Fabrication of Electrochemical Sensors for the Determination of Pharmaceuticals

Thesis Submitted to
Cochin University of Science and Technology
in partial fulfillment of the requirements
for the award of the degree of

Doctor of Philosophy
in
Chemistry

by

SINDHU ISSAC

Department of Applied Chemistry
Cochin University of Science and Technology
Kochi – 22

February **2011**

Department of Applied Chemistry
Cochin University of Science and Technology
Kochi – 22

Dr. K. Girish Kumar
Professor of Analytical Chemistry

Tel: 0484-2575804
E-mail: chem@cusat.ac.in

Date: 24th February 2011

Certificate

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

K. Girish Kumar
(Supervising Guide)

Declaration

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

Kochi -22 **Sindhu Issac**
24th February 2011

Acknowledgement

This thesis would not have been successful but for the sincere help of others. As such, it is only appropriate that I express my sincere gratitude to them at this juncture.

I would like to express my deep and sincere gratitude to my guide Dr. K. Girish Kumar, Professor of Analytical Chemistry, Department of Applied Chemistry, Cochin University of Science and Technology for his expertise, kindness, valuable guidance and above all for his patience and optimism which helped a lot in instilling confidence in me. I sincerely thank him for continuously orienting me in the correct research direction and guiding me to write this thesis through his constant encouragement and advice. He was very affectionate and was ready to help me even in the midst of his busy schedule and without his guidance this thesis would have been a distant possibility.

I would like to thank Dr. K. Sreekumar, Head of the Department, for providing me with the necessary facilities in the Department. My gratitude goes to all teachers of the department, who with their profound knowledge have enlightened me during my stint here as a research scholar. I owe immensely to all my teachers right from my schooling days to the post graduate level for having constantly kept my yearning for knowledge burning. I gratefully remember all the non-teaching staff of the department for the assistance and backup they offered me.

My sincere thanks to Dr. G. Devala Rao, Principal, KVSR College of Pharmacy, Vijayawada for providing me with pure sample of the drugs.

I am grateful to Dr. Anita I. of Maharaja's College, Ernakulam for her support and help.

My labmates have always been a source of support to me. I thank my senior lab mates Dr. Rema, Dr. Pearl, Dr. Sareena and Beena Chechi for their guidance, support and affection. I would also like to thank Litha, Theresa, Renjini, Leena, Sobhana Miss, Laina, Divya, Soumya, Jesni Chechi, Ajith, Rajitha and Zafna for their help and companionship. The fond memories of our times together will always remain with me.

I am extremely thankful to Theresa, Litha, Renjini, Laina and Divya for the concern and affection they bestowed on me. Their cheerful and refreshing company added colour to my research life. I remember that I had full freedom to approach them whenever I needed their help. Also, special thanks to Renjini and Laina for being my side during the days of my thesis work.

My sincere thanks to all my friends of the Polymer, Organic, inorganic and Physical lab. I thank Kannan Chettan, Mahesh Chettan, Anoop, Mangala, Navya, Manju, Nancy Miss and Jisha for their wonderful friendship.

Let me also take this opportunity to thank my family for their incessant love and co-operation. My Pappa, Mummy, Chechi, Chettai and my dearest Chinnu were always with me with a lot of patience and prayerful support. My appachan and amma gave their whole hearted support and encouragement during this period.

The immense moral support I received from my husband Renju helped me realize my dream. I am indebted to him for the understanding and love shown. He has been always ready with practical suggestions to help me.

I owe my thanks to the Council of Scientific and Industrial Research(CSIR) for the fellowship.I extend my thanks to Directorate of Extramural Research and Property Rights, DRDO, New Delhi for the financial assistance in the form of project.

I extend my thanks to the scientists of the Sophisticated Test and Instrumentation Centre, Kochi for the analysis.

I bow my head in humility before my Saviour and Lord for giving me the knowledge to complete the work and guiding me all through my life.

Sindhu Issac

PREFACE

Electrochemistry has many advantages making it an appealing choice for pharmaceutical analysis. Electrochemistry has always provided analytical techniques characterized by instrumental simplicity, moderate cost and portability. Electroanalytical techniques have introduced the most promising methods for specific applications. Due to similarity in the electrochemical and biological reactions; it can be assumed that the oxidation/reduction mechanisms taking place at the electrode and in the body share similar principles. Biologically important molecules can be investigated electroanalytically by voltammetry in order to determine the molecule in different ways. Additional applications of electrochemistry include the determination of electrode mechanisms. Redox properties of drugs can give insights into their metabolic fate in vivo redox processes or pharmacological activity. Further, the electroanalytical techniques have been shown to be excellent for the determination of pharmaceutical compounds in different matrices. Many of the active constituents of formulations, in contrast to excipients, can be readily oxidized / reduced. The selectivity of this method is normally excellent because the analyte can be readily identified by its voltammetric peak potential. The advance in experimental electrochemical techniques in the field of analysis of drugs is because of their simplicity, low cost and relatively short analysis time as compared to other techniques.

Voltammetric sensors are an important class of electrochemical sensors in which the analytical information is obtained from the measurement of current obtained as a result of electrochemical oxidation/reduction. This current is proportional to the concentration of the

analyte. Chemically modified electrodes (CMEs) have great significance as important analytical tools for the electrochemical determination of pharmaceuticals. The modification of electrode results in efficient determination of electro-active biomolecules at very lower potential without its major interferences. The operation mechanism of CMEs depends on the properties of the modifier materials that are used to promote selectivity and sensitivity towards the target analytes. Modified electrodes can be prepared by deposition of various compounds such as organic compounds, conducting polymers, metal oxides, etc. on the various electrode surfaces.

The thesis presents the development, electrochemical characterization and analytical application studies of eight voltammetric sensors developed for six drugs viz., Ambroxol, Sulfamethoxazole, PAM Chloride, Lamivudine, Metronidazole Benzoate and Nimesulide. The modification techniques adopted as part of the present work include Multiwalled Carbon Nanotube (MWCNT) based modification, Electropolymerisation and Gold Nanoparticle(AuNP) based modifications.

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 voltammetric sensors developed for different drugs.

Chapter 2 gives a brief sketch of the materials and methods used in the investigation. This chapter explains the procedure for the

fabrication of chemically modified electrodes as voltammetric sensors for the determination of various drugs. 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 multiwalled carbon nanotube- nafion based sensor for the quantitative determination of Ambroxol (AMB). The analytical applications of the developed sensor in the determination of the drug in pharmaceutical formulations and real sample like urine were also investigated.

Chapter 4 deals with the development of multiwalled carbon nanotube-nafion based sensor for the determination of the drug Sulfamethoxazole (SMX). The electrochemical response characteristics are described in detail and the application study of the developed sensor 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 poly (p-toluene sulphonic acid) based sensor for the determination of the drug PAM Chloride. The response parameters of the newly developed sensor as well as its analytical applications have been discussed in this chapter.

Chapter 6 presents the fabrication of poly (L-Cysteine) modified glassy carbon sensor for the drug Lamivudine (LAM). The analytical applications of the developed sensor in the determination of

pharmaceutical formulations and real samples have also been discussed in this chapter.

Chapter 7 deals with the development of two sensors for the drug Metronidazole benzoate (MBZ). The sensors fabricated include (i) poly (p-amino benzene sulphonic acid) modified glassy carbon sensor and (ii) AuNP/ poly (p-amino benzene sulphonic acid) modified glassy carbon sensor. Optimization studies of the developed sensors, response characteristics and analytical applications are dealt with in detail in this chapter.

Chapter 8 discusses the development and performance characteristics of two sensors for the drug Nimesulide (NIM) viz, (i) poly (Cystamine) modified glassy carbon sensor and (ii)AuNP/ poly (Cystamine) modified glassy carbon sensor. 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 2

MATERIALS AND METHODS ..... 49 - 60

Chapter 7

SENSORS FOR THE DETERMINATION OF METRONIDAZOLE BENZOATE ..... 137 - 166

Chapter 8

SENSORS FOR THE DETERMINATION OF NIMESULIDE 167- 194

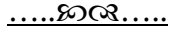
…..ꕥ…..

LIST OF TABLES

Page No

LIST OF FIGURES

Page No



Chapter 1

INTRODUCTION

CONTENTS

Analytical chemistry is the study of separation, identification and quantification of the chemical components of natural and artificial materials[1]. Qualitative analysis gives an indication of the identity of the chemical species in the sample and quantitative analysis determines the amount of one or more of these components. The separation of components is often performed prior to analysis. Thus, analytical chemistry is the science of obtaining, processing and communicating information about the composition and structure of matter. In other words, it is the art and science of determining what matter is and how much of it exists.

Analytical methods can be classified into classical and instrumental[2]. Classical methods (also known as wet chemistry methods) involve separations such as precipitation, extraction, distillation and qualitative analysis (colour, odour or melting point). Quantitative analysis is achieved by measurement of

weight (gravimetry) or volume (titrimetry). Instrumental methods use an apparatus to measure physical properties of the analyte such as light absorption, fluorescence or conductivity. The separation of materials is accomplished using chromatography or electrophoresis methods.

The first instrumental analysis was flame emissive spectrometry developed by Robert Bunsen and Gustav Kirchhoff who discovered Rubidium (Rb) and Cesium (Cs) in 1860 [3]. Most of the major developments in analytical chemistry took place after 1900. During this period, instrumental analysis became progressively dominant in this field. In particular many of the basic spectroscopic and spectrometric techniques were discovered in the early 20^{th} century and refined later[4].

Analytical chemistry has been important since the early days of chemistry, providing methods for determining the elements and chemicals present in the world around us. During this period significant analytical contributions were made to Chemistry, which include the development of systematic elemental analysis by Justus von Liebig and systematized organic analysis based on the specific reactions of functional groups.

Analytical chemistry is also focused on improvements in experimental design, chemometrics and the creation of new measurement tools to provide better chemical information. Analytical chemistry has applications in forensics, bioanalysis, clinical analysis, environmental analysis and material analysis.

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 determine the structures of chemical compounds. Chemical analysis generally consists of a chain of procedures

to identify and/or quantify one or several components in a sample of matter. The need 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.

Electroanalytical chemistry is that branch of chemical analysis that employs electrochemical methods to obtain information related to the amounts, properties and environment of chemical species. Isaac Maurits Kolthoff defined electroanalytical chemistry as the application of electrochemistry to analytical chemistry. It is preferable to consider electroanalytical chemistry as that area of analytical chemistry and electrochemistry in which the electrode is used as a probe, to measure something that directly or indirectly involves the electrode[5].

1.1 Electroanalytical Techniques

Electroanalytical techniques 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. These techniques are capable of producing exceptionally low detection limits and wealth of characterization information describing electrochemical addressable systems[6,7]. 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:

(a) 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 generally classified into three types. They are

1. Potentiometry
2. Coulometry
3. Voltammetry/Amperometry

1.3 Potentiometry

Potentiometric methods of analysis are based on measuring the potential of electrochemical cell without drawing appreciable current. In potentiometry the measuring set up always consists of two electrodes: the measuring electrode, also known as the indicator electrode and the

reference electrode. When placed in a solution together they produce a certain potential [7]. The equilibrium potential of the indicator electrode is measured against a selected reference electrode under a high impedence voltmeter. By judicious choice of electrode material, the selectivity to one particular ion can be increased, in some cases with very minimal interferences in the measured potential from other ions. Such electrodes are known as ion selective electrodes [8]. Direct Potentiometry and Potentiometric Titrations are the two major types of analytical techniques in potentiometry. In direct potentiometry, the cell potential is determined and related to the activity or concentration of the individual chemical species. In potentiometric titration the variation in cell potential is monitored as a function of the volume of reagent added[9].

1.4 Coulometry

Coulometry is an electroanalytical method which involves the measurement of the quantity of electricity (in coulombs) needed to convert the analyte quantitatively to a different oxidation state. Like gravimetric methods, coulometry has the advantage that the proportionality constant between the measured quantity (charge in coulombs) and the mass of analyte can be computed from known physical constants: thus, calibration or standardization is not usually necessary. Coulometric methods are often as accurate as gravimetric or volumetric procedures and they are usually faster and more convenient than gravimetric methods [7].

Coulometric analysis is an application of Faraday's first law of electrolysis- the extent of chemical reaction at an electrode is directly proportional to the quantity of electricity passing through the electrode. The

weight of substance produced or consumed in an electrolysis involving Q coulombs is given by the equation.

$$w = MQ/96487\ n$$

where M is the molecular mass of the substance liberated or consumed, n is the number of electrons involved in the reaction.

The fundamental requirement of a coulometric analysis is that the electrode reaction used for the determination should proceed with 100% efficiency, so that the quantity of substance reacted can be expressed by means of Faraday's law [10].

Two general techniques are used for coulometric analysis: controlled-potential (potentiostatic) coulometry and controlled-current (amperostatic) coulometry. In controlled-potential coulometry, the potential of working electrode is maintained at a constant level such that quantitative oxidation or reduction of the analyte occurs without involvement of less reactive species in the sample or solvent. In this method, the current is initially high but decreases rapidly and approaches zero as the analyte is removed from the solution.

Controlled-current coulometry uses a constant current, which passes through a cell until an indicator signals the completion of the analytical reaction. The quantity of charge required to reach the end point is calculated from the magnitude of the current and from the time that the current passes. This method is frequently referred to as coulometric titration.

1.5 Voltammetry/Amperometry

Voltammetry is an electroanalytical technique in which the current-potential behaviour at an electrode surface is measured. The potential is varied in some systematic manner to cause oxidation or reduction of the

electroactive chemical species at the electrode. The resultant current is proportional to the concentration of the electrochemical species.

A three-electrode system is used, which includes a working electrode, at which the oxidation or reduction process of interest occurs, a reference electrode, such as the SCE (Saturated Calomel Reference Electrode) or silver-silver chloride electrode and an auxiliary or counter electrode, which carries the bulk of the current (instead of reference electrode). The three electrodes are connected to the power source, which is a specially designed circuit for precise control of the potential applied to the working electrode and often called a potentiostat [11].

The voltammetric technique is based on the measurement of the current flowing through the working electrode dipped in a solution containing electro-active compounds, while a potential scanning is imposed on it. The working electrode could be made with several materials. Usually, it has a very small surface in order to quickly and accurately assume the potential imposed by the electrical circuit. The electrode can be solid (gold, platinum or glassy carbon) or formed by a drop of mercury hanging from a tip of a capillary[12].

The voltammetric techniques are based on Faraday's Law and Fick's Laws. In voltammetry the current corresponding to the quantity of material transported by diffusion and reacting at the electrode surface is usually measured. This current is proportional to the concentration of the electroactive component present in the test solution [11].

Amperometry is a voltammetric technique, which is based on the measurement of current at a fixed operating potential. If this potential is conveniently chosen then the magnitude of current is directly proportional

to concentration. This current is the resultant of electrochemical oxidation or reduction of the electroactive compound. Additionally, if steady state convection is employed, as in flowing streams and the concentration of electroactive species is uniform, then a constant current is measured [8,9].

1.6 Advantages of Electroanalytical techniques [13]

- Electroanalytical methods are well established and use relatively inexpensive equipment to produce unique characterization information for molecules and chemical systems: qualitative and quantitative analytical data, thermodynamic data and kinetic data.

- Electroanalytical methods are sensitive; they are able to detect submicro molar concentrations and subpicomole amounts of electroactive material.

- Electroanalytical methods are selective; they are able to control the potential of an electrode, which makes it possible to determine the electrochemical "spectrum" of electroactive species in solution, analogous to probing the energy states of a molecule with light via spectroscopy.

1.7 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 instrument. Sensors are designed to detect and respond to an analyte in the gaseous, liquid or solid state [14]. 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 etc and do not have a chemical interface. Chemical sensors rely on 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 and quantitative determination of the analyte [15]. A useful definition for a chemical sensor is a small device that as a 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.

1.8 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

1.8.1 Electrochemical Sensors

These include potentiometric sensors(ion selective electrodes, ion selective field effect transistors) and voltammetric/amperometric sensors including solid electrolyte gas sensors. [16].

1.8.2 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.

1.8.3 Mass Sensitive Sensors

These make use of the piezoelectric effect, 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 function of the amount of material absorbed on the surface.

1.8.4 Heat Sensitive Sensors

The heat of a chemical reaction involving the analyte is monitored with transducers such as 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 [17].

1.9 Voltammetric Sensors

Voltammetric sensor is an electrochemical sensor which functions by the measurement of the current response as a function of applied potential; in other words, it depends on the registering of current - potential profiles.

For this purpose, it is necessary that the species of interest is electroactive at the electrode material at a reasonable value of potential where neither solvent nor electrolyte decomposition occur. A special case of voltammetric sensor is amperometric sensor where a fixed potential is applied and the current is registered. The recording of current as a function of time (chronoamperometry) can give important and useful information.

Many species can undergo oxidation or reduction at a potential which is characteristic of the particular species. If the potential is fixed at the value appropriate to the reduction or oxidation of the species being determined, the amount of current which flows can be directly related to the concentration of the species.

1.9.1 Experimental set-up and instrumentation

The instrumentation for voltammetric sensors is more complex than that for potentiometric sensors. To control the applied potential and register the current at the working electrode a potentiostat is necessary- most voltammetric techniques are based on potential control.

The development of voltammetric techniques dates back to the pioneering work of Jaroslav Heyrovsky in 1922 which was acknowledged by the Nobel Prize in 1959.

The voltammetric cell incorporates the sample solution and a pair of electrodes of unequal size, as well as reference electrode. Three electrodes are usually necessary in order to avoid the passage of current through the reference electrode, which otherwise would alter its potential via changes in the activities of the various species. The electrical circuit, through which the current passes, is between the working (indicator) electrode and an auxiliary electrode. The reference electrode serves in a three - electrode

system to control the potential of the working electrode and hence the reactions which can occur there.

The working electrode is an ideally polarizable electrode, ie the electrode shows a large change in potential when an infinitesimally small current passes through. Reference electrode is a nonpolarizable electrodes i.e. an electrode of fixed potential. The auxiliary or counter-electrode is a current conducting electrode. The voltammetric measurements are usually performed in a quiescent solution in presence of a large excess of inert salt, called supporting electrolyte.

Control and data acquisition of the response can be conveniently done by computer through an adequate interface in a digitally based potentiostat. Analogue potentiostats and galvanostats are not widely available now and many modern voltammetric procedures are based on step functions which lends them directly to computer control. The digital waveform can be converted into an analogue waveform by a digital to analogue converter and the response redigitialised through an analogue to digital converter, if necessary.

1.9.2 Working electrode

The working electrode, is the electrode in an electrochemical system on which the reaction of interest is occurring. Depending on whether the reaction on the electrode is a reduction or an oxidation, the working electrode can be referred to as either cathodic or anodic. Common working electrodes consist of inert metals such as gold, silver or platinum to inert carbon such as glassy carbon or pyrolytic carbon and mercury drop and film electrodes.

1.9.2.1 Mercury Electrode

It is a liquid electrode and can be used in dropping, streaming or pool configurations that are impossible with solid electrodes. The dropping or streaming mercury electrodes have the special advantage of providing a continuously renewed surface, which helps to minimize the effects from adsorption of solution impurities or fouling of the electrode surface by films produced in the electrode reaction. In addition the surface is smooth and continuous and does not require the pretreatment and polishing that is common with solid electrodes. The advantages of the liquid surface and a large overpotential for hydrogen evolution make mercury the material of choice for cathodic processes. Mercury is readily oxidized, particularly in the presence of anions that precipitate or complex mercury(I) or mercury (II) ions, such as the halides, cyanide, thiosulfate, hydroxide or thiocyanate. For this reason, mercury is seldom used to study anodic processes.

1.9.2.2 Solid Inert Electrodes

Platinum and gold are the most commonly used metallic solid electrodes. These metals are obtained in high purity, machined easily and fabricated readily into a variety of geometric configurations - wires, rods, flat sheets and woven gauzes. Platinum has extremely small overpotentials for hydrogen evolution, which is the basis for its use in the construction of reversible hygrogen electrodes. Gold has a significantly larger overpotential, but it is much smaller than that for mercury. Platinum also adsorbs hydrogen readily and the amount of adsorbed hydrogen can be used to estimate the true surface area of the platinum. Gold does not appreciably adsorb hydrogen and this factor together with its larger overpotential for hydrogen evolution makes gold the metal of choice for the study of cathodic process.

1.9.2.3 Carbon electrodes

As an inert electrode material, carbon is useful for both oxidation and reduction in both aqueous and nonaqueous solutions. Several different forms of carbon have been used to make satisfactory electrodes including spectroscopic-grade graphite (usually impregnated with ceresin or paraffin wax), pyrolytic graphite (a high density highly oriented form of graphite), carbon paste (spectroscopic-grade graphite mulled in sufficient Nujol to form a stiff paste), graphite dispersed in epoxy resin or silicone rubber and vitreous or glassy carbon.

Vitreous or Glassy Carbon: The use of glassy carbon as an electrode material was suggested in 1962. Glassy carbon is an electrically conductive and gas impermeable material, highly resistant to chemical attack and obtainable in pure state. Some of its cited advantages relative to platinum are (1) low cost, (2) pretreatment by polishing with metallographic paper, (3) larger overpotential for production of hydrogen and dissolved oxygen and (4) increased reversibility for several redox couples and reactions that involve subsequent proton transfer. Glassy carbon has a unique advantage in the determination of trace metals by stripping voltammetry.

1.9.3 Reference Electrode

A reference electrode is an electrode which has a stable and well known electrode potential. Its potential should not vary when an external potential is applied in the working electrode and must be insensitive to the composition of the analyte solution. The electrodes commonly used as reference electrodes are standard hydrogen electrode (SHE), silver- silver chloride electrode, calomel electrode etc.

1.9.4 Auxiliary Electrode

In voltammetric studies the current flows between the working electrode and auxiliary electrode. If a two electrode system consisting of only reference and working electrodes is used, then current flow through the reference electrode will cause a change in its potential. So a three electrode system, incorporating a third electrode called the auxiliary electrode is used. The main condition for an electrode to act as auxiliary electrode is that it should not dissolve in the medium of the electrochemical cell and that the reaction product at the auxiliary electrode should not react at the working electrode. The electrode area of the auxiliary electrode must also be larger than that of working electrode to ensure that the area of the electrode does not control the limiting current. Platinum electrodes in the form of coils or thin foils are the most widely used auxiliary electrodes in aqueous, non aqueous and molten salt media. Carbon electrodes are also used in molten salt media.

1.9.5 Chemically Modified Electrodes (CMEs)

An active area of research in electrochemistry is the development of electrodes produced by chemical modification of various conductive substrates. Such electrodes have been tailored to accomplish a broad range of functions. Modifications include applying irreversibly adsorbing substances with desired functionalities, covalent bonding of components to the surface and coating the working electrode with polymer films or films of other substances. One of the most important properties of CMEs is their ability to catalyze the oxidation or reduction of solute species that exhibits high over voltages at unmodified surfaces. Thus CMEs play an important role in reducing the high overvoltage required for the voltammetric determination of analyte without its major interferences.

Modified electrodes have many applications. A primary interest has been in the area of electrocatalysis. Another application is in the production of electrochromic devices that change colour on oxidation and reduction. Finally the most important analytical use for such electrodes is as analytical sensors selective for particular species or functional groups.

Various substrates such as Carbon Nanotubes (CNT), Gold nanoparticles (AuNPs), Polymer films, Metalloporphyrins, Calixarenes etc are used for the modification of electrode surface. For the present study, the modification techniques adopted are CNT based modification, AuNPs based modification and electropolymerisation.

1.9.5.1 Modification based on Carbon Nanotubes

Carbon nanotubes (CNTs), discovered by Iijima [18], are an interesting class of nanomaterials offering high electrical conductivity, high surface area, significant mechanical strength and good chemical stability [19-25]. CNTs constitute a new structure of graphitic carbon consisting of one or several concentric tubules each with a helically wound hexagonal honeycomb lattice and can be divided into multi-wall carbon nanotubes (MWCNT) and single wall carbon nanotubes (SWCNT) according to the carbon atom layers in the wall of the nanotubes. CNTs have an aspect ratio ranging from 100 to 1000.

Carbon nanotubes have been known to promote electron transfer reactions when used as electrode modifying material [26-31]. Since the first use of the CNT-based electrode by Britto et al. [32] for the detection of dopamine, research on the application of both MWCNT and SWCNT for electrode modification has expanded into many areas. Electrode modification with CNTs usually involves their direct immobilization on the

surfaces of carbon electrodes (mostly glassy carbon electrodes, GCEs) via drop-dry [33] or by abrasive immobilization (mostly on pyrolytic graphite electrodes) [34, 35] methods.

1.9.5.2 Electropolymerisation

Since the discovery of the organic conducting polymers more than 20 years ago, these materials are finding an increasing use in various branches of technology, such as metallization of dielectrics, primary and secondary batteries, antistatic coatings, electromagnetic shielding, electrochromic systems, etc[36]. Organic conjugated polymers (conducting polymers) [37-39] are mainly organic compounds that have an extended p-orbital system, through which electrons can move from one end of the polymer to the other. Common classes [40] of organic conductive polymers include poly(acetylene)s, poly (pyrrole)s, poly(thiophene)s, poly(terthiophene)s, poly(aniline)s, poly (fluorine)s, poly(3-alkylthiophene)s, polytetrathiafulvalenes, polynapthalenes, poly(p-phenylene sulfide), poly(para-phenylene vinylene)s etc.

One of the most striking properties of conducting polymers is their ability to catalyze some electrode reactions. A thin layer of a conducting polymer, deposited onto the surface of substrate electrode, is able to enhance the kinetics of electrode processes[41].

In an electrochemical polymerization, the monomer, dissolved in an appropriate solvent, is oxidized at the surface of an electrode by application of an anodic potential (oxidation). The choice of the solvent and electrolyte is of particular importance in electrochemistry since both solvent and electrolyte should be stable at the oxidation potential of the monomer and provide an ionically conducting medium [42].

Electropolymerization is generally achieved by potentiostatic (constant-potential), galvanostatic (constant current) and potentiodynamic (potential scanning *i.e.* cyclic voltammetry) methods. These techniques are easier to be described quantitatively and therefore have been commonly utilized to investigate the nucleation mechanism and the macroscopic growth. Potentiodynamic techniques such as cyclic voltammetry have been mainly used to obtain qualitative information about the redox processes involved in the early stages of the polymerization reaction and examine the electrochemical behavior of the polymer film after electrodeposition[43].

Polymer-modified electrodes (PMEs) prepared by electropolymerization have received extensive interest in the detection of analytes because of their selectivity, sensitivity and homogeneity in electrochemical deposition, strong adherence to electrode surface and chemical stability of the film [44-50]. Selectivity of PMEs as sensors can be attained by different mechanisms such as size exclusion [51], ion exchange [52], hydrophobic interaction [53] and electrostatic interaction [54,55]. Information concerning the hydrophobicity of coating films can help in better evaluation of the sensitivity of the examined sensor to the desired analytes [56].

1.9.5.3 Modification based on Gold nanoparticles

The design of new nanoscale materials has acquired great importance in recent years owing to their wide-ranging applications in various fields. Among these materials, metallic nanoparticles are of great interest due to their important properties and their numerous possible applications [57, 58]. The metal nanoparticles have size-dependent unique chemical, electrical and optical properties and are very promising for practical applications in diverse fields such as multifunctional reagent and biosensors [59–65]. The nanoparticles are very different from bulk materials and their

electronic, optical and catalytic properties originate from their quantum scale dimensions (<2 nm) [66]. The electrocatalytic activity of metal nanoparticles is strongly dependent on their composition, size, surface area and surface morphology [67]. Noble metal nanoparticles have been extensively utilized in recent years, owing to their extraordinary catalytic activities for both oxidation and reduction reactions. To obtain high surface area, metal nanoparticle catalysts are usually dispersed in an organic polymer such as nafion [68, 69], colloids [70], surfactants [71] and porous substrates, which enable metal particles to be highly dispersed and stable.

In recent years, gold nanoparticles (AuNPs) have been extensively studied in electrochemistry because of their special physico-chemical characteristics [72–78]. The use of AuNPs superstructures for the creation of electrochemical devices is an extremely promising prospect[79]. Various methodologies have been used for the tailoring of AuNPs on electrode surfaces, which include the anchoring by electrostatic interaction, covalent linkage, electrochemical deposition etc.[80–83]. Electrostatic assembly utilizes the negatively charged citrate surface of the particles, while covalent attachment relies on the reaction of the Au surface with thiols or disulfides to form Au^0-S bonds [79]. The AuNPs can be immobilized as an organized mono - or multilayer on solid support with the help of short/long chain molecules having suitable functional groups like -NH_2, -SH at both ends [84]. The fabrication of sensors based on self-assembly AuNPs nanostructure is of recent technological interest [85–88]. Arrays of AuNPs have been utilized for electrochemical sensors as they exhibit excellent catalytic activity towards various reactions [89]. In these, the AuNPs function as ‘‘electron antennae’’, efficiently tunneling electrons between the electrode and electrolyte [80, 90].

The catalytic activity of gold nanoparticles is known to depend highly on their dispersity and surface properties. As usual, for many catalytic processes, a high degree of dispersity and large surface area are desirable. Conducting polymers are often considered to be useful matrices for the immobilization of the dispersed nano metal catalysts. Porous structure and high surface area of many conducting polymers favours their use as supporting material for the development of new catalytic and electrocatalytic materials. Because of a relatively high electric conductivity of some polymers, it is possible to shuttle the electrons through polymer chains between the electrode and dispersed metal nano particles, where the electrocatalytic reaction occurs. Thus, an efficient electrocatalysis can be achieved at these composite materials and a great deal of attention is paid to the use of conducting polymers as supporting matrices for the immobilization of catalytically active noble metal nano particles [41].

1.9.6 Electrode Process

A typical electrode reaction involves the transfer of charge between an electrode and a species in solution. The electrode reaction involves a series of steps:

- Reactant moves to the interface: this is termed as mass transport.
- Electron transfer can then occur via quantum mechanical tunneling between the electrode and reactant close to the electrode.
- The product moves away from the electrode to allow fresh reactant to the surface.

An electrode can donate or accept electrons only from species present in a layer of solution that is immediately adjacent to the electrode. For the description of any electrode process we have to consider the transport of

species to the electrode surface in addition to the electrode reaction. This transport can occur by any of the following processes.

- Diffusion – Transport of species due to concentration gradient.
- Convection – Transport due to mechanical motion of the solution as a result of stirring.
- Migration – Transport of ions due to the electrostatic attraction between the oppositely charged electrode and the ions.

In all the dynamic electroanalytical methods conditions are so created in solutions that the migration of the electroactive species can be neglected. This is achieved by the addition of a large excess of an inert electrolyte called the supporting electrolyte. If the solution is kept unstirred convection can also be eliminated, leaving the transport process taking place by diffusion alone. The current then achieved is called diffusion controlled limiting current.

1.9.7 Supporting Electrolyte

The supporting electrolyte is an inert soluble ionic salt added to the solvent, generally in 10-fold or 100-fold excess over the concentration of the species being studied. The inertness meant here is the ability to avoid oxidation or reduction at the indicator electrode during the course of electrochemical measurements.

There are three functions of the supporting electrolyte. First, it carries most of the ionic current of the cell since its concentration is much larger than that of the other species in solution. Thus it serves to complete the circuit of the electrochemical cell and keep the cell resistance at a low value. This is necessary only for non equilibrium electroanalytical measurements, in which the current that flows is not negligible. Second, it

maintains a constant ionic strength. This is necessary because the structure of the interphase region should not change significantly if a reaction occurs there. A stable structure is created on the electrolyte side by adding a high concentration of an inert salt. The maintenance of a constant or reproducible structure of the interphase region is necessary for both equilibrium and non equilibrium measurements alike, since the structure of the interphase region affects all of the electrical properties measurable by an external electrical circuit. Third, it suppresses the effect of the migration current. Migration current is the current that arises as a result of the movement of ions caused by an electric field; here the electric field is produced by the potential difference between the indicator and counter electrodes of the cell. This motion of charge can be detected as a current, the migration current. The net migration current observed is reduced by the presence of a large excess of ions that are not electrochemically active at the potentials in use, because they can carry an ionic current without permitting their conversion into electronic current at the electrodes.

KCl is the commonly used supporting electrolyte salt for two reasons. First and the most important, KCl is easily available in high purity form. Also the mobility of the potassium ion and that of the chloride ion is almost exactly equal. KNO_3 is an alternative when chloride cannot be used; the mobilities of potassium and nitrate ions are also similar. Tetra alkylammonium salts, buffer solutions etc are also used as supporting electrolytes.

1.9.8 Voltammetric Techniques

The most common voltammetric techniques are as follows:

(a) Polarography
(b) Linear Sweep Voltammetry (LSV)

(c) Cyclic Voltammetry (CV)

(d) Pulse Techniques

- Normal Pulse Voltammetry(NPV)
- Differential Pulse Voltammetry (DPV)
- Square Wave Voltammetry (SWV)

(e) Stripping Methods

- Anodic Stripping Voltammetry (ASV)
- Cathodic Stripping Voltammetry (CSV)

1.9.8.1 Polarography

Polarography is a voltammetric technique in which the working electrode is a Dropping Mercury Electrode (DME). The DME consists of a glass capillary through which mercury flows under gravity to form a succession of mercury drops. Each new drop provides a clean surface at which the redox process takes place, giving rise to an increase in current with increasing area as the drop grows and a decrease when the drop falls. In polarography the working electrode potential is varied in a linear manner from the initial to the final potential. The current versus potential response of a polarographic experiment has a sigmoid shape. The plateau of the sigmoid curve represents the limiting current and is related to the analyte concentration by the Ilkovic equation

$$I_d = 607\ n\ D^{1/2}\ m^{2/3}\ t^{1/6}\ C_0$$

where, D is the diffusion coefficient, m is the rate of flow of the Hg through the capillary, t is the drop time and C_0 is the bulk analyte concentration.

Even though polarography with the DME is the best technique for some analytical determinations, it has several limitations. Mercury is oxidized at potentials more positive than + 0.2V versus SCE, which makes it impossible to be used for any analyte in the positive range of potential. Another limitation is the residual current that results from charging of the electrode surface.

1.9.8.2 Linear Sweep Voltammetry (LSV)

LSV is the simplest voltammetric technique. At the working electrode, a rapid potential scanning that varies linearly (20-100 mV/s) is applied. Capacitive current increases when the velocity of scanning is increased and cannot be electronically compensated. Thus the performance of this technique is strongly restricted. Detection limits are in mg/l levels.

1.9.8.3 Cyclic Voltammetry(CV)

CV has become an important and widely used electroanalytical technique in many areas of chemistry. It is widely used for the study of redox processes, understanding reaction intermediates and obtaining stability of reaction products. This technique is based on varying the applied potential at a working electrode in both forward and reverse directions while monitoring the current.

The important parameters in a cyclic voltammogram are the peak potentials (E_{pc},E_{pa}) and peak currents (I_{pc}, I_{pa}) of the cathodic and anodic peaks respectively. If the electron transfer process is fast when compared to other processes (such as diffusion), the reaction is said to be electrochemically reversible and the peak separation is

$$\Delta E_p = E_{pa} - E_{pc} = 2.303\ RT / nF$$

Thus, for a reversible redox reaction at 25 °C with *n* electrons ΔE_p should be 0.0592/*n* V or about 60 mV for one electron. In practice this value is difficult to attain because of factors such as cell resistance. Irreversibility due to a slow electron transfer rate results in $\Delta Ep > 0.0592/n$ V, greater, say, than 70 mV for a one-electron reaction. The formal reduction potential (E_0) for a reversible couple is given by

$$E_o = (E_{pa} + E_{pc})/2$$

For a reversible reaction, the concentration is related to peak current by the Randles-Sevick expression (at 25^0C):

$$I_p = 2.69 \times 10^5 \, n^{3/2} \, A \, D^{1/2} \, C_0 \, \upsilon^{1/2}$$

where I_p is the peak current, A is the electrode area, D is the diffusion coefficient, C_0 is the concentration and υ is the scan rate.

1.9.8.4 Pulse Methods

Pulse voltammetric techniques, introduced by Barker and Jenkin, are aimed at lowering the detection limits of voltammetric measurements. These techniques permit convenient measurements upto 10^{-8}M concentration level. Because of their greatly improved performance, modern pulse techniques have largely replaced classical polarography in the analytical laboratory. The various pulse techniques are all based on a sampled current/ potential-step experiment. A sequence of such potential steps, each with duration of about 50 milli seconds, is applied onto the working electrode. After the potential is stepped, the charging current decays rapidly (exponentially) to a negligible value, while the faradaic current decays more slowly. Thus by sampling the current late in the pulse life, an effective discrimination against the charging current is achieved.

The difference between the various pulse voltammetric techniques is the excitation waveform and the current sampling regime.

a) Normal Pulse Voltammetry (NPV)

This technique uses a series of potential pulses of increasing amplitude. The current measurement is made near the end of each pulse, which allows time for the charging current to decay. It is usually carried out in an unstirred solution at either DME (called normal pulse polarography) or solid electrodes. The potential is pulsed from an initial potential *Ei*. The duration of the pulse, t, is usually 1 to 100 milli seconds and the interval between pulses typically 0.1 to 5 sec. The resulting voltammogram displays the sampled current on the vertical axis and the potential to which the pulse is stepped on the horizontal axis.

b) Differential Pulse Voltammetry (DPV)

This technique is comparable to normal pulse voltammetry in that the potential is scanned with a series of pulses. However, it differs from NPV because each potential pulse is fixed, of small amplitude (10 to 100 mV) and is superimposed on a slowly changing base potential. Current is measured at two points for each pulse, the first point just before the application of the pulse and the second at the end of the pulse. These sampling points are selected to allow for the decay of the nonfaradaic (charging) current. The difference between current measurements at these points for each pulse is determined and plotted against the base potential.

c) Square-Wave Voltammetry (SWV)

The excitation signal in SWV consists of a symmetrical square-wave pulse of amplitude E_{sw} superimposed on a staircase waveform, where

the forward pulse of the square wave coincides with the staircase step. The net current, i_{net}, is obtained by taking the difference between the forward and reverse currents ($i_{for} - i_{rev}$) and is centered on the redox potential. The peak height is directly proportional to the concentration of the electroactive species and direct detection limits as low as 10^{-8} *M* is possible.

Square-wave voltammetry has several advantages. Among these are its excellent sensitivity and the rejection of background currents and speed This speed, coupled with computer control and signal averaging, allows for experiments to be performed repetitively and increases the signal to noise ratio. Applications of square-wave voltammetry include the study of electrode kinetics, determination of some species at trace levels and its use with electrochemical detection in HPLC.

1.9.8.5 Stripping Voltammetry

Stripping voltammetry is a sensitive electroanalytical technique for the determination of trace amounts of metals in solution. The technique consists of three steps. First, metal ions are deposited onto an electrode which is held at a suitable potential. The solution is stirred during this step to maximize the amount of metal deposited. Second, stirring is stopped so that the solution will become steady. Third, the metal deposits are stripped from the electrode by scanning the potential. The observed current during the stripping step can be related to the amount of the metal in the solution.

The stripping step may consist of a positive or a negative potential scan, creating either an anodic or cathodic current respectively. Hence, Anodic Stripping Voltammetry (ASV) and Cathodic Stripping Voltammetry (CSV) are two specific stripping techniques.

1.10 Electroanalytical Techniques for Drugs

The drug analysis, an important branch of analytical chemistry, plays vital role in the quality control of drugs and has an extensive impact on public health. Therefore, the establishment of simple, fast, sensitive and accurate method for the determination of active ingredient is of great importance and interest. Electrochemical techniques are well suited for the determination of drugs in various samples such as pharmaceutical dosage forms including syrups, tablets, creams, suppositories and 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 procedures are 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 behavior of a given drug through mechanistic studies. Electrochemical techniques are 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 also the content of drugs in various media including biological liquids of the human and animal organisms, food products, waste waters of pharmaceutical plants, etc.

Voltammetric techniques are becoming increasingly important for therapeutic monitoring of drugs and metabolites in biological fluids. These

techniques are sensitive, reliable and simple and the redox reactions often provide selectivity for the compound sought in the presence of degradation products or metabolites. The present study is mainly concentrated on the voltammetric methods of drug analysis using chemically modified electrodes.

The potential range over which voltammetric techniques can be used depend on the solid electrode material, the solvent, the supporting electrolyte and pH of the studied solution. The selected working electrode should provide high signal-to-noise characteristics and reproducible responses. Thus, its selection depends on the redox behavior of the investigated compound and the background current over the potential region required for the measurement. In addition to these two main factors, the potential range, surface reproducibility, mechanical properties, cost, availability, electrical conductivity and toxicity are criteria for choosing the solid electrode material. Also the working electrode should possess the following requirements: electrochemical inertness over a broad interval of potentials; high overvoltage of hydrogen and oxygen evolution; low residual current and the possibility of a sufficiently simple regeneration of the surface. All these parameters and factors can be achieved by modifying the electrode surface with suitable substances so that high sensitivity, accuracy, reproducible results and low detection and quantification limits can be easily attained. The field of modified electrodes has become very popular with large number of applications in industry, quality control of drugs and food, determination in pharmaceutical dosage forms, environmental monitoring, etc.

1.11 A Brief Review on Voltammetric Sensors (based on CNTs, AuNPs and Polymer) 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. Voltammetric techniques have been shown to be excellent procedures for the sensitive determination of organic molecules, including drugs and related molecules in pharmaceutical dosage forms and biological fluids [91-101]. As a rule, in voltammetric analysis, many active compounds in dosage forms, in contrast to excipients, can be readily oxidized or reduced at the electrode surface on applying a potential. The advance in experimental voltammetric techniques in the field of analysis of drugs is due to their simplicity, low cost and relatively short analysis time compared with the other techniques. The specificity and selectivity of the voltammetric techniques are usually excellent because the analyte can be readily identified by its voltammetric peak potential. Chemically modified electrodes have attracted much interest in the study of the electrocatalytic reaction of important pharmaceuticals. Modified electrodes can be prepared by deposition of various compounds such as organic compounds, conducting polymers, nano particles, metal oxides, etc. on the various electrode surfaces.

D.Sun et al have developed a sensitive and convenient voltammetric method for the determination of 10-Hydroxycamptothecin (HCPT) using a MWCNT modified glassy carbon electrode (GCE). HCPT is a natural alkaloid possessing promising antitumour activity. Compared with the poor electrochemical signal at the unmodified GCE, the electrochemical response of HCPT at the MWCNT-modified GCE was greatly improved. A wide linear range from 1×10^{-8} to 4×10^{-6} M with a very low detection limit of 2×10^{-9}M was obtained using this modified electrode [102].

A single walled carbon nanotubes modified gold electrode was developed by B. Zeng et al for the voltammetric determination of rutin,

used in the treatment of a disease characterized by capillary bleeding. Here the working concentration range was found to be 2×10^{-8} to 5×10^{-6} M with a detection limit of 1×10^{-8}M [103].

A rapid, sensitive and simple electrochemical method was developed for the determination of trace level ofloxacin by K. J. Huang et al. A MWCNT/Nafion/GCE was constructed to study the electrochemical behavior of ofloxacin. MWCNT exhibited excellent electrocatalytic activity in the oxidation of ofloxacin characterized by the peak current enhancement. MWCNT/Nafion/GCE greatly improved the sensitivity of determination of ofloxacin [104].

The electrochemical behavior and detection of Daunomycin at MWCNT modified electrode was carried out by S. Lu. Compared with bare GCE, the MWCNT film coated GCE remarkably enhanced the sensitivity of determining daunomycin. This new procedure had the advantages such as ultrasensitivity, rapid response and excellent reproducibility [105].

Z. Wang et al developed a voltammetric method for the determination of hymecromone at a MWCNT/Cetyl Pyridine Bromine composite film modified GCE. The linear concentration range obtained for this developed electrode was in the range of $3.0 \times 10^{-7} – 2.0 \times 10^{-5}$M with a detection limit of 8.0×10^{-8}M. The proposed method was also successfully applied to assay hymecromone in pharmaceutical formulation with satisfactory results [106].

Isoniazid (pyridine-4-carboxylic acid hydrazide) is one of the most effective antituberculosis agents, which is usually used to prevent the development of clinical tuberculosis. A multi-walled carbon nanotube paste electrode (MWCPE) was developed by S. Shahrokhian et al as an

electrochemical sensor with high sensitivity and selectivity in responding to isoniazid [107].

Y. Tu et al have developed MWCNT-quantum dots (QDs) composite-modified glassy carbon electrode for the voltammetric determination of Levodopa. The modified electrode showed high electrocatalytic activity for levodopa with a standard heterogeneous rate constant of 0.595 cm s^{-1}, which was greatly increased compared with the values for bare GCE and individual MWCNTs modified GCE. [108].

Lincomycin is a well-established antibiotic drug used in human and veterinary medicine. Y.Wu et al have developed a very sensitive and simple voltammetric method based on MWCNT/GCE for the measurement of lincomycin. A sensitive linear voltammetric response for lincomycin was obtained in the concentration range of 4.5×10^{-7} to 1.5×10^{-4} M and the detection limit was 2×10^{-7} M. This proposed method possesses many advantages such as very low detection limit, fast response, low cost and simplicity [109].

A sensitive voltammetric method for the determination of a well known anti-hypertensive and sedative agent, Reserpine, was developed by H. Zhang et al. GCE modified with an MWCNT thin film was used by this group for the determination of reserpine. The electrochemical behavior of reserpine indicated that the MWCNT-film significantly increased the oxidation peak current of reserpine and consequently improved the sensitivity for reserpine determination remarkably [110].

A MWCNT-dihexadecyl hydrogen phosphate (DHP) film-coated glassy carbon electrode (GCE) was fabricated and the electrochemical behaviors of acyclovir on the MWCNTs-DHP film-coated GCE was

investigated by F.Wang et al. The oxidation peak current was proportional to the concentration of acyclovir from 8×10^{-8} to 1×10^{-5} M. The detection limit was about 3×10^{-8} M for 60 s accumulation at 0 V [111].

C. Yang has developed an electrochemical method based on SWCNT film coated GCE for the determination of tinidazole. Tinidazole gave a well defined reduction peak with a high peak current at SWCNT/GCE when compared with bare GCE. This developed sensor possesses several advantages like low detection limit, rapid response, excellent reproducibility, simplicity and low cost [112].

The electrochemical behavior of tryptophan at a GCE modified with MWCNT embedded cerium hexacyanoferrate was studied by B. Fang et al. The CeHCF/ MWCNT/GCE showed potent electrocatalytic activity towards the electrochemical oxidation of tryptophan in phosphate buffer solution (pH 7.0) with a decrease of the overpotential by 240mV. The anodic peak current increased linearly with the concentration of tryptophan in the range of 2×10^{-7} to 1×10^{-4}M with a detection limit of 2×10^{-8}M [113].

The electrochemical behavior of brucine at the MWCNT-modified GCE was studied using CV and SWV by S. F. Wang et al. The SWV currents of brucine at the modified electrode increased linearly with its concentration in the range of 1×10^{-6} to 1×10^{-4} M, with a detection limit of 2×10^{-7} M. The MWCNTs modified electrode was successfully applied for the determination of brucine in practical samples [114].

Dopamine (DA) is one of the most significant catecholamines and belongs to the family of excitatory chemical neurotransmitters. The electrochemical determination of DA, however, was restricted in an excess of Ascorbic acid (AA), which has a similar oxidation potential to DA.

Z.Wang et al have developed a MWCNT modified electrode which exhibited an attractive ability to separate DA and AA effectively and showed good stability and reproducibility [115].

The voltammetric determination of the anaesthetic halothane on a MWCNT modified glassy carbon electrode was reported by X. Dai et al. It was found that MWCNT exhibited excellent electrocatalytic effect on the voltammetric behavior of halothane [116].

An enzymeless sensor based on MWCNT-Dicetyl phosphate (DCP) film modified vitreous carbon electrode was developed by S.Lu for the voltammetric determination of hypoxanthine. The MWCNT-DCP film modified electrode showed a remarkable enhancement effect on the oxidation peak current of hypoxanthine. Under the optimized conditions the oxidation peak current was proportional to the concentration of hypoxanthine over the range from 5×10^{-7} - 2×10^{-4} M with a detection limit of 2×10^{-7}M. [27].

P. Xiao et al have developed a voltammetric sensor for promethazine, a phenothiazine derivative widely used as therapeutic agent for treating various mental disorders, using a MWCNT modified gold electrode. It was observed that under the optimized conditions, the anodic peak current was linear to promethazine concentration in the range from 5×10^{-8} to 1×10^{-5} M. The detection limit was found to be 1.0×10^{-8} M. This method was successfully applied for the determination of promethazine in medicine sample (i.e. Compound Reserpine) and the recovery obtained was 97.6-101.8% [117].

