DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR VARIOUS PHARMACEUTICALS

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by

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Certificate

Certified that the present work entitled **"Development of Electrochemical Sensors for Various Pharmaceuticals",** submitted by Ms. Renjini Joseph, 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 **"Development of Electrochemical Sensors for Various 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
11-01-2012

Renjini Joseph

Words of Gratitude…

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Preface

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. 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.

As part of the present investigations eight voltammetric sensors have been fabricated for seven drugs such as Metronidazole Benzoate, Sulfamethoxazole, Acyclovir, PAM Chloride, Trimethoprim, Tamsulosin Hydrochloride and Ceftriaxone Sodium. For the present study, the modification techniques adopted are Metalloporphyrin based modification, MWCNT based modification, SAM based modification and electropolymerisation.

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

Chapter 1 gives a detailed description about the various electroanalytical techniques and their application. It gives a brief description about the different types of chemical sensors and discusses in detail about electrochemical sensors. The chapter also gives a brief review of the important voltammetric sensors developed for different drugs.

Chapter 2 discusses in detail the materials and methods used in the investigation. It also describes the method for the fabrication of chemically modified electrodes as voltammetric sensors for the determination of various drugs. The chapter also discusses the procedure for the analysis of drug content in pharmaceutical formulations and also in real samples like urine. The instruments used in the present investigation are also discussed.

Chapter 3 presents the fabrication of 5,10,15,20-tetrakis(3-methoxy-4-hydroxyphenyl)porphyrinato Zinc(II) (TMHPP Zn(II)) based sensor for the quantitative determination of Metronidazole benzoate (MTZB). 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 5,10,15,20-tetrakis(3-methoxy-4-hydroxyphenyl) porphyrinato Copper(II) (TMHPP Cu(II)) based sensor for the determination of the drug Sulfamethoxazole (SM).

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 mercaptobenzothiazol and TMHPP Cu(II) based sensor for the determination of the drug Acyclovir (ACV). The response parameters of the newly developed sensor as well as its analytical applications have been discussed in this chapter. The analytical applications of the developed sensor in the determination of pharmaceutical formulations and real samples have also been discussed in this chapter.

Chapter 6 presents the fabrication of dodecane thiol and multiwalled carbon nanotube (MWCNT) modified gold sensor for the drug PAM Chloride. The response parameters of the newly developed sensor as well as its analytical application have been discussed in this chapter.

Chapter 7 deals with the development of poly (aniline) modified gold sensor for the drug Trimethoprim (TMP). Optimization studies of the developed sensor, response characteristics and analytical applications are dealt with in detail in this chapter.

Chapter 8 is devoted to the detailed description about the sensors developed for the drug Tamsulosin Hydrochloride (TAM). The sensors fabricated include (i) poly (pyrrole) modified gold sensor and (ii) poly (pyrrole) and MWCNT modified gold

sensor. The various response parameters of the developed sensors are discussed in detail.

Chapter 9 presents a detailed account of the development and performance characteristics of poly (o-aminophenol) modified gold sensor for the drug Ceftriaxone Sodium (CFS). The application studies of the developed sensor in the determination of the drug in pharmaceutical formulations and urine samples are also explained in the chapter.

Chapter 10 gives the summary and the conclusions of the work done. References are given as a separate section at the end of the thesis.

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Chapter 1

Introduction

Contents

Analytical chemistry deals with the analysis of material samples to gain an understanding of their chemical composition and structure. This differs from other sub disciplines of chemistry in that it is not intended to understand the physical basis for the observed chemistry as with physical chemistry and it is not intended to control or direct chemistry as is often the case in organic chemistry and it is not necessarily intended to provide engineering tactics as are often used in materials science. Analytical chemistry has significant overlap with other branches of chemistry (especially those that are focused on a certain broad class of chemicals), such as organic chemistry, inorganic chemistry or biochemistry and theoretical chemistry. Analytical chemistry and experimental physical chemistry are very unrelated in their mission but often share the most in common in the tools used in experiments.

The main objectives of analytical chemistry are to answer three basic questions; what is? (Qualitative analysis), how much is it? (Quantitative analysis) and in what form is it? (Structural analysis). The qualitative analysis yields information about the identity of atomic or molecular species or the functional groups in the sample where as quantitative analysis provides numerical information as to the relative amount of one or more of these components [1]. Structural analysis, as implied by its name, aims to elucidate chemical structures. In short qualitative analysis identifies a property of the analyte (or its reaction product) whereas quantitative analysis measures its numerical value and structural analysis interprets it.

The main techniques employed in quantitative analysis are based on

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

(ii) Appropriate electrical measurements

(iii) The measurement of certain spectroscopic properties

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

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

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

The titrimetric analysis is carried out by determining the volume of a solution of accurately known concentration which is required to react quantitatively with a measured volume of a solution of the substance to be determined. Volumetry measures the volume of gases involved in a chemical reaction [1].

The need for trace level analysis led to the development of chromatography, spectrophotometry and electroanalysis. Chromatography is a separation process employed for the separation of mixtures of substances. It is widely used for the identification of components of mixtures. It is often possible to make quantitative determination particularly when using gas chromatography and high performance liquid chromatography. Spectrophotometric methods of analysis depend on measuring the amount of radiant energy of a particular wavelength absorbed by the sample or measuring the amount of radiant energy of a particular wavelength emitted by the sample. The fundamental law that governs the spectrophotometry is the Beer's law. Atomic absorption spectroscopy (AAS), atomic fluorescence spectroscopy (AFS), flame emission spectroscopy (FES) and inductively coupled plasma (ICP) make use of absorption/emission spectroscopy.

1.1 Electroanalysis

Electroanalysis can be defined as the application of electrochemistry to solve real life analytical problems [2]. Each analytical technique has a specific

purpose and a range of applications. Electroanalytical measurements offer a number of important potential benefits [3]

a) Selectivity and specificity

b) Selectivity resulting from the choice of material

c) High sensitivity and low detection limit

d) Possibility of giving results in real time or close to real time

e) Application as miniaturized sensors in situations where other sensors may not be useful.

The principal criterion for any electroanalytical measurement is that the medium between the electrodes making up the electrical circuit has to be sufficiently conducting. Thus, electroanalysis is complementary to other analytical techniques. Electrochemical monitoring has many advantages; the detection limits achieved in electroanalysis make it a better alternative to the existing analytical techniques. Also, the advantage of distinguishing oxidation states is highly important. The electrochemical approach can give a rapid answer, without digestion, as to the labile fraction of a given element in a particular oxidation state, and the experiment can be performed on site in the field.

1.2 Types of Electroanalysis

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

a) Potentiometry

b) Coulometry

c) Voltammetry/Amperometry

1.3 Potentiometry

It is the technique of using a single measurement of electrode potential to determine the concentration of an ionic species in solution. The electrode whose potential is dependent upon the concentration of the ion to be determined is termed as the indicator electrode and the case where the ion to be determined is directly involved in the reaction, it is an electrode of the first kind. When the concentration of the ion to be determined is not directly concerned in the electrode reaction, it is an electrode of the second kind. The measurement is made at effectively zero current. The current paths between the electrodes can be highly resistive. By judicious choice of electrode material, the selectivity 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 [2]. Detection limits are of the order of 100 nanomoles per litre of the total concentration of the ion present in a particular oxidation state can be achieved. It is possible to measure 100 picomolar differences in concentration.

1.4 Coulometry

Coulometric methods of analysis are based on the measurement of quantity of electrical charge that passes through a solution during an electrochemical reaction. Coulometric method of analysis is an application of Faraday's first law of electrolysis - the mass of a substance liberated at the electrodes during electrolysis is directly proportional to the quantity of electrical charge (Q) that passed through the electrolyte.

If m is the mass of a substance deposited by a current of I amperes in t seconds, then according to the first law of electrolysis

$$m \alpha Q \quad \text{or} \quad m \alpha I \times t$$

Or m = ZIt where, Z is the proportionality constant. Thus, the first law relates the quantity of current passed and the extent of chemical change that took place.

Two general techniques are used for coulometric analysis, namely, constant current (amperostatic) and constant potential (potentiostatic) coulometry.

1.5 Voltammetry/Amperometry

Voltammetry is an electroanalytical technique which involve the application of a potential (E) to an electrode and monitoring the resulting current (i) flowing through the electrochemical cell. In many cases the applied potential is varied or the current is monitored over a period of time (t). Thus, all voltammetric techniques can be described as some function of E, i, and t. They are considered active techniques (as opposed to passive techniques such as potentiometry) because the applied potential forces a change in the concentration of an electroactive species at the electrode surface by electrochemically reducing or oxidizing it [4].

The analytical advantages of the various voltammetric techniques include excellent sensitivity with a very large useful linear concentration range for both inorganic and organic species (10 to 10^{-10} M), can be performed using a large number of useful solvents and electrolytes, works well on a wide range of temperatures, is rapid, simultaneous determination of several analytes is possible and kinetic and mechanistic parameters can be determined. Voltammetry has a well developed theory and thus can reasonably estimate the values of unknown parameters and can be easily executed with different potential waveforms even small currents can be measured.

A three electrode system is used, which includes a working electrode, a reference electrode and an auxiliary or counter 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 [1].

In amperometry, a fixed potential is applied to the electrode, which causes the species to be determined to react and a current to pass. Depending on the potential that is applied, the magnitude of the current is directly proportional to the concentration.

1.6 Chemical Sensors

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 [5]. 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. There are two parts to a chemical sensor – a region where selective chemistry takes place and the transducer. The chemical reaction produces a signal such as a colour change, emission of fluorescent light, change in electrical potential at the surface, flow of electrons, production of heat, or change in the oscillator frequeny of a crystal. The transducer responds to this signal and translates the magnitude of the signal into a measure of the amount of the analyte.

1.7 Classification of Chemical sensors

Based on the transducer type chemical sensors are classified as: Electrochemical, Optical, Mass sensitive and Heat sensitive sensors [6].

An overview of development of analytical chemistry demonstrates that **electrochemical sensors** represent the most rapidly growing class of chemical sensors. These include potentiometric sensors (ion selective electrodes and ion selective field effect transistors) and voltammetric /amperometric sensors including solid electrolyte gas sensors [7].

In the class of **optical sensors,** a spectroscopic measurement is associated with the chemical reaction. They are referred to as optodes.

Mass sensitive sensors make use of the piezoelectric effect. 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.

Heat sensitive sensors are also known as calorimetric sensors. Here, the transducer monitors the heat of a chemical reaction involving the analyte.

Electrochemical sensors are the leaders among the presently available sensors because of their remarkable detectability, experimental simplicity and low cost [8].

1.8 Voltammetric Sensors

The current-potential relationship of an electrochemical cell provides the basis for voltammetric sensors. Amperometric sensors, which are also based on the current-potential relationship of the electrochemical cell, can be considered as a subclass of voltammetric sensors. In amperometric sensors, a fixed potential is applied to the electrochemical cell, and a corresponding current, due to a reduction or oxidation reaction, is then obtained. This current can be used to quantify the species involved in the

reaction. The key consideration of an amperometric sensor is that it operates at a fixed potential. In general, voltammetric sensors examine the concentration effect of the detecting species on the current-potential characteristics of the reduction or oxidation reaction involved. The mass transfer rate of the detecting species in the reaction onto the electrode surface and the kinetics of the faradaic or charge transfer reaction at the electrode surface directly affects the current-potential characteristics. This mass transfer can be accomplished through (a) an ionic migration as a result of an electric potential gradient (b) a diffusion under a chemical potential difference or concentration gradient and (c) a bulk transfer by natural or forced convection. The electrode reaction kinetics and the mass transfer processes contribute to the rate of the faradaic process in an electrochemical cell. This provides the basis for the operation of the voltammetric sensor [9].

1.8.1 Instrumentation

Voltammetric technique was developed after the discovery of polarography in 1922 by the Czech chemist Jaroslav Heyrovsky, for which he received the Nobel Prize in chemistry in 1959. The electrochemical cell, where the voltammetric experiment is carried out, consists of a working (indicator) electrode, a reference electrode and usually a counter (auxiliary) 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 electrode and an auxiliary electrode. In a three electrode system, the reference electrode controls the potential of the working electrode and hence controls the reactions which can occur there.

The working electrode is an ideally polarizable electrode, i.e. the electrode shows a large change in potential when an infinitesimally small current passes through. Reference electrode is a nonpolarizable electrode i.e. an electrode of fixed potential. The auxiliary 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 lend 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.

1.8.2 Working Electrode

The working electrode is the electrode, where the electrode reaction under study is taking place. The working electrodes are of various geometries and materials, ranging from small mercury drops to flat platinum disks. Mercury is useful because it displays a wide negative potential range (because it is difficult to reduce hydrogen ion or water at the mercury surface), its surface is readily regenerated by producing a new drop or film and many metal ions can be reversibly reduced into it. Other commonly used electrode materials are gold, platinum and glassy carbon.

1.8.2.1 Mercury Electrode

Mercury electrode is a type of liquid electrode which was found to be very attractive as an electrode material for many years. It can be used in

dropping, streaming or pool configurations that are impossible with solid electrodes. Mercury may be considered as an excellent electrode material for studying reactions owing to its extended negative potential window and comparatively lesser electrode poisoning even in complex matrices. However oxidation of mercury in the presence of anions that precipitate or complex mercury (I) or mercury (II) ions limits its use to study anodic processes.

1.8.2.2 Solid Inert Electrodes

Platinum and gold are very popular as metallic solid electrode materials because of their versatile potential window, low background current, chemical inertness and suitability for various sensing and detection applications. Platinum has extremely small overpotentials for hydrogen evolution, which is the basis for its use in the construction of reversible hydrogen electrodes. Platinum and gold electrodes have extremely small overpotentials for hydrogen evolution compared to the liquid mercury electrode. This makes them better choice for the study of cathodic processes.

1.8.2.3 Carbon Electrodes

Carbon, being an inert electrode material is useful for both oxidation and reduction reaction in solutions. Electrodes made of 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 have been used.

Carbon Paste Electrode: Carbon paste electrodes (CPEs) belong to a group of heterogenous carbon electrodes. CPEs are represented by carbon paste, i.e, a mixture prepared from graphite powder and a suitable liquid binder packed into a suitably designed electrode body. Due to numerous advantages, properties and characteristics, these electrodes are widely used for potentiometry, voltammetry, amperometry and coulometry. Adams, the inventor of CPEs and his research group were the first to publish an extensive study on carbon pastes comprising numerous test measurements. Their investigations have been primarily focused on the characterization of CPEs with respect to their applicability in anodic and cathodic voltammetry.

1.8.3 Reference Electrode

Reference electrode is also known as the unpolarized electrode or unpolarizable electrode. An ideal reference electrode has a potential that is known, constant and completely insensitive to the composition of the solution under study. In addition this electrode should be easy to assemble and should maintain a constant potential even when there is a net current in the cell. The most widely used reference electrode consists of a silver electrode immersed in a solution of potassium chloride that has been saturated with silver chloride. Other commonly used reference electrodes are standard hydrogen electrode (SHE), calomel electrode etc.

1.8.4 Auxiliary Electrode

Auxiliary electrode is the electrode that serves as a source or sink for electrons so that current can be passed through the cell. Unlike the usual two electrode system, in voltammetric measurements a third electrode is required. If a two electrode system consisting of only reference and working electrode is used, then current flow through the reference electrode will cause a change in

its potential. Hence a three electrode system, incorporating a third electrode called the auxiliary electrode is used. The foremost 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. Platinum electrodes in the form of coils or thin foils are the most extensively used auxiliary electrodes.

1.8.5 Chemically Modified Electrodes

Chemically modified electrodes (CMEs) have continued to be of major concern during the past decade and a relatively large amount of electrochemical research has been devoted to the development and applications of different types of CMEs. According to Internatioal union of Pure and Applied Chemistry, a CME can be defined as "an electrode made of a conducting or semiconducting material that is coated with a film of a chemical modifier and that by means of Faradaic reactions or interfacial potential differences exhibits chemical, electrochemical, and/or optical properties of a film". CMEs can be fabricated by using various techniques which include electropolymerisation [10], dip-dry method [11], drop dry method [12], vapour deposition [13], spin coating [14], Langmuir–Blodgett [15] and the self assembled monolayer (SAM) technique. One of the most important properties of CMEs is their ability to catalyze the oxidation or reduction of analyte species that exhibit 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 major interferences.

Various modifications using Metalloporphyrins, Carbon Nanotubes (CNTs), Gold nanoparticles (AuNPs), Polymer films, SAM, Calixarenes etc.

has been used. For the present study, the modification techniques adopted are Metalloporphyrin based modification, CNT based modification, SAM based modification and Electropolymerisation.

1.8.5.1 Modification Based on Metalloporphyrins

Metalloporphyrins are organometallic complexes which belong to the large family of N_4 - macrocyclic compounds. They are all based on porphin, a heterocyclic ring system consisting of four pyrrole units which are linked by methine bridges. Metalloporphyrins as electrode modifying agents are very attractive because they are rather stable compounds and their properties can be finely tuned by simple modifications of their basic molecular structure. The coordinated metal, the peripheral substituents and the conformations of the macrocyclic skeleton influence the coordination and the related sensing properties of these compounds. They are used as biomimetric models for the study of several biological redox processes particularly in molecular oxygen transport (reduction) and catalytic activation to mimic monooxygenase enzymes of cytochrome P450. In oxidation reactions, they are well known as efficient materials for the catalytic degradation of various types of pollutants and residual waste materials. Furthermore, they have also been used extensively as catalysts, semiconductors, anticancer medicine etc.[16-19].

For at least three decades now, studies have demonstrated that many forms of metalloporphyrins can be applied successfully as electrocatalysts for a wide variety of electrochemical reactions. In fact there are many examples in nature to justify the interesting ability of macrocyclic organic N_4 complexes to catalyse general redox reactions involving gaseous molecules such as O_2, H_2 and N_2 etc. These include reactions found in enzymatic systems

such as sulfite reductase, nitrate reductase, cytochrome c oxidase, blue copper oxidases, pseudocatalase, photosystem II, nitrogenase and hydrogenase.These naturally occurring reactions must have generated enormous curiosity amongst scientists to further investigate the electrocatalytic properties of metalloporphyrins. The investigation has led to the application of metalloporphyrins for the oxidation of dopamine, thiols, H_2S, HS^-, reduced glutathione, L-cysteine, coenzyme A, pencillin, oxalic acid, hydroxylamine, hydrazine, nitrite, nitric oxide, cyanide, organic peroxides, hydrogen peroxide, ascorbic acid, catechol, sulfite etc. and in the reduction of molecular oxygen, hydrogen peroxide, carbon dioxide, L-cystine, disulfides etc.

1.8.5.2 Modification Based on Carbon Nanotubes

Carbon nanotubes (CNTs) have become the subject of intense research in the last decades because of their unique properties and the promising applications in many aspects of nanotechnology. CNTs are electrochemically inert materials similar to other carbon based materials used in electrochemistry, i.e. glassy carbon, graphite and diamond. CNTs possess sp^2 carbon units with several nanometers in diameter and many microns in length. Two groups of CNTs, multi-walled (MW) and single-walled (SW) can be synthesised by electrical arc discharge, laser vaporisation and chemical vapour deposition methods. CNTs behave as either metals or semiconductors, depending on the diameter and the degree of helicity [20]. They are suitable for the modification of various electrodes due to their high electronic conductivity for the electron transfer reactions and better electrochemical and chemical stabilities in both aqueous and non-aqueous solutions [21]. Furthermore, construction of efficient electrochemical sensors using the CNT modified electrodes is very

promising in that they promote electron transfer reactions in several small biologically important molecules and large biomolecules [22, 23].

1.8.5.3 Electropolymerisation

Three scientists, A.J. Heeger, A.G. Mac Diarmid and H. Shirakawa are credited for the discovery and development of electrically conducting polymers and they were awarded the Nobel Prize in Chemistry in 2000. Conducting polymers (CPs) are materials discovered just over 20 years ago. Electronic conducting polymers have many interesting features such as high electrical conductivity, mechanical flexibility and ability to be electrochemically switched between electronically insulating and conducting state that makes them ideal candidates for sensing devices. Consequently, they have numerous bioanalytical and technological applications. CPs contains conjugated π electron backbones which are the reason for their unusual electrochemical properties (high electrical conductivities, low ionisation potentials and high electron affinities) and optical properties (low energy optical transitions). CPs are easily synthesised and deposited onto the conductive surface of a given substrate from monomer solutions by electrochemical polymerisation. Electropolymerisation is a simple but powerful method in targeting selective modification of different type electrodes with desired matrices, because by adjusting the electrochemical parameters, we can control film thickness, permeation and charge transport characteristics. Polymer-modified electrodes have many advantages in the detection of analytes because of its selectivity, sensitivity and homogeneity in electrochemical deposition, strong adherence to electrode surface and chemical stability of the film [24]. Preparation of polymer films by oxidation and electropolymerisation of aromatic compounds (aniline, phenol, benzene and their derivatives) and of heteroaromatic compounds (pyrrole, thiophene and

their derivatives) has been widely used in electrode surface modification to obtain interesting electrode properties.

1.8.5.4 Self Assembled Monolayer

SAM technique is a recently developed technique which is simple and reproducible and the molecules are chemically bound to the electrodes. Notable advantages of SAM over other techniques for electrode modification are the high order and stability of the molecules on electrodes [25, 26]. The chemisorption of thiolates on gold through strong S–Au bonds are the most important class of SAM from the electrochemical point of view. By the strong S–Au bonds, the thiol molecules are linked to gold electrode surfaces, thus forming SAMs. These modified surfaces exhibit new electrochemical and physical properties that are from the organic monolayer. Apart from this, the application of functionalised SAMs has also been reported. Functionalised SAMs are used to fabricate very interesting devices that can be used as electrochemical and chemical sensors [27-29], in non-linear optics [27] and as biosensors [30, 31]. The SAM modified electrodes have certain advantages such as selectivity, sensitivity, stability, short response time, the possibility of introducing different chemical functionalities, ease of preparation and highly ordered molecules on the electrode. The unlimited applications of SAMs on gold surface play an increasingly important role which might surpass bulk gold in many technological aspects.

1.8.6 Mass Transport Processess

The fundamental movement of charged or neutral species in an electrochemical cell to the electrode surface is facilitated by three processes namely: diffusion, migration and convection.

Diffusion is mass transport resulting from the spontaneous movement of analyte species from regions of high concentrations to lower ones, with the aim of minimizing concentration differences. To minimize the concentration difference an electroactive species will diffuse from the bulk solution to the electrode surface (or from the electrode surface into the bulk solution).

Migration refers to the movement of a charged particle in a potential field. In most voltammetric experiments migration is undesirable but can be eliminated by the addition of a large excess of supporting electrolyte.

Finally **convection** is a mass transport achieved by some form of external mechanical energy acting on the solution or the electrode such as stirring the solution, solution flow or rotation and/or vibration of the electrode.

1.8.7 Supporting Electrolyte

The supporting electrolyte is an electrolyte solution, whose constituents are not electroactive in the range of applied potentials being studied, and whose ionic strength (and, therefore, contribution to the conductivity) is usually much larger than the concentration of an electroactive substance to be dissolved in it.

There are many functions of the supporting electrolyte. Supporting electrolyte increases the conductivity of solution. It maintains a constant ionic strength and there by ensures that the activity coefficient of the species under study does not change as the charge transfer reaction proceeds. If no supporting electrolyte were present, migration would transport a considerable fraction of the ionic active species across the solution to the electrode. This would change the concentration profiles as

defined by diffusion and thus alter the current. KCl, KNO_3, tetra alkyl ammonium salts, buffer solutions etc are used as supporting electrolytes.

1.8.8 Voltammetric Technique

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)

1.8.8.1 Polarography

The most extensively used voltammetric method is polarography, in which the working electrode is a Dropping Mercury Electrode (DME), which consists of a continuing series of minute droplets of mercury issuing under pressure from a glass capillary. Because of the frequent drop wise renewal of the electrode, the concentration of any reduced metals dissolved in the mercury can never reach significant levels; also contamination of the electrode surface by adsorption cannot accumulate beyond the life of each drop. 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, n denotes the number of Faradays, 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.

1.8.8.2 Linear Sweep Voltammetry (LSV)

Linear sweep voltammetry measures the current at a working electrode while the potential between the working electrode and a reference electrode is swept linearly in time. Oxidation or reduction of species is registered as a peak at the potential at which the species begins to be oxidized or reduced. LSV is similar to CV except that the potential range is scanned starting at the initial potential and ending at the final potential. In CV direction of the potential scan is reversed at the end of the forward scan and the potential range is scanned again in the reverse direction. Detection limits are in mg/l levels.

1.8.8.3 Cyclic Voltammetry (CV)

CV is the most extensively used electrochemical technique and is used to study electrochemical reactions as well as to provide information on the reversibility and kinetics of such reactions [32, 33]. During a cyclic voltammetry experiment the potential of an electrode is scanned linearly from an initial potential to a final potential and then back to the initial potential.The potential at which the peak current occurs is known as the peak potential (E_p). The magnitude of the Faradaic current (I_{pa} – anodic peak current) or (I_{pc} – cathodic peak current), gives an indication of the rate

at which electrons are being transferred between the redox species and the electrode. Cyclic voltammetric process could be reversible, quasi reversible and irreversible.

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

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

Theoretically, the potential difference between the oxidation and reduction peak is 59 mV for one electron reversible reactions. Irreversibility due to a slow electron transfer rate results in ΔEp > 0.0592/n V, say greater 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.

1.8.8.4 Pulse Methods

Many attempts have been made throughout the years to improve the sensitivity of polarographic methods. Many of the limitations were overcome by the development of pulse methods. The idea behind all pulse voltammetric methods is to measure the current at a time when the difference

between the desired faradaic curve and the interfering charging current is large [2]. These techniques permit convenient measurements up to 10^{-8}M concentration level. The difference between the various pulse voltammetric techniques is the excitation waveform and the current sampling regime.

a) Normal Pulse Voltammetry (NPV)

In this technique short potential pulses of increasing height are superimposed on a constant base potential. This base potential is chosen, where no faradaic reaction occurs i.e. where I = 0. Measurement of the current at the end of the pulse and plotting the succession of points against the potential of the applied pulses gives a voltammetric profile. An important advantage is that if irreversible adsorption of the product of the electrode reaction occurs, then this will be much reduced through the use of pulses.

b) Differential Pulse Voltammetry (DPV)

DPV measures the differences between two currents, just before the end of the pulse and just before pulse application. The difference between current measurements for each pulse is determined and plotted against the base potential.

c) Square Wave Voltammetry (SWV)

The square wave voltammetric wave form consists of a square wave superimposed on a stair case. The currents at the end of the forward and reverse pulses are both registered as a function of staircase potential. 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 detection limits as low as 10^{-8} M is possible.

The major advantage of square wave voltammetry is its speed. As a result, the analysis time is drastically reduced. A complete voltammogram can be recorded within a few seconds, as compared to 2-3 min in differential pulse voltammetry. In addition, SWV is more sensitive than DPV, this is because both forward and reverse currents are measured in the former, but only the forward currents are measured in the latter. 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. SWV method has also been used for the sensitive determination of many pharmaceuticals.

1.8.8.5 Stripping Voltammetry

Electroanalytical techniques, especially modern stripping voltammetry have been used for the sensitive determination of a wide range of pharmaceuticals. Such techniques enjoy the advantages that there is no need for derivatization and that these methods are less sensitive to matrix effects than other analytical techniques.

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.

a) Anodic Stripping Voltammetry

ASV is most widely used for trace metal determination and has a practical detection limit in parts per trillion ranges. This low detection limit is coupled with its ability to determine simultaneously four to six trace metals using relatively inexpensive instrumentation. Metal ions in the sample solution are concentrated into a mercury electrode during a given time period by application of a sufficiently negative potential. These amalgamated metals are then stripped (oxidized) out of the mercury by scanning the applied potential in a positive direction. The resulting peak current, I_p, is proportional to the concentration of each metal in the sample solution, with the position of the peak potential, E_p, specific to each metal. The use of mercury limits the working range for ASV to approximately 0 to –1.2 V versus SCE. The use of thin Hg films or Hg microelectrodes along with pulse techniques such as square-wave voltammetry can substantially lower the limits of detection of ASV. With more than one metal ion in the sample, the ASV signal may sometimes be complicated by formation of intermetallic compounds, such as ZnCu. This may shift or distort the stripping peaks for the metals of interest. These problems can often be avoided by adjusting the deposition time or by changing the deposition potential.

b) Cathodic Stripping Voltammetry

This method is the "mirror image" of ASV. CSV can be used to determine substances that form insoluble salts with the mercurous ion. Application of a relatively positive potential to a mercury electrode in a solution containing such substances results in the formation of an insoluble

film on the surface of the mercury electrode. A potential scan in the negative direction will then strip the deposited film into solution. This method has been used to determine inorganic anions such as halides, selenide and sulfide and oxyanions such as MoO_4^{2-} and VO_3^{5-}. In addition, many organic compounds, such as nucleic acid bases, also form insoluble mercury salts and may be determined by CSV.

1.9 Electroanalytical Techniques for the Assay of Pharmaceuticals

Till now, the commonly employed techniques for the determination of the drug in bulk form, pharmaceutical formulation and biological fluids are based on HPLC [34], LC/MS [35], spectroscopy [36] and microbiological assay [37]. Such techniques for the measurement of biological concentration are necessary in clinical environment to ensure that adequate drug levels can be maintained thus avoiding toxic concentrations of them. Since these techniques involve expensive instrumentation and running costs, the use of simpler, faster and cheaper, and sensitive electrochemical techniques can be interesting alternatives, especially those based on electroanalytical techniques.

Electrochemistry has many advantages making it an appealing choice for pharmaceutical analysis [38, 39]. Electrochemistry has always provided analytical techniques characterised by instrumental simplicity, moderate cost and portability. These techniques have introduced the most promising methods for specific applications [40-42]. 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. 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. The use of various electrodes viz., mercury, solids and modified electrodes for electroanalytical measurements has increased in recent years because of their applicability in the determination of active compounds that undergo oxidation reactions, which is a matter of great importance in the field of clinical and pharmaceutical analysis. The present study is mainly concentrated on the voltammetric methods of drug analysis using chemically modified electrodes.

1.10 A Brief Review on Voltammetric Sensors for Drugs

Quality assurance plays a central role in determining the safety and efficiency of medicines. Highly specific and sensitive analytical techniques hold the key to the design, development, standardisation and quality control of medicinal products. Modern physical methods of analysis are extremely sensitive and provide precise and detailed information from small samples of material. They can be rapidly applied and in general are readily amenable for automation [43]. In voltammetric analysis, many active compounds in dosage forms, in contrast to excipients, can be readily

oxidised or reduced at the electrode surface by applying a potential. The progress 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 various electrode surfaces.

D. W. Yang et al have developed a 5,10,15,20-tetrakis[2-(2,3,4,6-tetraacetyl-D-glucopyranosyl)-1-o-phenyl]porphyrin Mn(III)/chitosan modified glassy carbon electrode (GCE) for the amperometric determination of dimetridazole (DMZ). The modified electrode exhibited an effective catalytic response to the reduction of DMZ with good reproducibility and stability. At the modified electrode, the reduction potential of DMZ showed a decrease and the current response, a significant rise relative to that of bare GCE. Linear calibration curve was obtained over the range 2.7×10^{-9} M – 1.5×10^{-3} M with a detection limit of 2.7×10^{-9} M [44].

The electrochemical behavior of artemisinin (ARN) on AuNP/chitosan/ 5,10,15,20-tetrakis[2-(2,3,4,6-tetraacetyl-β-D-glucopyranosyl)-1-o-phenyl] porphyrin Fe(II) modified GCE was investigated by F. C. Gong et al. The modified electrode showed excellent selectivity and sensitivity towards ARN with respect to a number of interferents and exhibited stable current response. The cathodic peak current was linear to the ARN concentration in the range of 1.7×10^{-9} M - 1.8×10^{-7} M, with a detection limit of 1.7×10^{-9} M [45].

A novel film electrode for the voltammetric determination of acetaminophen (AMP) has been constructed by S. S. Huang et al based on Poly [tetra (p-aminophenyl) porphyrin Nickel] film on GCE. AMP effectively accumulated on the modified electrode and caused a pair of redox peaks at around 430 mV and 300 mV. The anodic peak current was linear to the AMP concentration in the range of 1.0×10^{-6} M - 2.0×10^{-4} M. The developed method was also successfully applied for the determination of AMP content in tablet samples with satisfactory results [46].

A metalloporphyrin, [5,10,15,20–tetrakis(4-methoxyphenyl) porphyrinato] Mn(III)chloride (TMOPPMn(III)Cl) coated GCE was prepared by L. Rajith et al for the electrocatalytic oxidation of trimethoprim (TMP). The electrochemical behaviour of TMP on TMOPPMn(III)Cl modified GCE was explored using DPV. The voltammograms showed enhanced oxidation response at the TMOPPMn (III)Cl modified GCE with respect to the bare GCE for TMP, attributable to the electrocatalytic activity of TMOPPMn(III)Cl. All of the experimental parameters were optimised and a direct electrochemical method was proposed for the determination of TMP. It was found that the oxidation peak current was proportional to the concentration of TMP over the range 6.0×10^{-8} M – 1.0×10^{-6} M with a very low detection limit of 3.0×10^{-9} M at two minutes open circuit accumulation. The developed method was a superior alternative and stands distinct from other works for the determination of TMP on account of the low detection limit apart from predominant characteristics such as ease, escalated speed of detection, cost effectiveness and excellent sensitivity for TMP. Applicability to assay the drug in urine and tablet samples has also been studied [47]

R. Cristescu et al have reported the deposition by matrix assisted pulsed laser evaporation (MAPLE) of (5,10,15,20-tetraphenyl)porphyrinato Mn(III) chloride thin films onto gold screen printed electrode. The modified electrode was characterised by atomic force microscopy and Raman spectroscopy. The metalloporphyrin thin film electrode was demonstrated to promote the electrochemical response of dopamine by CV. The electrode reaction of dopamine was significantly improved at the modified electrode [48].

A berberine (BE) sensitive optical fiber sensor was fabricated by X. B. Zhang et al, which was based on a composite active material consisting of tetraphenylporphine (TPP) and tetraphenyl porphyrinato Manganese(III) chloride (TPPMnCl) in a PVC matrix. The response of the sensor is based on the fluorescence quenching of TPP–TPPMnCl by BE. The developed sensor shows response to BE in the concentration range 7.5×10^{-7} M - 5.6×10^{-4} M. The method was applied successfully to the determination of BE in pharmaceutical sample [49].

K. K. Atkowska et al have developed a sensitive sensor for determination of l-histidine using gold electrode modified with Fe(III) porphyrin bearing three 2,6-di-tert-butyl phenol groups and one palmitoyl chain. Two methods of electrode modification were applied: direct chemisorption and embedment into dodecanethiol monolayer. Both types of electrodes were used for the determination of l-histidine using SWV. The determination of l-histidine with electrode modified by embedment technique was more precise, in comparison to that obtained by the direct chemisorption. Applicability of gold electrodes modified with Fe(III) porphyrin for the direct electrochemical determination of l-histidine was demonstrated using the artificial matrix mimicking human serum [50].

A novel voltammetric sensor was fabricated by X. Lu et al, which was based on 5-{[4-(4-mercapto)phenylmethoxy]phenyl}-10,15,20-tris(phenyl)porphyrin Cobalt(II) (MPPTPCo(II)). The properties of the modified electrode were investigated by scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and electrochemical methods. The MPPTP Co(II) modified electrode could be successfully used for the electrocatalytic oxidation of ascorbic acid. The current increased linearly with ascorbic acid concentration in the range of 1.2×10^{-8} M – 3.9×10^{-5} M. The detection limit was 2.6×10^{-9}M [51].

A fluorescence sensor based on the supramolecular recognition by glycosylated metalloporphyrin for levamisole (LEV) assay was developed by F. C. Gong et al. For the preparation of a LEV sensitive active material, 5, 10, 15, 20-tetrakis[2-(2, 3, 4, 6-tetraacetyl-β-D-glucopyranosyl)-1-o-phenyl] porphyrin and its metal complexes were synthesized and used in an optode membrane prepared by including glycosylated metalloporphyrin in chitosan matrice. The immobilised glycosylated metalloporphyrin was shown to be weakly fluorescent as a result of inhibition of the electron transfer by central metal ion. The fluorescence enhancement of the metalloporphyrin modified optode membrane by LEV was based on the complexation with the central metal moiety of metalloporphyrin and weakening the inhibition of the electron transfer for metalloporphyrin. The glycosylated metalloporphyrin/ chitosan optode membrane showed excellent selectivity towards LEV with respect to a number of interferents and exhibited stable response. The calibration graph obtained with the proposed sensor was linear over the range of $3.5x10^{-7}$- $1.3x10^{-5}$M with a detection limit of $3.5x10^{-7}$M for LEV. The prepared sensor is applied for the determination of LEV in pharmaceutical

preparations and the results agreed with the values obtained by the pharmacopia method [52].

Cobalt(II) phthalocyanine–Cobalt(II) tetra(5-phenoxy-10,15,20-triphenylporphyrin), (CoPc–$(CoTPP)_4$) pentamer, as a novel redox mediator on a GCE was successfully used for the amperometric determination of glucose by K. I. Ozoemena et al. GCE was first modified with the pentamer and then followed by the immobilisation of glucose oxidase (GOx) through cross-linking with glutaraldehyde in the presence of bovine serum albumin (BSA) and Nafion cation exchange polymer. The developed biosensor displayed good amperometric response characteristics to glucose exhibiting a low overpotential at 400 mV, very fast amperometric response time of 5 s, linear concentration range extended up to 1.1×10^{-4} M and 1.0×10^{-5} M detection limit [53].

