{S6}	***1.2***	***46.0***	***52.8, BEP***	***57.12***
NP_{S7}	1.8	63.5	34.7, BEP	60.2
NP_{S8}	2.0	40.8	57.2, BEP	47.6
NP_{S9}	1.0	49.5	49.5,BES	39.8
NP_{S10}	1.2	46.0	52.8, BES	35.6
NP_{S11}	1.8	63.5	34.7, BES	68.2
NP_{S12}	2.0	40.8	57.2, BES	40.9
NP_{S13}	1.0	49.5	49.5,DBS	64.1
NP_{S14}	1.2	46.0	52.8, DBS	67.5
NP_{S15}	1.8	63.5	34.7, DBS	37.5
NP_{S16}	2.0	40.8	57.2, DBS	47.3
NP_{S17}	1.0	49.5	49.5,BEA	46.8
NP_{S18}	1.2	46.0	52.8, BEA	47.2
NP_{S19}	1.8	63.5	34.7, BEA	44.6
NP_{S20}	2.0	40.8	57.2, BEA	46.7

Table 7.3 Optimization of composition of carbon paste sensor using NIM - MPA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	Graphite	Plasticizer	
NC_{M1}	2.0	33.0	65.0, BEP	53.4
NC_{M2}	***2.2***	***40.2***	***57.6, BEP***	***57.14***
NC_{M3}	2.4	42.0	55.6, BEP	49.9
NC_{M4}	2.0	33.0	65.0, DBP	48.3
NC_{M5}	2.2	40.2	57.6, DBP	51.6
NC_{M6}	2.4	42.0	55.6,DBP	63.5
NC_{M7}	2.0	33.0	65.0, DBS	71.2
NC_{M8}	2.2	40.2	57.6, DBS	64.7
NC_{M9}	2.4	42.0	55.6, DBS	42.4
NC_{M10}	2.0	33.0	65.0, BES	43.9
NC_{M11}	2.2	40.2	57.6, BES	38.9
NC_{M12}	2.4	42.0	55.6, BES	62.7
NC_{M13}	2.0	33.0	65.0, BEA	45.3
NC_{M14}	2.2	40.2	57.6, BEA	68.1
NC_{M15}	2.4	42.0	55.6, BEA	50.8

Table 7.4 Optimization of composition of carbon paste sensor using NIM - STA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	Graphite	Plasticizer	
NC_{S1}	***2.0***	***33.0***	***65.0, BEP***	***54.34***
NC_{S2}	2.2	40.2	57.6, BEP	47.5
NC_{S3}	2.4	42.0	55.6, BEP	52.6
NC_{S4}	2.0	33.0	65.0, DBP	50.8
NC_{S5}	2.2	40.2	57.6, DBP	48.9
NC_{S6}	2.4	42.0	55.6,DBP	50.6
NC_{S7}	2.0	33.0	65.0, DBS	46.7
NC_{S8}	2.2	40.2	57.6, DBS	62.6
NC_{S9}	2.4	42.0	55.6, DBS	46.9
NC_{S10}	2.0	33.0	65.0, BES	61.7
NC_{S11}	2.2	40.2	57.6, BES	43.4
NC_{S12}	2.4	42.0	55.6, BES	48.1
NC_{S13}	2.0	33.0	65.0, BEA	39.8
NC_{S14}	2.2	40.2	57.6, BEA	38.1
NC_{S15}	2.4	42.0	55.6, BEA	48.0

Table 7.5 Response characteristics of the developed sensors NP_{M8}, NC_{M2}, NP_{S6} and NC_{S1}

Parameter	Response Characteristics			
	NP_{M8}	NC_{M2}	NP_{S6}	NC_{S1}
Slope (mV per decade)	55.60	57.14	57.12	54.34
Linear range (M)	1.0×10^{-2} - 1.0×10^{-6}	1.0×10^{-2} - 2.04×10^{-5}	1.0×10^{-1} - 3.63×10^{-6}	1.0×10^{-1} - 1.26×10^{-5}
pH range	5 - 8	5 - 8	5 - 7	5 - 7
Detection limit (M)	6.7×10^{-6} M	8.32×10^{-6}	8.91×10^{-7}	3.38×10^{-6}
Response time (s)	<45	< 30	< 35	< 25
Shelf life	4 weeks	3 weeks	2 weeks	3 weeks

Table 7.6 Selectivity coefficient values of various interfering species, K^{pot}

Interfering Species	$K^{pot}_{A,B}$			
	NP_{M8}	NC_{M2}	NP_{S6}	NC_{S1}
NH_4^+	1.9×10^{-3}	2.3×10^{-3}	1.5×10^{-3}	1.6×10^{-3}
K^+	5.0×10^{-2}	6.8×10^{-3}	5.9×10^{-3}	4.4×10^{-3}
Na^+	5.0×10^{-2}	4.8×10^{-3}	6.3×10^{-3}	5.7×10^{-3}
Mg^{2+}	5.6×10^{-2}	6.3×10^{-3}	6.1×10^{-3}	5.1×10^{-3}
Co^{2+}	3.2×10^{-2}	3.9×10^{-2}	3.6×10^{-2}	2.8×10^{-3}
Ca^{2+}	8.9×10^{-3}	7.2×10^{-3}	8.5×10^{-3}	8.8×10^{-3}
Ni^{2+}	1.4×10^{-3}	3.1×10^{-2}	1.9×10^{-2}	2.5×10^{-2}
Zn^{2+}	4.3×10^{-3}	4.2×10^{-3}	3.3×10^{-4}	5.4×10^{-3}
Ascorbic acid	3.6×10^{-2}	2.7×10^{-2}	3.1×10^{-2}	2.2×10^{-3}
Glycine	1.7×10^{-2}	1.4×10^{-3}	2.1×10^{-3}	1.9×10^{-3}
Lactose	5.2×10^{-3}	4.6×10^{-3}	5.3×10^{-3}	5.8×10^{-3}
Starch	4.8×10^{-2}	3.9×10^{-2}	4.5×10^{-2}	5.3×10^{-2}
Urea	3.8×10^{-4}	3.6×10^{-3}	5.3×10^{-4}	4.7×10^{-3}
Talc	6.2×10^{-4}	7.1×10^{-3}	6.6×10^{-3}	6.9×10^{-3}

Table 7.7 Determination of NIM in pharmaceutical formulation

Sample	Declared Amount (mg/tablet)	Method adopted	Found * (mg/tablet)	SD*	CV*
Nimulase (Kniss Laboratories PVT Ltd, India)	100	NP_{M8}	97	0.90	0.93
		NC_{M2}	98	0.92	0.94
		NP_{S6}	99	0.91	0.92
		NC_{S1}	98	0.95	0.97
		Standard Method	98	0.85	0.87

*Average of six replicates

Table 7.8 Determination of NIM in urine sample using the developed sensors

Drug added (M)	Sensor	Drug found* (M)	Recovery %
2.00×10^{-3}	NP_{M8}	1.98×10^{-3}	99.0
	NC_{M2}	1.98×10^{-3}	99.0
	NP_{S6}	1.97×10^{-3}	98.5
	NC_{S1}	1.99×10^{-3}	99.5

*Average of six replicates

Figure 7.1 Structure of Nimesulide

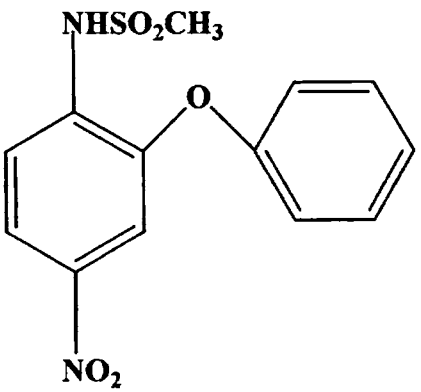


Figure 7.2 Calibration graph for NIM selective PVC membrane sensor based on NIM - MPA ion association (NP_{M8})