A β-cyclodextrin-coated electrode incorporating carbon nanotubes was constructed and applied to the detection of uric acid in the presence of high concentration of ascorbic acid by Z. Wang et al. The CNT/β-

cyclodextrin modified electrode could effectively separate the anodic peaks of uric acid and ascorbic acid by about 400 mV. The practical analytical application was illustrated by a selective measurement of uric acid in human urine without any preliminary treatment [118].

K. Wu et al. developed an electrochemical method for the determination of thyroxine using MWCNT/GCE. It was reported that this novel MWCNT film could remarkably enhance the sensitivity of determination of thyroxine [119].

Based on the unique properties of MWCNT thin film, a new rapid, convenient and sensitive voltammetric method was described for the determination of procaine in pharmaceutical preparations by K. Wu et al. The MWCNT-coated GCE exhibited electrocatalytic activity to the oxidation of procaine as characterized by the significant peak current enhancement and lowering of oxidation overpotential. The various experimental parameters such as solution pH value, the amount of MWCNT, accumulation conditions and scan rate were optimized for the determination of procaine and this method gave a detection limit of 2×10^{-7}M [120].

R.N. Hedge et al have developed a simple and rapid electrochemical method for the determination of trace-level trazodone. The cyclic voltammetric studies revealed the electrocatalytic activity of MWCNT towards the oxidation of trazodone in neutral solutions. The concentration range and detection limit for trazodone under optimized conditions, was found to be 0.2-10 μM and 24 nM, respectively. The reported method was successfully applied to trazodone determination in pharmaceutical samples [121].

A rapid and sensitive voltammetric sensor based on the reduction of betamethasone was developed using single wall carbon nanotube modified

edge plane pyrolytic graphite electrode (SWNT/EPPGE) by N. Goyal et al. The reduction of betamethasone occurred in a well-defined, pH dependent peak. Linear calibration curve was obtained in the range 1 to 25 nM with the limit of detection as 0.50 nM. The developed sensor could successfully sense the drug in human body fluids and was applied for the determination of betamethasone content in several commercially available pharmaceutical preparations indicating the excellent analytical applicability of the developed sensor [122].

B. Rezaei et al have studied the electrochemical behavior and determination of hydrochlorothiazide, a thiazide diuretic and antihypertensive drug, on MWCNT modified GCE by adsorptive stripping voltammetry. The MWCNTs remarkably enhanced the oxidation of hydrochlorothiazide in wide pH range of 2-9.5 and allowed the development of a highly sensitive voltammetric sensor for the determination of hydrochlorothiazide in pharmaceutical and urine samples [123].

A. A. Ensafi et al have developed a p-aminophenol- MWCNT paste electrode for the simultaneous determination of cysteamine and tryptophan. It was reported that the MWCNT- p-aminophenol paste electrode could successfully measure cysteamine and tryptophan in mixture independently from each other with a potential difference of 600 mV [124].

A MWCNT modified GCE as voltammetric sensor was developed the determination of noscapine in blood and pharmaceutical sample by B. Rezaei et al. The linear concentration range obtained in this study was 4×10^{-7}–1×10−4 M with a detection limit of 8.0×10^{-8} M [125].

L. Lonappan et al have developed a voltammetric sensor based on MWCNT-Nafion modified Platinum electrode for the determination of

Pyridine – 2- aldoxime methochloride (PAM Chloride). The modified electrode exhibited excellent electrocatalytic effect on the determination of PAM Chloride, characterized by the peak current enhancement and lowering of the oxidation potential. The concentration of Pyridine-2-Aldoxime Methochloride showed excellent linear relationships with the oxidation peak current in the range 1×10^{-6}-1×10^{-3} M, with a detection limit of 3.03×10^{-7} M for 90 s accumulation [126].

The voltammetric determination of 2-mercaptobenzimidazole was studied by J. P. Fan et al using a GCE coated with polymeric nickel and copper tetraaminophthalocyanine (poly-NiTAPc and poly-CuTAPc) membrane. The polymeric membrane decreased the overpotential of oxidation of MBI by 136.2 and 115.0 mV and increased the oxidation peak current by about 3.4 and 3.3 times, while the reduction peak potential shifted positively by 113.0 and 84.1 mV and the peak current increased by about 10 and 7 times for poly-NiTAPc and poly-CuTAPc, respectively, compared to the unmodified GCE [127].

M. R. Majidi et al have investigated the voltammetric behavior of isoniazid at an overoxidized polypyrrole (OPPy) modified GCE. The OPPy-modified GCE exhibited catalytic activity towards the electro-oxidation of isoniazid, which appeared as a reduced overpotential in a wide operational pH range of 1–13 [128].

A. Liu et al fabricated a novel uric acid electrochemical sensor for uric acid based on the electropolymerization of 4-(2-pyridylazo)-resorcinol on GCE. The modified electrode not only improved the electrochemical catalytic oxidation of uric acid (UA), but also resolved the overlapping oxidation peaks of UA and AA into two well-defined peaks [129].

A poly(thionine) thin film modified electrode was successfully assembled on the surface of the GCE by means of electrochemical polymerization by H.Y.Huo et al. The modified electrode was used for the determination of heparin [130].

G. Yang et al have developed a poly- amidosulfonic acid modified GCE for the determination of isoniazid. The modified electrode showed an excellent electrocatalytical effect on the oxidation of isoniazid. There was a good linear relationship between anodic peak current and isoniazid concentration in the range of 5×10^{-8}– 1×10^{-5} M with a detection limit of 1×10^{-8} M. This developed method had been applied to the direct determination of isoniazid in injection and tablet samples with satisfactory results [131].

A polyaniline–MWCNT (PANI–MWCNTs) composite modified electrode was fabricated and the electrochemical behavior of acetaminophen on the PANI–MWCNTs composite modified electrode was investigated using cyclic voltammetry (CV), single-potential step chronocoulometry and square-wave voltammetry (SWV) by M. Li et al. The developed electrode exhibited excellent electrocatalytic effects on the redox behavior of acetaminophen. The oxidation peak current was proportional to the concentration of acetaminophen from 1×10^{-6} -1×10^{-4} M and the detection limit was 2.5×10^{-7} M [132].

M. Aslangolu have developed a cyclic voltammetric method based on a β-cyclodextrine doped poly (2,5-diaminobenzenesulfonicacid) modified GCE for the determination of levodopa. β-cyclodextrine doped poly(2,5-diaminobenzenesulfonic acid)/GCE exhibited electrocatalytic activity to the oxidation of levodopa and provided voltammetric monitoring of levodopa in presence of ascorbic acid [133].

A voltammetric method using a poly (1-methylpyrrole) modified GCE was developed by M. Aslangolu et al for the quantification of adrenaline. The modified electrode exhibited stable and sensitive current responses towards adrenaline. Compared with bare GCE, the modified electrode exhibited a remarkable shift of the oxidation potentials of adrenaline in the cathodic direction and a drastic enhancement of the anodic current response [134].

An electropolymerized film of eriochrome black T (EBT) has been prepared by H. Yao et al at GCE by CV. The poly (EBT) membrane at GCE exhibited an excellent electrocatalytic activity towards the oxidation of epinephrine (EP), ascorbic acid (AA) and uric acid (UA) in acidic solution and reduced the overpotential for the oxidation of EP. The poly (EBT)-coated electrode could separately detect EP, AA and UA in their mixture with the potential differences of 180 and 160 mV for EP-AA and UA-EP, respectively, which are large enough to allow for determination of EP in the presence of AA and UA [135].

A novel L-cysteine film modified electrode has been fabricated by C.Wang et al by means of an electrochemical oxidation procedure and it was successfully applied to the electrochemical determination of acetaminophen. Linearity between the oxidation peak current and the acetaminophen concentration was obtained in the range of $1\times10^{-4} - 2\times10^{-7}$ M with a detection limit of 5×10^{-8} M [136].

J. Guan et al have developed a voltammetric method for the determination of sinomenine using cystic acid modified GCE based on the electrochemical oxidation of L- Cysteine. The low-cost modified electrode possessed good sensitivity, selectivity, stability and had been

successfully applied to the determination of sinomenine in pharmaceutical formulations [137].

A poly (L-Cysteine) modified electrode was developed by X. Zheng et al for the voltammetric determination of ascorbic acid. The poly (L-cysteine) film exhibited highly-efficient catalytic ability to the oxidation of ascorbic acid since it greatly lowered the oxidation overpotential and remarkably enhanced the oxidation peak current [138].

Z. Yan et al have studied the electrochemical behavior of uric acid at a Cysteine modified GCE by CV and DPV. The results indicated that the modified electrode exhibited efficient electrocatalytic oxidation for uric acid. The anodic overpotential was reduced nearly 200mV compared with the result obtained at a bare electrode [139].

A poly (p-toluene sulphonic acid) modified GCE was fabricated by P. F. Huang et al for the simultaneous electrochemical detection of dopamine and ascorbic acid. The poly(p-toluene sulphonic acid)/GCE could separate the dopamine and ascorbic acid oxidation potential by about 192 mV and was used for the selective determination of dopamine in presence of high concentration of ascorbic acid [140].

A novel film electrode for the voltammetric determination of tyrosine has been constructed by C.Li based on the electropolymerization of L-serine on GCE. Voltammetric behaviour of tyrosine on the poly-L-serine film electrode was investigated with CV and LSV and electrochemical parameters were calculated from chronocoulometry. A linearity between the oxidation peak current and the tyrosine concentration was obtained in the range of 3×10^{-7} to 1×10^{-4} M with a detection limit of 1×10^{-7} M. The determination of tyrosine in commercial amino acid oral solution

demonstrated that the developed electrode has good selectivity and high sensitivity [141].

Z. Yang et al have developed a voltammetric method for the determination of Hydroquinone using a GCE modified with p-aminobenzene sulfonic acid through electropolymerization. The anodic current was obtained as a linear function of concentration in the range from 1.1×10^{-6} to 2.0×10^{-3} M and the detection limit was determined to be 4.0×10^{-7}M. At the developed electrode, no interference was observed in the presence of comparable amounts of catechol and resorcinol [142].

A molecularly imprinted poly pyrrole film was prepared by S. P. Ozkorucuklu et al and was used for the determination of sulfamethoxazole. The voltammetric behaviour of sulfamethoxazole on imprinted and non-imprinted (NIP) films was investigated by differential pulse voltammetry (DPV). The MIP electrode exhibited the best reproducibility and highest sensitivity. The polypyrrole (PPy) electrodes showed low response time, good mechanical stability and simple to construct [143].

Electrochemical behavior of tryptophan at the poly p-aminobenzene sulphonic acid modified GCE was investigated by Y. Ya et al with voltammetry. The electrochemical response of tryptophan was improved significantly in the presence of poly p-aminobenzene sulphonic acid film. Compared with bare GCE, the poly p-aminobenzene sulphonic acid film electrode remarkably enhanced the irreversible oxidation peak current of tryptophan. The oxidation peak current was proportional to tryptophan concentration in the range of 1×10^{-7} to 1×10^{-6} M and 2×10^{-6} to 1×10^{-5} M with a detection limit of 7×10^{-8} M [144].

L.Wang et al have modified the GCE with electropolymerized films of *p*-toluene sulfonic acid for the electrochemical detection of uric acid in presence of ascorbic acid. The polymer modified electrode could separate the oxidation peak potentials of uric acid and ascorbic acid present in the same solution by about 300 mV, though the bare electrode gave a single broad response. Also the modified electrode successfully eliminated the fouling effect by the oxidized product of ascorbic acid on the response of uric acid. The detection limit of uric acid in the presence of ascorbic acid at millimolar level was 5.0×10^{-7} M [145].

A stable electroactive thin film of poly (caffeic acid) was deposited on the surface of a GCE by potentiostatic technique. The voltammetric behavior of epinephrine at this modified electrode was studied by cyclic voltammetry by W. Ren et al. The poly (caffeic acid) modified electrode exhibited a promotion effect on the oxidation of epinephrine. In a pH 7.4 phosphate buffer, the anodic current increased linearly with the concentration of epinephrine in the range from 2.0×10^{-6} to 3.0×10^{-4} M and the detection limit for EP was 6.0×10^{-7} M. The proposed method was also applied for the determination of epinephrine in practical injection samples with simplicity, rapidness and accurate results [146].

A voltammetric sensor based on poly aniline modified GCE was developed by P. J. O'Connell et al. The developed sensor could selectively catalyze the oxidation of L-ascorbic acid at low potentials (+100 mV). Due to this low anodic potential of L-Ascorbic acid at the developed sensor, the effect of many common electrochemical interferents was minimized [147].

The electrochemical behavior of a corticosteroid methylprednisolone, used for doping, was studied at gold nanoparticles (AuNPs) modified

indium tin oxide electrode by R.N. Goyal et al. The AuNPs modified indium tin oxide electrode exhibited an effective catalytic response towards its oxidation and lowered its oxidation potential by 127mV when compared with bare indium tin oxide electrode. Linear concentration curves were obtained over the concentration range 0.01–1.0 μM with a detection limit of 2.68×10^{-7}M at AuNPs modified indium tin oxide [148].

A simple, rapid and sensitive electrochemical method was developed by M. Behpour for simultaneous determination of acetaminophen and atenolol on a AuNPs modified carbon paste electrode.The modified electrode exhibited electrocatalytic properties toward acetaminophen and atenolol oxidation with a peak potential of 20 and 50 mV lower than that at the bare carbon paste electrode, respectively. Also the enhanced peak current response is a clear evidence of the catalytic activity of the AuNPs modified carbon paste electrode towards oxidation of acetaminophen and atenolol [149].

R. N. Goyal et al have developed a nanogold modified indium tin oxide electrode for the determination of paracetamol. The electrode exhibited an effective catalytic response to the oxidation of paracetamol with good reproducibility and stability. At the modified electrode, the oxidation potential of paracetamol was lowered by approximately 110 mV and current response enhanced significantly relative to the situation prevailing when a bare ITO electrode was used. Linear calibration curve was obtained over the range 2.0×10^{-7}–1.5×10^{-3} M with a detection limit of 1.8×10^{-7} M [150].

The electrochemical behavior of rutin on a AuNPs/ ethylenediamine /multi-wall carbon nanotubes modified glassy carbon electrode was

investigated by S. Yang et al. Rutin effectively accumulated on the modified electrode and caused a pair of redox peaks at around 487 mV and 432 mV. The anodic peak current was linear to the rutin concentration in the range of 4.8×10^{-8} M to 9.6×10^{-7}M. The developed method was also successfully applied for the determination of rutin content in tablet samples with satisfactory results [151].

J. B. Raoof et al have reported a AuNPs/self-assembled monolayer (SAM)/ modified gold electrode for the electrochemical determination of ascorbic acid (AA) and dopamine (DA) in aqueous media. The result showed that the AuNPs modified electrode could clearly resolve the oxidation peaks of AA and DA, with a peak-to-peak separation (ΔEp) of 110 mV enabling determination of AA and DA in the presence of each other [152].

A novel electrochemical sensor was fabricated by electrodeposition of AuNPs on pre-synthesized polypyrrole nanowire, forming an Au/PPy composite matrix on glassy carbon electrode (Au/PPy/GCE). As an electrochemical sensor, the Au/PPy/GCE exhibited strongly catalytic activity toward the oxidation of hydrazine and hydroxylamine. The sensor showed excellent sensitivity, selectivity, reproducibility and stability properties [153].

L.Wang et al have developed gold nano/SAM modified gold electrode for the voltammetric sensing of epinephrine. The gold nano electrode was demonstrated to promote the electrochemical response of epinephrine by cyclic voltammetry. The electrode reaction of epinephrine was significantly improved at the gold nano modified electrode which results in a large increase in the voltammetric peak current with a detection

limit of 6×10^{-8} M. The gold nano modified electrode showed high electrocatalytic activity and excellent sensitivity property [154].

AuNPs-immobilized GCE was developed by L. Zhang et al for the electrocatalytic oxidation of ascorbic acid, reducing the overpotential by about 200 mV with obviously increased current response. Due to its strong electrocatalytic activity towards ascorbic acid, the AuNPs modified electrode could resolve the overlapped voltammetric waves of ascorbic acid and dopamine into two well-defined voltammetric peaks with peak-to-peak separation in potentials of about 300 mV. This modified electrode allowed the selective determination of ascorbic acid in presence of dopamine. The modified electrode showed good selectivity, stability and anti-fouling properties. The developed method was used for the selective determination of ascorbic acid in presence of dopamine and for the simultaneous determination of both in their mixtures with satisfactory results [155].

AuNPs immobilized on an amine-terminated SAM on a polycrystalline gold electrode was successfully used for the selective determination of dopamine in presence of ascorbate by C. R. Raj et al. Well-separated voltammetric peaks were observed for dopamine and ascorbate at the AuNPs -immobilized electrode. The oxidation potential of ascorbic acid was shifted to less positive potential due to the high catalytic activity of AuNPs. The reversibility of the electrode reaction of DA was significantly improved at the gold nano electrode, which resulted in a large increase in the square-wave voltammetric peak current with a detection limit of 0.13 mM. The coexistence of a large excess of AA did not interfere the voltammetric sensing of DA. The gold nano modified electrode showed excellent sensitivity, good selectivity and antifouling properties [156].

AuNPs modified carbon paste electrode was used for the determination of atenolol in drug formulations by M.Behpour et al using CV, DPV and chronocoulometric methods. The modified electrode showed an electrocatalytic activity toward the anodic oxidation of atenolol by a marked enhancement in the current response. The anodic peak potential shifted by -80 mV when compared with the potential using bare carbon paste electrode. A linear analytical curve was observed in the range of 1.96×10^{-6} to 9.09×10^{-4} M. The detection limit for this method was found to be 7.3×10^{-8} M. The method was then successfully applied to the determination of atenolol in tablets and human urine [157].

A novel voltammetric sensor was fabricated by J. Yu et al, which was based on MWCNTs dispersed in AuNPs colloid. On the composite film coated electrode, the electrochemical behavior of mefenamic acid was studied and an irreversible anodic peak was obtained, which was much larger than that on a bare GCE, due to the co-effect of MWCNTs and AuNPs on the electrochemical oxidation of mefenamic acid. The anodic peak current was linear to the concentration of mefenamic acid in the range of 1×10^{-7} to 2×10^{-5} M with a detection limit of 1.0×10^{-8} M. The method was applied successfully to the determination of mefenamic acid in medicine sample [158].

J. Zhong et al have developed a sensor based on AuNP/DNA modified gold electrode for the voltammetric determination of phenothiazine drugs namely promethazine and chlorpromazine. This modified electrode demonstrated good sensitivity and stability towards the oxidation of these drugs. A linear dependence between the concentration and peak current was obtained in the range $2 \times 10^{-5} - 1.6 \times 10^{-4}$M with a detection limit of 1×10^{-5} M [159].

A new type of AuNPs attached indium tin oxide (ITO) electrode was made by S. Li et al. This modified electrode was used for the determination of pirarubicin in urine by cyclic voltammetry. Compared to a bare ITO electrode, the modified electrode exhibited a marked enhancement in the current response. Liner calibration curves are obtained in the range 5×10^{-9} -1.5×10^{-6} M with a detection limit of 1×10^{-9} M [160].

M. Hosseini et al have developed AuNP/polyaniline film on titanium electrode for the voltammetric determination of ascorbic acid. The electrochemical behavior and electro-catalytic activity of AuNP/poly aniline/Ti electrode were characterized by cyclic voltammetry. The electro-oxidation of ascorbic acid was found to proceed more facile on AuNP/poly aniline/Ti electrode than on bare gold electrode. The irreversible oxidation current of ascorbic acid exhibited a linear dependence on the ascorbic acid concentration in the range of 1–5 mM [161].

1.12 Scope of the Present Investigation

The quality of a drug is determined after establishing its authenticity by testing its purity and quality of the pure substance in the drug and its formulations. A number of methods including physical, chemical, physico-chemical and biological ones are employed for determining the quality of the drugs. Among the physico-chemical methods, electrochemical methods are the most widely used one. In continuation to the development of spectrophotometric [162-166] and electrochemical methods[167-176] for the determination of drugs in our laboratory, the present investigation involve the fabrication of voltammetric sensors for drugs such as Ambroxol, Sulfamethoxazole, PAM Chloride, Lamivudine, Metronidazole Benzoate and Nimesulide. For all the sensors developed, the various

parameters studied include linear response range, detection limit, effect of pH, scan rate study and interference study. The developed sensors have been applied for the determination of the drugs in pharmaceutical formulations and in real samples.

…..ꕥ…..

Chapter 2

MATERIALS AND METHODS

CONTENTS

A brief sketch of the materials and methods used in the investigations is presented in this chapter. The general method for the fabrication of the modified electrodes is 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. Double distilled water was used through out the studies. Multiwalled Carbon nanotube (MWCNT), Nafion, Alumina, Cystamine dihydrochloride and PAM Chloride were purchased from Sigma Aldrich Corporation, USA. Except MWCNT, other chemicals were used as

received. L-Cysteine(L-Cys), Lithium perchlorate ($LiClO_4$) and Tetra – n-butyl ammonium chloride were obtained from Lancaster, UK and were used as such without any further purification. Chloroauric acid was purchased from SRL Chemicals, India. Sodium dihyrogen orthophosphate (NaH_2PO_4) and disodium hydrogen orthophosphate (Na_2HPO_4) were purchased from Merck, Germany and were used as received. All other common reagents used for the studies were obtained from s.d. fine chemicals, India. Pure drugs such as Ambroxol Hydrochloride (AMB), Sulfamethoxazole (SMX), Lamivudine (LAM), Metronidazole Benzoate (MBZ), Nimesulide (NIM) and Trimethoprim (TMP) were obtained as gift samples. Pharmaceutical formulations containing the drugs were purchased from local drug stores.

2.2 Instruments Used

All the electrochemical measurements were made on BAS Epsilon Electrochemical analyzer (Bioanalytical system, USA) interfaced to a PC. A conventional three electrode system, including working electrode, counter electrode and reference electrode was employed. Working electrode used was glassy carbon electrode (GCE) modified with suitable chemical modifications, counter electrode used was platinum wire and Ag/AgCl was used as the reference electrode. All voltammograms were recorded in the polarographic mode. The pH measurements were carried out in a Metrohm pH meter. Electrode cleaning was carried out in an Ultrasonicator(Oscar Ultrasonics, Pvt. Ltd. Mumbai). Scanning Electron Microscopic (SEM) images were recorded using JOEL 6300 LV at Sophisticated Test and Instrumentation Centre (STIC), Kochi. FTIR spectra was recorded on JASCO-4100 FTIR Spectrometer using KBr discs.

2.3 Cleaning of GCE

First the GCE was mechanically polished with alumina on a polishing pad and was cleaned thoroughly with double distilled water. This was then sonicated in methanol, 1:1 HNO_3 solution, acetone and double distilled water sequentially to obtain the cleaned GCE.

2.4 Fabrication of Chemically modified electrodes (CMEs) as sensors for the determination of various pharmaceuticals

2.4.1 Fabrication of MWCNT/Nafion modified GCE

MWCNT was first refluxed in 100 mL 6 M HNO_3 for 10 hours. The resulting suspension was then diluted with 200 mL water. MWCNT was then filtered and washed with double distilled water. The washed nanotube was collected and dried.

MWCNTs were generally insoluble in common solvents; therefore the key step was to disperse insoluble MWCNTs in suitable solvents to perform the electrochemical measurements by using the MWCNT modified electrodes. However nafion, a sulfonated tetra fluoro ethylene copolymer, was found to effectively disperse MWCNTs. Nafion possess several advantages such as excellent ion exchange characteristics, thermal stability, chemical inertness and mechanical strength and has been widely used for the MWCNT modification of electrodes [177]. Hence Nafion was selected as the solvent to disperse MWCNT into water.

5 mg of the treated MWCNT was added to 2 mL of 0.5 % nafion water solution and then sonicated for about 1 h with an ultrasonicator to get a stable and homogenous suspension. Then the GCE was coated with an adequate amount of the resulting MWCNT/Nafion suspension and the solvent was evaporated at room temperature in air to get MWCNT/Nafion modified GCE.

2.4.2 Fabrication of poly(p-toluene sulphonic acid)/GCE

1mM p-toluene sulphonic acid (p-TSA) was prepared in 0.1 M NaCl solution. 10 mL of this solution was then pipetted into the electrochemical cell. The cleaned GCE, reference electrode and auxiliary electrode were then immersed in the p-TSA solution. 20 cyclic scans were performed between -2.0 to +2.5 V at a scan rate of 0.1 Vs^{-1}[178]. GCE was then taken out and washed several times with double distilled water and kept in air. A uniform blue film was found to be formed on the GCE surface.

2.4.3 Fabrication of poly(L-Cysteine)/GCE

5mM L-Cysteine (L-Cys) was prepared in 0.1M phosphate buffer solution (PBS). 10 mL of this solution was then pipetted into the electrochemical cell. The cleaned GCE, reference electrode and auxiliary electrode were then immersed in the L-Cys solution. The film was grown on the electrode surface by 30 segments of cyclic voltammetric scans between –0.8 and 2.0V [179]. After immobilization, the film was washed with ethanol to remove the remaining L-Cys monomers. It was observed that after drying in air, a blue thin film was formed at the electrode surface.

2.4.4.1 Fabrication of poly(p-amino benzene sulphonic acid)/GCE

2mM p-amino benzene sulphonic acid (p-ABSA) was prepared in 0.1M HNO_3 solution. 10 mL of this solution was then pipetted into the electrochemical cell with glassy carbon as the working electrode, Ag/AgCl as the reference electrode and platinum wire as the counter electrode. The electrochemical deposition of p-ABSA film was carried out by cyclic voltammetry between -0.5 and 2.0V at 0.1Vs^{-1} for 30 cycles [180]. The resulting film was washed with doubly distilled water

and dried in air. After drying in air, a thin blue film was observed at the electrode surface. Thus poly (p-ABSA) film was electrochemically deposited on the GCE surface.

2.4.4.2 Fabrication of AuNP/poly(p-amino benzene sulphonic acid)/GCE

The poly (p-ABSA) modified electrode was immersed in 0.05M H_2SO_4 solution containing 1mM $HAuCl_4$. 20 cyclic scans were carried out between 1.3 and 0 Vat a scan rate of $0.1Vs^{-1}$ [181]. This resulted in the electrochemical deposition of gold nanoparticle (AuNP) on the electrode surface.

2.4.5.1 Fabrication of poly(Cystamine)/GCE

1mM Cystamine hydrochloride (CA) was prepared in 0.1M PBS. 10 mL of this solution was then pipetted into the electrochemical cell. The cleaned GCE, reference electrode and auxiliary electrode were then immersed in the CA solution. The film was grown on the electrode surface by 20 segments of cyclic voltammetric scans between –1.2 and 2.5V [182]. The resulting film was washed with doubly distilled water and dried in air. After drying in air, a blue thin film of poly-CA was formed at the electrode surface.

2.4.5.2 Fabrication of AuNP/poly-CA/GCE

The poly-CA modified electrode was immersed in 0.05M H_2SO_4 solution containing 1mM $HAuCl_4$ solution. 20 cyclic scans were carried out between 1.3 and 0Vat a scan rate of $0.1Vs^{-1}$ to deposit AuNPs electrochemically on the electrode surface.

2.5 Preparation of the drug solutions

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

2.5.1 Ambroxol solution

4.146g of AMB was dissolved in methanol and the solution was made upto the volume in a 100mL titrimetric flask.

2.5.2 Sulfamethoxazole solution

2.530g of SMZ was dissolved in methanol in a 100mL volumetric flask and made upto the mark.

2.5.3 PAM Chloride solution

1.726g of PAM Chloride was dissolved in water in a 100mL volumetric flask and made upto the mark.

2.5.4 Lamivudine solution

2.293g of LAM was dissolved in water in a 100mL volumetric flask and made upto the mark.

2.5.5 Metronidazole benzoate solution

2.753g of MBZ was dissolved in methanol in a 100mL volumetric flask and made upto the mark.

2.5.6 Nimesulide solution

3.083g of NIM was dissolved in acetone in a 100mL volumetric flask. The solution was made upto 100mL using acetone.

2.6 Preparation of Buffer Solutions

Phosphate buffer solution (PBS) was used as the supporting electrolyte for carrying out the electrochemical measurements.

2.6.1 Preparation of phosphate buffer solution (PBS)

PBS of different pH was prepared by mixing NaH_2PO_4 and Na_2HPO_4 in varying amounts.

a) pH 2

1.3799g of NaH_2PO_4 and 0.0001g of Na_2HPO_4 was dissolved in 100mL double distilled water to get PBS of pH 2.

b) pH 3

1.379g of NaH_2PO_4 and 0.0003g of Na_2HPO_4 was dissolved in 100mL double distilled water to get PBS of pH 3.

c) pH 4

1.378g of NaH_2PO_4 and 0.0036g of Na_2HPO_4 was dissolved in 100mL double distilled water to get PBS of pH 4.

d) pH 5

1.3615g of NaH_2PO_4 and 0.036g of Na_2HPO_4 was dissolved in 100mL double distilled water to get PBS of pH 5.

e) pH 6

1.2143g of NaH_2PO_4 and 0.3218g of Na_2HPO_4 was dissolved in 100mL double distilled water to get PBS of pH 6.

f) pH 7

0.5836g of NaH_2PO_4 and 1.5466g of Na_2HPO_4 was dissolved in 100mL double distilled water to get PBS of pH 7.

g) pH 8

0.094g of NaH_2PO_4 and 2.497g of Na_2HPO_4 was dissolved in 100mL double distilled water to get PBS of pH 8.

h) pH 9

0.01g of NaH_2PO_4 and 2.6605g of Na_2HPO_4 was dissolved in 100mL double distilled water to get PBS of pH 9.

2.7 Voltammetric determination of pharmaceuticals

Voltammetric measurements were carried out in a BAS Epsilon Electrochemical analyzer with a 3 electrode system. To the stock solution of drug, appropriate amount of supporting electrolyte (PBS) was added to get the drug solution of suitable concentration and was taken in the electrochemical cell. Voltammogram was recorded and the peak current corresponding to the oxidation/reduction of the drug was measured. The current obtained was plotted as a function of concentration. A linear relationship between the peak current and concentration of the drug could be observed.

2.8 Preparation and analysis of Pharmaceutical Formulations

2.8.1 Ambroxol Formulation – Ambrodil HCl

Ten tablets ('Ambrodil HCl', Aristo, India) were weighed and ground to a fine powder. An adequate amount of this powder corresponding to the concentration 1×10^{-3}M was weighed and transferred to a beaker. The powder was dissolved in methanol and filtered to a titrimetric flask (50 mL) through a Whatman 41 filter paper. The beaker was washed several times and the washings were collected in the volumetric flask and then it was quantitatively diluted. Solutions of different concentrations were prepared by serial dilution of the stock with supporting electrolyte. CV was measured and the unknown concentrations were determined from the calibration graph.

2.8.2 Sulfamethoxazole Formulation – Bactrim and Septra

Ten tablets of each type ('Bactrim', Piramal Healthcare, India and 'Septra', Burroughs Wellcome, India) were weighed and finely powdered. Then required amount of the powdered drug was weighed to prepare a solution of 5×10^{-3} M and was transferred to a beaker. The powder was

dissolved in methanol and filtered to a titrimetric flask (50 mL) through a Whatman 41 filter paper. The beaker was washed several times and the washings were collected in the volumetric flask and then it was quantitatively diluted. Solutions of different concentrations were prepared by serial dilution of the stock with supporting electrolyte. Differential Pulse Voltammogram was recorded and the unknown concentrations were determined from the calibration graph.

2.8.3 Lamivudine Formulation - Lamivir

Ten tablets ('Lamivir', Cipla, India) were accurately weighed and finely powdered. An adequate amount of this powder corresponding to the concentration 1×10^{-2}M was weighed and transferred to a beaker. The powder was dissolved in double distilled water and filtered to a titrimetric flask (100 mL) through a Whatman 41 filter paper. The beaker was washed several times with water and the contents were filtered into the flask. The solution was made upto the mark and shaken well. Solutions of different concentrations were prepared by serial dilution of the stock with supporting electrolyte. The sample solution was then taken in the electrochemical cell and the voltammetric studies were carried out.

2.8.4 Metronidazole Formulations – Metrogyl and Flagyl

Ten tablets of each type ('Flagyl', Nicholas Piramal India Ltd and 'Metrogyl', J.B. Chemicals & Pharmaceuticals, India) were weighed and finely powdered. Then required amount of the powdered drug was weighed to prepare a solution of 1×10^{-2} M and was transferred to a beaker. The powder was dissolved in methanol and filtered to a titrimetric flask (50 mL) through a Whatman 41 filter paper. The beaker was washed several times and the washings were collected in the titrimetric flask and then it was

quantitatively diluted. Solutions of different concentrations were prepared by serial dilution of the stock with supporting electrolyte. Differential Pulse Voltammogram was recorded and the unknown concentrations were determined from the calibration graph.

2.8.5 Nimesulide Formulation – Nise

The mass of ten tablets ('Nise', Dr. Reddy's, India) were taken and then powdered well. The mass of powder required to prepare 1×10^{-2} M solution was weighed. It was then transferred to a beaker and dissolved in acetone. The clear solution was transferred to the titrimetric flask through a Whatman 41 filter paper. Then the residue in the beaker was washed several times with acetone and the washings were collected in the titrimetric flask. The solution was then made upto the mark. Solutions of different concentrations were prepared by serial dilution of the stock with supporting electrolyte. The sample solution was then taken in the electrochemical cell and the voltammetric studies were carried out.

2.9 Analysis of Urine Sample

The developed sensors were applied for the determination of the drug in spiked urine samples. Different quantities of drug were added to a fixed volume of urine sample and then quantitatively diluted using the supporting electrolyte. The voltammograms were recorded and the unknown concentrations were determined from the calibration graph.

2.10 Standard Methods

2.10.1 Ambroxol [183]

Ten tablets of 'Ambrodil HCl' were accurately weighed and ground to a fine powder. An adequate amount of this powder containing 0.3g of AMB was weighed. It was dissolved in 70 mL of alcohol and 5mL of 0.01 M HCl was

added. It was made upto the volume in a 100mL titrimetric flask. Potentiometric titration was carried out using 0.1M NaOH. The volume added between the two points of inflexion was recorded. 1mL of 0.1 M NaOH is equivalent to 41.46 mg of AMB.

2.10.2 Sulfamethoxazole [184]

Ten tablets of each type 'Bactrim' and 'Septra', were weighed and finely powdered An adequate amount of this powder containing 0.2g of SMX was weighed. It was dissolved in 50mL of 2M HCl and 3g of KBr was added to it. Then it was ice cooled and titrated against 0.1 M sodium nitrite. The end point was determined potentiometrically.

2.10.3 PAM Chloride [185]

0.5 g of PAM Chloride was accurately weighed and dissolved in 250 mL water. 5mL of this solution was then diluted to 100mL with water. 5mL of this solution was then transferred to a 50mL titrimetric flask. Required amount of urine sample was added and then diluted to 40 mL with water. To this 5 mL of 1M NaOH was added and made up to the mark. The absorbance of the prepared solution was measured. Similarly solutions of various concentrations were prepared and the absorbance was measured.

2.10.4 Lamivudine [186]

Ten tablets of 'Lamivir' were accurately weighed and finely powdered. The mass equivalent to the mass of one tablet was accurately wighed and dissolved in distilled water and made up to the volume in a 100mL titrimetric 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)

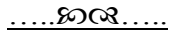
was added to the mixture which reduces the unreacted NBS. The residual iron (III) is complexed with orthophenanthroline. The absorbance is measured at 510nm. The amount of NBS reacted corresponds stoichiometrically to the amount of LAM.

2.10.5 Metronidazole [184]

Ten tablets of each type 'Flagyl' and 'Metrogyl', were weighed and finely powdered. An adequate amount of this powder containing 0.08g of MBZ was accurately weighed and dissolved in methanol. 10mL of this solution was diluted to 100mL with methanol and further 10mL of this solution was diluted to 100mL with methanol. The absorbance of the resulting solution was measured.

2.10.6 Nimesulide [183]

The mass of ten tablets of 'Nise' were taken and then powdered well. An adequate amount of this powder containing 0.24g of NIM was accurately weighed and dissolved in 30mL of acetone. Then 20mL of double distilled water was added to it and titrated against 0.1M NaOH solution. The end point was determined potentiometrically.



Chapter 3

SENSOR FOR THE DETERMINATION OF AMBROXOL

CONTENTS

The fabrication of a carbon nanotube based sensor for the quantitative determination of AMB is discussed in detail in this chapter. The electrochemical determination of AMB has been carried out at a Multi Walled Carbon Nanotube-Nafion modified Glassy Carbon Electrode ((MWCNT/Nafion/GCE) by CV and LSV. AMB gave a well-defined oxidation peak at 1.06 V in 0.1M pH 4 phosphate buffer solution. The electrochemical parameters for the developed sensor have been studied in detail. The analytical applications of the developed sensor in the determination of AMB in pharmaceutical formulations and real sample like urine were also clearly investigated.

3.1 Introduction

Ambroxol(Figure **3.1**) is trans - 4- (2 - amino-3, 5 - dibromo benzyl amino) cyclohexanol. It is a white to yellowish crystalline powder with a molecular mass of 414.56 and a molecular formula of $C_{13}H_{19}Br_2ClN_2O$. It is slightly soluble in water and ethanol; soluble in dimethylformamide and methanol, and insoluble in chloroform and benzene. It is an expectoration improver and a clinically proven systemically active mucolytic agent. It is used in the treatment of acute and chronic disorders characterized by the production of excess or thick mucus. It works to decrease mucus viscosity by altering its structure[187]. AMB is administered as a hydrochloride salt in daily doses of 30-120 mg using mostly oral formulations like tablets and syrups. When administered orally onset of action occurs after about 30 minutes. The breakdown of acid mucopolysaccharide fibers makes the sputum thinner and less viscous and therefore more easily removed by coughing. Although sputum volume eventually decreases, its viscosity remains low for as long as treatment is maintained [188,189].

AMB also provides pain relief in acute sore throat. Pain in sore throat is the hallmark of acute pharyngitis. Sore throat is usually caused by a viral infection. The infection is self limited and the patient recovers normally after a few days. What is most bothering for the patient is the continuous pain in the throat maximized when the patient is swallowing. Thus the main goal of the treatment is to reduce pain. The most important property of AMB for treating sore throat is the local anaesthetic effect.

The importance of this drug has thus prompted the development of many methods for its determination. Several analytical methods have been reported for the determination of AMB in pharmaceutical formulations or

biological samples. It mainly includes High Performance Liquid chromatography (HPLC) [190-192] UV-Visible Spectrophotometry [193,194] flow injection analysis [195] gas chromatography [196] and capillary zone electrophoresis [197]. Some of these reported methods require time-consuming sample preparation or expensive instrumentation. Hence it is of immense importance to develop a technique for the determination of AMB with high degree of selectivity, sensitivity and low detection limit. In this context, voltammetric techniques are found to be highly attractive because these techniques are relatively simple and the required sensitivity and selectivity for drug analysis can be easily achieved [198].

As already mentioned, the development of chemically modified electrodes (CMEs) has continued to be of major concern in voltammetric determinations. The operation mechanism of CMEs depends on the properties of the modifier materials that are used to promote selectivity and sensitivity towards the target analytes [199]. Modification of electrode surface with Carbon nanotubes (CNTs) has gained much attention recently [101-126,200]. The performance of CNT has been found to be superior to other carbon electrodes in terms of reaction rate.

This chapter presents the detailed results of development and analytical applications of MWCNT/Nafion based sensor for the determination of the drug AMB. MWCNTs were dispersed into nafion solution via ultrasonication, and then the resulting homogenous dispersion of MWCNT was dropped on to GCE. The MWCNT/Nafion /GCE was obtained by evaporating the solvent. The modified GCE exhibited significant enhancement in the oxidation peak current compared with bare GCE. The enhancement of peak current clearly indicates that nanotubes are effective electro catalysts [118]. The developed sensor has been

successfully applied for the determination of AMB in commercially available tablets and urine sample.

3.2 Treatment of MWCNT

MWCNT was refluxed in 100 mL 6 M HNO_3 for 10 h to eliminate metal oxide catalysts within the nanotubes, to introduce carboxyl groups [201] and segment MWCNT for easier and better dispersion. The resulting suspension was then diluted with 200 mL of water and MWCNT was filtered and washed with double distilled water. The washed nanotube was collected and dried. The FTIR spectrum of the treated nanotube was recorded. Peaks were obtained at 1703 cm^{-1} and 1564 cm^{-1} which proved that carboxy and carboxylate groups were present on the surface of MWCNTs. Thus the acid treatment caused segmentation and carboxylation of MWCNT at their terminus.

Scanning Electron Microscopic (SEM) image of the treated MWCNTs were recorded and is shown in Figure **3.2**. From the SEM image, it could be observed that MWCNT retained their nanosized tubular shape even after pretreatment.

3.3 Preparation of MWCNT / Nafion modified GCE

Prior to modification, the GCE was cleaned as described in section 2.3 of Chapter 2. A detailed procedure for the fabrication of MWCNT/Nafion modified GCE is given in section 2.4.1.

6 μL of the resulting MWCNT/Nafion suspension was coated on the surface of GCE. The electrode was then dried in air to get a uniform thin film of nanotube on GCE.

SEM images of both bare and MWCNT modified GCE were taken to study the surface morphology (Figure **3.3**). These images provided a clear evidence for the effective modification of GCE with MWCNT.

3.4 Electrochemical measurement

Electrochemical measurements were made on BAS Epsilon Electrochemical analyzer (Bioanalytical system, USA) interfaced to a PC. A conventional three electrode system, including MWCNT/Nafion modified GC working electrode, a platinum wire counter electrode and an Ag/AgCl reference electrode were employed.

Stock solution of AMB was prepared as described in section 2.5.1 of Chapter 2. Standard solutions of the analyte (1×10^{-3}- 1×10^{-5} M) were prepared by serial dilution of stock solution with appropriate supporting electrolyte (pH4 PBS). Sample solution was taken in the electrochemical cell and then accumulated under an open circuit for 4 min. Cyclic voltammogram was then recorded from 0 to 1.2 V at a scan rate of $0.1Vs^{-1}$. Firstly, an attempt was made to study the electrochemical behavior of AMB at Carbon Paste Electrode (CPE). Unfortunately at CPE, the anodic peak of AMB was obtained at a high potential (1.12 V) with a low peak current. The linear concentration range was found to be 1×10^{-3}- 1×10^{-4} M only. When GCE was used, a much better peak than with CPE was obtained for AMB. However at MWCNT/Nafion modified GCE, the electrochemical response of AMB was found to be enhanced tremendously. The MWCNT modified sensor yielded a well defined oxidation peak for AMB at 1.06 V with high peak current of 0.1231 mA justifying the choice.

During the first potential sweep from 0 to 1.2 V, AMB gave an oxidation peak at 1.06 V at MWCNT/Nafion/GCE, but during the reverse

sweep no reduction peak was obtained. This clearly indicates that the electrochemical response of AMB is irreversible. For electrode regeneration, several cyclic scans were carried out in the blank electrolyte solution until a stable voltammogram was obtained.

The cyclic voltammograms of 1×10^{-3} M AMB at MWCNT/Nafion /GCE, Nafion/GCE and bare GCE are shown in Figure **3.4**. Though the oxidation potential obtained in all these cases are almost the same, the oxidation peak current of AMB obtained at the MWCNT/Nafion modified electrode is 0.1231 mA, at Nafion/GCE is 0.0258mA and that obtained at bare GCE is only 0.0232 mA. As can be seen, a poorly defined oxidation peak of AMB with very low current was observed at bare GCE. At Nafion/GCE also the peak current was low and the peak was broad due to the slow electron transfer. However, at the MWCNT/Nafion/GCE, its response has improved considerably, and the peak current has greatly increased. The remarkable peak current enhancement undoubtedly proved the electro catalytic activity of MWCNT/Nafion/GCE towards the oxidation of AMB. The ability of MWCNT to promote electron transfer also resulted in the tremendous enhancement of peak current.

3.4.1 Surface Area Study

The observed enhancement in the peak current for AMB can also be attributed to the increase in surface area of GCE when modified with MWCNT. Surface area study provided a clear cut evidence for this.

2 mM $K_3Fe(CN)_6$ was taken in the voltammetric cell and CV was recorded for both MWCNT modified GCE and bare GCE at different scan rates say 10,20,30, 40, 50 and 60 mVs^{-1}. The current obtained for both electrodes was recorded. The readings are tabulated in Table **3.1** and **3.2**.

Graphs were plotted with current vs square root of scan rate (Ip vs $\upsilon^{1/2}$) for both electrodes. From the slope of these linear graphs, surface areas can be calculated using the Randles Sevick equation [202]

$$I_p = 2.69 \times 10^5 \, A \, n^{3/2} \, D_R^{1/2} \, c \, \upsilon^{1/2}$$

where Ip refers to the peak current, n is the number of electrons transferred, A is the surface area of electrode, D_R is diffusion coefficient, c is the concentration of $K_3Fe(CN)_6$ and υ refers to the scan rate. For $K_3Fe(CN)_6$, n = 1, and $D_R = 7.6 \times 10^{-6}$ cms^{-1}. For bare GCE the surface area was found to be 0.0828 cm^2. There was an enhancement in the effective surface area to 0.10854 cm^2 when GCE was modified with MWCNT. This enlargement in the effective surface area provided an additional proof for the modification of bare electrode with MWCNT.

3.5 Performance Characteristics of the Developed Sensor

The functional potential of the developed sensor depends on many factors including effect of pH, effect of the amount of MWCNT/Nafion dispersion, effect of scan rate, effect of accumulation time, range of linear response, detection limit and interference study. Each of these parameters are discussed in the next section.

3.5.1 Effect of pH

The effect of pH on the anodic peak current of 1×10^{-3} M AMB at the MWCNT/Nafion /GCE was investigated by CV. The pH range studied was from 2-9. However in solutions with pH above 7, AMB gets precipitated. A well-defined oxidation peak and a high peak current was obtained at pH 4. So pH 4 was selected as the optimal pH. (Figure **3**.**5**).

3.5.2 Effect of the amount of MWCNT-Nafion dispersion

The effect of the amount of MWCNT dispersion on the anodic peak current of 1×10^{-3} M AMB was studied. (Figure **3.6**). As the amount of MWCNT dispersion was increased up to 6 μL, the oxidation peak current greatly enhanced. The enhancement of the current indicates that the specific surface area and the number of catalytic sites increased with an increase of MWCNT. When the amount of MWCNT dispersion was more than 6 μL, the oxidation peak current decreased slightly. This indicates that the excess of MWCNT blocked the electron transfer of AMB. So the amount of MWCNT dispersion was fixed to be 6 μL.

3.5.3 Effect of accumulation time

As for any techniques employing preconcentration, the accumulation time is of significant importance for the voltammetric signal. The effect of accumulation time on the peak current of 1×10^{-3} M AMB was investigated by open circuit potential without stirring. The anodic peak current increased within the first 4 min and then leveled off (Figure **3.7**). This indicates that the MWCNT modified electrode surface is almost saturated with AMB after the accumulation for 4 min. From this it can be inferred that a saturation accumulation is reached only after 4 minutes.

3.5.4 Effect of scan rate

The oxidation peak current of 1×10^{-3} M AMB at different scan rates ranging from 50-250 mV/s was measured by LSV using the same modified electrode. It was found that the anodic peak current increased with an increase in the scan rate (υ) (Figure **3.8**). The oxidation peak current varied linearly with square root of scan rate, indicating that the oxidation of AMB at the MWCNT/Nafion/ GCE is diffusion controlled.

According to the Laviron's conclusion [203] the relationship between the peak potential (E_p) and υ was examined. It was found that E_p varied linearly with ln υ (Figure **3.9**) which is a characteristic curve for the diffusion controlled process [204]. The no. of electrons involved in the reaction can be calculated from the slope of the plot according to $b= RT/ \alpha n_a F$, where b is the slope. α of the totally irreversible electrode process is assumed as 0.5. The obtained value for n_a is around 1, which indicates that only one electron is involved in the oxidation of AMB. Therefore a mechanism (Figure **3.10**) can be proposed which involves 1 e^- oxidation of the amino group of AMB to a radical cation which then binds together to give dimeric compound [205].

3.5.5 Calibration curve

The relationship between the anodic peak current of AMB and its concentration was investigated by LSV. The working concentration range for the developed sensor was found to be 1×10^{-2} M-1×10^{-5} M. At regions of very low concentrations the sensor becomes insensitive. The detection limit was obtained from the graph and was found to be 6×10^{-6} M. (Figure **3.11**)

The reproducibility of the MWCNT/Nafion/GCE was examined by repetitive measurement of oxidation peak current of 1×10^{-3} M AMB using the same electrode. After each determination, the used modified electrode surface was regenerated by repetitive cyclic scan in the supporting electrolyte. After several successive measurements, only slight deviation of the peak current (RSD is 3.6%) was observed suggesting that the MWCNT/Nafion/GCE has excellent reproducibility.