The catalytic properties of the macrocyclic compounds of meso-tetrakis(o-nitrophenyl)tetrabenzoporphyrin (TBP) with Fe(III), Mn(III), Ni(II) and Co(II) were examined for the electrochemical oxidation of ascorbic acid by J. Ren et al. The different coordination compounds were deposited on GCE. The catalytic activity increased in the order TBP Co(II)>TBP Mn(III)>TBP Fe(III)>TBP Ni(II). Kinetic measurements of the processes were carried out. The Tafel slopes determined are 59.5 mV for TBP Fe(III), 72.86 mV for TBP Mn(III), 66.80 mV for TBP Ni(II), and 68.52 mV for TBP Co(II)[54].

A multi walled carbon nanotube (MWCNT) and dodecanethiol (DDT) modified gold electrode was prepared and characterised by F. Zhao et al. When the gold electrode was modified with a DDT self assembled monolayer (SAM) followed by a MWCNT film, the electrode area

increased by a factor of about 4. At the same time, the blocking action of the DDT with respect to electron transfer disappeared. At the modified electrode, prochlorperazine exhibited two anodic peaks at about 690 mV and 880 mV. The peak at about 690 mV was suitable for prochlorperazine determination. After an accumulation time of 180 s in an open circuit, the peak current was linear to prochlorperazine concentration over the range of 5.0×10^{-7} M – 1.5×10^{-5} M. The detection limit was 1.0×10^{-7} M [55].

A polypyrrole(PPy)/4-hydroxy-6-methyl-2-mercaptopyrimidine (HMMP) co modified gold electrode (PPy/HMMP/Au) was prepared and characterised by M. Chen et al. The polymerisation mechanisms of pyrrole at a bare gold electrode, dodecanethiol modified gold electrode and HMMP modified gold electrode were compared and discussed. With ascorbic acid as a probe, various factors affecting the electrochemical characteristics of the PPy/HMMP/Au electrode were investigated. Both the kinetics of oxidation and the reproducibility of the electrochemical response were enhanced at the co modified electrode compared to that of an unmodified gold electrode or the PPy modified gold electrode in the determination of ascorbic acid [56].

Dipyrromethene Cu(II) derivatives possessing two dodecane alkyl chains have been used for the modification of gold electrodes by B. Saraswathyamma et al. Electroactive host molecules have been incorporated into a lipophilic dodecanethiol (SAM) deposited onto gold electrodes through hydrophobic and Van der Waals interactions (embedment technique). The presence of dipyrromethene Cu(II) redox centers on the electrode surface was proved by CV and SWV. The gold electrodes incorporating redox active dipyrromethene Cu(II)/SAM were used for the direct voltammetric determination of paracetamol in human plasma. A linear calibration curve was

obtained over the range 2.0×10^{-4} M - 1.5×10^{-3} M of paracetamol. The detection limit estimated based on the linearization method was $1.2 \pm 0.07 \times10^{-4}$ M [57].

The 5-[p-(mercaptopropyloxy)-phenyl]-10,15,20-triphenylporphine (SAM) modified gold electrode have been used for the electrocatalytic oxidation of ascorbic acid by X. Lu et al. A large decrease in the overpotential for the oxidation of ascorbic acid has been observed at the SAM modified gold electrode. Catalytic current increased linearly with ascorbic acid concentration in the range 8.2×10^{-8} M –3.9×10^{-4} M. The detection limit was 4.6×10^{-9} M. The modified electrode exhibited good sensitivity and stability. The developed method was applied for the determination of ascorbic acid in real samples [58].

Tetraoctylammonium bromide stabilised AuNPs (TOAB-AuNPs) attached to 1,6-hexanedithiol (HDT) modified gold electrode was used for the simultaneous determination of paracetamol (PA) and ascorbic acid at physiological pH by S. S. Nair et al. The attachment of TOAB/AuNPs on HDT modified gold surface was confirmed by attenuated total reflectance (ATR), FT-IR spectroscopy and atomic force microscope (AFM). Interestingly, TOAB/AuNPs modified electrode shifted the oxidation potential of PA towards less positive potential by 70 mV and enhanced its oxidation current twice when compared to bare gold electrode. In addition, the modified electrode separated the oxidation potentials of ascorbic acid and PA by 210 mV, whereas the bare gold electrode failed to resolve them. The amperometric current of PA was increased linearly from 1.5×10^{-7} M - 1.3×10^{-5} M and the lowest detection limit was found to be 2.6×10^{-9} M. The present method was successfully used for the determination of PA in human blood plasma and commercial drugs [59].

J. B. Raoof et al have reported a AuNP/SAM modified gold electrode for the electrochemical determination of ascorbic acid and dopamine (DA) in aqueous media. The result showed that the AuNP/SAM modified electrode could clearly resolve the oxidation peaks of ascorbic acid and DA, with a peak to peak separation (ΔEp) of 110 mV, enabling determination of ascorbic acid and DA in the presence of each other [60].

L.Wang et al have developed SAM/AuNP modified gold electrode for the voltammetric sensing of EP. The SAM/AuNP electrode was demonstrated to promote the electrochemical response of EP by CV. The electrode reaction of EP was significantly improved at the modified electrode which resulted in a large increase in the voltammetric peak current with a detection limit of 6.0×10^{-8} M. The SAM/AuNP modified electrode showed high electrocatalytic activity and excellent sensitivity [61].

AuNPs immobilised on an amine-terminated SAM (AuNP/SAM) on a polycrystalline gold electrode was successfully used for the selective determination of DA in presence of ascorbic acid by C. R. Raj et al. Well separated voltammetric peaks were observed for DA and ascorbic acid at the AuNPs immobilised 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 AuNP/SAM electrode, which resulted in a large increase in the square wave voltammetric peak current with a detection limit of 1.3×10^{-4} M. The co existence of a large excess of ascorbic acid did not interfere in the voltammetric sensing of DA. The AuNP/SAM modified electrode showed excellent sensitivity, good selectivity and antifouling properties [62].

The electrochemical behavior and determination of phenylephrine (PHE) at MWCNT modified GCE was carried out by Z. Y. Hai et al. Experimental results showed that the oxidation of PHE at the modified electrode was a one electron process controlled by diffusion and the modified electrode exhibited excellent behavior for PHE determination. Here the working concentration range was found to be 1.0×10^{-7} M - 7.0×10^{-6} M with a detection limit of 3.0×10^{-8}M [63].

S.Shahrokhian et al developed a voltammetric sensor for the determination of tryptophan (TRP) at a MWCNT/Cobalt salophen composite film modified CPE. The linear concentration range obtained for the developed sensor was in the range 5.0×10^{-7} M – 5.0×10^{-5} M with a detection limit of 1.0×10^{-7} M. High sensitivity and selectivity together with sub micromolar detection limit of the electrode response make it very suitable for the determination of trace amounts of TRP in pharmaceutical formulation with satisfactory results [64].

Edge plane pyrolytic graphite electrode modified with single walled carbon nanotube (SWCNT) was developed by R. N. Goyal et al for the voltammetric determination of triamcinolone, abused by athletes for doping. Here the working concentration range was found to be 1.0×10^{-10} M – 2.0×10^{-8} M with a detection limit of 8.9×10^{-10} M [65].

A SWCNT modified gold electrode was developed by B. Zeng et al for the voltammetric determination of rutin, used in the treatment of a disease characterised by capillary bleeding. Here the working concentration range was found to be 2.0×10^{-8} M – 5.0×10^{-6} M with a detection limit of 1.0×10^{-8} M [66].

A 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 characterised by the peak current enhancement. MWCNT/Nafion /GCE greatly improved the sensitivity of determination of ofloxacin [67].

A MWCNT modified CPE was used to study the electrochemical behaviour of bergenin by Q. Zhuang et al. The modified electrode showed an excellent electrocatalytic activity by lowering the anodic potential and remarkable enhancement of the anodic current of bergenin if compared with the electrochemical performances obtained at bare CPE [68].

A MWCNT modified GCE as voltammetric sensor was developed for the determination of noscapine in blood and pharmaceutical sample by B. Rezaei et al. The linear concentration range obtained in this study was 4.0×10^{-7} M – 1.0×10^{-4} M with a detection limit of 8.0×10^{-8} M [69].

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, characterised by the peak current enhancement and lowering of the oxidation potential. The concentration of PAM Chloride showed excellent linear relationship with the oxidation peak current in the range 1.0×10^{-6} M - 1.0×10^{-3} M, with a detection limit of 3.0×10^{-7} M [70].

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 optimised conditions, was found to be 2.0×10^{-7}M - 1.0×10^{-5}M and 2.4×10^{-8}M, respectively. The reported method was successfully applied to trazodone determination in pharmaceutical samples [71].

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 4.5×10^{-7} M - 1.5×10^{-4} M and the detection limit was 2.0×10^{-7} M. This proposed method possesses many advantages such as very low detection limit, fast response, low cost and simplicity [72].

A MWCNT modified GCE (MWCNT/GCE) was fabricated and the electrochemical behaviour of sulfamethoxazole (SM) was examined by CV and DPV by S. Issac et al. Compared with bare GCE, the MWCNT/GCE exhibits excellent enhancement effect on the electrochemical oxidation of SM. A well defined oxidation peak of SM occurs at 740 mV. The experimental parameters were optimised and a direct electrochemical method for the determination of SM was proposed. Under optimum conditions the oxidation peak current was linear to the concentration of SM in the range of 5.0×10^{-5} M - 1.0×10^{-2} M with a detection limit of 1.0×10^{-5} M. The MWCNT/GCE showed good stability, selectivity and it can be used to quantify SM in pharmaceutical formulations and urine sample [73].

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 optimised conditions, the anodic peak current was linear to promethazine concentration in the range 5.0×10^{-8} M – 1.0×10^{-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 [74].

A voltammetric method was developed for the determination of tetracycline (TC) using 1–octyl–3–methylimidazolium–hexafluorophosphate/ MWCNT film coated GCE by G. Guo et al. Under the optimised experimental conditions, the peak current was linear to TC concentration in the range of 1.1×10^{-7} M - 2.2×10^{-5} M. The developed sensor had good reproducibility. It was successfully applied to the determination of TC in pharmaceutical samples [75].

Based on the unique properties of MWCNT/chitosan thin film, a new rapid, convenient and sensitive voltammetric method was described for the determination of levodopa and serotonin by A. Babaei et al. The MWCNT/chitosan coated GCE exhibited electrocatalytic activity to the oxidation of levodopa and serotonin as characterised by the significant peak current enhancement. Under the optimum conditions the electrode provides a linear response versus levodopa and serotonin concentrations in the range of 2.0×10^{-6} M –2.2×10^{-4} M and 5.0×10^{-7} M –1.3 $\times10^{-4}$ M respectively, using DPV. The modified electrode was satisfactorily used for determination of levodopa and serotonin in human serum and urine with satisfactory results [76].

A rapid and sensitive voltammetric sensor based on the reduction of betamethasone was developed using SWCNT modified edge plane pyrolytic

graphite electrode (SWCNT/EPPGE) by N. Goyal et al. The reduction of betamethasone gave a well defined, pH dependent peak. Linear calibration curve was obtained in the range 2.5×10^{-10} M -1.0×10^{-9}M with the limit of detection as 5.0×10^{-10} M. 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 [77].

A ultrahigh sensitive and selective electrochemical detection of nanomolar concentrations of dopamine (DA) in the presence of ascorbic acid by S. Komathi et al. The modified electrode was fabricated with a new functional nanocomposite, comprising of MWCNT grafted silica network (silica NW) and AuNPs (MWCNT/silica NW/AuNPs). The fabrication of modified electrode involves two steps: covalent functionalisation of MWCNT with silica NW and deposition of AuNP. CV and DPV experiments were performed for the individual and simultaneous electrochemical detection of DA (in nanomolar concentrations) and ascorbic acid. Differential pulse voltammograms at the modified electrode revealed that the current response is linear for DA in the concentration range of 1.0×10^{-10} M – 3.0×10^{-9}M with a detection limit of 1×10^{-10}M. This is the lowest detection limit reported for DA [78].

The voltammetric determination of rutin was studied by D. P. Santos et al using a poly (glutamic acid) modified GCE. The modified electrode exhibited stable and sensitive current response towards rutin. Square wave voltammogram at 410 mV for the modified GCE was found to be several orders of magnitude lower than that on a bare GCE. The square wave voltammetric currents of rutin at the modified electrode increased linearly

with its concentration in the range 7.0×10^{-7} M – 1.0×10^{-5}M. The method was successfully applied to the determination of rutin in pharmaceutical formulation without any pretreatment [79].

Amidosulfonic acid was electropolymerised by CV onto the surface of GCE by G. Yang et al to fabricate the chemically modified electrode, which showed high stability, good selectivity and reproducibility for the determination of isoniazid. The modified electrode showed an excellent electrocatalytic effect on the oxidation of isoniazid. Under the optimum experimental conditions, there was a good linear relationship between anodic peak current and isoniazid concentration in the range of 5.0×10^{-8} M - 1.0×10^{-5}M, and a detection limit of 1.0×10^{-8}M. The developed method has been applied for the direct determination of isoniazid in injection and tablet samples with satisfactory results [80].

S. Issac et al have fabricated a poly (p-toluene sulfonic acid) (p-TSA) modified GCE by electropolymerisation using CV. This chemically modified electrode showed high stability, good selectivity and reproducibility for the determination of PAM chloride- a cholinesterase reactivator, used in military as an antidote in the treatment of organophosphate poisoning and to counteract the effects of overdosage by anticholinesterases used in treating myasthenia gravis. 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 is proportional to the concentration of PAM chloride in the range 1.0×10^{-7} M - 1.0×10^{-3} M with a detection limit of 3.0×10^{-8} M. The developed sensor was also successfully applied for the determination of PAM chloride in body fluids [81].

Electrochemical behavior of TRP at the poly (p-aminobenzene sulfonic acid) modified GCE was investigated by Y. Ya et al with voltammetry. The electrochemical response of TRP was improved significantly in the presence of poly (p-aminobenzene sulfonic acid) film. Compared with bare GCE, the poly (p-aminobenzene sulfonic acid) film electrode remarkably enhanced the irreversible oxidation peak current of TRP. The oxidation peak current was proportional to TRP concentration in the range of 1.0×10^{-7} M to 1.0×10^{-6} M with a detection limit of 7.0×10^{-8} M [82].

Preparation of a molecularly imprinted polymer (MIP) film and its recognition properties for SM was investigated by S. P. Ozkorucuklu et al. The overoxidized polypyrrole (OPPy) film was prepared by the cyclic voltammetric deposition of pyrrole (PPy) in the presence of supporting electrolyte (tetrabutyl ammonium perchlorate) with and without a template molecule (SM) on a pencil graphite electrode (PGE). The voltammetric behaviour of SM on imprinted and non imprinted (NIP) films was investigated by DPV. The MIP electrode exhibited best reproducibility and highest sensitivity. The calibration curve for SM at MIP electrode has a linear concentration range of 2.5×10^{-6} M to 7.5×10^{-4} M. The detection limit of SM was found to be 3.5×10^{-4} M. The developed method was simple, low response time and good mechanical stability [83].

A polyaniline/polypyrrole (PANI/PPy) polymer film modified GCE was used for the determination of furazolidone drug by D. C. Tiwari et al using CV. Two well defined reduction peaks (- 358 mV and - 784 mV) and one oxidation peak (306 mV) were observed. The results, compared with that of a bare GCE, indicate that reduction peak potential of furazolidone shifts in negative direction and reduction peak current increases significantly at PANI/PPy modified polymer film electrode. PANI/PPy polymer electrode

was characterised by FT-IR spectroscopy, UV- Vis spectroscopy and SEM. The modified electrode was found to be stable and produce a pronounced response in long pH range (5-12) [84].

The voltammetric behavior of paracetamol at poly(3,4-ethylenedioxythiophene) (PEDOT) modified electrode was studied by CV by S. Mehretie et al. The cyclic voltammetric study indicates that the PEDOT modified electrode shows a very good electrocatalytic activity by reducing the overpotential by 200 mV for paracetamol oxidation. In a pH 7 phosphate buffer, the anodic current increased linearly with the concentration of paracetamol in the range from 1.5×10^{-8} M to 2.5×10^{-6} M and the detection limit was 1.1×10^{-6} M. The proposed method was applied for paracetamol determination in commercial tablets with a mean recovery of (101 ± 6)% [85].

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 [86].

A novel GCE modified with Poly (malachite Green) was investigated by C. Fang et al. The modified electrode can be used to determine dopamine (DA) and ascorbic acid. The anodic peaks for ascorbic acid and dopamine were separated (ΔEpa about 200 mV) at the poly (malachite green) modified electrode. Thus, DA can be determined in the presence of ascorbic acid [87].

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 AMP. Linearity between the oxidation peak current and the AMP concentration was obtained in the range of 2.0 $\times 10^{-7}$ M - 1.0$\times 10^{-4}$ M with a detection limit of 5.0 $\times 10^{-8}$ M [88].

An electropolymerised 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, ascorbic acid and uric acid in acidic solution and reduced the overpotential for the oxidation of epinephrine. The poly (EBT) coated electrode could separately detect epinephrine, ascorbic acid and uric acid in their mixture with the potential differences of 180 mV and 160 mV for epinephrine-ascorbic acid and uric acid - epinephrine, respectively, which are large enough to allow for determination of epinephrine in the presence of ascorbic acid and uric acid [89].

1.11 Scope of the present investigation

Voltammetric sensors have been becoming one of the effective and powerful means for analytical scientists in the determination of drug substances and are playing an increasing role in pharmaceutical analysis. Many methods have been employed in our laboratory for the quantitative analysis of drugs in pure form as well as in dosage forms [90-101]. Among all the developed methods (physicochemical and biological), electrochemical methods are cost effective, easy to prepare and rapidly manipulated. Thus it was aimed to develop voltammetric sensors for drugs namely, Metronidazole benzoate, Sulfamethoxazole, Acyclovir, PAM chloride, Trimethoprim, Tamsulosin hydrochloride and Ceftriaxone sodium. For all the sensors, the various parameters studied include effect of pH, effect of

scan rate, supporting electrolyte study, effect of concentration and interference study. The developed sensors have been successfully applied for the determination of drug in pharmaceutical formulations and also in real samples like urine.

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Chapter 2

Materials and Methods

Contents

This chapter discusses in detail the materials and methods used in the investigations. The general method for the fabrication of the modified electrodes is described in this chapter. The method for the synthesis of metalloporphyrins used in the fabrication of the modified electrodes is also described in this chapter. Details about the general reagents and the instruments used in the investigations are discussed in this chapter. The chapter also discusses the general procedure for the analysis of the pharmaceutical formulations and real samples employed in the studies.

2.1 Reagents

All reagents and solvents used were of analytical grade and double distilled water was used throughout the studies. Graphite powder, Multiwalled Carbon nanotube (MWCNT), Nafion, Alumina and PAM Chloride were purchased from Sigma Aldrich Corporation, USA. Except MWCNT, other chemicals were used as received. Aniline, Pyrrole, Zinc acetate and 3-methoxy-4-hydroxy benzaldehyde were purchased from SRL Chemicals, India. Aniline and Pyrrole were freshly distilled prior to use. Sodium dihydrogen 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 Metronidazole Benzoate (MTZB), Sulfamethoxazole (SM), Acyclovir (ACV), Trimethoprim (TMP), Tamsulosin hydrochloride (TAM) and Ceftriaxone sodium (CFS) 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 carbon paste/gold electrode modified with suitable chemical modifications, counter electrode used was platinum wire and Ag/AgCl was used as the reference electrode. 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 were recorded on JASCO-4100 FTIR Spectrometer using KBr discs. The UV-Visible spectra were recorded using Spectro UV-Visible Double Beam UVD-3500 instrument. AAS was recorded using Thermo AA spectrometer. ^{1} H NMR spectra were recorded using JEOL GSX 400 NB FT NMR spectrometer. Elemental analyses were performed with VarioEL III CHNS analyzer.

2.3 Synthesis and characterisation of porphyrin and metalloporphyrins

The ligand [5,10,15,20-tetrakis(3-methoxy-4-hydroxyphenyl) porphyrin (TMHPP) and its metal complexes (Zinc and Copper) were prepared. They have been characterised by the elemental analysis, IR, UV-Visible and NMR spectroscopic methods and were found to be in good agreement with the reported values.

2.3.1 Synthesis of TMHPP

The synthesis was performed according to Alder method [102]. Freshly distilled pyrrole (1.04 ml, 15 mmol) and 3-methoxy-4-hydroxy benzaldehyde (2.282 g, 15 mmol) were added to 30 ml of boiling propionic acid. The mixture was refluxed for 30 min and was allowed to cool for a few minutes. The filter cake was washed thoroughly with methanol. The resulting purple crystals were further purified by column chromatography. The yield was found to be 10%. Formation of TMHPP was confirmed by elemental analysis and spectroscopic techniques.

CHN analysis

Calcd (%): C, 72.18; H, 4.76; N, 7.01

Found (%): C, 72.08; H, 4.66; N, 6.97

Spectroscopic analysis

IR (KBr), ν (cm^{-1}): 3363 (NH); 3000 (CH); 3539 (OH)

UV-Visible spectrum in CH_2Cl_2, λ (nm): 411, 445, 514, 647

^1H NMR (500 MHz, $CDCl_3$) ppm: δ = 8.9 (s, 8H, pyrrollic –β- H), 5.9 (s, 4H, OH), 3.9 (s, 12H, OCH_3), -2.7 (s, 2H, NH), 8.2 - 7.3 (m, 12H, aromatic)

2.3.2 Synthesis of TMHPP Zn(II)

0.037 g of Zn $(OAc)_2 .2H_2O$ (0.17 mmol) in methanol (10 mL) was added to 0.050 g of TMHPP (0.0626 mmol) in chloroform (10 mL) at room temperature. It was then acidified with a drop of glacial acetic acid and refluxed for 12 hours. TMHPP Zn(II) was obtained as a red powder and it was filtered and washed with water. The yield was found to be 5%. TMHPP Zn(II) was characterised by elemental analysis and spectroscopic techniques.

CHN analysis

Calcd (%): C, 66.86; H, 4.17; N, 6.50

Found (%): C, 66.75; H, 4.07; N, 6.45

Spectroscopic analysis

IR (KBr), ν (cm^{-1}): 3363 (NH); 3000 (CH); 3539 (OH); 455 (M-N)

UV-Visible spectrum in DMSO, λ (nm): 411, 530

2.3.3 Synthesis of TMHPP Cu(II)

The ligand TMHPP (2.5 g, 3 mmol) and $CuCl_2.2H_2O$ (1.6 g, 9 mmol) were refluxed in 500 mL N, N-dimethylformamide for half an hour. The solvent was then stripped off and the residue was then extracted into 250

mL water. A red precipitate was formed immediately, which was isolated by filtration, washed with 250 mL of distilled water and air dried. The yield was found to be 4%. The product was confirmed by elemental analysis and spectroscopic techniques.

CHN analysis

Calcd (%): C, 68.00; H, 5.05; N, 6.10.

Found (%): C, 67.60; H, 4.68; N, 5.65; Cu, 6.66.

Spectroscopic analysis

IR (KBr), ν (cm^{-1}): 3363 (NH); 3000 (CH); 3539 (OH); 455 (M-N).

UV-Visible spectrum in DMSO, λ (nm): 405, 481, 530, 621.

2.4 Cleaning of CPE

CPE was sonicated in methanol, acetone and double distilled water successively to obtain the cleaned CPE.

2.5 Cleaning of gold electrode (GE)

The working GE was mechanically polished with aqueous slurries of alumina (50 nm) on a flat pad prior to surface modification. After polishing, it was rinsed ultrasonically with absolute ethanol to remove residual alumina particles from the surface and then cleaned with a piranha solution (H_2O_2:H_2SO_4 = 1:3, v/v) for 10 minutes. Following this mechanical process, an electrochemical cleaning process was carried out using cyclic voltammetry performed from 0 to 1500 mV in 0.5 M sulphuric acid solution at a scan rate of 100 mVs^{-1} until a stable cyclic voltammogram was obtained.

2.6 Preparation of chemically modified electrodes for pharmaceutical analysis

2.6.1 Preparation of TMHPP Cu(II) / TMHPP Zn(II) modified CPE

The TMHPP Cu(II)/TMHPP Zn(II) modified CPE was prepared by mixing different percentages of graphite powder, paraffin liquid and TMHPP Cu(II)/TMHPP Zn(II). This mixture was mixed in a mortar for at least 10 minutes to become homogeneous. This paste was packed into one end of a teflon holder in which electrical contact was made with a copper rod that runs through the center of the electrode body. Appropriate packing and a smooth surface was achieved by pressing the surface against a smooth filter paper. The prepared CPE is then left unused for a certain time (1-2 hours) to allow their final homogenization to proceed. Also the electrode surface was polished using a butter paper to produce reproducible working surface. Electrochemical behavior of these different electrodes was studied using cyclic voltammetric technique. The optimized electrode composition was then used for the electrochemical studies.

2.6.2 Preparation of MBZ / TMHPP Cu(II) modified GE

SAM was formed by immersing the cleaned GE in an ethanolic 1×10^{-3}M MBZ solution for 24 hours at room temperature. Finally the MBZ modified electrode was rinsed with ethanol in order to remove the unbonded thiol and it was immersed in TMHPP Cu(II) solution in dimethyl formamide for another period of 24 hours. It was observed that after drying in air, a uniform film was formed at the electrode surface.

2.6.3 Preparation of dodecane thiol / MWCNT modified GE

SAM was formed by dipping the cleaned GE in an ethanolic 1×10^{-3}M dodecane thiol (DT) solution for 24 hours at room temperature.

Then the modified electrode was washed with ethanol. Further modification of the DT modified electrode was carried out by dropping adequate amount of MWCNT onto it. Prior to this, the required pretreatment of MWCNT was carried out.

Acid treatment of MWCNT was carried out as described in the literature [103]. As a result of this treatment, length of MWCNT got shortened and they gained functional groups which made them hydrophilic. MWCNTs are generally insoluble in common solvents; therefore the key step is to disperse insoluble MWCNTs in suitable solvents to perform the electrochemical measurements by using MWCNT modified electrodes. However Nafion, a sulfonated tetrafluoro ethylene copolymer was found to effectively disperse MWCNTs. So Nafion was selected as the appropriate solvent for this study. 5 mg of the acid treated MWCNT was added to 2 mL of 0.5 % Nafion water solution and then sonicated for about 1 hour to get a homogenous suspension.

The DT modified GE was coated with an adequate amount of the MWCNT/Nafion suspension. Finally the DT/MWCNT/Nafion modified GE was kept in air at room temperature, so that the solvent was evaporated to get the modified GE.

2.6.4 Preparation of Poly (aniline) modified GE

The cleaned GE, reference electrode and auxiliary electrode were immersed in 0.5 M H_2SO_4 solution containing 0.1 M aniline. It was then subjected to 10 potential scans between -1.0 to +1.0 V at 100 mVs^{-1}[104]. The resulting film was washed with double distilled water and dried in air. It was observed that after drying in air, a uniform green film was formed at the electrode surface.

2.6.5 Preparation of Poly (pyrrole) modified GE

The cleaned GE, reference electrode and auxiliary electrode were immersed in 1.0 M KNO_3 solution containing 0.1 M pyrrole. The film was grown on the electrode surface by 20 cycles of cyclic voltammetric scans between 0.0 to 0.6 V at 100 mVs^{-1}[105]. After immobilization, film was washed with double distilled water and dried in air. It was observed that after drying in air, a uniform yellow film was found to be formed at the electrode surface.

2.6.6 Preparation of Poly (pyrrole)/ MWCNT modified GE

The poly (pyrrole) modified GE was coated with required amount of MWCNT/Nafion suspension. Solvent was evaporated at room temperature in air to get the modified GE.

2.6.7 Preparation of Poly (2-aminophenol) modified GE

0.002 M 2-aminophenol was prepared in 0.1M $HClO_4$ solution. 10 mL of this solution was then pipetted into the electrochemical cell and the electrochemical deposition of 2-aminophenol was carried out by cyclic voltammetry between -0.2 and 0.8 V at 100 mVs^{-1} for 30 cycles [106]. The resulting film was washed with doubly distilled water and dried in air. After drying in air, a film was observed at the electrode surface.

2.7 Preparation of the drug solutions

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

2.7.1 Metronidazole benzoate solution

2.753 g of MTZB was accurately weighed and dissolved in methanol. It was filtered into a 100 mL volumetric flask and the solution was made upto volume.

2.7.2 Sulfamethoxazole solution

2.530 g of SM was accurately weighed and dissolved in methanol. It was filtered into a 100 mL volumetric flask and the solution was made upto volume.

2.7.3 Acyclovir solution

2.2521 g of ACV was accurately weighed and dissolved in methanol. It was filtered into a 100 mL volumetric flask and the solution was made upto volume.

2.7.4 Trimethoprim solution

2.9032 g of TMP was accurately weighed and dissolved in methanol. It was filtered into a 100 mL volumetric flask and the solution was made upto volume.

2.7.5 PAM Chloride solution

1.726 g of PAM Chloride was accurately weighed and dissolved in water. It was filtered into a 100 mL volumetric flask and the solution was made upto volume.

2.7.6 Tamsulosin Hydrochloride solution

4.449 g of TAM was accurately weighed and dissolved in water. It was filtered into a 100 mL volumetric flask and the solution was made upto volume.

2.7.7 Ceftriaxone sodium solution

6.615 g of CFS was accurately weighed and dissolved in water. It was filtered into a 100 mL volumetric flask and the solution was made upto volume.

2.8 Preparation of buffer solutions

Buffer solutions were used to adjust the pH of the test solutions. Phosphate buffer solution (PBS) and Acetate buffer solution (ABS) were used as the supporting electrolyte for carrying out the electrochemical measurements.

2.8.1 Preparation of phosphate buffer solution (PBS)

The buffer solutions of different pH were prepared by mixing NaH_2PO_4 and Na_2HPO_4 in different amounts.

a) pH 2

To 100 mL double distilled water, 1.3799 g of NaH_2PO_4 and 0.0001 g of Na_2HPO_4 were added to give the PBS having pH 2.

b) pH 3

To 100 mL double distilled water, 1.379 g of NaH_2PO_4 and 0.0003 g of Na_2HPO_4 were added to give the PBS having pH 3.

c) pH 4

To 100 mL double distilled water, 1.378 g of NaH_2PO_4 and 0.0036 g of Na_2HPO_4 were added to give the PBS having pH 4.

d) pH 5

To 100 mL double distilled water, 1.3615 g of NaH_2PO_4 and 0.036 g of Na_2HPO_4 were added to give the PBS having pH 5.

e) pH 6

To 100 mL double distilled water, 1.2143 g of NaH_2PO_4 and 0.3218 g of Na_2HPO_4 were added to give the PBS having pH 6.

f) pH 7

To 100 mL double distilled water, 0.5836 g of NaH_2PO_4 and 1.5466 g of Na_2HPO_4 were added to give the PBS having pH 7.

g) pH 8

To 100 mL double distilled water, 0.094 g of NaH_2PO_4 and 2.497 g of Na_2HPO_4 were added to give the PBS having pH 8.

h) pH 9

To 100 mL double distilled water, 0.01 g of NaH_2PO_4 and 2.6605 g of Na_2HPO_4 were added to give the PBS having pH 9.

2.8.2 Preparation of acetate buffer solution (ABS)

The buffer solutions of different pH were prepared by mixing CH_3COONa and CH_3COOH in different amounts.

a) pH 2

To 100 mL double distilled water, 0.00242 g of CH_3COONa and 0.59943 g of CH_3COOH were added to give the ABS having pH 2.

b) pH 3

To 100 mL double distilled water, 0.02378 g of CH_3COONa and 0.59001 g of CH_3COOH were added to give the ABS having pH 3.

c) pH 4

To 100 mL double distilled water, 0.20548 g of CH_3COONa and 0.50984 g of CH_3COOH were added to give the ABS having pH 4.

d) pH 5

To 100 mL double distilled water, 0.87113 g of CH_3COONa and 0.21614 g of CH_3COOH were added to give the ABS having pH 5.

e) pH 6

To 100 mL double distilled water, 1.28854 g of CH_3COONa and 0.03197 g of CH_3COOH were added to give the ABS having pH 6.

f) pH 7

To 100 mL double distilled water, 0.00336 g of CH_3COONa and 1.35339 g of CH_3COOH were added to give the PBS having pH 7.

g) pH 8

To 100 mL double distilled water, 0.00034 g of CH_3COONa and 1.36024 g of CH_3COOH were added to give the ABS having pH 8.

h) pH 9

To 100 mL double distilled water, 0.00003 g of CH_3COONa and 1.36092 g of CH_3COOH were added to give the ABS having pH 9.

2.9 Analysis of the pharmaceutical formulations

2.9.1 Tablets for Metronidazole benzoate (Metrogyl and Flagyl)

The weight of ten tablets of each type ('Flagyl', Rhone-Plc, India and 'Metrogyl', Unique, India) was accurately determined and powdered well. The mass of powder required to prepare 5×10^{-3} M solution was dissolved in methanol. The clear solution was transferred to the titrimetric flask (50 mL) through a Whatman 41 filter paper. The beaker was washed several times with methanol and the contents were filtered into the flask. The solution was then made upto the mark. Solutions of different concentrations were prepared by serial dilution of the stock with supporting electrolyte. DPV

was recorded and the unknown concentrations were determined from the calibration graph.

2.9.2 Tablets for Sulfamethoxazole (Septra)

Ten tablets ('Septra', Burroughs Wellcome, India) were weighed, crushed and finely powdered. An adequate amount of this powder corresponding to the concentration 5×10^{-4} 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 with methanol 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.

2.9.3 Tablets for Acyclovir (Acivir and Zovirax)

Ten tablets ('Acivir', Cipla, India and 'Zovirax', Burroughs Wellcome, India) were weighed and finely powdered. Then sufficient amount of the powdered drug was weighed to prepare a solution of 5×10^{-4} M and was transferred to a beaker. The beaker was washed a number of times with methanol 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. The sample solution was then taken and the voltammetric studies were carried out.

2.9.4 Tablet for Trimethoprim

Tablet of TMP was prepared by mixing TMP and SM in the ratio 2:1. An adequate amount of this powder, corresponding to the concentration 1×10^{-4} M was taken, dissolved in dilute methanol and filtered into a 100 mL titrimetric flask. Solutions of different concentrations were prepared by serial dilution of the stock with supporting electrolyte. SWV was recorded and the unknown concentrations were determined from the calibration graph.

2.9.5 Tablet for Tamsulosin Hydrochloride (Veltam)

The mass of ten tablets ('Veltam', Intas pharmaceuticals, India) were taken and then powdered well. The mass of powder required to prepare 5×10^{-3} M solution was weighed. It was then transferred to a beaker and dissolved in water. 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 water and the washings were collected in the 100 mL 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.

2.9.6 Tablet for Ceftriaxone Sodium (Trixon)

Ten tablets of type ('Trixon', Lincoln, India) were weighed and finely powdered. An adequate amount of this powder, corresponding to the concentration 5×10^{-4} M was dissolved in water and made up in a 100mL titrimetric flask. Solutions of different concentrations were prepared by serial dilution of the stock with supporting electrolyte. The sample solution was then taken and the voltammetric studies were carried out.

2.10 Analysis of urine sample

The developed sensors were used for the determination of the corresponding drug in spiked urine samples. Standard addition method was employed for this purpose. Various amounts 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.

2.11 Standard methods

2.11.1 Metronidazole benzoate [107]

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

2.11.2 Sulfamethoxazole [107]

The mass of ten tablets of 'Bactrim' and 'Septra', were taken and then powdered well. An adequate amount of this powder containing 0.2 g of SM was weighed and dissolved in 50 mL of 2 M HCl. 3 g of KBr was added to the mixture. It was then cooled and titrated against 0.1 M sodium nitrite. The end point was determined potentiometrically.

2.11.3 Acyclovir [107]

Ten tablets of 'Acivir' and 'Zovirax' were accurately weighed and finely powdered. 0.15 g of this was weighed and dissolved in 60 mL of anhydrous acetic acid. Then it was titrated against 0.1 M perchloric acid. The end point was determined potentiometrically.

2.11.4 Trimethoprim [107]

0.25 g of trimethoprim was dissolved in 50 mL of acetic acid. 20 mL of this solution was taken in a beaker. It was then titrated with 0.1 M perchloric acid determining the end point potentiometrically.

2.11.5 PAM Chloride [108]

0.5 g of PAM Chloride was accurately weighed and dissolved in 250 mL water. 5mL of this solution was made up in a 100 mL titrimetric flask. 5 mL of this solution was then taken in a 50 mL titrimetric flask. Requisite amount of urine sample was added and then quantitatively diluted to 40 mL with water. 5 mL of 1M NaOH was added to this mixture and made up to the mark. The absorbance of the resulting solution as well as solutions of various concentrations was measured.

2.11.6 Tamsulosin Hydrochloride [109]

0.3 g of 'Veltam' tablet was dissolved in 5 mL of formic acid, 75 mL of a mixture of glacial acetic acid and acetic anhydride. Titrate immediately with 0.1 N perchloric acid and determine the end point potentiometrically.