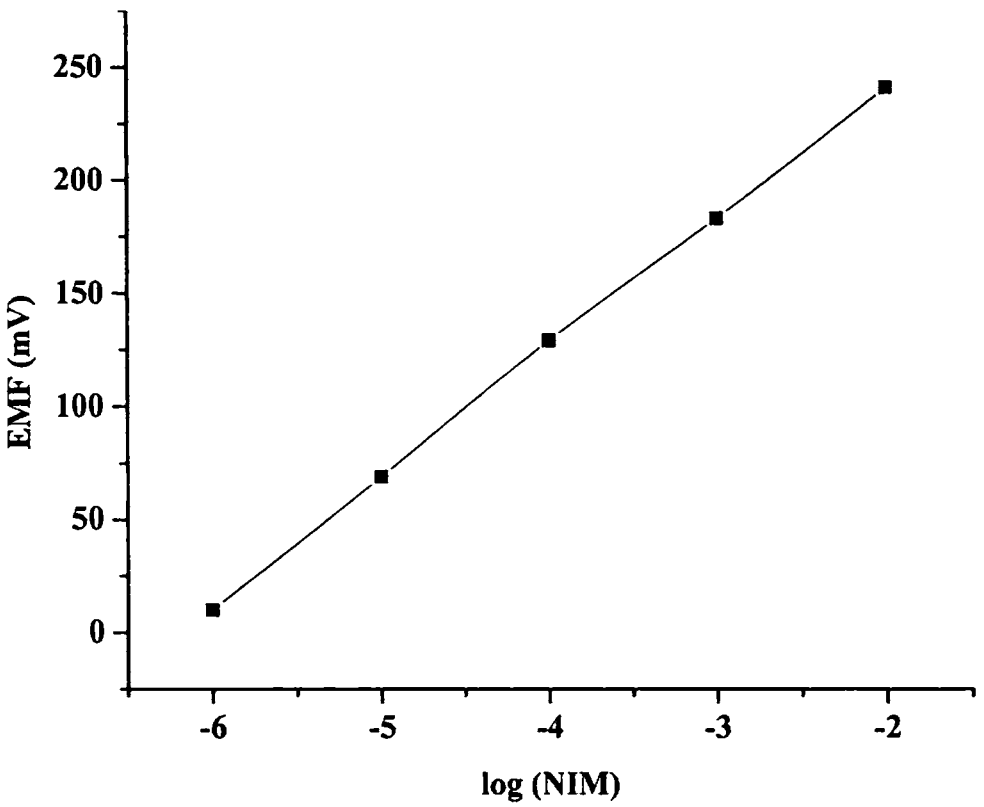


Figure 7.3 Calibration graph for NIM selective PVC membrane sensor based on NIM - STA ion association (NP_{S6})

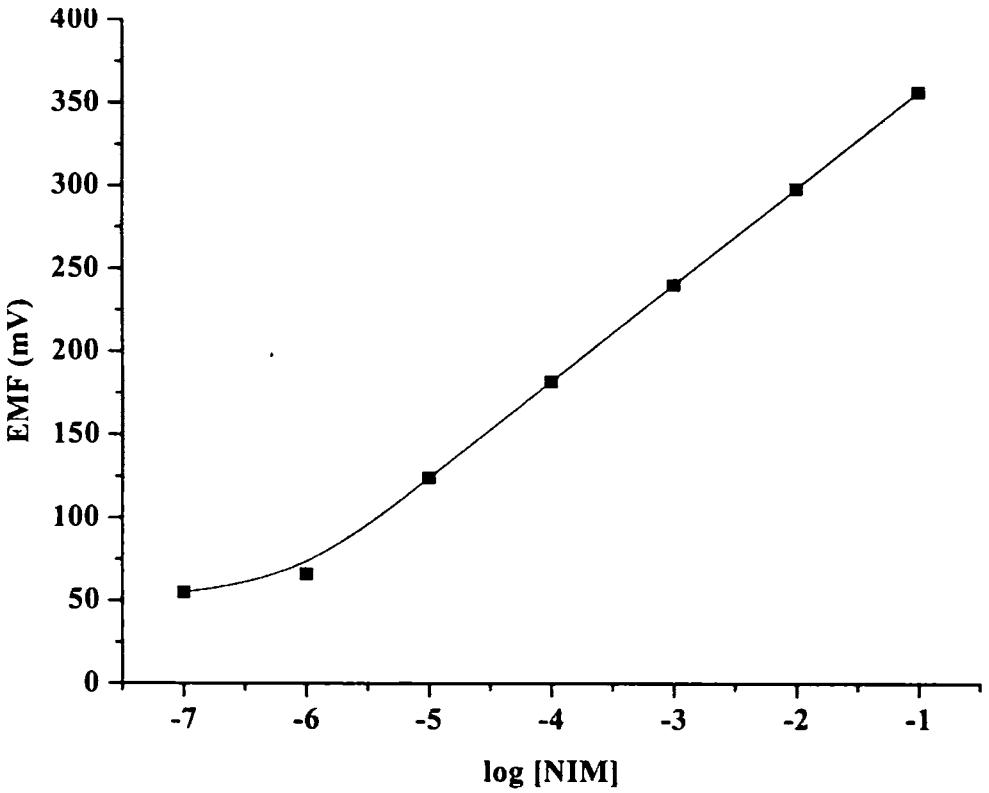


Figure 7.4 Calibration graph for NIM selective carbon paste sensor based on NIM - MPA ion association (NC_{M2})

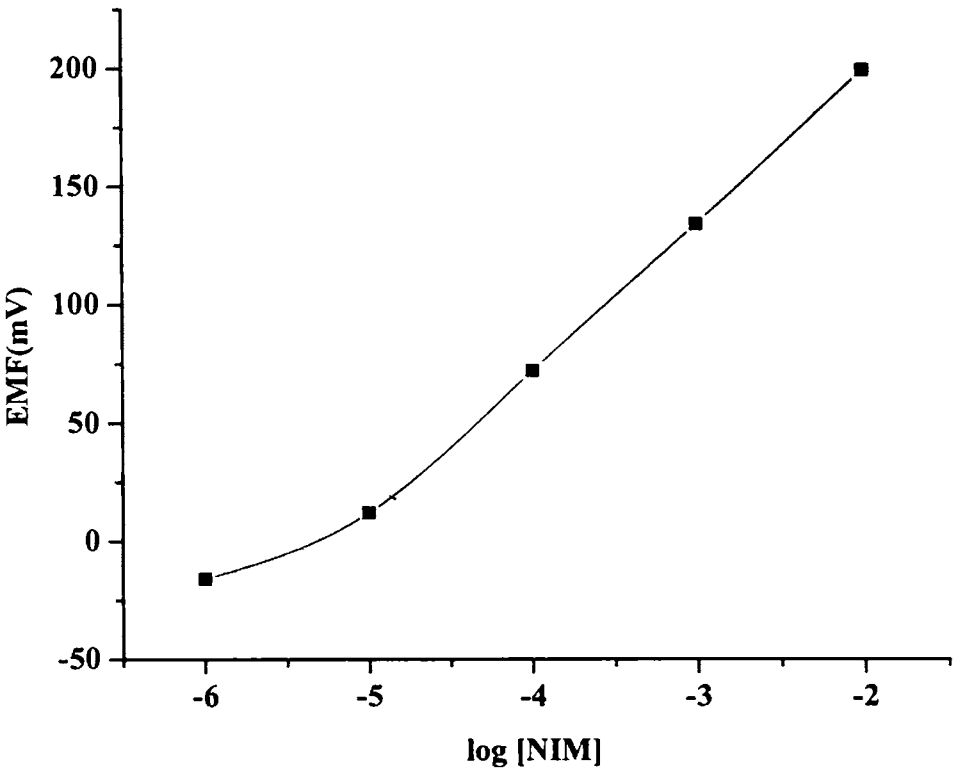


Figure 7.5 Calibration graph for NIM selective carbon paste sensor based on NIM - STA ion association (NC_{S1})