3.5.6 Interference study

Several organic and inorganic species can interfere with the determination of AMB using the developed sensor. The selectivity of the

sensor is determined by measuring the change in the sensor response in the presence of foreign compounds. It was found that a 100-fold excess molar concentration of glucose, Na^+, Cl^-, K^+, SO_4^{2-}, glutamic acid, lactose, and citric acid almost did not influence the current response of 5×10^{-4}M AMB (signal change below 5%). However ascorbic acid was found to interfere with the determination of AMB using the developed sensor. The results are tabulated in Table **3.3**

3.6 Analytical Applications

The sensor developed for AMB was employed for its determination in tablet form. The utility of the developed sensor in the determination of AMB in real sample like urine was also studied.

3.6.1 Determination of AMB in Pharmaceutical Formulations (Tablets)

The developed sensor was applied to the determination of AMB in pharmaceutical formulation commercialized as Ambrodil hydrochloride (Aristo, India) containing 30 mg of the drug, as declared by the company.

The AMB content in the tablet was determined using the developed sensor by calibration method. A detailed procedure for the determination is given in section 2.8.1 of Chapter 2. The results obtained are summarized in Table **3.4**. The results obtained from the measurements are found to be in satisfactory agreement with the declared amount. The close agreement of the found values with the declared amount is indicative of non-interference of the other ingredients and excipients that are present in the formulation.

In order to test the reliability of this method, the results obtained by the MWCNT/Nafion/GCE method were compared with those obtained by

the standard pharmacopoeial method [183].The results are illustrated in Table **3.4**. The results show that the developed sensor is highly reliable for the determination of AMB in pharmaceutical formulations.

3.6.2 Determination of AMB in urine sample

The developed sensor was applied for the determination of the AMB in spiked urine samples. 5 mL of urine samples were taken in a series of 25 mL standard flasks. Different quantities of AMB (ranging from 4-12 mg) were added to these urine samples and then quantitatively diluted using the supporting electrolyte. The voltammograms were recorded and the unknown concentrations were determined from the calibration graph. The results are shown in Table **3.5.** The average % recovery of AMB obtained using the developed sensor was found to be 100.9

3.7 Conclusion

A voltammetric sensor was developed for the selective determination of AMB by LSV and CV. AMB gave a well defined oxidation peak at MWCNT/Nafion/GCE. The linear range was found to be 1×10^{-2} M-1×10^{-5} M and the detection limit was found to be 6×10^{-6} M. MWCNTs showed electrocatalytic action for the oxidation of AMB, characterized by the enhancement of the peak current which was probably due to the fast electron transfer ability of MWCNT, increase in the number of reaction sites and also due to the larger effective surface area of MWCNTs. The fabricated sensor was successfully applied for the determination of AMB in pure form and dosage forms. The developed sensor gave very close values to the declared amount of 30 mg per tablet. The sensor was also applied for the determination of the drug in urine sample.

Table 3.1 Surface Area Study on bare GCE using 2mM $K_3[Fe(CN)_6]$

Sl.No	Scan rate(mV/s)	Current(μA)
1	10	4.3244
2	20	5.9022
3	30	7.1299
4	40	8.2309
5	50	9.5705
6	60	10.8705

Table 3.2 Surface Area Study on MWCNT/Nafion/GCE using 2mM $K_3[Fe(CN)_6]$

Sl.No	Scan rate(mV/s)	Current(μA)
1	10	8.3833
2	20	10.5562
3	30	12.2164
4	40	14.2309
5	50	16.5705
6	60	18.8705

Table 3.3 Study on the effect of foreign species on the oxidation peak current of 5×10^{-4}M AMB

Substance	Concentration, M	Current (mA)	Signal change, %
AMB	5×10^{-4}	0.0300	-
Lactose	1×10^{-2}	0.0298	0.67
Na^+	1×10^{-2}	0.0289	3.98
Cl^-	1×10^{-2}	0.0289	3.98
K^+	1×10^{-2}	0.029	3.33
SO_4^{2-}	1×10^{-2}	0.029	3.33
Glutamic acid	1×10^{-2}	0.0312	3.64
Ascorbic acid	1×10^{-2}	0.0273	9.30
Glucose	1×10^{-2}	0.0287	4.67
Citric acid	1×10^{-2}	0.0294	2.33

Table 3.4 Determination of AMB in tablets

Sample	Declared Amount (mg/tablet)	Method Adopted	Found* (mg/tablet)	S.D*	C.V*
Ambrodil HCl	30	Developed Sensor	30.0	0.82	2.73
		Standard Method	31.4	1.01	3.21

*Average of six replicates

Table 3.5 Analysis of AMB in urine sample

Added (mg)	Found (mg)	Recovery %
5.0	5.3	106.0
7.0	7.0	100.0
9.0	8.7	96.7

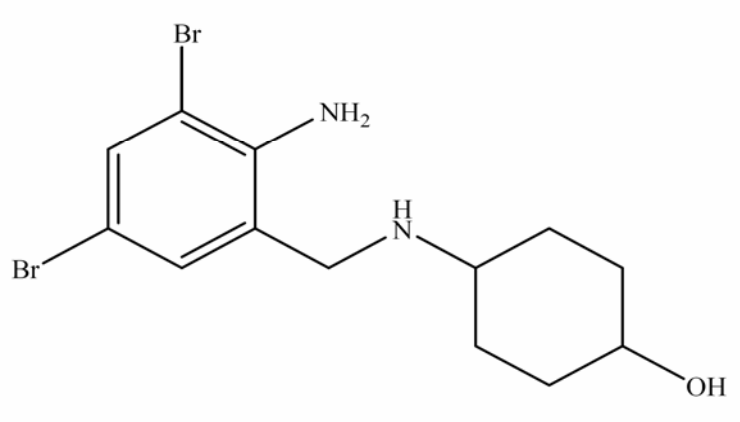


Figure 3.1 Structure of AMB

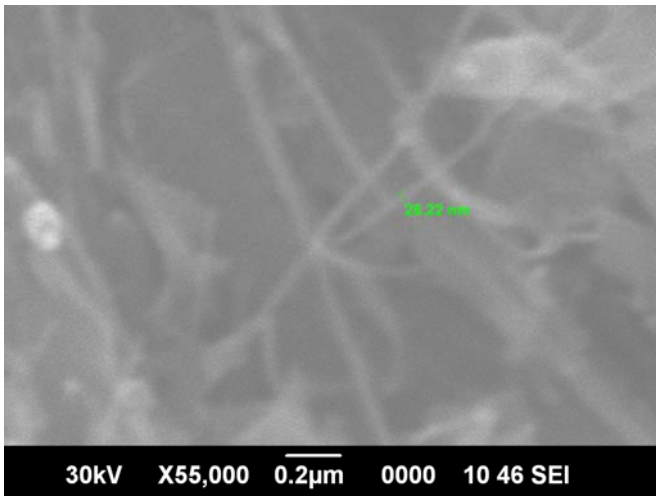
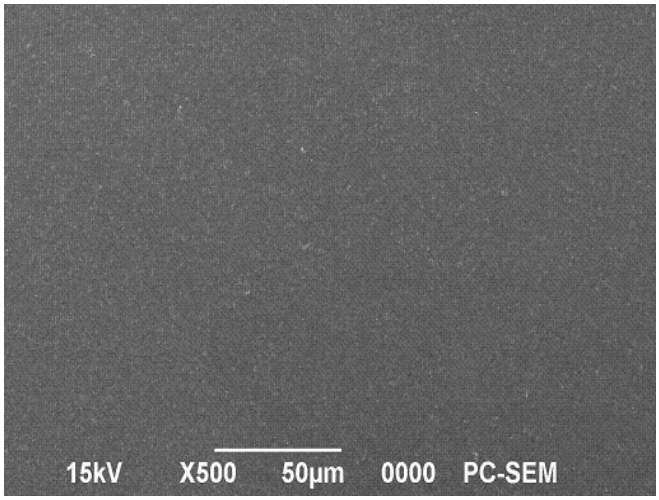
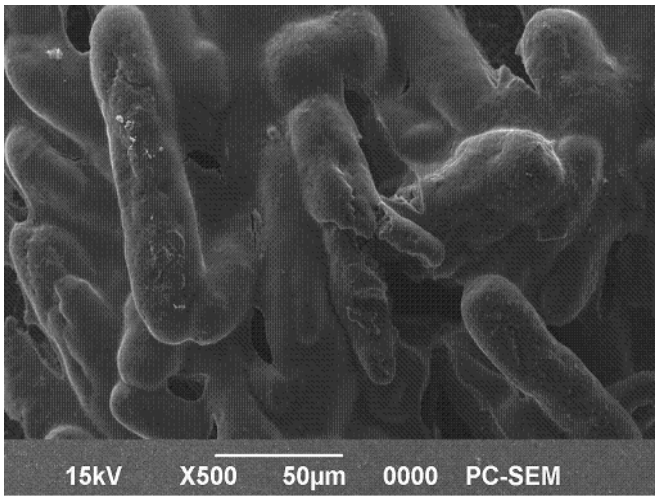


Figure 3.2 SEM image of MWCNT after acid treatment.



(a)



(b)

Figure 3.3 SEM images of (a) bare GCE and (b) MWCNT/Nafion modified GCE

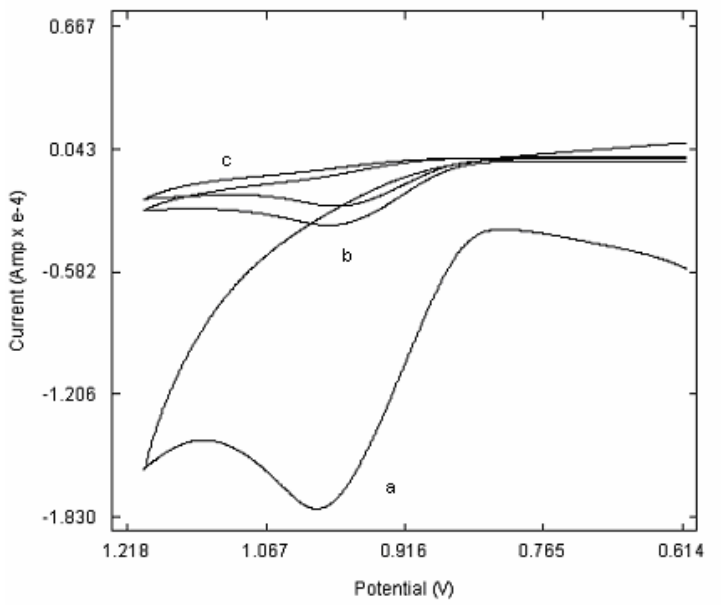


Figure 3.4 Cyclic Voltammogram of AMB at (a) MWCNT/ Nafion / GCE (b) Nafion/GCE and (c) bare GCE

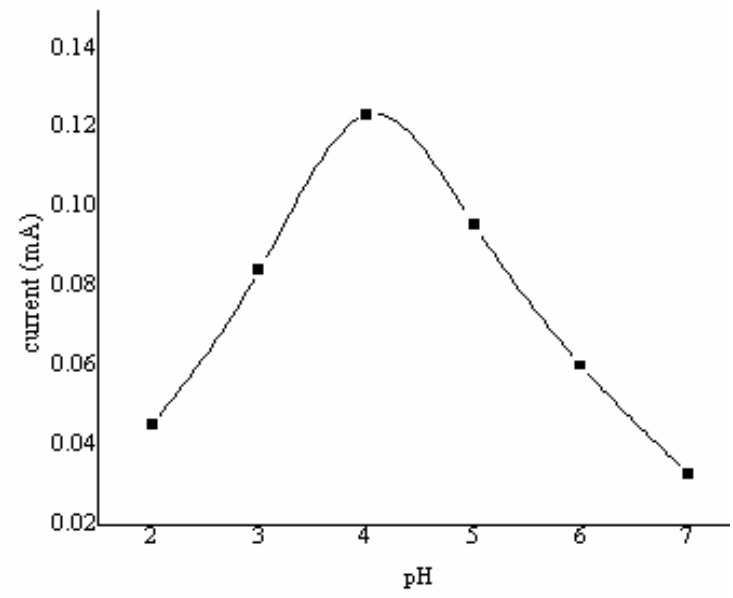


Figure 3.5 Effect of pH on the oxidation peak current of AMB

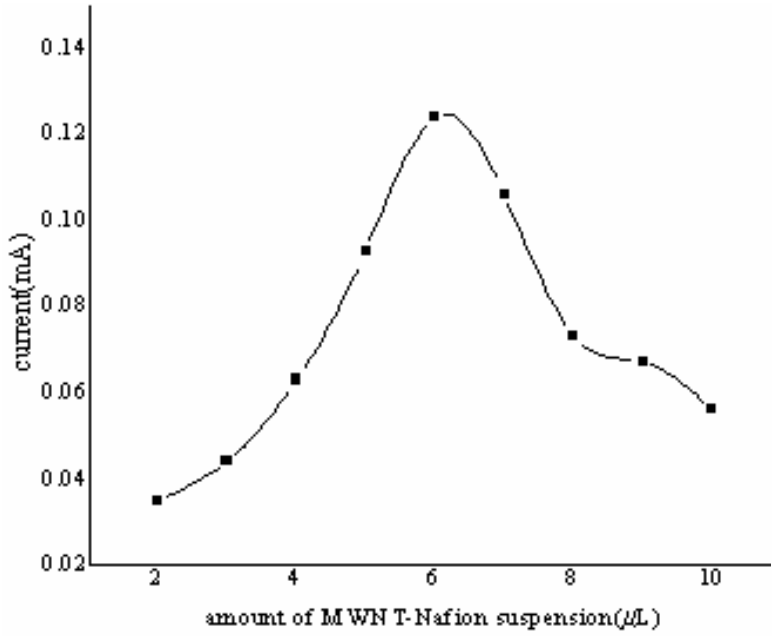


Figure 3.6 Effect of the amount of MWCNT- Nafion Suspension

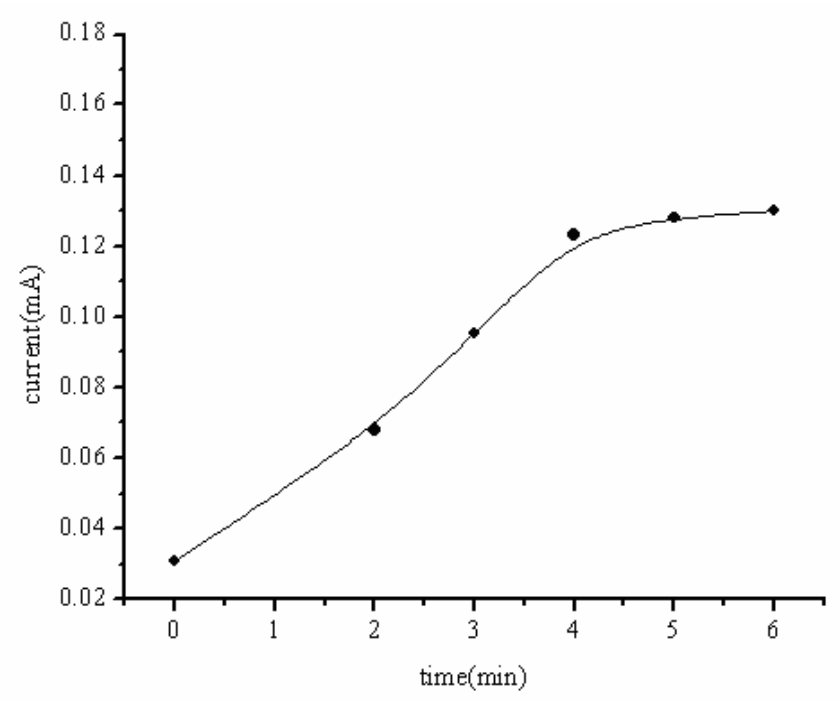


Figure 3.7 Effect of accumulation time

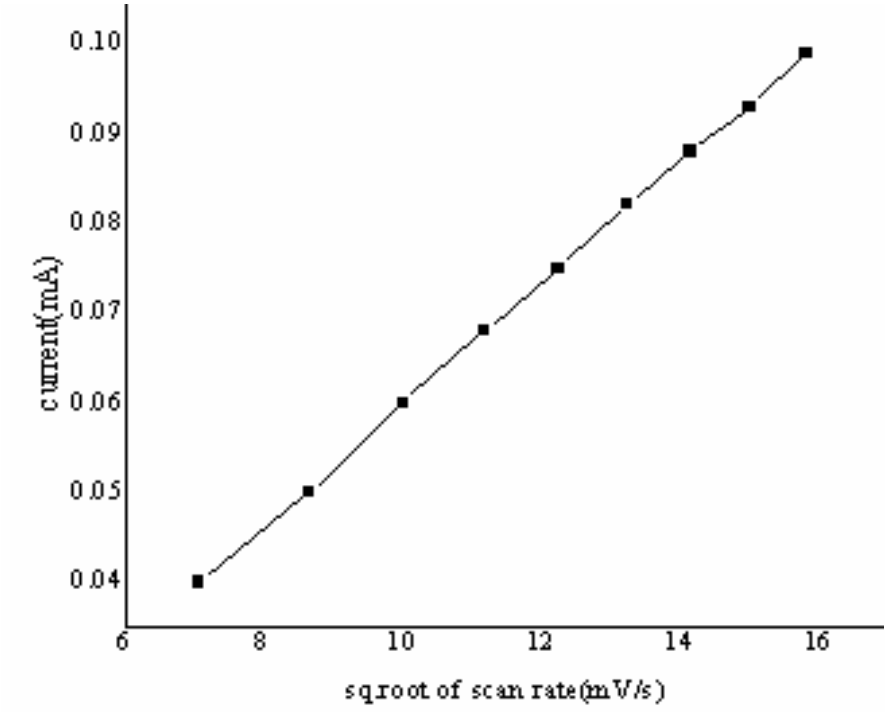


Figure 3.8 Effect of scan rate

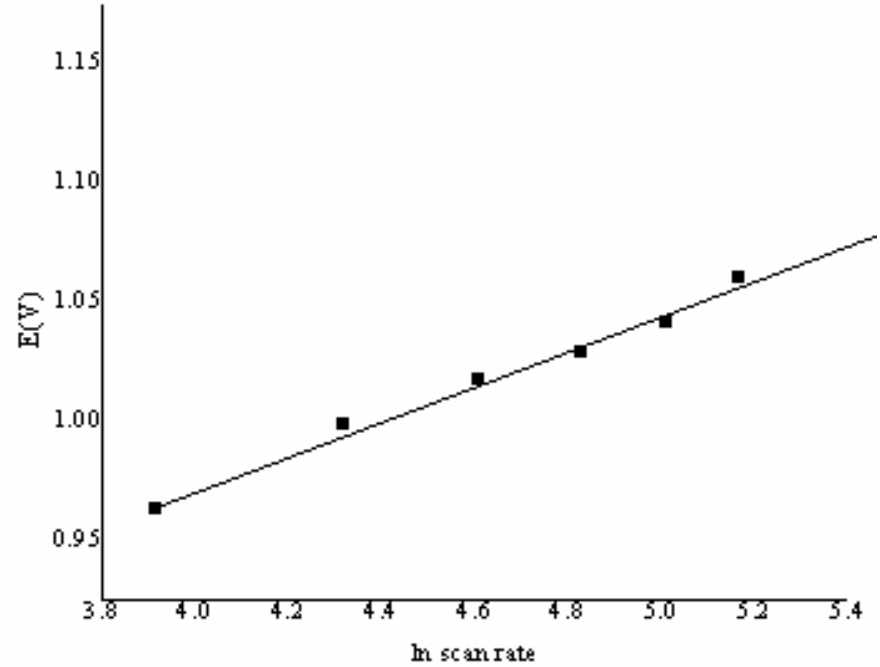


Figure 3.9 Plot of peak potential against ln (scan rate)

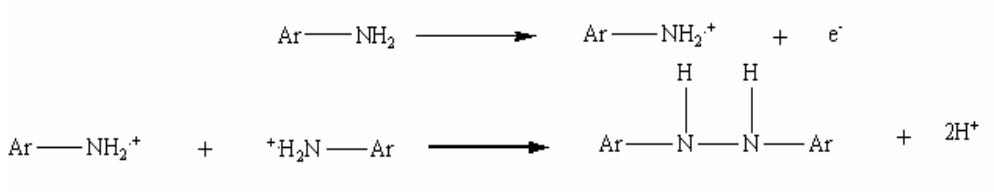


Figure 3.10 Mechanism of oxidation of amino group in AMB to a dimeric compound.

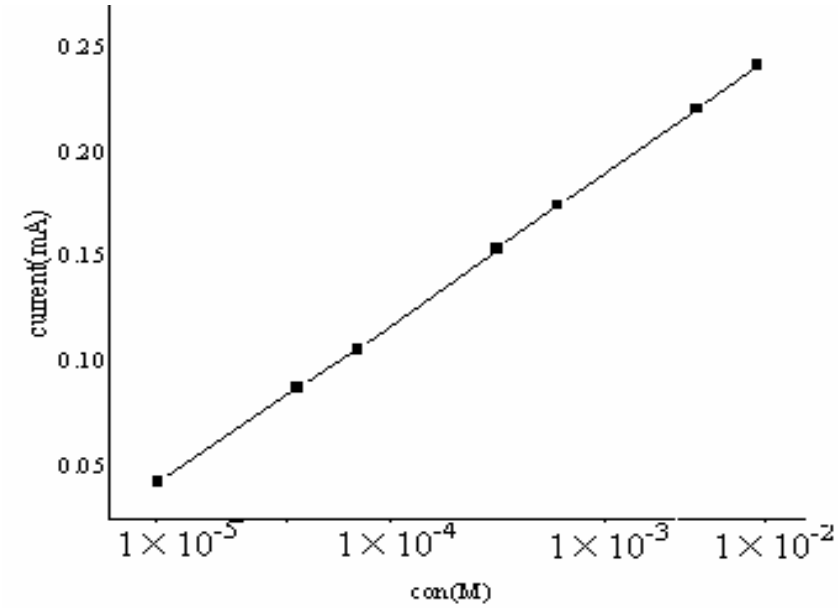


Figure 3.11 Calibration Curve

Chapter 4

SENSOR FOR THE DETERMINATION OF SULFAMETHOXAZOLE

CONTENTS

This chapter deals with the development of an electrochemical sensor for the determination of Sulfamethoxazole (SMX) at MWCNT/Nafion modified GCE. SMX gave a well defined oxidation peak at 0.74 V in 0.1 M phosphate buffer solution (PBS) of pH 8.0. The experimental parameters such as the amount of MWCNT -Nafion suspension, pH of the supporting electrolyte and scan rate were optimized and a direct electrochemical method for the determination of SMX was developed. Under optimum conditions the oxidation peak current was linear to the concentration of SMX in the range 1×10^{-2} - 5×10^{-5} M with a detection limit of 1×10^{-5} M. The developed sensor showed good stability, selectivity and was successfully used to quantify SMX in pharmaceutical formulations and urine sample.

4.1 Introduction

Sulfamethoxazole, 4-amino-N-(5-methylisoxazol-3-yl)-benzenesulfo namide, (Figure **4.1**) is a sulfonamide bacteriostatic antibiotic. The molecular formula of SMX is $C_{10}H_{11}N_3O_3S$. It is almost white, odorless and tasteless compound with a molecular mass of 253. SMX competitively inhibits the conversion of *p*-aminobenzoic acid to dihydropteroate, which bacteria need for folic acid synthesis and ultimately for purine and DNA synthesis. Thus it prevents the formation of dihydrofolic acid, a compound that bacteria needs in order to survive. Humans do not synthesize folic acid but acquire it in their diet, so their DNA synthesis is less affected. SMX is often used as a part of synergistic combination with trimethoprim in a 5:1 ratio. Its primary activity is against susceptible forms of Streptococcus, Staphylococcus aureus, Escherichia coli, Haemophilus influenza and oral anaerobes. It is commonly used to treat urinary tract infections. In addition it can be used as an alternative to amoxicillin based antibiotics to treat sinusitis [206,207].

Due to the vital importance of SMX determination in pharmaceutical preparations and in biological fluids, several analytical methods have been developed for the quantitative determination of SMX. These include spectrophotometric method [208,209], flow injection spectrophotometric method [210], the Bratton–Marshall method [211,212], titrimetric assay method [213,214], gas chromatography and gas chromatography-mass spectrometry [215], high performance liquid chromatography [216] and high performance thin layer chromatography [217]. However, most of these methods require expensive and sophisticated instruments and are time consuming. Hence a voltammetric technique, which is relatively simple, cost effective and sensitive, has been developed here for the analysis of SMX.

This chapter describes the fabrication of MWCNT/Nafion modified glassy carbon sensor for the determination of SMX in pure form and in dosage forms. A uniform suspension of MWCNT was prepared in nafion-water mixture via ultrasonication. This suspension was then dropped on the surface of a clean GCE and dried in air to get a film of MWCNT modified electrode. The electrochemical behavior of SMX was studied at this modified electrode by differential pulse voltammetry (DPV). A remarkable peak current enhancement as well as a significant reduction in the oxidation potential of SMX was observed using the modified electrode. The performance characteristics of the developed sensor were also studied. The developed sensor was then successfully applied for the determination of SMX in tablets as well as its recovery from urine samples.

4.2 Fabrication of MWCNT/Nafion/GCE

First, acid treatment of MWCNT was carried out as explained under section 3.2 of Chapter 3.

The detailed procedure for the fabrication of MWCNT/Nafion/GCE is given in section 2.4.1 of Chapter 2. From the homogenous suspension of MWCNT in nafion water mixture, 4 μL was taken using a micro pipette and then dropped on to the surface of the cleaned GCE. The electrode was then allowed to dry in air to obtain the MWCNT/Nafion/GCE. SEM images of the bare and modified electrode (shown in figure **3.2** of Chapter 3) showed clearly that the electrode surface was effectively modified with nanotubes.

4.3 Electrochemical determination of SMX

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a PC-controlled BAS Epsilon Electrochemical

workstation. A three-electrode system was employed with MWCNT/Nafion /GCE as working electrode, Pt wire as counter electrode and Ag/AgCl as reference electrode.

The stock solution of SMX was prepared in methanol as explained under section 2.5.2 of Chapter 2. The dilution series were prepared by serial dilution of the stock solution with supporting electrolyte (PBS, pH 8). The standard solution of SMX (5×10^{-3}M) was taken in the electrochemical cell. Then its electrochemical behavior at bare GCE and MWCNT/Nafion/GCE was studied by DPV. SMX gave a well defined oxidation peak at 0.74 V with a peak current of 0.0476 mA at MWCNT/Nafion/GCE and an oxidation peak at 0.83 V for bare GCE. Compared with bare GCE, the oxidation peak potential of SMX at MWCNT/Nafion/GCE had shifted negatively by about 100 mV. Also, the anodic peak current of SMX was greatly increased at the MWCNT/Nafion/GCE (Figure **4.2**). Thus by modifying GCE with MWCNT, the electrode reactivity was found to be tremendously increased as indicated by the enhancement in peak current as well as lowering of peak potential. The remarkable peak current enhancement and the decrease in oxidation potential undoubtedly proved the electrocatalytic activity of MWCNT/Nafion/GCE towards the oxidation of SMX. The ability of MWCNT to promote electron transfer resulted in the reduction of peak potential and enhancement of peak current of SMX at MWCNT/Nafion/GCE. Also, the increase in effective surface area of GCE when modified with MWCNT, resulted in the enhanced sensitivity towards SMX. The anodic peak was due to the oxidation of electrochemically active amino group on SMX [218]. No reduction peak was observed for SMX in the reverse sweep of Cyclic Voltammetry (CV) indicating an irreversible electrochemical process.

4.3.1 Surface Area Study

The surface areas of both bare and MWCNT/Nafion/GCE were determined as detailed in section 3.4.1 of chapter 3. The effective surface area of the modified electrode was found to be higher than that of the bare electrode (section 3.4.1). This enhancement in surface area resulted in the remarkable catalytic activity of the modified electrode characterized by the increase in peak current and reduction in peak potential.

4.4 Optimisation Studies

The response characteristics of the developed sensor depend on various parameters such as effect of supporting electrolyte, pH, scan rate, amount of MWCNT-Nafion dispersion and concentration.

4.4.1 Effect of supporting electrolyte

The electrochemical behavior of SMX (1×10^{-3}M) in various media such as 0.1 M solutions of PBS, H_2SO_4, NaOH, tetra- n butyl ammonium chloride and KNO_3 were studied by DPV. The obtained oxidation peak was best defined in 0.1 M PBS. So 0.1 M PBS was taken as the experimental medium for SMX.

4.4.2 Effect of pH

The influence of pH on the anodic peak current of SMX (1×10^{-3}M) at the MWCNT/Nafion/GCE was studied by DPV (Figure **4.3**). The pH range studied was from 4-9. The oxidation peak current increased with an increase in the pH and thereafter decreased. It was found that a high peak current as well as a well-defined oxidation peak was obtained at pH 8. So PBS of pH 8 was chosen as the electrolyte for the determination of SMX.

4.4.3 Influence of the thickness of MWCNT-Nafion film

The MWCNT/Nafion cast film thickness is determined by the amount of MWCNT/Nafion suspension. It was found that the anodic peak current of SMX (5×10^{-3}M) is dependent on the thickness of MWCNT/Nafion film (Figure **4.4**). When 4 μL of MWCNT/Nafion suspension was used to cast on the surface of the electrode, the anodic peak current reached its maximum value. The enhancement of the current indicates that the specific surface area and the number of catalytic sites increase with an increase of MWCNT. Further increase in the amount of MWCNT/Nafion suspension caused a decrease in the anodic peak current. This indicates that the excess of MWCNT blocks the electron transfer of SMX. This is because Nafion is an insulator and blocks electron transfer and so the peak current decreases when the MWCNT/Nafion film is too thick. So the amount of MWCNT dispersion was fixed to be 4 μL.

4.4.4 Influence of scan rate

Influence of scan rate on the anodic peak current of 1×10^{-3}M SMX at the MWCNT/Nafion/GCE was investigated in the range of 10–80 mV/s by DPV. It was found that the anodic peak current increased with an increase in the scan rate. A linear graph was obtained for the plot of anodic peak current vs square root of scan rate (Figure **4.5**). Thus it can be concluded that the electrochemical oxidation of SMX at the MWCNT/Nafion modified GCE is diffusion controlled.

4.4.5 Concentration Study

Under optimum conditions, the anodic peak currents of SMX increased linearly with its concentration. The experimental results showed that the anodic peak current was linear with the concentration of SMX in

the range 1×10^{-2}- 5×10^{-5} M (Figure **4.6**).The detection limit was found to be 1×10^{-5} M.

The MWCNT modified electrode surface after each determination was regenerated by repetitive cycling in the supporting electrolyte. For five successive determination of 1×10^{-3} M SMX with the same electrode regenerated after every determination, the RSD of the peak current was 4.2%. This indicated that the MWCNT/Nafion/GCE has excellent reproducibility.

4.4.6 Interference Study

Under optimised experimental conditions, the effects of some foreign species on the determination of SMX (1×10^{-3} M) were evaluated in detail. The results are given in Table **4.1**. 100-fold concentration of glucose, lactose, citric acid, Na^+, K^+, SO_4^{2-} and Cl^- have almost no influence on the current response of SMX (signal change below 5 %). Since SMX is often used as a part of synergistic combination with trimethoprim in tablets, the influence of trimethoprim on the oxidation peak current of SMX was studied. It was found that a 10-fold concentration of trimethoprim almost did not interfere in the determination of SMX. This showed that the developed sensor could be used for the selective determination of SMX.

4.5 Application Studies

The developed sensor was employed for the determination of the drug content in its tablet form. The application of the developed sensor in the determination of SMX in real sample like urine was also studied.

4.5.1 Drug analysis

The developed sensor was successfully applied for the determination SMX in commercially available pharmaceutical formulations such as Bactrim (Piramal Healthcare, India) and Septra (Burroughs Wellcome, India).

A detailed procedure for the determination of SMX in tablets is given in section 2.8.2 of Chapter 2. The results are shown in Table **4.2**. The results obtained are in good agreement with the declared SMX content and showed a high degree of precision [coefficient of variation (C.V) is 0.28 %].

The results were compared with those obtained by the standard method (potentiometric titration) [184] reported in United States Pharmacopoeia. The results show that there is a satisfactory agreement between the SMX content determined by the developed method and the standard method.

4.5.2 Determination of SMX in urine sample

The developed sensor was also applied for the determination of SMX in biological fluids. The sensor was applied to the recovery of SMX from urine samples. 5 mL of urine samples were taken in a series of 25 mL standard flasks. Different quantities of SMX (ranging from 4-20 mg) was added to these urine samples and then quantitatively diluted using the supporting electrolyte (PBS, pH 8). The electrochemical behavior of the prepared solutions on MWCNT/Nafion/GCE was studied by DPV and the unknown concentrations were determined from the calibration graph. The results are shown in Table **4.3**. The results showed that the developed sensor can determine the investigated drug in urine samples with high accuracy and high % recovery. The recovery obtained was in the range of 98 – 102 %.

4.6 Conclusion

Voltammetric determination of SMX was carried out at a MWCNT/ Nafion modified sensor by DPV and CV. MWCNT acted as an efficient electrocatalyst in the determination of SMX as it caused an enhancement in the peak current and reduction in the peak potential. SMX gave an oxidation peak at 0.74 V at MWCNT/Nafion/GCE and the oxidation was found to be an irreversible process. Under optimum conditions the oxidation peak current was proportional to the concentration of SMX in the range 1×10^{-2} M to 5×10^{-5} M with a detection limit of 1×10^{-5} M. Thus MWCNT/Nafion /GCE provided a good platform for the determination of SMX in pure form, dosage forms and in urine sample. The developed method is a good alternative for the determination of SMX because it is simple, fast and cost effective and it has sufficient precision, accuracy and sensitivity.

Table 4.1 Interference study

Substance	Concentration (M)	Current(mA)	Signal change, %
SMZ	0.001	0.0302	
Glucose	0.05	0.0297	1.65
Lactose	0.05	0.0306	1.32
Citric acid	0.05	0.0312	3.33
Trimethoprim	0.01	0.0282	4.97
Na^+	0.05	0.0296	1.65
K^+	0.05	0.0298	1.32
Cl^-	0.05	0.0296	1.65
SO_4^{2-}	0.05	0.0298	1.32
CH_3COO^-	0.05	0.0318	1.25

Table 4.2 Determination of SMX in tablets

Sample	Declared Amount (mg/tablet)	Method Adopted	Found* (mg/tablet)	S.D*	C.V*
Bactrim (Piramal Healthcare, India)	800	Developed sensor	800	2.0	0.25
		Standard Method	802	1.8	0.22
Septran (Burroughs Wellcome, India)	400	Developed sensor	402	1.2	0.31
		Standard Method	398	1.6	0.4

* Average of six replicates

Table 4.3. Determination of SMX in urine sample

Added (mg)	Found (mg)	Recovery (%)
6.0	5.9	98.2
10.0	10.2	102.2
14.0	13.8	98.8

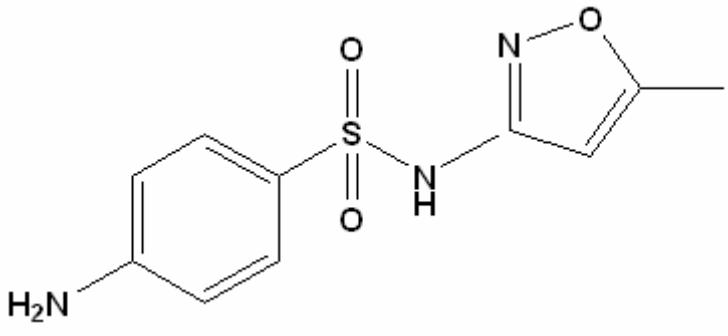


Figure 4.1 Structure of Sulfamethoxazole

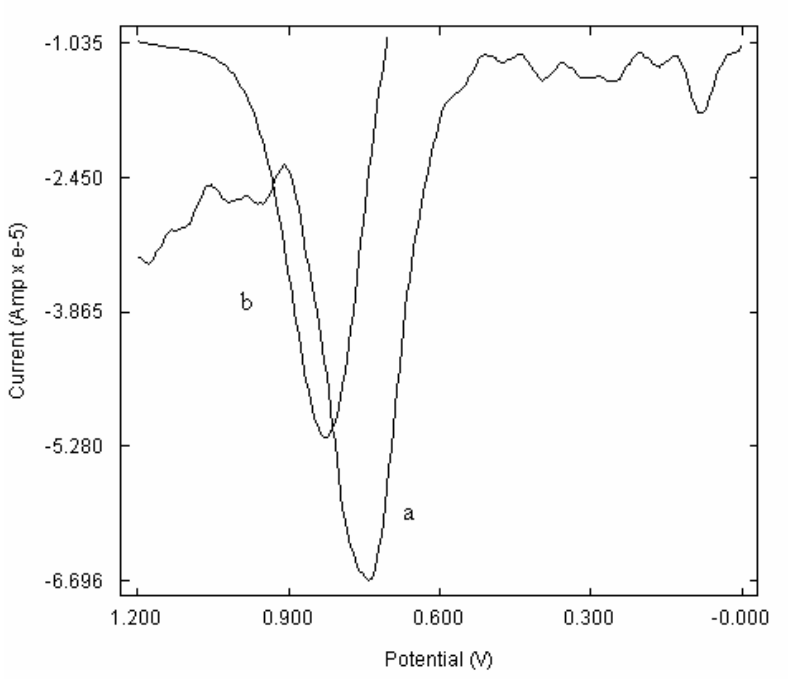


Figure 4.2 Differential pulse voltammogram of SMX at (a) MWCNT /GCE and (b) bare GCE

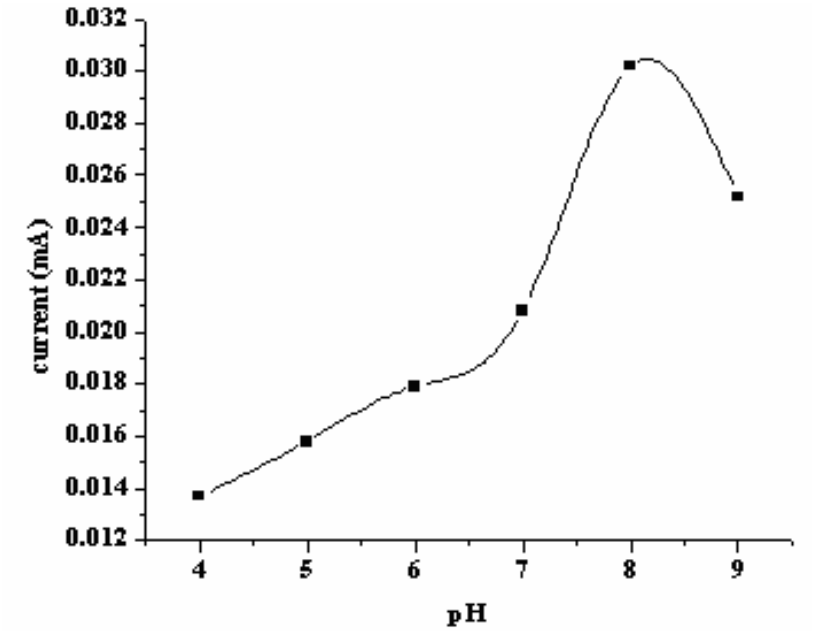


Figure 4.3 Effect of pH on the anodic current of 1×10^{-3}M SMX.

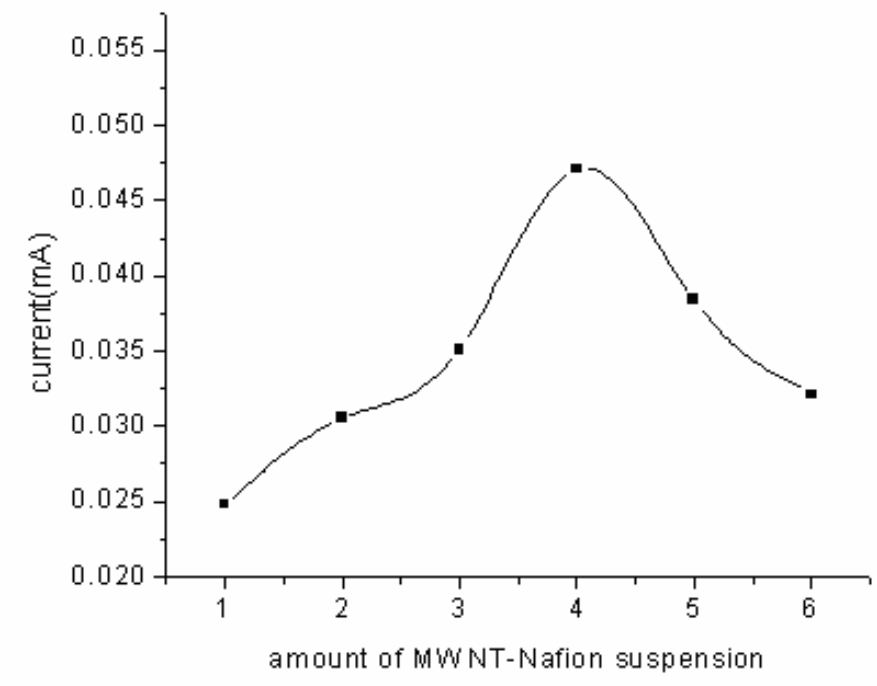


Figure 4.4 Effect of the amount of MWCNT-Nafion suspension.

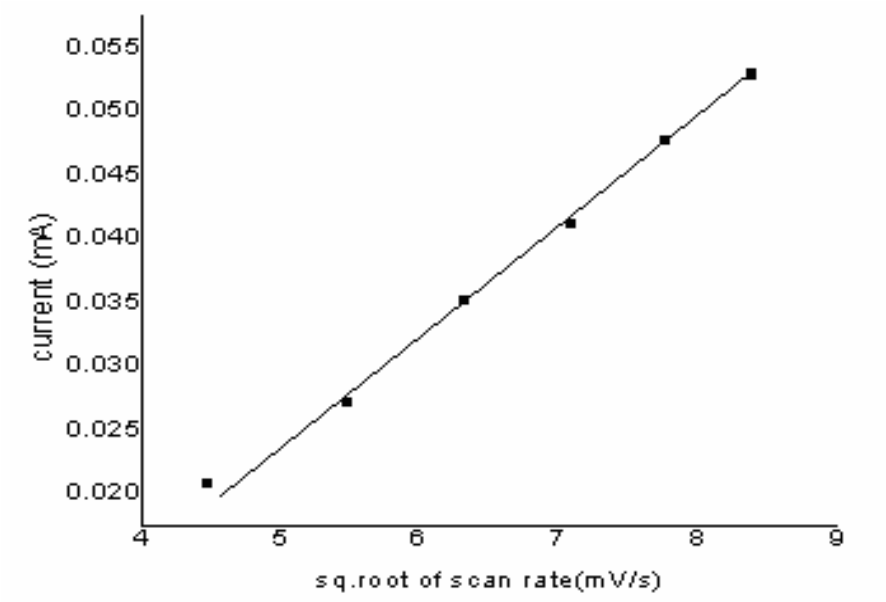


Figure 4.5 Effect of scan rate

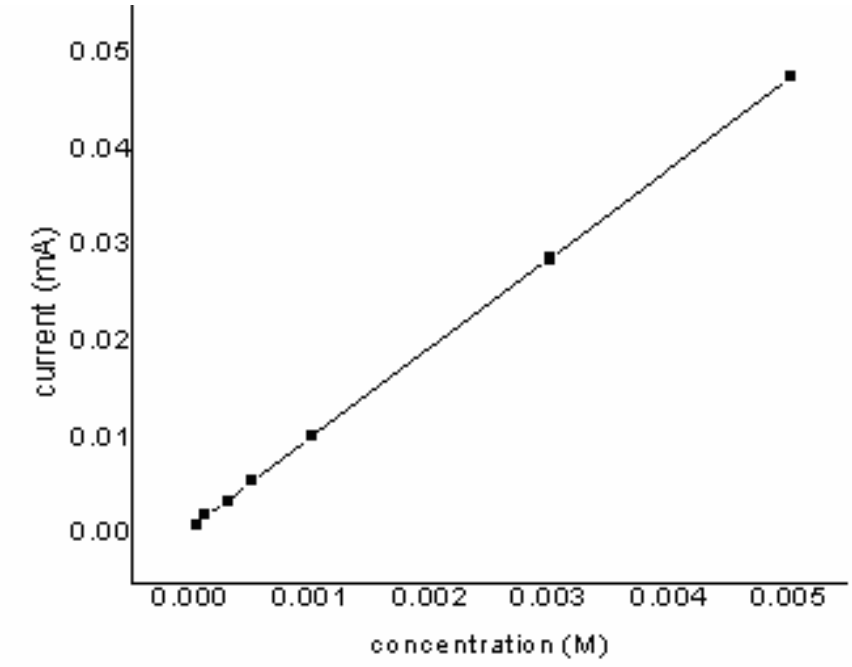


Figure 4.6 Calibration graph for SMX at MWCNT modified GCE at 25^0C

…..ഇ൹ര…..

Chapter 5

SENSOR FOR THE DETERMINATION OF PAM CHLORIDE

CONTENTS

The construction and performance characteristics of a poly p-toluene sulfonic acid [poly (p-TSA)] modified glassy carbon sensor for the drug Pyridine-2-Aldoxime Methochloride (PAM Chloride) has been discussed in this chapter. The poly (p-TSA) modified GCE was fabricated by electropolymerisation using CV. The electrochemical behavior of PAM Chloride at this modified electrode has been studied by CV and SWV. Compared with bare GCE, the modified electrode showed an enhancement in the oxidation peak current as well as a reduction in the oxidation potential of PAM Chloride. Under optimum conditions the oxidation peak current was proportional to the concentration of PAM Chloride in the range 1×10^{-3} M to 1×10^{-7} M with a detection limit of 3×10^{-8} M. The developed sensor was also successfully applied for the determination of PAM Chloride in body fluids.

5.1 Introduction

PAM Chloride (2-formyl-1 methylpyridinium chloride oxime) belongs to a family of compounds called oximes (Figure **5.1**). The principal action of PAM Chloride is to reactivate cholinesterase (mainly outside the central nervous system) which has been inactivated by phosphorylation due to an organophosphate pesticide or related compound [219,220].

An understanding of the enzyme acetylcholine esterase (AChE) has led to the development of drugs for the treatment of myasthenia gravis and Alzheimer's disease. Regrettably, exploitation of this enzyme in a very different direction has also occurred. In 1936, German chemists recognized that minute amounts of certain organophosphate molecules could induce a cholinergic crisis. This spurred development of these molecules for use as chemical weapons.

Organophosphates are well established as agricultural insecticides. However, the organophosphates developed for military use are approximately 100,000 times more potent. While they are commonly referred to as nerve gas, "nerve agents" is the preferred term for these poisons, which are dispersed as aerosol and form vapor under normal atmospheric conditions [221].

The four nerve agents that have received the greatest military attention are tabun, sarin and soman (among the so-called G agents), and VX. The two clinically significant characteristics that distinguish the nerve agents from one another are the primary mode of absorption and the pharmacokinetics involved in the interaction between the agents and AChE.

When a nerve agent initially binds with AChE, the interaction is reversible; the undocking of the poison will restore normal function to the enzyme. With time, however, these agents lose an alkyl group, which changes the kinetics of the enzyme-poison complex, permanently deactivating the enzyme. This process, termed aging, occurs at different rates. Aging of the soman-enzyme complex is the fastest; it is 50% complete in as little as two minutes. VX and organophosphate insecticides age slowly over several days.

Organophosphates inhibit cholinesterase by phosphorylation of the enzyme. The most important role of PAM Chloride is to reactivate the cholinesterase by removing the phosphoryl group that is bound to the ester group. In this reaction both the organophosphate and the pralidoxime are mutually inactivated. These products undergo rapid metabolism, leading to the removal of the organophosphate.

PAM Chloride is also used to treat overdose of medicines, such as ambenonium, neostigmine, and pyridostigmine that are used to treat myasthenia gravis. Poisoning with these chemicals or medicines causes our muscles, including the muscles that help us breathe, to become weak. PAM Chloride help us to get back strength in our muscles.

Despite its pivotal role in military purpose, only a few analytical methods have been reported so far for the determination of PAM Chloride. It mainly includes spectrophotometric method [222], liquid chromatographic method [223] and isotachophoretic method [224]. But these reported methods 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. Hence a simple,

selective, eco-friendly and cost effective technique is required for the routine analysis of PAM Chloride in pure form as well as in biological fluids.