2.11.7 Ceftriaxone Sodium [110]

Ten tablets of 'Trixon' was accurately weighed and finely powdered. 0.01g of the powder was weighed and dissolved in 10 mL water. 1 mL of the solution was taken in a 10 mL volumetric flask and the volume was made upto the mark with 0.1 M NaOH. The absorbance of the resulting solution as well as solutions of various concentrations were measured.

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Chapter 3

Development of Sensor for Metronidazole Benzoate

Contents

The development of a metalloporphyrin based sensor for the determination of metronidazole benzoate (MTZB) is discussed in detail in this chapter. The electrochemical determination of MTZB has been carried out at a [5,10,15,20-tetrakis(3-methoxy-4-hydroxyphenyl)porphyrinato]Zinc(II) modified carbon paste electrode (TMHPP Zn(II)/CPE) by cyclic voltammetry (CV), differential Pulse voltammetry (DPV) and linear sweep voltammetry (LSV). MTZB gave a well-defined reduction peak at -0.713 V in 0.1 M phosphate buffer solution of pH around 7. All of the experimental parameters were optimized and a direct electrochemical method was developed for the determination of MTZB. Applicability to assay the drug in urine and tablet samples has also been studied.

3.1 Introduction

Metronidazole benzoate (Figure **3.1**) is 1-(2-benzyloxy ethyl)-5-nitro methylimidazole. MTZB, one of the nitroimidazole derivative, is well known for its antimicrobial properties. MTZB blocks some of the functions within the bacterial cells and the parasites, resulting in their death. MTZB is effective against parasitic infections including giardia infections of the small intestine, amoebic liver abscess and amoebic dysentery, bacterial vaginosis and trichomonas vaginal infections. MTZB is used alone or in combination with other antibiotics in treating abscesses in the liver, pelvis, abdomen and brain caused by susceptible anaerobic bacteria. MTZB is used in treating infection of the colon caused by a bacterium called C. difficile. C. difficile is an anaerobic bacterium that can infect the colon and leads to inflammation of the colon (pseudomembranous colitis) with severe diarrhea and abdominal pain. MTZB is also used in combination with other drugs to treat Helicobacter pylori (*H. pylori*) that causes stomach or intestinal ulcers [111]. MTZB can be administered intravenously to treat serious infections. The liver is primarily responsible for eliminating MTZB from the body, and doses may need to be reduced in patients with liver disease and abnormal liver function.

Due to the vital importance of MTZB determination in pharmaceutical preparations and in biological fluids, several analytical methods have been developed for the quantitative determination of MTZB. These include kinetic spectrophotometric method [112], spectrophotometry [113], titrimetry, thin layer chromatography, gas chromatography [114], high performance liquid chromatography [115] and voltammetry [116]. However the above mentioned methods do not have sufficient selectivity for MTZB

determination. Hence it is of prior importance to develop an alternative method for MTZB determination with high degree of selectivity.

Chemically modified carbon paste electrodes (CMCPEs) have continued to be of major concern during the past decade and a relatively large amount of electrochemical research has been devoted to the development and applications of different types of CMCPE [117-120]. Modification of carbon paste electrodes with suitable materials facilitates the electrochemical reactions of the redox compounds to proceed without hindrance [121, 122]. This phenomenon generally results in increased selectivity and sensitivity of the determinations [123-125]. It has been known for a number of years that certain transition metal complexes with porphyrins [126-127] can catalyze the electrooxidation/electroreduction of some chemically and biologically important compounds.

As explained in chapter 1 metalloporphyrins as electrode modifying agents are very attractive because they are rather stable compounds and their properties can be finely tuned by simple modifications of their basic molecular structure.

As part of the present investigation a novel voltammetric sensor based TMHPP Zn(II) has been developed for the selective determination of MTZB. The prepared MTZB sensor was characterized in terms of selectivity, sensitivity and reproducibility. A relatively low applied potential and improved selectivity for MTZB determination has been realized. The developed sensor has been successfully applied for the determination of MTZB in commercially available tablets and urine sample.

3.2 Synthesis of TMHPP and TMHPP Zn(II)

The detailed procedure for the synthesis has been discussed in section 2.3.1 and 2.3.2 of chapter 2. The structure of TMHPP is shown in Figure **3.2**

3.3 Preparation of TMHPP Zn(II) modified CPE

Firstly, the carbon paste electrode was cleaned as explained in section 2.4 of chapter 2. Bare CPE was prepared by thoroughly mixing analytical grade graphite and paraffin liquid (plasticizer) in a 70:30 (w/w %) ratio. The TMHPP Zn(II) modified CPE was prepared by mixing different percentages of graphite powder, paraffin liquid and TMHPP Zn(II). Detailed procedure for the preparation of TMHPP Zn(II)/CPE is given in section 2.6.1 of chapter 2.

3.4 Optimization studies

The optimization of carbon paste composition is very important because the sensitivity and selectivity of the developed sensor is highly influenced by the amount of graphite powder, amount of modifier and nature of plasticizer. Five different plasticizers viz., Bis(2-ethyl hexyl) phthalate, Di-n-butyl phthalate, Di-butyl sebacate, Bis(2-ethyl hexyl) adipate and Paraffin liquid were employed to study their effect on the electrochemical behaviour of MTZB. Of the five different plasticizers used, paraffin liquid was found to give best response. Hence, the sensor with paraffin liquid was selected for further studies. Different ratios of carbon paste composition were employed to evaluate their effects on the response characteristics of the developed sensor. Electrochemical behaviour of MTZB at these different electrodes was studied using cyclic voltammetric technique. Best results were obtained at 70:22:8 (w/w %) ratio of graphite

powder, paraffin liquid and TMHPP Zn(II). This optimized electrode composition was then used for the voltammetric determination of MTZB.

3.5 Electrochemical behaviour of MTZB

Electrochemical measurements were carried out with BAS Epsilon Electrochemical analyzer (Bioanalytical system, USA) interfaced to a PC. A conventional three electrode system, including TMHPP Zn(II) modified carbon paste working electrode, a platinum wire counter electrode and Ag/AgCl reference electrode were employed.

Stock solution of MTZB was prepared as described in section 2.7.1 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 (PBS, pH 7). Sample solution was taken in the electrochemical cell and deaerated with N_2 for 10 minutes. Cyclic voltammogram was then recorded from 0.2 to –1.0 V at a scan rate of 100 mV/s. During the first potential sweep from 0.2 to –1.0 V, MTZB gave a reduction peak at -0.713 V at TMHPP Zn(II)/CPE, but during the reverse sweep no oxidation peak was obtained. This clearly indicates that the electrochemical response of MTZB is irreversible. For electrode regeneration, several cyclic scans were carried out in the blank electrolyte solution until a stable voltammogram was obtained.

Firstly, an attempt was made to study the electrochemical behaviour of nimesulide. At bare CPE, cathodic peak of nimesulide was obtained at a potential of -0.630 V with a peak current of 0.0084 mA. Unfortunately at TMHPP Zn(II)/CPE peak was obtained at -0.645 V with a peak current of 0.0011 mA. Thus compared to bare CPE, the peak current of nimesulide showed a marked decrease and the peak potential showed a positive

deviation. The various experimental parameters such as pH, supporting electrolyte and carbon paste composition were varied and their effects on the results were studied. However this hardly had any impact on the improvement of results.

The electrochemical response characteristics of hesperidin methyl chalcone were next studied at bare CPE and TMHPP Zn(II)/CPE. At bare CPE, hesperidin methyl chalcone gave a reduction peak at -0.928 V. However at TMHPP Zn(II)/CPE, no voltammetric response was obtained.

Also the voltammetric behaviour of the drug tinidazole was investigated. The electroreduction of tinidazole occurs at a potential of -0.840 V at bare CPE, but at TMHPP Zn(II)/CPE reduction peak was obtained at -0.856 V.

Finally the electrochemical behaviour of MTZB at bare CPE and TMHPP Zn(II)/CPE was studied by CV. The cyclic voltammograms of 1×10^{-3} M MTZB at TMHPP Zn(II)/CPE and bare CPE are shown in Figure **3.3.** At bare CPE, MTZB yields a reduction peak at -0.830 V. Under the same conditions, a well defined reduction peak appears at -0.713 V for MTZB at TMHPP Zn (II) modified CPE resulting shift in reduction potential by 117 mV. Compared with bare CPE, there was also an enhancement of reduction peak current for MTZB from 0.0100 mA to 0.0305 mA at TMHPP Zn(II) modified CPE. The decrease of potential and remarkable peak current enhancement undoubtedly proved the electrocatalytic activity of TMHPP Zn(II) modified CPE towards the reduction of MTZB. No oxidation peak was observed for MTZB in the reverse sweep of CV indicating an irreversible electrochemical process.

Here the cathodic peak arises due to the electrochemical reduction of MTZB. The reduction of MTZB is a complex process in which nitro group can receive up to six electrons until complete reduction to amino group. Under anaerobic conditions or low oxygen pressure, the reduction of MTZB occurs in a similar way to the reduction of nitrobenzene [128]. In the present case one reduction peak was obtained and this is attributed to the four electron reduction of nitro group in MTZB to the corresponding hydroxylamine (Figure **3.4**) according to the currently accepted mechanism for the electroreduction of aromatic and heteroaromatic compounds containing nitrogroup [129, 130].

3.6 Factors affecting the developed sensor

The developed sensor depends on various factors such as influence of supporting electrolyte, influence of pH, influence of scan rate and influence of concentration.

3.6.1 Influence of pH

The electrochemical studies of 1×10^{-3} M MTZB in PBS were carried out in the pH range of 4 to 10 using CV. Figure **3.5** illustrates the effect of pH on the cathodic peak current of 1×10^{-3} M of MTZB at the TMHPP Zn(II)/CPE. The best reduction response was obtained in pH 7 as the peak current is the highest. Thus pH 7 was fixed as optimal pH.

3.6.2 Influence of supporting electrolyte

The voltammetric behaviour of MTZB was studied in 0.1 M concentrations of different supporting electrolytes such as phosphate buffer, acetate buffer, H_2SO_4, NaOH and KNO_3. When 0.1 M acetate buffer solution was used as the supporting electrolyte, MTZB gave a reduction peak at -0.74 V with a peak current of 0.0115 mA. With KNO_3, the peak

was obtained at -0.750 V with a current of 0.0100 mA. When H_2SO_4 was used as the supporting electrolyte, no peak was obtained for MTZB. In NaOH, the reduction peak was observed at -0.734 V with a peak current of 0.0222 mA. However in PBS solution (pH 7) MTZB gave a peak at -0.713 V with a peak current of 0.0305 mA. Thus the reduction peak obtained was best defined in 0.1 M phosphate buffer (pH 7), justifying the choice.

3.6.3 Influence of scan rate

The reduction peak current of 1×10^{-3} M MTZB at different scan rates ranging from 50-250 mV/s was measured by LSV, using the same modified electrode. It was found that cathodic peak current increases with an increase in the scan rate. The results are illustrated in Figure **3.6**. The reduction peak current varies linearly with square root of scan rate indicating that the reduction of MTZB at the TMHPP Zn(II) modified electrode is diffusion controlled (Figure **3.7**).

According to the Laviron's equation [131] the relationship between the peak potential (E_p) and scan rate (υ) was examined at the developed sensor. It was found that E_p varies linearly with ln υ (Figure **3.8**). 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, 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 is 3.61 (around 4). This confirms that 4 electrons are involved in the reduction of MTZB.

3.6.4 Influence of concentration

The relationship between the cathodic peak current of MTZB and its concentration was investigated by DPV (Figure **3.9**). A linear concentration range was found to occur from 1.0×10^{-3} M to 1.0×10^{-5} M. The detection limit was obtained from the graph and was found to be 4.0×10^{-6} M (Figure **3.10**). Comparison of the developed sensor with the previously reported methods demonstrates that the presently developed sensor method is a better method for the determination of MTZB (Table **3.1**).

The stability of the modified CPE was investigated by measuring the voltammetric current once in every day. The results indicated that the developed sensor retained its original current response in the first continuous eight days. The reproducibility of the TMHPP Zn(II) modified CPE was examined by repetitive measurement of the reduction peak current of 1×10^{-3} M MTZB using the same electrode. After several successive measurements, stable electrochemical responses were obtained suggesting that the TMHPP Zn (II)/CPE have excellent reproducibility.

3.6.5 Interference study

To evaluate the interference of various species in the determination of MTZB, a systematic study was carried out with various interferents in deaerated medium to avoid interference from dissolved oxygen. Cyclic voltammograms were recorded and the results are given in Table **3.2**. Glycine, sodium chloride, ascorbic acid, dextrose and norfloxacin has no influence on the determination of MTZB, even at a 100 fold molar excess concentration (signal change below 0.5%). The results reveal that the developed sensor is selective to MTZB in presence of the foreign compounds tested.

3.7 Analytical applications

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

3.7.1 Analysis of pharmaceutical formulations for determination of MTZB

The developed sensor was employed for the assay of MTZB content in pharmaceutical formulations commercialized as 'Metrogyl' (Unique, India) and 'Flagyl' (Rhone-Plc, India) containing 400 mg and 200 mg of the drug respectively, as declared by the company.

The MTZB content in the tablet was determined using the developed sensor by calibration method. A detailed procedure is given in section 2.10.1 of Chapter 2. The results given in Table **3.3** clearly reveal that the results obtained from the measurements are found to be in satisfactory agreement with the declared amount. The close agreement between the observed value and declared value is indicative of non-interference of the other ingredients that are present in the formulation.

The results obtained using TMHPP Zn(II)/CPE was compared with those obtained by the standard pharmacopoeial method [107]. The results are illustrated in Table **3.3.** The results show that there is a satisfactory agreement between the MTZB content determined by the developed method and the standard method. The developed sensor could be used for the determination of MTZB in tablets with high accuracy and precision.

3.7.2 Analysis of urine sample for determination of MTZB

5 ml of urine samples were taken in different 25 ml standard flasks. Different quantities of MTZB were added to these urine samples and quantitatively diluted using the supporting electrolyte.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 MTZB and the unknown concentrations were determined from the calibration graph. The results are summarised in Table **3.4.**

3.8 Conclusion

Voltammetric behaviour of MTZB was investigated at TMHPP Zn (II)/CPE by CV, DPV and LSV. The reduction of MTZB was found to be an irreversible process. The TMHPP Zn(II)/CPE showed electrocatalytic action for the reduction of MTZB, characterised by the enhancement of the peak current and the reduction of peak potential. The developed method is a better method for the analytical determination of MTZB, because it is simple, fast and it has sufficient precision, accuracy and sensitivity. The developed sensor has been successfully applied for the voltammetric determination of MTZB in pharmaceutical formulations and in urine sample.

Table 3.1 Comparison of major characteristics of some analytical methods used in the determination of MTZB

Analytical method	Linear range (M)	Detection limit (M)	Reference
Spectrophotometry	5.0×10^{-3} - 2.5×10^{-2}	1.0×10^{-3}	[113]
Kinetic spectrophotometric method	5.5×10^{-4} - 3.3×10^{-2}	2.4×10^{-4}	[112]
Gas chromatography	5.0×10^{-2} - 6.0×10^{-1}	2.5×10^{-3}	[114]
High performance liquid chromatography	2.0×10^{-4} - 8.0×10^{-1}	3.0×10^{-5}	[115]
Voltammetry	2.0×10^{-6} - 6.0×10^{-4}	1.1×10^{-6}	[116]
Voltammetry (Present method)	1.0×10^{-3} - 1.0×10^{-5}	4.0×10^{-6}	

Table 3.2 Effect of foreign substances on the reduction peak current of 1×10^{-3} M MTZB

MTZB taken – 1.00×10^{-3} M

Foreign species	Tolerance limit (M)	Signal change (%)
Glycine	1.00×10^{-1}	0.25
Sodium Chloride	1.00×10^{-1}	0.26
Norfloxacin	1.00×10^{-1}	0.12
Ascorbic acid	1.00×10^{-1}	0.15
Dextrose	1.00×10^{-1}	0.13

Table 3.3 Determination of MTZB in pharmaceutical formulations

Sample	Declared Amount (mg/tablet)	Method Adopted	Found* (mg/tablet)	S.D*	C.V*
Metrogyl (Unique, India)	400	TMHPP Zn(II)/CPE	402	1.6	0.41
		Standard Method	402	7.07	0.02
Flagyl (Rhone-Plc, India)	200	TMHPP Zn(II)/CPE	199	1.40	0.71
		Standard Method	199	5.65	0.02

*average of six replicates

Table 3.4 Determination of MTZB in urine sample

Added (M)	Found (M)	Recovery (%)
8.00×10^{-4}	7.88×10^{-4}	98.5
1.00×10^{-3}	9.87×10^{-4}	98.7
3.00×10^{-3}	2.93×10^{-3}	97.6

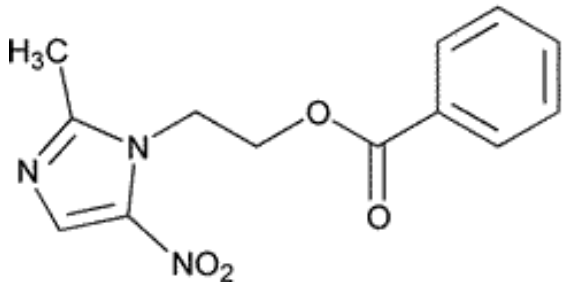

Figure 3.1 Structure of MTZB

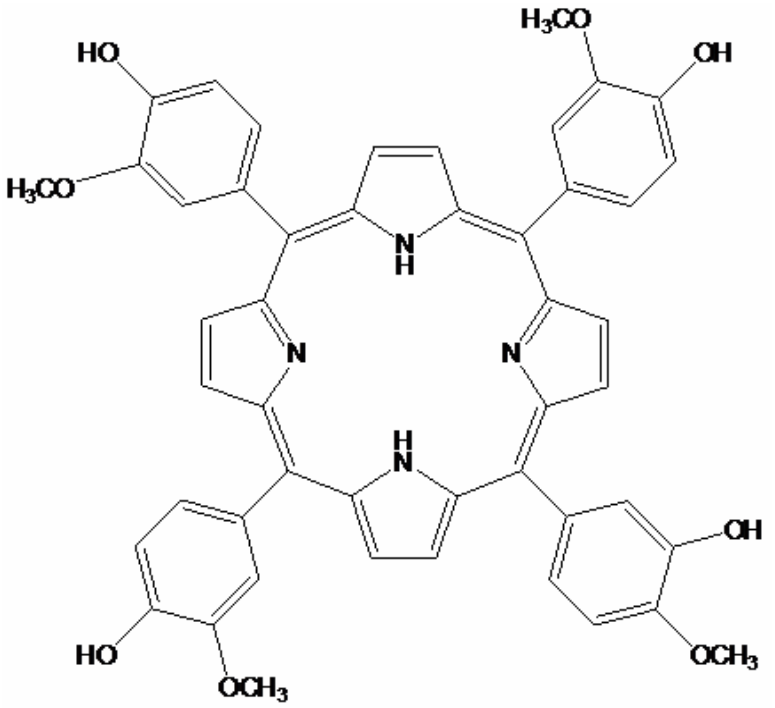

Figure 3.2 Structure of 5,10,15,20- tetrakis (3-methoxy-4-hydroxy phenyl) porphyrin

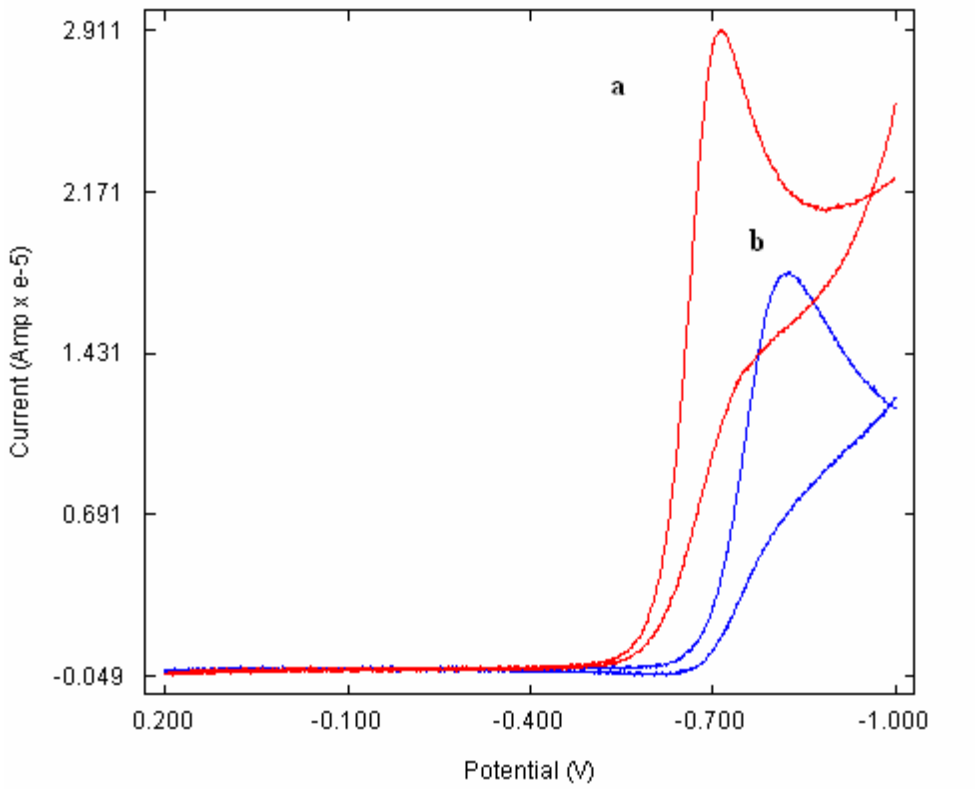

Figure 3.3 Cyclic voltammogram of MTZB at (a) TMHPP Zn(II) modified CPE (b) Bare CPE

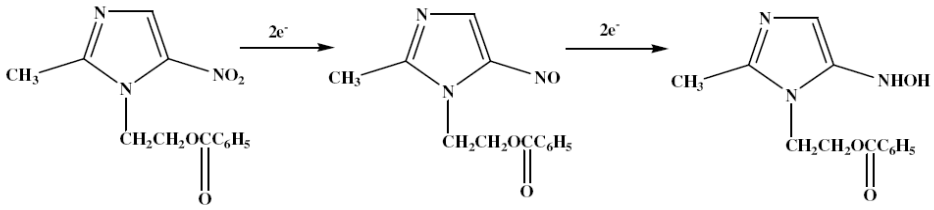

Figure 3.4 Mechanism of reduction of nitrogroup to hydroxylamine derivative

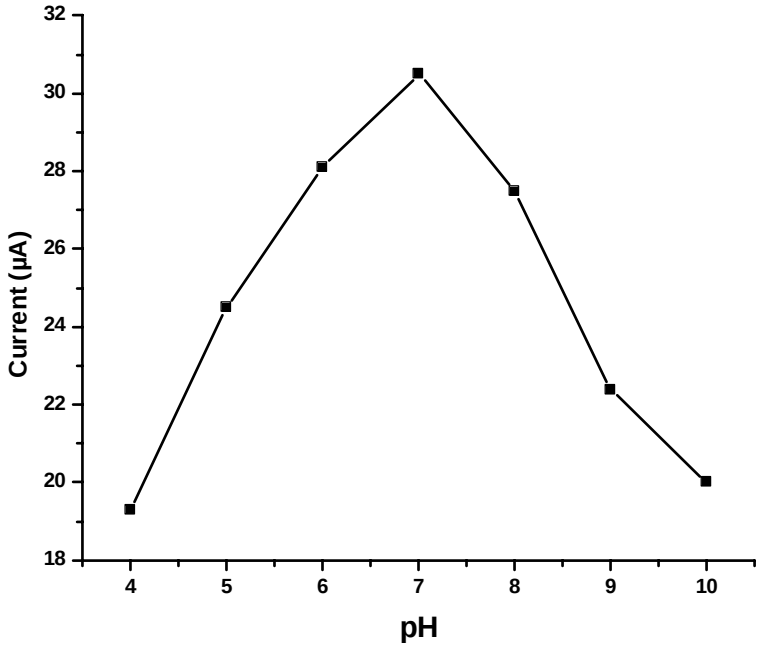

Figure 3.5 Effect of pH

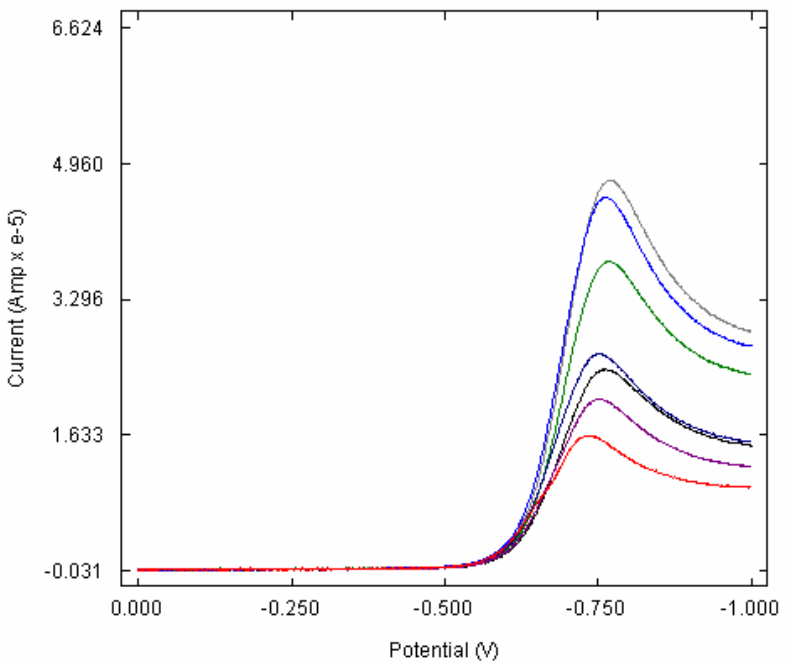

Figure 3.6 Overlay of Linear Sweep Voltammograms of MTZB at different scan rates in phosphate buffer solution (pH 7).

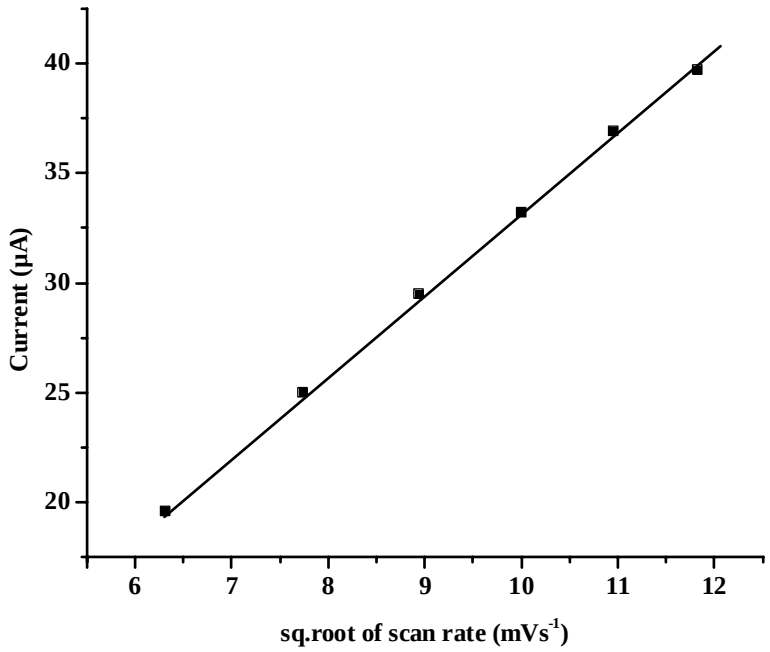

Figure 3.7 Effect of scan rate

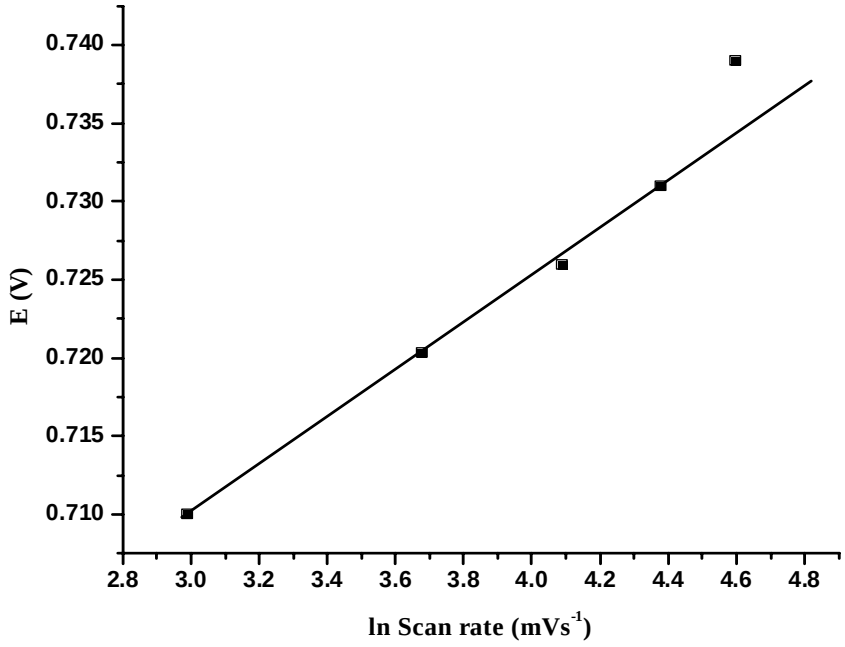

Figure 3.8 Plot of peak potential against ln scan rate

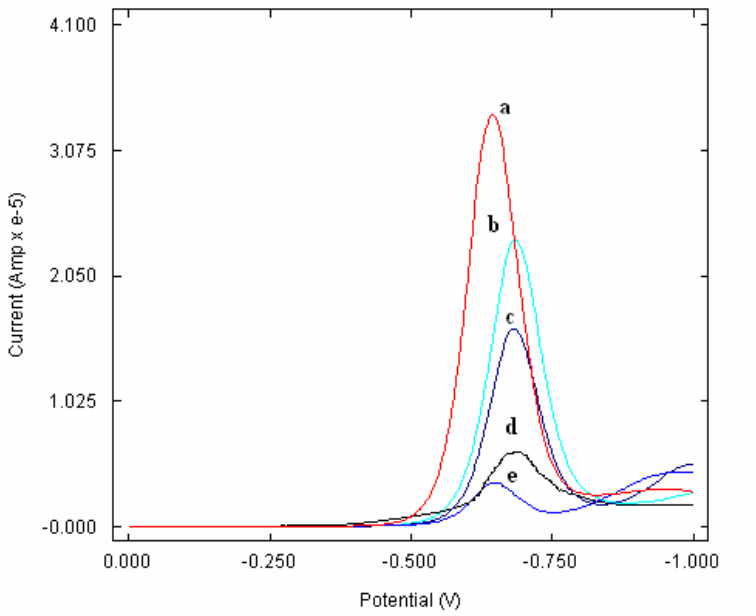

Figure 3.9 Differential pulse voltammogram of MTZB of different concentrations a) 5×10^{-2} M b) 3×10^{-4} M c) 9×10^{-5} M d) 1×10^{-5} M e) 8×10^{-6} M

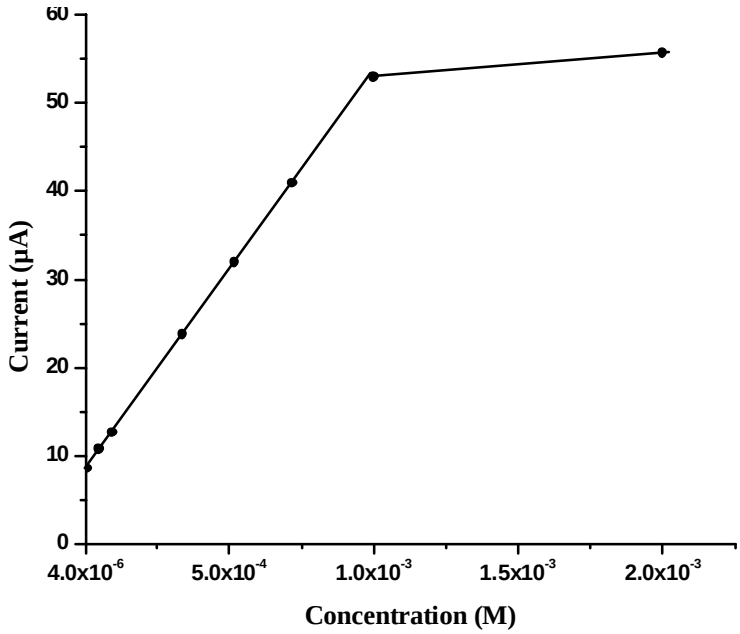

Figure 3.10 Calibration graph for MTZB at TMHPP Zn(II)/CPE.

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Chapter 4

Development of Sensor for Sulfamethoxazole

Contents

This chapter illustrates the fabrication of a metalloporphyrin based sensor for sulfamethoxazole (SM). The metalloporphyrin used was [5,10,15,20-tetrakis (3-methoxy-4-hydroxyphenyl)porphyrinato]copper (II) [TMHPP Cu(II)]. The electrochemical behavior of SM has been studied at a TMHPP Cu(II) modified CPE (TMHPP Cu(II)/CPE). The electrochemical response characteristics of the developed sensor was studied in detail. The utility of the developed sensor was investigated in the determination of the drug in pharmaceutical formulations and also for the determination of the drug from urine samples.

4.1 Introduction

Sulfamethoxazole, 4-amino-N-(5-methylisoxazol-3-yl)-benzenesulfonamide, (Figure **4.1**) is a sulfonamide used in the treatment of urinary tract infections, pneumocystis pneumonia, chronic bronchitis, meningococcal meningitis, acute otitis media and toxoplasmosis. SM inhibits bacterial synthesis of dihydrofolic acid by competing with para aminobenzoic acid (PABA). SM binds to dihydropteroate synthetase which catalyses this reaction. SM is normally given in combination with trimethoprim. Studies have shown that bacterial resistance develops more slowly with the combination of the two drugs than with either trimethoprim or SM alone. Both SM and trimethoprim exist in the blood as unbound, protein bound and metabolized forms. They are now available as widely used pharmaceutical products and veterinary practices [132].

At present, the standard methods for the determination of SM are the spectrophotometric method [133], flow injection spectrophotometric method [134], bratton marshall method [134-136], titrimetric assay method [137,138], gas chromatography and gas chromatography mass spectrometry [139], capillary electrophoresis [136], high performance liquid chromatography [135], high performance thin layer chromatography [140], liquid chromatography-mass spectrometry [141] and voltammetry [142, 73]. Among these, electrochemical detection is one of the promising approaches.

Chemical modification of electrodes improves the electrocatalytic current necessary for sensitive determination of target analytes. Electrocatalysis amplifies the detection signal of an analyte resulting in faster electrode reactions at a lower potential in comparison to the bare electrode. As

mentioned earlier, metalloporphyrins have a wide range of properties and have been used in sensor applications.

In the present work a voltammetric sensor based TMHPP Cu(II) has been developed for the selective determination of SM. The electrochemical behavior of SM suggests that TMHPP Cu(II)/CPE exhibits obvious electrocatalytic activity to the oxidation of SM, since it lowers its oxidation potential compared to bare CPE. After optimizing the experimental parameters, a differential pulse voltammetric method was developed for the direct measurement of SM. The developed sensor has been successfully applied for the determination of SM in commercially available tablets and urine sample. This newly developed method posses several advantages such as high sensitivity, rapid response, low cost and simplicity.

4.2 Synthesis of TMHPP and TMHPP Cu(II)

The complete method for the synthesis has been discussed in section 2.3.1 and 2.3.3 of chapter 2.

4.3 Construction of TMHPP Cu(II) modified CPE

At first, cleaning of CPE was carried out as explained under section 2.4 of chapter 2. Bare CPE was prepared as described in section 3.3 of chapter 3. The TMHPP Cu(II) modified CPE was constructed by mixing different percentages of graphite powder, paraffin liquid and TMHPP Cu(II). Detailed method for the preparation of TMHPP Cu(II)/CPE is given in section 2.6.1 of chapter 2.

4.4 Optimization of the carbon paste composition

The amount of graphite powder, amount of modifier and nature of plasticizer influences the response characteristics of the developed sensor.

So these factors are carefully varied to arrive at an optimum carbon paste composition. Since the type of plasticizer used for the CPE construction plays a very important role in the response of the electrode, five different types of plasticizers were chosen for the study. These include Bis(2-ethyl hexyl) phthalate, Di-n-butyl phthalate, Di-butyl sebacate, Bis(2-ethyl hexyl)adipate and Paraffin liquid. In the present investigation paraffin liquid was found to be the optimum available plasticizer for the developed sensor. Several different carbon paste compositions that contained different percentages of modifier were investigated. The results revealed that best composition was 70:21:9 (w/w %) ratio of graphite powder, paraffin liquid and TMHPP Cu(II). This optimized electrode composition was chosen for further studies.

4.5 Electrochemical response of SM

All electrochemical experiments were performed on an Electrochemical analyzer (BAS Epsilon Bioanalytical system, U.S.A), interfaced to a PC. A three electrode system which consists of a TMHPP Cu(II) modified CPE as working electrode, an Ag/AgCl reference electrode and a Platinum wire auxiliary electrode were employed.

Stock solution of SM was prepared as explained in section 2.7.2 of Chapter 2. Standard solutions of the analyte (1×10^{-3} M - 1×10^{-8} M) were prepared by serial dilution of the stock solution using supporting electrolyte. Sample solution was taken in the electrochemical cell. The solution was then deaerated with N_2 for 10 minutes. Differential pulse voltammograms from 0.900 to -1.500 V at 20 mV/s were recorded and finally the oxidation peak current at -0.140 V was measured for SM. For electrode regeneration several cyclic scans were carried out in the blank electrolyte solution until a stable voltammogram was obtained.