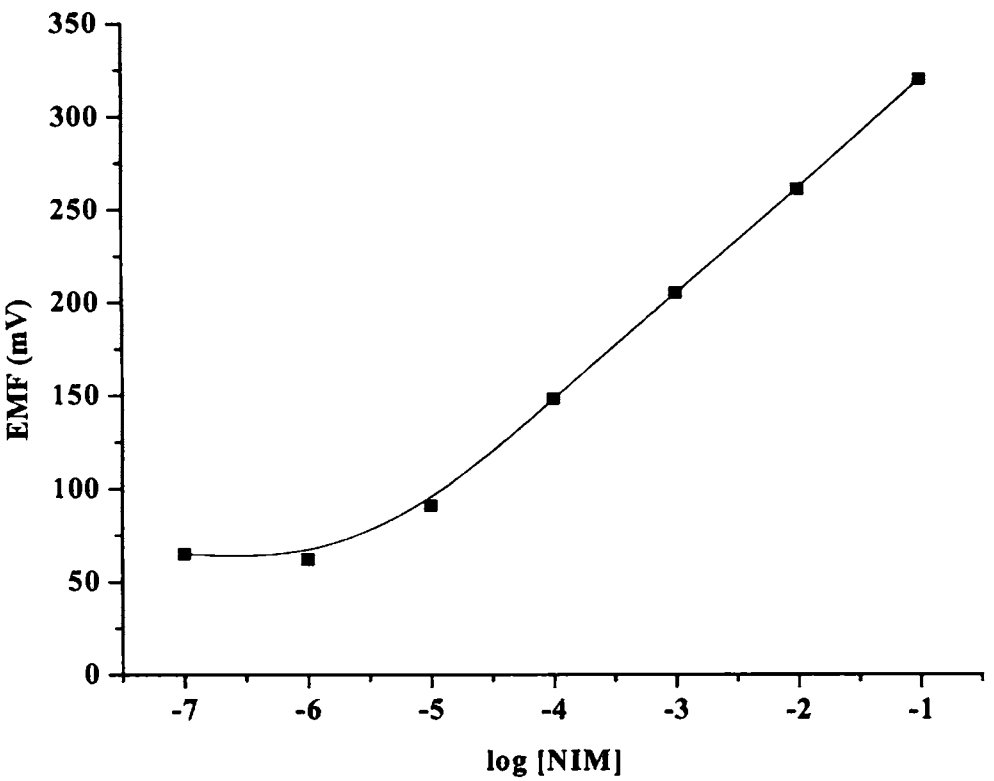


Figure 7.6 SEM image of the polymeric membrane of $\mathbf{NP_{M8}}$ sensor

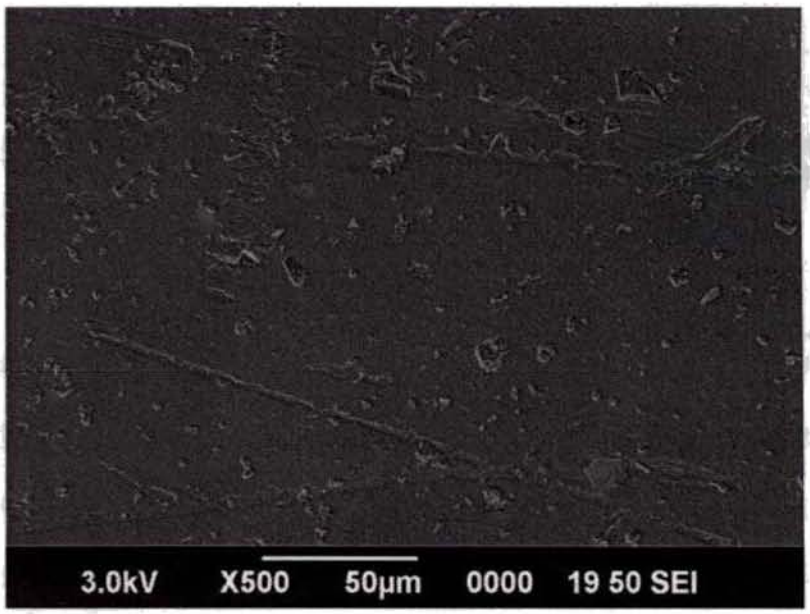


Figure 7.7 SEM image of the polymeric membrane of $\mathbf{NP_{S6}}$ sensor

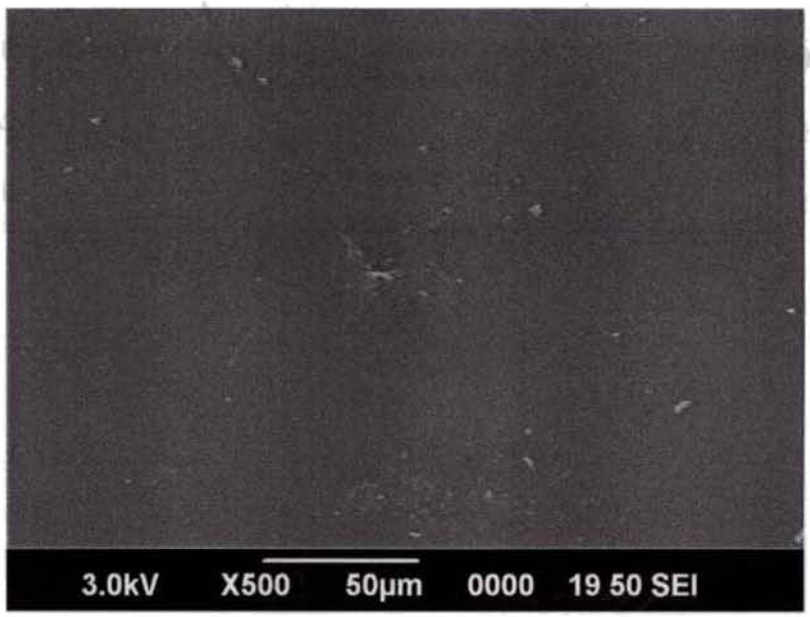


Figure 7.8 Effect of pH on the cell potential of NIM selective PVC membrane sensor (NP_{M8}) at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

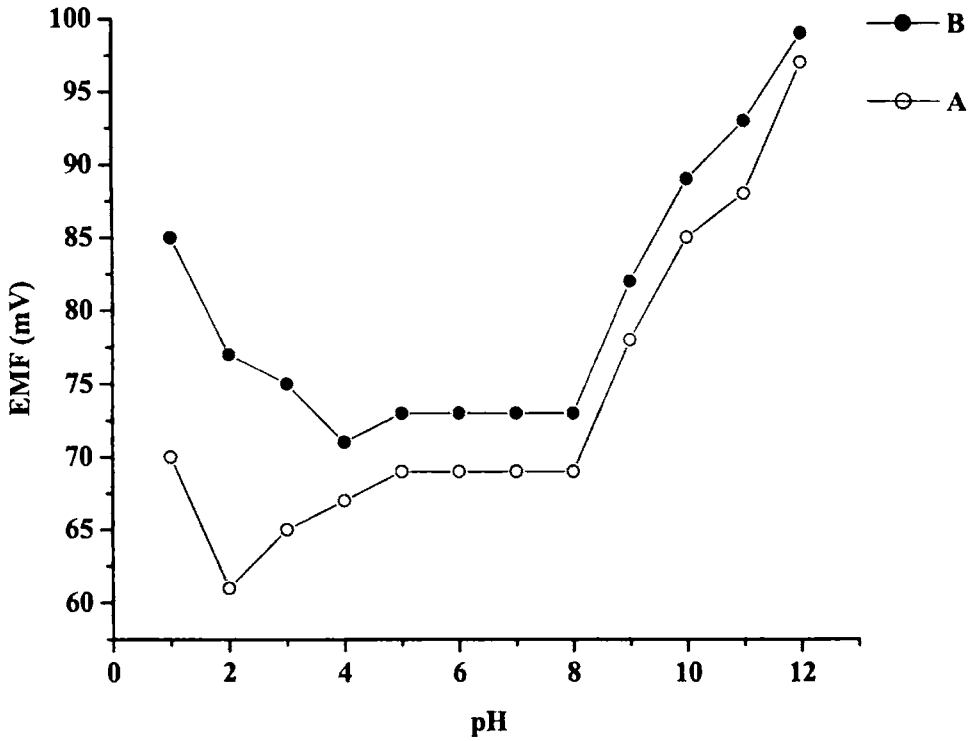


Figure 7.9 Effect of pH on the cell potential of NIM selective carbon paste sensor (NC_{M2}) at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

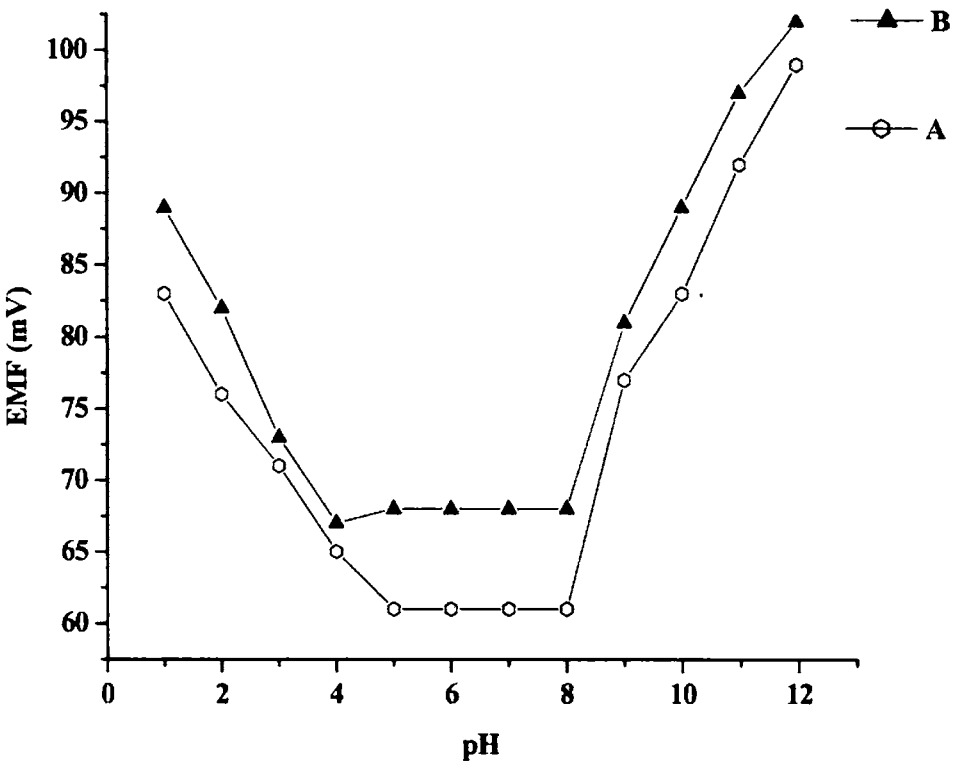


Figure 7.10 Effect of pH on the cell potential of the NIM selective PVC membrane sensor (NP_{S6}) at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