The presence of an electrochemically active aldoxime group prompted us to develop an electrochemical technique for the determination of PAM Chloride. Also, electrochemical methods are highly attractive as these techniques are found to be simple, fast, convenient and cost effective.

The modification of electrode surface with suitable materials results in efficient determination of electro-active biomolecules at very lower potential without its major interferences [225,226]. Recently, lots of polymer films have been widely utilized in the electroanalytical field because they are easier to generate on the electrode surface than monolayers. As it is easy to control the film thickness, permeation and charge transport characteristics by adjusting the electrochemical parameters, electropolymerisation remains as a good approach to immobilize polymers to prepare Polymer Modified Electrodes (PMEs) [180]. The present work explains the electropolymerisation of p-TSA on the surface of GCE by CV to form a poly (p-TSA) film.

As part of the present investigation the electrochemical behaviour of PAM Chloride at poly (p-TSA) modified GCE was studied in detail. The poly (p-TSA) modified GCE was fabricated by electropolymerisation of p-TSA using Cyclic Voltammetry (CV). PAM Chloride gave a well defined oxidation peak at 0.6V at this developed sensor. Compared with bare GCE, there was an increase in the peak current and reduction in the peak potential of PAM Chloride at the modified electrode. The developed sensor was also successfully applied for the determination of PAM Chloride in real sample like urine.

5.2 Preparation of poly (p-TSA) modified GCE

The poly (p-TSA)/GCE was prepared electrochemically using a BAS Epsilon electrochemical workstation with a conventional three electrode system, including GC working electrode, a platinum wire counter electrode and an Ag/AgCl reference electrode.

Firstly the GCE was cleaned as explained under section 2.3 of Chapter 2. The poly (p-TSA)/GCE was prepared by electropolymerisation of p-TSA on GCE. The detailed procedure for the fabrication of poly (p-TSA)/GCE is given in section 2.4.2 of Chapter 2.

With the potential scanning from -2.0 to 2.5 V, two strong reduction peaks at -0.539 V and -1.646V and one oxidation peak at 1.5 V were observed (Figure **5.2**). In the subsequent cycles larger peaks were observed upon continuous scanning, which reflected the continuous growth of the film [178]. These facts indicated p-TSA was deposited on the surface of GCE by electropolymerization. The electrode was then electroactivated by CV from -0.8 to +0.8 V at 0.1V s^{-1} in phosphate buffer (pH 7).

Surface morphology of bare GCE and poly (p-TSA)/GCE were studied by Scanning Electron Microscopy (SEM). SEM images (Figure **5.3**) clearly indicated that effective modification of GCE surface has taken place after electropolymerisation.

5.2.1 Surface Area Study

Further evidence for the effective modification of GCE was obtained from the surface area studies. 2 mM $K_3Fe(CN)_6$ was taken as a probe to measure the effective surface areas of both poly (p-TSA) modified GCE and bare GCE by CV at different scan rates (Figure **5.4**) . The current

obtained for both electrodes was recorded. Graphs were plotted with current vs square root of scan rate (Ip vs $\upsilon^{1/2}$) for both electrodes. From the slope of these linear graphs, surface areas can be calculated using the Randles Sevick equation [202].

$$I_p = 2.69 \times 10^5 \, A \, n^{3/2} \, D_R^{1/2} \, c \, \nu^{1/2}$$

From the slope of I_p vs. $\nu^{1/2}$ plot, the areas of bare GCE and poly (p-TSA) modified GCE were estimated and were found to be about 0.0795 cm^2 and 0.1026 cm^2 respectively. There was about 29% enhancement in the effective surface area when GCE was modified with poly (p-TSA) which is a strong evidence for the successful modification of GCE with polymer film.

5.3 Electrochemical behavior of PAM Chloride

Stock solution of PAM Chloride was prepared as described in section 2.5.3 of Chapter 2. Standard solutions of the analyte (1×10^{-3}- 1×10^{-7} M) were prepared by serial dilution of stock solution with the supporting electrolyte. Sample solution was then taken in the electrochemical cell. The solution was then de-aerated with nitrogen for 5 min. SWV was performed at poly (p-TSA) modified GCE and the voltammograms were recorded from 0 to 0.8 V. An oxidation peak around 0.6 V was obtained for PAM Chloride and the corresponding peak current was measured. For electrode regeneration several cyclic scans were carried out in the blank electrolyte solution until a stable voltammogram was obtained.

Figure **5.5** shows the square wave voltammogram of PAM Chloride at bare GCE and poly (p-TSA) modified GCE. As can be seen, the oxidation peak of PAM Chloride at bare GCE was broad suggesting slow

electron transfer, presumably due to the fouling of the electrode surface by the oxidation product. At the poly (p-TSA) modified GCE, its response has improved considerably, and the peak current has greatly increased.

PAM Chloride gave an oxidation peak at 0.67 V at bare GCE and the peak current was around 10 μA. However at the poly (p-TSA) modified GCE, the oxidation peak appeared at 0.6V and the peak current has increased to 84 μA. Compared with bare GCE, the oxidation potential of PAM Chloride has shifted negatively by about 0.07 V and current has increased by 8 folds at poly (p-TSA)/GCE.

The surface charge states of PMEs are crucial to their behavior and potential applications. In p-TSA molecule there is high electron density of sulphonic acid group and hence poly (p-TSA) film has a negatively charged surface [178]. From the structure it is clear that PAM Chloride is positively charged. The enhanced sensitivity of PAM Chloride at the modified electrode could be attributed to the electrostatic attraction between positively charged PAM Chloride and the high electron density of sulphonic group of poly (p-TSA). Further there is a possibility for the formation of intermolecular hydrogen bonding between oxygen of sulphonic acid group and hydrogen of the aldoxime group. Such interactions would lead to an increase in the concentration of PAM Chloride around the surface of the modified electrode (Figure **5.6**). [143, 227]

Here the oxidation peak arises due to the electrochemical oxidation of the aldoxime group of PAM Chloride to carbonyl group [228]. The mechanism involves, first the oxidation of aldoxime group to iminoxy radical which then undergoes oxidation to give hydroxyl nitroso intermediate. This then undergoes dimerisation followed by decomposition

to give carbonyl compound and hyponitrous acid. The whole process involves 2 e^- transfer. The detailed mechanism is shown in Figure **5.7**.

5.4 Optimisation Studies

5.4.1 Effect of supporting electrolyte

The electrochemical behavior of PAM Chloride in various media such as 0.1 M solutions of phosphate buffer, H_2SO_4, NaOH, NaCl and KNO_3 were studied by SWV. When 0.1 M NaCl solution was used as the supporting electrolyte, PAM Chloride gave an oxidation peak at 0.74 V with a peak current of 29 μA. With KNO_3, the peak was obtained at 0.76V with a current of 30 μA. No peak was obtained for PAM Chloride when H_2SO_4 was used as the supporting electrolyte. However in PBS solution (pH 7) PAM Chloride gave a peak at 0.6V with a peak current of 84 μA. Thus the oxidation peak obtained was best defined in 0.1 M phosphate buffer (pH 7), justifying the choice.

5.4.2 Effect of pH

The effect of solution pH on the peak potentials and the peak currents of PAM Chloride were studied and the result is shown in Figure **5.8**. The pH range studied was from 2-9. With the increase of pH value, the peak potentials shifted negatively. Also the experimental results showed that pH had a significant influence on the anodic peak current of PAM Chloride at poly (p-TSA) modified GCE (Figure **5.9**). It was found that the oxidation peak current of PAM Chloride increased with an increase in pH from 2-7. At higher pH the peak current was found to decrease. Obviously, the maximum response of anodic peak current appeared at pH 7. So pH 7 was selected as the optimal pH.

5.4.3 Effect of scan rate on the peak current and peak potential

The effect of scan rate on the anodic peak current 1×10^{-3} M PAM Chloride at poly (p-TSA)/GCE in phosphate buffer solution (pH 7) was investigated by SWV (Figure **5.10**). The anodic peak current was found to be linear to the square root of scan rate (see Figure **5.11**), which indicates a diffusion controlled oxidation process of PAM Chloride at poly (p-TSA)/GCE.

The experimental results showed a good linear dependence of the oxidation peak potential upon the logarithm of the scan rate (ln υ) (Figure **5.12**). The no. of electrons (n_a) involved in the reaction was calculated from the slope of the plot according to the relation, $b= RT/ \alpha n_a F$ [203]. Here b is the slope, R is the Universal Gas Constant, T is the temperature, α is a constant (for a totally irreversible electrode process the value of α is assumed to be 0.5) and F is 96500 C. The obtained value for n_a was around 2, which confirmed that 2 electrons are involved in the oxidation of PAM Chloride.

5.4.4 Effect of the number of scan cycles of electropolymerization on the electrocatalytic ability of poly (p-TSA)/GCE

Effect of film thickness on the oxidation peak current of 1×10^{-3} M PAM Chloride was investigated. The oxidation peak current gradually increased with increase of scan cycles of electropolymerization. When the cycles were beyond 20, the peak current decreased, may be due to the decreased electron transfer rate of PAM Chloride with an increase in film thickness. Also, the repeatability and stability for the film modified electrode were poor when the voltammetric sweeping segments were less than 20. Hence the film thickness obtained with 20 segment sweeping was selected as the optimal condition.

5.4.5 Calibration curve

The dependence of peak current on the concentration of PAM Chloride was investigated by SWV (Figure **5.13**). The oxidation peak current was found to increase with an increase in the concentration over the range 1×10^{-7}M to 1×10^{-3}M. However a linear response was found to obtain from 2×10^{-7}M – 2×10^{-6}M (Figure **5.14**) with a detection limit of 3×10^{-8}M.

To estimate the repeatability of the developed method, the relative standard deviation (RSD) of five measurements of 1×10^{-3}M PAM Chloride at the same modified electrode was measured. The obtained RSD is 4.1% which showed the excellent reproducibility of the modified electrode.

5.4.6 Interference Study

The selectivity of the sensor was also examined by studying the effects of foreign species on the determination of PAM Chloride (1×10^{-4} M). It was found that the major interfering species like ascorbic acid, citric acid, urea, glucose, lactose, Na^+, K^+, SO_4^{2-} and Cl^- did not cause any observable interference when present in 100 fold excess than PAM Chloride (signal change below 5 %). This is due to the high selectivity of the electrode surface coated with poly (p-TSA) for the determination of PAM Chloride. The results are given in Table **5.1**.

5.5 Analytical Application

The application of the developed sensor in the determination of PAM Chloride in urine sample was studied.

5.5.1 Determination of PAM Chloride in urine sample

The developed sensor was applied for the determination of PAM Chloride in urine sample. 2 mL of urine sample and 8 mL phosphate

buffer was taken in the cell. Suitable aliquot of PAM Chloride was added to the above solution. The solution was then thoroughly stirred. SWV was then recorded and the anodic peak current obtained was measured. This procedure was repeated for several additions of PAM Chloride and the unknown concentrations were determined from the calibration graph. The recoveries obtained are in the range of 99 – 102%. The recovery obtained with the developed sensor was compared with that obtained using the standard method (spectrophotometric method) [185]. The results are shown in Table **5.2** and Table **5.3.** It was found that there is a satisfactory agreement between the PAM Chloride content determined by the developed sensor and by the reported standard method.

5.6 Conclusion

A voltammetric sensor was fabricated for the determination of the drug PAM Chloride. The sensor developed was based on the electropolymerization of p-TSA on GCE surface. The poly (p-TSA) film significantly improved the electron transfer, decreased the oxidation potential and enhanced the oxidation peak current of PAM Chloride. Performance characteristics of the developed sensor reveal low detection limit, high sensitivity, good selectivity and fast response. The developed sensor was applied to the determination of PAM Chloride in biological fluids like urine. In general the poly(p-TSA) sensor is sufficiently simple and specific for the quantitative determination of the drug. Moreover, it is cost effective and can be used for the direct determination of PAM Chloride in complex matrix without the need for prior separation.

Table 5.1 Study of the effect of foreign species on the oxidation peak current of 1×10^{-4}M PAM Chloride

Substance	Concentration, M	Current(μA)	Signal change, %
PAM Chloride	1×10^{-4}	17.0047	-
Urea	1×10^{-2}	16.8713	0.78
Na^{+}	1×10^{-2}	17.8500	4.97
Lactose	1×10^{-2}	16.9650	0.23
K^{+}	1×10^{-2}	16.9100	0.55
Glucose	1×10^{-2}	17.2732	1.58
Cl^{-}	1×10^{-2}	17.8500	4.97
Ascorbic acid	1×10^{-2}	16.8942	0.64
SO_4^{2-}	1×10^{-2}	16.9100	0.55
Citric acid	1×10^{-2}	16.5742	2.53

Table 5.2 Determination of PAM Chloride in urine sample

Added(mg)	Found(mg)	Recovery (%)
4.0	4.1	102.5
6.0	6.1	101.7
8.0	7.9	98.8

Table 5.3 Comparison of the results obtained with the developed sensor and the standard method

Developed Sensor		Standard Method	
Recovery(%)*	CV*	Recovery*	CV*
101	1.85	99.4	1.91

*average of six replicates

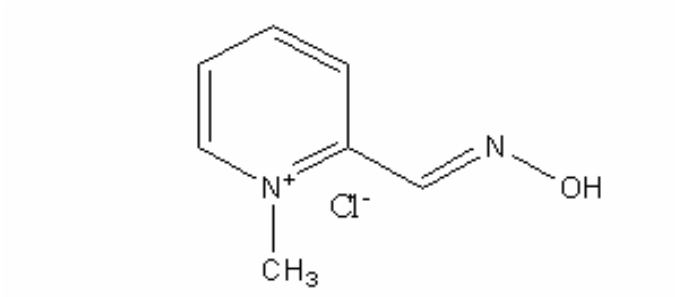


Figure 5.1 Structure of PAM Chloride

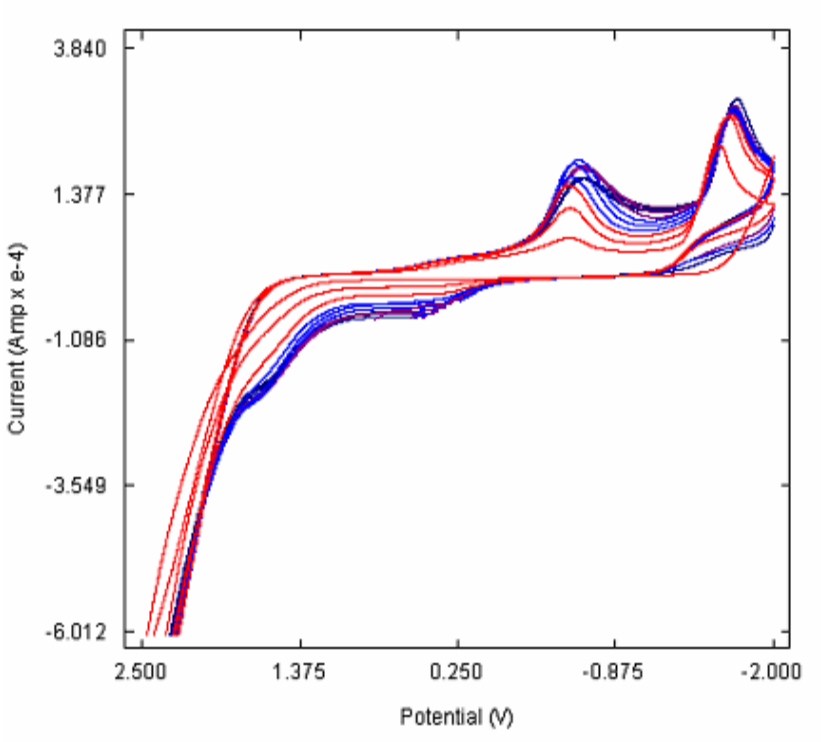
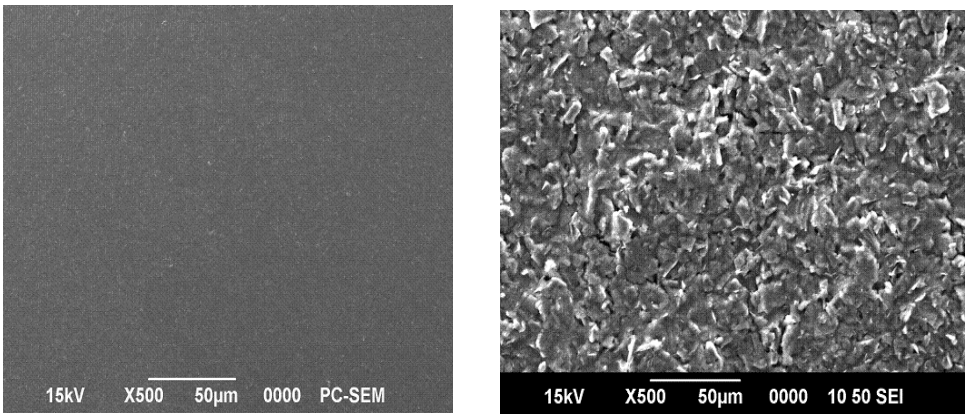
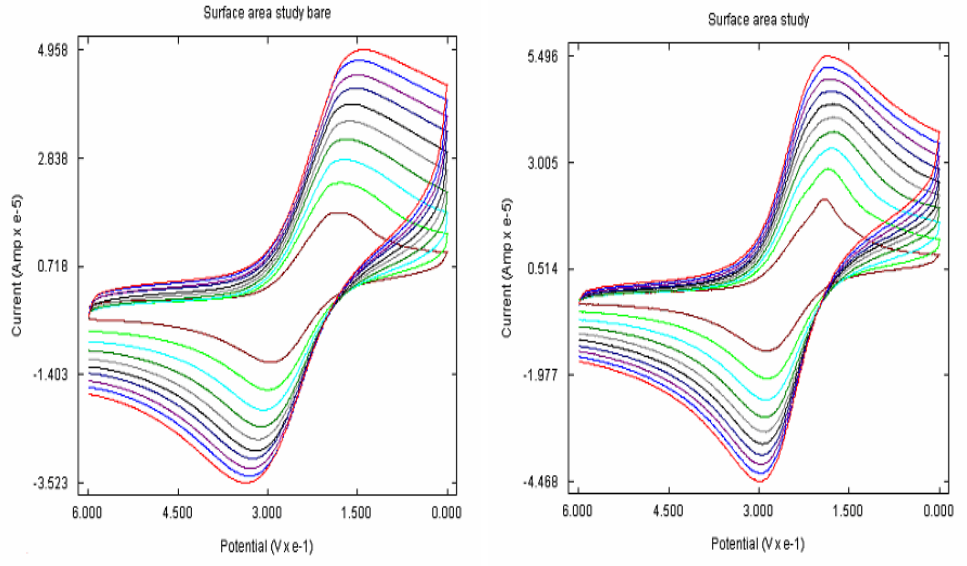


Figure 5.2 Electropolymerization of pTSA at GCE



(a) **(b)**

Figure 5.3 SEM images of (a) bare GCE and (b) poly (p-TSA) modified GCE



(a) **(b)**

Figure 5.4 Surface area study at a) bare GCE and b) poly (p-TSA)/GCE

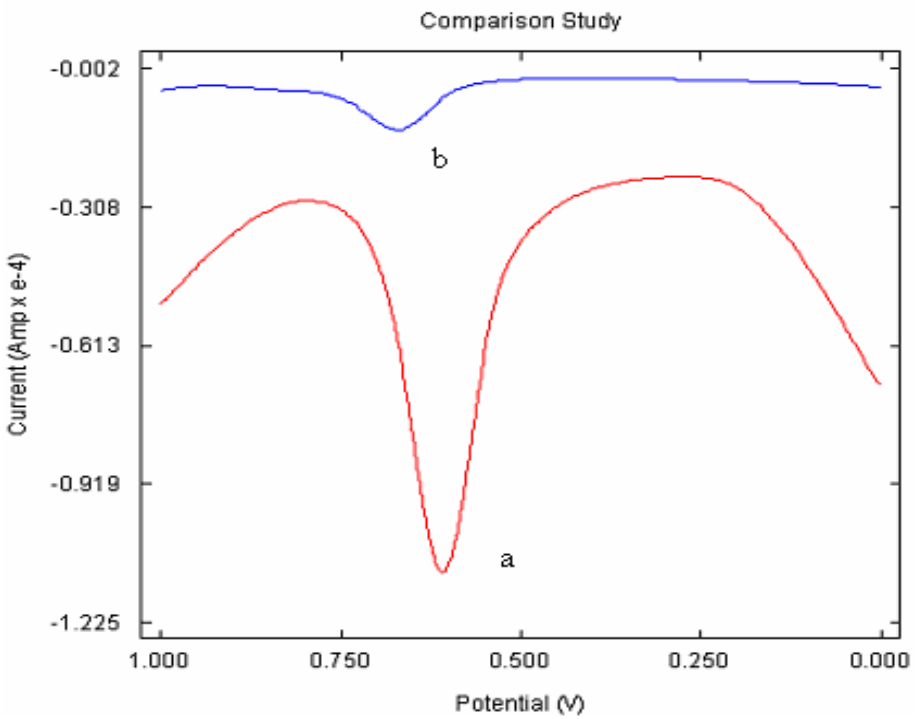


Figure 5.5 Overlay of Square Wave Voltammogram of PAM Chloride at (a) poly (p-TSA) modified GCE and (b) bare GCE at a scan rate of 0.06Vs^{-1} from 0 – 1.0 V

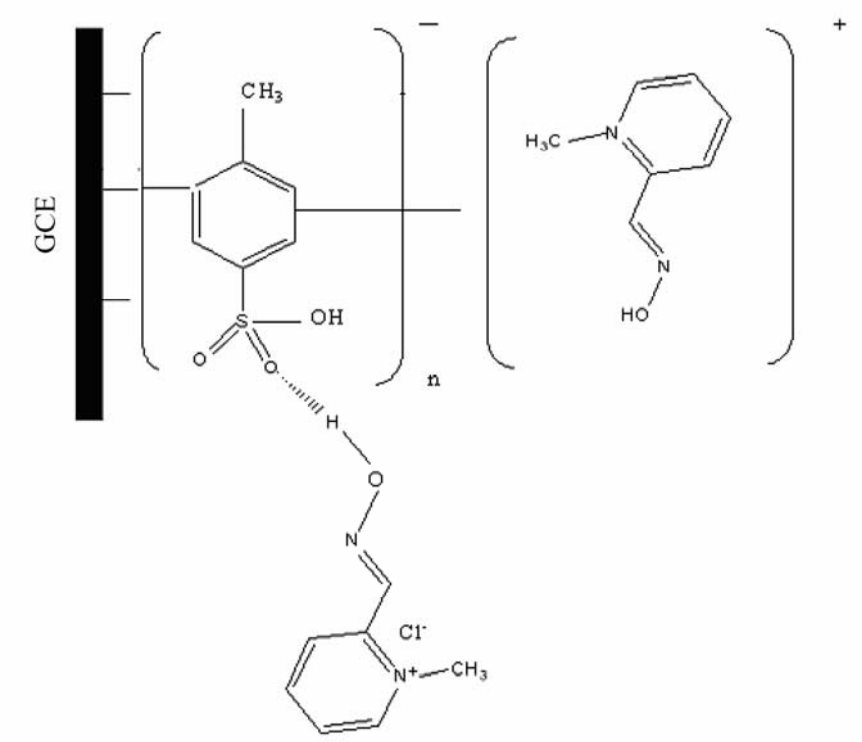


Figure 5.6 Electropolymerization of p-TSA at GCE and the attachment of PAM Chloride at this modified surface

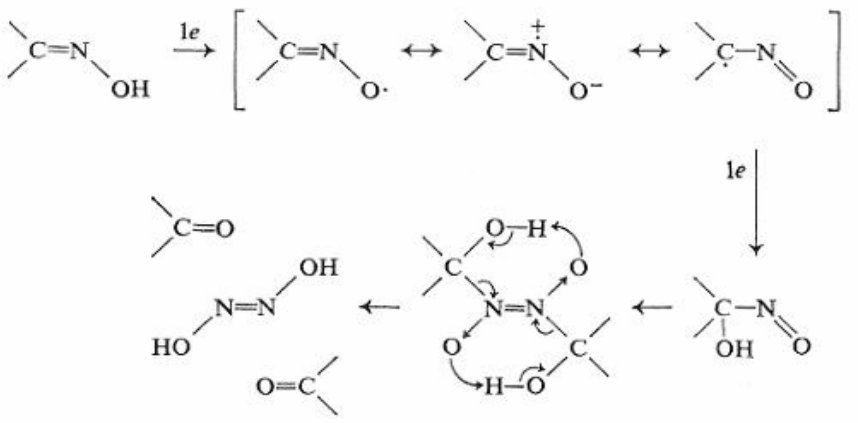


Figure 5.7 Mechanism of oxidation of oxime group in PAM Chloride to carbonyl group

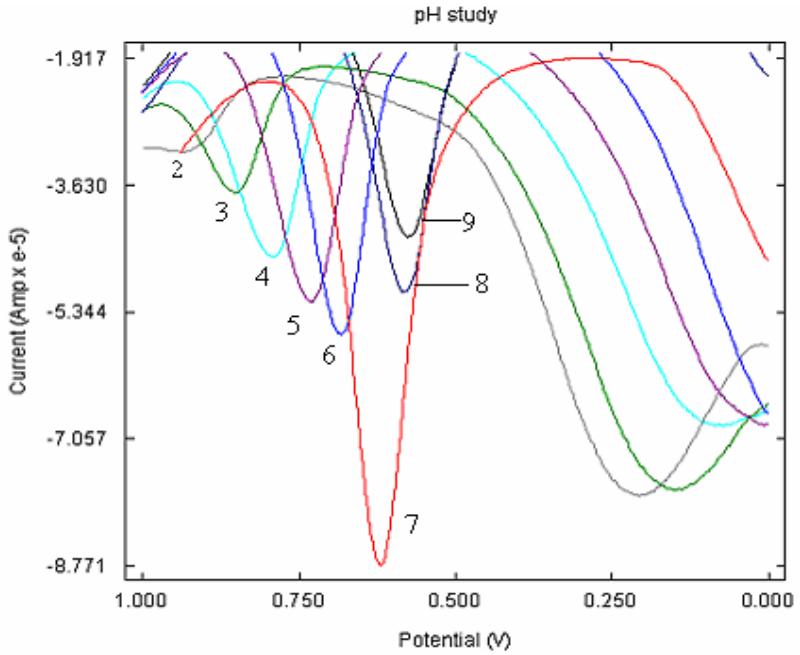


Figure 5.8 Overlay of SWVs of PAM Chloride at poly (p-TSA)/GCE in 0.1M phosphate buffer containing 1×10^{-3}M PAM Chloride at different pH 2, 3, 4, 5, 6, 7, 8and 9.

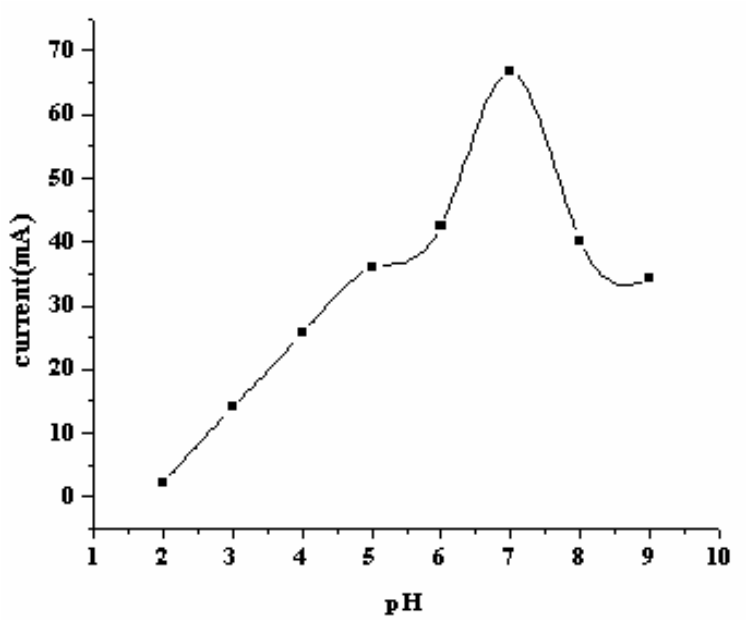


Figure 5.9 Effect of pH on the anodic peak current of 1×10^{-3}M PAM Chloride

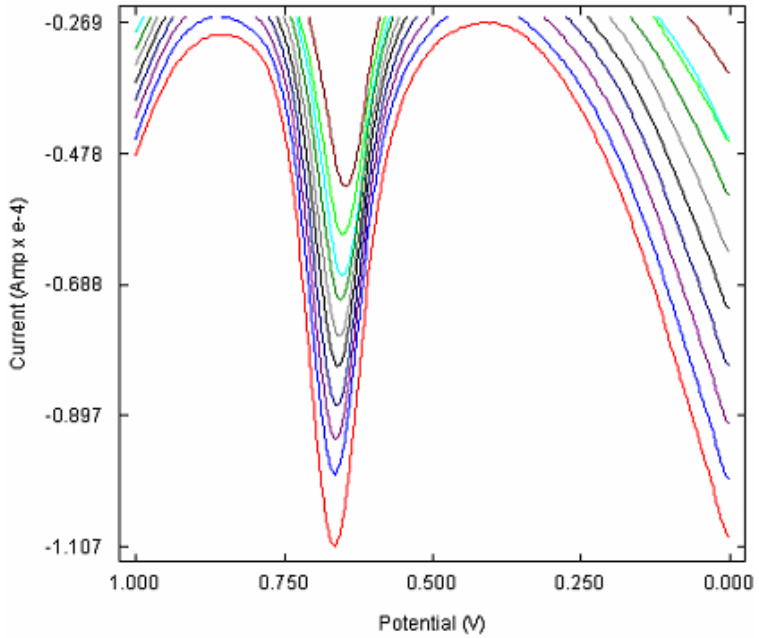


Figure 5.10 Overlay of SWVs of PAM Chloride at poly (p-TSA)/GCE in 0.1M phosphate buffer (pH 7) containing 1×10^{-3}M PAM Chloride at different scan rates a) 40 b) 60 c) 80 d) 100 e) 120 f) 140 g) 160 h) 180 i) 200 mVs^{-1}.

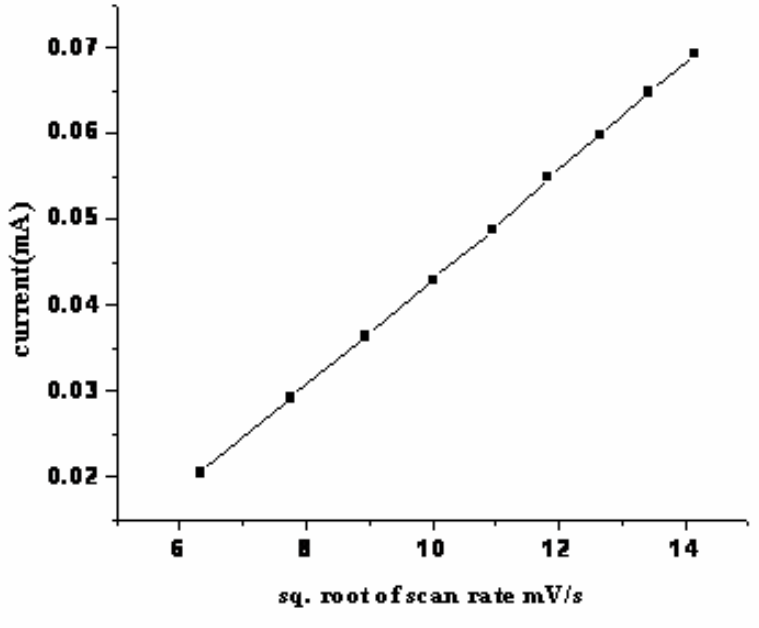


Figure 5.11 Effect of scan rate

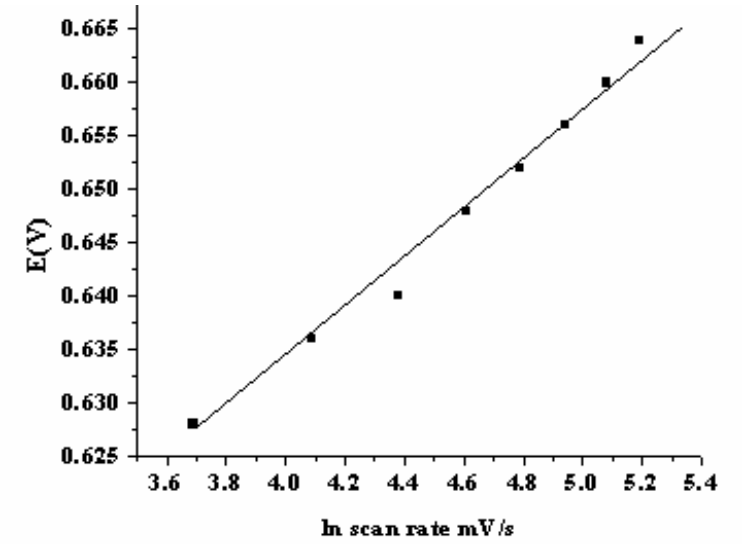


Figure 5.12 Plot of peak potential against ln (scan rate)

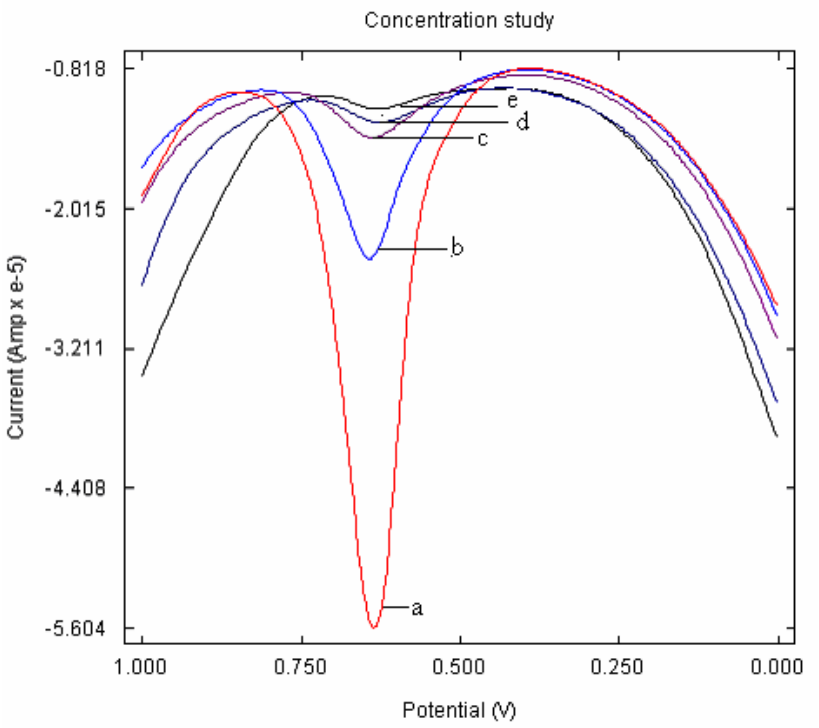


Figure 5.13 Square Wave Voltammogram of PAM Chloride of different concentrations a) 1×10^{-3}M b) 1×10^{-4}M c) 1×10^{-5}M d) 1×10^{-6}M e) 1×10^{-7}M at poly (p-TSA) modified GCE at 25^{0}C

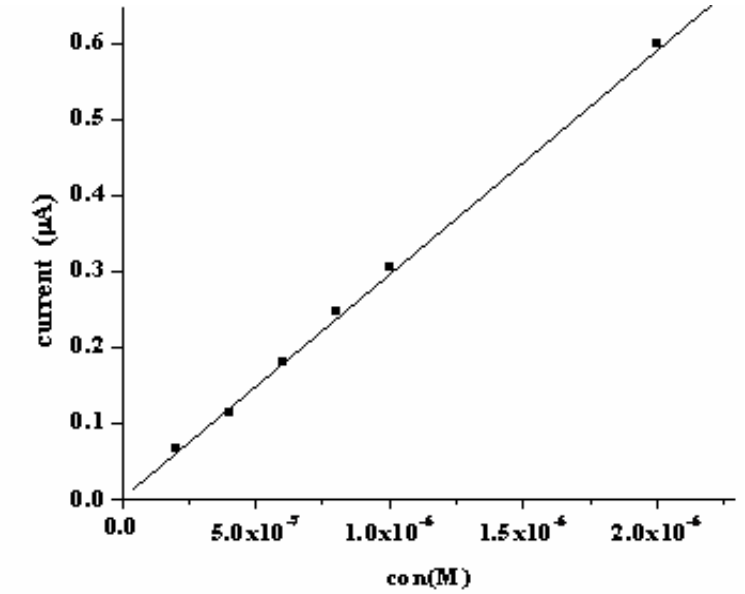


Figure 5.14. Calibration curve for PAM Chloride at poly (p-TSA) modified GCE at 25^0C

Chapter 6

SENSOR FOR THE DETERMINATION OF LAMIVUDINE

CONTENTS

This chapter deals with the fabrication of a poly L-Cysteine (L-Cys) modified glassy carbon sensor for the determination of Lamivudine (LAM). The electrochemical behavior of LAM at this electrode was examined by CV, DPV and SWV. At poly (L-Cys) modified GCE, LAM gave a well defined reduction peak at -1.592V and a peak current of 0.081mA in 0.1M phosphate buffer solution (PBS) of pH 7. Compared with bare GCE, the poly (L-Cys) modified GCE significantly enhanced the cathodic peak current of LAM as well as lowered its reduction potential. The experimental parameters such as the effect of supporting electrolyte, the pH values of the supporting electrolyte and scan rate were optimized and a direct electrochemical method for the determination of LAM was developed. Under optimum conditions the cathodic peak current was linear to the concentration of LAM in the range of 1×10^{-3} - 5×10^{-5} M. The developed sensor was successfully applied for the determination of LAM in pharmaceutical formulations and urine sample.

6.1 Introduction

Lamivudine (Figure **6.1**) 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) [229-232]. Furthermore LAM shows activity against hepatitis B virus [233-235].

HIV-1, the agent that causes AIDS, is found in blood, spleen, lymph nodes, brain, saliva, cervicovaginal secretions and semen of infected male patients [236-238]. 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. LAM and zidovudine are two commonly used drugs of this class. The use of zidovudine plus LAM combination therapy causes a decrease of HIV-1 in blood plasma.

LAM is 2',3'-dideoxy-3-thiacytidine and as mentioned earlier is a potent reverse transcriptase inhibitor (NARTI). It is also called 3TC. It is an analogue of cytidine. LAM has been used for the treatment of chronic hepatitis B at a lower dose than for treatment of HIV. In combination with other antiretroviral agents, LAM 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 LAM unfortunately leads to emergence of a resistant hepatitis B virus mutant. Despite this, LAM 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 [241] and its high selectivity was also observed in liver cells, a critical target site for anti hepatitis drugs. 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.

LAM 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. LAM was approved by the Food and Drug Administration (FDA) on Nov 17, 1995 for use with Zidovudine 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 [240]. An HPLC assay for the determination of LAM in human serum has been described by Harker et al [241]. Furthermore, an HPLC methodology for the quantitative determination of LAM in perfusion solutions from isolated perfused rat kidney studies was described by Hysu and Lloyd [242]. A radioimmuno assay for the quantitation of LAM was described, which may be of use for the determination of intracellular phosphorylated LAM [243]. High Throuhput Analysis of LAM in pharmaceutical preparations using monolithic silica HPLC column was described by Hassan et al [244]. A rapid HPLC method with UV detection for the analysis of LAM in commercial pharmaceutical

dosage forms (tablets and oral solutions) and human serum was developed by Sibel and Bengi [245]. Also few spectrophotometric[246,247] and titrimetric techniques[248] have also been reported for the determination of LAM.

Most of the reported methods are time consuming and also require expensive and sophisticated instruments. Hence it is worthwhile to develop a simple and sensitive method for the analysis of this drug. Electroanalytical techniques satisfy many of the requirements for environmental monitoring, clinical assays or process control particularly owing to their inherent specificity, speed of response, sensitivity and simplicity of preparation. Till date, the only electrochemical method available for the determination of LAM in the literature is with hanging mercury electrode [249, 250]. However, the use of mercury electrodes will contaminate the environment because of their environmental toxicity, if mercury is not handled with special care. Carbon electrodes, especially glassy and paste electrodes, are widely used in electrochemical investigations because of their low background current, wide potential windows (anodic and cathodic), chemical inertness, and suitability for detection of various organic and biological compounds [251].

This chapter presents the detailed results of the development and analytical applications of poly (L-Cys)/GCE for the determination of LAM. To do so, (L-Cys) was electrochemically polymerized at the surface of GCE and the electrochemical behavior of LAM at this developed sensor was investigated. It was observed that the modified GCE exhibited significant enhancement in the reduction peak current as well as lowered the reduction potential of LAM compared with bare GCE. The developed sensor has also been successfully applied for the determination of LAM in tablets and urine sample.

6.2 Preparation of poly (L-Cys) modified GCE

Prior to modification, the GCE was cleaned as described in section 2.3 of Chapter 2. The poly L-Cys/GCE was prepared by electropolymerization of L-Cys on GCE. The detailed procedure for the fabrication of poly (L-Cys)/GCE is given in section 2.4.3 of Chapter 2.

The film was grown on the electrode surface by 30 segments of cyclic voltammetric scans. An oxidation peak was observed at 1.30V in the first anodic scan, and a reduction peak was observed at -0.52V in the reverse scan (Figure **6.2**). The reduction peak current increased with the increase in the cyclic number of voltammetric scans, indicating that an electroconductive film is formed on the electrode surface [179]. After immobilization, the film was washed with ethanol to remove the remaining L-Cys monomers. After drying in air, a blue thin film could be seen at the electrode surface. The thickness of the film could be controlled by the cyclic number of voltammetric scans and the concentration of L-Cys.

Surface Area study gave a strong evidence for the effective modification of GCE with poly (L-Cys) film. 2 mM $K_3Fe(CN)_6$ was taken as a probe[202] to measure the effective surface areas of both poly (L-Cys)/GCE and bare GCE by CV at different scan rates (Figure **6.3**). From the slope of I_p vs. $\nu^{1/2}$ plot, the areas of bare GCE and poly (L-Cys) modified GCE were estimated and was found to be about 0.0815 cm^2 and 0.1176 cm^2 respectively. There was about 44% enhancement in the effective surface area when GCE was modified with poly (L-Cys) which is a strong evidence for the successful modification of GCE with polymer film.

Surface morphology of bare GCE and poly (L-Cys)/GCE were studied by Scanning Electron Microscopy (SEM). SEM images (Figure **6.4**) clearly indicated that effective modification of GCE surface has taken place after electropolymerization.

6.3 Electrochemical behaviour of LAM

Stock solution of LAM was prepared as given in section 2.5.4 of Chapter 2. Standard solutions of the analyte (5×10^{-3}- 1×10^{-5} M) were prepared by serial dilution of stock solution with PBS (pH 7). Sample solution was then taken in the electrochemical cell. The solution was then de-aerated with nitrogen for 5 minutes. SWV was used to investigate the electrochemical behavior of LAM at poly (L-Cys)/GCE. As shown in the Figure **6.5,** no peak was obtained at the modified electrode in 0.1 M phosphate buffer solution. After addition of 5×10^{-3} M LAM to the PBS, a reduction peak appeared at -1.592V with a peak current of 81 μA during the cathodic sweep from -1.2 V to -1.8 V. The SW voltammogram of 5×10^{-3} M LAM at bare GCE under identical conditions is given for comparison (Figure **6.6**). At bare GCE, the reduction peak was obtained at -1.7 V with a peak current of 10 μA. Compared with bare GCE, the reduction potential of LAM has shifted negatively by about 0.1V and current has increased by 8 folds at poly (L-Cys) modified GCE. The comparison of curves shows that the poly (L-Cys) film enhances the reduction peak current of LAM remarkably suggesting that poly (L-Cys) film can accelerate the electron transfer of LAM.

Here the cathodic peak arises due to the electrochemical reduction of LAM. The mechanism involves the reduction of C=N bond followed by deamination [249]. The whole process involes 2 e^- transfer. The mechanism proposed for the reduction of LAM is represented in Figure **6. 7**

6.4 Optimisation Studies

The response characteristics of the developed sensor depend on various parameters such as effect of supporting electrolyte, pH, scan rate, film thickness and concentration.

6.4.1 Effect of supporting electrolyte

The electrochemical behavior of LAM in various media such as 0.1 M solutions of phosphate buffer, acetate buffer, citrate buffer, H_2SO_4, NaOH, NaCl and KNO_3 were studied by SWV. It was observed that, when 0.1 M solutions of NaCl, KNO_3, H_2SO_4 and NaOH were used as the supporting electrolyte, no response was obtained for LAM. In acetate buffer, a reduction peak at -1.6V with a peak current of 0.0495mA was obtained. In citrate buffer, the cathodic peak was observed at -1.5 V with a peak current of 0.05mA. But in this buffer, the peak obtained was a broad one and was not well defined. Also, the linear concentration range obtained in the citrate buffer was from 1×10^{-3}- 1×10^{-4} M. So citrate buffer could not be selected as the supporting electrolyte. However in PBS solution (pH 7) LAM gave a reduction peak at -1.592V with a peak current of 0.081mA. Moreover, the detection limit was found to be extended to 1×10^{-5}M. Thus the reduction peak obtained was best defined in 0.1 M phosphate buffer (pH 7), justifying the choice.

6.4.2 Effect of pH

The effect of pH on the cathodic peak current of LAM at poly (L-Cys) modified GCE was investigated by SWV (Figure **6.8**). The pH range studied was from 3-9. A well-defined peak and a high peak current was obtained at pH 7. So pH 7 was selected as the optimal pH.

6.4.3 Effect of scan rate

The cathodic peak current of 1×10^{-3} M LAM at different scan rates ranging from 20-180 mV/s was measured by SWV using the same modified electrode. The results are illustrated in Figure **6.9**. It was found that the cathodic peak current increased with an increase in the scan rate (υ). The current varied linearly with square root of scan rate (Figure **6.10**), indicating that the reduction of LAM at poly(L-Cys) modified GCE is diffusion controlled.

According to the Laviron's conclusion [203], the relationship between the peak potential (E_p) and υ was examined. It was found that E_p varies linearly with ln υ and is shown in Figure **6.11**. The no. of electrons (n_a) involved in the reaction can be calculated from the slope of the plot according to the relation, $b= RT/ \alpha n_a F$, where b is the slope. α of the totally irreversible electrode process is assumed to be 0.5. The obtained value for n_a is 1.7 (almost around 2), which confirms that 2 electrons are involved in the reduction of LAM.

6.4.4 Effect of film thickness

Effect of film thickness on the cathodic peak current of 1×10^{-3} M LAM was investigated. The cathodic peak current gradually increased with increase of scan cycles of electropolymerization. When the cycles were beyond 30, the peak current decreased, may be due to the decreased electron transfer rate of LAM with an increase in film thickness. Also, the repeatability and stability for the film modified electrode were poor when the voltammetric sweeping segments were less than 30. Hence the film thickness obtained with 30 segment sweeping was selected as the optimal condition.

6.4.5 Calibration curve

The relationship between the cathodic peak current of LAM and its concentration was investigated by DPV (Figure **6.12**). The working concentration range for the developed sensor was found to be 1×10^{-3} M - 5×10^{-5} M. At regions of very low concentrations the sensor becomes insensitive. The detection limit was obtained from the graph and was found to be 1×10^{-5} M. (Figure **6.13**).

The poly (L-Cys) modified electrode surface after each determination was regenerated by repetitive cycling in the supporting electrolyte. For five successive determination of 1×10^{-3} M LAM with the same electrode regenerated after every determination, the RSD of the peak current was 3.2%. This indicated that the poly (L-Cys) has excellent reproducibility.

6.4.6 Interference study

Several organic and inorganic species can interfere with the determination of LAM using the developed sensor. The selectivity of the sensor was determined by measuring the change in the sensor response in the presence of foreign compounds. It was found that a 100-fold excess molar concentration of glucose, Na^+, Cl^-, K^+, SO_4^{2-}, lactose, ascorbic acid and citric acid almost did not influence the current response of 1×10^{-4}M LAM (signal change below 5%). The results are tabulated in Table **6.1.**

6.5 Analytical Applications

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

6.5.1 Determination of LAM in Pharmaceutical Formulations (Tablets)

The developed sensor proved to be useful for the determination of LAM content of a pharmaceutical preparation by DPV. It was used to analyze the LAM content of a tablet Lamivir (Cipla, India), containing 100mg of the drug as declared by the company. The detailed procedure for the determination is given in section 2.8.3 of Chapter 2. DPV was recorded and the unknown concentrations were determined from the calibration curve. The results are shown in Table **6.2**. The results obtained are in good agreement with the declared LAM content and showed a high degree of precision [coefficient of variation (C.V) is 1.37 %].