The electrochemical response of sparfloxacin, ambroxol, trimethoprim, metronidazole benzoate, acyclovir, ceftriaxone sodium and SM were studied at bare CPE and TMHPP Cu(II) modified CPE by DPV. At bare CPE, sparfloxacin gave an oxidation peak at 0.890 V with a peak current of 0.0044 mA in 0.1 M PBS (pH 6) solution. At TMHPP Cu(II) modified CPE, an oxidation peak at 1.060 V, with a peak current of 0.0011 mA was obtained. Since the results were not encouraging, experimental parameters such as carbon paste composition, supporting electrolyte and pH were changed and their effects on the results were studied. However compared to bare electrode the peak current of sparfloxacin showed a marked decrease and the peak potential showed a positive deviation.

Further the electrochemical behaviour of ambroxol was studied by DPV. At bare CPE, an oxidation peak was obtained at 0.928 V, with a peak current of 0.0192 mA in 0.1 M PBS (pH 6) solution. However at TMHPP Cu(II) modified CPE, no voltammetric response was obtained. Also the effect of carbon paste composition, supporting electrolyte and pH were studied. But no voltammetric response was obtained.

The electrochemical oxidation of trimethoprim, metronidazole benzoate and acyclovir at bare CPE and modified CPE was investigated by DPV in 0.1 M PBS (pH 6) solution. It was observed that there is an increase of peak potential by about 0.150 V for the electrooxidation of trimethoprim and considerable decrease of peak current at TMHPP Cu(II) modified CPE than at bare CPE under any of the conditions studied. For metronidazole benzoate and acyclovir, no voltammetric response was obtained at bare CPE and TMHPP Cu(II) modified CPE.

The electrochemical response characteristic of ceftriaxone sodium was then studied by DPV. The response of the drug at the modified CPE was compared with that at bare CPE. At bare CPE an oxidation peak was obtained at 1.003 V with a peak current of 0.0030 mA. At TMHPP Cu(II) modified CPE an oxidation peak was obtained at 1.060 V with a peak current of 0.0020 mA. Thus with the modified electrode there was an increase of peak potential and decrease of peak current.

Finally the electrochemical behavior of SM at a TMHPP Cu(II)/CPE has been investigated using DPV. Figure **4.2** displays the comparison of oxidation peak of 1×10^{-3} M SM in PBS (pH 6) at bare CPE and TMHPP Cu(II) modified CPE. At bare CPE, SM yields an oxidation peak at 0.816 V. Under the same conditions, a well defined oxidation peak appears at -0.140 V for the TMHPP Cu(II) modified CPE. The tremendous decrease in oxidation potential was remarkable when the CPE electrode is modified with TMHPP Cu(II). This behavior, which was observed at different concentrations of SM and at several potential scan rates, clearly demonstrates that the mediator functions electrocatalytically towards SM. No reduction peak was observed for SM in the reverse sweep of CV indicating an irreversible electrochemical process.

Para amino substituted sulfonamides can be electrochemically oxidized at the $-NH_2$ group, but the reduction of the $-SO_2$ group is very difficult to achieve. The group attached to –NH functional group has little or no influence on the oxidation potential [143]. Previous investigations [144-146] have addressed the electrochemical behaviour of sulfonamides and proposed an irreversible two-electron pH dependent reaction for their oxidation in aqueous solutions. In the present case one oxidation peak is

obtained and this is attributed to the two electron oxidation of amino group in SM to the corresponding iminobenzoquinone according to the currently accepted mechanism [146] and is shown in Figure **4.3**.

4.6 Optimization of experimental parameters

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

4.6.1 Influence of supporting electrolyte

The supporting electrolyte plays an important role in the electrochemical response of SM. Its choice can modify the thermodynamics and kinetics of electrochemical processes as well as mass transfer within the cell.Therefore 0.1 M concentrations of PBS, sulfuric acid, hydrochloric acid, potassium chloride, acetate buffer, tetra-n-butyl ammonium chloride and sodium hydroxide were tested as supporting electrolytes for SM oxidation by DPV. It was observed that the peak current is highest and the peak shape is well defined in PBS. Hence PBS was chosen as the experimental medium for the voltammetric studies of SM.

4.6.2 Influence of pH

The influence of pH on the anodic peak current of 1×10^{-3} M SM at the TMHPP Cu(II) modified CPE was investigated by DPV. The pH range was studied from 3-10. Figure **4.4** clearly depict the effect of pH on the anodic peak current of SM at the TMHPP Cu(II) modified CPE. The best oxidation response was obtained in pH 6 as the peak current is the highest. Thus pH 6 was fixed as optimal pH.

4.6.3 Influence of scan rate

The influence of scan rate on the oxidation peak current was studied by DPV. It was found that oxidation peak current of 1×10^{-3} M SM shows a linear relationship with scan rate in the range 10-150 mV/s. The results are illustrated in Figure **4.5**. The oxidation peak current varies linearly with square root of scan rate (Figure **4.6**) indicating that the oxidation of SM at the TMHPP Cu(II) modified electrode is diffusion controlled.

The experimental investigations showed a good linear dependence of the oxidation peak potential upon the logarithm of the scan rate (ln υ) (Figure **4.7**). The no. of electrons (n_a) involved in the reaction was calculated from the slope of the plot according to the relation, $b= RT/ \alpha n_a F$. Thus, the calculated n_a value is 2.05. It indicates that two electrons are involved in the oxidation process of SM.

4.6.4 Influence of concentration

Under the optimized experimental conditions, the relationship between the anodic peak current of SM and its concentration was investigated by DPV. The results are illustrated in Figure **4.8**. The oxidative peak current has a linear relationship with the concentration in the range 1.0×10^{-8} M – 1.0×10^{-6} M (Figure **4.9**). The detection limit of SM was 1.0×10^{-9} M. Table **4.1** represents the comparative study of characteristics of the developed method to some of the reported methods. The developed method showed better linear concentration range and detection limit in comparison to the other methods reported in the literature.

The storage stability of TMHPP Cu(II) modified CPE was evaluated by measuring the current response of SM once in every day. The results

show that the developed sensor retained its original current response in the first continuous six days. The reproducibility of the electrode was examined by repetitive measurement of 1.0×10^{-3} M SM using the same TMHPP Cu(II) modified CPE. After several successive measurements, comparable results were obtained suggesting that the TMHPP Cu(II) modified CPE has good reproducibility.

4.6.5 Interference study

The possible interfering species examined are listed in Table **4.2**. Glycine, sodium chloride, potassium chloride, dextrose, lactose, urea and trimethoprim have no influence on the determination of SM, even at a 100 fold molar excess concentration. Since trimethoprim is often used as a part of synergistic combination with SM in tablets, the influence of trimethoprim on the oxidation peak current of SM was studied. It was found that same concentration of trimethoprim did not interfere in the determination of SM. This result showed that the TMHPP Cu(II) modified CPE has good selectivity for the determination of SM.

4.7 Analytical applications

The sensor developed for SM was employed for the determination of SM in tablets and in urine samples.

4.7.1 Analysis of pharmaceutical formulations for determination of SM

The developed sensor was applied for the determination of SM content in tablet (Septran, Burroughs Wellcome, India). The SM content in the tablet was determined by calibration method. The detailed procedure for the determination is given in section 2.10.2 of chapter 2.The results obtained are summarized in Table **4.3**. The results shown in Table **4.3** are in good

agreement with the declared SM content and showed a high degree of precision.

The results were compared with those obtained by the standard method [107].The results show that the developed method is comparable to the standard method.

4.7.2 Determination of SM in urine sample

The developed sensor was applied for the determination of the drug in urine samples. Urine samples of 5 ml were taken in different 25 ml standard flasks. An adequate amount of SM corresponding to 1×10^{-3} M was added to the urine sample. This solution was quantitatively diluted using PBS to obtain various concentrations. The prepared solution was analysed for SM using TMHPP Cu(II) modified CPE by DPV method and the unknown concentrations were determined from the calibration graph. The results are shown in Table **4.4**.

4.8 Conclusion

A voltammetric sensor based on TMHPP Cu(II) has been developed for the determination of SM. The oxidation of SM was found to be an irreversible process. The TMHPP Cu(II) modified CPE showed electrocatalytic action for the oxidation of SM, characterized by the reduction of peak potential. The TMHPP Cu(II) modified CPE was successfully applied as a selective and very sensitive voltammetric sensor for the determination of nanomolar amounts of SM in pharmaceutical formulations and in urine sample. The performance characteristics of the modified electrode as well as the simplicity of its preparation and the renewability of its surface by simply polishing demonstrate its analytical utility as a sensor for the determination of SM.

Table 4.1 Comparison of major characteristics of some analytical methods used in the determination of SM

Analytical method	Linear range (M)	Detection limit (M)	Reference
Spectrophotometry	7.0×10^{-3} - 1.0×10^{-2}	3.0×10^{-5}	[133]
Capillary electrophoresis	1.3×10^{-7} - 1.0×10^{-2}	1.0×10^{-7}	[136]
Voltammetry	6.1×10^{-6} - 6.0×10^{-5}	1.1×10^{-6}	[142]
Voltammetry	5.0×10^{-5} - 1.0×10^{-2}	1.0×10^{-5}	[73]
Voltammetry (Present method)	1.0×10^{-8} - 1.0×10^{-6}	1.0×10^{-9}	

Table 4.2 Interference study

SM taken – 1.00×10^{-3} M

Foreign species	Tolerance limit (M)	Signal change (%)
Glycine	1.00×10^{-1}	2.05
Sodium chloride	1.00×10^{-1}	2.00
Potassium chloride	1.00×10^{-1}	2.09
Trimethoprim	1.00×10^{-1}	1.98
Dextrose	1.00×10^{-1}	2.10
Lactose	1.00×10^{-1}	2.13
Urea	1.00×10^{-1}	1.99
Ascorbic acid	1.00×10^{-1}	2.56

Table 4.3 Determination of SM in tablet

Sample	Declared Amount (mg/tablet)	Method Adopted	Found* (mg/tablet)	S.D*	C.V*
Septran (Burroughs Wellcome, India)	400	Developed sensor	396	1.41	0.003
		Standard Method	399	2.39	0.599

*Average of six replicates

Table 4.4 Determination of SM in urine sample

Added (M)	Found (M)	Recovery (%)
1.00×10^{-6}	1.01×10^{-6}	101.0
4.00×10^{-6}	3.90×10^{-6}	97.5
8.00×10^{-6}	8.02×10^{-6}	100.2

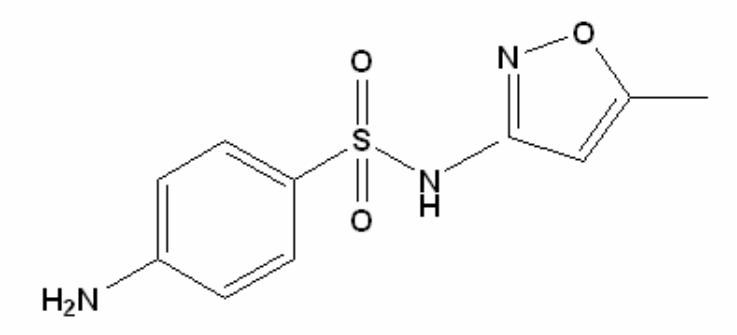

Figure 4.1 Structure of SM

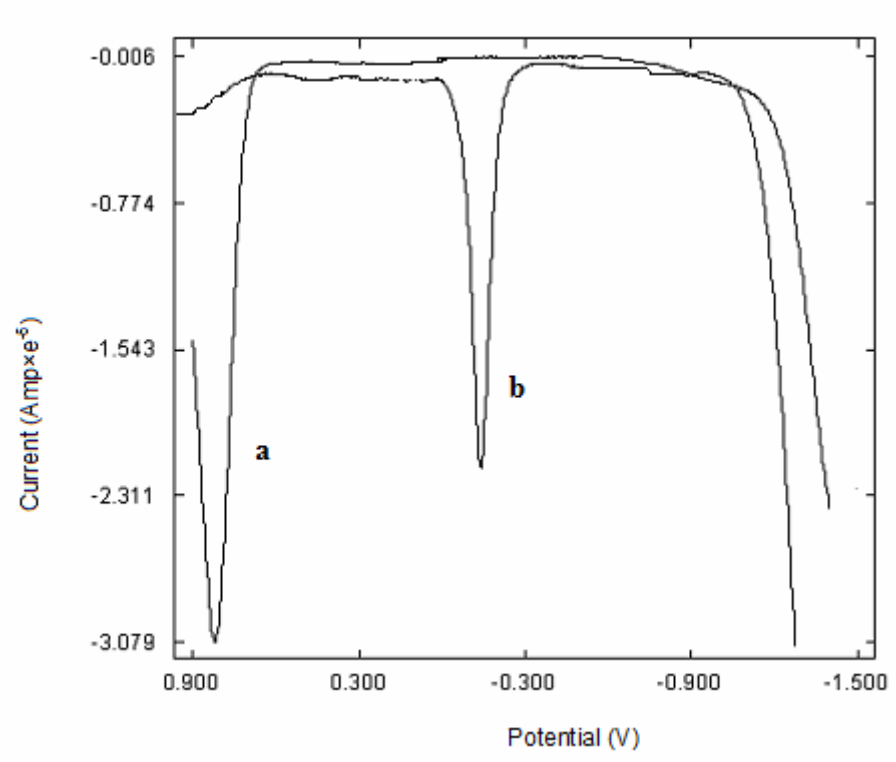

Figure 4.2. Differential pulse voltammogram of SM at (a) Bare CPE (b) TMHPP Cu(II) modified CPE .

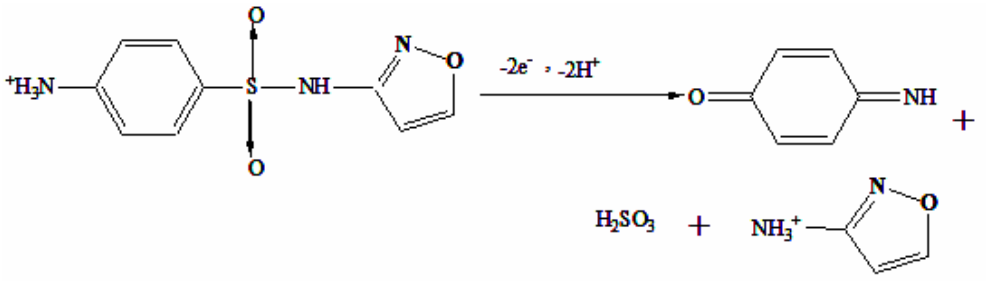

Figure 4.3 Mechanism of oxidation of amino group in SM to iminobenzoquinone

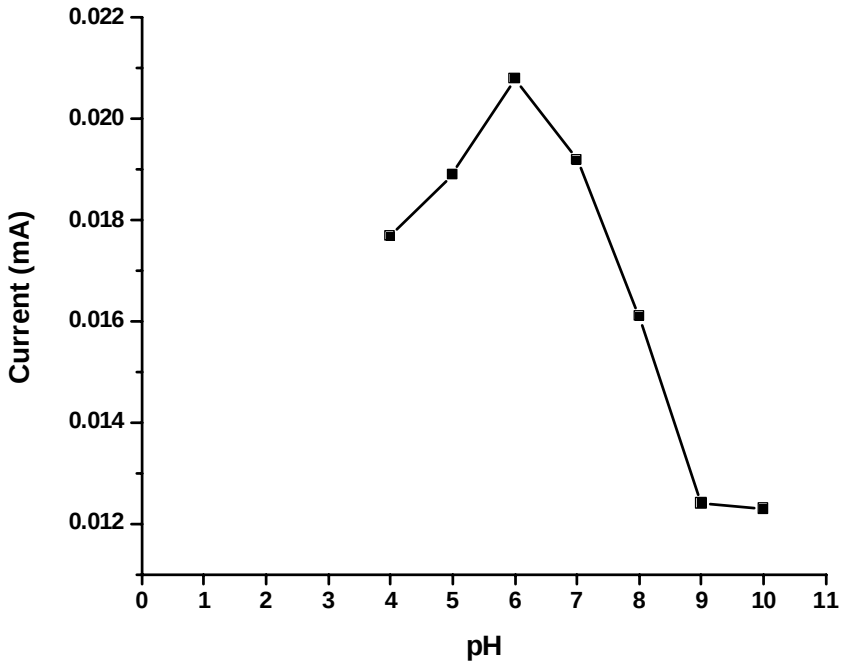

Figure 4.4 Influence of pH

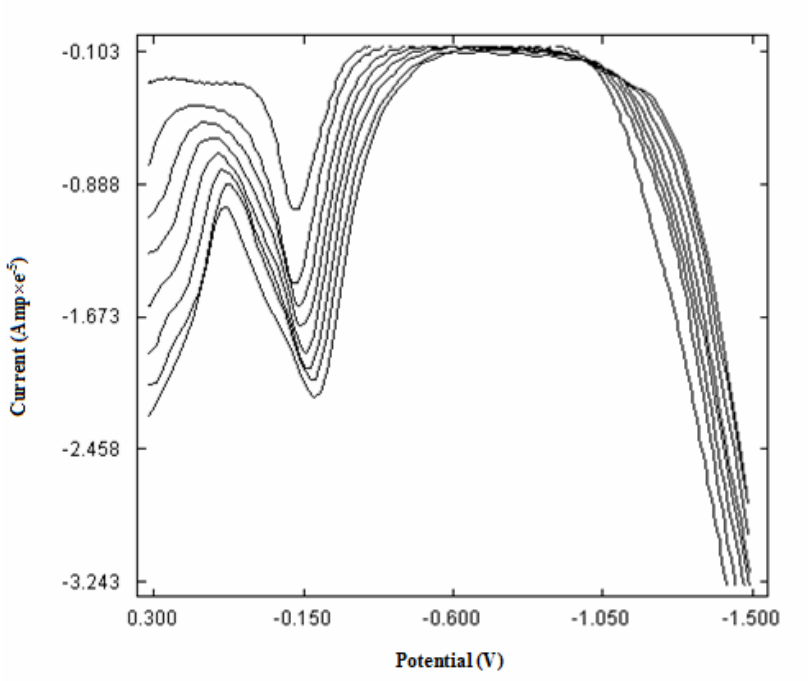

Figure 4.5 Overlay of differential pulse voltammograms of SM at different scan rates in phosphate buffer solution (pH 6).

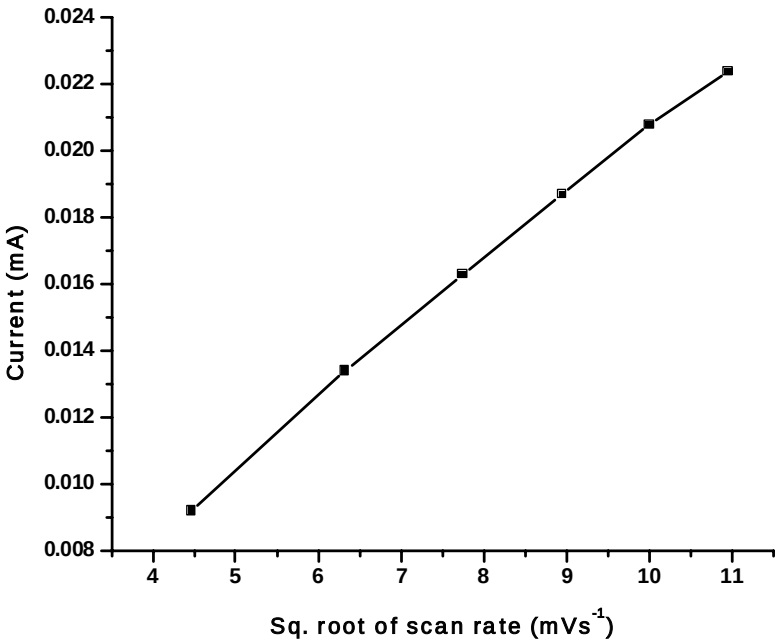

Figure 4.6 Influence of scan rate

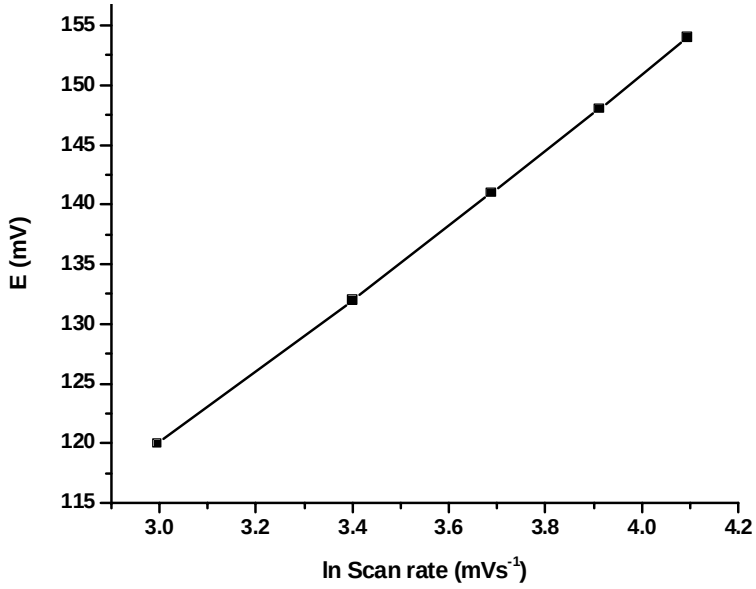

Figure 4.7 Plot of peak potential against ln scan rate

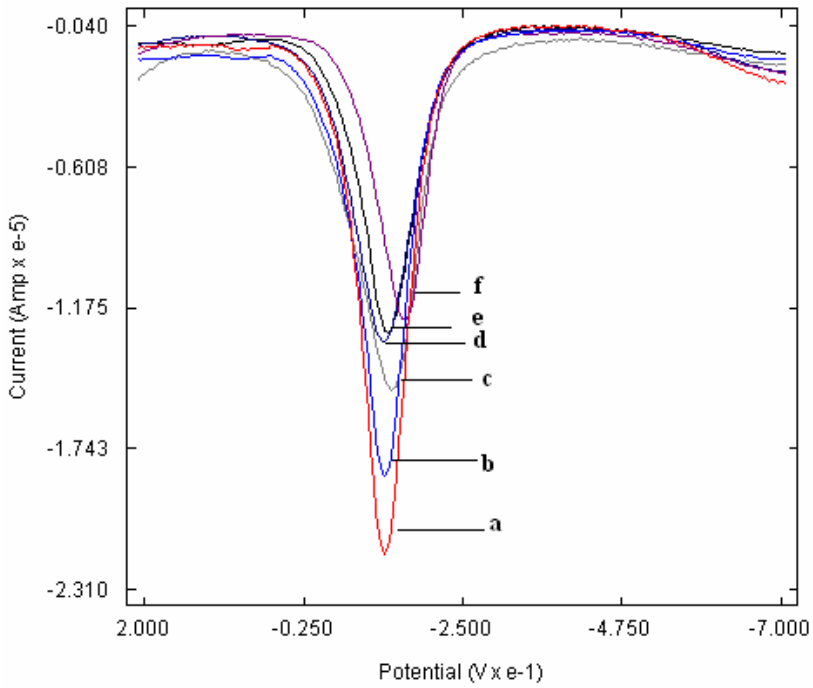

Figure 4.8 Differential pulse voltammogram of SM of different concentrations a) 5×10^{-2} M b) 3×10^{-4} M c) 9×10^{-5} M d) 1×10^{-5} M e) 8×10^{-6} M f) 1×10^{-6} M

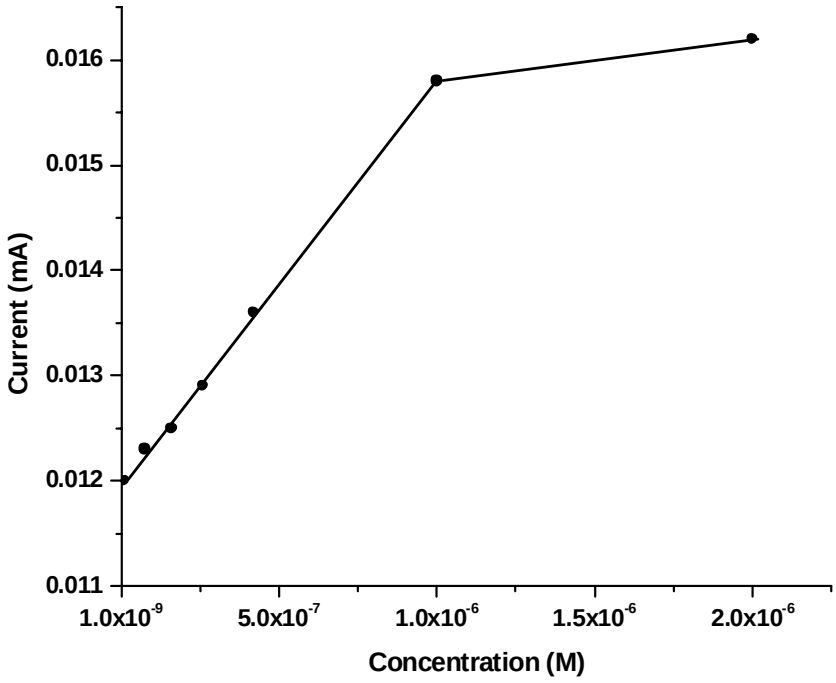

Figure 4.9 Influence of concentration

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Chapter 5

Development of Sensor for Acyclovir

Contents

This chapter deals with the fabrication of electrochemical sensor for the selective determination of Acyclovir (ACV) using gold electrode (GE) modified with self assembled monolayer of 2-mercaptobenzothiazol (MBZ) and [5,10,15,20-tetrakis(3-methoxy-4-hydroxyphenyl)porphyrinato]copper(II) (TMHPP Cu(II)) by SWV. The self assembled films are stable and showed blocking characteristics towards the faradaic processes such as gold surface oxidation and under potential deposition of copper. The optimized conditions obtained for the MBZ/TMHPP Cu(II) modified GE were 0.1 M phosphate buffer solution (pH 7.0), square wave frequency of 15 Hz and square wave amplitude of 25 mV. Under these optimum conditions, the resultant peak current increases linearly with the concentration of ACV in the range of 6.0×10^{-8} to 1.0×10^{-6} M. The MBZ/TMHPP Cu(II) modified GE showed good stability, selectivity and it can be used to quantify ACV in pharmaceutical formulations and urine sample.

5.1 Introduction

Acyclovir, 2-amino-9-((2-hydroxyethoxy)methyl)-1H-purin-6(9H)-one (ACV) (Figure **5.1**) is the most commonly used guanine analogue antiviral drug. It is primarily used for the treatment of herpes simplex as well as herpes zoster infections. It is also used to treat outbreaks of genital herpes. In people with frequent outbreaks, ACV is used to reduce the number of future episodes. ACV is an antiviral drug. However, it is not a cure for these infections. The viruses that cause these infections continue to live in the body even between outbreaks. ACV decreases the severity and length of these outbreaks. It helps the sores heal faster, keeps new sores from forming and decreases pain and itching. In addition, in people with weak immune system, ACV can decrease the risk of the virus spreading to other parts of the body and causing serious infections. It is also efficacious in preventing HSV infections in renal allograft recipients [147]. Based on the above description the quantitative determination of ACV has become very important and hence been widely studied.

In recent years, various methods have been reported for the determination of ACV. It includes high performance liquid chromatography [148], radioimmunoassay [149,150], fluorimetry [151], spectrophotometry [152], high-performance capillary electrophoresis [153] chemiluminescence [154] and voltammetry [155-157]. But these methods suffer low sensitivity and narrow linear concentration range. We adopt electrochemical methods based on chemically modified electrode (CME), as it is very simple, convenient and inexpensive. The modification of electrode surface with suitable materials results in efficient determination of electroactive biomolecules at very lower potential without its major interferences [158, 159].

Self-assembled monolayer (SAM) provides one simple route to functionalize electrode surfaces by organic molecules (both aliphatic and aromatic) containing free anchor groups such as thiols, disulphides, amines, silanes or acids. The monolayer produced by self-assembly allows tremendous flexibility with respect to several applications depending upon their terminal functionality (hydrophilic or hydrophobic control) or by varying the chain length (distance control) [160]. Metalloporphyrins have attracted a great deal of interest owing to the diversity of their structures and interaction between the central metal and the analyte. Metalloporphyrin macrocycle is almost planar molecule and it can easily form stable complexes with uncharged molecules possessing donor atoms such as oxygen or nitrogen. Synthesis of thiol derivatised metalloporphyrin is tedious and requires toxic chemicals. But there are reports on immobilising metalloporphyrin to surface confined monolayer of thiol formed on GE [161]. The advantages of SAM modified GE using different metallophthalocyanine complexes have been reported by Nyokong and co workers for the determination of several analytes [162, 163]. As part of our investigations, we report strategies to link metalloporphyrin, TMHPP Cu(II) to the MBZ modified GE through adsorption. To the best of our knowledge, the studies on the electrochemical behaviour of ACV and its quantitative determination at MBZ/TMHPP Cu(II) modified GE have not been reported yet.

5.2 Synthesis of TMHPP and TMHPP Cu(II)

The complete method for the synthesis has been discussed in section 2.3.1 and 2.3.3 of chapter 2.

5.3 Preparation of MBZ/TMHPP Cu(II) modified GE

Firstly the GE was cleaned as explained under section 2.5 of Chapter 2. SAM was formed by immersing the cleaned GE in an ethanolic solution of different thiol compounds such as MBZ, 1-dodecane thiol, 3-mercaptopropionic acid and 2-mercaptoethanol. The effect of concentration as well as time for monolayer formation using the above monolayer was also studied. But a better voltammetric response was obtained by dipping GE in MBZ ($1x10^{-3}$M) solution for 24 hours at room temperature and by further modification with TMHPP Cu(II). So we selected MBZ as the base monolayer for the attachment of TMHPP Cu(II). The detailed procedure for the fabrication of MBZ/TMHPP Cu(II) modified GE is given in section 2.6.2 of Chapter 2.

5.4 Electrochemical determination of ACV

The stock solution of ACV was prepared as given in section 2.7.3 of chapter 2.10 mL of phosphate buffer (pH 7) was taken in the electrochemical cell and required volume of stock drug solution was added to it. The solution was then de-aerated with nitrogen for 5 minutes. SWV was performed at MBZ/TMHPP Cu(II) modified GE and the voltammograms were recorded from 0 to 0.8 V. For electrode regeneration five cyclic scans were carried out from 0 to 0.8 V at a scan rate of 100 mVs^{-1} in the blank electrolyte solution until a stable voltammogram was obtained.

Square wave voltammetry was used to explore the electrochemical behaviour of sparfloxacin, ambroxol, trimethoprim, ceftriaxone sodium, PAM chloride, tamsulosin hydrochloride, sulfamethoxazole and ACV at bare GE and MBZ/TMHPP Cu(II) modified GE in 0.1 M PBS (pH 7) solution. The experimental result show that at bare GE, the anodic peak

corresponding to the oxidation of drug sparfloxacin occurs at 1.067 V with a peak current of 5.1923 µA. At MBZ/TMHPP Cu(II) modified GE, the peak was obtained at 1.097 V with a peak current of 4.2443 µA.

Thus compared to bare GE, the drug show considerable increase in oxidation peak potential and decrease in peak current. Also the effect of supporting electrolyte and pH was studied. But the results show no improvement.

The electrochemical behaviour of ambroxol was studied at bare GE and MBZ/TMHPP Cu(II) modified GE. At bare GE, ambroxol gave an oxidation peak at 0.998 V with a peak current of 4.2387 µA. However at MBZ/TMHPP Cu(II) modified GE, voltammetric response was obtained at 1.050 V with a peak current of 4.1087 µA.

Further the electrochemical response of trimethoprim was studied by SWV. No voltammetric response was obtained for trimethoprim at bare GE and MBZ/TMHPP Cu(II) modified GE.

The electrochemical response characteristics of ceftriaxone sodium, PAM chloride, and tamsulosin hydrochloride at bare GE and MBZ/TMHPP Cu(II) modified GE was investigated by SWV. It was observed that there is an increase of oxidation peak potential for ceftriaxone sodium, PAM chloride and tamsulosin hydrochloride and considerable decrease of peak current at MBZ/TMHPP Cu(II) modified GE than at bare GE under any of the conditions studied.

Also the voltammetric behaviour of the drug sulfamethoxazole was investigated. The oxidation of sulfamethoxazole occurs at a potential of 0.940 V at bare GE, but at modified GE peak was obtained at 0.922 V.

The electrochemical behaviour of ACV at bare GE, TMHPP Cu(II) and MBZ/TMHPP Cu(II) modified GE has been investigated using CV and SWV. No response was found to obtain for bare GE and TMHPP Cu(II) modified GE. But at MBZ/TMHPP Cu(II) modified GE, an oxidation peak was obtained for ACV. Figure **5.2** displays the comparison of square wave voltammograms of 1×10^{-3} M ACV in PBS (pH 7) at bare GE, MBZ modified GE and MBZ/TMHPP Cu(II) modified GE. At bare GE, ACV exhibits no voltammetric response. Also ACV does not generate a voltammetric peak on MBZ modified GE. Under the same conditions, a well defined and sensitive oxidation peak appears at 0.22 V for the MBZ/TMHPP Cu(II) modified GE. The electrochemical behaviour reveals that the MBZ/TMHPP Cu(II) exhibits obvious electrocatalytic activity to the oxidation of ACV. Compared to cyclic voltammetric technique, square wave voltammetric technique was found to be more sensitive for ACV determination. A very low detection limit of the order of 1×10^{-9} M was obtained by using square wave voltammetric technique. So square wave voltammetric technique was used for further studies.

The anodic current generated for the ACV electrooxidation process may be due to the oxidation of one electro reactive functional group in multiple steps or simultaneous oxidation of electro reactive functional groups present in the acyclovir structure. Herein, one oxidation peak is obtained and this is attributed to the two electron oxidation of ACV to the corresponding oxoguanine analog according to the mechanism [164] shown in Figure **5.3.**

5.5 Formation and performance of MBZ/TMHPP Cu(II) modified GE

Figure **5.4** shows the proposed scheme for the immobilization of TMHPP Cu(II) on the MBZ modified GE surface. Initially, the surface of

the gold electrode was chemically modified with MBZ to form ordered molecular assemblies. By the strong S-Au covalent bond the MBZ molecule was linked to the gold electrode surface. Subsequently, TMHPP Cu(II) was immobilized on the MBZ monolayer through chemisorption. The Cu(II) complex coordinates with the nitrogen present in MBZ. MBZ/TMHPP Cu(II) catalyzes the oxidation of ACV to the corresponding oxoguanine analog.

5.6 Surface study

Cyclic voltammograms of different electrodes in $K_3Fe(CN)_6$ solution are shown in Figure **5. 5**. $K_3Fe(CN)_6$ exhibits a pair of reversible peaks at bare GE (ΔEp is 0.075 V) . When it is modified with MBZ the peaks become almost invisible (ΔEp is 0.200 V). However, when it is further modified by TMHPP Cu(II), a pair of reversible peaks occurs (ΔEp is 0.064 V). Furthermore, the peaks current are much higher than those of bare GE. This may be attributed to the electrostatic interaction between negatively charged $[Fe(CN)_6]^{3-}$ and Cu^{+2} redox centre of the metalloporphyrin, causing a fast electron transfer which leads to a sharper and better defined peak. The small difference in ΔEp value of bare GE and MBZ/TMHPP Cu(II) modified GE indicates the reversibility of the process.

2 mM $K_3Fe(CN)_6$ was taken to measure the microscopic areas of the MBZ/TMHPP Cu(II) modified GE and bare GE. Cyclic Voltammograms were recorded at different scan rates (Figure **5.6**). For a reversible system the relationship between the current and scan rate is given by the Randles-Sevick equation [165].

$$Ip = 2.69 \times 10^5 A n^{3/2} Dr^{½} C v^{½}$$

Where Ip refers to the peak current, n is the number of electron transferred, A is the surface area of the electrode, Dr is the diffusion coefficient, C is the concentration of $K_3Fe(CN)_6$ and v refers to the scan rate. For $K_3Fe(CN)_6$, n =1 and Dr = 7.6×10^{-6} cm s^{-1}. The surface area can be calculated from the slope of the Ip versus $v^{1/2}$. Surface area for bare electrode was found to be 1.005 cm^2. On modification with MBZ/TMHPP Cu(II) the effective surface area was increased to 3.015 cm^2, i.e., there is a threefold increase in surface area. This is a strong evidence for the successful and effective modification of GE using MBZ/TMHPP Cu(II).

Further evidence for the modification of GE was obtained from the surface morphology studies using Scanning Electron Microscope (SEM). SEM images of both bare GE, MBZ modified GE and MBZ/TMHPP Cu(II) modified GE are shown in Figure **5.7**. The SEM image of MBZ modified GE showed that SAM was formed on the surface of GE and that of MBZ/TMHPP Cu(II) modified GE showed that the SAM modified electrode surface contains layers of TMHPP Cu(II).

5.7 Factors influencing the developed sensor

The influence of various experimental parameters such as effect of supporting electrolyte, pH, scan rate and concentration on the functional potential of the developed sensor was studied.