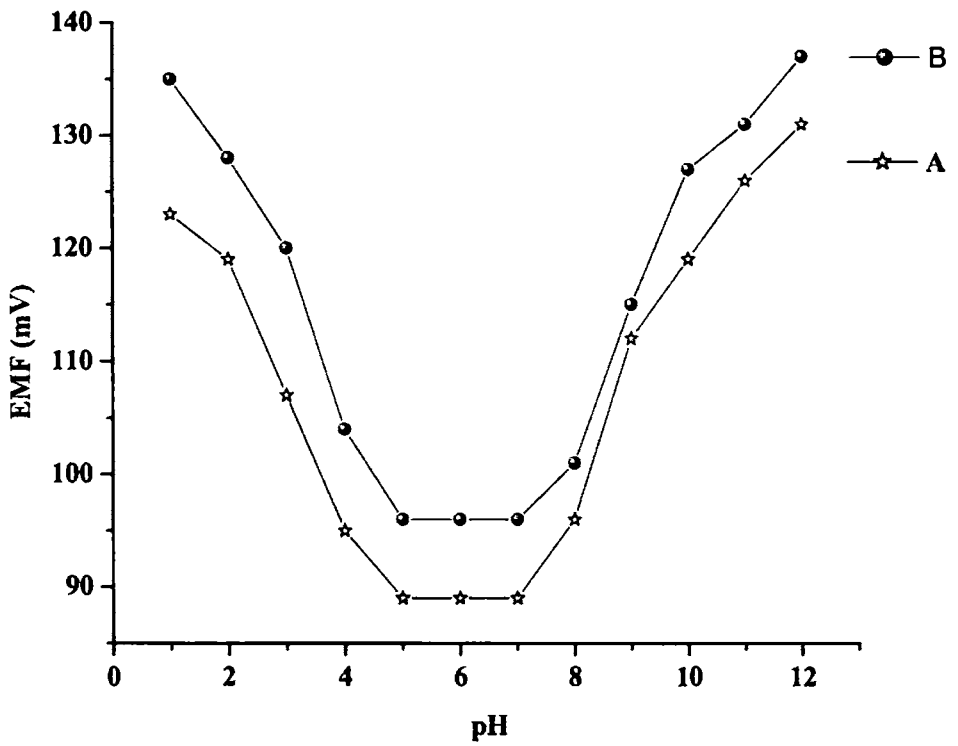
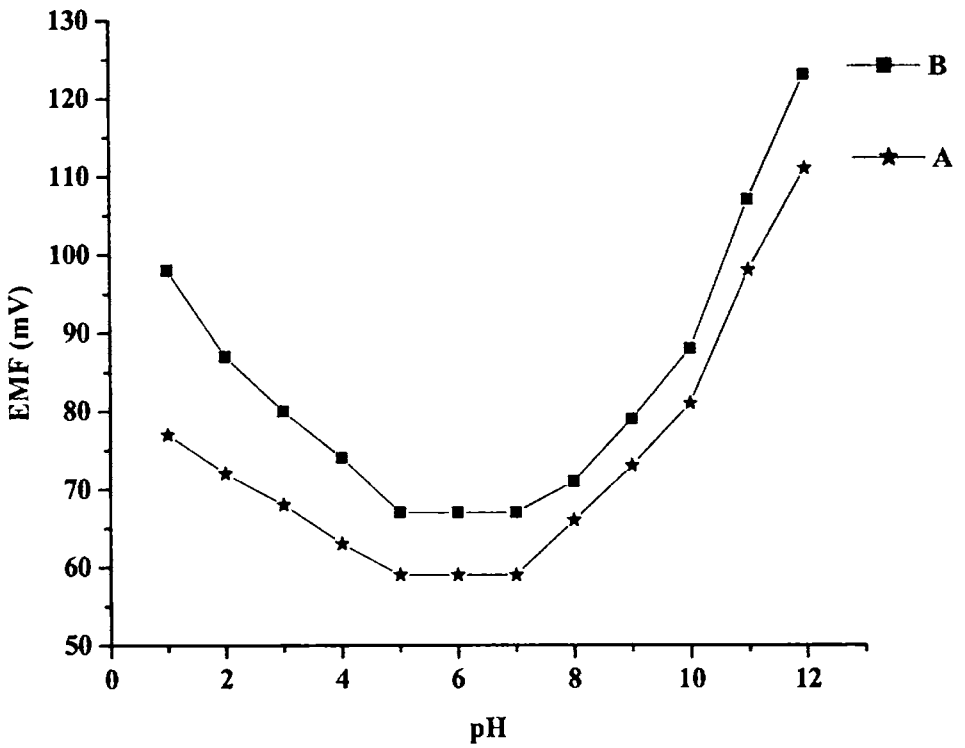


Figure 7.11 Effect of pH on the cell potential of the NIM selective carbon paste sensor (NC_{S1}) at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)



Chapter 8

SENSORS FOR THE DETERMINATION OF LOMEFLOXACIN

This chapter details the fabrication of two novel electrochemical sensors for the quantitative determination of the drug Lomefloxacin (LOM) based on LOM - STA (silicotungstic acid) and LOM - MPA (molybdophosphoric acid) ion pairs as the electroactive materials. Different performance characteristics of the sensors including slope, concentration range, detection limit, response time, pH range and shelf life have also been explained in detail in this chapter. The applicability of the developed sensors in the determination of the drug in pharmaceutical formulations such as tablets was investigated. Also their applicability in the determination of LOM has been tested in real samples like urine by the standard addition method.

8.1 Introduction

Lomefloxacin hydrochloride (Figure 8.1) is a fluoroquinolone antibiotic, used to treat bacterial infections including bronchitis and urinary tract infections. Quinolones constitute a large class of synthetic antimicrobial agents that are highly effective in the treatment of many types of infectious diseases, particularly those caused by bacteria. New quinolones are continually being developed as bacterial species develop resistance to existing quinolones. Quinolone antibiotics were once considered relatively safe, but several side effects have become evident with experience. For example, numerous case reports have implicated their use since 1965 in

spontaneous tendon ruptures or damage, especially with the concurrent use of a systemic corticosteroid. In the beginning of 2004, the Food and Drug Administration upgraded the warnings found within the package inserts for all drugs within this class regarding such serious adverse reactions.

Fluoroquinolone antibiotics have exhibited high activity against gram-positive and gram-negative bacteria by inhibiting activity of their DNA gyrase[339,340]. They are widely used in the treatment of urinary or respiratory infections[341]. Up to now, many techniques, such as spectrophotometry[342], spectrofluorometry[343-346], HPLC[347-349], electrochemical analysis[350-354] and chemiluminiscence method[355,356] have been utilized for the determination of fluoroquinolone derivatives in pharmaceutical formulations and biological fluids.

Lomefloxacin is one of the synthetic antibacterial fluoroquinolone agents of the third generation. It is 1-ethyl-6,8-dyfluro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid[357]. It is a white to pale yellow powder with a molecular weight of 387.8. It is slightly soluble in water and practically insoluble in alcohol. Lomefloxacin HCl is stable to heat and moisture but is sensitive to light in dilute aqueous solution. It is a synthetic broad spectrum antimicrobial agent for oral administration. Lomefloxacin is also used to prevent urinary tract infections prior to surgery. It is used as a prophylactic or preventative treatment to prevent urinary tract infections in patients undergoing transrectal or transurethral surgical procedures.

Flouroquinolones such as lomefloxacin possess excellent activity against gram-negative aerobic bacteria such as E.coli and Neisseria gonorrhoea as well as gram-positive bacteria including Salmonella pneumoniae and Staphylococcus aureus[358]. They also posses effective activity against shigella, salmonella,

campylobacter, gonococcal organisms, and multi drug resistant pseudomonas and enterobacter. The bactericidal action of lomefloxacin results from interference with the activity of the bacterial enzymes DNA gyrase and topoisomerase IV, which are needed for the transcription and replication of bacterial DNA. DNA gyrase appears to be the primary quinolone target for gram-negative bacteria. Topoisomerase IV appears to be the preferential target in gram-positive organisms. Interference with these two topoisomerases results in strand breakage of the bacterial chromosome, supercoiling, and resealing. As a result DNA replication and transcription is inhibited.

Lomefloxacin is extensively used because of its strong and prolonged antibacterial activity. Therefore, the detemination of lomefloxacin in capsules, injections and blood samples requires a simple, quick and sensitive analytical method. A number of analytical methods for the determination of lomefloxacin, for example, UV/Visible spectrophotometry[359-361], spectrofluorometry[362], flow injection analysis[363], capillary electrophoresis[364], sensitized fluorometry[365], and high performance liquid chromatography[366] have been proposed. However, most of these methods require sophisticated instrumentation, prohibitive cost, or technical difficulty. Because of these considerations, potentiometry with ion selective electrodes seems attractive for determination of lomefloxacin in pharmaceutical substances. Potentiometric sensors or so-called ion selective electrodes (ISEs) are the subject of continuous research efforts. This group of chemical sensors is characterized as simple in preparation, robust in operation, and moderately selective in analytical performance. In the last three decades, being commercially available and not expensive, ion selective electrodes have become an item of general equipment of analytical work. This result happens because ion selective electrodes have rapid, simple, low cost and give accurate

measurements of ionic species. The key to constructing such an electrode is to produce a sensitive and selective membrane that responds to a particular drug. Such a membrane is usually prepared by incorporating an appropriate ion exchanger and solvent mediator into a poly (vinyl chloride) membrane matrix[367].