The results were compared with those obtained by the standard method (potentiometric titration) [186]. The results show that there is a satisfactory agreement between the LAM content determined by the developed method and the standard method.

6.5.2 Determination of LAM in urine sample

The developed sensor was applied for the determination of LAM in urine sample. 2 mL of urine sample and 8 mL phosphate buffer was taken in the cell. Suitable aliquot of LAM was added to the above solution. The solution was then thoroughly stirred. DPV was then recorded and the cathodic peak current obtained was measured. This procedure was repeated for several additions of LAM and the unknown concentrations were determined from the calibration graph. The results are summarised in Table **6.3.** The recoveries obtained are in the range of 98 – 101%.

6.6 Conclusion

A poly(L-Cys) modified glassy carbon sensor was fabricated for the voltammetric determination of LAM by CV, SWV and DPV. LAM gave a well defined reduction peak at -1.592V with a high peak current of 81μA at the poly(L-Cys)/GCE. The linear range was found to be 1×10^{-3} M-5×10^{-5} M and the detection limit was found to be 1×10^{-5} M. Compared with bare electrode, the electrochemical response of LAM at the modified electrode was tremendously increased, characterized by the enhancement of the peak current and reduction in the peak potential. The fabricated sensor was successfully applied for the determination of LAM in pure form and dosage forms. The developed sensor gave very close values to the declared amount of 100 mg per tablet. The sensor was also applied for the determination of the drug in urine sample.

Table 6.1 Study of the effect of foreign species on the cathodic peak current of 1×10^{-4}M LAM

Substance	Concentration, M	Current(μA)	Signal change, %
LAM	1×10^{-4}	0.0307	-
Urea	1×10^{-2}	0.0313	1.95
Na^+	1×10^{-2}	0.0308	0.33
K^+	1×10^{-2}	0.0314	2.88
Glucose	1×10^{-2}	0.0320	4.23
Cl^-	1×10^{-2}	0.0308	0.33
Ascorbic acid	1×10^{-2}	0.0292	4.88
SO_4^{2-}	1×10^{-2}	0.0314	2.28
Citric acid	1×10^{-2}	0.0312	1.63

Table 6.2 Determination of LAM in tablet

Sample	Declared Amount (mg/tablet)	Method Adopted	Found* (mg/tablet)	S.D*	C.V*
Lamivir (Cipla, India)	100	Developed sensor	99.90	1.37	1.37
		Standard Method	99.50	1.64	1.65

* Average of six replicates

Table 6.3 Determination of LAM in urine sample.

Added (mg)	Found (mg)	Recovery (%)
3.0	3.1	100.0
5.0	4.9	98.0
7.0	7.1	100.9
9.0	8.9	98.9

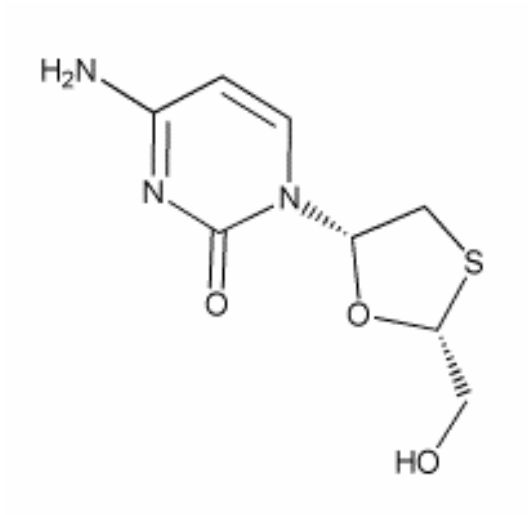


Figure 6.1 Structure of LAM

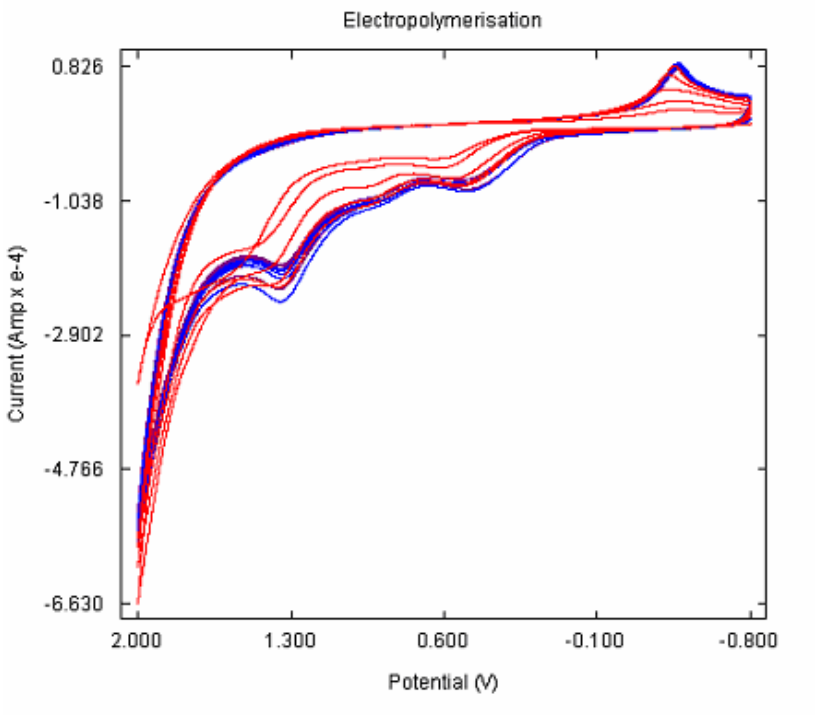
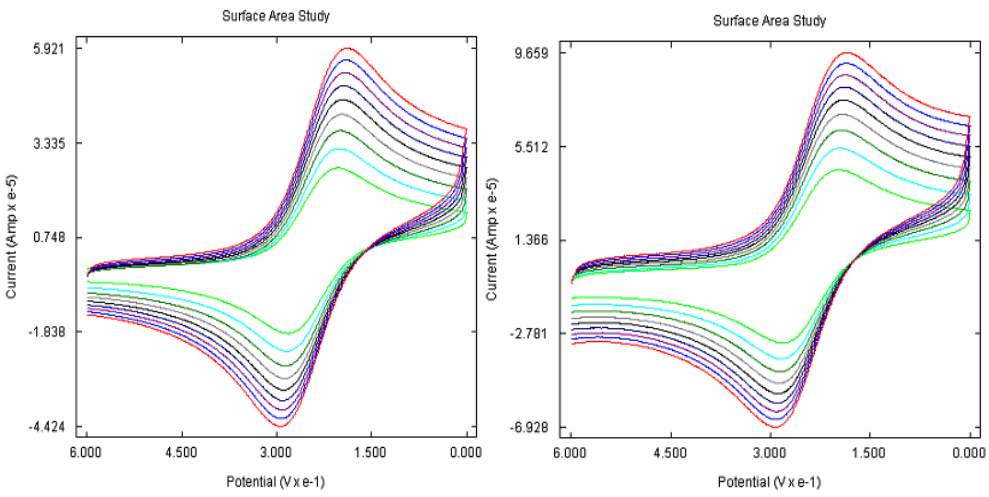
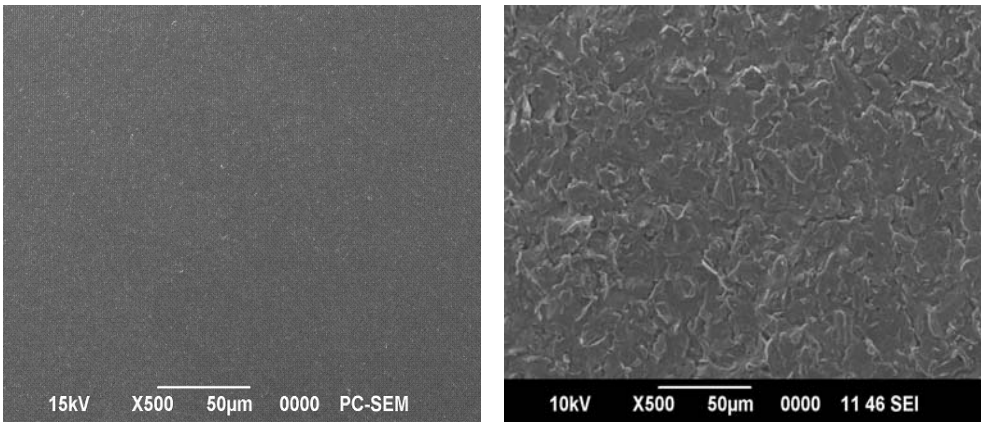


Figure 6.2 Electropolymerization of L-Cys at GCE



(a) **(b)**

igure 6.3 Surface area study at a) bare GCE and b) poly (L-Cys)/GCE



(a) **(b)**

Figure 6.4 SEM images of (a) bare GCE and (b) poly (L-Cys) modified GCE

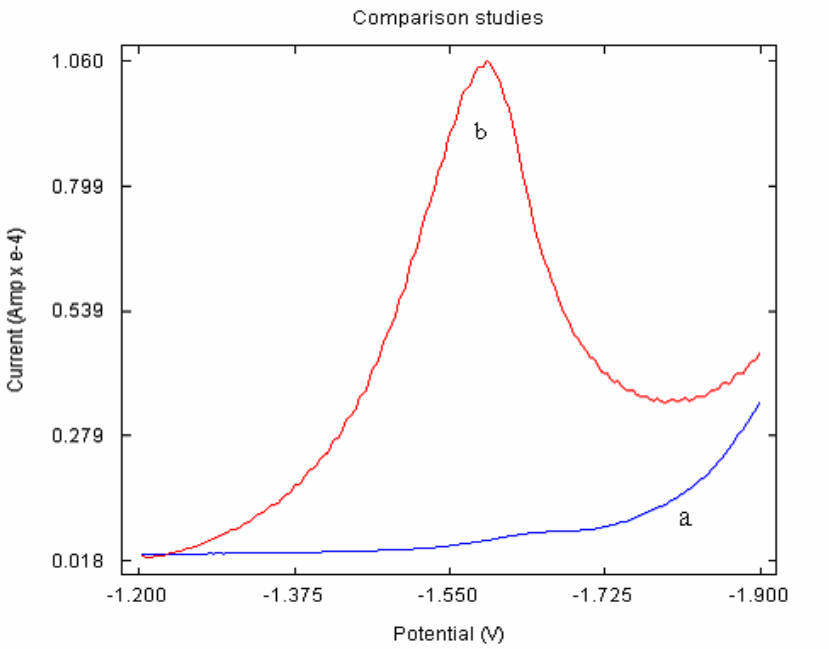


Figure 6.5 Square Wave Voltammogram showing the voltammetric behaviour of poly (L-Cys) /GCE in PBS pH 7 and without (a) LAM with (b) LAM at a scan rate of 0.06Vs^{-1} from -1.2 V – -1.8 V.

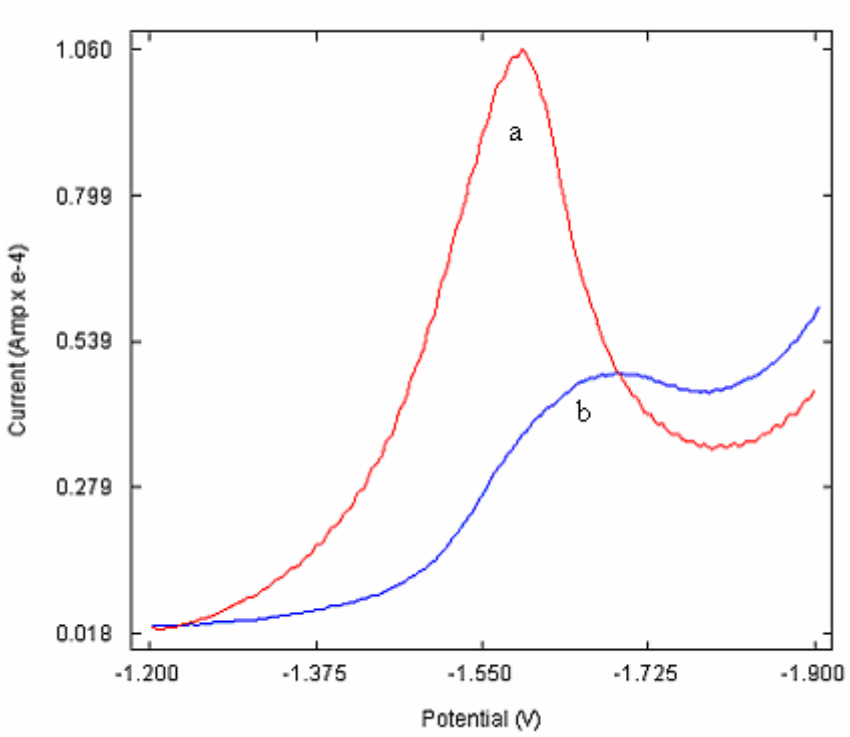


Figure 6.6 Overlay of Square Wave Voltammogram of LAM at (a) poly (L-Cys) modified GCE and (b) bare GCE at a scan rate of $0.06Vs^{-1}$ from -1.2 – -1.8 V

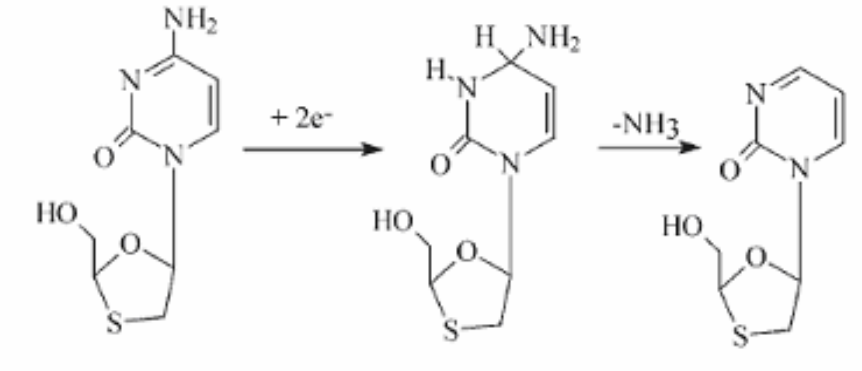


Figure 6.7 Mechanism showing the $2e^-$ reduction of LAM

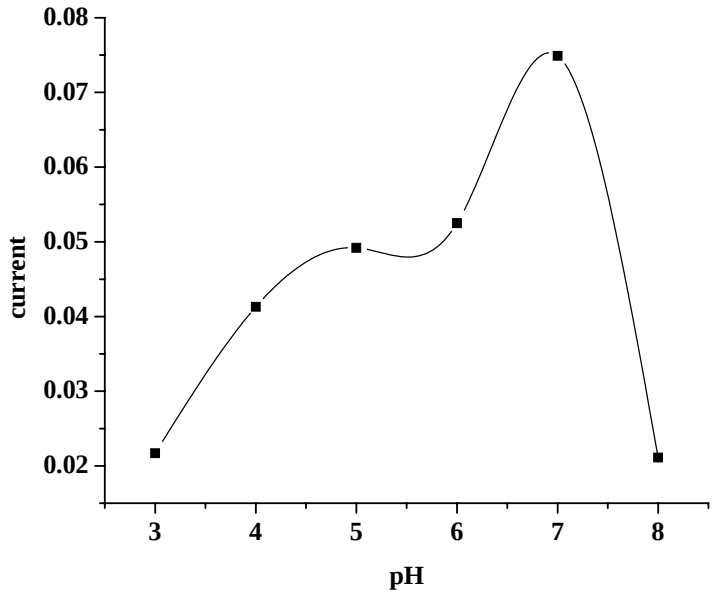


Figure 6.8 Effect of pH on the cathodic peak current of 5×10^{-3}M LAM

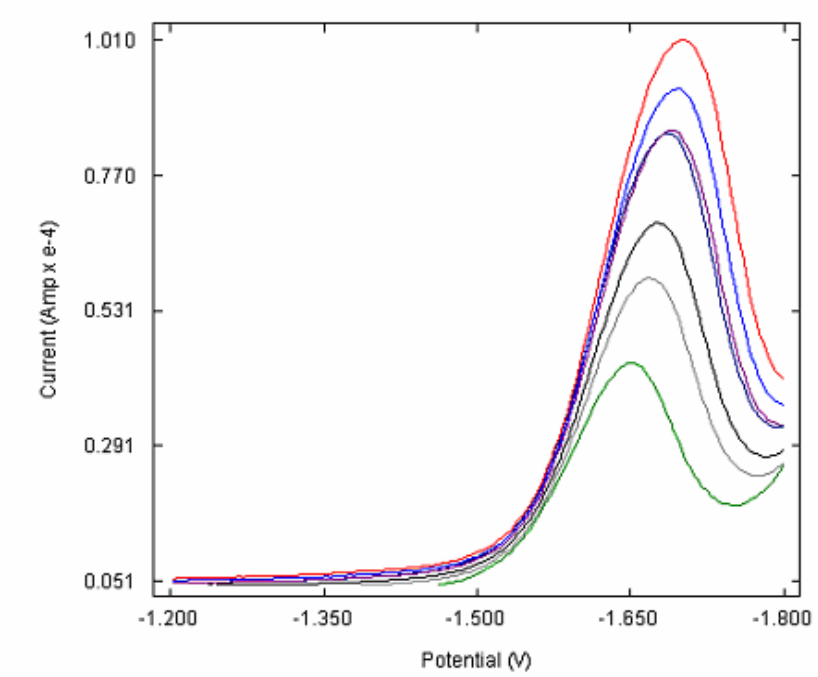


Figure 6.9 Overlay of SWVs of LAM at poly (L-Cys)/GCE in 0.1M phosphate buffer (pH 7) containing 5×10^{-3}M LAM at different scan rates a) 40 b) 60 c) 80 d) 100 e) 120 f) 140 g) 160 mVs^{-1}.

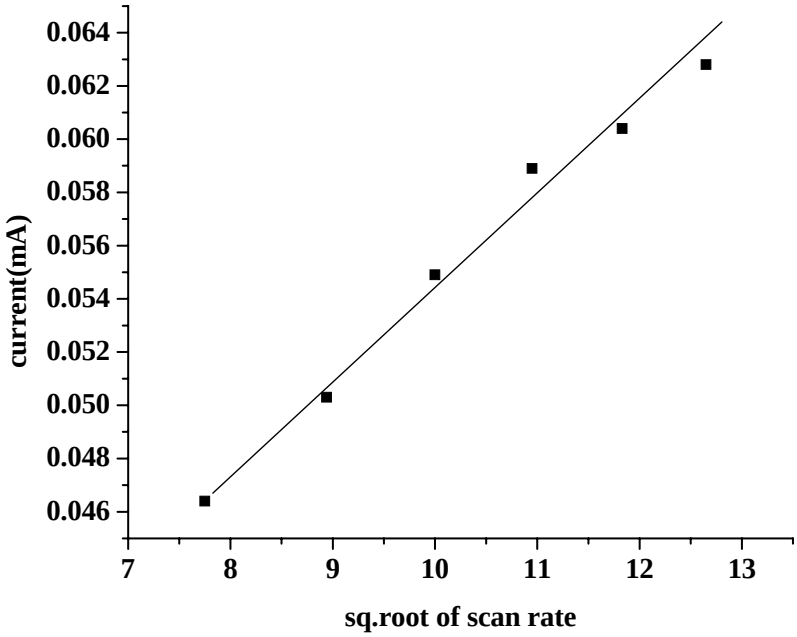


Figure 6.10 Effect of scan rate

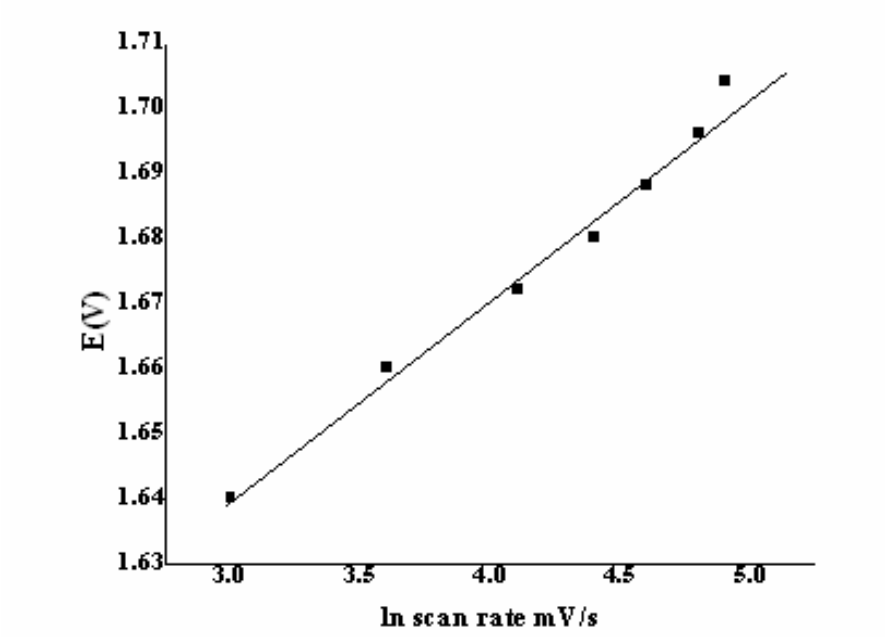


Figure 6.11 Plot of peak potential against ln (scan rate)

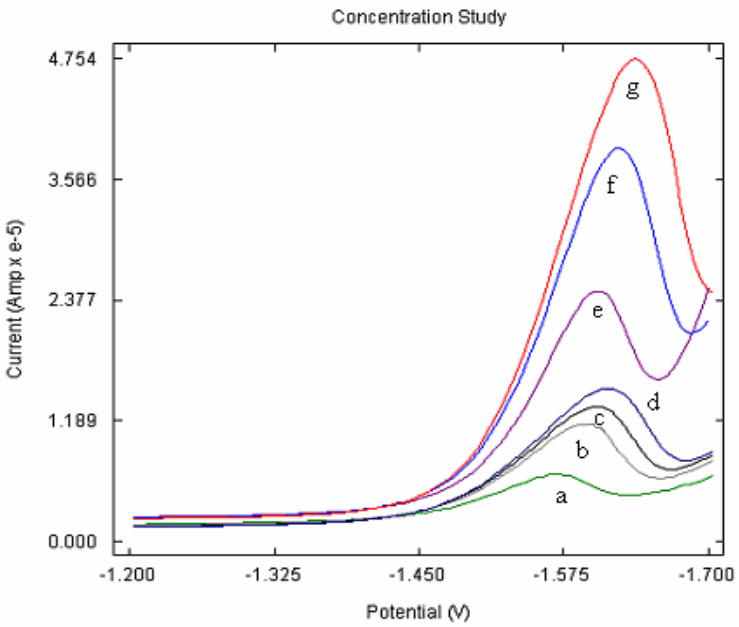


Figure 6.12 Differential Pulse Voltammogram of LAM of different concentrations a) 1×10^{-5}M b) 3×10^{-5}M c) 5×10^{-5}M d) 7×10^{-5}M e) 1×10^{-4}M f) 3×10^{-4}M g) 5×10^{-4}M at poly (L-Cys) modified GCE at 25^{0}C

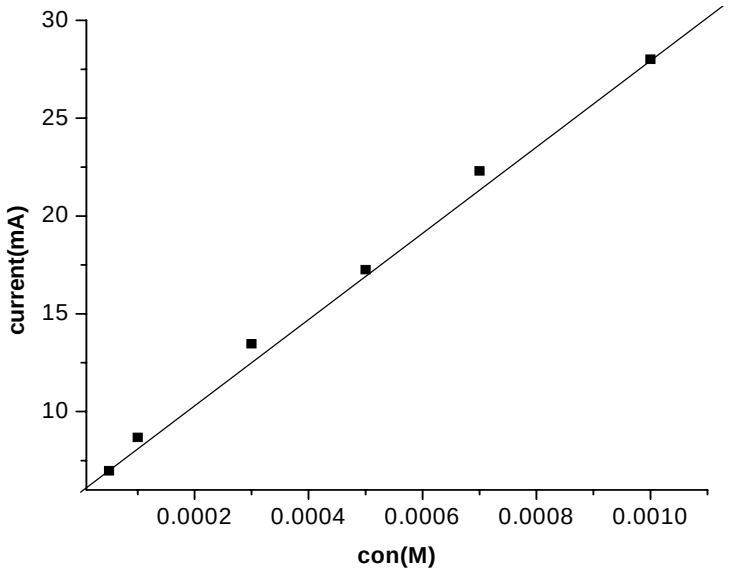


Figure 6.13 Calibration curve for LAM at poly (L-Cys) modified GCE at 25^{0}C

Chapter 7

SENSORS FOR THE DETERMINATION OF METRONIDAZOLE BENZOATE

CONTENTS

This chapter deals with the fabrication of two novel electrochemical sensors for the selective determination of Metronidazole Benzoate (MBZ). The sensors include a poly p-amino benzene sulphonic acid (poly p-ABSA) modified glassy carbon sensor and gold nanoparticle(AuNP)/poly p-amino benzene sulphonic acid (poly p-ABSA) modified glassy carbon sensor. A thin film of poly p-ABSA was obtained on the surface of GCE by electropolymerisation using CV. AuNP/ poly p-ABSA modified glassy carbon sensor was prepared by electrochemical deposition of AuNPs onto the surface of poly p-ABSA coated GCE. The response characteristics of the developed sensors have been studied. Analytical applications of the developed sensors in the determination of the drug in pharmaceutical formulations were investigated. The sensors were also applied for the determination of MBZ in real samples like urine.

7.1 Introduction

Metronidazole benzoate (Figure **7.1**) is chemically 1-(2-benzyloxy ethyl)-5-nitro methylimidazole. MBZ is an antibiotic, effective against anaerobic bacteria and certain parasites. MBZ selectively blocks some of the functions within the bacterial cells and the parasites, resulting in their death.

MBZ is used to treat parasitic infections including Giardia infections of the small intestine, amoebic liver abscess and amoebic dysentery, bacterial vaginosis and trichomonas vaginal infections. MBZ is used alone or in combination with other antibiotics in treating abscesses in the liver, pelvis, abdomen and brain caused by susceptible anaerobic bacteria. MBZ is also used in treating infection of the colon caused by a bacterium called C. difficile. Many commonly-used antibiotics can alter the type of bacteria that inhabit the colon. C. difficile is an anaerobic bacterium that can infect the colon when the normal types of bacteria in the colon are inhibited by common antibiotics. This leads to inflammation of the colon (pseudomembranous colitis) with severe diarrhea and abdominal pain. MBZ is also used in combination with other drugs to treat Helicobacter pylori (*H. pylori*) that causes stomach or intestinal ulcers [252].

MBZ may be taken orally with or without food and can also be administered intravenously to treat serious infections. The liver is primarily responsible for eliminating MBZ from the body, and doses may need to be reduced in patients with liver disease and abnormal liver function.

A simple and rapid stability-indicating HPLC assay procedure for the determination of MBZ has been described by Bempong et al [253]. Shahid Ali et al have developed an HPLC method for the simultaneous

determination of MBZ, methylparaben and propylparaben in oral suspension formulation [254]. A reverse phase high performance liquid chromatography method was developed for the simultaneous estimation of diloxanide furoate and MBZ in formulation by Danao et al [255]. Farhadi et al have developed a kinetic- spectrophotometric method for the accurate and sensitive determination of MBZ [256]. A High performance thin-layer chromatography (HPTLC) method for the simultaneous determination of MBZ and miconazole nitrate was described by Meshram et al [257]. Several other methods have also been reported for the determination of MBZ, including spectrophotometry, [258,259] titrimetry, thin layer chromatography and gas chromatography [260]. However, the above mentioned methods do not have sufficient selectivity for MBZ determination. Hence, it is of primary importance to develop an alternative method for MBZ determination with a high degree of selectivity and sensitivity.

Voltammetric sensors have the advantage of low cost, ease of use and maintenance and also simplicity and speed of assay procedure. The reliability of analytical information makes them very attractive for the assay of pharmaceutical products. Renjini et al have developed a voltammetric method for the determination of MBZ at a metalloporphyrin modified carbon paste electrode [261].

As explained earlier, PMEs have attracted considerable attention recently owing to their high sensitivity, selectivity, ease of preparation, homogeneity in electrochemical deposition and chemical stability of the film [45, 46]. Films of electronically conducting polymers are generally deposited onto the electrode surface by electropolymerization of the corresponding monomer in presence of an electrolyte solution to get the

PMEs. Among the conducting polymers, polyaniline (PANI) and its derivatives have been the focus of much attention. Sulfonated polyaniline is of interest because of its unusual physical properties, improved processability, and potential industrial applications [262, 263]. The solubility of polyaniline in aqueous solutions and in most common organic solvents is greatly improved by the presence of $–SO_3–$ groups [264]. The environmental stability of the parent polyaniline is also further improved in sulfonated polyaniline. The conductivity of sulfonated polyaniline is independent of external protonation in a broad pH range. Sulfonated polyaniline was found to have better thermal stability than its parent polyaniline [265]. Sulfonated polyaniline is electrochemically active in wide pH range 1–12 rather than PANI. In the present study, (p-amino benzene sulfonic acid) was selected for the modification of electrode surface via electropolymerisation to get poly(p-amino benzene sulfonic acid) modified GCE.

The unique properties of gold nanoparticles (AuNPs), such as good conductivity, useful electrocatalytic ability and biocompatibility, have attracted several researchers to fabricate electrochemical sensors and biosensors based on AuNPs [266–269]. The AuNPs dispersed on various substrates have been reported, such as carbon paste electrode, self-assembled monolayer and conducting and non-conducting polymers. Among them, conducting polymers are suitable host matrices because they have the advantages of permitting a facile electronic charge flow through the polymer matrix in electrochemical processes [270].

This chapter describes the construction of both poly p-ABSA modified glassy carbon sensor and (AuNP)/ poly p-ABSA modified glassy carbon sensor for the determination of MBZ. The poly (p-ABSA) modified

GCE was fabricated by electropolymerisation of p-ABSA using CV. The (AuNP)/ poly p-ABSA modified electrode was fabricated electrochemically using CV in 0.05M H_2SO_4 solution containing 1mM $HAuCl_4$. The performance characteristics of the developed sensors were studied in detail. The sensors were successfully applied for the determination of MBZ 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.

7.2 Preparation of poly (p-ABSA) modified GCE

Firstly, the GCE was cleaned as described in section 2.3 of Chapter 2. The poly p-ABSA/GCE was prepared by electropolymerization of p-ABSA on GCE. Detailed procedure for the fabrication of poly p-ABSA/GCE is given in section 2.4.4(i) of Chapter 2.

The film was grown on the electrode surface by 30 segments of cyclic voltammetric scans. As shown in Figure **7.2**, an anodic peak at 1.057 V and a cathodic peak at −0.136 V were observed in the first scan. From the second scan, an anodic peak appeared at +0.50 Vand a cathodic peak at -0.386V [180]. The oxidation and reduction peak currents increase with an increase in the cyclic number of voltammetric scans, indicating that an electroconductive polymer film has been formed on the electrode surface. After immobilization, the film was washed thoroughly with double distilled water and dried in air. A blue thin film was found to be formed on the electrode surface.

Surface Area study gave a strong evidence for the effective modification of GCE with poly (p-ABSA) film. 2 mM $K_3Fe(CN)_6$ was taken as a probe[202] to measure the effective surface areas of both poly

(p-ABSA)/GCE and bare GCE by CV at different scan rates (Figure **7.3**). From the slope of I_p vs. $\nu^{1/2}$ plot, the areas of bare GCE and poly (p-ABSA) modified GCE were estimated and was found to be 0.0911 cm^2 and 0.1876 cm^2 respectively. There was about 50% enhancement in the effective surface area when GCE was modified with poly (p-ABSA) which is a strong evidence for the successful modification of GCE with polymer film.

SEM images (Figure **7.4**) clearly indicated that effective modification of GCE surface has taken place after electropolymerization.

7.3 Preparation of AuNP/poly (p-ABSA) modified GCE

AuNP/poly(p-ABSA) modified electrode was fabricated as explained under section 2.4.4(ii) of Chapter 2.

AuNPs were deposited on the surface of poly (p-ABSA)/GCE by 20 segments of cyclic voltammetric scans [181]. (Figure**7.5**). With the potential scanning from 1.3V to 0 V, a reduction peak was observed at 0.780V and an oxidation peak was observed at 1.120V. In the subsequent cycles larger peaks were observed upon continuous scanning, which indicated that AuNPs were deposited on the electrode surface.

The effective surface area of AuNP/ poly (p-ABSA)/GCE was measured using CV technique (Figure**7.6)**. The surface area obtained for AuNP/poly (p-ABSA)/GCE was 0.2145cm^2. Compared with both bare as well as poly (p-ABSA)/GCE, there was an increase in the surface area for AuNP/ poly (p-ABSA)/GCE which is a strong evidence for the successful modification.

SEM image (Figure **7.7 (a)**) of AuNP/poly (p-ABSA)/GCE clearly showed that AuNPs were distributed in an almost homogeneous manner at

the surface of the poly(p-ABSA) film and Figure **7.7(b)** showed the size of AuNPs.

7.4 Electrochemical behavior of MBZ

7.4.1 Poly (p-ABSA)/GCE

Stock solution of MBZ was prepared as described in section 2.5.5 of Chapter 2. Standard solutions of the analyte (1×10^{-3}- 1×10^{-7} M) were prepared by serial dilution of stock solution with the supporting electrolyte (PBS, pH 7). Sample solution (1×10^{-3}M) was then taken in the electrochemical cell. The solution was then de-aerated with nitrogen for 5 min. SWV was performed at both bare GCE as well as at poly (p-ABSA) modified GCE and the voltammograms were recorded from -0.1 to -0.8 V (Figure **7.8**). A reduction peak at -0.652 V was obtained for MBZ with a peak current of 0.0516mA at poly (p-ABSA)/GCE. At bare GCE, the reduction peak was obtained at -0.704V with a peak current of 0.0363mA. Compared with bare GCE, the reduction potential has shifted negatively by about 0.05V and the peak current has also increased. This clearly depicted the electrocatalytic activity of poly (p-ABSA) film on the voltammetric determination of MBZ.

7.4.2 AuNP/poly (p-ABSA)/GCE

1×10^{-3}M MBZ solution was taken in the electrochemical cell and SWV was recorded from -0.1V to -0.8V at a scan rate of $0.06Vs^{-1}$ at AuNP/poly(p-ABSA)/GCE. MBZ gave a well defined reduction peak at -0.62V with a peak current of 0.0937mA. Compared with bare GCE, there was a reduction in the cathodic potential by about 0.1V and 3 times enhancement in the peak current at AuNP/ poly (p-ABSA)/GCE (Figure **7.9**). Also, when compared with poly (p-ABSA)/GCE, there was a reduction in

the potential by about 0.03V and an increase in the peak current at AuNP/poly (p-ABSA)/GCE. An attempt was also made to study the voltammetric behavior of MBZ at AuNP modified GCE, which was prepared electrochemically by immersing GCE in $HAuCl_4$ solution and carrying out cyclic voltammetry for 20 scans. The cathodic peak for MBZ at AuNP/GCE was obtained at -0.64V with a peak current of 0.0430mA. However the reproducibility of the results obtained at AuNP/GCE were very poor. All these factors clearly revealed that polymer film supported AuNPs have enhanced catalytic activity towards the electrochemical reduction of MBZ. A comparison of voltammetric behavior of MBZ at bare GCE, poly(p-ABSA)/GCE and AuNP/poly(p-ABSA)/GCE is shown in Figure **7.10**

7.5 Performance Characteristics of the Developed Sensor

The functional potential of the developed sensors depend on many factors including effect of pH, effect of the supporting electrolyte, effect of scan rate, range of linear response, detection limit and interference study. Each of these parameters are discussed in detail in the next section.

7.5.1 Effect of supporting electrolyte

The electrochemical behavior of MBZ in various media such as 0.1 M solutions of phosphate buffer, acetate buffer, citrate buffer, H_2SO_4, and NaCl were studied by SWV at both the developed sensors. It was observed that, when 0.1 M solution of H_2SO_4 was used as the supporting electrolyte, no response was obtained for MBZ at both the sensors. In acetate buffer, a reduction peak at -0.576V with a peak current of 0.0463 mA was obtained for MBZ at AuNP/poly (p-ABSA)/GCE and a reduction peak at -0.62V with a peak current of 0.032mA was obtained at poly (p-ABSA)/GCE. In citrate buffer, the cathodic peak was observed at -0.76 V with a peak

current of 0.0123mA at AuNP/poly (p-ABSA)/GCE. At poly (p-ABSA)/GCE, MBZ gave a reduction peak at -0.8 V with a peak current of 10 μA in citrate buffer. The cathodic peak for MBZ was obtained at -0.728V and -0.75V at AuNP/poly (p-ABSA)/GCE and poly (p-ABSA) /GCE resprctively in NaCl solution. However in PBS solution (pH 7), MBZ gave a reduction peak at -0.62V with a peak current of 0.0937mA at AuNP/poly (p-ABSA)/GCE. At poly (p-ABSA)/GCE, the reduction peak for MBZ was obtained at -0.65V with a peak current of 0.0516mA in PBS of pH 7. Thus the reduction peak obtained for MBZ at both the developed sensors was best defined in 0.1 M PBS (pH 7), justifying the choice.

7.5.2 Effect of pH

The influence of pH on the cathodic peak current of MBZ (1×10^{-3}M) at AuNP/poly (p-ABSA)/GCE and poly (p-ABSA)/GCE was studied by SWV (Figure **7.11** and **7.12)**. The pH range studied was from 3-9. The reduction peak current increased with an increase in the pH and thereafter decreased at both the developed sensors. It was found that a high peak current as well as a well-defined reduction peak was obtained at pH 7. So PBS of pH 7 was chosen as the electrolyte for the determination of MBZ.

7.5.3 Effect of scan rate

The cathodic peak current of 1×10^{-3} M MBZ at different scan rates ranging from 30-130 mV/s was measured by SWV at both the developed sensors. The results are illustrated in Figure **7.13** and Figure **7.14**. It was found that the cathodic peak current increased with an increase in the scan rate (υ). The current varied linearly with square root of scan rate (Figure **7.15** and Figure **7.16**), indicating that the reduction of MBZ at AuNP/poly(p-ABSA)/GCE and poly(p-ABSA)/GCE is diffusion controlled.

According to the Laviron's conclusion [203] the relationship between the peak potential (E_p) and υ was examined at both the developed sensors. It was found that E_p varies linearly with ln υ and is shown in Figure **7.17** and Figure **7.18**. The no. of electrons (n_a) involved in the reaction can be calculated from the slope of the plot according to the relation, $b= RT/ \alpha n_a F$, where b is the slope. α of the totally irreversible electrode process is assumed to be 0.5. The obtained value for n_a at AuNP/poly(p-ABSA)/GCE is 2.05 and at poly(p-ABSA)/GCE is 1.83 (around 2). This confirms that 2 electrons are involved in the reduction of MBZ.

The cathodic peak obtained for MBZ at AuNP/poly(p-ABSA)/GCE and poly(p-ABSA)/GCE is attributed to the 2 e^- reduction of nitro group to nitrosyl group[271] (Figure **7.19**).

7.5.4 Effect of film thickness of poly(p-ABSA)

Effect of film thickness of poly(p-ABSA) on the cathodic peak current of 1×10^{-3} M MBZ was investigated. The cathodic peak current gradually increased with increase of scan cycles of electropolymerization. When the cycles were beyond 30, the peak current decreased, may be due to the decreased electron transfer rate of MBZ with an increase in film thickness. Also, the repeatability and stability for the film modified electrode were poor when the voltammetric sweeping segments were less than 30. Hence the film thickness obtained with 30 segment sweeping was selected as the optimal condition.

7.5.5 Effect of number of scan cycles on the electrodeposition of AuNPs

The optimized condition for the deposition of AuNPs on poly (p-ABSA) was investigated. Through changing the cycle number (N) in

electrodeposition process, the amount and the size of the deposited AuNPs can be controlled. The optimum current was obtained when N was 20. When N was below 20, the response current of MBZ increased with increasing N, however, when N was above 20, the currents decreased gradually. On the other hand, the larger N may result larger gold nanostructures. The size of the nanoparticles significantly influenced the catalytic efficiency. Considering the results, 20 cycles was chosen as the optimum cycle number in gold electrodeposition process.

7.5.6 Calibration curve

The relationship between the cathodic peak current of MBZ and its concentration was investigated by DPV (Figure **7.20**) at AuNP/poly(p-ABSA)/GCE. The working concentration range for the developed sensor was found to be 1×10^{-3} M – 1×10^{-7} M. However a linear response was found to obtain from 4×10^{-7}M – 1×10^{-5}M (Figure **7.21**) with a detection limit of 4×10^{-8}M.

The dependence of peak current on the concentration of MBZ at poly(p-ABSA)/GCE was investigated by DPV (Figure **7.22**). The reduction peak current was found to increase with an increase in the concentration over the range 5×10^{-6}M to 1×10^{-4}M (Figure **7.23**). The detection limit was found to be 1×10^{-6}M.

7.5.7 Stability and reproducibility of developed sensors

The storage ability of AuNP/poly (p-ABSA)/GCE was examined. It was found that the current response of MBZ at AuNP/poly (p-ABSA)/GCE showed no apparent decrease in the first continuous 5 days. After everyday use the electrode was stored in 0.1M PBS (pH 7.0). The high stability of the

modified electrode can be related to the poly (p-ABSA) matrix, which increases the effective surface area and stabilizes the activity of AuNPs.

In addition, a series of repetitive measurements were carried out in 1×10^{-3}M MBZ solution to characterize the reproducibility of the AuNP/poly (p-ABSA)/GCE. A relative standard deviation (R.S.D.) of 2.5% for 8 successive determinations of MBZ was observed. This indicated that the modified electrode has excellent reproducibility and good anti-fouling ability.

For poly (p-ABSA) modified electrode also the storage ability was examined. It was observed that the electrode surface showed stability for only 1 day. Also, for testing the reproducibility of poly (p-ABSA) modified electrode, the electrode surface after each determination was regenerated by repetitive cycling in the supporting electrolyte. For five successive determination of 1×10^{-3} M MBZ with the same electrode regenerated after every determination, the RSD of the peak current was 3.2%. This indicated that the poly (p-ABSA)/GCE has excellent reproducibility.

7.5.8 Interference Study

The selectivity of AuNP/poly (p-ABSA)/GCE was examined by studying the effects of foreign species on the determination of MBZ (1×10^{-5} M). It was found that the major interfering species like ascorbic acid, urea, glucose, lactose, Na^+, K^+, SO_4^{2-} and Cl^- did not cause any observable interference when present in 100 fold excess than MBZ (signal change below 5 %). This is due to the high selectivity of the electrode surface coated with AuNP/poly (p-ABSA) for the determination of MBZ. The results are given in Table **7.1**.

Also, the selectivity of poly (p-ABSA)/GCE towards MBZ was determined by measuring the change in the sensor response in the presence of foreign compounds. It was found that a 100-fold excess molar concentration of glucose, Na^+, Cl^-, K^+, SO_4^{2-}, lactose, ascorbic acid and urea almost did not influence the current response of 5×10^{-5}M MBZ (signal change below 5%). The results are tabulated in Table **7.2.**

7.6. Analytical Applications

The sensors developed for MBZ were employed for its determination in tablet forms. The utility of the developed sensors in the determination of MBZ in real sample like urine was also studied.

7.6.1 Determination of MBZ in Pharmaceutical Formulations (Tablets)

The developed sensors were applied for the determination of MBZ in pharmaceutical formulations commercialized as 'Metrogyl' (J.B. Chemicals & Pharmaceuticals, India) and 'Flagyl' (Nicholas Piramal India Ltd) containing 400 mg and 200 mg of the drug respectively, as declared by the company.

The MBZ content in the tablet was determined using the developed sensors by calibration method. A detailed procedure for the determination is given in section 2.8.4 of Chapter 2. The results obtained are summarized in Table **7.3**. The results obtained from the measurements are found to be in satisfactory agreement with the declared amount. The close agreement of the found values with the declared amount is indicative of non-interference of the other ingredients and excipients that are present in the formulation.

In order to test the reliability of this method, the results obtained using AuNP/poly (p-ABSA)/GCE and poly (p-ABSA)/GCE were compared with those obtained by the standard pharmacopoeial method

[184]. The results are illustrated in Table **7.3**. The results show that the developed sensors are highly reliable for the determination of MBZ in pharmaceutical formulations.

7.6.2 Determination of MBZ in urine sample

The developed sensors were applied for the determination of MBZ in urine sample. 2 mL of urine sample and 8 mL phosphate buffer was taken in the cell. Suitable aliquot of MBZ was added to the above solution. The solution was then thoroughly stirred. DPV was then recorded and the cathodic peak current obtained was measured. This procedure was repeated for several additions of MBZ and the unknown concentrations were determined from the calibration graph. The results are summarised in Table **7.4** and Table **7.5.**

7.7 Conclusion

Two voltammetric sensors were developed for the drug MBZ viz, poly (p-ABSA)/GCE and AuNP/poly (p-ABSA)/GCE. Voltammetric determination of MBZ at these developed sensors was carried out by CV, SWV and DPV. MBZ gave a well defined cathodic peak at both these developed sensors. Compared with bare electrode, the voltammetric response at both these sensors was found to be tremendously enhanced. At AuNP/poly (p-ABSA)/GCE the reduction peak was obtained at -0.62V with a peak current of 0.0937mA. The linear concentration range obtained at this sensor was 4×10^{-7}M – 1×10^{-5}M with a detection limit of 4×10^{-8}M. At poly (p-ABSA)/GCE, MBZ gave a reduction peak at -0.652V with a peak current of 0.0516mA. The linear range was found to be 5×10^{-6} M-1×10^{-4} M and the detection limit was found to be 1×10^{-6}M. The fabricated sensors were successfully applied for the determination of MBZ in pure form and dosage forms. The developed sensors were also applied for the determination of the drug in urine sample.