5.7.1 Supporting electrolyte study

The electrochemical behaviour of $1x10^{-3}$M ACV was studied by SWV in 0.1 M concentration of different electrolytes such as PBS, sulfuric acid, hydrochloric acid, potassium chloride, acetate buffer, tetra-n-butyl ammonium chloride and sodium hydroxide. As ACV gave the best oxidation peak in PBS solution, it was used as the supporting electrolyte throughout the experiment.

5.7.2 pH study

The effect of supporting electrolyte pH on the response performance of the developed sensor was investigated by recording the oxidation peak current of ACV in a concentration of 1×10^{-3} M over a pH range of 3-10. Figure **5.8** clearly depict the effect of pH on the current response of the developed sensor. The anodic current increased up to a value of pH 7, and decreased gradually after that. Thus, pH 7 was selected for the subsequent experiments.

5.7.3 Scan rate study

The effect of scan rate on the electrochemistry of ACV at the MBZ / TMHPP Cu(II) film modified GE was investigated by SWV. It was found that the oxidation peak current of 1×10^{-3}M ACV increases with increase in scan rate in the range of 10-150 mVs^{-1}. The results are given in Figure **5.9.** The oxidation peak current varies linearly with square root of scan rate (Figure **5.10**) indicating that the oxidation of ACV at the MBZ/TMHPP Cu(II) modified GE is diffusion controlled.

The experimental investigations showed a good linear dependence of the oxidation peak potential upon the logarithm of the scan rate (ln V) (Figure **5.11**). The no. of electrons (n_a) involved in the reaction was calculated from the slope of the plot according to the relation, $b=RT/\alpha n_aF$. The obtained value of n_a was around 4, which confirmed that 4 electrons are involved in the oxidation of ACV.

5.7.4 Concentration study

The effect of concentration of the drug solution on the anodic peak current of ACV was critically investigated by SWV. The results are shown in Figure **5.12**. The oxidation peak current was found to increase with increase

in the concentration (Figure **5.13**). Linear response was found to obtain from 6.0×10^{-8} M – 1.0×10^{-6} M with a detection limit of 1.0×10^{-9} M.

The Table **5.1** gives a comparative study of response characteristics of the newly developed method with some of the already reported methods. It is obvious that the developed method is superior in terms of linear concentration range and detection limit.

5.7.5 Interference study

In order to investigate the selectivity of the method, the interference effects of some biologically important compounds on the electrochemical oxidation of 1×10^{-3} M ACV have been evaluated. The results are given in Table **5.2**. It was found that 1×10^{-1} M glycine, sodium chloride, potassium chloride, dextrose, lactose, urea and uric acid have almost no influence on the current response of 1×10^{-3} M ACV (Signal change below 5%). However, ascorbic acid and guanine interfere severely with the oxidation signal of ACV.

5.8 Stability of MBZ/TMHPP Cu(II) modified GE

The stability of MBZ/TMHPP Cu(II) modified GE was investigated by measuring the voltammetric response of 1.0×10^{-3} M ACV in 0.1 M PBS (pH 7.0) every day over 4 weeks. The results showed that there was apparent loss of activity after 18 days. The stability can be attributed to the high level orientation of MBZ/TMHPP Cu(II) on GE.

5.9 Analytical applications

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

5.9.1 Determination of ACV in Pharmaceutical formulations (tablets)

The high sensitivity and wide concentration range of the developed method made their utilisation possible for the determination of ACV in pharmaceutical formulations (Acivir, Cipla, India and Zovirax, Burroughs wellcome, India). The detailed method for the determination is given in section 2.10.3 of chapter 2. The results obtained are presented in Table **5.3**. The results consolidated in Table **5.3** are in good agreement with the declared ACV content and showed a high degree of precision. The standard method [107] was then used for the comparison and validation of results. The results reveal that there is a close agreement between the ACV content determined by the developed method and the standard method.

5.9.2 Determination of ACV in urine sample

The feasibility of the developed sensor for ACV in urine sample was analyzed. Various concentration of the drug solution in PBS containing fixed volume (5 ml) of urine sample was prepared. The electrochemical behaviour of the prepared solution was studied by SWV. The results are given in Table **5.4** and the unknown concentrations were determined from the calibration graph. The recovery percentage is in the range 100-102.

5.10 Conclusion

TMHPP Cu(II) was immobilized on MBZ modified GE to construct a novel sensor for the determination of ACV. In the presence of TMHPP Cu(II), the electrode area enlarged and the blocking action of MBZ disappeared. ACV generates one anodic peak at 0.22 V in 0.1M phosphate buffer solution (pH 7). The developed method can reduce the applied potential for the oxidation of ACV. The simplicity, sensitivity and low analytical cost demonstrate its analytical utility as a sensor for the determination of ACV.

Table 5.1 Comparison of major characteristics of some analytical methods used in the determination of ACV.

Analytical method	Linear range (M)	Detection limit (M)	Reference
High performance liquid chromatography	5.0×10^{-4} - 1.0×10^{-1}	5.0×10^{-5}	[148]
Spectrophotometry	5.0×10^{-3} - 5.5×10^{-2}	1.6×10^{-3}	[152]
Voltammetry	2.7×10^{-7} - 5.2×10^{-8}	2.6×10^{-6}	[155]
Voltammetry	8.0×10^{-8} - 1.0×10^{-5}	3.0×10^{-8}	[156]
Voltammetry	2.0×10^{-6} - 1.0×10^{-4}	3.5×10^{-7}	[157]
Voltammetry (Present method)	6.0×10^{-8} - 1.0×10^{-6}	1.0×10^{-9}	

Table 5.2 Study of the effect of foreign species on the anodic peak current of ACV

ACV taken - 1.00×10^{-3} M

Foreign species	Tolerance limit (M)	Signal change (%)
Glycine	1.00×10^{-1}	2.05
Sodium chloride	1.00×10^{-1}	2.00
Potassium chloride	1.00×10^{-1}	2.09
Dextrose	1.00×10^{-1}	2.10
Lactose	1.00×10^{-1}	2.13
Urea	1.00×10^{-1}	1.99
Uric acid	1.00×10^{-1}	2.00
Guanine	1.00×10^{-1}	30.0
Ascorbic acid	1.00×10^{-1}	22.4

Table 5.3 Determination of ACV in tablets

Sample	Declared Amount (mg/tablet)	Method Adopted	Found* (mg/tablet)	S.D*	C.V*
Acivir (Cipla, India)	400	MBZ/TMHPP Cu(II)/GE	399	4.65	1.17
		Standard Method	402	7.07	0.02
Zovirax (Burroughs wellcome, India)	200	MBZ/TMHPP Cu(II)/GE	198	4.94	2.49
		Standard Method	199	5.65	0.02

*Average of six replicates

Table 5.4 Determination of ACV in urine sample using MBZ/TMHPP Cu(II) modified GE

Added (M)	Found (M)	Recovery (%)
3.00×10^{-6}	3.07×10^{-6}	102
8.00×10^{-6}	8.02×10^{-6}	100
2.00×10^{-7}	2.02×10^{-7}	101

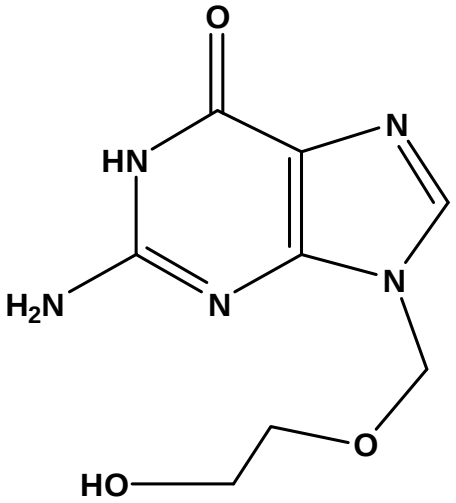


Figure 5.1 Structure of ACV

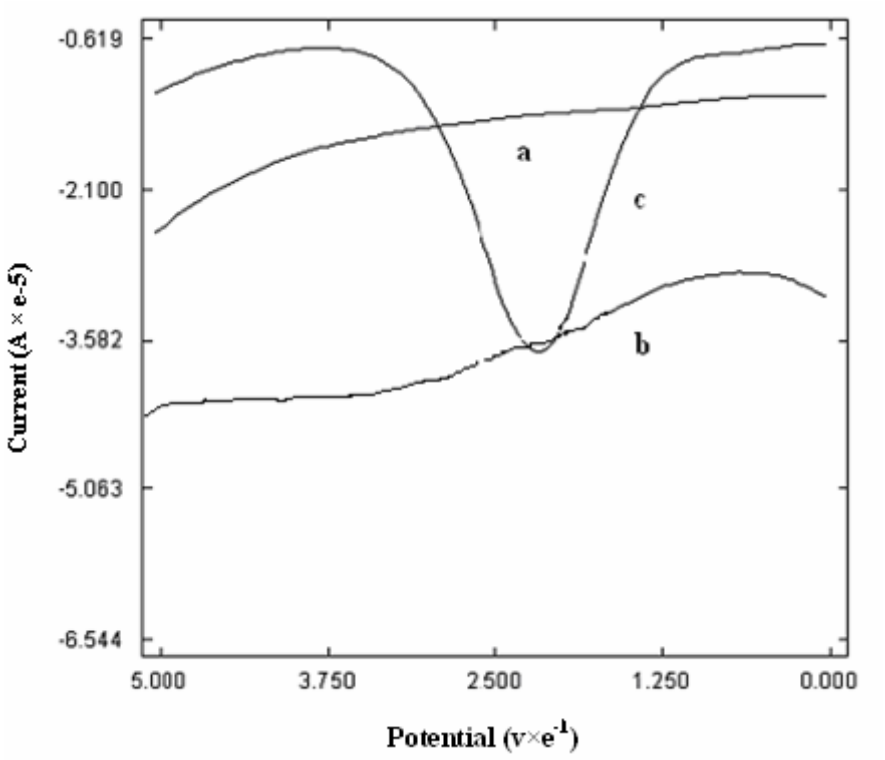


Figure 5.2 Square wave voltammogram of ACV at (a) Bare GE (b) MBZ modified GE and (c) MBZ/TMHPP Cu(II) modified GE

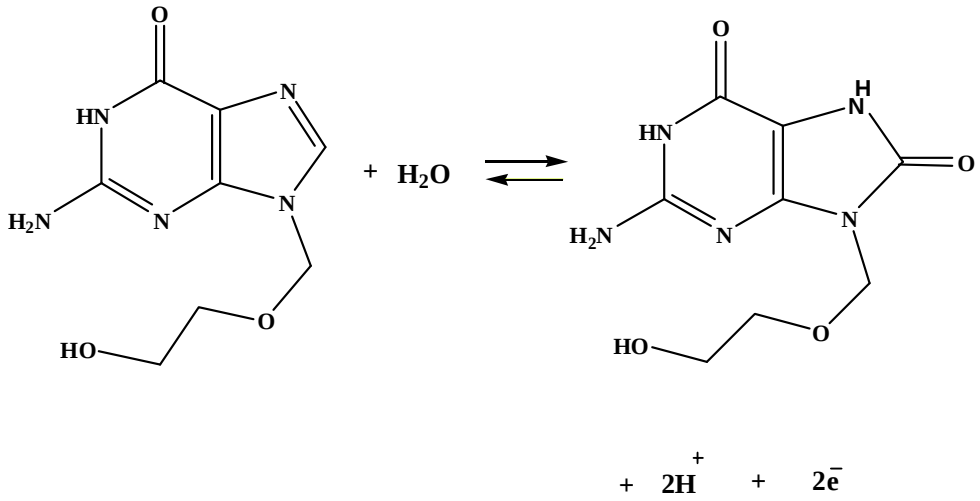


Figure 5.3 Mechanism showing the oxidation of ACV to the corresponding oxo-guanine analog.

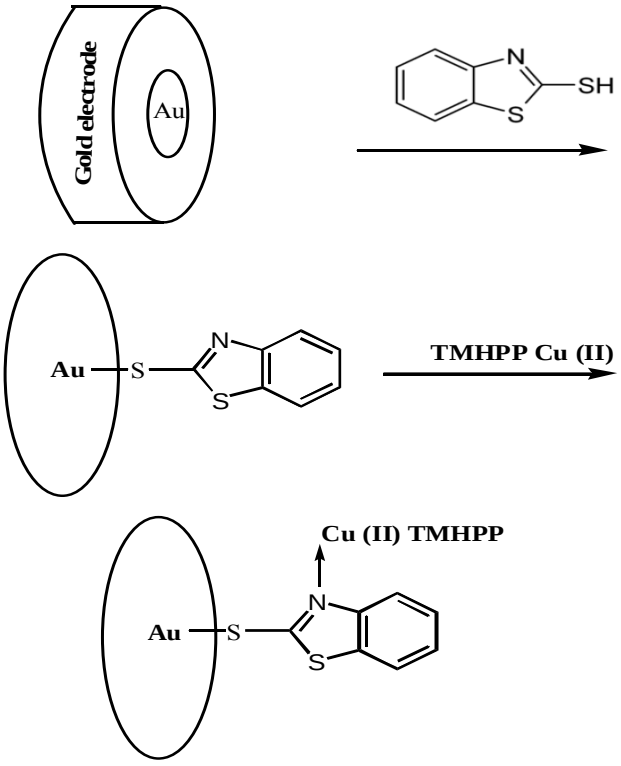


Figure 5.4 Schematic representation for the immobilization of TMHPP Cu (II) on the MBZ modified GE surface.

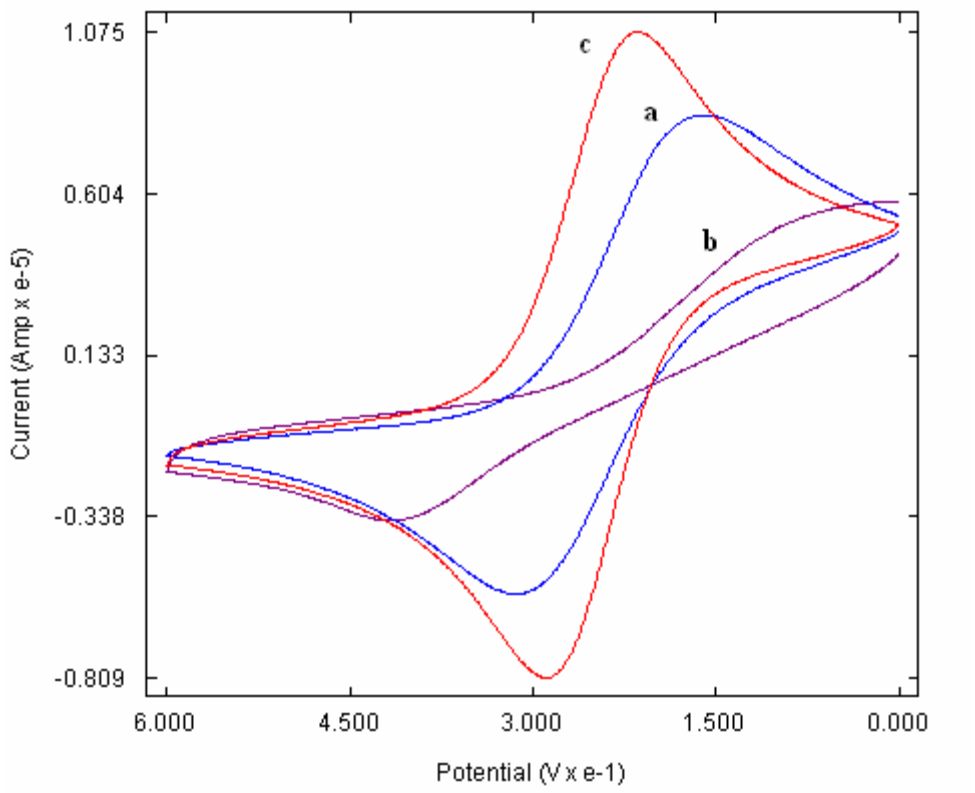
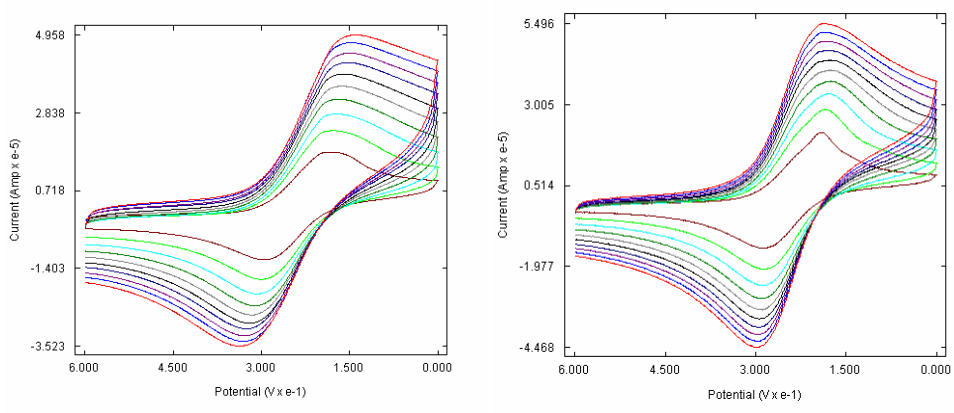
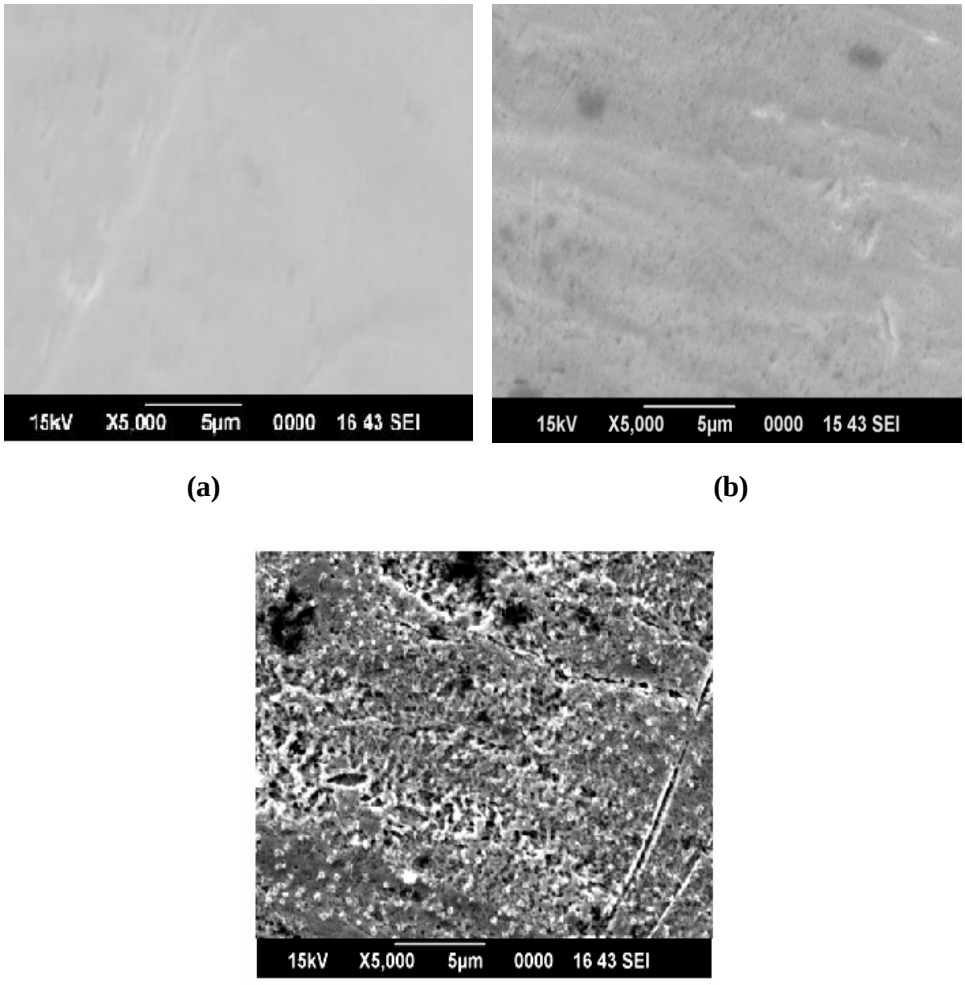


Figure 5.5 Cyclic voltammograms of (a) bare GE (b) MBZ modified GE (c) MBZ/TMHPP Cu(II) modified GE in $K_3Fe(CN)_6$ solution.



(a) **(b)**

Figure 5.6 Surface area study at (a) bare GE and (b) MBZ/TMHPP Cu(II) modified GE in $K_3Fe(CN)_6$ solution.



(a) **(b)**

(c)

Figure 5.7 SEM images of (a) bare GE (b) MBZ modified GE (c) MBZ/TMHPP Cu(II) modified GE.

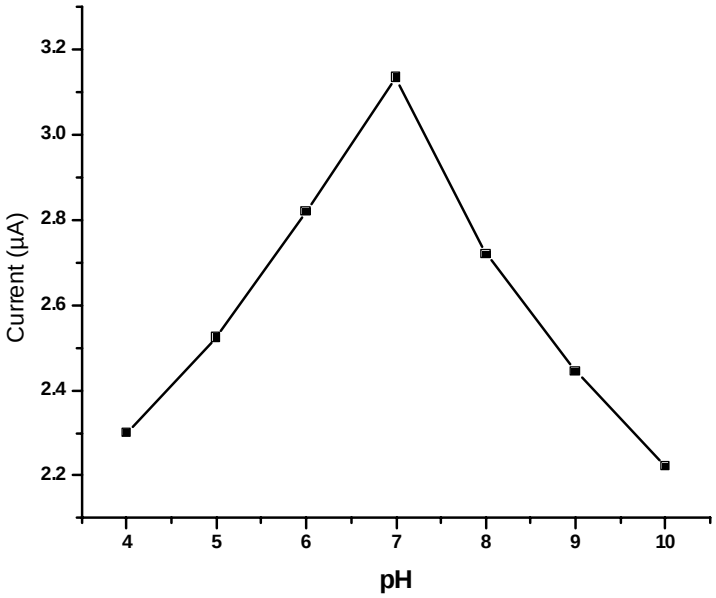


Figure 5.8 Effect of pH on the oxidation peak current of ACV.

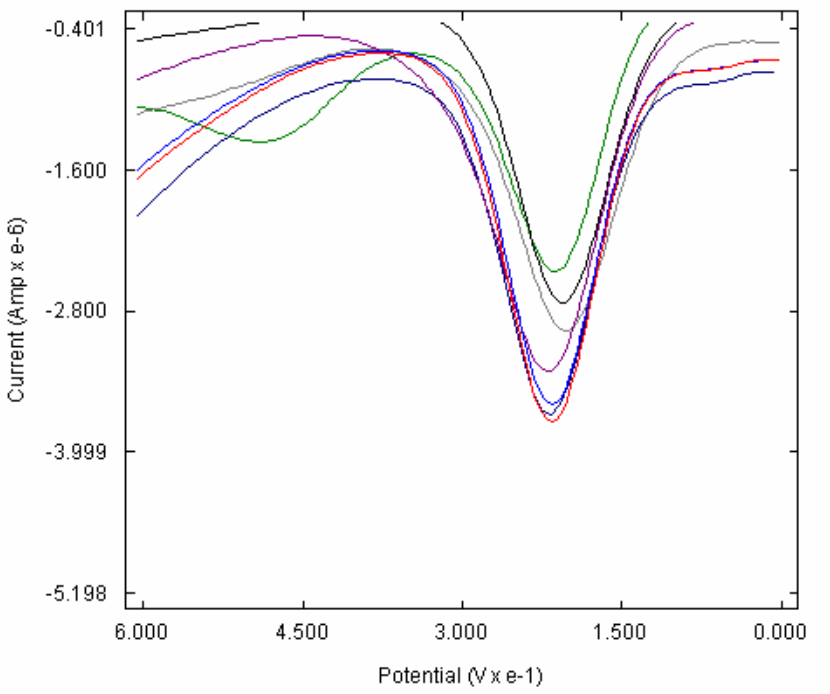


Figure 5.9 Overlay of square wave voltammograms of ACV at different scan rates in phosphate buffer solution (pH 7).

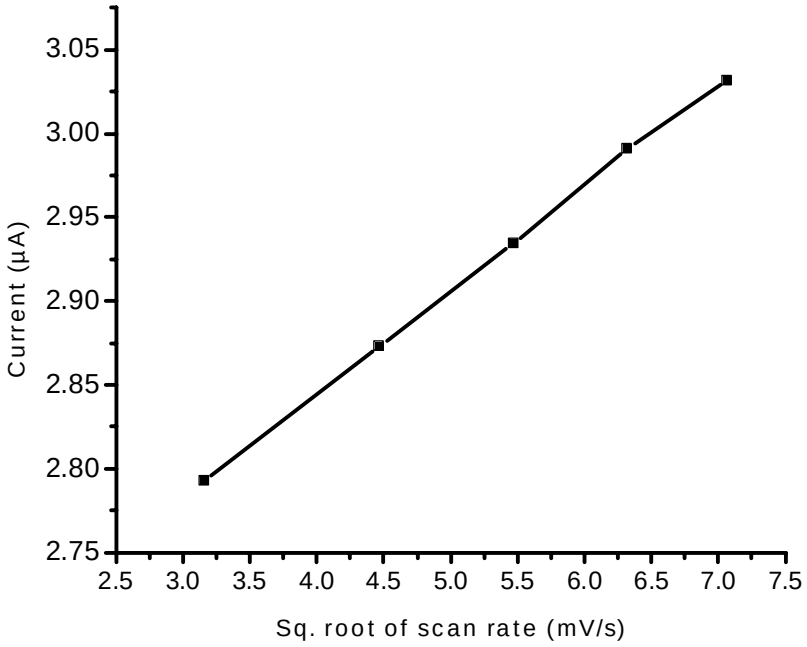


Figure 5.10 Effect of scan rate at MBZ/TMHPP Cu(II) modified GE.

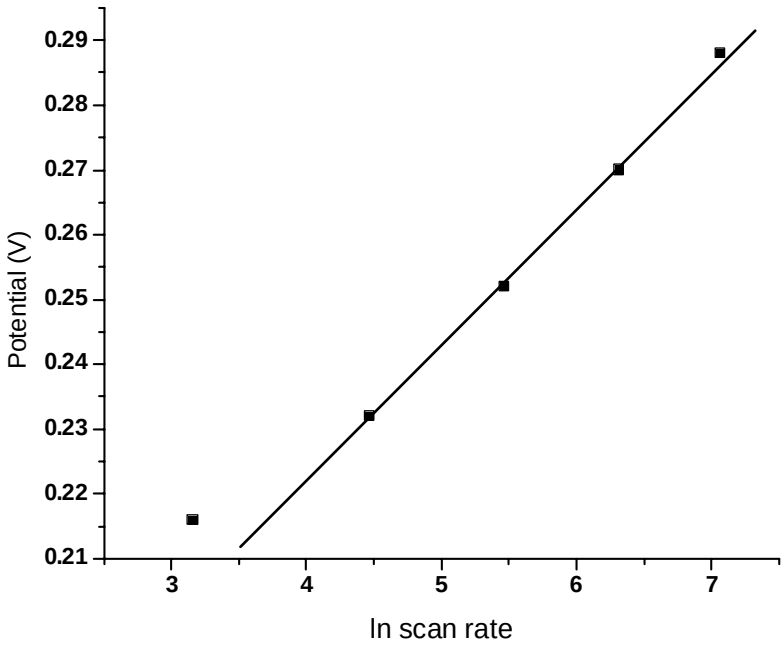


Figure 5.11 Plot of peak potential against ln scan rate

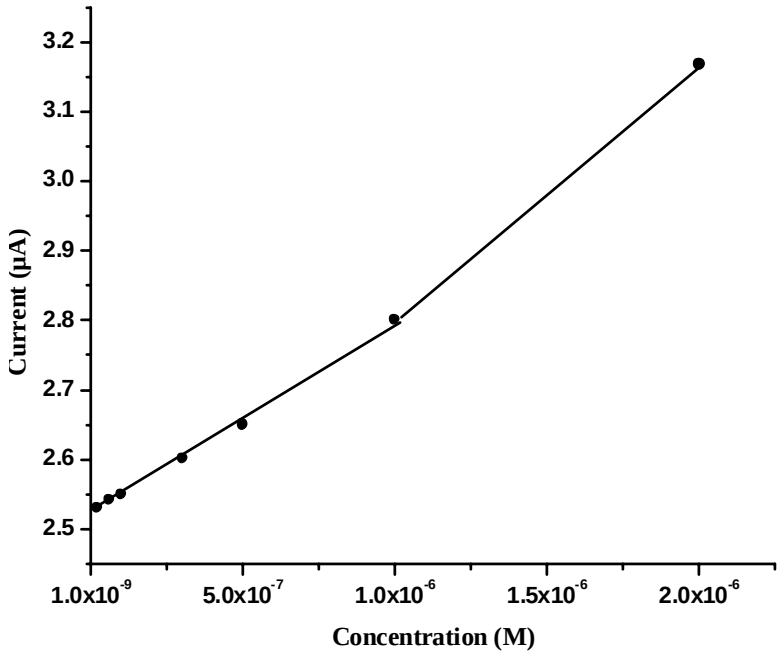


Figure 5.12 Effect of concentration

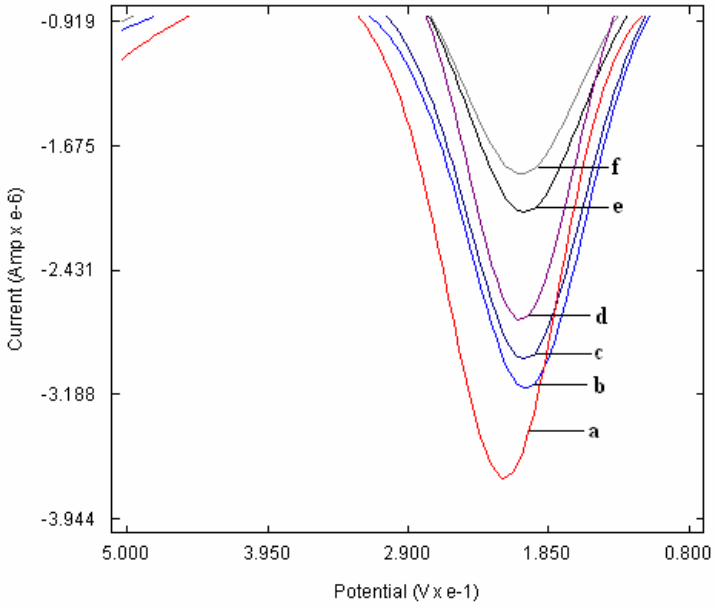


Figure 5.13 Overlay of square wave voltammograms of ACV of different concentrations (a) 5×10^{-6} M (b) 3×10^{-6} M (c) 3×10^{-7} M (d) 1×10^{-7}M (e) 4×10^{-8} M (f) 1×10^{-8} M

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Chapter 6

Development of Sensor for PAM Chloride

Contents

The details about the fabrication and the electrochemical response characteristics of the sensor for PAM chloride is discussed in this chapter.The sensor was fabricated using gold electrode modified with self assembled monolayer of dodecane thiol (DT) and MWCNT. The electrochemical behavior of PAM chloride at the modified electrode was examined by DPV. The electrode thus prepared was then characterized by SEM and surface area study. The DT/MWCNT modified electrode showed good stability and selectivity and can be used to quantify PAM chloride in urine sample.

6.1 Introduction

PAM chloride, chemically 2-formyl-1 methylpyridinium chloride oxime (Figure **6. 1**), is an antidote approved for reactivation of inhibited acetylcholinesterase (AChE) in organophosphate poisoning [166]. Nerve agents are highly toxic, man made compounds that have been manufactured for use in chemical warfare. The four principal nerve agents that have received the greatest military attention are sarin, soman and tabun. They inhibit the enzyme cholinesterase from hydrolyzing the neurotransmitter acetylcholine. Consequently, acetylcholine accumulates and causes prolonged stimulation of the affected tissues resulting in involuntary urination and defecation, muscle twitching/fasciculation seizures, coma and ultimately death. The bond between the nerve agent and the enzyme can be cleaved easily by PAM chloride. 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 breath, to become weak. PAM Chloride helps us to get back strength in our muscles.

There are various analytical methods for the determination of PAM chloride in pharmaceutical formulations. It mainly includes spectrophotometry [167], capillary zone electrophoresis [168], isotachophoresis [169] and voltammetry [70, 81]. Electrochemical methods based on chemically modified electrodes have attracted much attention because of quick response, high sensitivity and selectivity in the determination of trace level analytes. The slow electron transfer kinetics on bare (unmodified) electrode is substantially changed by modifying the surface of the bare electrode which speeds up the electron transfer kinetics [170-172].

Since carbon nanotubes (CNTs) were discovered in 1991, they have attracted a great deal of attention among researchers [173]. CNTs have many advantages such as high conductivity, high surface area and high mechanical strength. They have been widely used in many fields and are expected to be applied in more areas, including nanoelectronic devices, electron field emission sources and potential hydrogen storage material. In the electroanalytical field, CNTs are widely used as electrode modified materials and to prepare sensors etc. [174]. Sensors based on CNTs have improved the voltammetric response (lower overvoltages and higher peak currents) of a variety of clinical, biological and environmental compounds.

The substrate electrodes used for preparing CNT modified electrodes were generally glassy carbon electrodes and graphite electrodes. Metal substrate electrodes were rarely utilized probably due to the inconvenience of immobilizing CNTs on metal surfaces. Among the few examples, Wang et al. directly dropped minor amounts of singlewalled carbon nanotubes onto gold electrodes to prepare modified electrodes [175]. The modified electrode was used to study the electrochemical behavior of uric acid. However, the CNT modified gold electrodes prepared in this way were not stable. In order to fabricate more stable CNT modified metal electrodes, a chemical covalent method was adopted, but the preparation procedure was relatively complicated and time-consuming.

Molecular self assembled monolayers (SAMs) have witnessed tremendous growth in the last two decades. Self-assembled monolayer (SAM) formation induced by the strong chemisorption between the substrate and head group of selected organic molecule provides one of the most elegant approaches towards making ultrathin organic films of controlled thickness [176]. The monolayer formation in this work will be

on gold surface since thiols chemisorb as thiolates on gold surface. The choice of gold is due to the fact that gold is an inert material, i.e. does not form stable oxide under ambient conditions [177]. In the present study, DT was left to assemble on a gold electrode and MWCNT was further immobilized on the DT modified GE surface. Thus a novel DT/MWCNT modified GE was fabricated. Using the modified electrode, the electrochemical behaviour of PAM chloride was investigated.

6.2 Functionalisation of MWCNT

MWCNT was refluxed in 100 mL 6 M nitric acid for 10 hours to eliminate metal oxide catalyst within the nanotubes, to introduce carboxyl groups [178] and segment MWCNT for easier and better dispersion. The resulting suspension was diluted with 200 mL of water and it was filtered, washed with double distilled water 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 of MWCNT and generation of –COOH groups at their terminus.

SEM image of the treated MWCNTs is presented in Figure **6.2**. The SEM image demonstrates that MWCNT retained their nanosized tubular shape even after pretreatment.

6.3 Fabrication of DT/MWCNT modified GE

Prior to modification, GE was cleaned as explained under section 2.5 of Chapter 2. SAM was formed by soaking the cleaned GE in an ethanolic solution of suitable thiol compounds such as MBZ, DT, 3-mercaptopropionic acid and 2-mercaptoethanol. The effect of concentration as well as time for monolayer formation using the above thiol compounds was also studied. But

a better voltammetric response was obtained by dipping GE in DT (1×10^{-3} M) solution for 24 hours at room temperature and by further modification with MWCNT. So we selected DT as the base monolayer for the attachment of MWCNT. 4 μL of MWCNT/Nafion suspension was deposited onto the surface of DT modified GE. The composite was then dried in air before it was used as a working electrode in the experiment. A uniform thin film of nanotube was formed on GE. The detailed procedure for the fabrication of DT/MWCNT modified GE is given in section 2.6.3 of Chapter 2.

The SEM images of the bare GE, DT modified GE and DT/MWCNT modified GE are given in the Figure **6.3**. The modified electrode has many spherical particles on the surface which is not detected in the bare GE. These images provided a strong evidence for the successful modification of GE with DT/MWCNT.

6.4 Estimation of surface area of the electrode

To investigate the electrochemical properties of the modified layer, a square wave voltammetric study for the DT/MWCNT modified GE was performed utilizing the redox probe $K_3Fe(CN)_6$. Figure **6.4** shows cyclic voltammograms of different electrodes in $K_3Fe(CN)_6$ solution. $K_3Fe(CN)_6$ could exhibit a pair of reversible peak at the bare GE. When it is modified with DT, no peak was obtained. However, when it is further modified by MWCNT a pair of reversible peak occurs with the peak current much higher than those of bare GE. This should be attributed to the conductivity of MWCNT imbedded into the DT. This may be due to hydrophobic interaction between MWCNT and DT. Under the same conditions, with the scan rate increases from 40 to 400 mVs^{-1}, the peaks of $K_3Fe(CN)_6$ grew for

both bare GE and DT/MWCNT modified GE (Figure **6.5**). For a reversible system, the peak current should follow Randles Sevick equation

$$I_p = 2.69\times10^5 n^{3/2} A D^{1/2} C_0 v^{1/2}$$

From the slope of I_p vs. $v^{1/2}$ plot, the area of bare GE and DT/MWCNT modified GE were estimated and were found to be about 1.005 cm^2 and 1.9932 cm^2 respectively. The area thus obtained for modified GE was almost double the effective area of the bare GE. Therefore in comparison with the bare GE, DT/MWCNT modified GE could give more sensitive response to electro active species. The CV of MWCNT modified GE was also recorded. Under the same conditions, the peak current was smaller than those of DT/MWCNT modified GE. This is related to the difference in dispersion of MWCNT at bare GE and the DT/MWCNT modified GE, which affects the electrode area. Owing to the hydrophobic interaction between DT and MWCNT, MWCNT was more strongly adhered to DT modified GE than to bare GE [179].