The present chapter describes the preparation and electrochemical characterization of simple polymeric membrane potentiometric sensors for the determination of LOM in pharmaceutical formulations. They are based on the use of silicotungstic acid (STA) and molybdophosphoric acid (MPA) with the drug lomefloxacin (LOM) in the formation of ion association species. These species were used as electroactive materials in plasticized poly (vinyl chloride) matrix membranes. The ISEs based on these membranes were prepared, characterized, compared, and used for rapid and accurate selective determination of LOM in pure samples as well as in pharamaceutical preparations and biological samples like urine.

8.2 Synthesis of the Ion Associations

The ion association complexes were prepared by mixing 75 mL of 10^{-2}M LOM with 25 mL of 10^{-2}M STA and 25 mL of 10^{-2}M MPA solutions. The precipitates formed were stirred well. The precipitates were filtered through a Whatman filter paper and washed with distilled water several times. The obtained precipitates were dried at room temperature and stored in a desiccator. These ion associations were used for the fabrication of polymeric membrane sensors for the determination of LOM.

The compositions of both ion association complexes were confirmed by the elemental analysis to be 3:1 (LOM: STA and LOM: MPA). The elemental analysis data obtained for the ion associations are as follows:

LOM-STA ion association

Found (%) – C - 15.38, H - 1.34, N - 3.26

Calculated (%) – C - 15.56, H - 1.45, N - 3.20

LOM-MPA ion association

Found (%) – C - 18.63, H - 1.63, N - 3.56

Calculated (%) – C - 18.48, H - 1.72, N - 3.80

8.3 Fabrication of the LOM Membrane Sensor

The membrane sensors were fabricated using the synthesized ionophores. The detailed procedure for the fabrication of the polymeric membrane sensors is given in Chapter 2. The sensors were constructed according to the method of Cragg et al. In this method at first, the ionophore, PVC and plasticizer are dissolved in about 5- 7 mL of THF. The solution was then poured into glass rings struck onto a glass plate. The homogeneous cocktail was covered with a filter paper and allowed to stand overnight, to allow solvent evaporation at room temperature. After the slow evaporation of solvent the sensing membrane is formed. A transparent membrane about 1 mm in thickness was obtained, from which a disk of about 12 mm diameter was cut. It was then glued to one end of a glass tube. The electrode bodies were filled with a solution that was 1.0×10^{-1}M in NaCl and 1.0×10^{-3}M in LOM. Before use, the membrane electrodes were preconditioned overnight in 1.0×10^{-3}M LOM solution.

8.4 Potential Measurement and Calibration

The potential measurements were carried out at 25±1 °C on a Metrohm 781 ion meter.

The electrochemical cell assembly may be represented as follows:

Saturated calomel electrode | internal filling solution (1×10^{-1} M NaCl solution + 1×10^{-3}M drug solution) | PVC membrane | test solution | saturated calomel electrode.

A saturated calomel reference electrode was used in conjunction with the developed sensor. A stock solution of 1.0×10^{-1}M LOM was prepared. The working solutions were prepared by appropriate dilution of the stock solution with water. The membrane electrodes were immersed in each of the solutions having different concentrations. The performances of the electrodes were investigated by measuring the emf values between 1.0×10^{-2}M and 1.0×10^{-8}M concentrations of the respective LOM solutions. The potential readings were recorded after stabilization and were plotted as a function of log [LOM]. The calibration graph was used for subsequent determination of unknown LOM concentrations.

8.5 Performance Characteristics of the Developed Sensors

Any potentiometric ion selective sensor can be evaluated based on its response parameters. These electrochemical response characteristics of a newly developed sensor depends on various factors such as membrane composition, choice of a suitable plasticizer etc. The next section describes in detail the response characteristics of the two fabricated sensors.

8.5.1 Optimization of the Membrane Composition

The operating characteristics of ion selective electrodes can be significantly modified by changing the relative proportions of the components of the electrode membrane, which essentially comprises the ionophore and plasticizer. A total of fifteen different sensors were prepared

for both the ion associations by varying the ratios of ionophore, plasticizer and PVC. It is well known that the construction of PVC based ISEs requires the use of a plasticizer which mainly acts as a fluidizer allowing homogeneous dissolution and diffusional mobility of the ion pair inside the membrane. The nature and/or the amount of plasticizer must be properly controlled in order to minimize electrical asymmetry of the membrane and to limit fouling of the sensor. In addition, the proper selection of the plasticizer allows one to control the value of the electrode/solution distribution ratio of the particular C^+A^- ion pair employed as an ion exchanger. The analytical performance of such electrode is strongly dependent on a suitable ratio of plasticizer and electroactive material. Hence the present work involved the study of the influence of the plasticizer type and also their concentration on the performance characteristics of the sensors. Accordingly five different types of plasticizers were chosen for the study which involve 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).

In the present study several membrane compositions were investigated in which the percentage of ion exchanger ranged from 1.0 to 1.4% (Table 8.1). For the membrane electrode incorporating LOM-STA as the ionophore, the best result was obtained with the sensor having 1.4 wt% of the ionophore. The optimized composition of the corresponding sensor was, ion association: PVC: plasticizer (DBP) as 1.4: 32.6: 66.0 wt% (LO_{S3}). Of the five different plasticizers used, DBP was found to give a near Nernstian response. The sensor LO_{S3} gave a near Nernstian slope of 58.2 mV/decade for a

concentration range $1.0 \times 10^{-2} - 1.0 \times 10^{-6}$ (Figure 8.2). Hence the subsequent studies were performed using the LO_{S3} sensor.

The membrane composition of the sensors incorporating the ion association LOM-MPA was also varied (Table 8.2). It was found that the sensor having 1.2 wt% ion pair gave a near Nernstian slope of 54.9 mV/decade (Figure 8.3). Out of the five plasticizers used, BEP gave the best result in terms of the slope. The optimized membrane composition can be represented as ion association: PVC: plasticizer as 1.2: 50.2: 48.6 wt% (LO_{M8}).

8.5.2 Effect of Concentration of Internal Filling Solution

The influence of the concentration of the internal filling solution on the potential response of the LOM selective membrane sensors were studied. The LOM concentration was varied from 1.0×10^{-4} to 1.0×10^{-2} M. In all the cases, the EMF vs. log [LOM] plot was obtained. It was found that the variation of the concentration of the internal filling solution does not cause any significant difference in the electrode response. Hence a 1.0×10^{-3}M concentration of internal filling solution was selected for further assays. The optimum conditioning time for the membrane sensors in 1.0×10^{-3}M LOM solution was approximately 12 hours for LOM-STA sensor and around 24 hours for LOM-MPA sensor. The electrodes then generate stable potentials when placed in contact with LOM solutions. The electrodes were rinsed with distilled water before each measurement and stored in the preconditioning solution when not in use.

8.5.3 Working Concentration Range, Slope and Response Time

The sensor LO_{S3} had a working concentration range between 1.0×10^{-2} - 1.0×10^{-6}M with a slope of 58.2 mV/decade. In the case of the LO_{M8}

sensor, a linear response was obtained in the concentration range 1.0×10^{-2} - 5.0×10^{-5}M. This was chosen as the working concentration range of the sensor. The sensor showed a near Nernstian slope of 54.9 mV/decade within this working concentration range. The limit of detection, as determined from the intersection of the two extrapolated linear segments of the calibration graph, was 2.69×10^{-6} M and 5.23×10^{-5}M for LO_{S3} and LO_{M8} respectively.

The time required for all constructed sensors to reach values within ±1 mV of the final equilibrium potential after immersion in LOM solutions were investigated. The response time of the sensor LO_{S3} was found to be <20 s whereas in the case of LO_{M8} the response time was found to be <30 s.

The response characteristics of both the sensors, LO_{S3} and LO_{M8} are summarized in Table 8.3.

The SEM analysis was conducted inorder to know the surface morphology of the developed membranes. Figures 8.4 and 8.5 give the scanning electron microscopic images of the surfaces of the membranes LO_{S3} and LO_{M8}.These images give an idea of the homogeneity of the membranes which is a main factor affecting the response characteristics of the sensor. One of the disadvantages of a PVC based membrane is that, it is not possible to control the uniformity distribution of the electroactive material in the membrane. From the figures, it is clear that a smooth and homogeneous surface is obtained for the membrane LO_{S3}.The better response parameters of LO_{S3} compared to LO_{M8} may be due to its much homogeneous and uniform membrane surface. The high sensitivity as indicated by the slope, a lower detection limit and a fast response time is presented by the sensor LO_{S3}.