Table 7.1 Study of the effect of foreign species on the cathodic peak current of 1×10^{-5}M MBZ at AuNP/poly (p-ABSA) /GCE

Substance	Concentration, M	Current (µA)	Signal change %
MBZ	1×10^{-5}	13.3272	-
Urea	1×10^{-3}	13.3148	0.09
Na^{+}	1×10^{-3}	13.9151	4.51
K^{+}	1×10^{-3}	13.4584	0.98
Glucose	1×10^{-3}	13.4586	0.99
Cl^{-}	1×10^{-3}	14.0051	4.51
Ascorbic acid	1×10^{-3}	12.8578	3.58
SO_4^{2-}	1×10^{-3}	13.4584	0.98
Lactose	1×10^{-3}	13.2864	0.31

Table 7.2 Study of the effect of foreign species on the cathodic peak current of 5×10^{-5}M MBZ at poly (p-ABSA)/GCE

Substance	Concentration, M	Current (µA)	Signal change, %
MBZ	5×10^{-5}	17.7096	-
Urea	1×10^{-3}	18.1094	2.25
Na^+	1×10^{-3}	16.9079	3.51
K^+	1×10^{-3}	17.4533	1.45
Glucose	1×10^{-3}	18.2546	3.08
Cl^-	1×10^{-3}	16.6079	3.51
Ascorbic acid	1×10^{-3}	17.1465	3.18
SO_4^{2-}	1×10^{-3}	17.4533	1.45
Lactose	1×10^{-3}	17.0425	3.76

Table 7.3 Determination of MBZ in tablets

Sample	Declared Amount (mg/tablet)	Method Adopted	Found* (mg/tablet)	S.D*	C.V*
Metrogyl (J.B. Chemicals & Pharmaceuticals, India)	400	AuNP/poly (p-ABSA) /GCE	404	3.21	0.79
		Poly (p-ABSA) /GCE	401	4.72	1.17
		Standard Method	402	3.84	0.95
Flagyl (Nicholas Piramal India Ltd)	200	AuNP/poly (p-ABSA) /GCE	202	1.90	0.95
		Poly (p-ABSA) /GCE	202	2.24	1.11
		Standard Method	199	1.84	0.92

*Average of 6 replicates

Table 7.4 Determination of MBZ in urine sample using AuNP /poly (p-ABSA)/GCE

Added (mg)	Found (mg)	Recovery (%)
2.0	2.1	105.0
4.0	4.0	100.0
6.0	5.8	96.7
8.0	7.9	98.8

Table 7.5 Determination of MBZ in urine sample using poly(p-ABSA)/GCE

Added (mg)	Found (mg)	Recovery (%)
6.0	6.1	101.7
8.0	7.9	98.8
10.0	9.8	98.0
12.0	11.9	99.2

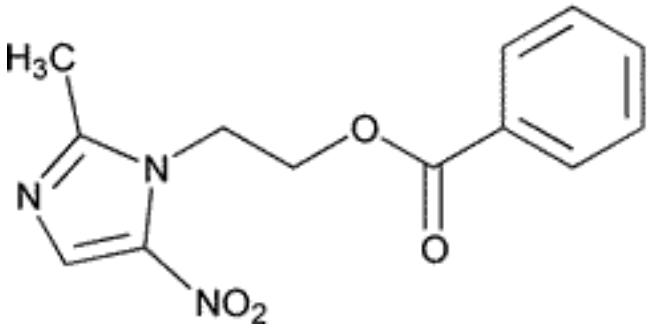


Figure 7.1 Structure of MBZ

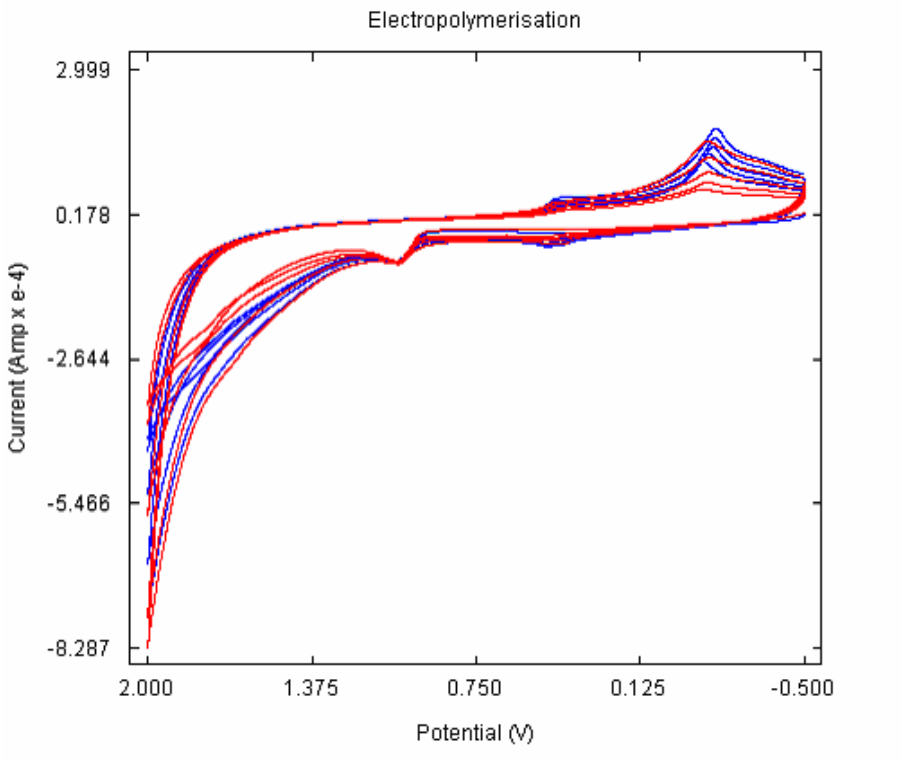
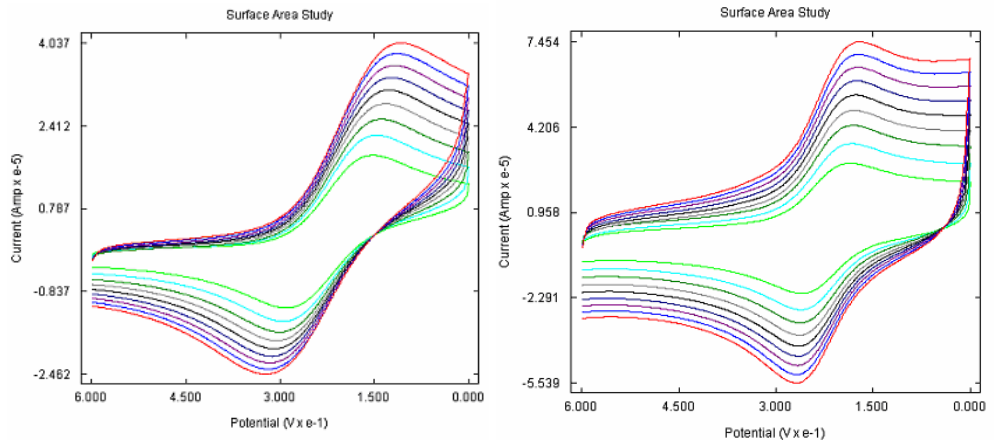
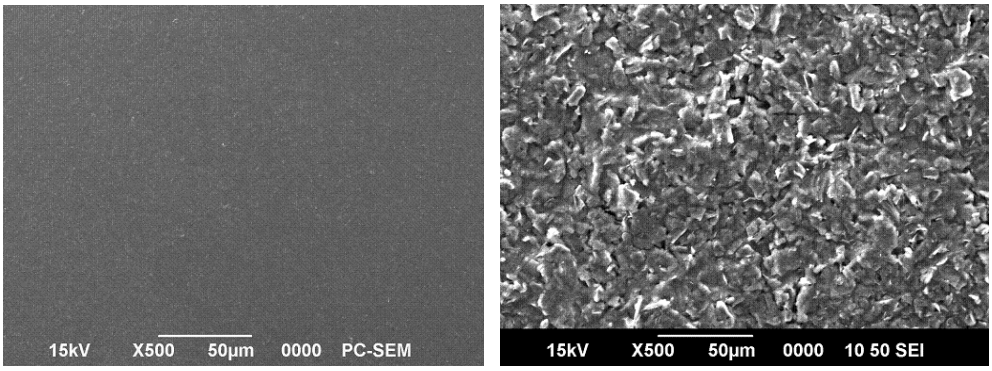


Figure 7.2 Electropolymerization of p-ABSA at GCE



(a) **(b)**

Figure 7.3 Surface area study at a) bare GCE and b) poly(p-ABSA)/GCE



(a) **(b)**

Figure 7.4 SEM images of (a) bare GCE and (b) poly (p-ABSA)/GCE

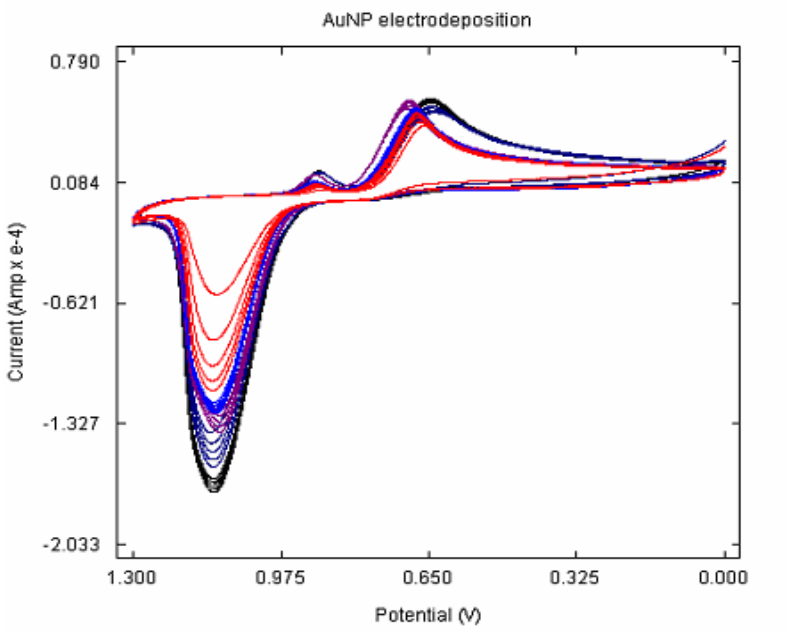


Figure 7.5 Electrodeposition of AuNP at poly(p-ABSA)/GCE

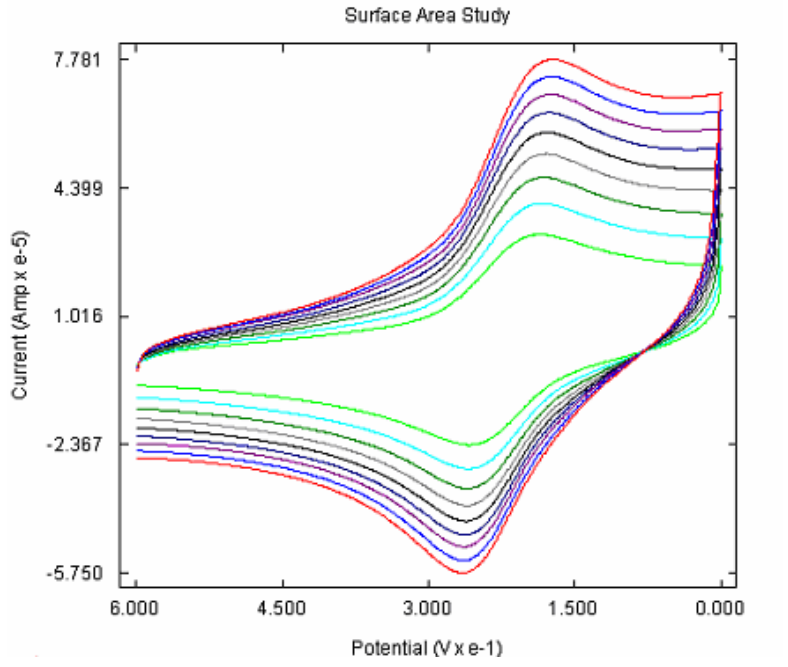


Figure 7.6 Surface area study at AuNP/poly(p-ABSA)/GCE

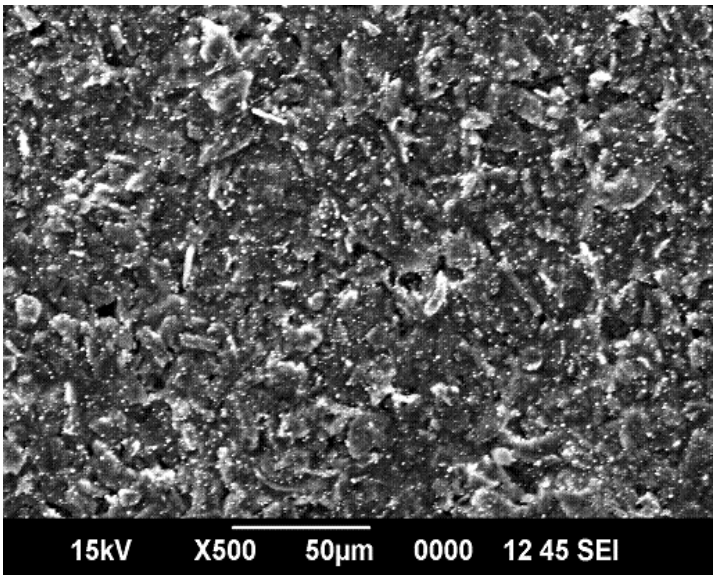


Figure 7.7(a) SEM image of AuNP/poly (p-ABSA)/GCE

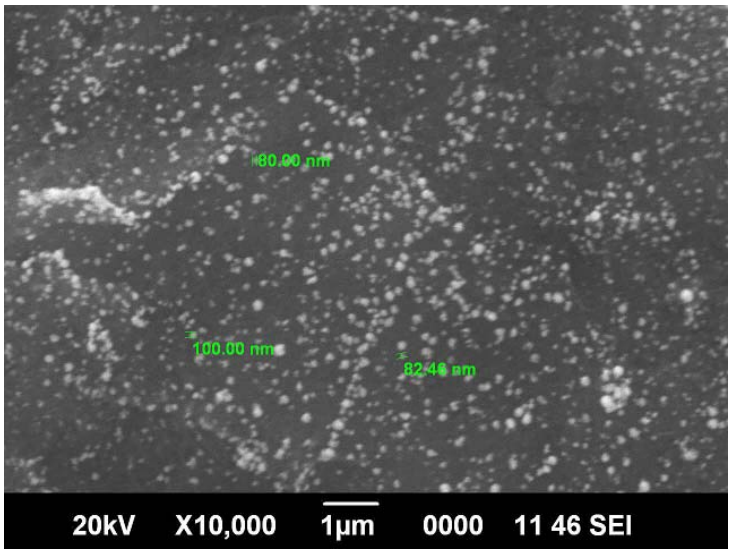


Figure 7.7(b) SEM image of AuNP/poly (p-ABSA)/GCE showing the size of AuNPs

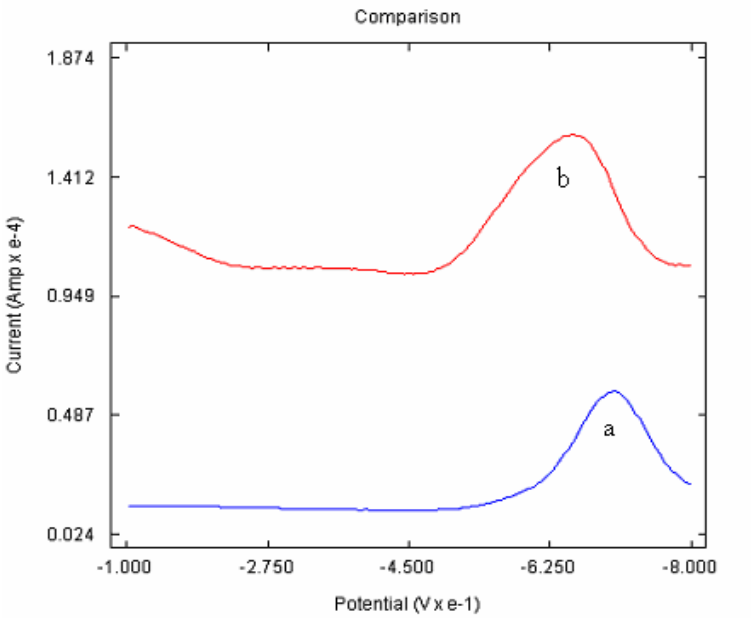


Figure 7.8 Overlay of Square Wave Voltammogram of MBZ at (a) bare GCE (b) poly (p-ABSA)/GCE at a scan rate of 0.06Vs^{-1} from -0.1V to -0.8 V

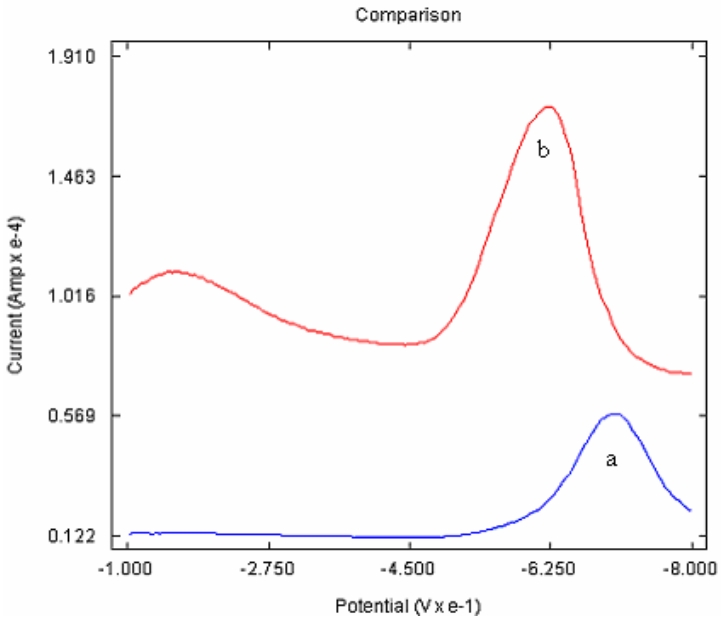


Figure 7.9 Overlay of Square Wave Voltammogram of MBZ at (a) bare GCE (b) AuNP/poly (p-ABSA)/GCE at a scan rate of 0.06Vs^{-1} from -0.1V to -0.8 V

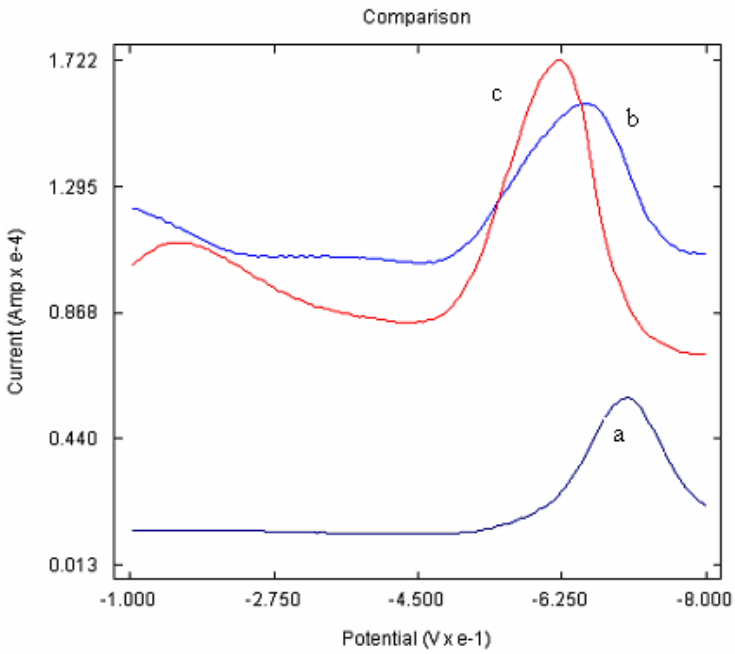


Figure 7.10 Overlay of Square Wave Voltammograms of MBZ at (a) bare GCE (b) poly (p-ABSA)/GCE and (c)AuNP/ poly (p-ABSA)/GCE at a scan rate of 0.06Vs^{-1} from -0.1V to -0.8 V

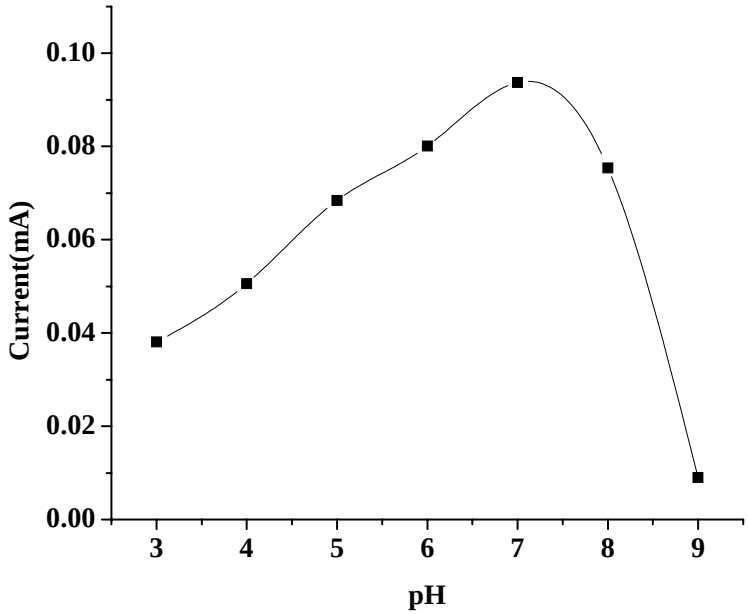


Figure 7.11 Effect of pH on the cathodic peak current of 1×10^{-3}M MBZ at AuNP/ poly (p-ABSA)/GCE

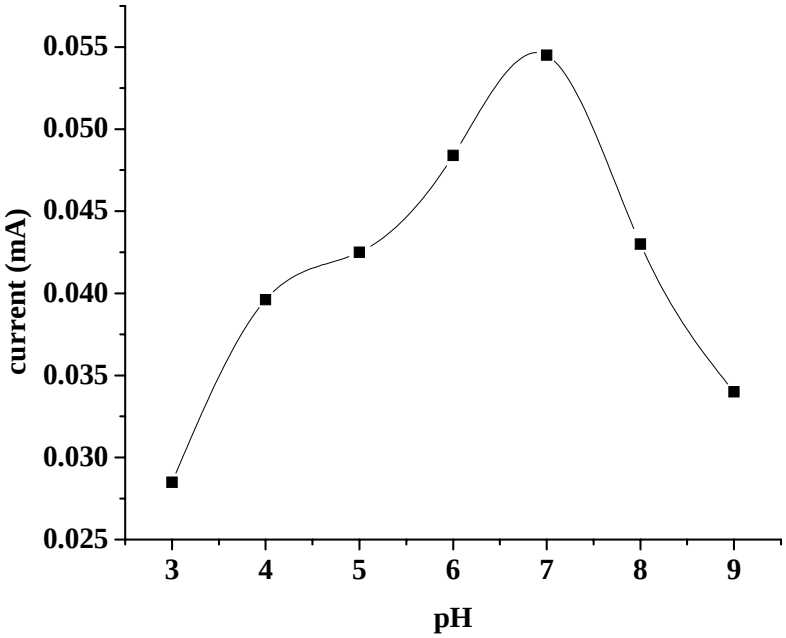


Figure 7.12 Effect of pH on the cathodic peak current of 1×10^{-3}M MBZ at poly (p-ABSA)/GCE

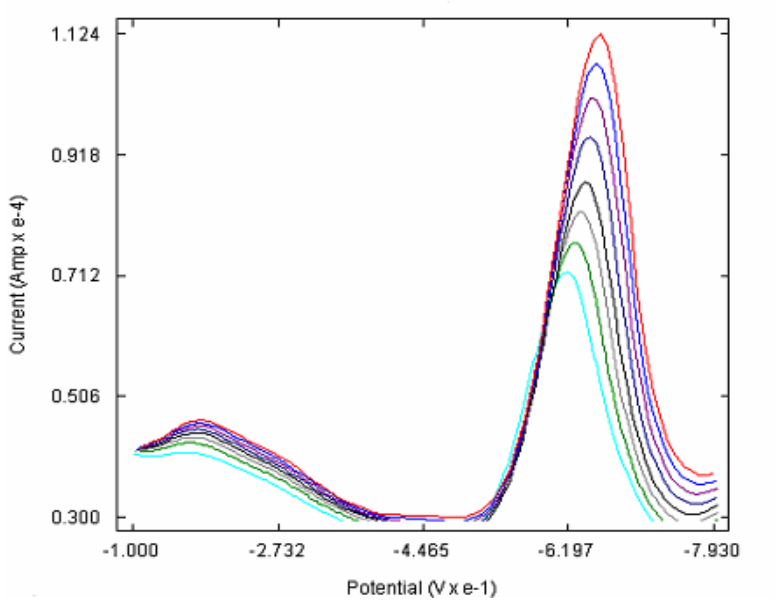


Figure 7.13 Overlay of SWVs of MBZ at AuNP/poly (p-ABSA)/GCE in 0.1M phosphate buffer (pH 7) containing 1×10^{-3}M MBZ at different scan rates a) 30 b) 45 c) 60 d) 75 e) 90 f) 105 g) 120 h) 135 mVs^{-1}.

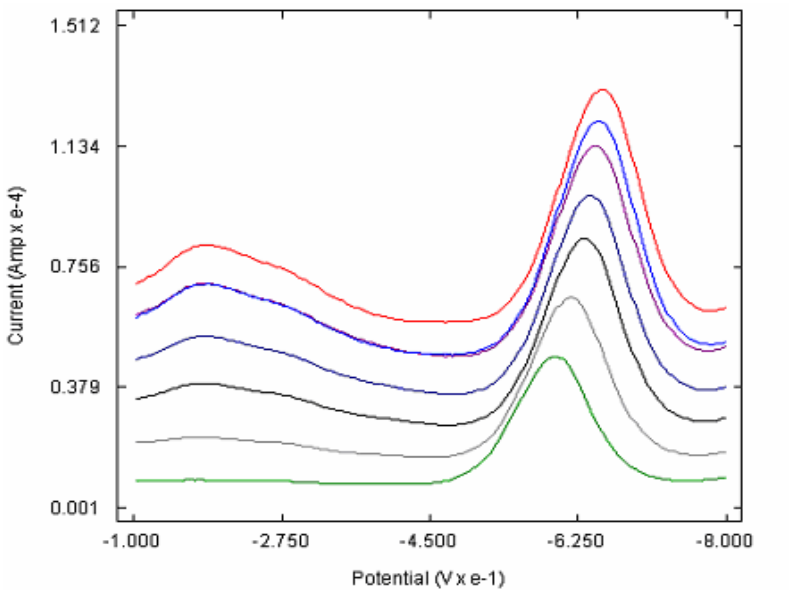


Figure7.14 Overlay of SWVs of MBZ at poly (p-ABSA)/GCE in 0.1M phosphate buffer (pH 7) containing 1×10^{-3}M MBZ at different scan rates a) 30 b) 45 c) 60 d) 75 e) 90 f) 105 g) 120 h) 135 mVs^{-1}.

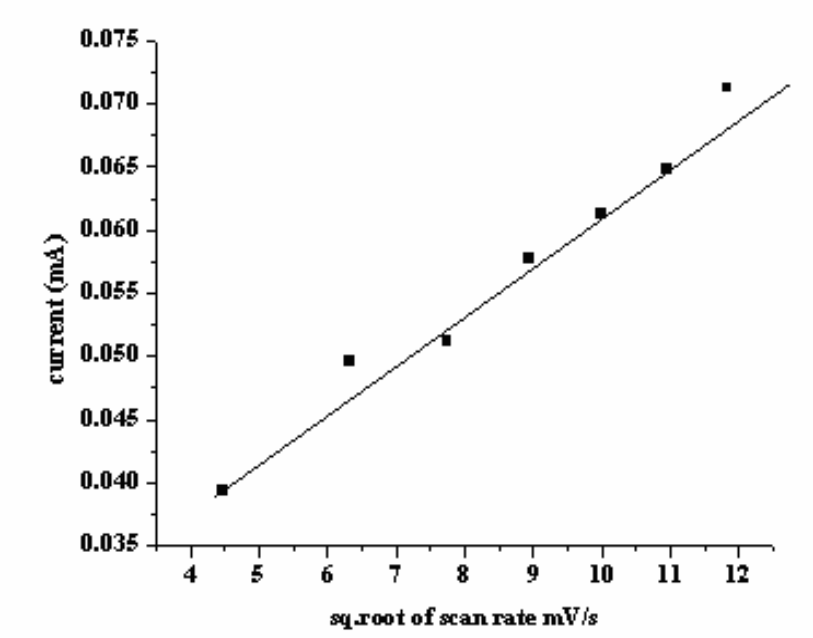


Figure 7.15 Effect of scan rate at AuNP/poly(p-ABSA)/GCE

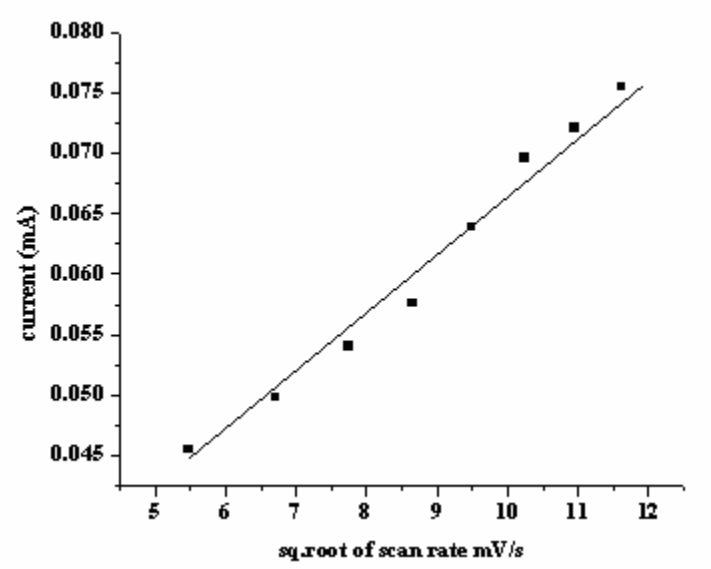


Figure 7.16 Effect of scan rate at poly(p-ABSA)/GCE

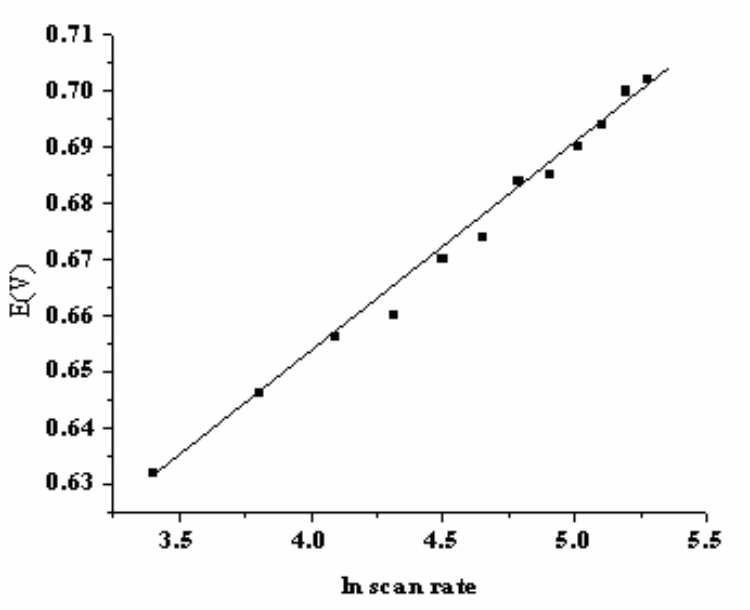


Figure 7.17 Plot of peak potential against ln (scan rate) at AuNP / poly(p-ABSA)/GCE

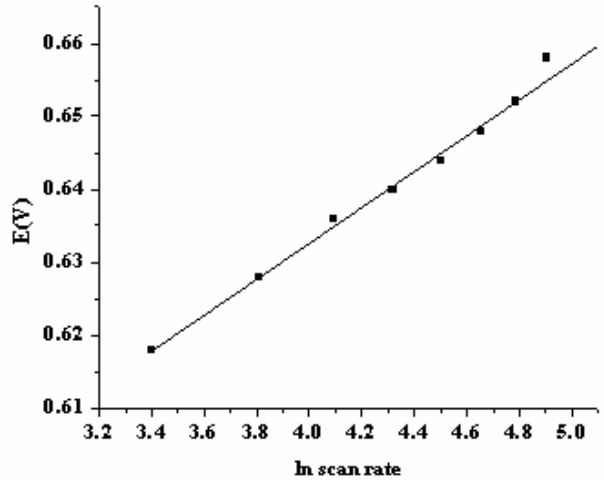


Figure 7.18 Plot of peak potential against ln (scan rate) at poly (p-ABSA)/GCE

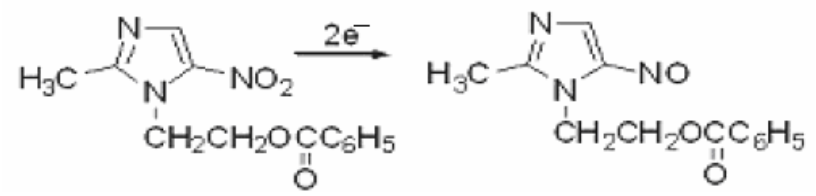


Figure 7.19 Mechanism showing the $2e^-$ reduction of MBZ

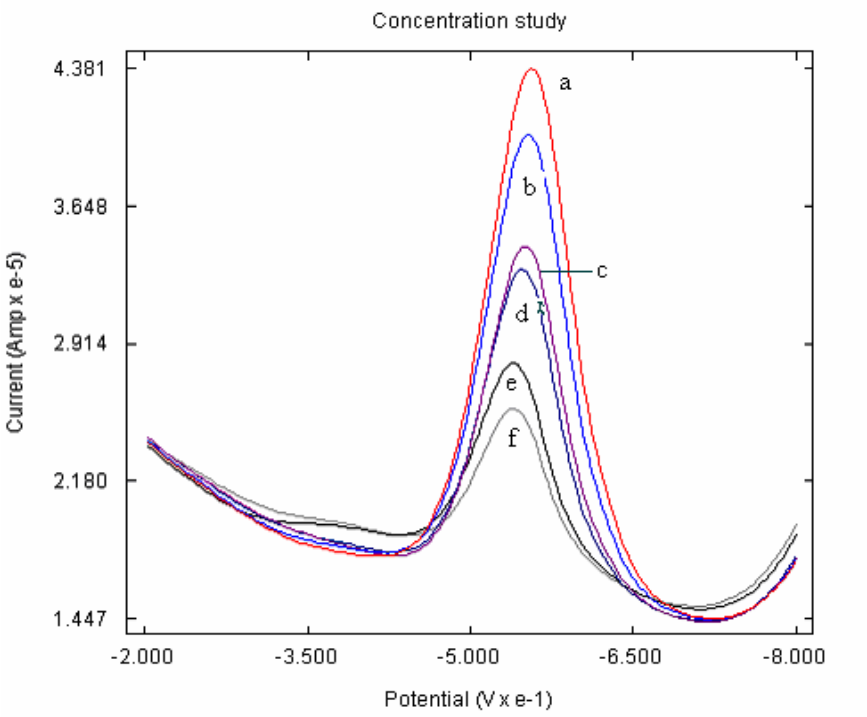


Figure 7.20 Differential Pulse Voltammogram of MBZ of different concentrations a) 1×10^{-5}M b) 8×10^{-6}M c) 5×10^{-6}M d) 2×10^{-6}M e) 8×10^{-7}M f) 4×10^{-7}M at AuNP/poly (p-ABSA) modified GCE at 25^0C

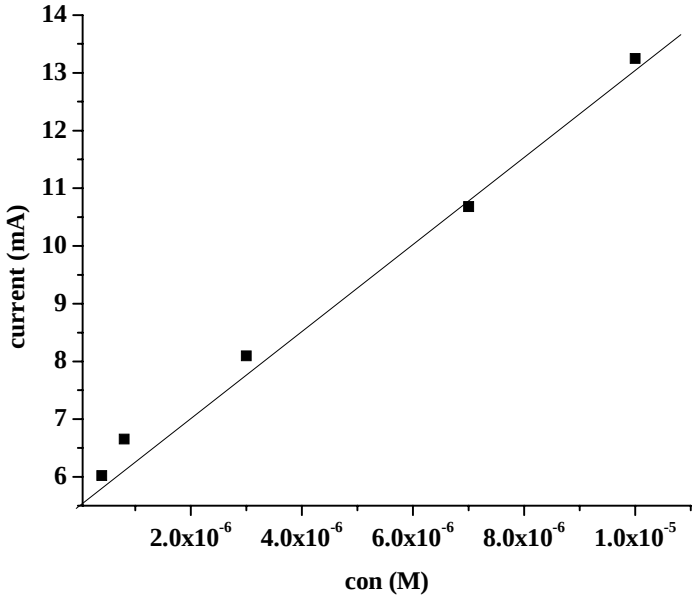


Figure 7.21 Calibration curve for MBZ at AuNP/poly (p-ABSA)/ GCE at 25^0C

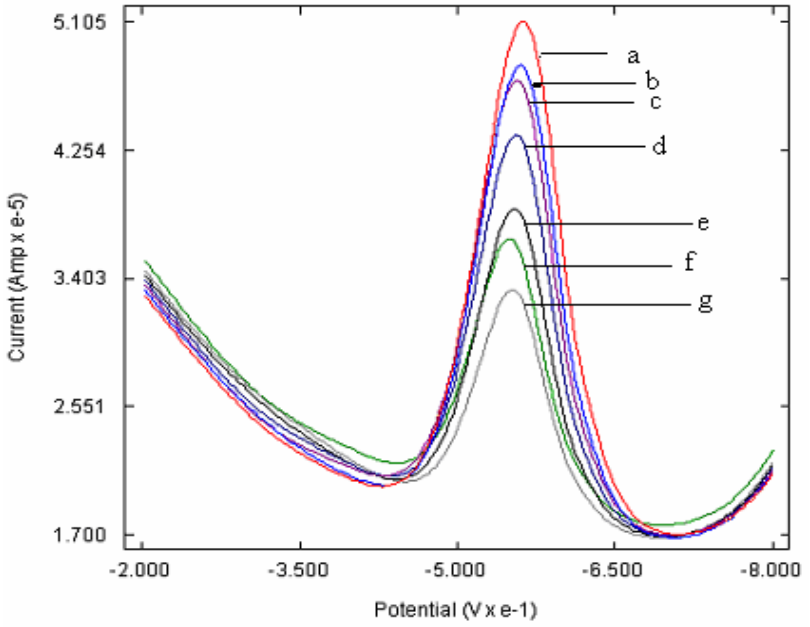


Figure 7.22 Differential Pulse Voltammogram of MBZ of different concentrations a) 1×10^{-4}M b) 6×10^{-5}M c) 2×10^{-5}M d) 9×10^{-6}M e) 7×10^{-6}M f) 5×10^{-6}M at poly (p-ABSA) modified GCE at 25^0C

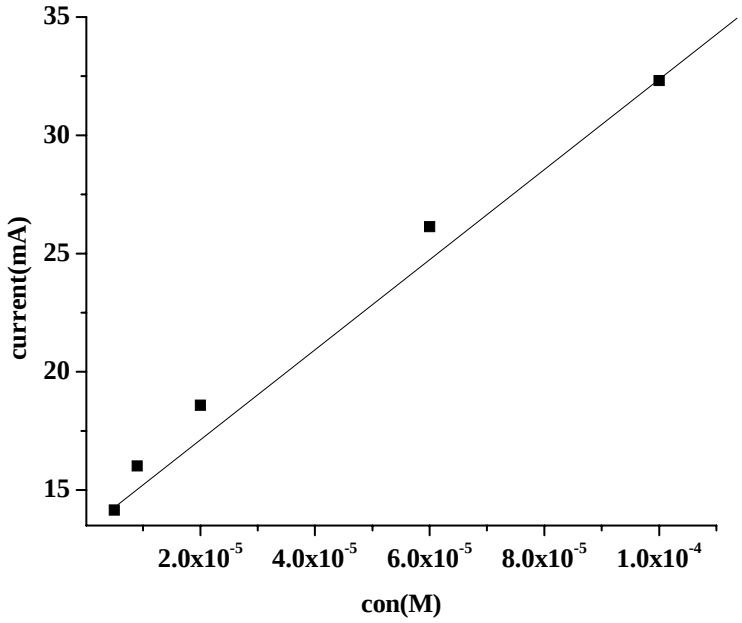


Figure 7.23 Calibration curve for MBZ at poly (p-ABSA)/GCE at 25^0C

Chapter 8

SENSORS FOR THE DETERMINATION OF NIMESULIDE

CONTENTS

The construction and performance characteristics of two types of sensors for the drug Nimesulide (NIM) have been discussed in this chapter. The sensors include a poly cystamine dihydrochloride (poly-CA) modified glassy carbon sensor and gold nanoparticle (AuNP)/ poly-CA modified glassy carbon sensor. The poly-CA/GCE was fabricated by electropolymerisation of 1×10^{-3}M CA on GCE surface by CV. AuNP/poly-CA/GCE was fabricated by the electrochemical deposition of $HAuCl_4$ onto the surface of poly-CA/GCE. The electrodes thus prepared were then characterized by surface morphology studies and surface area studies. Along with their response characteristics, the present study has covered the applicability of the newly developed sensors in the determination of NIM in pharmaceutical formulations and real samples like urine.

8.1 Introduction

Nimesulide, N-(4-Nitro-2-phenoxyphenyl) methane sulfonamide ($C_{13}H_{12}N_2O_2S$), is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic activity [272] (Figure **8.1**). It acts as a cyclooxygenase- 2(COX-2)inhibitor and also has other novel pharmacological features which account for its effect in the control of pain and inflammation. It has become a leading NSAID in over 50 countries worldwide. NIM provides relief from primary dysmenorrhoea in adolescents and osteoarthritis.

NIM is chemically different from other drugs in this class because of the sulfonanilide moiety. Like all NSAIDs, NIM acts by inhibiting the synthesis of prostaglandins as a consequence of blockade of the enzyme COX. NIM has potent analgesic, anti-inflammatory and antipyretic activities on oral and rectal administration. NIM possesses a much lower risk for gastroduodenal lesions in comparison to classical NSAIDs [273].

Several analytical methods have been reported for the quantitative determination of NIM, of which HPLC methods for the determination of NIM in biological fluids are the most important [274-280]. Other methods include spectrophotometry[281-283], capillary zone electrophoresis[284], ion association method[285], reverse phase HPLC method[286] etc. For biological fluid samples, a thin layer chromatographic method was used for the determination of NIM in plasma[287]. Electrochemical determination 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 electrochemical methods available

for the determination of NIM in literature including polarography[288], differential pulse polarography[289], potentiometry[290], adsorptive stripping voltammetry[291] and amperometry[292]. The present work describes the fabrication of differential pulse voltammetric sensors for the determination of NIM.

As part of the present investigations, two sensors were fabricated viz. poly-CA/GCE and AuNP/ poly-CA/GCE for the voltammetric determination of NIM. The poly- CA/GCE was fabricated by electropolymerisation of CA using CV. The (AuNP)/ poly-CA modified electrode was fabricated by the electrochemical deposition of AuNP on to the surface of GCE from a 0.05M H_2SO_4 solution containing 1mM $HAuCl_4$. The performance characteristics of the developed sensors were studied in detail. The sensors were successfully applied for the determination of NIM in pure form, pharmaceutical preparations and real sample like urine and the results obtained are in good agreement with that obtained by the official method.

8.2 Preparation of poly-CA/GCE

Firstly, the GCE was cleaned as described in section 2.3 of Chapter 2. The poly-CA/GCE was prepared by electropolymerization of Cystamine hydrochloride (CA) on GCE. Detailed procedure for the fabrication of poly-CA/GCE is given in section 2.4.5(i) of Chapter 2.

The film was grown on the electrode surface by 20 segments of cyclic voltammetric scans. As shown in Figure **8.2**, an anodic peak at 1.26 V and a cathodic peak at −0.536 V were observed. The oxidation and reduction peak currents increase with an increase in the cyclic number of voltammetric scans, indicating that an electroconductive polymer film has

been formed on the electrode surface. After immobilization, the film was washed thoroughly with double distilled water and dried in air. A blue thin film was found to be formed on the electrode surface.

Surface Area study gave a strong evidence for the effective modification of GCE with poly-CA film. 2 mM $K_3Fe(CN)_6$ was taken as a probe[202] to measure the effective surface areas of both poly-CA/GCE and bare GCE by CV at different scan rates (Figure **8.3**). From the slope of I_p vs. $\nu^{1/2}$ plot, the areas of bare GCE and poly–CA/GCE were estimated and was found to be 0.0911 cm^2 and 0.1476 cm^2 respectively. There was an enhancement in the effective surface area when GCE was modified with poly-CA which is a strong evidence for the successful modification of GCE with polymer film.

SEM images (Figure **8.4**) clearly indicated that effective modification of GCE surface has taken place after electropolymerization.

8.3 Preparation of AuNP/poly-CA modified GCE

AuNP/poly-CA modified electrode was fabricated as explained under section 2.4.5(ii) of Chapter 2.

AuNPs were deposited on the surface of poly-CA/GCE by 20 segments of cyclic voltammetric scans [181]. (Figure **8.5**). With the potential scanning from 1.3V to 0 V, 2 reduction peaks were observed at 0.935V and 0.716V and an oxidation peak was observed at 1.09V. In the subsequent cycles larger peaks were observed upon continuous scanning, which indicated that AuNPs were deposited on the electrode surface.

The effective surface area of AuNP/poly-CA/GCE was measured using CV technique (Figure **8.6)**. The surface area obtained for AuNP/poly-

CA/GCE was 0.1945cm^2. Compared with both bare electrode as well as poly-CA/GCE, there was an increase in the surface area for AuNP/ poly-CA/GCE which is a strong evidence for the successful modification.

SEM image (Figure **8.7**(a) and (b)) of AuNP/poly-CA/GCE clearly showed that AuNPs were distributed in an almost homogeneous manner at the surface of the poly-CA film.

8.4 Electrochemical behavior of NIM

8.4.1 Poly-CA/GCE

Stock solution of NIM was prepared as described in section 2.5.6 of Chapter 2. Standard solutions of the analyte (1×10^{-4}- 1×10^{-6} M) were prepared by serial dilution of stock solution with the supporting electrolyte (PBS, pH 6). Sample solution (1×10^{-4}M) was then taken in the electrochemical cell. The solution was then de-aerated with nitrogen for 5 min. First CV was recorded at both bare electrode and poly-CA/GCE from -0.2 V to -0.8 V at a scan rate of 0.1Vs^{-1}. A single reduction peak was obtained at both the electrodes, indicating that the electrochemical response of NIM is irreversible. However, compared with CV, NIM gave a better response using Square Wave Voltammetric technique at both the electrodes. Hence, SWV was performed at both bare GCE and poly-CA/GCE and the voltammograms were recorded from -0.2 to -0.8 V (Figure **8.8**). A reduction peak at -0.64V with a peak current of 8.9837μA was obtained at bare GCE. Also, the reduction peak obtained at bare GCE was a broad one suggesting slow electron transfer, presumably due to the fouling of the electrode surface by the reduction product. But at poly-CA/GCE, NIM gave a well defined reduction peak at -0.60V with a peak current of 19.2691 μA. Compared with bare GCE, the reduction potential has shifted negatively by about 0.04V and the peak current has also

increased. This clearly depicted the electrocatalytic activity of poly-CA film on the voltammetric determination of NIM.

8.4.2 AuNP/poly –CA/GCE

Firstly an attempt was made to modify the poly-CA/GCE with gold nanoparticles, synthesized by the well known citrate reduction method (Turkevich method). Au has a strong specific interaction with sulfur that allows the formation of monolayers. Cystamine has got S atoms which can form covalent bond with Au atom.

The poly-CA/GCE was immersed in a solution of AuNP for 10h and then the electrode was taken out and washed thoroughly with double distilled water. The modified electrode thus prepared was then used to study the voltammetric behavior of NIM. However, at the modified electrode thus prepared, NIM did not show any remarkable change in the voltammetric response when compared with that at the poly-CA/GCE. The experiment was repeated by varying the dipping time, but unfortunately no change in the voltammetric behavior of NIM was observed.

Then an effort was made to deposit the AuNPs electrochemically on the surface of poly-CA/GCE. The AuNP modified electrode thus prepared electrochemically showed observable changes in the voltammetric behavior of NIM. There was remarkable change in the peak current (increased) and the reduction potential (lowered) of NIM at the AuNP modified electrode than at the poly-CA/GCE.

The electrochemical behavior of 1×10^{-4}M NIM at AuNP/poly-CA/GCE was studied by CV and SWV. NIM gave a single reduction peak at AuNP/poly-CA/GCE using CV technique. No peak was obtained in the

reverse sweep of CV indicating that the electrode process is irreversible. But a more defined peak for NIM was obtained at AuNP/poly-CA/GCE using SWV. Hence, SW voltammogram was recorded from -0.2V to -0.8V at a scan rate of $0.06Vs^{-1}$. NIM gave a well defined reduction peak at -0.576V with a peak current of 28.2567 μA at AuNP/poly-CA/GCE. Compared with bare GCE, there was a reduction in the cathodic potential by about 0.07V and 3 times enhancement in the peak current at AuNP/poly-CA/GCE (Figure **8.9**). Also, when compared with poly- CA/GCE, there was a reduction in the potential by about 0.03V and an increase in the peak current at AuNP/poly-CA/GCE (Figure **8.10**). All these factors clearly revealed that polymer film supported AuNPs have enhanced catalytic activity towards the electrochemical reduction of NIM. A comparison of voltammetric behavior of NIM at bare GCE, poly-CA/GCE and AuNP/poly-CA/GCE is shown in Figure **8.11.**

8.5 Optimisation Studies

The response characteristics of the developed sensors depend on various parameters such as effect of supporting electrolyte, pH, scan rate, film thickness and concentration.

8.5.1 Effect of supporting electrolyte

The electrochemical behavior of NIM in various media such as 0.1 M solutions of phosphate buffer, acetate buffer, citrate buffer, H_2SO_4, NaOH, NaCl and KNO_3 were studied by SWV at both the developed sensors. The obtained reduction peak was best defined in 0.1 M PBS. So 0.1 M PBS was taken as the experimental medium for NIM.

8.5.2 Effect of pH

The influence of pH on the cathodic peak current of NIM (1×10^{-4}M) at poly-CA/GCE and AuNP/poly-CA/GCE was studied by SWV

(Figure **8.12** and **8.13)**. The pH range studied was from 3-9. The reduction peak current increased with an increase in the pH and thereafter decreased at both the developed sensors. It was found that a high peak current as well as a well-defined reduction peak was obtained at pH 6. So PBS of pH 6 was chosen as the electrolyte for the determination of NIM.

8.5.3 Effect of scan rate

The cathodic peak current of 1×10^{-4} M NIM at different scan rates ranging from 20-180 mV/s was measured by SWV at both the developed sensors. The results are illustrated in Figure **8.14** and Figure **8.15**. It was found that the cathodic peak current increased with an increase in the scan rate (υ). The current varied linearly with square root of scan rate (Figure **8.16** and Figure **8.17**), indicating that the reduction of NIM at poly-CA/GCE and AuNP/poly-CA/GCE is diffusion controlled.