6. 5 Electrochemical study

Stock solution of PAM Chloride was prepared as explained in section 2.7.5 of Chapter 2. Standard solutions of the analyte (1×10^{-3} M - 1×10^{-7} M) were prepared by serial dilution of the stock solution using supporting electrolyte. Sample solution was taken in the electrochemical cell. The solution was then deaerated with N_2 for 10 minutes. A potential scan between 0 - 0.700 V was triggered, and the anodic peak was recorded using DPV. An oxidation peak at 0.408 V was measured for 10^{-3}M drug solution. The electrode was regenerated by successive potential scan between 0 - 0.700 V in a blank electrolyte solution until a stable voltammogram was obtained.

The electrochemical behaviour of tamsulosin hydrochloride, ceftriaxone sodium, trimethoprim, acyclovir, sparfloxacin, sulfamethoxazole and PAM chloride were studied at bare GE and DT/MWCNT modified GE by DPV. Firstly an attempt was made to study the electrochemical behaviour of TAM. Unfortunately at bare GE, the anodic peak of TAM was obtained at a high potential of 1.160 V with a low peak current of 4.3915 µA. When DT/MWCNT modified GE was used, anodic peak was obtained at 1.2234 V with a current of 3.3022 µA. Thus compared to bare GE, the peak current of tamsulosin hydrochloride showed a marked decrease and the peak potential showed a positive deviation. The various experimental parameters such as pH, supporting electrolyte and the amount of MWCNT were varied and their effects on the results were studied. Also, different thiol compounds were tried on the electrode to study their effect on the results. However this hardly had any impact on the improvement of results.

Under the same experimental conditions at bare GE, an oxidation peak was obtained at 1.064 V for ceftriaxone sodium. But at DT/MWCNT an oxidation peak was obtained at 1.2344 V. Here also various experimental parameters were changed to get better results. But the results were not satisfactory.

Further, the electrochemical behaviour of trimethoprim and acyclovir was investigated. Both of them gave no voltammetric response at bare GE and DT/MWCNT modified GE.

The electrochemical response characteristics of sparfloxacin were next studied at bare GE and DT/MWCNT modified GE. At bare GE sparfloxacin gave an oxidation peak at 0.980 V. However at DT/MWCNT modified GE, no voltammetric response was obtained.

Also the voltammetric behaviour of the drug sulfamethoxazole was investigated. The electrooxidation of sulfamethoxazole occurs at a potential of 0.910 V at bare GE, but no response was obtained on modified GE.

Finally the electrochemical behaviour of PAM chloride at bare GE, DT modified GE and DT/MWCNT modified GE was studied by DPV (Figure **6.6**). The electrochemical response of PAM Chloride at MWCNT modified GE was also investigated. PAM Chloride gave an oxidation peak at 0.592 V at the bare GE. At DT modified electrode, no voltammetric response was obtained. At MWCNT modified GE, oxidation peak was obtained at 0.490 V. However at DT/MWCNT modified GE, PAM chloride exhibited a well defined and sensitive oxidation peak at 0.408 V, which was 0.184 V less than for the bare GE and 0.102 V less than for the MWCNT modified GE. This may be attributed to the rapid electron transfer due to the conductivity of MWCNT wires imbedded onto the DT modified GE which can promote the electron transfer. The absence of a reduction peak for PAM chloride in the reverse sweep establishes the irreversibility of the electrochemical process.

The reaction mechanism for PAM chloride oxidation at the modified electrode is given in Figure **6.7**. The mechanism involves one-electron oxidation of aldoxime to the iminoxy radical, which is followed by a second one electron oxidation step to the α-hydroxy nitroso compound. Dimerization and elimination of hyponitrous acid gives the carbonyl compound [180]

6.6 Optimization of operational parameters

The experimental parameters that affect the PAM Chloride signal were carried out in order to establish optimum conditions.

6.6.1 Effect of varying supporting electrolyte

The electrochemical oxidation signals of PAM chloride at DT/MWCNT coated GE has been examined in various electrolytes. The supporting electrolytes include KNO_3, H_2SO_4, NaOH, NaCI, tetra-n-butyl ammonium chloride, acetate buffer and PBS (each 0.1 M).The results showed that a well defined voltammogram was obtained in PBS. Therefore 0.1 M PBS was used as the supporting electrolyte for the determination of PAM Chloride.

6.6.2 Effect of varying solution pH

In order to optimize the response of the DT/MWCNT modified GE for PAM Chloride oxidation, the effect of pH on the electrochemical oxidation was investigated using DPV. The plot of anodic peak current versus pH of the supporting electrolyte in the range of 3 to 10 for PAM Chloride is presented in Figure **6.8**. With the increase of pH from 3 to 8, the peak current increased significantly, and then decreased as the pH increases. The best oxidation response was obtained in pH 8 as the peak current is the highest. Thus pH 8 was fixed as optimal pH.

6.6.3 Effect of varying scan rate

The effect of scan rate on the linear sweep voltammetric response was investigated at different scan rates ranging from 50-250 mV/s. It was found that anodic peak current increases with an increase in the scan rate. The results are depicted in Figure **6.9**. The oxidation peak current varies linearly with square root of scan rate (Figure **6.10**). This indicates that the electrode process is controlled by diffusion.

According to the Laviron's equation the relationship between the peak potential (E_p) and scan rate (υ) was examined. It was found that E_p

varies linearly with ln υ (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, 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 is 2.10. This confirms that 2 electrons are involved in the oxidation of PAM Chloride.

6.6.4 Effect of amount of MWCNT-nafion on peak current

When the amount of MWCNT-nafion suspension was 1-4 µL (5 mg of MWCNT in a mixture of 300 µL nafion and 2 mL water), the peak current increased gradually (Figure **6.12**) as PAM chloride got adsorbed on the surface. The enhancement of the current indicates that the specific surface area and the number of catalytic sites increase with an increase of MWCNT. However, nafion is an insulator and prohibit the charge transfer of PAM Chloride at the film electrode. So when the amount exceeded 4 µL the oxidation peak current decreased as too much of nafion retarded the electron transfer and mass transportation of PAM Chloride.

6.6.5 Effect of varying concentration

The effect of varying concentration of PAM Chloride on the peak current at DT/MWCNT modified GE in 0.1 M PBS (pH 8) solution was investigated by DPV (Figure **6.13**). A linear response was found to obtain from 8.0×10^{-7} M to 1.0×10^{-3} M (Figure **6.14**) with a detection limit of 1.0×10^{-8} M. Table **6.1** represents the comparative study of the characteristics of the developed method with some of the reported methods. The method showed good linear concentration range and detection limit with respect to the other methods reported in literature.

The stability of the modified GE was investigated by measuring the voltammetric current once in every day. The results showed that the developed sensor retained its original current response in the first continuous nine days. For five successive determination of 1×10^{-3} M PAM Chloride with the same electrode regenerated after every determination, the RSD of the peak current was 3.1%. This indicated that the DT/MWCNT modified GE has excellent reproducibility.

6.6.6 Interference study

Six possible interfering substrates were used to evaluate the selectivity of the sensor. The results of the interference investigation are listed in Table **6.2**. It was found that glycine, sodium chloride, potassium chloride, dextrose, lactose and urea do not cause any interference under the experimental conditions. These results indicated that the film electrode has good selectivity for the determination of PAM chloride. However, ascorbic acid interfere with the oxidation signal of PAM Chloride.

6.7 Analytical application

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

6.7.1 Determination of PAM Chloride in urine sample

The applicability of DT/MWCNT electrode for PAM chloride determination in urine was investigated. Various concentrations of the drug solution in PBS containing fixed amount of urine sample was prepared. The electrochemical behaviour of the prepared solution on DT/MWCNT modified GE was studied by DPV and the unknown concentrations were determined from the calibration graph. The recoveries obtained are in the

range of 100 – 101%. The recovery obtained with the developed sensor was compared with that obtained using the standard method (spectrophotometric method) [108]. The results are shown in Table **6.3** and Table **6.4.** 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.

As an antidote for nerve agents in chemical warfare, PAM chloride is not available commercially and so tablet study could not be included in our work.

6.8 Conclusion

A novel DT/MWCNT modified gold electrode was fabricated and it exhibited a highly selective and enhanced electroanalytical response towards PAM Chloride. The designed sensor also showed ease of preparation, quick regeneration, good reproducibility, high stability and anti-fouling property due to high water stability and high mechanical strength of MWCNT. The effects of foreign species were studied and were found to be insignificant. The sensor was successfully utilized for the determination of PAM Chloride in urine sample.

Table 6.1 Comparison of major characteristics of some analytical methods used in the determination of PAM Chloride.

Analytical method	Linear range (M)	Detection limit (M)	Reference
Capillary zone electrophoresis	3.0×10^{-4} - 2.0×10^{-1}	1.0×10^{-7}	[168]
Isotachophoresis	1.0×10^{-5} - 7.0×10^{-6}	1.5×10^{-7}	[169]
Spectrophotometry	1.0×10^{-4} - 1.0×10^{-2}	1.0×10^{-5}	[167]
Voltammetry	1.0×10^{-6} - 2.0×10^{-5}	3.0×10^{-7}	[70]
Voltammetry	2.0×10^{-7} - 2.0×10^{-6}	3.0×10^{-8}	[81]
Voltammetry (Present method)	8.0×10^{-7} - 1.0×10^{-3}	1.0×10^{-8}	

Table 6.2 Interference study

PAM Chloride taken – 1.00×10^{-3} M

Foreign species	Tolerance limit (M)	Signal change (%)
Glycine	1.00×10^{-1}	1.23
Sodium chloride	1.00×10^{-1}	1.36
Potassium chloride	1.00×10^{-1}	1.64
Dextrose	1.00×10^{-1}	0.27
Lactose	1.00×10^{-1}	2.73
Urea	1.00×10^{-1}	2.32
Ascorbic acid	1.00×10^{-1}	7.56

Table 6.3 PAM Chloride determination in urine sample

Added (M)	Found (M)	Recovery (%)
8.00×10^{-5}	8.03×10^{-5}	100
4.00×10^{-5}	4.04×10^{-5}	101
8.00×10^{-6}	8.10×10^{-6}	101

Table 6.4 Comparison of the developed method with the standard method

Developed Sensor		Standard Method	
Recovery(%)*	CV*	Recovery*	CV*
101	1.85	99.8	1.01

* average of six replicates

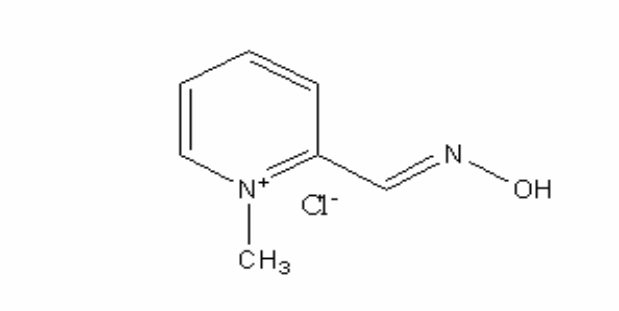

Figure 6.1 Structure of PAM Chloride

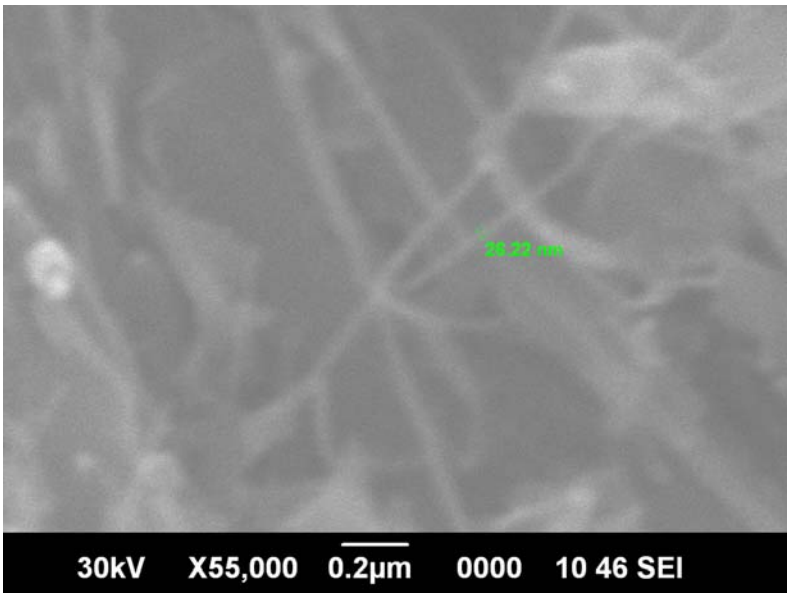

Figure 6.2 SEM image of MWCNT after acid treatment

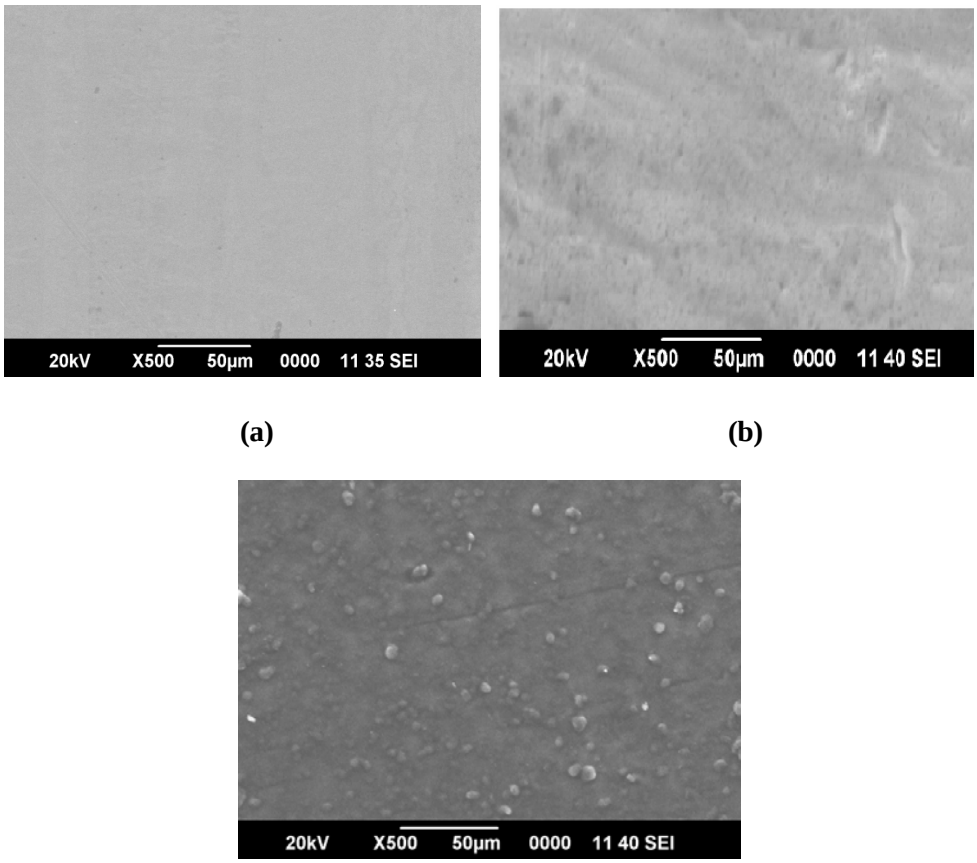

Figure 6.3 SEM image of (a) bare GE (b) DT modified GE (c) DT/MWCNT modified GE

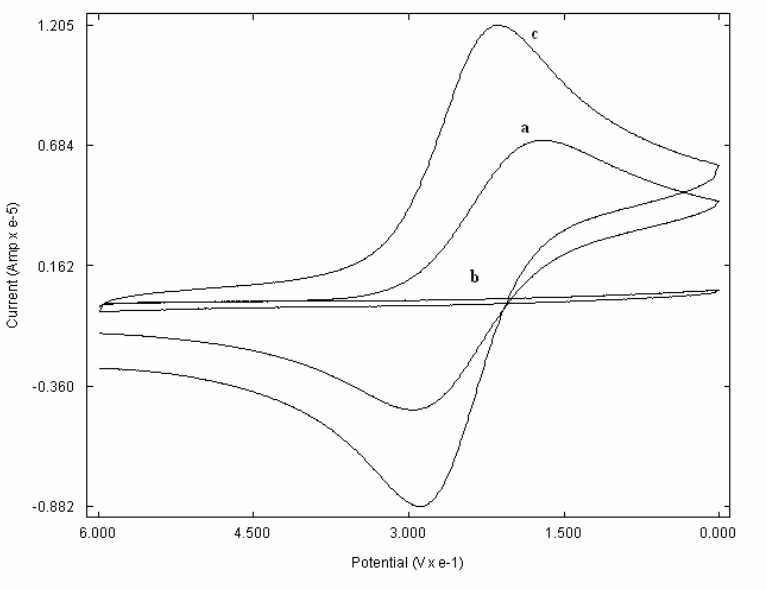

Figure 6.4 Cyclic voltammograms of 2 mM $K_3Fe(CN)_6$ solution at (a) bare GE (b) DT modified GE (c) DT/MWCNT modified GE.

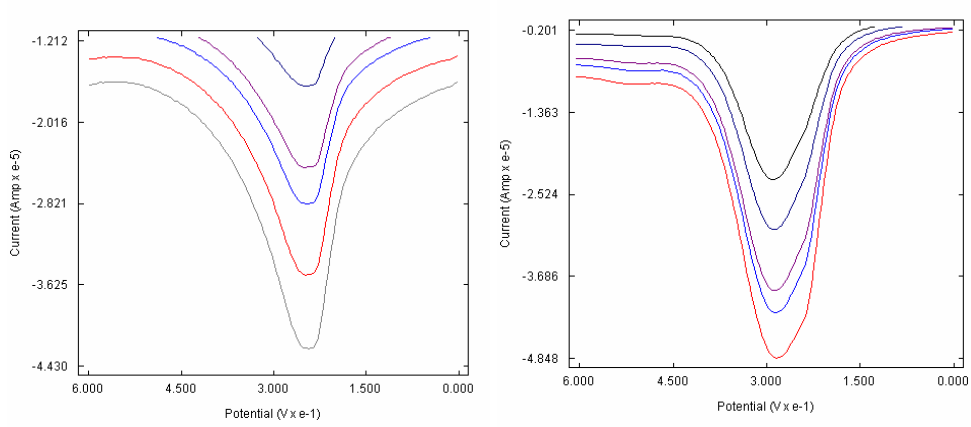

Figure 6.5 Study of electrode surface area at (a) bare GE (b) DT/MWCNT modified GE.

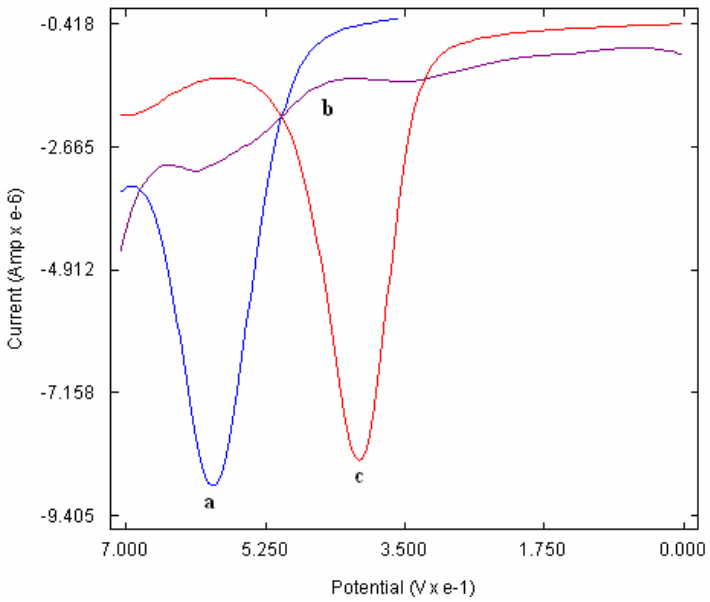

Figure 6.6 Differential pulse voltammogram of 1×10^{-3} M PAM Chloride at (a) Bare GE (b) DT modified GE and (c) DT/MWCNT modified GE

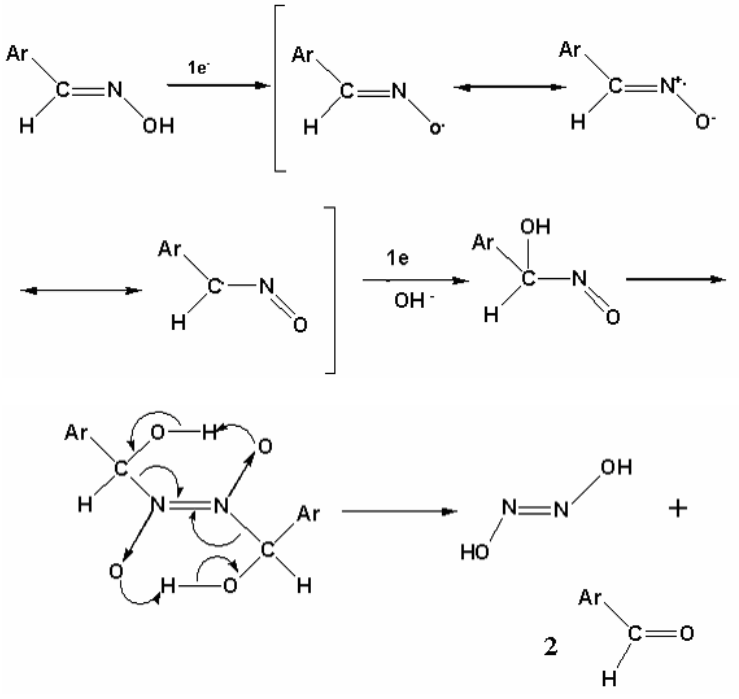

Figure 6.7 Reaction mechanism of conversion of oxime group in PAM Chloride to carbonyl group

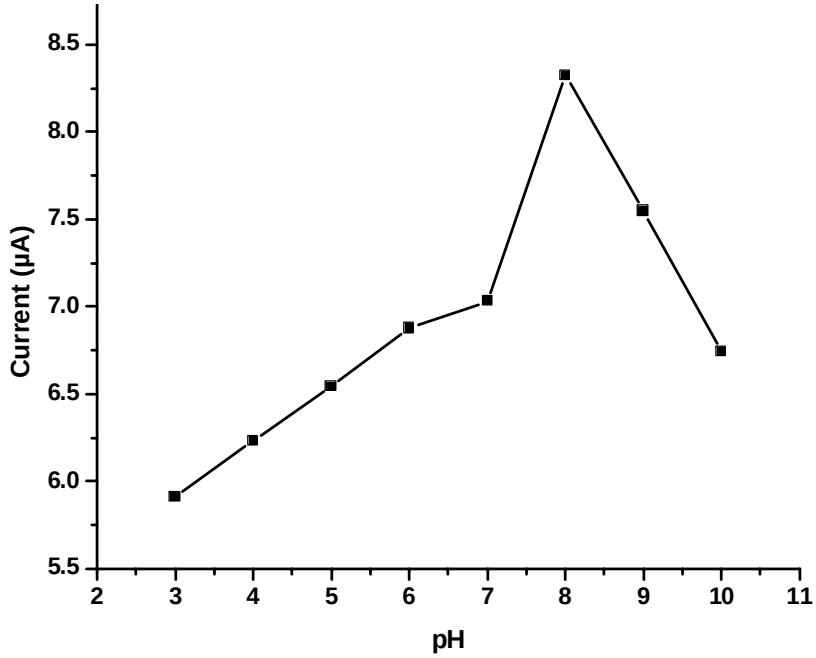

Figure 6.8 Effect of varying pH on the anodic current of PAM Chloride

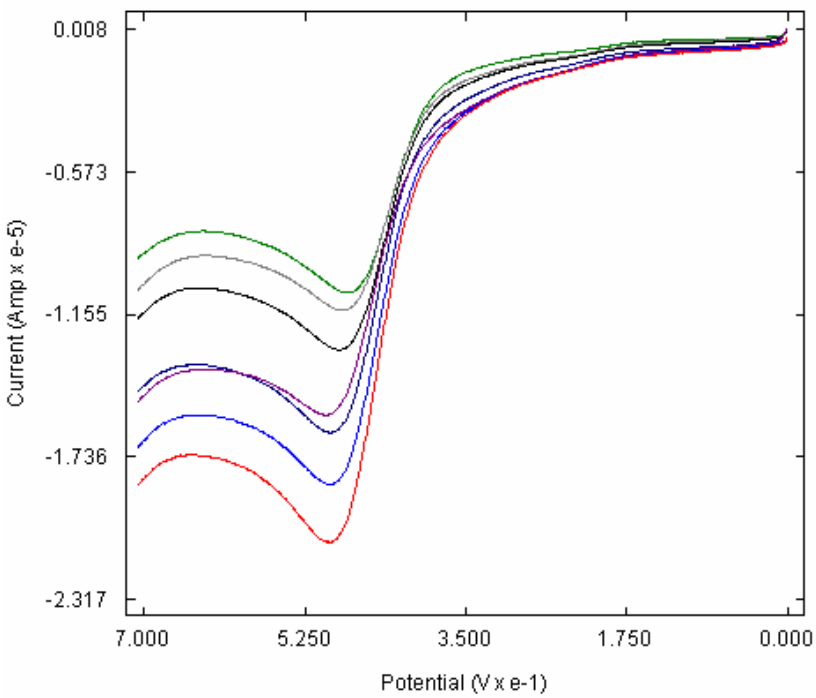

Figure 6.9 Linear sweep voltammograms of PAM Chloride at different scan rates in phosphate buffer solution (pH 8).

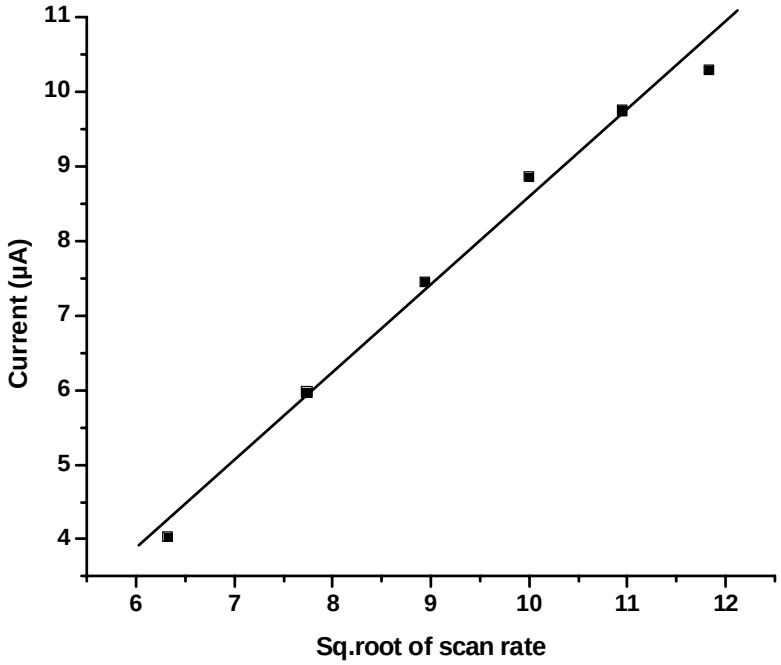

Figure 6.10 Effect of varying scan rate

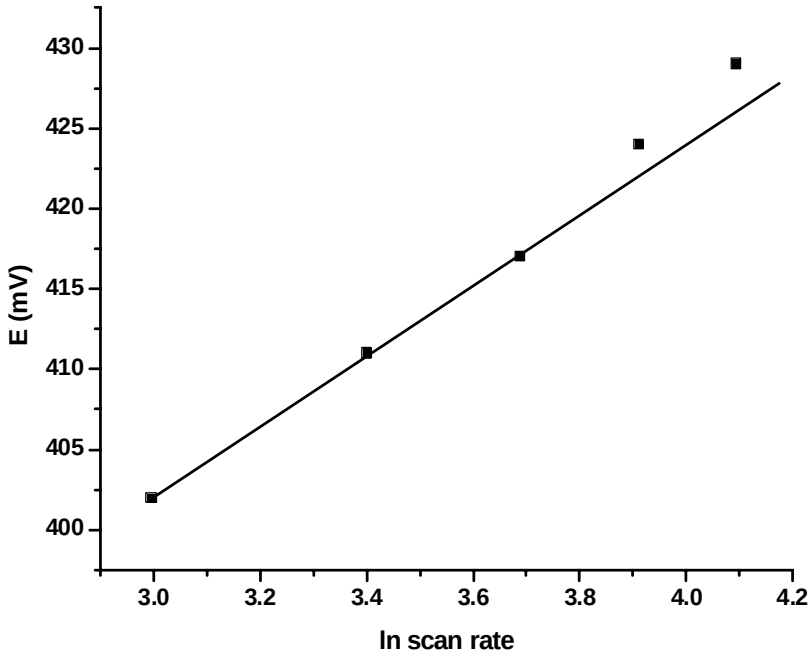

Figure 6.11 Dependence of peak potential (E) on ln scan rate

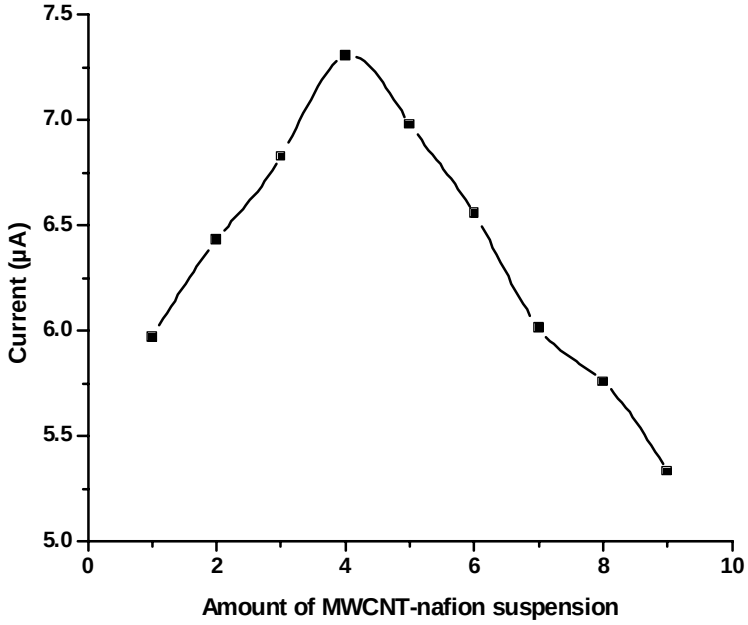

Figure 6.12 Effect of varying amount of MWCNT-nafion dispersion

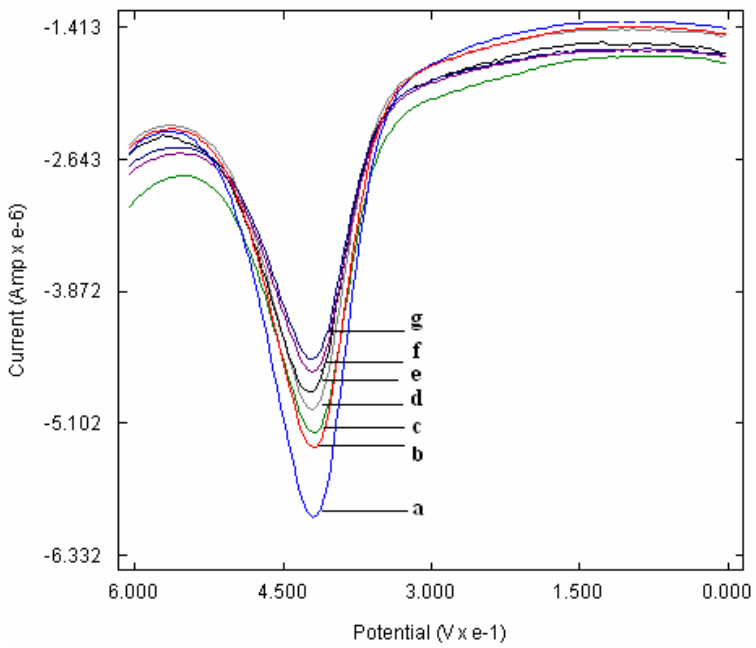

Figure 6.13 Dependence of anodic peak current on PAM Chloride of different concentrations (a) 9×10^{-4} M (b) 5×10^{-4} M (c) 3×10^{-4} M (d) 1×10^{-4} M (e) 8×10^{-5} M (f) 6×10^{-5} M (g) 4×10^{-5} M

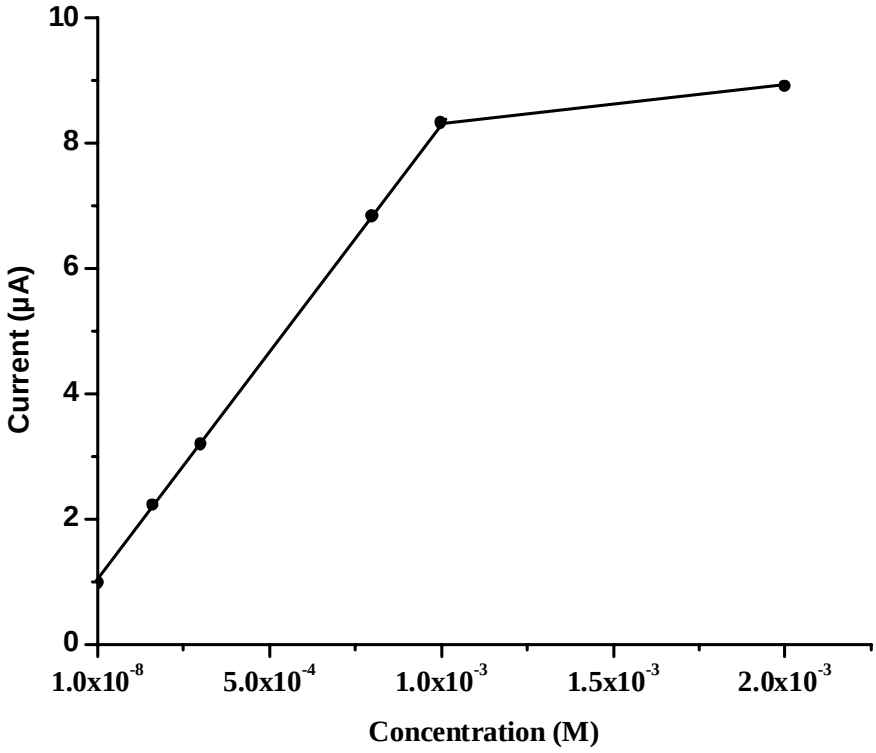

Figure 6.14 Effect of varying concentration

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Chapter 7

Development of Sensor for Trimethoprim

Contents

The development and performance characteristics of polyaniline [PANI] modified gold electrode sensor for the drug trimethoprim (TMP) has been discussed in this chapter. Cyclic voltammetry was employed in the process of electropolymerization. The electrochemical behaviour of TMP at PANI modified GE was explored using square wave voltammetry (SWV). A well defined oxidation peak of TMP occurs at 0.192 V in 0.1M acetate buffer solution (ABS) of pH 4. The oxidation peak current was proportional to the concentration of TMP in the range 1×10^{-3} M to 2×10^{-7} M, with a detection limit of 1×10^{-8} M. The developed sensor was also applied for the determination of TMP in pharmaceutical preparations and in body fluids.

7.1 Introduction

TMP, 5-(3,4,5-trimethoxybenzyl)pyrimidine-2,4-diamine (Figure **7.1**), belongs to the class of chemotherapeutic agents known as dihydrofolate reductase inhibitors. It is used in the prophylaxis treatment and in urinary tract infections. TMP is a synthetic antibiotic that interferes with the production of tetrahydrofolic acid, by inhibiting the enzyme responsible for making tetrahydrofolic acid from dihydrofolic acid. TMP was commonly used in a 1:5 combination with sulfamethoxazole. This combination is known as co-trimoxazole. The combination use has been declining due to reports of bone marrow toxicity, lack of greater efficacy in treating common urine and chest infections and side effects of antibacterial sulfonamides. As a consequence, the use of co-trimoxazole was restricted in 1995 following the availability of trimethoprim (not in combination) in 1980. With its greater efficacy against a limited number of bacteria, co-trimoxazole remains indicated for some infections viz; it is used as prophylaxis in patients at risk for Pneumocystis jirovecii pneumonia and as therapy in Whipple's disease [181]. Thus the determination of TMP is of immense importance and various methods have been developed. They include spectrophotometry [182], molecular imprinting chemiluminiscence [183], polarography [184], liquid chromatography [185] and voltammetry [186]. But most of these methods lack selectivity and sensitivity for the determination of TMP. Hence it is of great importance to develop a technique for the determination of TMP with high degree of selectivity, sensitivity and low detection limit.

Conducting electroactive polymers (CPs) are materials discovered just over 20 years ago which have aroused considerable interest on account of their electronic conducting properties and unique chemical and

biochemical properties. Consequently, they have numerous analytical and technological applications. CPs are easily synthesized and deposited onto the conductive surface of a given substrate from monomer solutions by electrochemical polymerization with precise electrochemical control of their formation rate and thickness [187].