8.5.4 Effect of pH

It is necessary to find the optimum pH range when the electrode functions without interference from the hydrogen and hydroxide ions. The pH dependence of the test solution on the electrode potential was tested over the range 1 to 12 at two different concentrations viz., 1.0×10^{-3} M and 1.0×10^{-4} M of LOM solutions. The pH of the test solutions was adjusted by using appropriate buffer solutions. In the case of LO_{S3} it is clear from Figure 8.6 that the potentials were found to stay constant from pH 6 to 8, beyond which the potential changes considerably. Figure 8.7 gives the pH-potential profile for LO_{M8}. The pH profile shows that, for LO_{M8}, the potential remained constant in the pH range 7 - 9. The observed potential drift at lower pH values may be attributed to the membrane response to hydrogen ions. The decrease in potential readings at pH > 9.0, on the other hand, can be probably attributed to the interference of the hydroxide ions and also to the decrease in concentration of the LOM ions.

8.5.5 Potentiometric Selectivity

Selectivity is one of the most important characteristics of a membrane sensor, because it helps one to determine whether a reliable measurement in the target sample is possible or not. To investigate the selectivity of the membrane sensors proposed, their potential response were investigated in the presence of various interfering ions. Selectivity is measured in terms of potentiometric selectivity coefficient $K_{A,B}^{pot}$. The potentiometric selectivity coefficients measure the response of the electrode for the primary ion in the presence of foreign ions. The extent of interference of various interfering species was studied by the fixed interference method[368]. The resulting selectivity coefficients are summarized in Table 8.4. The results reveal high

selectivity for LOM in the presence of other common species tested. Common excipients used in pharmaceutical preparations like lactose, glycine, etc, did not interfere.

8.5.6 Shelf Life or Life Time

The calibration curve was periodically plotted with standard solutions and the response slopes were calculated inorder to determine the shelf life of the fabricated sensors. It was observed that the investigated electrode LO_{S3} exhibited good stability in terms of slope in the linear domain of concentration and it could be used continuously for about 3 weeks without considerable variation in its slope value. The sensor LO_{M8} could be used continuously for about four weeks without considerable change in its slope value. After this period it appeared that the response characteristics were altered, and the sensors could not be used for analytical purposes.

8.6 Analytical Applications

The prepared sensors have been successfully used for the determination of LOM in pharmaceutical formulations and real samples like urine.

8.6.1 Determination of LOM in Pharmaceutical Formulations (Tablets)

The developed sensors LO_{S3} and LO_{M8} were applied to the determination of LOM in pharmaceutical formulations commercialized as Lomedon (Indon, India) and Lomegen (Genix, India). The detailed procedure for the determination is given in section 2.9.6 of Chapter 2. The LOM content was determined by the proposed ion selective sensors using the calibration method. Determination of LOM present in its tablets using the proposed method and by the standard spectrophotometric method[189] was carried out. The results are summarized in Table 8.5. The results clearly indicate a satisfactory agreement between the

LOM content determined by the proposed membrane sensors and by the standard method, as well as the declared amounts in the drug preparation used. This is indicative of non interference of other ingredients and excipients, which are present in the pharmaceutical formulations.

8.6.2 Recovery of LOM from Urine Sample

In order to investigate the applicability of the proposed LOM membrane sensors to the determination of the drug in the biological fluids, it was applied to the recovery of 2.00×10^{-3}M solution of the drug in urine sample. The sensors proved useful for the determination of LOM content in urine sample using the standard addition method which has been discussed in detail in Chapter 2. The results are illustrated in Table 8.6. As is obvious from the table, acceptable recoveries are obtained by the proposed membrane electrodes. The percentage recoveries of LOM obtained using the sensors LO_{S3} and LO_{M8} were 99.0 and 102.0 respectively.

8.7 Conclusion

Novel polymeric membrane sensors were developed for the drug LOM based on the ion pair complexes LOM - STA and LOM - MPA. The proposed sensors exhibited long lifetime, good stability, sensitivity, precision, accuracy and selectivity. The sensors are cost effective, easy to prepare and to use. Its usefulness for LOM determination in real samples, particularly for some commercial pharmaceutical preparations was demonstrated suggesting its use as a reliable and advantageous alternative to the most other previously reported methods in the routine control of LOM concentration in these samples. The sensors were also applied to the determination of the drug in real samples like urine. The analytical method proposed proved to be a simple, rapid and accurate method.

Table 8.1 Optimization of composition of PVC membrane sensor using LOM - STA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	PVC	Plasticizer	
LO_{S1}	1.0	42.4	56.6, DBP	61.5
LO_{S2}	1.2	50.2	48.6, DBP	50.4
LO_{S3}	***1.4***	***32.6***	***66.0, DBP***	***58.2***
LO_{S4}	1.0	42.4	56.6, BES	39.8
LO_{S5}	1.2	50.2	48.6, BES	50.7
LO_{S6}	1.4	32.6	66.0, BES	48.1
LO_{S7}	1.0	42.4	56.6, BEP	52.5
LO_{S8}	1.2	50.2	48.6, BEP	60.5
LO_{S9}	1.4	32.6	66.0, BEP	50.8
LO_{S10}	1.0	42.4	56.6, DBS	53.2
LO_{S11}	1.2	50.2	48.6, DBS	64.7
LO_{S12}	1.4	32.6	66.0, DBS	44.3
LO_{S13}	1.0	42.4	56.6, BEA	64.1
LO_{S14}	1.2	50.2	48.6, BEA	49.2
LO_{S15}	1.4	32.6	66.0, BEA	50.6

Table 8.2 Optimization of composition of PVC membrane sensor using LOM - MPA ion association

Sensor	Composition % (w/w)			Slope mV/decade
	Ion pair	PVC	Plasticizer	
LO_{M1}	1.0	42.4	56.6, DBP	49.1
LO_{M2}	1.2	50.2	48.6, DBP	52.7
LO_{M3}	1.4	32.6	66.0, DBP	47.6
LO_{M4}	1.0	42.4	56.6, BES	64.3
LO_{M5}	1.2	50.2	48.6, BES	45.1
LO_{M6}	1.4	32.6	66.0, BES	52.4
LO_{M7}	1.0	42.4	56.6, BEP	52.3
LO_{M8}	***1.2***	***50.2***	***48.6, BEP***	***54.9***
LO_{M9}	1.4	32.6	66.0, BEP	62.7
LO_{M10}	1.0	42.4	56.6, DBS	42.7
LO_{M11}	1.2	50.2	48.6, DBS	61.4
LO_{M12}	1.4	32.6	66.0, DBS	67.1
LO_{M13}	1.0	42.4	56.6, BEA	35.5
LO_{M14}	1.2	50.2	48.6, BEA	50.9
LO_{M15}	1.4	32.6	66.0, BEA	48.5

Table 8.3 Response characteristics of the developed sensors LO_{S3} and LO_{M8}

Parameter	PVC membrane senor LO_{S3}	PVC membrane senor LO_{M8}
Slope (mV per decade)	58.2	54.9
Linear range (M)	1.0×10^{-2} - 1.0×10^{-6}	1.0×10^{-2} - 5.0×10^{-5}
pH range	6 - 8	7 - 9
Detection limit (M)	2.69×10^{-6}	5.23×10^{-5}
Response time (s)	< 20	< 30
Shelf life	3 weeks	4 weeks

Table 8.4 Selectivity coefficient values of various interfering species, K^{pot}

Interfering Species	$K^{pot}_{A,B}$	
	PVC membrane senor LO_{S3}	PVC membrane senor LO_{M8}
NH_4^+	2.6×10^{-2}	3.1×10^{-2}
K^+	1.5×10^{-3}	2.8×10^{-3}
Na^+	7.3×10^{-2}	5.4×10^{-2}
Mg^{2+}	4.3×10^{-3}	4.1×10^{-2}
Co^{2+}	6.4×10^{-2}	5.7×10^{-2}
Ca^{2+}	3.5×10^{-3}	4.9×10^{-3}
Ni^{2+}	8.6×10^{-3}	7.3×10^{-3}
Zn^{2+}	6.7×10^{-3}	8.4×10^{-3}
Ascorbic acid	5.6×10^{-2}	4.8×10^{-2}
Starch	6.1×10^{-2}	4.5×10^{-2}
Talc	3.9×10^{-2}	4.8×10^{-2}
Glycine	3.8×10^{-3}	5.3×10^{-3}
Lactose	2.9×10^{-2}	4.1×10^{-2}

Table 8.5 Determination of LOM in pharmaceutical formulations

Sample	Declared Amt (mg/tablet)	Method adopted	Found * (mg/tablet)	SD	CV
Lomedon (Indon, India)	400	LO_{S3}	398	0.96	0.24
		LO_{M8}	397	0.85	0.21
		Standard Method	397	0.91	0.23
Lomegen (Genix, India)	400	LO_{S3}	397	0.94	0.24
		LO_{M8}	397	0.93	0.23
		Standard Method	398	0.97	0.24

* Average of six replicates.