According to the Laviron's conclusion [203] the relationship between the peak potential (E_p) and υ was examined at both the developed sensors. It was found that E_p varies linearly with ln υ and is shown in Figure **8.18** and Figure **8.19**. The no. of electrons (n_a) involved in the reaction can be calculated from the slope of the plot according to the relation, $b= RT/ \alpha n_a F$, where b is the slope. α of the totally irreversible electrode process is assumed to be 0.5. The obtained value for n_a at poly-CA/GCE is 1.97 and at AuNP/poly-CA/GCE is 2.23. This confirms that 2 electrons are involved in the reduction of NIM.

The cathodic peak obtained for NIM at poly-CA/GCE and at AuNP/poly-CA/GCE is attributed to the 2 e^- reduction of nitro group to nitrosyl group[293] (Figure **8.20**).

8.5.4 Effect of film thickness of poly-CA

The influence of film thickness on the cathodic peak current of 1×10^{-4} M NIM was studied. The cathodic peak current gradually increased with increase in the number of scan cycles of electropolymerization. When the cycles were beyond 20, the peak current decreased, may be due to the decreased electron transfer rate of NIM with an increase in film thickness. Also, the repeatability and stability for the film modified electrode were poor when the voltammetric sweeping segments were less than 20. Hence the film thickness obtained with 20 segment sweeping was selected as the optimal condition.

8.5.5 Effect of number of scan cycles for the electrodeposition of AuNPs

The influence of the number of scan cycles for the electrodeposition of AuNPs on the cathodic peak current of NIM was investigated. The amount and the size of the deposited AuNPs can be controlled by changing the cycle number (N) in electrodeposition process. The optimum current was obtained when N was 20. When N was below 20, the response current of NIM increased with increasing N, however, when N was above 20, the current decreased gradually. Considering the results, 20 cycles was chosen as the optimum cycle number in gold electrodeposition process.

8.5.6 Calibration curve

The dependence of peak current on the concentration of NIM at poly-CA/GCE was investigated by DPV (Figure **8.21**). The reduction peak current was found to increase with an increase in the concentration over the range 1×10^{-5}M to 1×10^{-3}M (Figure **8.22**). The detection limit was found to be 2.29×10^{-6}M.

To estimate the repeatability of the developed method, the relative standard deviation (RSD) of five measurements of 1×10^{-4}M NIM at the same modified electrode was measured. The obtained RSD is 3.7% which showed the excellent reproducibility of the modified electrode.

Also, at AuNP/poly-CA/GCE, the dependence of cathodic peak current of NIM on its concentration was investigated by DPV (Figure **8.23**). A linear response was found to obtain from 1×10^{-6}M – 1×10^{-4}M (Figure **8.24**) with a detection limit of 3.25×10^{-7}M.

The AuNP/poly-CA/GCE modified electrode surface after each determination was regenerated by repetitive cycling in the supporting electrolyte. For five successive determination of 1×10^{-4} M NIM with the same electrode regenerated after every determination, the RSD of the peak current was 2.5%. This indicated that the AuNP/poly-CA/GCE has excellent reproducibility.

8.5.7 Interference Study

The selectivity of the poly-CA/GCE was also examined by studying the effects of foreign species on the determination of NIM (1×10^{-4} M). It was found that the major interfering species such as ascorbic acid, citric acid, urea, glucose, lactose, Na^+, K^+, SO_4^{2-} and Cl^- did not cause any observable interference when present in 100 fold molar excess when compared with the concentration of NIM(signal change below 5 %). This is due to the high selectivity of the electrode surface coated with poly-CA for the determination of NIM. The results are given in Table **8.1**.

Under optimised experimental conditions, the effects of some foreign species on the determination of NIM (1×10^{-4}M) using AuNP/poly-CA/GCE

were evaluated in detail. The results are given in Table **8.2**. 100-fold concentration of glucose, lactose, citric acid, Na^+, K^+, SO_4^{2-} and Cl^- have almost no influence on the current response of NIM (signal change below 5 %).

8.6 Application Studies

The developed sensors were employed for the determination of the drug content in its tablet form. The application of the developed sensors in the determination of NIM in real sample like urine was also studied.

8.6.1 Determination of NIM in Pharmaceutical Formulations (Tablets)

The developed sensors were successfully applied for the determination NIM in commercially available pharmaceutical formulation, 'Nise' (Dr. Reddy's, India) containing 100 mg of the drug as declared by the company.

The NIM content in the tablet was determined using the developed sensors by calibration method. A detailed procedure for the determination is given in section 2.8.5 of Chapter 2. The results obtained are summarized in Table **8.3**. The results obtained from the measurements are found to be in satisfactory agreement with the declared amount. The close agreement of the found values with the declared amount is indicative of non-interference of the other ingredients and excipients that are present in the formulation.

The results were compared with those obtained by the standard method (potentiometric titration) [184]. The results (shown in Table **8.3**) show that there is a satisfactory agreement between the NIM content determined by the developed methods and the standard method.

8.6.2 Determination of NIM in urine sample

The developed sensors were successfully applied for the determination of NIM in urine sample. 2 mL of urine sample and 8 mL phosphate buffer was taken in the cell. Suitable aliquot of NIM was added to the above solution. The solution was then thoroughly stirred. DPV was then recorded and the cathodic peak current obtained was measured. This procedure was repeated for several additions of NIM and the unknown concentrations were determined from the calibration graph. The results are summarised in Tables **8.4** and **8.5.**

8.7 Conclusion

Voltammetric determination of the drug NIM was carried out using two developed sensors viz, poly-CA/GCE and AuNP/poly-CA/GCE. Compared with bare electrode, the electrochemical response of NIM was found to be tremendously enhanced at both these developed sensors. Among the two developed sensors, the AuNP/poly-CA/GCE showed a better response towards NIM than poly-CA/GCE. At AuNP/poly-CA/GCE, NIM(1×10^{-4} M) gave a well defined reduction peak at -0.576V with a peak current of 28.2567 μA and the linear respose range was found to be 1×10^{-4} M – 1×10^{-6} M with a detection limit of 3.25×10^{-7} M. However, at poly-CA/GCE the reduction peak was obtained only at -0.6V with a peak current of 19.2691 μA. The linear working range was found to be 1×10^{-4} M – 1×10^{-5} M and the detection limit was only 2×10^{-6}M. The combined catalytic activity of AuNPs and the polymer film attributed to the enhanced sensitivity of AuNP/poly-CA/GCE. The fabricated sensors were successfully applied for the determination of NIM in pure form and dosage forms. The developed sensors were also applied for the determination of the drug in urine sample.

Table 8.1 Study of the effect of foreign species on the cathodic peak current of 1×10^{-4}M NIM at poly-CA/GCE

Substance	Concentration, M	Current(μA)	Signal change, %
NIM	1×10^{-4}	19.2691	-
Urea	1×10^{-2}	18.4167	4.42
Na^+	1×10^{-2}	19.9546	3.55
K^+	1×10^{-2}	18.4584	4.20
Glucose	1×10^{-2}	19.6587	2.02
Cl^-	1×10^{-2}	19.9546	3.55
Ascorbic acid	1×10^{-2}	18.8578	2.13
SO_4^{2-}	1×10^{-2}	18.4584	4.20
Lactose	1×10^{-2}	20.0864	4.24

Table 8.2 Study of the effect of foreign species on the cathodic peak current of 1×10^{-4}M NIM at AuNP/poly-CA/GCE

Substance	Concentration, M	Current(μA)	Signal change, %
NIM	1×10^{-4}	28.2567	-
Urea	1×10^{-2}	29.6384	4.88
Na^+	1×10^{-2}	27.6272	2.23
K^+	1×10^{-2}	29.1886	3.29
Glucose	1×10^{-2}	28.4586	0.71
Cl^-	1×10^{-2}	27.6272	4.88
Ascorbic acid	1×10^{-2}	29.0578	2.83
SO_4^{2-}	1×10^{-2}	29.1886	3.29
Lactose	1×10^{-2}	28.2864	0.11
Citric acid	1×10^{-2}	28.7456	1.73

Table 8.3 Determination of NIM in tablet

Sample	Declared Amount (mg/tablet)	Method Adopted	Found* (mg/tablet)	S.D*	C.V*
Nise (Dr. Reddy's, India)	100	AuNP/poly-CA/GCE	102	1.31	1.29
		Poly-CA/GCE	103	2.72	2.64
		Standard Method	102	1.84	1.80

*Average of six replicates

Table 8.4 Determination of NIM in urine sample using poly-CA/GCE

Added (mg)	Found (mg)	Recovery (%)
5.0	5.2	104.0
7.0	6.9	98.6
9.0	9.1	101.1
11.0	10.9	99.1

Table 8.5 Determination of NIM in urine sample using AuNP/ poly-CA/GCE

Added (mg)	Found (mg)	Recovery (%)
2.0	2.0	100.0
4.0	3.9	97.5
6.0	5.8	96.7
8.0	8.2	102.5

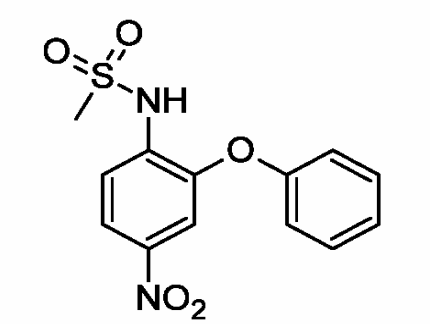


Figure 8.1 Structure of NIM

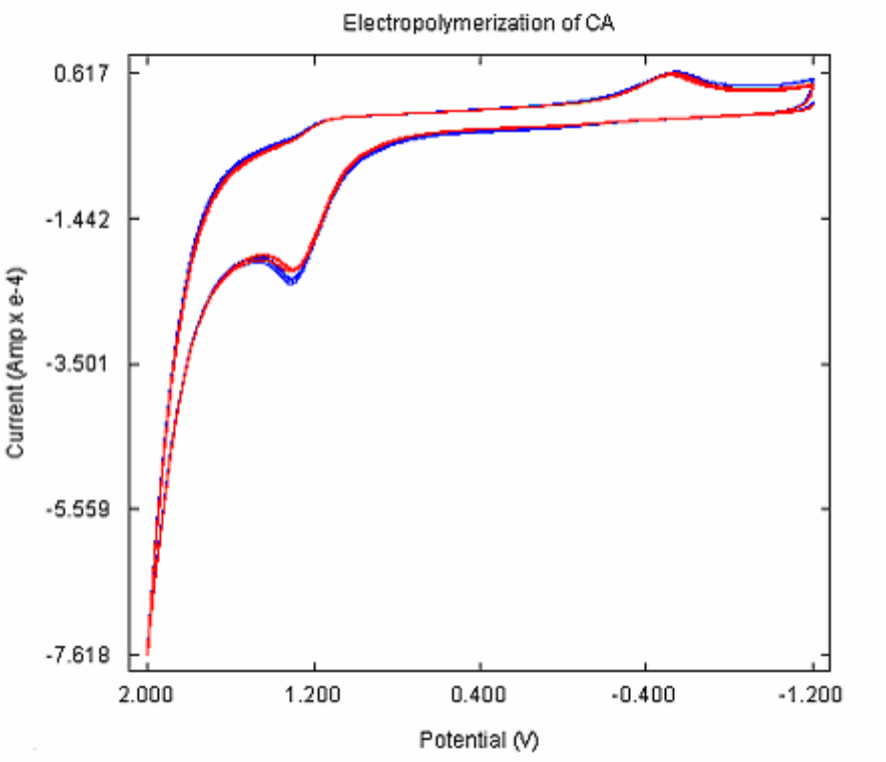
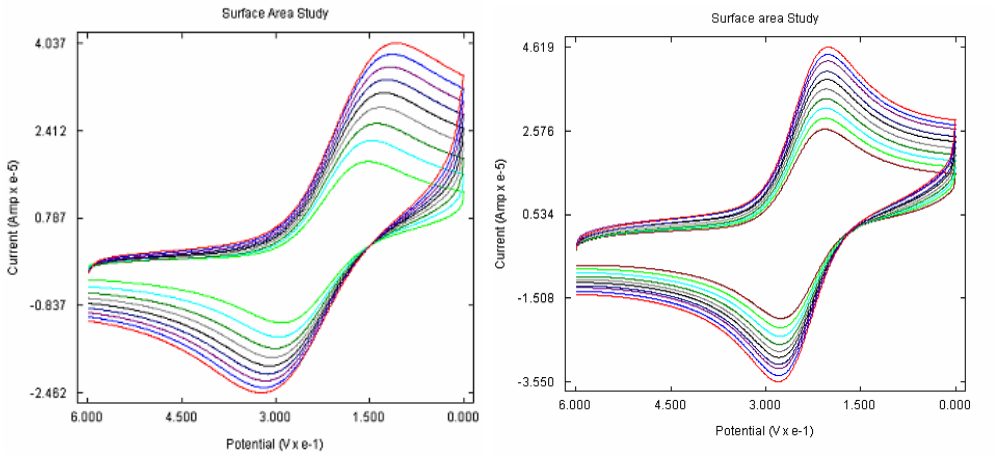
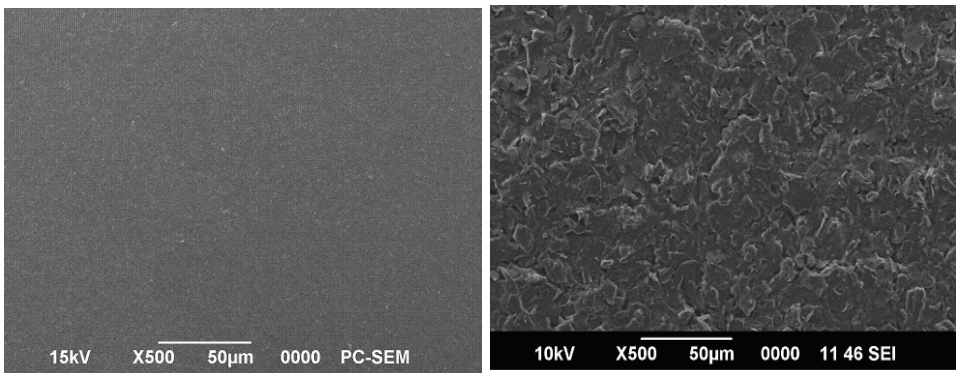


Figure 8.2 Electropolymerization of CA at GCE



(a) **(b)**

Figure 8.3 Surface area study at a) bare GCE and b) poly- CA/GCE



(a) **(b)**

Figure 8.4 SEM images of (a) bare GCE and (b) poly -CA/GCE

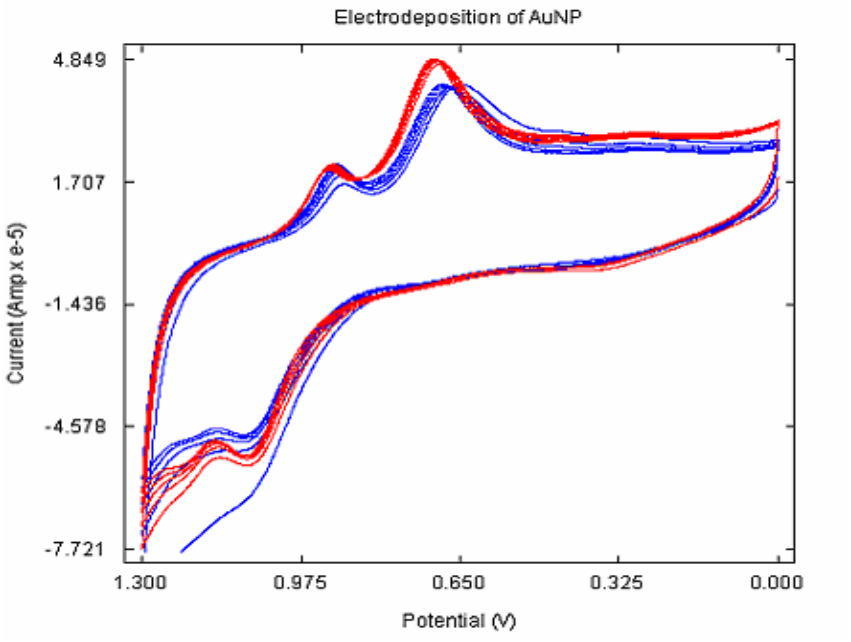


Figure 8.5 Electrodeposition of AuNP at poly-CA/GCE

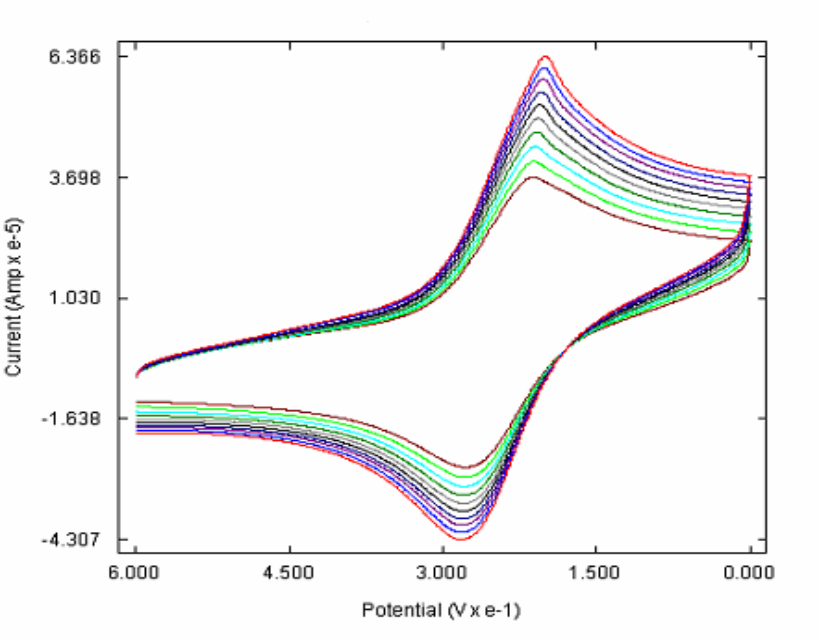


Figure 8.6 Surface area study at AuNP/poly-CA/GCE

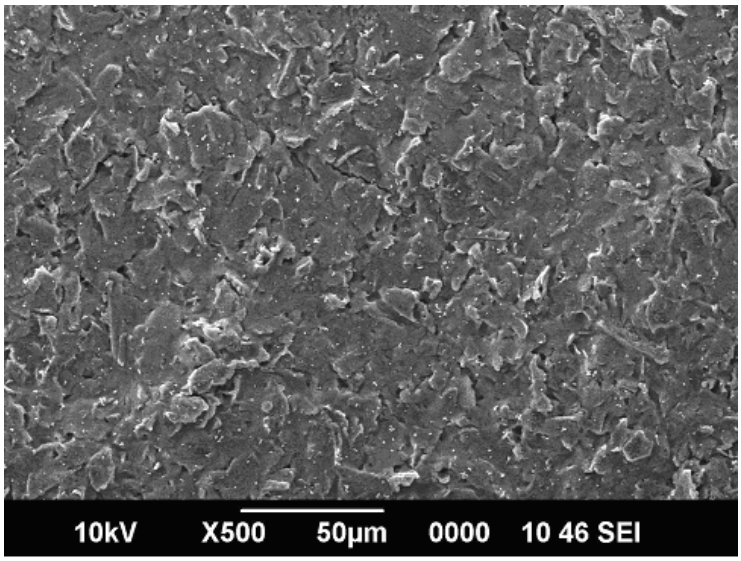


Figure 8.7 (a) SEM image of AuNP/poly –CA/GCE

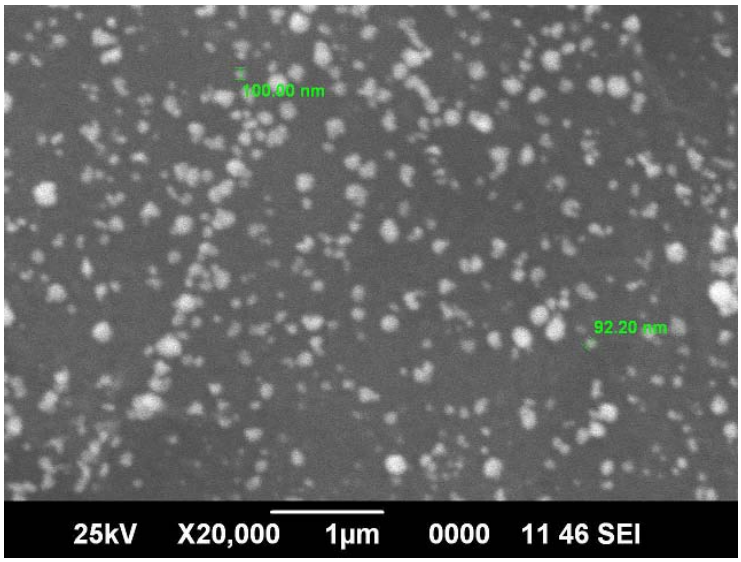


Figure 8.7 (b) SEM image of AuNP/poly –CA/GCE showing the size of AuNPs

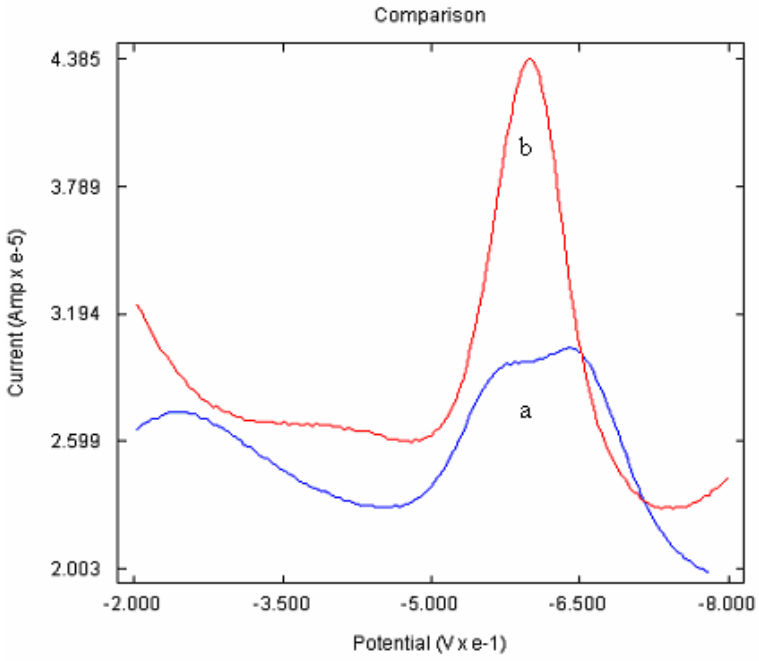


Figure 8.8 Overlay of Square Wave Voltammogram of NIM at (a) bare GCE (b) poly-CA/GCE at a scan rate of 0.06Vs^{-1} from -0.2V to -0.8 V

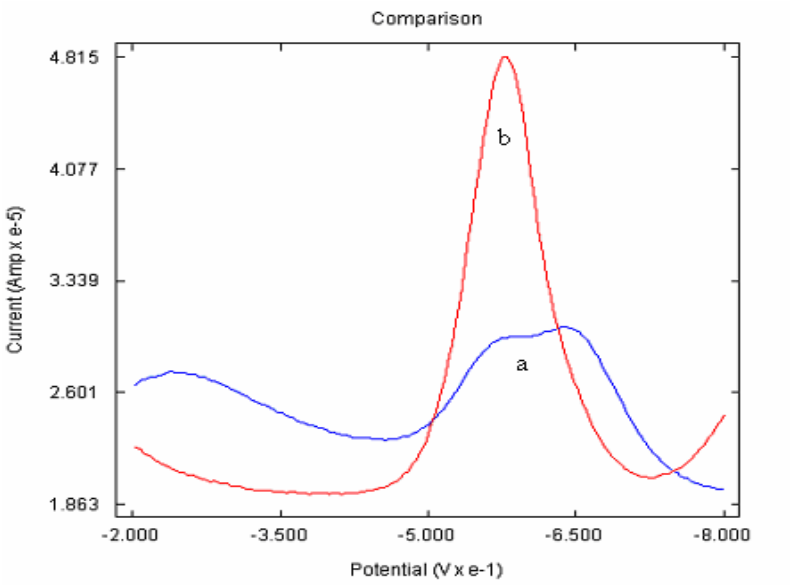


Figure 8.9 Overlay of Square Wave Voltammogram of NIM at (a) bare GCE (b) AuNP/poly-CA/GCE at a scan rate of 0.06Vs^{-1} from -0.2V to -0.8 V

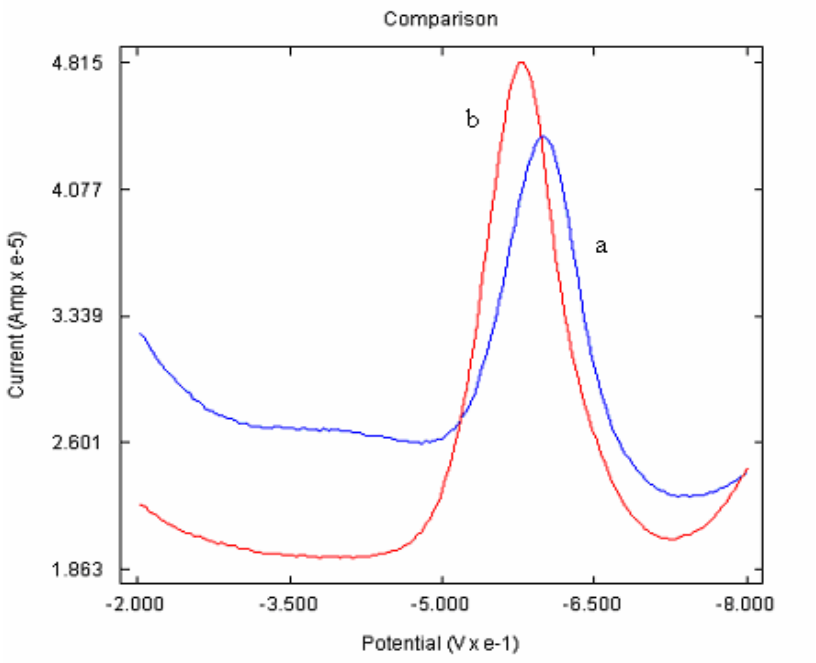


Figure 8.10 Overlay of Square Wave Voltammogram of NIM at (a) poly-CA/GCE (b) AuNP/poly-CA/GCE at a scan rate of 0.06Vs^{-1} from -0.2V to -0.8 V

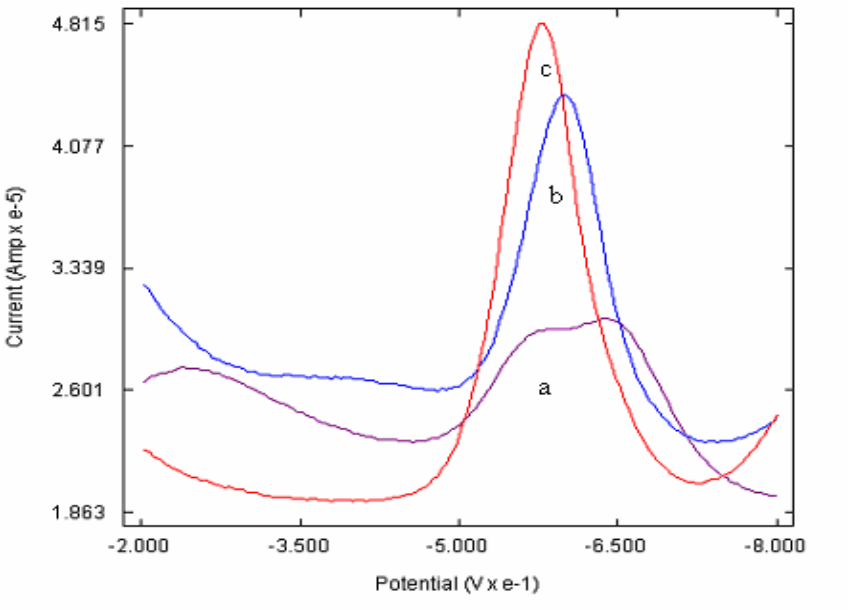


Figure 8.11 Overlay of Square Wave Voltammograms of NIM at (a) bare GCE (b) poly –CA/GCE and (c)AuNP/ poly-CA/GCE at a scan rate of 0.06Vs^{-1} from -0.2V to -0.8 V

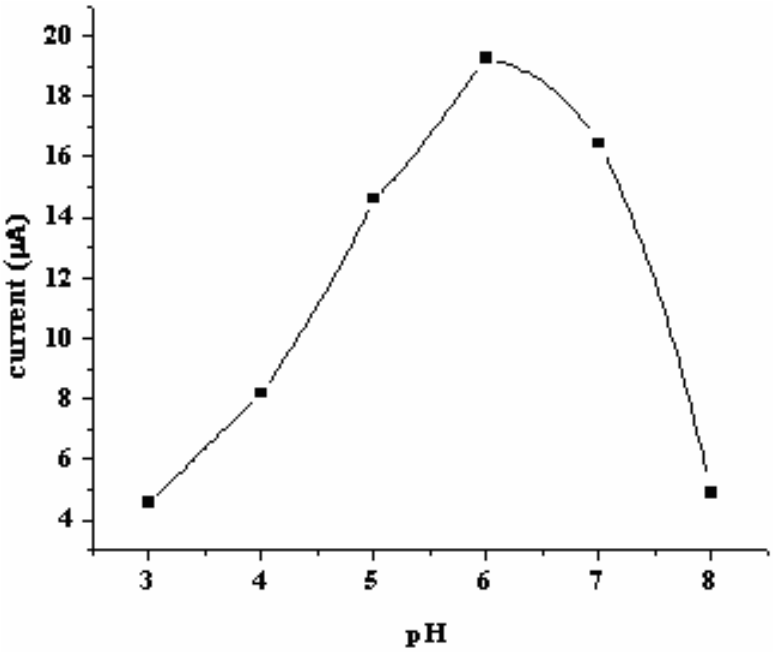


Figure 8.12 Effect of pH on the cathodic peak current of 1×10^{-4}M NIM at poly- CA/GCE

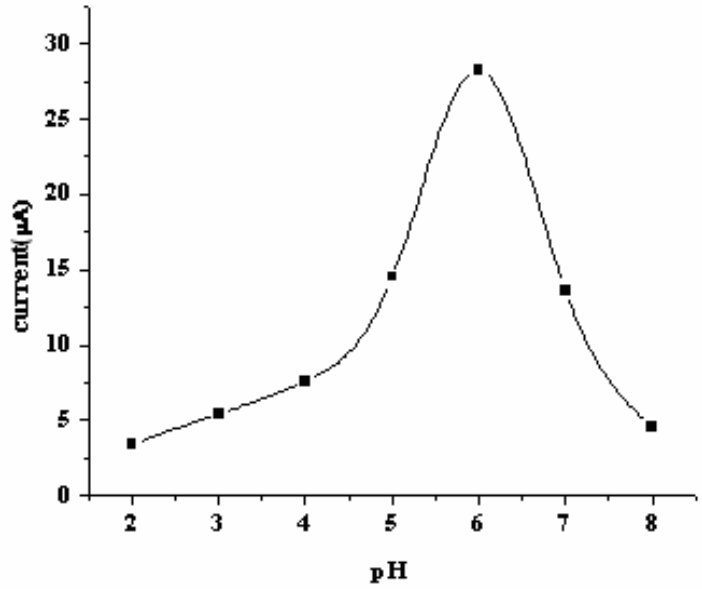


Figure 8.13 Effect of pH on the cathodic peak current of 1×10^{-4}M NIM at AuNP/ poly- CA/GCE

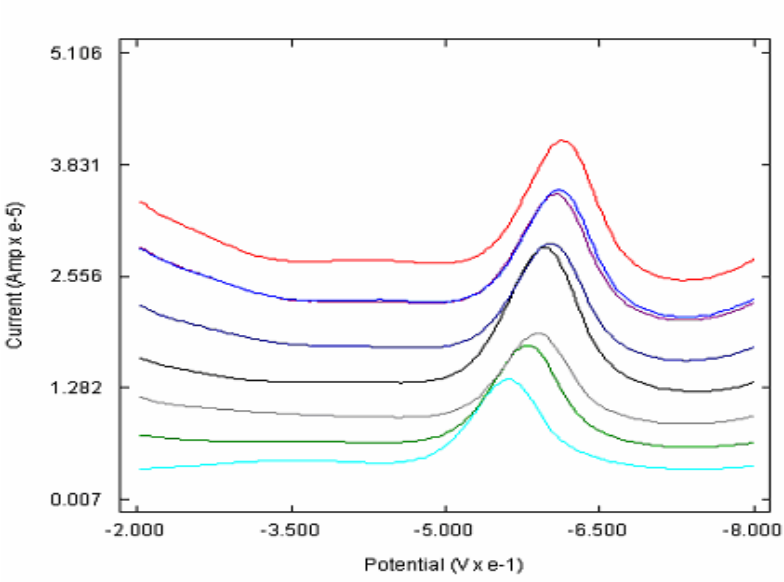


Figure 8.14 Overlay of SWVs of NIM at poly -CA/GCE in 0.1M phosphate buffer (pH 6) containing 1×10^{-4}M NIM at different scan rates a) 20 b) 40 c) 60 d) 80 e) 100 f) 120 g) 140 h) 160 mVs^{-1}.

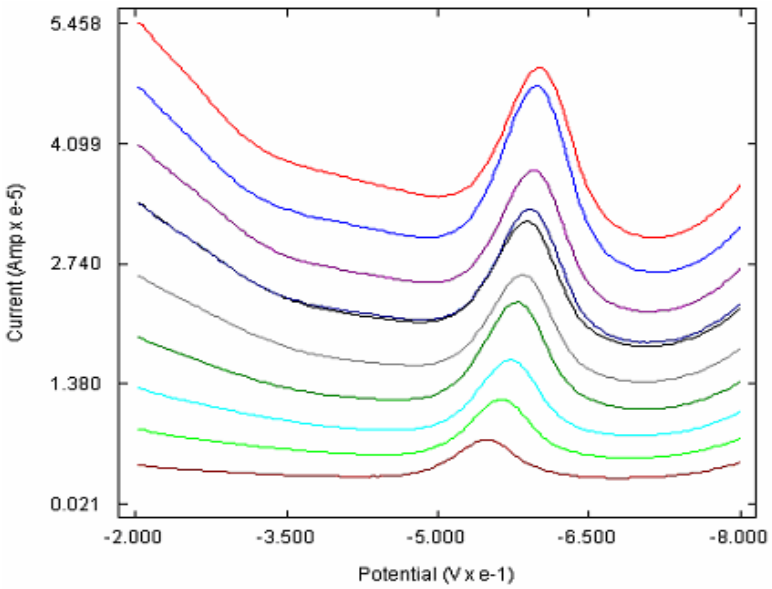


Figure 8.15 Overlay of SWVs of NIM at AuNP/poly -CA/GCE in 0.1M phosphate buffer (pH 6) containing 1×10^{-4}M NIM at different scan rates a) 20 b) 40 c) 60 d) 80 e) 100 f) 120 g) 140 h) 160 i) 180 j) 200 mVs^{-1}.

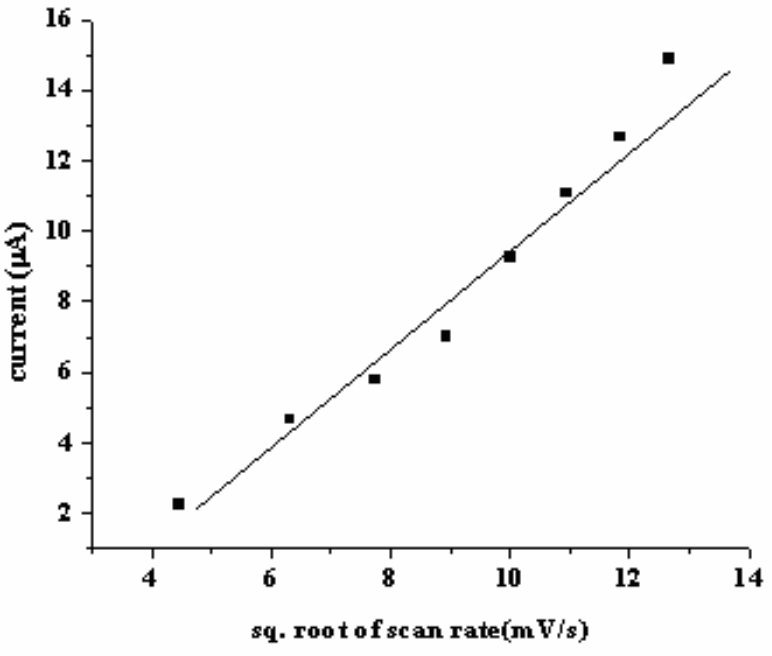


Figure 8.16 Effect of scan rate at poly-CA/GCE

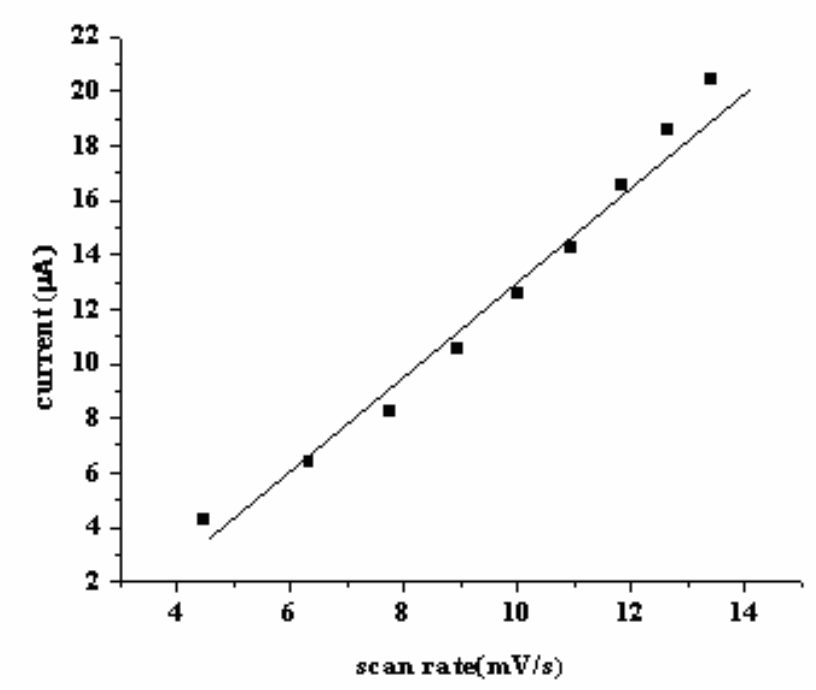


Figure 8.17 Effect of scan rate at AuNP/poly-CA/GCE

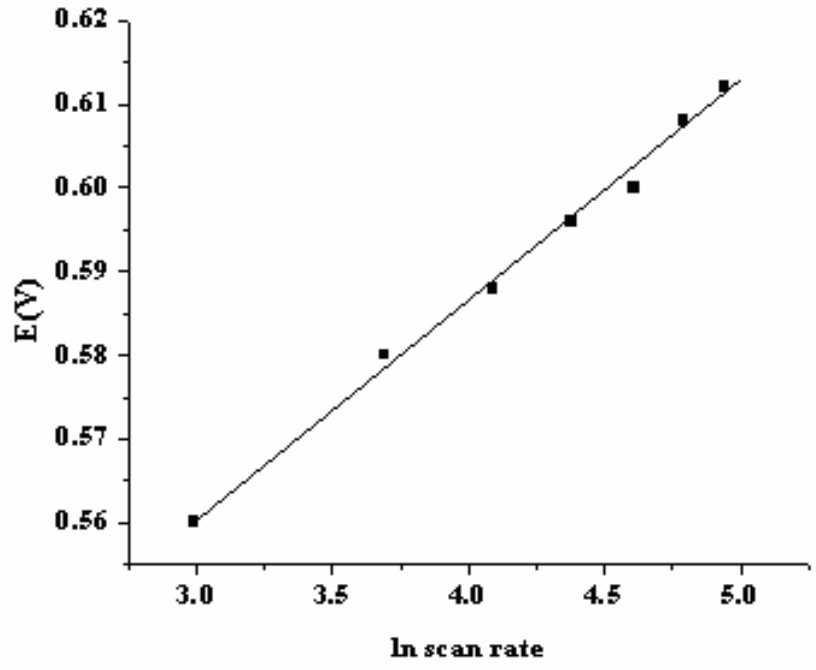


Figure 8.18 Plot of peak potential against ln (scan rate) at poly-CA/GCE

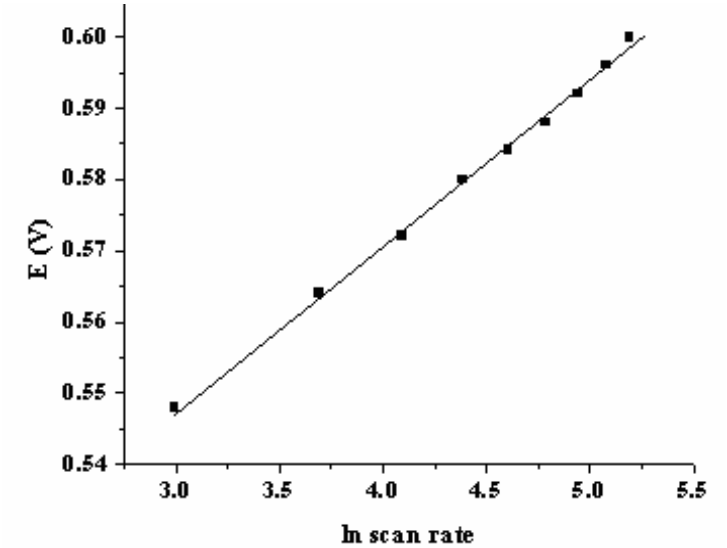


Figure 8.19 Plot of peak potential against ln (scan rate) at AuNP /poly-CA/GCE

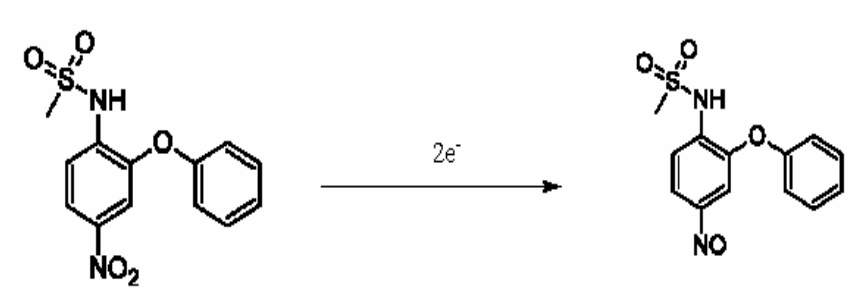


Figure 8.20 Mechanism showing the $2e^-$ reduction of NIM

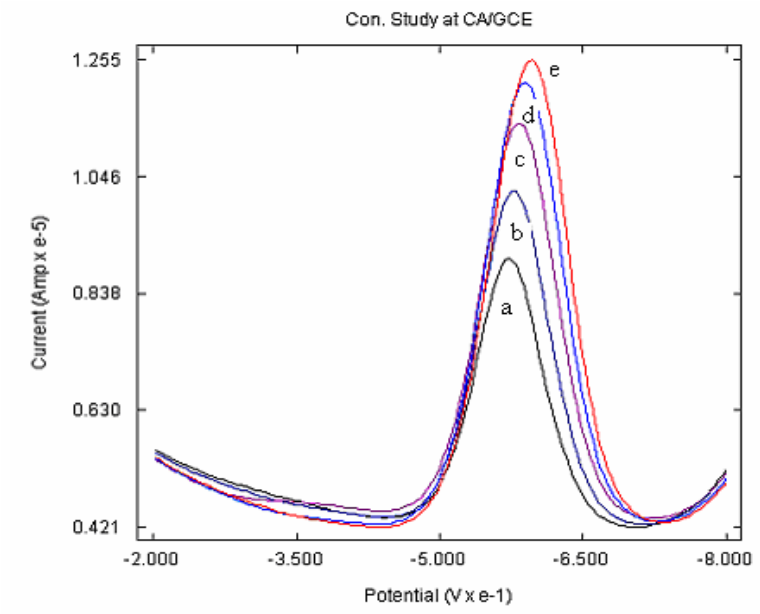


Figure 8.21 Differential Pulse Voltammogram of NIM of different concentrations a) 1×10^{-5}M b) 3×10^{-5}M c) 5×10^{-5}M d) 7×10^{-5}M e) 1×10^{-4}M at poly-CA/GCE at 25^0C

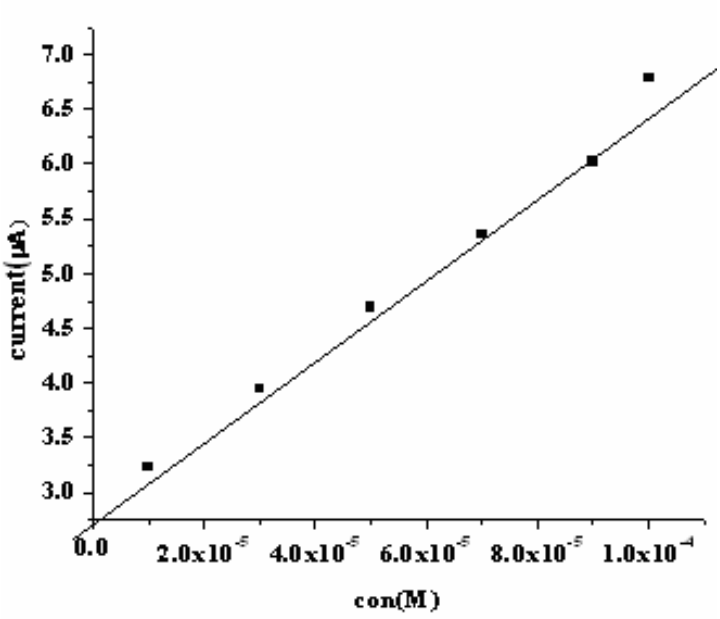


Figure 8.22 Calibration curve for NIM at poly-CA/GCE at 25^0C

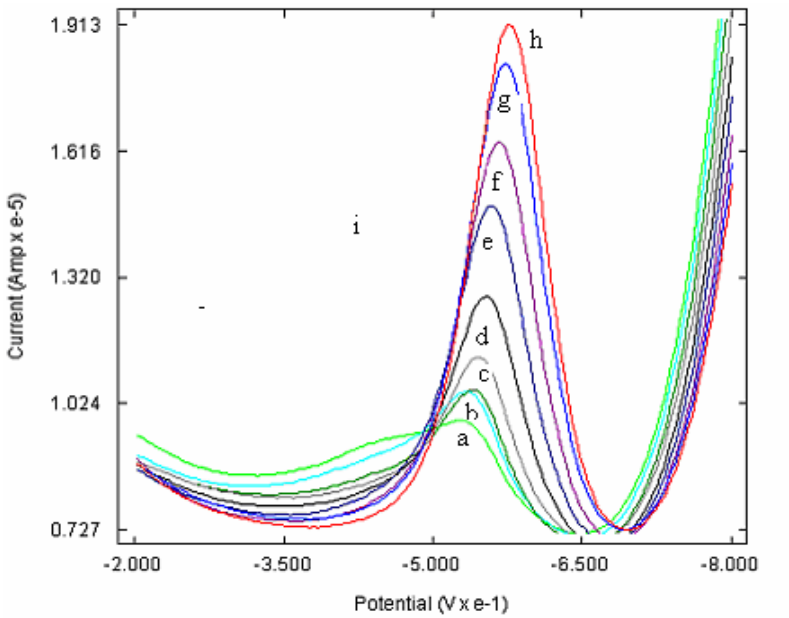


Figure 8.23 Differential Pulse Voltammogram of NIM of different concentrations a) 1×10^{-6}M b) 5×10^{-6}M c) 7×10^{-6}M d) 9×10^{-6}M e) 2×10^{-5}M f) 6×10^{-5}M g) 8×10^{-5}M h) 1×10^{-4}M at AuNP/poly-CA/GCE at 25^0C

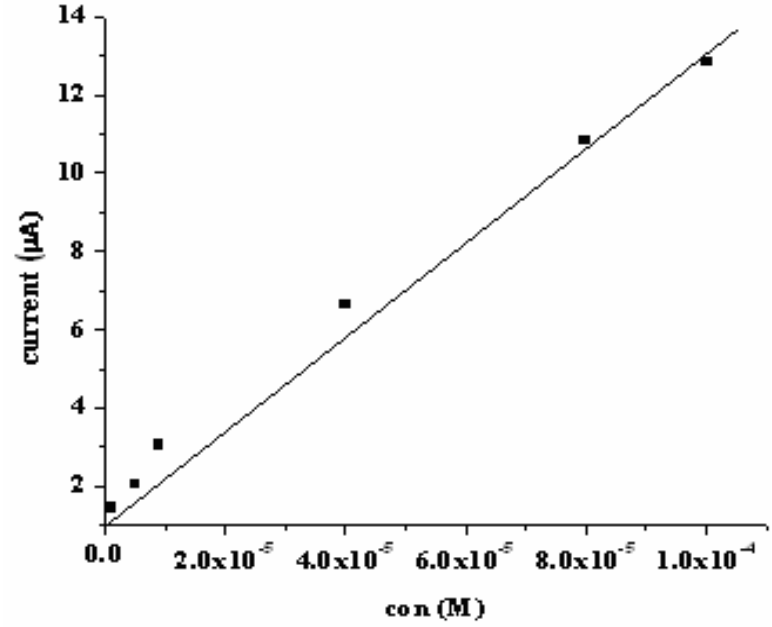


Figure 8.24 Calibration curve for NIM at AuNP/poly-CA/GCE at 25^0C

Chapter 9

CONCLUSIONS

A brief summary of the important findings and results of the present investigations are discussed in this chapter.