PANI is one of the many conducting polymers that have been studied quite extensively [188–190]. This is because it is easily prepared by either chemical or electrochemical methods, reasonably stable under ambient conditions and has relatively high conductivity. Its properties can be easily controlled by varying experimental parameters during electrochemical preparation. Electropolymerization is a good method to immobilize polymers to prepare polymer modified electrodes (PMEs), because it is simple, reproducible and it can be carried out potentiostatically or amperometrically or with a cyclic scan of the potential. The formation rate can be regulated and the whole process takes only a few seconds. Preparation of polymer films by electropolymerization of aromatic compounds has been widely used in electrode surface modification to obtain interesting electrode properties. In the present work PANI modified GE was used to study the electrochemical behaviour of TMP.

The PANI modified GE was prepared by electropolymerisation of aniline using cyclic voltammetric technique. At bare GE, no voltammetric response was obtained. But on modification of GE with PANI, a well defined and sensitive oxidation peak was obtained at 0.192 V for TMP. The developed sensor has also been successfully applied for the determination of TMP in pharmaceutical preparation and in urine sample.

7.2 Preparation of PANI modified GE

Firstly GE was cleaned as explained under section 2.5 of Chapter 2. The PANI modified GE was prepared by electropolymerization of aniline on GE. The detailed procedure for the fabrication of PANI modified GE is given in section 2.6.4 of Chapter 2. Figure **7.2** shows cyclic voltammograms obtained during electropolymerization of PANI on GE in 0.5 M H_2SO_4 solution containing 0.1 M aniline at a scan rate of 100 mVs^{-1}. First anodic peak occurring at a potential of about 0.25 V could be attributed to the doping of sulfate anions via transition of leucoemeraldine form of PANI to emeraldine salt, while further increase of the potential above 0.65 V denotes transition of emeraldine salt to pernigraniline salt [191]. Between these two well defined anodic peaks, small peak at potential of about 0.45 V could be assigned to degradation reaction of PANI [192, 193]. In the subsequent cycles larger peaks were observed upon continuous scanning, which reflected the continuous growth of the film. These facts indicated that PANI was deposited on the surface of GE by electropolymerization. A uniform adherent green polymer was formed on the GE surface. After modification, the PANI modified electrode was carefully rinsed with water and stored in air before use.

Surface area study gave a clear evidence for the effective modification of GE with PANI film. 2 mM $K_3Fe(CN)_6$ was taken as a probe to measure the effective surface areas of both PANI modified GE and bare GE by CV at different scan rates (Figure **7.3**).

For a reversible system, the peak current should follow Randles Sevick equation

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

From the slope of I_p vs. $\nu^{1/2}$ plot, the areas of bare GE and PANI modified GE were estimated and was found to be 1.0050 cm^2 and 2.1689 cm^2 respectively. Thus, there was an enhancement in the effective surface area when GE was modified with PANI which is a strong evidence for the successful modification of GE with polymer film.

Surface morphology of bare GE and PANI modified GE were studied by SEM. SEM images (Figure **7.4**) clearly indicated that effective modification of GE surface has taken place after electropolymerization.

7.3 Electrochemical behaviour of TMP

Stock solution of TMP was prepared as described in section 2.7.4 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 taken in the electrochemical cell and then de-aerated with nitrogen for 5 minutes. SWV was executed at PANI modified GE and the voltammograms were recorded from -0.8 to 0.8 V. An oxidation peak at 0.192 V was obtained for TMP 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.

Square wave voltammetric technique was carried out to realize the electrochemical behaviour of sulfamethoxazole, PAM Chloride, ceftriaxone sodium, tamsulosin hydrochloride, acyclovir, alendronate sodium, diclofenac sodium and TMP in 0.1 M ABS (pH 4) solution. The anodic peak potential and current of each of the drug was investigated at bare GE and PANI modified GE. The experimental results show that at bare GE, the anodic peak potential corresponding to the oxidation of drug

sulfamethoxazole and PAM Chloride were observed at 1.030 V and 0.698 V with peak currents 3.3254 µA and 4.6570 µA respectively. The electrochemical behaviour of the drugs were then studied at PANI modified GE. It was found that the oxidation peak corresponding to sulfamethoxazole and PAM Chloride were observed at 1.045 V and 0.748 V. The peak currents were found to be 3.2754 µA and 4.1320 µA. Compared to bare GE, the drugs show considerable increase in oxidation peak potential and decrease in peak current. Also the effect of supporting electrolyte, pH and film thickness was studied. But the results show no improvement.

The electrochemical response characteristics of ceftriaxone sodium and tamsulosin hydrochloride were next studied at bare GE and PANI modified GE. At bare GE, ceftriaxone sodium and tamsulosin hydrochloride gave an oxidation peak at 1.001 V and 1.208 V. However at PANI modified GE, voltammetric response was obtained at 1.020 V and 1.228 V.

The voltammetric behaviour of alendronate sodium and diclofenac sodium was then studied by SWV. The response of the drugs at the modified GE was compared with that at bare GE. At bare GE, oxidation peak was obtained at 1.040 V and 0.641 V for alendronate sodium and diclofenac sodium respectively. The peak currents were found to be 5.2340 µA and 3.2443 µA. At PANI modified GE, oxidation peak was obtained at 1.052 V and 0.681 V with a peak current of 5.0421 µA and 2.8443 µA. Thus with the modified electrode there was an increase of peak potential and decrease of peak current.

Under the same experimental conditions at bare GE, no voltammetric response was obtained for TMP. But a well defined and sensitive oxidation

peak appears at 0.192 V for TMP at PANI modified GE. Figure **7.5** displays the square wave voltammogram of TMP at bare GE and PANI modified GE. The electrochemical behaviour of TMP suggests that PANI modified GE exhibits obvious electrocatalytic activity to the oxidation of TMP. The anodic peak generated for the TMP electrooxidation process may be due to the $4e^-$, $4H^+$ oxidation of the amino group present in TMP to the mononitroso derivative according to the mechanism shown in Figure **7.6**. The amino group at position 4 of the pyrimidine ring may be more easily oxidized than the one at position 2 which is a part of stable pyrimidine system [194].

No reduction peak was observed for TMP in the reverse sweep of CV indicating an irreversible electrochemical process.

7.4 Optimization of analytical parameters

The performance of the developed sensor depends on many analytical parameters including effect of supporting electrolyte, effect of pH, effect of scan rate, effect of film thickness and interference study.

7.4.1 Effect of supporting electrolyte

The effect of supporting electrolyte was explored for optimum analytical performance. The electrochemical properties of TMP in various media such as 0.1 M solutions of phosphate buffer, H_2SO_4, NaOH, NaCl, acetate buffer and KNO_3 were checked by SWV. The results showed that a well defined voltammogram was obtained in ABS. Therefore 0.1 M ABS was used as supporting electrolyte for the determination of TMP.

7.4.2 Effect of pH

pH of the solution has a profound effect on the response of the developed sensor. Experimental results of TMP concentration of 1×10^{-3} M in 0.1 M ABS at different pH values from 2 to 9 are shown in Figure **7.7**. The anodic current increased up to a value of pH 4, and decreased gradually after that. Thus, ABS at pH 4 was selected for further work because it not only gave the highest peak current but also gave the best peak shape.

7.4.3 Effect of scan rate

To determine the type of controlled process of TMP at PANI modified GE, the effect of scan rate on the oxidation peak current was studied in the range 50-200 mV/s by SWV. The results are illustrated in Figure **7.8**. It was found that the anodic peak current increased with an increase in the scan rate. The oxidation peak current varied linearly with square root of scan rate (Figure **7.9**), indicating that the oxidation of TMP is diffusion controlled.

Number of electrons (n_a) involved in the reaction can be calculated from the scan rate study using the Laviron's equation. It was found that potential (E) varies linearly with the logarithm of scan rate (v) (Figure **7.10**). The slope of this plot (b) is equal to $RT/\alpha n_a F$, where α of the totally irreversible electrode process is assumed to be 0.5. For the irreversible oxidation of TMP, n_a value was calculated to be 4.3. It indicates that four electrons are involved in the oxidation process of TMP.

7.4.4 Effect of film thickness

Optimization of the PANI film thickness on the oxidation peak current of 1×10^{-3} M concentration of TMP was also investigated. With the polymerization cycles increasing, the electrochemical response of TMP

increased at first, but when the polymerization cycles was more than 10, the current begins to fall. This may be due to the fact that increase in thickness of the film would prevent the electron transfer. Also, the repeatability and stability for the film modified electrode were poor when the voltammetric sweeping cycles were less than 10. Therefore, 10 cycles was used in the experiments.

7.4.5 Calibration curve

In the present work, quantification of TMP was based on the extent of the dependence of the peak current upon its concentration in the analyzed solution (Figure **7.11**). Validation of the present voltammetric method for the assay of TMP was examined via linearity, limit of detection and reproducibility. Under optimized conditions, with TMP concentrations varying in the range of 2.0×10^{-7} M – 1.0×10^{-3} M, the oxidation peak current varied linearily with the concentration of the drug. The detection limit of TMP was found to be 1.0×10^{-8} M (Figure **7.12**). Table **7.1** represents comparative study of characteristics of the developed method with some of the reported methods. The presently developed method is highly comparable with all the reported methods.

The stability of PANI modified GE was investigated by measuring the voltammetric response of 1.0×10^{-3} M TMP every day over 10 days. The results showed that there was apparent loss of activity after 10 days. The determination of TMP at a concentration level of 1×10^{-3} M was analyzed repeatedly at a PANI coated GE for several times. The relative standard deviation (RSD) of peak current was obtained as 1.64 %. This result demonstrated the good reproducibility of the developed sensor.

7.4.6 Interference study

The influence of some foreign compounds in the TMP oxidation was tested. The results (Table **7.2**) showed that upto 1×10^{-1}M concentration of glycine, sodium chloride, potassium chloride, dextrose, lactose, urea and sulfamethoxazole did not interfere with the determination of 1×10^{-3} M concentration of TMP. These results indicated that the film electrode has good selectivity for the determination of TMP.

7.5 Analytical applications

The analytical utility of the developed sensor in the determination of TMP in pharmaceutical preparations and in urine sample was investigated.

7.5.1 Determination of TMP in pharmaceutical preparation

The developed sensor proved to be useful for the determination of TMP content of a pharmaceutical preparation by SWV. The detailed procedure for the determination is given in section 2.10.4 of Chapter 2. SWV was recorded and the unknown concentrations were determined from the calibration curve. The results are shown in Table **7.3**. The results obtained are in good agreement with the declared TMP content and showed a high degree of precision [coefficient of variation (C.V) is 0.196].

The results were compared with those obtained by the standard method [107]. The results show that there is a good agreement between the TMP content determined by the developed method and the standard method.

7.5.2 Determination of TMP in urine sample

The developed sensor was applied for the determination of TMP in urine sample. Various concentrations of the drug solution in ABS containing fixed amount of urine sample was prepared. The electrochemical behaviour of the prepared solution on modified GE was studied by SWV (Table **7.4**) and the unknown concentrations were determined from the calibration graph. The recoveries obtained are in the range of 100 – 105%.

7.6 Conclusion

A voltammetric sensor for the determination of TMP has been developed based on the electropolymerization of PANI on GE. Fast electron transfer, high selectivity and excellent sensitivity for the oxidation of TMP are achieved at the PANI modified electrode. The electrode is stable and does not undergo surface fouling during the measurements. The increased sensitivity, ease of fabrication and regeneration of the electrode surface make this sensor a better alternative over the existing methods for the determination of TMP.

Table 7.1 Comparison of major characteristics of some analytical methods used in the determination of TMP

Analytical method	Linear range (M)	Detection limit (M)	Reference
Spectrophotometry	2.9×10^{-3}- 2.3×10^{-2}	1.0×10^{-4}	[182]
Molecular imprinting chemiluminiscence	5.0×10^{-4} - 1.0×10^{-1}	1.0×10^{-4}	[183]
Polarography	1.0×10^{-3} - 1.0×10^{-2}	7.5×10^{-4}	[184]
Liquid chromatography	3.0×10^{-4} - 3.2×10^{-2}	1.5×10^{-4}	[185]
Voltammetry	3.0×10^{-4} - 3.2×10^{-2}	1.5×10^{-4}	[186]
Voltammetry (Present method)	2.0×10^{-7} - 1.0×10^{-3}	1.0×10^{-8}	

Table 7.2 Effect of foreign species on the square wave voltammetric response for 1×10^{-3} M TMP at PANI modified GE

TMP taken – 1.00×10^{-3} M

Foreign species	Tolerance limit (M)	Signal change (%)
Glycine	1.00×10^{-1}	3.05
Sodium chloride	1.00×10^{-1}	2.00
Potassium chloride	1.00×10^{-1}	3.85
Dextrose	1.00×10^{-1}	2.16
Lactose	1.00×10^{-1}	2.93
Urea	1.00×10^{-1}	1.99
Sulfamethoxazole	1.00×10^{-1}	4.10
Ascorbic acid	1.00×10^{-1}	3.98

Table 7.3 Determination of TMP in pharmaceutical preparation

Declared Amount (mg/tablet)	Method Adopted	Found* (mg/tablet)	S.D*	C.V*
800	Developed sensor	803	1.58	0.196
	Standard Method	801	1.41	0.158

*average of six replicates

Table 7.4 Determination of TMP in urine sample

Added (M)	Found (M)	Recovery (%)
2.00×10^{-6}	2.10×10^{-6}	105
8.00×10^{-6}	8.02×10^{-6}	100
1.00×10^{-5}	1.02×10^{-5}	102

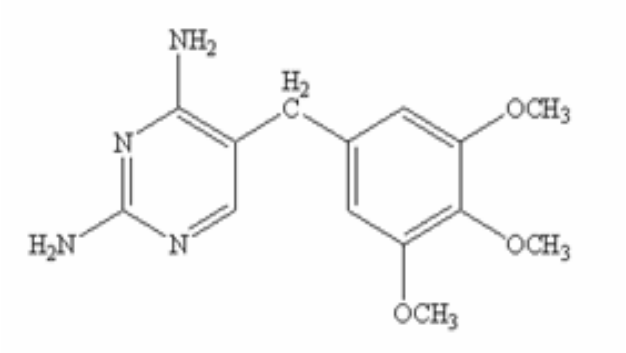


Figure 7.1 Structure of TMP

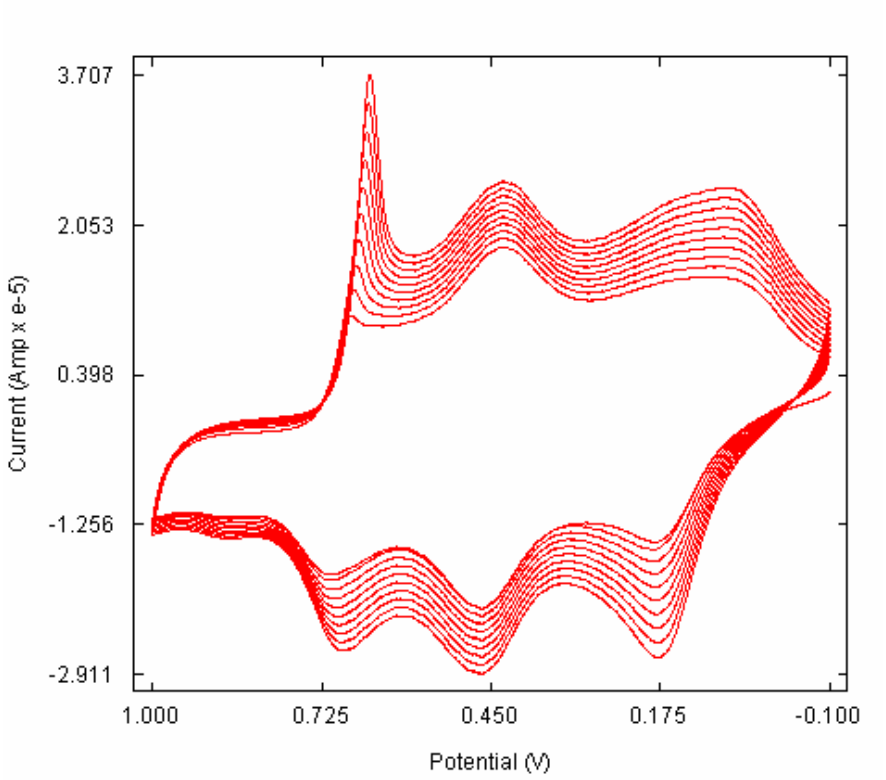
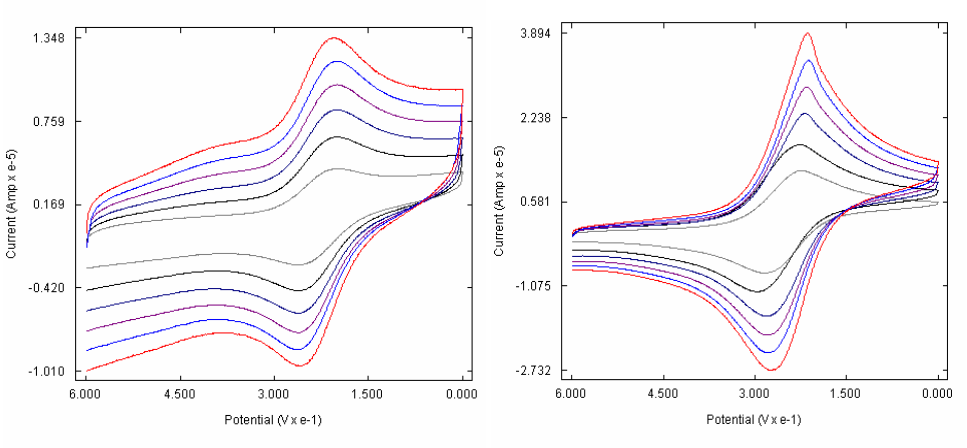
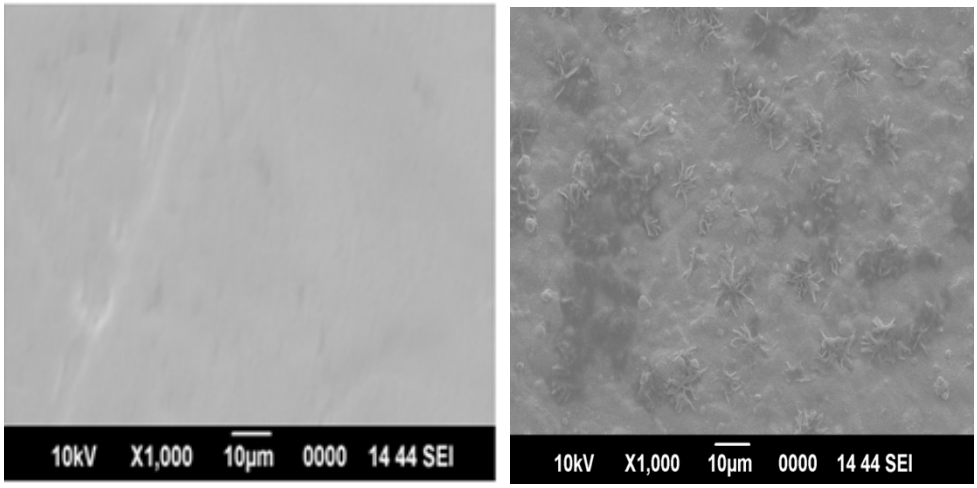


Figure 7.2 Cyclic voltammograms obtained during electropolymerisation in 0.5 M H_2SO_4 solution containing 0.1 M aniline



(a) **(b)**

Figure 7.3 Overlay of cyclic voltammogram of 2 mM $K_3 Fe(CN)_6$ solution at (a) bare GE (b) PANI modified GE



(a) **(b)**

Figure 7.4 SEM image obtained at (a) bare GE (b) PANI modified GE

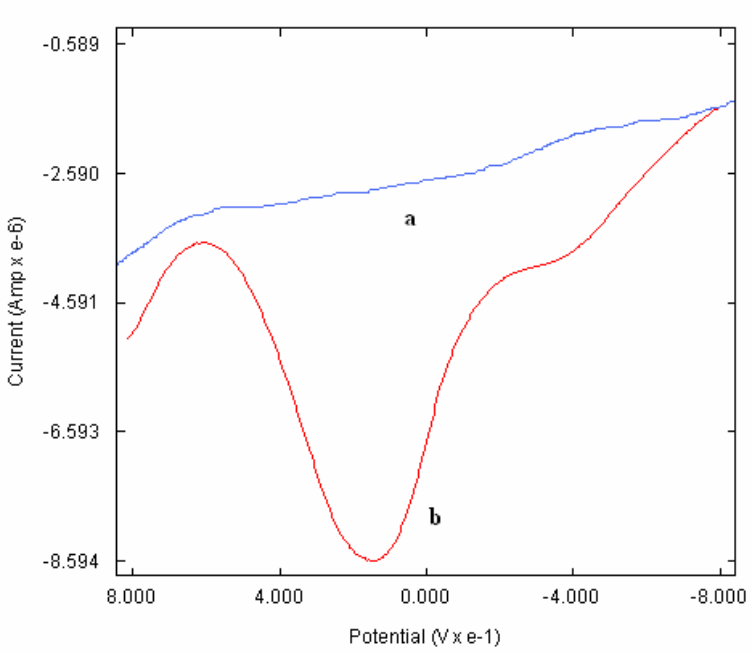


Figure 7.5 Overlay of square wave voltammogram of TMP at (a) bare GE (b) PANI modified GE in 0.1 M ABS (pH 4) at a scan rate of $0.06Vs^{-1}$

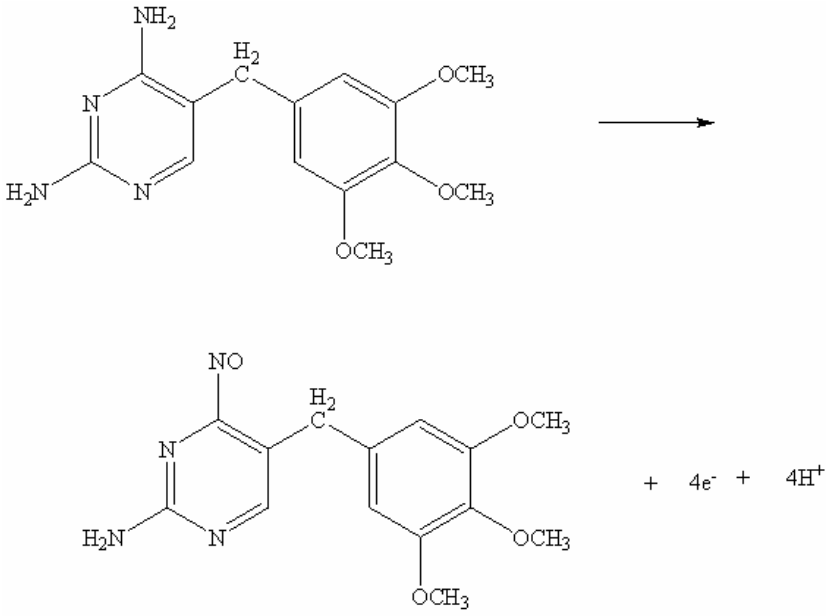


Figure 7.6 Oxidation mechanism of TMP

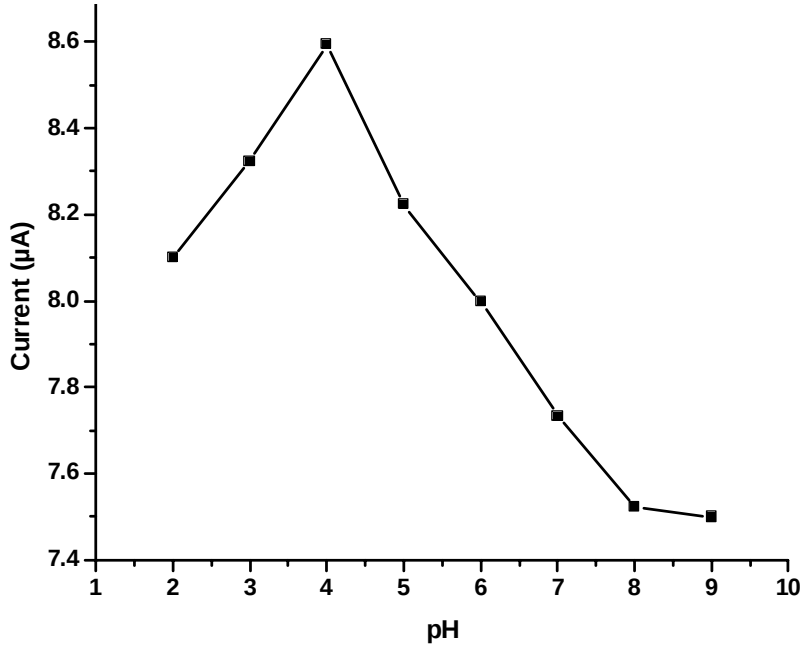


Figure 7.7 The relation between peak current and pH

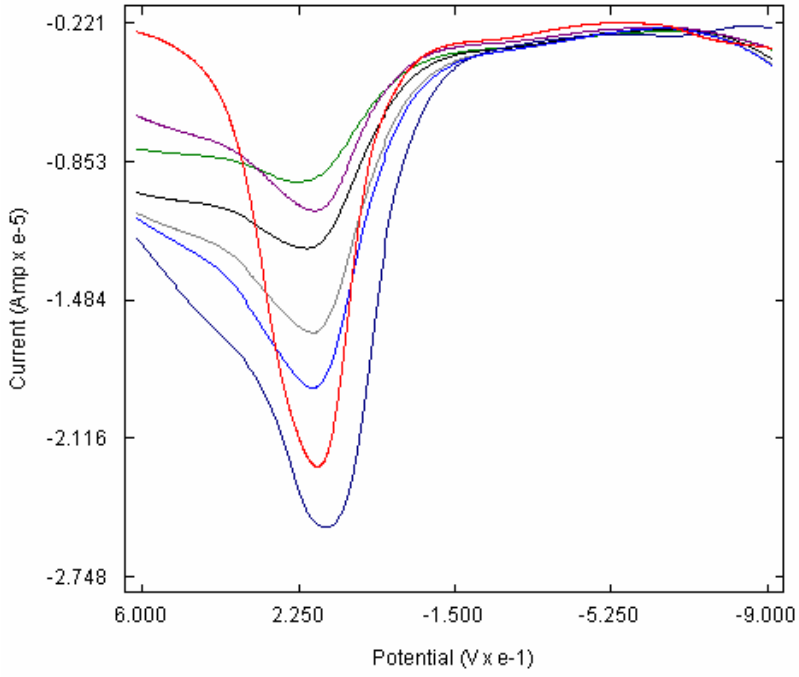


Figure 7.8 Overlay of square wave voltammogram of 1×10^{-3} M TMP at different scan rates

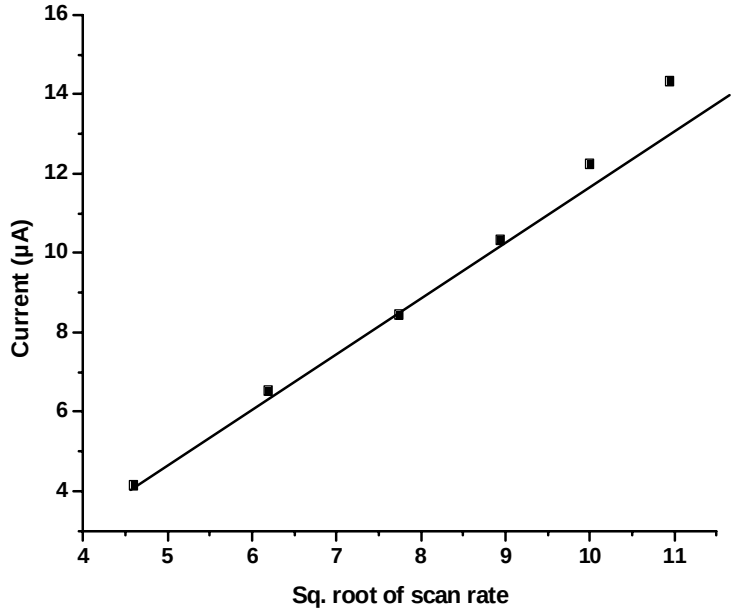


Figure 7.9 Plot of peak current versus square root of scan rate for the electrochemical oxidation of TMP at PANI modified GE

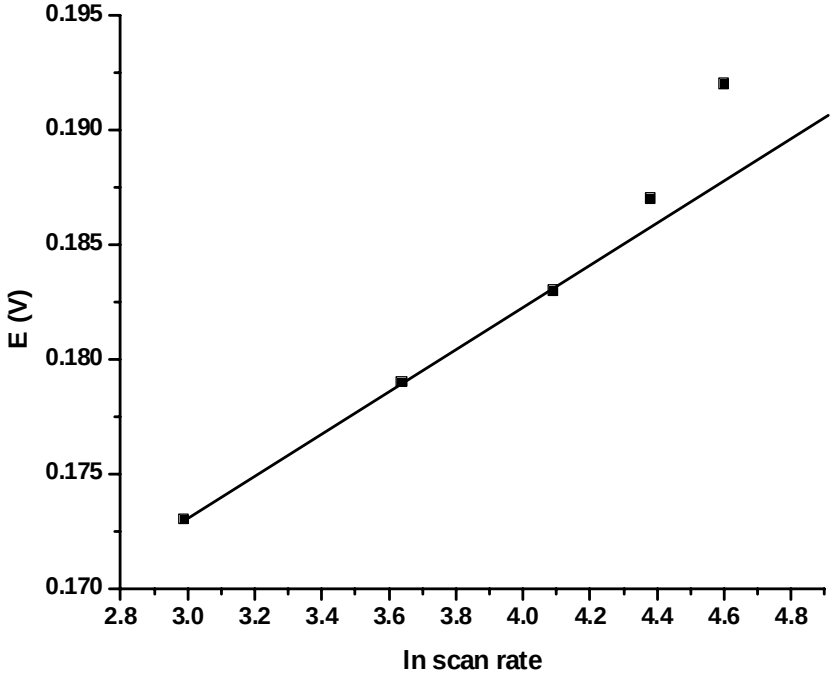


Figure 7.10 Dependence of peak potential (E) on ln scan rate

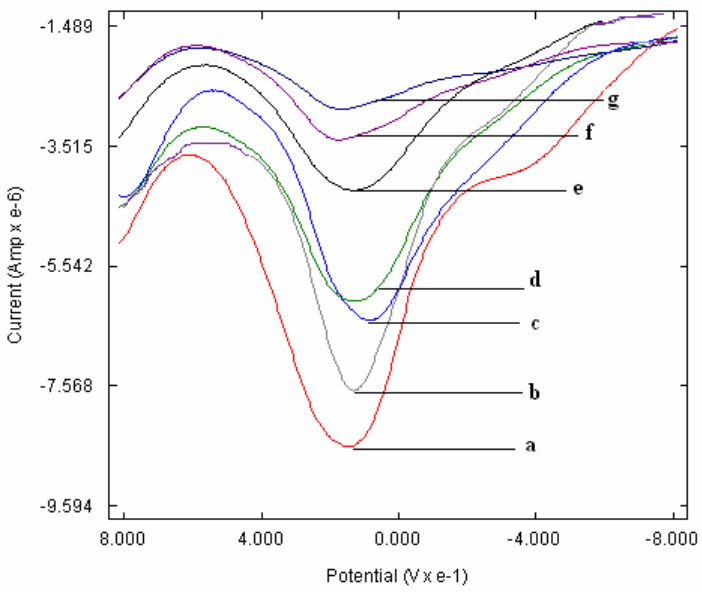


Figure 7.11 Overlay of square wave voltammogram of TMP of different concentrations
(a)1×10^{-3}M (b) 8×10^{-4}M (c) 3×10^{-4}M (d) 9×10^{-5}M (e) 8×10^{-6}M (f) 3×10^{-6}M (g) 1×10^{-6}M

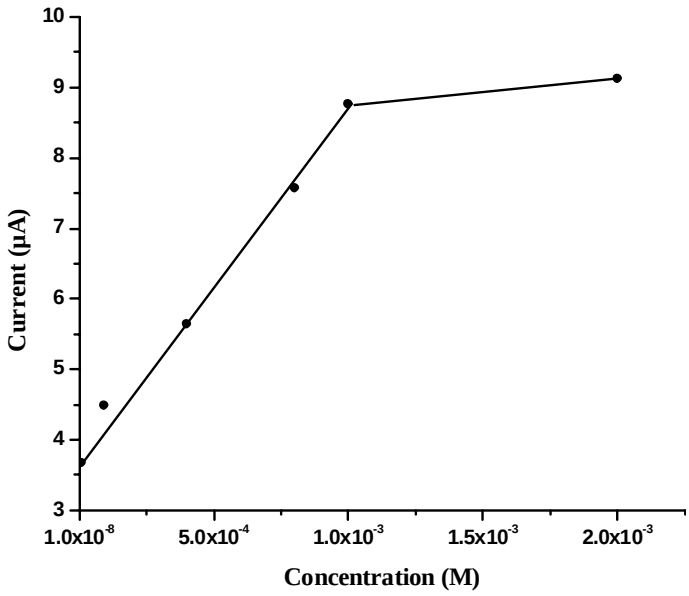


Figure 7.12 Dependence of peak current on concentration

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Chapter 8

Development of Sensors for Tamsulosin Hydrochloride

Contents

This chapter gives details about the electrochemical characteristics of two novel sensors developed for tamsulosin hydrochloride (TAM). The sensors comprise a polypyrrole (PPy) and PPy/MWCNT modified gold electrode. The electrochemical behaviour of TAM at bare GE, PPy modified GE and PPy/MWCNT modified GE are compared and discussed. A uniform film of PPy was formed on the surface of GE by electropolymerization and MWCNT was dropped onto the PPy modified GE surface to form PPy/MWCNT modified GE. The developed sensors worked over a fairly wide concentration range. The developed sensors were found to be useful in the determination of TAM in pharamaceutical formulations and in urine sample.

8.1 Introduction

Tamsulosin hydrochloride, 5-[(2R)-2-[2-(2-ethoxyphenoxy)ethylamino] propyl]-2-methoxybenzenesulfonamide hydrochloride (Figure **8.1**) is a structurally new type of highly selective α_1 -adrenergic receptor antagonist with efficacy in benign prostatic hyperplasia. The α_1-adrenoceptor antagonist activity of TAM has been found to be more potent than other drugs such as prazosin. TAM, an α_1-adrenoceptor blocking agent, exhibits selectivity for α_1 receptors in the human prostate. Molecular and pharmacological studies have led to the division of α_1 -adrenoceptors into three subtypes: α_{1A}, α_{1B}, and α_{1D} adrenoceptors. In in vitro studies, TAM shows a 12 to 20 fold greater affinity for α_{1A}-adrenoceptors than for α_{1B}- and α_{1D}-adrenoceptors respectively [195, 196] and an approximately 12-fold greater affinity for α_1-adrenoceptors in the human prostate than in the human aorta. Approximately 70% of the α_1 adrenoceptors in human prostate are of the α_{1A} subtype. TAM is not intended for use as an antihypertensive agent. It is absorbed from the gastrointestinal tract and is almost completely bioavailable. The extent and rate of absorption are reduced in the presence of food. TAM is given by mouth as the hydrochloride salt. In benign prostatic hyperplasia it is administered in a modified release formulation.

Several analytical methods have been reported for the analysis of TAM. These include high performance liquid chromatography [197, 198], liquid chromatography- mass spectrometry [199], spectrofluorimetry [200], spectrophotometry [201] and voltammetry [202]. Major drawbacks of these reported methods includes potential loss of drug in the re-extraction procedure, lengthy, tedious and time-consuming plasma sample preparation and extraction processes and interference of endogenous substances. Also these methods require sophisticated and expensive instrumentation. In this

circumstance electrochemical methods are often advantageous since it offers high sensitivity, ease of use, portability and relatively inexpensive equipment.

Among the numerous conducting polymers prepared to date, PPy is by far the most extensively studied. The reasons for this intense focus on PPy certainly lie in the fact that the monomer (pyrrole) is easily oxidized and commercially available. Hence, PPy presents several advantages including environmental stability, good redox properties and the ability to give high electrical conductivities [203–205]. As a result of its good intrinsic properties, PPy has proven promising for several applications including batteries, supercapacitors, electrochemical sensors, conductive textiles and fabrics, mechanical actuators, electromagnetic interference shielding, anti static coatings and drug delivery systems. The intrinsic properties of PPy are highly dependent on electropolymerization conditions.

Carbon nanotubes have attracted increasing interest for the construction of new types of electrochemical sensors because of their excellent electrode performance. It is well known that the electronic properties of carbon nanotubes are highly dependent on the surface structures (e.g. the number of defects and functional groups as well as the attachment of adsorbates). As a consequence, carbon nanotubes can serve as excellent substrates for the development of sensors and/or as modifier to promote electron transfer reactions between many biologically important species with the underlying electrode [206-208]. Recently, conducting polymer/CNT composites have been intensively studied to improve the conductivity, electron transport and electromagnetic properties.

The present work describes the preparation of both PPy modified gold electrode sensor and PPy/MWCNT modified gold electrode sensor

and investigate its performance for the electrocatalytic determination of TAM in 0.1 M ABS solution (pH 5). The analytical performance of the developed sensors was evaluated in detail. The sensors were successfully applied for the determination of TAM in pure solutions, pharmaceutical preparations and real sample like urine.