Table 8.6 Determination of LOM in urine sample using the developed sensors

Drug taken (M)	Sensor	Drug found* (M)	Recovery %
2.00×10^{-3}	LO_{S3}	1.98×10^{-3}	99.0
	LO_{M8}	2.04×10^{-3}	102.0

* Average of six replicates.

Figure 8.1 Structure of Lomefloxacin

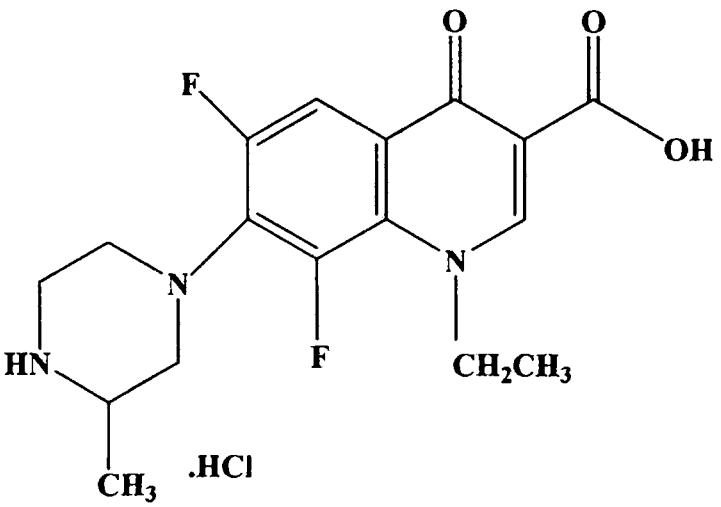


Figure 8.2 Calibration graph for LOM selective PVC membrane sensor based on LOM - STA ion association (LO_{S3})

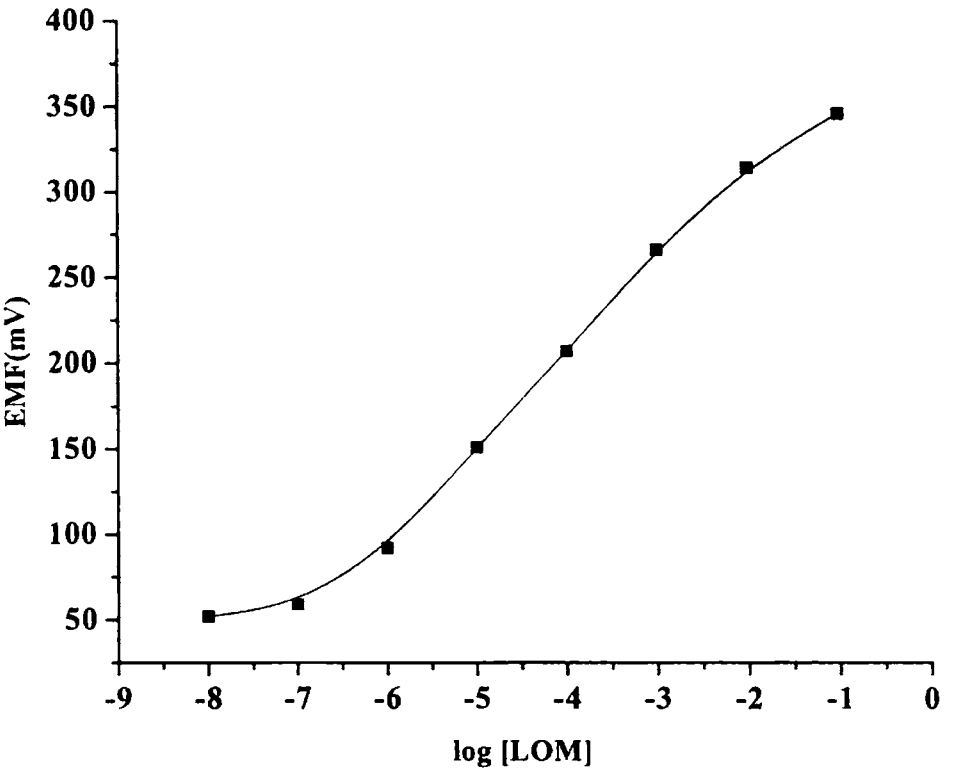


Figure 8.3 Calibration graph for LOM selective PVC membrane sensor based on LOM - MPA ion association (LO_{M8})

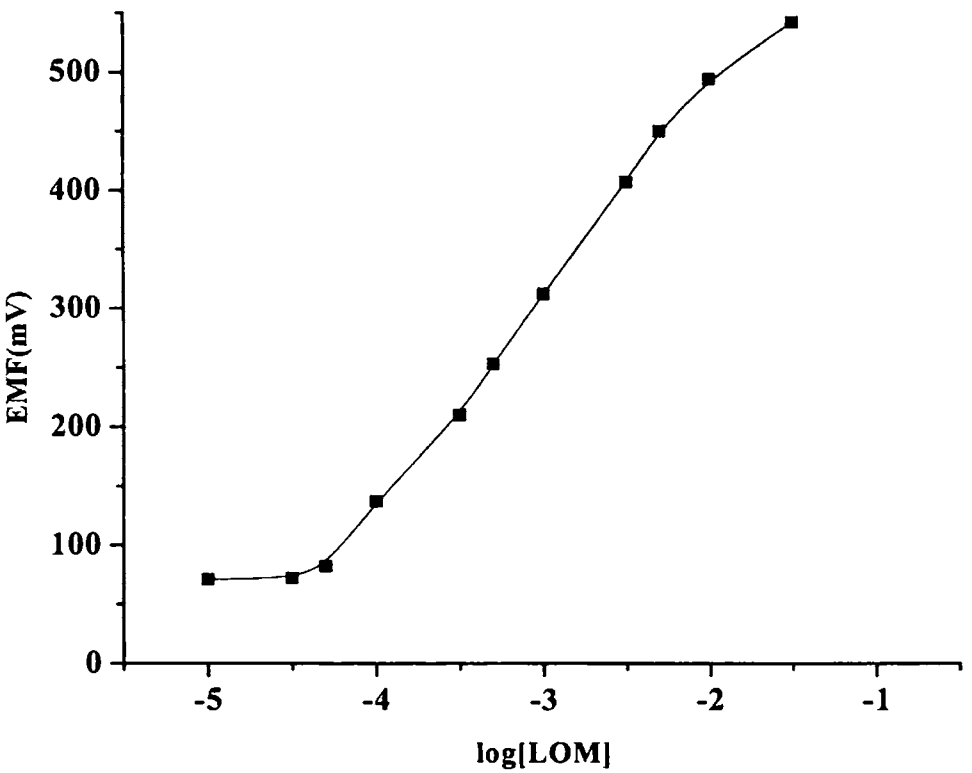


Figure 8.4 SEM image of the polymeric membrane of $\mathbf{LO_{S3}}$ sensor

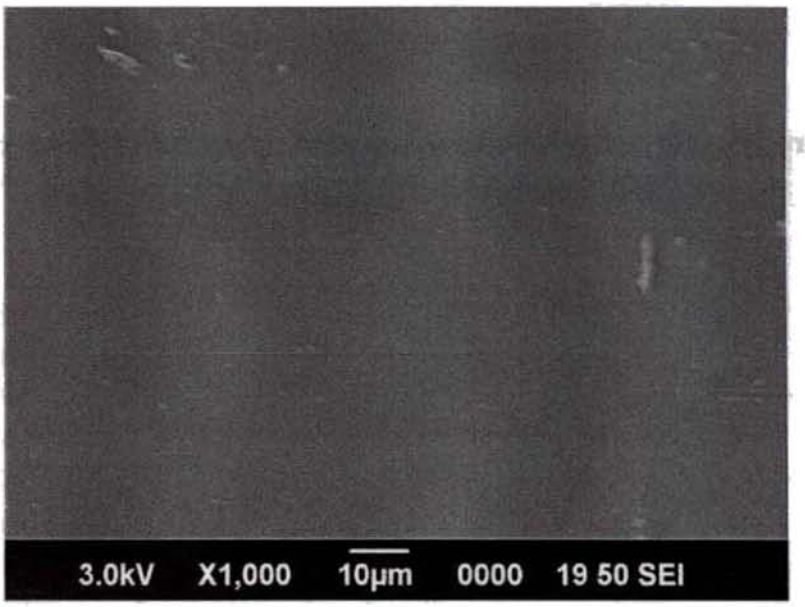


Figure 8.5 SEM image of the polymeric membrane of $\mathbf{LO_{M8}}$ sensor

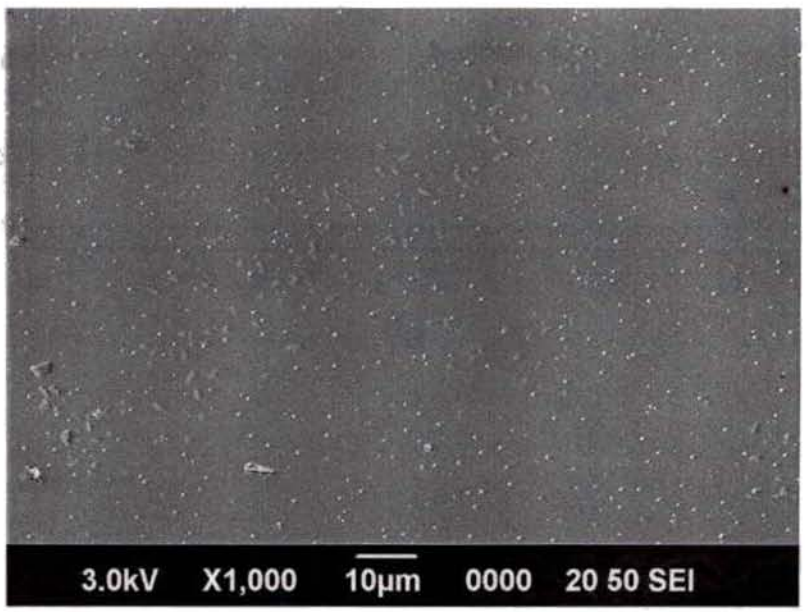


Figure 8.6 Effect of pH on the cell potential of the LOM selective PVC membrane sensor $\mathbf{LO_{S3}}$ at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)