Voltammetric chemical sensors, an important class of electrochemical sensors are widely used in pharmaceutical analysis because of their inherent advantages. These techniques are well suited for the determination of drugs in various samples, such as, raw material, pharmaceutical dosage forms and even those involving a complex matrix such as syrups, tablets, creams, suppositories and ointments or else in biological fluids. The principal advantage of the modern voltammetric methods is that the excipients do not interfere, and generally the separation and extraction procedure is not necessary. The development of chemically modified electrodes (CMEs) continues to be an area of great interest in pharmaceutical analysis due to their numerous applications in electrocatalysis. One of the most important properties of CMEs is their ability to catalyze the oxidation or reduction of solute species that exhibits high over voltages at unmodified surfaces. Thus CMEs play an important role in reducing the high overvoltage required for the voltammetric determination of analyte without any major interferences.

As part of the present investigations, a total of 8 sensors have been fabricated for the drugs Ambroxol, Sulfamethoxazole, Pyridine-2-Aldoxime Methochloride (PAM Chloride), Lamivudine, Metronidazole Benzoate and Nimesulide. While one sensor each was developed for the first four drugs, two sensors each were developed for the last two drugs.

The details of the modifiers are as follows:

Sl.No.	Drug	Sensor
1.	Ambroxol (AMB)	Multi walled Carbon Nanotube/Nafion modified glassy carbon sensor [MWCNT/Nafion/GCE]
2.	Sulfamethoxazole (SMX)	Multi walled Carbon Nanotube/Nafion modified glassy carbon sensor [MWCNT/Nafion/GCE]
3.	PAM Chloride	Poly (p-toluene sulphonic acid) modified glassy carbon sensor [Poly(p-TSA)/GCE]
4.	Lamivudine (LAM)	Poly (L-Cysteine) modified glassy carbon sensor. [Poly(L-Cys)/GCE]
5.	Metronidazole benzoate(MBZ)	Poly (p-amino benzene sulphonic acid) modified glassy carbon sensor [Poly(p-ABSA)/GCE]
6.	Metronidazole benzoate(MBZ)	Gold nanoparticle/ poly(p-amino benzene sulphonic acid) modified glassy carbon sensor [AuNp/poly(p-ABSA)/GCE]
7.	Nimesulide (NIM)	Poly (Cystamine) modified glassy carbon sensor [Poly-CA/GCE]
8.	Nimesulide (NIM)	Gold nanoparticle/ poly (Cystamine) modified glassy carbon sensor. [AuNP/poly-CA/GCE]

Different stages involved in the development of these sensors are:

1) Modification of electrode surface
2) Surface area calculation as an evidence for modification of the electrode surface
3) Study of the surface morphology of the modified electrodes using SEM
4) Fabrication of different types of sensors
5) Study of the response characteristics of the developed sensors such as scan rate, linear range, detection limit, effect of pH, effect of supporting electrolyte and interference study.

6) Analytical applications of the developed sensors in pharmaceutical formulations and real samples

The results of the whole investigations are summarized in the following table.

Sl.No.	Drug	Sensor	Detection Limit	Concentration range	Difference in overpotential / peak current w.r.t the bare electrode
1.	AMB	MWCNT/ Nafion/GCE	6×10^{-6} M	1×10^{-2}M- 1×10^{-5} M	Peak current increased by 100µA
2.	SMX	MWCNT/ Nafion/GCE	1×10^{-5} M	1×10^{-2} M - 5×10^{-5} M	Peak potential lowered by 100mV
3.	PAM Chloride	Poly (p-TSA)/GCE	3×10^{-8} M	1×10^{-3} M - 1×10^{-7} M	Peak potential lowered by 70mV and peak current increased by 74 µA
4.	LAM	Poly (L-Cys)/GCE	1×10^{-5} M	1×10^{-3}M- 5×10^{-5} M	Peak potential lowered by 100mV and peak current increased by 70 µA
5.	MBZ	Poly (p-ABSA)/GCE	1×10^{-6} M	1×10^{-4}M- 5×10^{-6} M	Peak potential lowered by 50mV and peak current increased by 16 µA
6.	MBZ	AuNP/poly (p-ABSA)/GCE	4×10^{-8}M	1×10^{-5}M- 4×10^{-7}M	Peak potential lowered by 100mV and peak current increased by 60 µA
7.	NIM	Poly-CA/GCE	2×10^{-6}M	1×10^{-4} M – 1×10^{-5} M	Peak potential lowered by 40mV and peak current increased by 10 µA
8.	NIM	AuNP/ poly-CA/GCE	3×10^{-7} M	1×10^{-4} M – 1×10^{-6} M	Peak potential lowered by 70mV and peak current increased by 20 µA

The development and application of CME based voltammetric sensors continue to be exciting and expanding areas of analytical research. The principal developments in this area focus on reducing the detection limit to true trace levels and there are important advances in the areas of materials and active components design. Clearly the results of the present investigations are highly promising and fruitful.

…..ൠ…..

REFERENCES

[1] F. J. Holler, D. A. Skoog and D. M. West, *Fundamentals of analytical chemistry*, Saunders College Publishing, Philadelphia (1996).

[2] T. A. Nieman, D. A. Skoog and F. J. Holler, *Principles of instrumental analysis*, Pacific Grove, California (1998).

[3] Y. Arikawa, *Anal.Sci.,* **17,** i571 (2001)

[4] http://en.wikipedia.org/wiki/Analytical_chemistry

[5] J. A. Plambeck, *Electroanalytical Chemistry, Basic Principles and Application,* Wiley Interscience Publication, USA (1982).

[6] A. J. Bard and L. R. Faulkner, *Electrochemical methods, fundamentals and applications,* Wiley, New York (1980).

[7] D. A. Skoog, F. J. Holler and S. R. Crouch, *Principles of instrumental analysis,* Thomson Brooks/Cole Publishing, Belmont CA (2007).

[8] C. M. A. Brett and A. M. O. Brett, *Electroanalysis,* Oxford University Press, Oxford (1998).

[9] R. Kellner, J. M. Mermet, M.Otto, M. Valcarcel and H.M. Widmer, *Analytical Chemistry,* Wiley- VCH, Weinheim (2004).

[10] J. Mendham, R. C. Denney, J. D. Barnes and M. Thomas, *Vogel's Text Book of Quantitative Chemical Analysis,* 6th Edn., Pearson Education Ltd., Singapore (2002).

[11] D. A. Skoog, D. M. West and F. J. Holler, *Fundamentals of Analytical Chemistry,* Saunders College Publishing, Fort Worth (1992).

[12] H. H. Willard, L. L. Merrit Jr., J. A. Dean and F. A. Settle Jr., *Instrumental Methods of Analysis,* 6th Edn., D. Van Nostrand, Princeton (1981).

[13] D. T. Sawyer, A. Sobkowiak and J. L. Roberts, *Electrochemistry For Chemists (IInd Edition),* Wiley Interscience Publication, USA (1995).

[14] J. Janata, *Anal. Chem.,* **150A,** 73 (2001).

[15] R. W. Cattrall, *Chemical Sensors,* Oxford Chemistry Premiers, New York (1997).

[16] J. R. Stetter, W. R. Penrose and S. Yao, *J .Electrochem. Soc.,* **150,** 11 (2003).

[17] N. R. Stradiotto, H. Yamanaka and M. V. B Zanoni, *J. Braz. Chem. Soc.,***14,** 159 (2003).

[18] S. Iijima, *Nature*, **354,** 56 (1991).

[19] N. Terui, B. Fugetsu and S. Tanaka, *Anal. Sci.,* **22,** 895 (2006).

[20] W. Qu, K. Wu and S. Hu, *J. Pharm. Biomed. Anal.,* **36,** 631 (2004).

[21] C. Li, *Microchim. Acta,* **157,** 21 (2007).

[22] R. F. Service, *Science,* **281,** 940 (1998).

[23] B. I. Yakobson and R. E. Smalley, *Am. Sci.,* **85,** 324 (1997).

[24] J. Wang, M. Li, Z. Shi, N. Li and Z. Gu, *Electroanal.,* **14,** 225 (2002).

[25] H. B. Ray, A. A. Zakhidov and W. A. de Heer, *Science,* **297,** 787 (2002).

[26] Y. Wu, S. Ye and S. Hu, *J. Pharm. Biomed. Anal.,* **41,** 820 (2006).

[27] S. Lu, *Anal. Sci.,* **19,** 1309 (2003).

[28] S. Sotiropoulou and N. A. Chaniotakis, *Anal. Bioanal. Chem.*, **375**, 103 (2003).

[29] R. R. Moore, C. E. Banks and R. G. Compton, *Anal. Chem.* **76,** 2677 (2004)**.**

[30] C. E. Banks, R. R. Moore, T. J. Davies and R. G. Compton, *Chem. Commun.,* **16,** 1804 (2004).

[31] C. E. Banks, T. J. Davies, G. G.Wildgoose and R.G. Compton, *Chem. Commun.,* **7,** 829 (2005).

[32] P. J. Britto, K. S. V Santhanam and P. M. Ajayan, *Bioelectroch. Bioener.,* **41,** 121 (1996).

[33] A. Salimi, A. Noorbakhsh and M. Ghadermarz, *Anal. Biochem.,* **344** 16 (2005).

[34] A. Salimi, R. G. Compton and R .Hallaj, *Anal. Biochem.,* **333,** 49 (2004).

[35] A. Salimi and R. Hallaj, *Talanta*, **66,** 967 (2005).

[36] X. Qi, Y. Du, G. Zhang, P. Zhao and J. Lu, *Front. Chem. China*, **3,** 345 (2006).

[37] S. M. Park, *Electrochemistry of* p-*Conjugated Polymers. In: Handbook of Organic Conductive Molecules and Polymers*, Nalwa, H. S., Ed., Chichester, Wiley, **3,** 429 (1997).

[38] A. E. Guiseppi, G. G. Wallace and T. Matsue, *In: Handbook of Conducting Polymers,* 2nd ed.; Skotheim, T. A.; Elsenbaumer, R.; Reynolds, J. R., Eds.; Marcel Dekker; New York, (1998).

[39] C. Arbizzani, M. Mastragostino and B. Scrosati, *In: Handbook of Organic Conductive Moleculesand Polymers,* Nalwa, H. S., Ed., Chichester, Wiley, **4,** Chapter 11, 595 (1997).

[40] A. Rahman, P. Kumar, D. S. Park and Y. B. Shim, *Sensors,* **8,** 118 (2008).

[41] A. Malinauskas, *Synth. Met.,* **107,** 75 (1999).

[42] S. Sadki, P. Schottland, N. Brodie*c* and G. Sabouraud *Chem. Soc. Rev.,* **29,** 283 (2000).

[43] A. S. N. Murty and K. S. Reddy, *Electrochim. Acta,* **28,** 473 (1983).

[44] Y. Ohnuki, T. Ohsaka, H. Matsuda and N. Oyama*, J. Electroanal. Chem.*, **158,** 55 (1983)

[45] A. Volkov, G. Tourillon, P. C. Lacaze and J. E. Dubois, *J. Electroanal. Chem.,* **115** 279 (1980).

[46] S. Cosnier, *Anal. Bioanal. Chem.,* **377,** 507 (2003).

[47] L. Xu, W. Chen, A. Mulchandani and Y. Yan, *Angew. Chem. Int. Ed.*, **44**, 6009 (2005).

[48] G. Y. Jin, Y. Z. Zhang and W. X. Cheng, *Sens. Actuat. B*, **2005**, *107*, 528

[49] Y. X. Sun, B. X. Ye, Y. Wang, X. R. Tang and X. Y. Zhou, *J. Microchemical,* **58** 182 (1998).

[50] C. M. A. Brett, G. Inzelt and V. Kertesz, *Anal. Chim. Acta,* **385,** 119 (1999).

[51] J. Wang, S. P. Chen and M. S. Lin, *J. Electroanal. Chem.,* **273,** 231 (1989).

[52] M. Pontie, C. Gobin, T. Pauporte, F. Bedioui and J. Devynck, *Anal. Chim. Acta,* **411,** 175 (2000).

[53] E. Ekinci, G. Erdogdu and A. E. Karagozler, *J. Appl. Polym. Sci.*, **79,** 327 (2001).

[54] H. Zhao, Y. Z. Zhang and Z. B. Yuan, *Electroanal.*, **14**, 445 (2002).

[55] H. Zhao, Y. Z. Zhang and Z. B. Yuan, *Anal. Chim. Acta*, **454**, 75 (2002).

[56] P. R. Roy, T. Okajima and T. Ohsaka, *Bioelectrochem.*, **59,**11 (2003).

[57] M. Starowicz, B. Stypuła and J. Ban'as, *Electrochem. Commun.,* **8,** 227 (2006).

[58] C. M. Welch and R. G. Compton, *Anal. Bioanal. Chem.,* **384,** 601(2006).

[59] R. Siegel, *Nano Struct. Mater.,* **3,**1(1993).

[60] M. A. Hayat, *Colloidal gold: principles, methods and applications, vol. 1.* Academic NewYork (1989).

[61] K. C. Graer, G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.,* **67,** 735 (1995).

[62] F. L. Leibowitz, W. Zheng, M. M. Maye and C. J. Zheng, *Anal. Chem.,* **71,** 5076 (1999).

[63] A. Doron, E. Katz and I .Willner, *Langmuir,* **11,** 1313 (1995).

[64] A. N. Shioway, E. Katz and I. Willner, *Chem. Phys. Chem.,* **1,** 18 (2000).

[65] K. R. Brown, A. P. Fox and M. J. Natan, *J. Am. Chem. Soc.,* **118,** 1154 (1996).

[66] A. P. Alivisatos, *Science,* **271,** 933 (1996).

[67] H. Zhang, Y. Guo, L. Wan and C. Bai, *Chem. Commun.,* **24,** 3022 (2003).

[68] S. Duron, R. R. Noriega, P. Nkeng, G. Poillerat and O. F. Solorza*, J. Electroanal. Chem.*, **566,** 281(2004).

[69] A. J. Dickinson, L. P. L Carrette, J. A. Collins, K. A. Friedrich and U. Stimming *Electrochim. Acta,* **47,** 3733 (2002).

[70] S. N. Pronkin, G. A. Tsirlina, O. A .Petrii and S. Y. Vassiliev, *Electrochim. Acta,* **46***,* 2343 (2001).

[71] Z. Liu, J. Y. Lee, M. Han, W. Chen and L. M. Gan, *J. Mater. Chem.,* **12,** 2453 (2002).

[72] Y. Zhuo, R. Yuan, Y. Q. Chai, Y. Zhang, X. L. Li, Q. Zhu and N. Wang, *Anal. Chim. Acta,* **548,** 205 (2005).

[73] Z. M. Liu, Y. Yang, H. Wang, Y. L. Liu, G. L. Shen and R. Q. Yu, *Sensor. Actuat. B: Chem.,* **106,** 394 (2005).

[74] S. S. Kumar, J. Mathiyarasu and K. L. Phani, *J. Electrochem. Chem.,* **578,** 95 (2005).

[75] L. Zhang, X. Jiang, E. K. Wang and S. J. Dong, *Biosens. Bioelectron.*, **21,** 337 (2005).

[76] X. Dai, O. Nekrassoya, M. E. Hyde and R. G. Compton, *Anal. Chem.,* **76,** 5924 (2004).

[77] L. Wang and E. K. Wang, *Electrochem. Commun.,* **6,** 49 (2004).

[78] V. V. Shumyantseva, S. Carrara, V. Bavastrello, D. J. Riley, T. V. Bulko, K.G. Skryabin, A. I. Archakov and C. Nicolini, *Biosens. Bioelectron.,* **21,** 217 (2005).

[79] A. N. Shipway, M. Lahav and I. Willner, *Adv. Mater.,* **12,** 993 (2000).

[80] A. R. Raj, T. Okajima and T. Ohsaka, *J. Electrochem. Chem.,* **543,** 127 (2003).

[81] J. S. Li, Z. S. Wu, H. Wang, G. L. Shen and R. Q. Yu, *Sensor. Actuat.B: Chem.,* **110,** 327 (2005).

[82] C. Y. Tsai, T. L. Chang, C. C. Chen, F. H. Ko and P. H. Chen, *Microelectron. Eng.,* **546,** 78 (2005).

[83] Y. Li and G. Shi, *J. Phys. Chem. B,* **109,** 23787(2005).

[84] C. R. Raj, A. I. Abdelrahman and T. Ohsaka, *Electrochem. Commun.,* **7,** 888 (2005).

[85] Y. H. Yang, Z. J. Wang, M. H. Yang, M. M. Guo, Z. Y. Wu, G. L. Shen and R. Q. Yu, *Sensor. Actuat. B: Chem.,* **114,** 1 (2006).

[86] Y. Y. Xu, C. Bian, S. F. Chen and S. H. Xia, *Anal. Chim. Acta*, **561**, 48 (2006).

[87] Y. Z. Fu, R. Yuan, L. Xu, Y. Q. Chai, X. Zhong and D. P. Tang, *Biochem. Eng. J.,* **23,** 37 (2005).

[88] X. Zhong, R. Yuan, Y. Q. Chai, Y. Liu, J. Y. Dai and D. P. Tang, *Sensor. Actuat. B: Chem.,* **104,** 191(2005).

[89] Y. Z. Xian, H. T. Wang, Y. Y. Zhou, D. M. Pan, F. Liu and L. T. Jin, *Electrochem. Commun.,* **6,** 1270 (2004).

[90] K. R. Brown, A. P. Fox and M. J. Natan, *J. Am. Chem. Soc.,* **118**, 1154 (1996).

[91] L. Liu and J. Song, *Anal. Biochem.,* **354**, 22 (2006).

[92] A. M. M. Ali, *J. Pharm. Biomed. Anal.,* **18,**1005 (1999)

[93] L. Fernandez-Llano, M. C. Blanco-Lopez, M. J. Lobo-Castanon, A. J. Miranda- Ordieres and P. Tunon-Blanco, *Electroanal.,* **19**, 1555(2007)

[94] A. Radi, *Electroanal.,* **10,** 103(1998)

[95] M. Hajjizadeh, A. Jabbari, H. Heli, A. A. Moosavi-Movahedi and S. Haghgoo, *Electrochim. Acta,* **53,**1766 (2007).

[96] M. Houshmand, A. Jabbari, H. Heli, M. Hajjizadeh and A. A. Moosavi-Movahedi, *J. Solid State Electrochem.,* **12,** 1117 (2008).

[97] S. Majdi, A. Jabbari and H. Heli, *J. Solid State Electrochem.,* **11,** 601 (2007).

[98] H. Yadegari, A. Jabbari, H. Heli, A. A. Moosavi-Movahedi and S. Majdi, *J. Braz. Chem. Soc.,* **19,** 1017 (2008).

[99] H. Yadegari, A. Jabbari, H. Heli, A. A. Moosavi-Movahedi, K. Karimian and A. Khodadadi, *Electrochim. Acta,* **53,** 2907 (2008).

[100] M. Hajjizadeh, A. Jabbari, H. Heli and A. A. Moosavi-Movahedi, *Chem. Anal. Warsz.,* **53**, 429 (2008).

[101] M. Hajjizadeh, A. Jabbari, H. Heli, A.A. Moosavi-Movahedi, A. Shafiee and K. Karimian, *Anal. Biochem.,* **373,** 337 (2008).

[102] D. Sun, H. Wang and K. Wu, *Microchim. Acta,* **152,** 255 (2006).

[103] B. Zeng, S. Wei, F. Xiao and F. Zhao, *Sensor. Actuat. B Chem.,* **115,** 240 (2006).

[104] K. J. Huang, X. Liu, W. Z. Xie and H. X. Yuan, *Microchim. Acta,* **162,** 227 (2008).

[105] S. Lu, *Anal. Lett.,* **36,** 2597 (2003).

[106] Z. Wang and C. Li, *Russ. J. Electrochem.,* **43,** 1364 (2007).

[107] S. Shahrokhian and M. Amiri, *Microchim. Acta,* **157,** 149 (2007).

[108] Y. Tu, Q. Xu, Q. J. Zou, Z. H. Yin, Y. Y. Sun and Y. D. Zhao, *Anal. Sci.,* **23,** 1321 (2007)

[109] Y. Wu, S. Ye and S. Hu, *J. Pharm. Biomed. Anal.*, **41**, 820 (2006).

[110] H. Zhang and K. Wu*, Microchim. Acta,* **149,** 73 (2005).

[111] F. Wang, L. Chen, X. Chen and S. Hu, *Anal. Chim. Acta,* **576,** 17 (2006).

[112] C. Yang, *Anal. Sci.,* **20,** 821(2004).

[113] B. Fang, Y. Wei, M. Li, G. Wang and W. Zhang, *Talanta,* **72,** 1302 (2007).

[114] S. F. Wang and Q. Xu, *Anal. Lett.,* **38**, 657 (2005).

[115] Z. Wang, J. Liu, Q. Liang, Y. Wang and G. Luo, *Analyst*, **127**, 653 (2002).

[116] X. Dai, G. G. Wildgoose and R. G. Compton, *Analyst,* **131,** 901 (2006).

[117] P. Xiao, W. Wu, J.Yu and F. Zhao, *Int. J. Electrochem. Sci.,* **2,** 149 (2007).

[118] Z. Wang, Y. Wang and G. Luo, *Analyst*, **127**, 1353 (2002).

[119] K. Wu, X. Ji, J. Fei and S. Hu, *Nanotechnology,* **15,** 287 (2004).

[120] K. Wu, H. Wang, F. Chen and S. Hu, *Bioelectrochem.,* **68,** 144 (2006).

[121] R. N. Hegde, N. P. Shetti and S. T. Nandibewoor, *Talanta,* **79,** 361 (2009).

[122] R. N. Goyal, B. Sunita and R. S. R Anoop, *Comb. Chem. High Throughput Screen.,* **13,** 610 (2010).

[123] B. Rezaei and S. Damiri, *Sensors Journal*, **8,** 1523 (2008).

[124] A. A. Ensafi and H. K. Maleh, *Electroanal.,* **22,** 2558 (2010).

[125] B. Rezaei and S. Z. M. Zare, *Sensor Actuat. B-Chem.,* **134,** 292 (2008).

[126] L. Lonappan and K. Girish Kumar, *Sensor Lett.,* (2010) in press.

[127] J. P. Fan, X. M. Zhang and M.Ying, *J. Solid State Electrochem.,* **12,** 1143 (2008).

[128] M. R. Majidi, A. Jouyban and K. A. Zeynali, *J. Electroanal. Chem.,* **589**, 32 (2006).

[129] A. Liu, W. Chen, L. Huang and X. Lin, *Chem. Pharm. Bull.,* **56,** 1665 (2008).

[130] H. Y. Huo, H. Q. Luo and N. B. Li, *Microchim. Acta,* **167,** 195 (2009).

[131] G. Yang, C. Wang, R. Zhang, C. Wang, Q. Qu and X. Hu, *Bioelectrochem.,* **73,** 37 (2008).

[132] M. Li and L. Jing, *Electrochim. Acta,* **52**, 3250 (2007).

[133] M. Aslanoglu, A. Kutluay, S. Goktas and S. Karabulut, *J. Chem. Sci.,* **121,** 209 (2009).

[134] M. Aslanoglu, A. Kutluay, S. Karabulut and S. Abbasoglu, *J. Chem. Sci.,* **55,** 794 (2008).

[135] H. Yao, Y. Sun, X. Lin, Y. Tang, A. Liu, G. Li, W. Li and S. Zhang, *Anal. Sci.,* **23,** 677 (2007).

[136] C. Wang, C. Li, F. Wang and C. Wang, *Microchim. Acta,* **155,** 365 (2006).

[137] J. Guan, Z. Wang, C. Wang, Q. Qu, G. Yang and X. Hu, *Int. J. Electrochem. Sci.,* **2,** 572 (2007).

[138] X. Zheng, D. Zhou, D. Xiang, W. Huang and S. Lu, *Russ. J. Electrochem.,* **45,** 1183 (2009).

[139] Z. Yan, J. R. Zhang and H. Q. Fang, *Anal. Lett.,* **32,** 223 (1999).

[140] P. F. Huang, L. Wang, J. Y. Bai, H. J. Wang, Y. Q. Zhao and S. D. Fan, *Microchim. Acta,* **157**, 41 (2007).

[141] C. Li, *Colloid. Surface. B,* **50,** 147 (2006).

[142] Z. Yang, G. Hu, Y. Liu, J. Zhao and G. Zhao, *Can. J. Anal. Sci. Spect.,* **52,** 11 (2006).

[143] S. P. Özkorucuklu, Y. Şahin, and G. Alsancak, *Sensors*, **8**, 8463 (2008).

[144] Y. Ya, D. Luo, G. Zhan and C. Li, *Bull. Korean Chem. Soc*., **29,** 928 (2008).

[145] L. Wang, P. Huang, J. Bai1, H. Wang, X. Wu and Y. Zhao, *Int. J. Electrochem. Sci.* **1**, 334 (2006).

[146] W. Ren, H. Q. Luo and N. B. Li, *Sensors,* **6,** 80 (**2006**).

[147] P. J. O'Connell, C. Gormally, M. Pravda and G. G. Guilbault, *Anal. Chim. Acta,* **431,** 239 (2001).

[148] R. N. Goyal, M. Oyama, A. A. Umar, A. Tyagi and N. Bachheti, *J. Pharm.Biomed. Anal.,* **44**, 1147 (2007).

[149] M. Behpour, S. M. Ghoreishi and E. Honarmand, *Int. J. Electrochem. Sci.,* **5,** 1922 (2010).

[150] R. N. Goyal, V. K. Gupta, M. Oyama and N. Bachheti, *Electrochem. Commun.,* **7**, 803 (2005).

[151] S. Yang, L. Qu, G. Li, R. Yanga and C. Liu, *J. Electroanal. Chem.,* **645**, 115 (2010).

[152] J. B. Raoof, A. Kiani, R. Ojani, R. Valiollahi and S. R. Nadimi, *J. Solid State Electrochem.,* **14,** 1171(2010).

[153] J. Li and X. Lin, *Sensor. Actuat. B Chem.,* **126,** 527 (2007).

[154] L. Wang, J. Bai, P. Huang, H. Wang, L. Zhang and Y. Zhao, *Electrochem. Commun.,* **8,** 1035 (2006).

[155] L. Zhang and X. Jiang, *J. Electroanal. Chem.,* **583,** 292 (2005).

[156] C. R. Raj, T. Okajima and T. Ohsaka, *J. Electroanal. Chem.,* **543**, 127 (2003).

[157] M. Behpour, E. Honarmand and S. M. Ghoreishi, *Bull. Korean Chem. Soc.,* **31**, 845 (2010).

[158] J. Yu, J. Li, F. Zhao and B. Zeng, *J. Braz. Chem. Soc.*, **19,** 849 (2008).

[159] J. Zhong, Z. Qi, H. Dai, C. Fan, G. Li and N. Matsuda, *Anal. Sci.,* **19,** 653 (2003).

[160] S. Li, C. Wei, J. Hu and Q. Li, *Anl. Lett.,* **41,** 582 (2008).

[161] M. Hosseini, M. M. Momeni and M. Faraji, *J. Mater. Sci.*, **45,** 2365 (2010).

[162] K. Girish Kumar and R. Jayasree, *J. Pharm. Biomed. Anal.,* **11,** 165 (1993).

[163] K. Girish Kumar and L. Karpagaselvi, *Analyst,* **119,** 1375 (1994).

[164] K. Girish Kumar and R. Letha, *J. Pharm. Biomed. Anal.,* **15,** 1725 (1997).

[165] K. Girish Kumar, K. P. R. Chowdhary and G. Devala Rao, *Ind. J. Phar. Sci.,* **61,** 394, 1999

[166] G. D. Rao, K. Girish Kumar and K. P. R. Chowdhary, *Ind. J. Phar. Sci.,* **63,** 161 (2001).

[167] K. Girish Kumar, S. John, R. Poduval and P. Augustine, *Chin. Pharm. J.,* **57,** 29 (2005)

[168] K.Girish Kumar, P. Augustine, R. Poduval and S. John, *Pharmazie,* **61,** 353 (2006).

[169] K. Girish Kumar, S. John, P. Augustine, R. Poduval and S. Beena, *Anal. Sci.,* **23,** 291 (2007)

[170] K. Girish Kumar, P. Augustine, S. John, J. Radecki and H. Radecka, *Anal. Lett.,* **41**, 1144 (2008).

[171] S. Beena, M. Pajak, J. Radecki, W. Maes, W. Dehaen, K. Girish Kumar and H. Radecka, *Electroanal.,* **20,** 2009 (2008).

[172] S. Beena, I. Grzybowska, Cz. Orlewska, J. Radecki, W. Dehaen, K. Girish Kumar and H. Radecka, *Electroanal.,* **20,** 2317 (2008).

[173] K. Girish Kumar, P. Augustine and S. John, *J. Appl. Electrochem.,* **40,** 65 (2010).

[174] R. Joseph and K. Girish Kumar, *Drug Test. Analysis,* **2,** 278 (2010).

[175] L. Rajith and K. Girish Kumar, *Drug Test. Analysis,* **2,** 436 (2010).

[176] R. Joseph and K. Girish Kumar, *Anal. Sci.,* (2010) in press**.**

[177] Y. Tsai, J. Chen, S. Li and F. Marken, *Electrochem. Commun.,* **6,** 917 (2004).

[178] P. F. Huang, L. Wang, J. Y. Bai, H. J. Wang, Y. Q. Zhao and S. D. Fan, *Microchim. Acta,* 2007, **157,** 41.

[179] C. Wang, C. Li, F. Wang and C. Wang, *Microchim. Acta,* **155,** 365 (2006).

[180] S. A. Kumar and S. M. Chen, *J. Mol. Catal. A-Chem.,* **278,** 244 (2007).

[181] X. Dai and R. G. Compton, *Anal. Sci.,* **22,** 567 (2006).

[182] H. Cheng, C. Chen and S. Zhang, *Anal. Sci.,* **25,** 1221 (2009).

[183] European Pharmacopoeia 4th ed. France **1531** (2002).

[184] United States Pharmacopoeia, United States Pharmacopoeial Convention Inc, Rockville, **209** (2005).

[185] Pharmacopoeia of India, 3rd Edition, Controller of Publications, Government of India, Delhi, **407** (1985).

[186] K. Basavaiah and B. C. Somashekhar, *Bulg. Chem. Commun.,* **38,** 145 (2006).

[187] http://www.chemicalland21.com/lifescience/phar

[188] http://www.drugs.com/ambroxol.html

[189] http://en.wikipedia.org/wiki/Ambroxol

[190] M. Heinanen and C. Barbas, *J. Pharm. Biomed. Anal.,* **24,** 1005 (2001).

[191] V. Brizzi and U. Pasetti, *J. Pharm. Biomed. Anal.,* **8,** 107 (1990).

[192] M. Nobilis, J. Pasters, D. Svoboda, J. Kvetina and K. Macek, *J. Chromatogr. Biomed. Appl.,* **581,** 251 (1992).

[193] G. Indrayanto and R. Handajani, *J. Pharm. Biomed. Anal.,* **11,** 781 (1993).

[194] T. Perez-Ruiz, C. Martinez-Lozano, A. Sanz and M. T. Sanmiguel, *Talanta,* **43,** 1029 (1996).

[195] P. Benli and M. Tuncel, *Pharmazie,* **53,** 203 (1998).

[196] J. Schmid, *J. Chromatogr.,* **414,** 65 (1987).

[197] T. Perez-Ruiz, C. Martinez-Lozano, A. Sanz and E. Bravo, *J. Chromatogr. B,* **742,** 205 (2000).

[198] A. R. Solangi, M. Y. Khuhawar and M. I. Bhanger, *J. Food Drug Anal.,* **13,** 201 (2005).

[199] K. Kalcher, *Electroanal.,* **2,** 419 (1990).

[200] R. P. Deo, S. N. Lawrence and J. Wang, *Analyst,* **124,** 1076 (2004).

[201] Y. M. Lee, O. Y. Kwon, Y. J. Yoon and K. Ryu, *Biotechnol. Lett.,* **28,** 39 (2006).

[202] L. S. Duan, F. Xie, F. Zhou and S. F. Wang, *Anal. Lett.,* **40,** 2653 (2007).

[203] E. Laviron, *J. Electroanal. Chem.,* **101,** 19 (1979).

[204] Y. D. Zhao, W. D. Zhang, Q. M. Luo and S. F. Y. Li, *Microchem. J.,* **75,** 189 (2003).

[205] B. T. Demircigil, B. Uslu, Y. Ozkan, S. A. Ozkan and Z. Senturk, *Electroanal.,* **15,** 230 (2003).

[206] http://en.wikipedia.org/wiki/Sulfamethoxazole

[207] http://www.medicinenet.com/sulfamethoxazole/article.htm

[208] M. Y. Issa and S. A. Amin, *Anal. Lett.,* **27,** 1147 (1994)**.**

[209] P. Nagaraja, S. D. Naik, A. K. Shreshta and A. Shivakumar, *Acta Pharm.,* **57,** 333 (2007).

[210] J. Fan, Y. Chen, S. Feng, C. Ye and J. Wang, *Anal. Sci.*, **19,** 419 (2003).

[211] R. Whelpton, G. Watkins and S. H. Curry, *Clin. Chem.*, **27,** 1911 (1981)

[212] T. You, X. Yang and E. Wang, *Analyst,* **123,** 2357 (1998).

[213] United States Pharmacopoeia, XXIth revision, National formulary XVIth ed., US Pharmacopeial Convention, Rockville, (1985)**.**

[214] K. Girish Kumar and P. Indrasenan, *Analyst,* **113,** 1369 (1988).

[215] B. Chiavarino, M. E. Crestoni, A. D. Marzio and S. Fornarini, *J. Chromatogr. Biomed. Appl.,* **706,** 269 (1998)

[216] N. A. Epshtein, *Pharm. Chem. J.,* **36,** 675 (2002).

[217] G. Knupp, H. Pollmann and D. Jonas, *Chromatographia,* **22,** 1 (1986).

[218] M. V. Astrid, E. Maria, B. Carrera, V. B. Dietrich and B. Carls, *Anal. Chim. Acta,* **159,** 119 (1984).

[219] http://www.health.state.ny.us/environmental/emergency/chemical_terrorism/nerve_agents_tech.htm

[220] http://en.wikipedia.org/wiki/Pralidoxime

[221] http://www.emedmag.com/html/pre/ter/CT1102.asp

[222] K. K. Rajic, B. Stankovic, A. Granov and Z. Binenfeld, *J. Pharm. Biomed. Anal.*, **6,** 773 (1988).

[223] M. Gyenge, H. Kalasz, A. G. Petroianu, R. Laufer, K. Kuca and K. Tekes, *J. Chromatogr. A,* **1161,** 146 (2007).

[224] M. Bodiroga , D. Agbaba, D. Zivanov-Stakic and R. Popovic, *J. Pharm. Biomed. Anal.,* **12,** 127 (1994).

[225] R. W. Murray, in: A. J. Bard (Ed.), *Electroanalytical Chemistry*, vol. 13, Marcel Dekker, New York, 191–368 (1984)

[226] E. Laviron, in: A. J. Bard (Ed.), *Electroanalytical Chemistry*, vol. 12, Marcel Dekker, New York, 53–157 (1982).

[227] L. Ozcan, M. Sahin and Y. Sahin, *Sensors,* **8,** 5792 (2008).

[228] W. Bird and D. G. M. Diaper, *Can. J. Chem.,* **47,** 145 (1969).

[229] R. V. Leeuwen, C. Katlama, V. Kitchen, C. A. Boucher, R. Tubiana, M. Mcbride, D. Ingrand, J. Weber, A. Hill and H. Mc Dade, *J. Infect. Dis.,* **171,** 1166 (1995).

[230] J. M. Pluda, T. P. Cooley, J. S. G. Montaner, L. E. Shay, N. E. Reinholter, S. N. Warthan, J. Ruedy, H. M. Hirst, C. A. Vicar and J. B. Quinn, *J. Infect. Dis.,* **171,** 1438 (1995).

[231] D. Ingrand, J. Weber, C. A. B. Boucher, C. Loveday, C. Robert, A. Hill and N.Cammack, *AIDS,* **9,** 1323 (1995).

[232] L. L. Lewis, D. Venzon, J. Church, M. Farley, S. Wheeler, A. Keller, M. Rubin, G. Yuen, S. Mueller, M. Sloas, L. Wood, F. Balis, G. M. Shearer, P. Brouwers, J. Goldsmith and P. A. Pizzo*, J. Infect. Dis.,* **174,** 16 (1996).

[233] J. L. Dienstag, R. P. Perrillo, E. R. Schiff, M. Bartholomew, C. Vicary and M. Rubin, *N. Engl.J.Med.,* **333,** 1657 (1995).

[234] Y. Benhamou, E. Dohin, F. Lunel-Fabiani, T. Poynard, J. M. Huraus, C. Katlama, P. Opolon and M. Gentilini, *Lancet,* **345,** 396 (1995).

[235] R. M. W. Hoetelmans, M. Profij, P. L. Meenhors, J. W. Mulder and J. H. Beijnen, *J. Chromatogr. B,* **713,** 387 (1998).

[236] J. K. Wong, C. C. Ignacio, F. Torriani, D. Havlir, N. J. Fitch and D. D. Richman, *J. Virol.,* **71,** 2059 (1997).

[237] P. L. Vernazza, B. L. Gilliam, J. Dyer, S. A. Fiscus, J. J. Eron, A. C. Frank and M. S. Cohen, *AIDS,* **11,** 987 (1997).

[238] I. L. Ajaka, L. Mandelbrot, M. C. Delmas, H. Bastian, J. L. Benifla, I. Farfara, I. de Vincenzi, S. Matheron, F. Simon and F. B.Vezinet, *AIDS,* **11,** 1575 (1997).

[239] N. F. Plessen, H. C. Michelis, H. Foth and G. F. Kahl, *J. Chromatogr.,* **534,** 101 (1990).

[240] D. M. Morris and K. Selinger, *J. Pharm. Biomed. Anal.,* **12,** 255 (1994).

[241] A. J. Harker, G. L. Evans, A. E. Hawley and D. M. Morris, *J. Chromatogr. B,* **657,** 227 (1994).

[242] P. H. Hysu and T. L. Lloyd, *J. Chromatogr. B*, **655,** 253 (1994).

[243] S. A. Wring, R. M. O'Neill, J. L. Williams, W. N. Jenner, M. J. Daniel, M. R. D. Gray, J. J. Newman, G. N. Wells and D. R. Sutherland, *J. Pharm. Biomed. Anal.,* **12,** 1573 (1994).

[244] H. Y. Aboul- Enien and M. M. Hefnawy, *Anal. Lett.,* **36,** 2527 (2003).

[245] S. A. Ozkan and B. Uslu, *J. Liq. Chrom. Rel. Tech.,* **25,** 1447 (2002).

[246] K. Basavaiah and B. C. Somashekhar, *Indian J. Chem. Tech.,* **13,** 7 (2006).

[247] D. G. Sankar, M. V. V. N. Reddy, J. M. Rajendra Kumar and T. K. Murthy, *Indian J. Pharm. Sci.,* **64,** 504 (2002).

[248] K. Basavaiah, B. C. Somashekhar and V. Ramakrishna, *J. Anal. Chem.,* **62,** 542 (2007).

[249] B. Dogan, B. Uslu, S. Suzen and S. A. Ozkan, *Electroanal.,* **17,** 1886 (2005).

[250] M. Singh, Y. Kumar, O. Meena, A. Garg and R. Pandey, *Int. J. Chem Tech Res.*, **2,** 1738 (2010).

[251] S. Shahrokhian and M. Amiri, *J. Solid State Electrochem.,* **11**,1133 (2007).

[252] http://www.medicinenet.com/metronidazole/article.htm

[253] D. K. Bempong, R. G. Manning, T. Mirza and L. Bhattacharyya, *J. Pharm Biomed. Anal.,* **38,** 776 (2005).

[254] M. Shahid Ali, R. S. Chaudhary and M. A. Takieddin, *Drug Dev. Ind. Pharm.***, 25,** 1143 (1999).

[255] K. R. Danao, S. M. Hiradeve, R. S. Moon, A.V. Kasture and P. G. Yeole, *IJPLS,* **1,** 82 (2010).

[256] K. Farhadi and S. Bahar, *J. Chin. Chem. Soc.,* **54,** 1521 (2007).

[257] D. B. Meshram, S. B. Bagade and M. R. Tajne, *Pak. J. Pharm. Sci.,* **22,** 323 (2009).

[258] P. Parimoo, C. V. N. Prasad and R. Vineeth, *J. Pharm. Biomed. Anal.,* **14**, 289 (1996).

[259] P. Nagarajia, K. R. Sunitha, R. A. Vasath and H. S. Yathirajan, *J. Pharm. Biomed. Anal.,* **28**, 527 (2002).

[260] S. C. Bhatia and V. D. Shanbhag, *J. Chromatogr.,* **305,** 325 (1984).

[261] R. Joseph and K. Girish Kumar, *Anal. Lett.,* **42,** 2309 (2009).

[262] J. Yue and A. J. Epstein, *J. Am. Chem. Soc.,* **112,** 2800 (1990).

[263] J. Yue, Z. H. Wang, K. R. Cromack, A. J. Epstein and A.G. MacDiarmid, *J. Am. Chem. Soc.* **113**, 2665 (1991).

[264] S. Shimizu, T. Saitoh, M. Uzawa, M. Yuasa, K. Yano, T. Maruyama, K.Watanabe, *Synth. Met.,* **85**, 1337(1997).

[265] J. Yue, A. J. Epstein, Z. Zhong, P. K. Gallogher and A. G. MacDiarmid, *Synth. Met.,* **41,** 765 (1991).

[266] N. Zhou, J. Wang, T. Chen, Z. G. Yu and G. X. Li, *Anal. Chem.*, **78,** 5227 (2006).

[267] Y. C. Liu, C. C. Yu and K. H. Yang, *Electrochem. Commun.,* **8**, 1163 (2006).

[268] F. N. Crespilho, M. E. Ghica, M. Florescu, F. C. Nart, O. N. Oliveira and C. M. A. Brett, *Electrochem. Commun.,* **8,**1665 (2006).

[269] L. Agui, J. Manso, P. Yanez-Sedeno and J. M. Pingarron, *Sens. Actuators B,* **113,** 272 (2006).

[270] P. Santhosh, A. Gopalan and K. P. Lee, *J. Catal.,* **238,** 177 (2006).

[271] J. W. Larsen, M. Freund, K.Y. Kim, M. Sidovar and J. L. Stuart, *Carbon*, **38,** 655 (2000).

[272] http://en.wikipedia.org/wiki/Nimesulide

[273] S. K. Kulkarni, *Current Science*, **83,** 1442 (2002).

[274] S. F. Chang, A. M. Miller and R. E. Ober, *J. Pharm. Sci.,* **66,** 1700(1977).

[275] D. Castoldi, V. Monzani and O. Tofanetti, *J. Chromatogr. B,* **69,** 413(1988).

[276] D. J. Jowarowicz Jr., M. T. Filopowski and K. M. K. Boje, *J. Chromatogr. B,* **723,** 293(1999).

[277] G. Khaska and N. Udupa, *J. Chromatogr. B,* **727,** 241(1999).

[278] M. C. Carrasco-Portugal, V. Granasos – Soto, G. A. Camacho Vieyra, J. Perez- Vrizar and F. J. Flores- Morrieto, *J. Liq. Chromatogr.,* **23,** 2237 (2000).

[279] P. Ptacek, J. Macek and K. Kilma, *J. Chromatogr. B.,* **758,** 183 (2001).

[280] A. Nonzioli, G. Luque and C. Fernandez, *J. High Resolut. Chromatogr.,* **12,** 413 (1989).

[281] A. S. R. Lakshimi and M. N. Reddy, *Microchim. Acta,* **132,** 1 (1999).

[282] S. Chandran, S. Saggar, K. P. Priya and R. N. Saha, *Drug Dev. Ind. Pharm.,* **26,** 229 (2000).

[283] S. Altinoz and O. O. Dursun, *J. Pharm. Biomed. Anal.,* **22,** 175 (2000).

[284] D. Dogrukol-Ak, M. Tuncel and H. Y. Aboul- Enein, *J. Sep. Sci.,* **24,** 743 (2001).

[285] I. C. Constantinescu, M. Florea, C. C. Arama, A. Nedelcu and C. M. Monciu, *Farmacia*, **57,** 267 (2009).

[286] P. R. Battu, *Int. J. PharmTech Res.* **1,** 206 (2009).

[287] A. Miljkovic, B. Brzakovic, I. Kovacevic, D. Agbaba and M. Pokrajac, *J. Plan. Chromatogr- Modern TLC,* **16,** 211 (2003).

[288] R. Reddy and P. Reddy, *Indian J. Pharm. Sci.,* **58,** 96 (1996).

[289] A.Wang, X. Shao, Q. Liu, Q. Qu, G. Yang and X. Hu, *J. Pharm. Biomed. Anal.,* **42,** 237 (2006).

[290] K. K.Girish Kumar, P. Augustine and S. John, *Port. Electrochim. Acta,* **253,** 381(2007)

[291] S. Furlanetto, S. Orlandini, G. Aldini, R. Gotti, E. Dreassi and S. Pinzauti, *Anal. Chim. Acta,* **413,** 229 (2000).

[292] R. I. L. Catarino, M. B. Q. Gracia and J. L. F. C. Lima, *Port. Electrochim. Acta,* **19,** 227 (2001).

[293] J. Zhang, X. Tan, D. Zhao, Z. Huang, Y. Mi and Z. Huang, *Electrochim. Acta,* **55,** 2522 (2010).

RESEARCH PAPERS PUBLISHED

INTERNATIONAL JOURNALS

- Sindhu Issac and K. Girish Kumar, Voltammetric study of Pyridine-2-Aldoxime Methochloride at poly (p-toluene sulphonic acid) modified glassy carbon sensor and its analytical applications, *Anal. Methods,* **2,** 1484 (2010).
- Sindhu Issac and K. Girish Kumar, Voltammetric determination of sulfamethoxazole at a multiwalled carbon nano tube modified glassy carbon sensor and its application studies, *Drug Test. Analysis,* **1**, 350 (2009).

CONFERENCE PAPERS

- "Electrochemical sensor for the determination of Nimesulide based on AuNP/poly cystamine modified glassy carbon electrode". (1st Kerala Women's Science Congress, St.Teresa's College Ernakulam, August 2010)
- "Development of AuNP/poly PABSA modified glassy carbon sensor for the voltammetric determination of Metronidazole benzoate". ("National Convention of Electrochemists – 15", VIT University, Vellore February 2010)
- "Development of Voltammetric sensor for Lamivudine based on L-Cysteine modified glassy carbon electrode" ("International conference on Materials for the Millenium", Cochin University of Science & Technology, January 2010).

- **"**Development of poly(p-toluene sulphonic acid) based voltammetric sensor for the determination of PAM Chloride**''** ("Current Advances in Chemical Sciences", Sacred Heart College Thevara. (2008))
- **"**Development of a carbon nanotube based voltammetric sensor for the determination of sulfamethoxazole in pure form, dosage forms and in urine sample." ("Current Trends in Cemistry''Cochin University of Science and Technology, Kerala. 2008)
- "Development of a carbon nanotube based voltammetric sensor for the determination of ambroxol in pharmaceutical formulations and urine sample." ("The 8th Workshop on Biosensors and Bioanalytical μ-Techniques in Environmental and Clinical Analysis", BITS Pilani, Goa Campus, 2007)