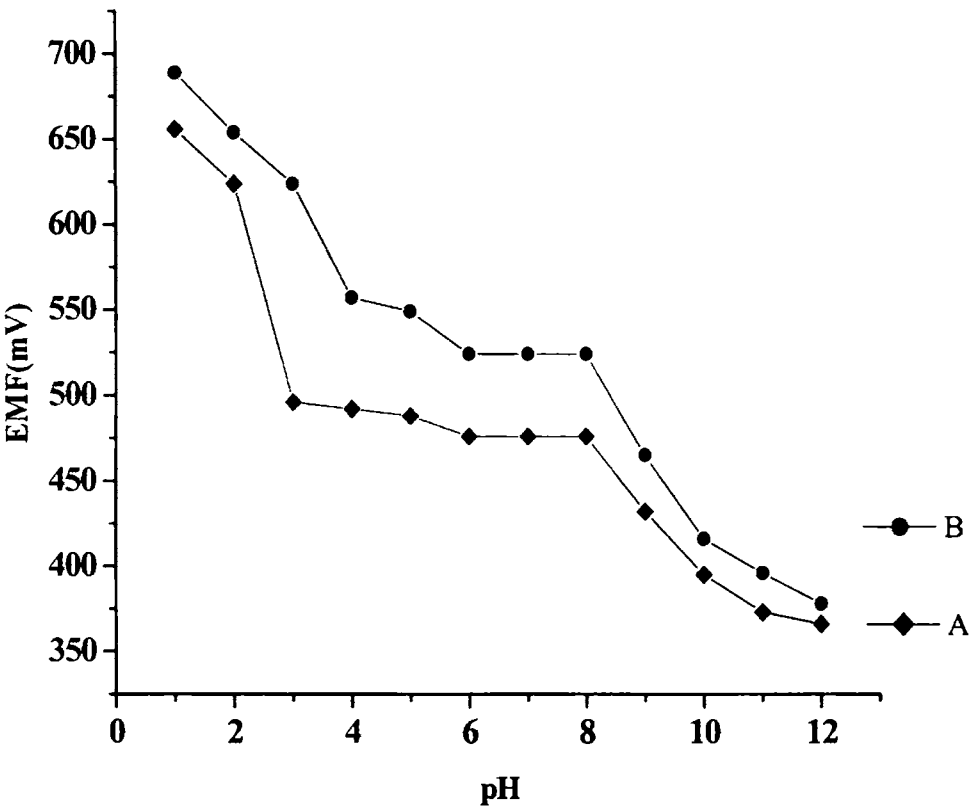
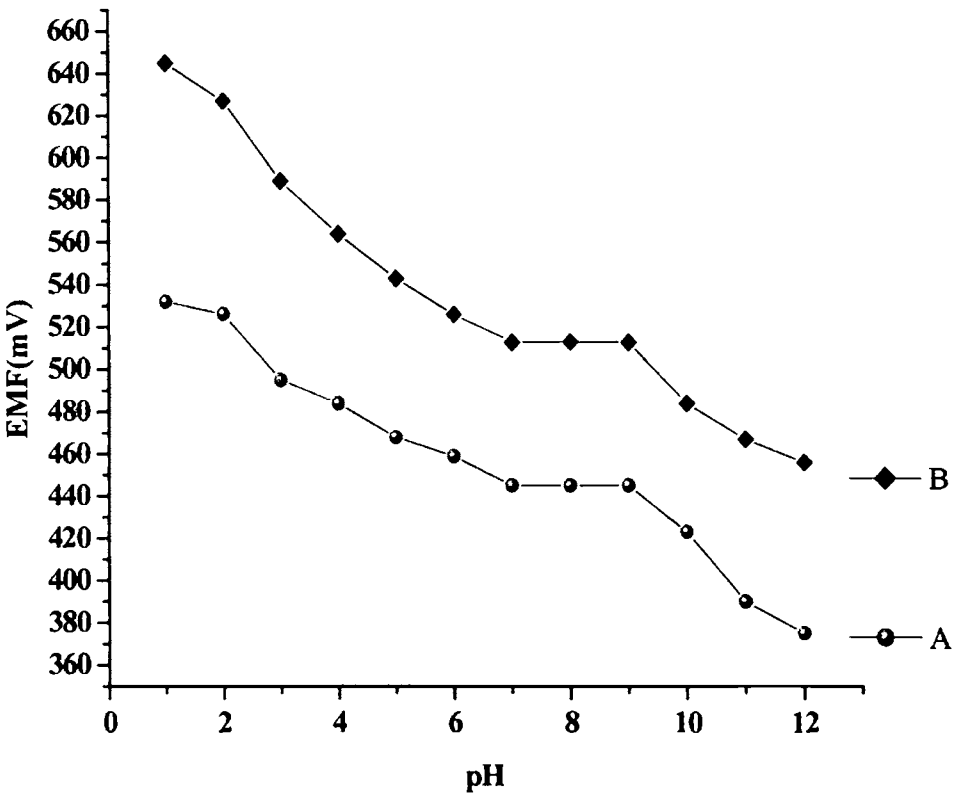


Figure 8.7 Effect of pH on the cell potential of the LOM selective PVC membrane sensor $\mathbf{LO_{M8}}$ at 1.0×10^{-4} M (A) and 1.0×10^{-3} M (B)



Chapter 9

CONCLUSIONS

A brief summary of the important findings and results of the work is discussed in detail in this chapter. Also an outline of the different stages involved in the work is given in brief in the chapter.

The work mainly focused on the fabrication of potentiometric ion selective sensors for six drugs. These drugs include Trimethoprim, Ketoconazole, Lamivudine, Domperidone, Nimesulide and Lomefloxacin. As part of the study, a total of sixteen different sensors have been developed, which includes polymeric membrane sensors and carbon paste sensors. In each of the two types of sensors, the main component was the electroactive substance or the ionophore. The sixteen sensors fabricated can be listed as follows:

Trimethoprim - 2 carbon paste sensors based on the ion associations of the drug with MPA and PTA

Ketoconazole - 1 PVC membrane sensor and 1 carbon paste sensor based on the ion association of the drug with MPA

Lamivudine - 2 PVC membrane sensors and 2 carbon paste sensors based on the ion associations of the drug with MPA and PTA

Domperidone - 1 PVC membrane sensor and 1 carbon paste sensor based on the ion association of the drug with PTA

Nimesulide - 2 PVC membrane sensors and 2 carbon paste sensors based on the ion associations of the drug with MPA and STA

Lomefloxacin - 2 PVC membrane sensors based on the ion associations of the drug with STA and MPA

The main stages involved in the work are:

(1) Synthesis of the ion association complexes

(2) Characterization of the ion association complexes using elemental analysis

(3) SEM analysis of the membranes formed in the case of PVC membrane sensors

(4) Fabrication of different types of sensors

(5) Optimization of the sensor matrix composition

(6) Study of the response parameters of the developed sensors such as slope, linear range, detection limit, effect of concentration of the internal filling solution, effect of pH, shelf life, response time, and selectivity.

(7) Analytical applications of the developed sensors

The utility of any ion selective sensor is based upon the ability to fabricate the ion selective membrane in such a way that it is able to retain its properties over a long period of time. Attempts are being made to discover novel materials which can replace the PVC based matrix inorder to fabricate

sensors which exhibit superior properties in terms of lower ionophore leakage, less plasticizer requirements and better overall selectivities and lifetime. Very often fabrication technologies can determine whether a given ionophore has desirable quality to be widely employed for their intended applications. The development and application of ion selective electrodes (ISEs) continue to be exciting and expanding areas of analytical research. Clearly, the ability to make direct or indirect measurements in complex samples without concern about sample colour or turbidity and the fact that such measurements require relatively inexpensive equipment make ISE based techniques attractive to scientists in many disciplines.

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