8.2 Preparation of PPy modified GE

Prior to modification, the GE was cleaned as described in section 2.5 of Chapter 2. The PPy modified GE was prepared by electropolymerization of pyrrole on GE. Detailed method for the fabrication of PPy modified GE is given in section 2.6.5 of Chapter 2. PPy thin film was successfully assembled on the surface of a GE by means of electrochemical polymerization, which was carried out with cyclic voltammetric sweeping in the potential range of 0.0 to 0.6 V (Figure **8.2**). The redox potential varies to a certain extent with repetitive potential cycling on the PPy modified GE, indicating that some restructuring of the PPy layer is taking place. In the first scanning, the doping anion dedoped from the polymer,so anodic peak current of the second scan is less than that of the first scan. However, with continuous scanning, the amounts of anion doping and dedoping come into balance and ultimately stable voltammograms are obtained.

Surface area study gave a clear evidence for the successful modification of GE with PPy film. The real surface area of bare GE and PPy modified GE can be determined by SWV in 2 mM $K_3Fe(CN)_6$ solution (Figure **8.3**). From the slope of I_p vs. $\nu^{1/2}$ plot, the area of bare GE and PPy modified GE was estimated and was found to be 1.0050 cm^2 and 2.2102 cm^2 respectively. There was an enhancement in the effective surface area

when GE was modified with PPy which is a strong evidence for the successful modification of GE with polymer film.

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

8.3 Preparation of PPy/MWCNT modified GE

PPy/MWCNT modified electrode was prepared as explained under section 2.6.6 of Chapter 2. MWCNT was immobilized on the surface of PPy modified GE by dropping 3 µL of MWCNT/Nafion suspension (5 mg of MWCNT in a mixture of 300 µL nafion and 2 mL water). Solvent was then evaporated at room temperature to get the PPy/MWCNT modified GE.

The surface area of PPy/MWCNT modified GE was calculated using SWV technique (Figure **8.5)**. The surface area of PPy/MWCNT modified GE was found to be 3.1344 cm^2. Thus, compared with both bare GE as well as PPy modified GE, there was an increase in the surface area for PPy/MWCNT modified GE which is a strong evidence for the successful modification.

SEM images (Figure **8.6**) clearly showed that the electrode surface was effectively modified with PPy and MWCNT.

8.4 Electrochemical performance of TAM at PPy modified GE and PPy/MWCNT modified GE

Stock solution of TAM was prepared as described in section 2.7.6 of Chapter 2. Standard solutions of the analyte (1×10^{-3}- 1×10^{-8} M) were prepared by serial dilution of stock solution with the supporting electrolyte (ABS, pH 5). Sample solution (1×10^{-3}M) was taken in the electrochemical cell and then de aerated with nitrogen for 5 minutes.

Square wave voltammetric technique was used to investigate the electrochemical behaviour of ampicillin, ceftriaxone sodium, trimethoprim, acyclovir, sparfloxacin, sulfamethoxazole, PAM chloride and TAM at bare GE, PPy modified GE and PPy/MWCNT modified GE in 0.1 M ABS (pH 5). Initially an attempt was made to study the electrochemical behaviour of ampicillin. Unfortunately at bare GE, the anodic peak of ampicillin was obtained at a high potential of 1.010 V with a low peak current of 4.3915 μA. When PPy and PPy/MWCNT modified GE was used, anodic peak was obtained at 1.089 V and 1.1434 V with peak current of 3.4022 μA and 3.6072 μA respectively. Thus compared to bare GE, the peak current of ampicillin showed a marked decrease and the peak potential showed a positive deviation. The various experimental parameters such as pH, supporting electrolyte, film thickness and the amount of MWCNT were varied and their effects on the results were studied. However this hardly had any impact on the progress of results.

Under the same experimental conditions at bare GE, an oxidation peak was obtained at 1.060 V for ceftriaxone sodium. But at PPy and PPy/MWCNT modified GE, oxidation peak was obtained at 1.2143 V and 1.2014 V. Here also various experimental parameters were altered to get better results. But the results were not good.

Further, the electrochemical behaviour of trimethoprim and acyclovir was investigated. Both of them gave no voltammetric response at bare GE, PPy modified GE and PPy/MWCNT modified GE.

The electrochemical characteristics of sparfloxacin were next studied using bare GE, PPy modified GE and PPy/MWCNT modified GE. At bare

GE, sparfloxacin gave an oxidation peak at 0.985 V. However at PPy and PPy/MWCNT modified GE, no voltammetric response was obtained.

Also the voltammetric behaviour of the drug sulfamethoxazole and PAM Chloride were investigated. The electrooxidation of sulfamethoxazole occurs at a potential of 0.918 V and that of PAM Chloride occurs at a potential of 0.614 V at bare GE. But no response was obtained at PPy and PPy/MWCNT modified GE for sulamethoxazole and PAM Chloride.

Finally the electrochemical behaviour of the drug TAM was investigated. SWV was carried out from 0.0 to 1.4 V at bare GE, PPy modified GE and PPy/MWCNT modified GE (Figure **8.7** and Figure **8.8)**. At bare GE, the oxidation peak was obtained at 1.220 V. An oxidation peak at 1.094 V was obtained for TAM at PPy modified GE. Compared with bare GE, the oxidation peak potential has shifted negatively by about 0.126 V and this behaviour clearly demonstrated the electrocatalytic activity of PPy film on the electrochemical determination of TAM.

At PPy/MWCNT modified GE, TAM gave a well defined oxidation peak at 0.990 V. Compared with bare GE, there was a significant negative shift in the anodic peak potential by about 0.230 V at PPy/MWCNT modified GE. Also, when compared with PPy modified GE, there was a reduction in the potential by 0.104 V at PPy/MWCNT modified GE. The electrochemical behaviour of TAM was also studied at MWCNT modified GE. An oxidation peak was obtained at 1.026 V for MWCNT modified GE. But the reproducibility of the results were very poor at MWCNT modified GE. Thus it is obvious that polymer film supported MWCNT have better catalytic activity towards the electrochemical oxidation of TAM. Figure **8.9**

displays the voltammetric behaviour of TAM at bare GE, PPy modified GE and PPy/MWCNT modified GE.

8.5 Optimization of Experimental Parameters

The performance of the developed sensors depends on many experimental parameters such as effect of supporting electrolyte, effect of pH, effect of scan rate, effect of film thickness, effect of concentration and interference study. Each of these parameters has to be optimized to get best voltammetric response at the modified electrodes.

8.5.1 Effect of supporting electrolyte

The electrochemical response of TAM in various media such as 0.1 M solutions of phosphate buffer, acetate buffer, H_2SO_4, NaOH and tetra -n-butyl ammonium chloride were studied by SWV. The best oxidation response was obtained in ABS as the peak current is the highest. So 0.1 M acetate buffer was taken as the experimental medium for the determination of TAM.

8.5.2 Effect of pH

The electrochemistry of TAM is generally pH dependent. Thus, the electrochemical behaviour of TAM at PPy and PPy/MWCNT modified GE was studied at different pH values using SWV. The pH range studied was from 3-10. The oxidation peak current increased with an increase in the pH and thereafter decreased at both the developed sensors. It was observed that a high peak current as well as a well defined oxidation peak was obtained at pH 5. The results are presented in Figure **8.10** and Figure **8.11**. So ABS of pH 5 was chosen as the electrolyte for the determination of TAM.

8.5.3 Effect of scan rate

The effect of potential sweep rate was investigated in ABS solution (pH 5) containing TAM using SWV. The oxidation peak current of 1×10^{-3} M TAM at different scan rates ranging from 50-250 mV/s was measured. It was found that anodic peak current increases with an increase in the scan rate. The results are illustrated in Figure **8.12** and Figure **8.13**. The oxidation peak current varies linearly with square root of scan rate indicating that the oxidation of TAM at the modified electrodes is diffusion controlled (Figure **8.14** and Figure **8.15**).

According to the Laviron's equation the relationship between the peak potential (E_p) and scan rate (υ) was examined at the developed sensors. It was found that E_p varies linearly with ln υ (Figure **8.16** and Figure **8.17**). The no. of electrons (n_a) involved in the reaction can be calculated from the slope of the plot according to the relation, $b= RT/\alpha n_a F$. The obtained value for n_a at PPy modified GE is 2.03 and at PPy/MWCNT modified GE is 1.98 (around 2). This confirms that 2 electrons are involved in the oxidation of TAM.

The anodic oxidative behaviour of TAM is due to 2 electron oxidation of alkoxybenzene group to quinone [209, 210] (Figure **8.18**).

8.5.4 Effect of film thickness

To investigate the effect of film thickness on the electrochemical response towards TAM, the parameters influencing the film thickness including the number of cycles during electropolymerization and the amount of MWCNT/Nafion suspension were studied. It was found that the maximum current was obtained when the number of cycles was 20. The anodic peak current slowly 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 with an increase in film thickness. Also, the repeatability and stability for the film modified electrode were not good when the cycles were less than 20. The relationship between the amount of MWCNT- nafion and the oxidation peak current of TAM was also studied. When the amount of MWCNT-nafion supension was 3 μL, the peak current reached its maximum value (Figure **8.19**). The enhancement of the current indicates that the specific surface area and the number of catalytic sites increase with an increase of MWCNT. But, nafion is an insulator and forbid the charge transfer of TAM at the film electrode. So when the amount exceeded 3 μL the oxidation peak current decreased as too much of nafion retarded the electron transfer of TAM.

8.5.5 Effect of concentration

Under the optimized experimental conditions, the effect of concentration of TAM on the peak current at PPy modified GE was investigated by SWV (Figure **8.20**). A linear response was found to obtain from 4.0×10^{-8} M – 1.0×10^{-6} M (Figure **8.21**) with a detection limit of 9.0×10^{-9} M.

The dependence of peak current on the concentration of TAM at PPy/MWCNT modified GE was studied by SWV (Figure **8.22**). The oxidation peak current was found to increase with an increase in the concentration over the range 3.0×10^{-8} M to 1.0×10^{-6} M (Figure **8.23**). The detection limit was found to be 5.0×10^{-9} M. Table **8.1** represents the comparative study of the characteristics of the developed method with some of the reported methods. The method showed good linear concentration range and detection limit with respect to the other methods reported in literature.

8.5.6 Stability and reproducibility

The storage stability of PPy modified GE was evaluated by measuring the current response of TAM once in every day. The results show that the developed sensor retained its original current response in the first continuous 5 days. The modified electrode was reused by stored it in ABS of pH 5. The reproducibility of peak current of PPy modified GE was checked by repeating the experiments with 1×10^{-3} M TAM concentration. The relative standard deviation (RSD) of peak current for six successive determinations of TAM was obtained as 2.7 %. This shows that the modified electrode has excellent reproducibility.

The storage stability of PPy/MWCNT modified GE was also examined. It was found that the electrode surface showed stability for 7 days. The stability of the modified electrode can be related to the PPy film, which stabilizes the activity of MWCNT. The reproducibility was investigated with six successive determinations of 1×10^{-3} M TAM concentration. The RSD was 3.1% for the response current to TAM, demonstrating that the fabrication procedure was reliable and the modified electrode has good reproducibility.

8.5.7 Interference Study

The selectivity of PPy modified GE was investigated by examining the effects of foreign species on the determination of TAM (1×10^{-3} M). It was observed that some possible interfering species like glycine, sodium chloride, potassium chloride, dextrose, lactose and urea did not cause any interference when present in 100 fold excess than TAM (signal change below 5 %). However ascorbic acid interferes severely. The results are given in Table **8.2**.

The selectivity of PPy/MWCNT modified GE was also evaluated by studying the interference of foreign compounds on the determination of TAM at the 1×10^{-3} M level. It was found that glycine, sodium chloride, potassium chloride, dextrose, lactose and urea almost did not interfere with the current response of the drug (signal change below 5 %), however ascorbic acid interferes severely (Table **8.3**).

8.6 Analytical Applications

The developed sensors were applied for the determination of TAM in pharmaceutical formulation. The practical utility of the developed sensors in the determination of TAM in urine sample was also studied.

8.6.1 Analysis of Pharmaceutical Formulations for Determination of TAM

The developed sensors were applied for the determination of TAM content in tablet (Veltam, Intas pharmaceuticals, India). The TAM content in the tablet was determined by calibration method. The detailed procedure for the determination is given in section 2.10.5 of chapter 2. The results obtained are summarized in Table **8.4**. The results shown in Table **8.4** are in good agreement with the declared TAM content and showed a high degree of precision.

The results obtained by using PPy modified GE and PPy/MWCNT modified GE were compared with those obtained by the standard method [109]. The results show that the developed method is comparable to the standard method.

8.6.2 Determination of TAM in urine sample

The developed sensors were employed for the determination of the drug in urine samples. Urine samples of 5 ml were taken in different 25 ml standard flasks. An adequate amount of TAM corresponding to 1×10^{-3} M

was added to the urine sample. This solution was quantitatively diluted using ABS to obtain various concentrations. The prepared solution was analysed for TAM using PPy modified GE and PPy/MWCNT modified GE by SWV method and the unknown concentrations were determined from the calibration graph. The results are shown in Table **8.5** and Table **8.6**.

8.7 Conclusions

Two novel voltammetric sensors were fabricated for the determination of TAM using PPy modified GE and PPy/MWCNT modified GE. Of the two sensors, the PPy/MWCNT modified GE showed a better voltammetric response towards TAM than PPy modified GE. TAM gave a well defined oxidation peak at 1.094 V on PPy modified GE and the linear respose range was found to be 4.0×10^{-8}M to 1.0×10^{-6} M with a detection limit of 9.0×10^{-9} M. But, at PPy/MWCNT modified GE the oxidation peak was obtained only at 0.990 V and the linearity was observed over the range of 3.0×10^{-8} – 1.0×10^{-6} M with a detection limit of 5.0×10^{-9} M. The combined electrocatalytic activity of PPy film and the MWCNT was responsible for the enhanced sensitivity of PPy/MWCNT modified GE. The developed sensors were successfully applied for the determination of TAM in pharmaceutical formulation and in urine sample.

Table 8.1 Comparison of various analytical methods

Analytical method	Linear concentration range (M)	Detection limit (M)	Reference
High performance liquid chromatography	4.0×10^{-8} - 4.0×10^{-6}	4.0×10^{-8}	[197]
Reverse phase high performance liquid chromatography	5.0×10^{-4} - 5.0×10^{-3}	1.1×10^{-4}	[198]
Liquid chromatography - Electronspray ionization mass spectrometry	2.0×10^{-8}- 3.0×10^{-6}	1.0×10^{-8}	[199]
spectrofluorimetry	5.0×10^{-3} - 3.0×10^{-2}	1.3×10^{-3}	[200]
Spectrophotometry	7.5×10^{-3} - 2.2×10^{-2}	3.0×10^{-3}	[201]
Voltammetry	2.0×10^{-6} - 4.0×10^{-4}	3.3×10^{-7}	[202]
Voltammetry (present methods)	4.0×10^{-8} - 1.0×10^{-6}	9.0×10^{-9}	
	3.0×10^{-8} - 1.0×10^{-6}	5.0×10^{-9}	

Table 8.2 Study of the effect of foreign substances on the anodic peak current of TAM at PPy modified GE

TAM taken – 1.00×10^{-3} M

Foreign species	Tolerance limit (M)	Signal change (%)
Glycine	1×10^{-1}	3.05
Sodium chloride	1×10^{-1}	2.00
Potassium chloride	1×10^{-1}	3.85
Dextrose	1×10^{-1}	2.16
Lactose	1×10^{-1}	2.93
Urea	1×10^{-1}	1.99
Ascorbic acid	1×10^{-1}	12.3

Table 8.3 Study of the effect of foreign substances on the anodic peak current of TAM at PPy/MWCNT modified GE

TAM taken – 1.00×10^{-3} M

Foreign species	Tolerance limit (M)	Signal change (%)
Glycine	1×10^{-1}	2.82
Sodium chloride	1×10^{-1}	2.13
Potassium chloride	1×10^{-1}	3.14
Dextrose	1×10^{-1}	2.98
Lactose	1×10^{-1}	2.45
Urea	1×10^{-1}	1.32
Ascorbic acid	1×10^{-1}	12.1

Table 8.4 Determination of TAM in pharmaceutical formulation

Sample	Declared Amount (mg/tablet)	Method Adopted	Found* (mg/tablet)	S.D*	C.V*
Veltam (Intas pharmaceuticals, India)	200	PPy/GE	204	2.54	1.24
		PPy/ MWCNT/GE	205	4.35	2.12
		Standard Method	202	3.86	1.91

* Average of six replicates

Table 8.5 Determination of TAM in urine sample using PPy modified GE

Added (M)	Found (M)	Recovery (%)
1.00×10^{-6}	1.05×10^{-6}	105
4.00×10^{-7}	4.17×10^{-7}	104
1.00×10^{-7}	1.04×10^{-7}	104

Table 8.6 Determination of TAM in urine sample using PPy/MWCNT modified GE

Added (M)	Found (M)	Recovery (%)
1.00×10^{-6}	1.01×10^{-6}	101
4.00×10^{-7}	3.96×10^{-7}	99.0
1.00×10^{-7}	1.03×10^{-7}	103

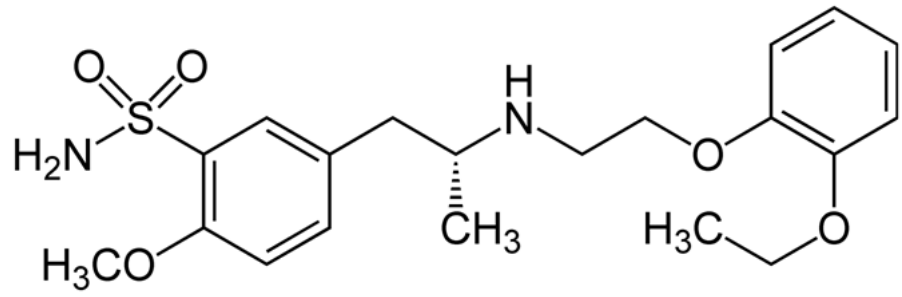


Figure 8.1 Structure of TAM

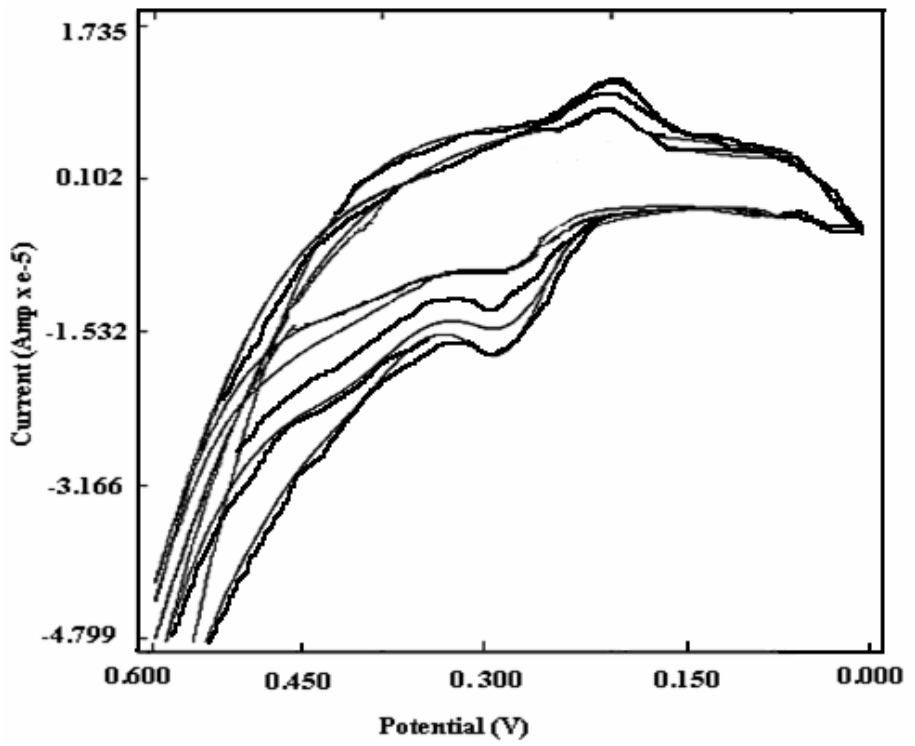
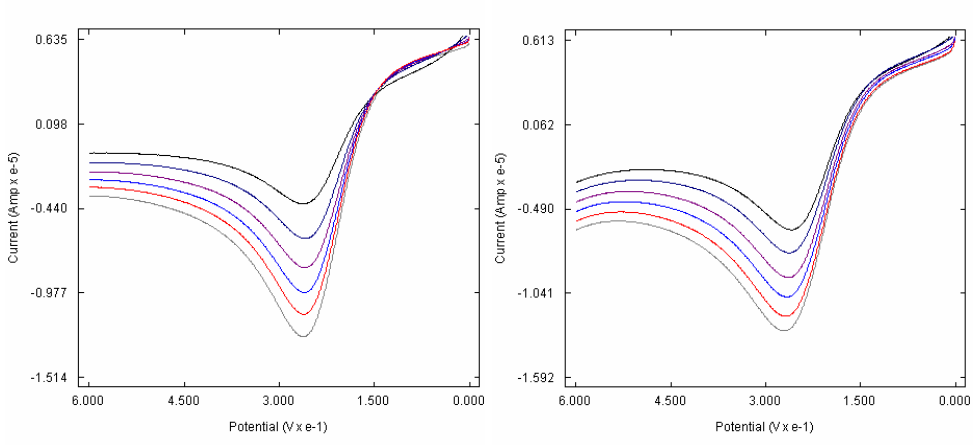
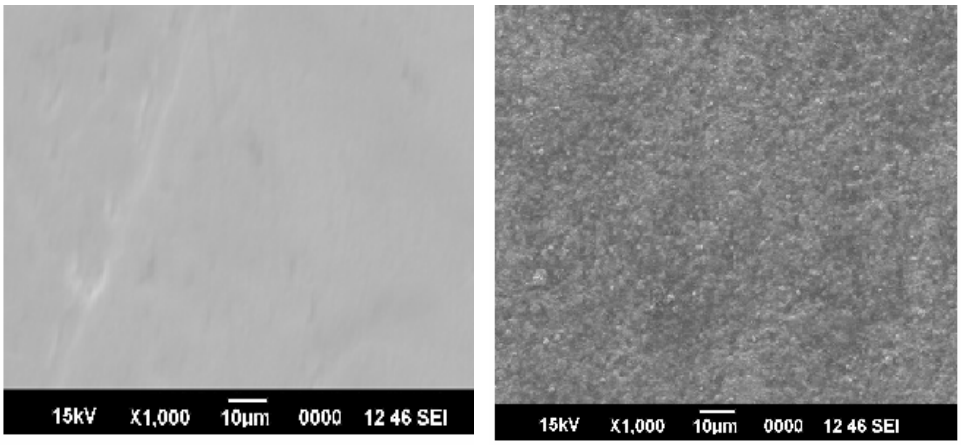


Figure 8.2 Cyclic voltammograms obtained during electropolymerisation of PPy on GE



(a) **(b)**

Figure 8.3 Overlay of square wave voltammogram of 2 mM K_3 $Fe(CN)_6$ solution obtained at (a) bare GE (b) PPy modified GE



(a) **(b)**

Figure 8.4 SEM image of (a) bare GE (b) PPy modified GE

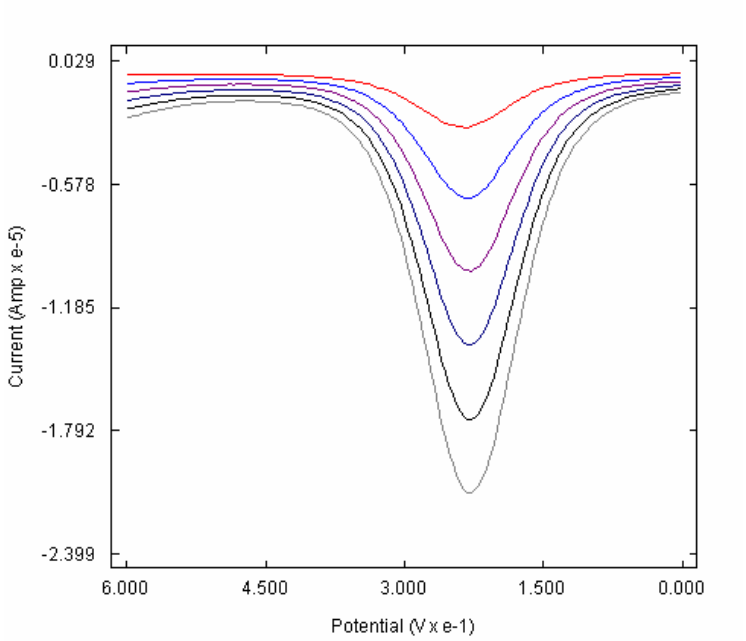


Figure 8.5 Overlay of square wave voltammogram of 2 mM $K_3Fe(CN)_6$ solution obtained at PPy/MWCNT modified GE

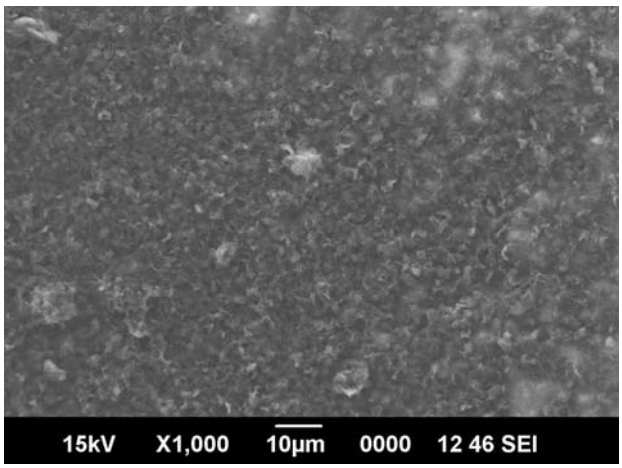


Figure 8.6 SEM image of PPy/MWCNT modified GE

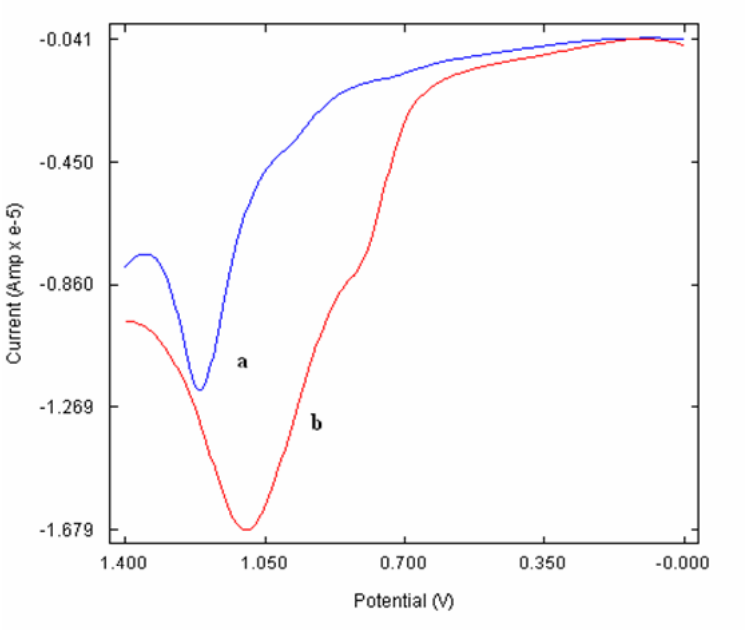


Figure 8.7 Square wave voltammmogram of $1x10^{-3}$ M TAM at (a) bare GE (b) PPy modified GE

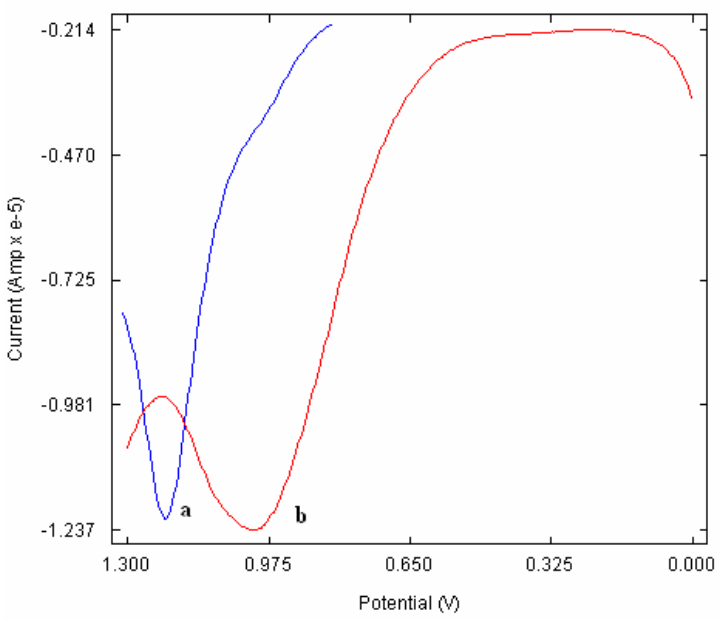


Figure 8.8 Square wave voltammmogram of $1x10^{-3}$ M TAM at (a) bare GE (b) PPy/MWCNT modified GE

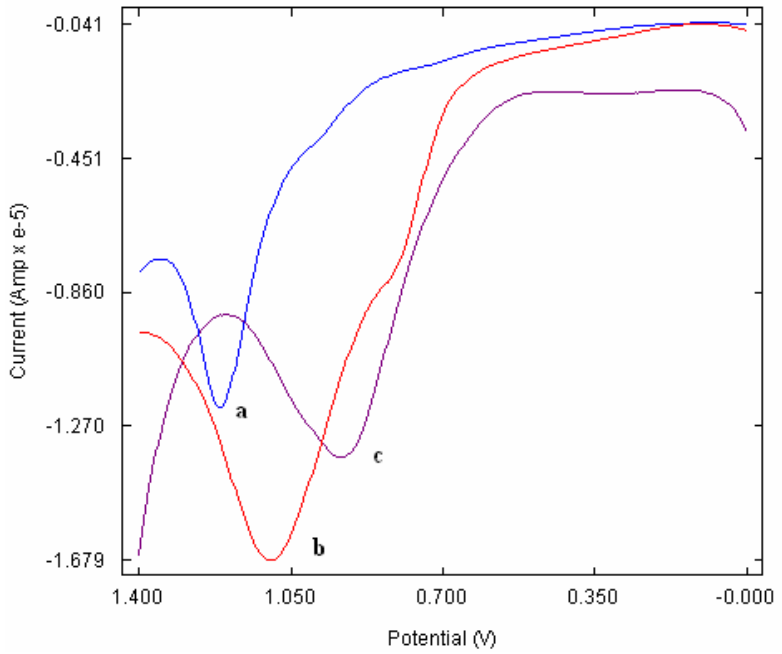


Figure 8.9 Overlay of square wave voltammmogram of $1x10^{-3}$ M TAM at (a) bare GE (b) PPy modified GE (c) PPy/MWCNT modified GE

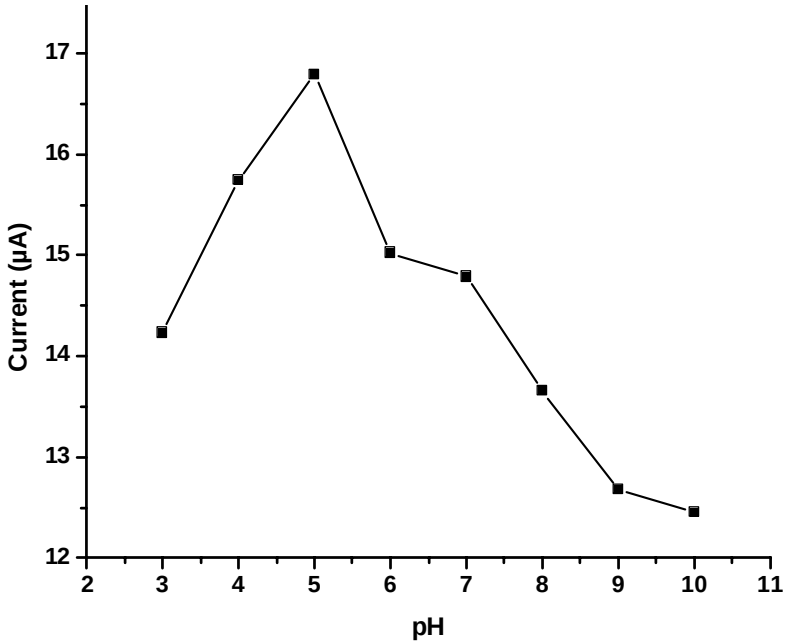


Figure 8.10 Effect of pH on the oxidation peak current of TAM at PPy modified GE

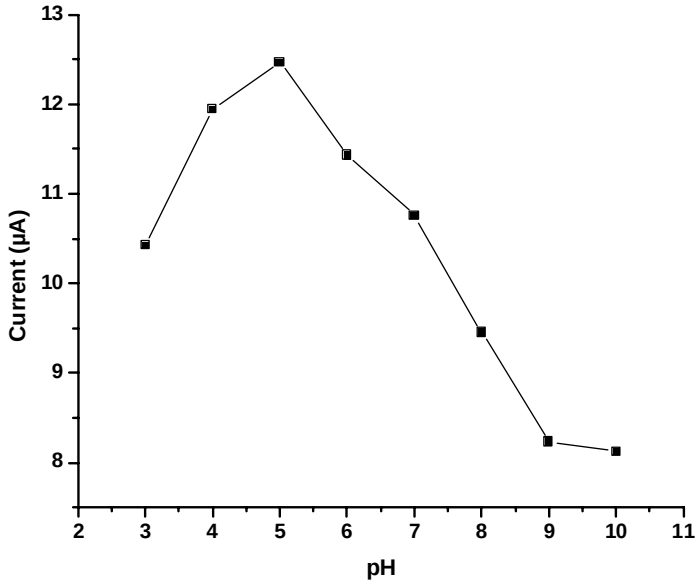


Figure 8.11 Effect of pH on the oxidation peak current of TAM at PPy/MWCNT modified GE

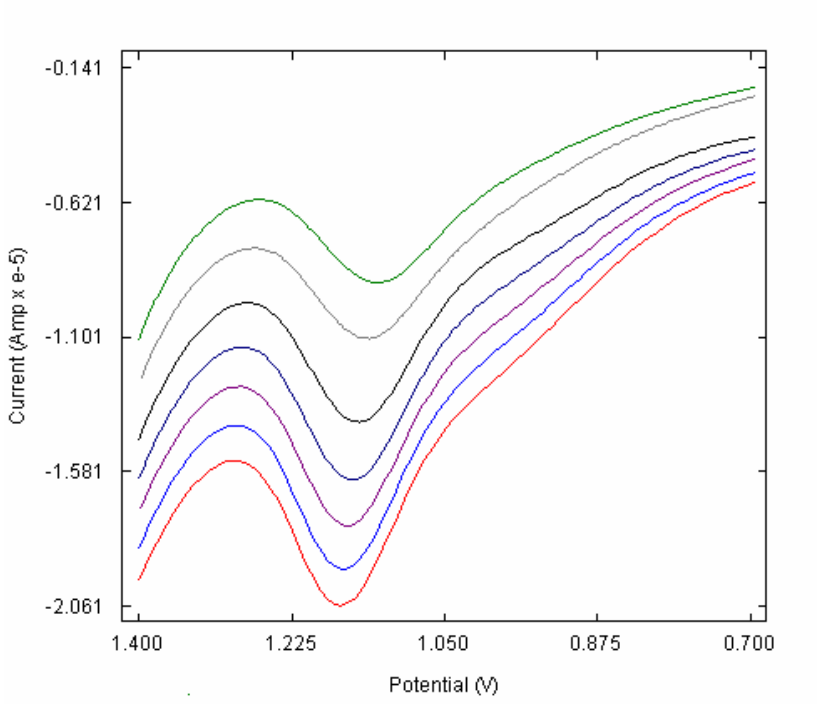


Figure 8.12 Overlay of square wave voltammograms of TAM at PPy modified GE in acetate buffer solution (pH 5) at different scan rates

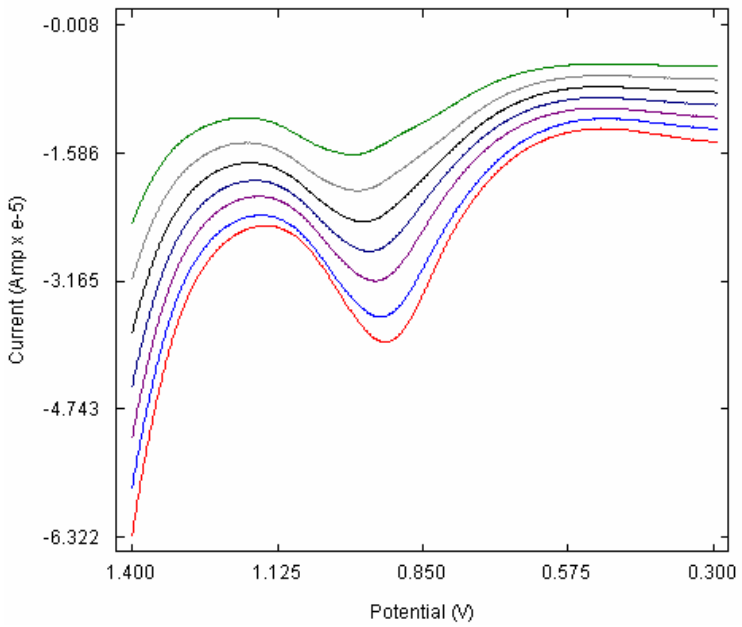


Figure 8.13 Overlay of square wave voltammograms of TAM at PPy/MWCNT modified GE in acetate buffer solution (pH 5) at different scan rates

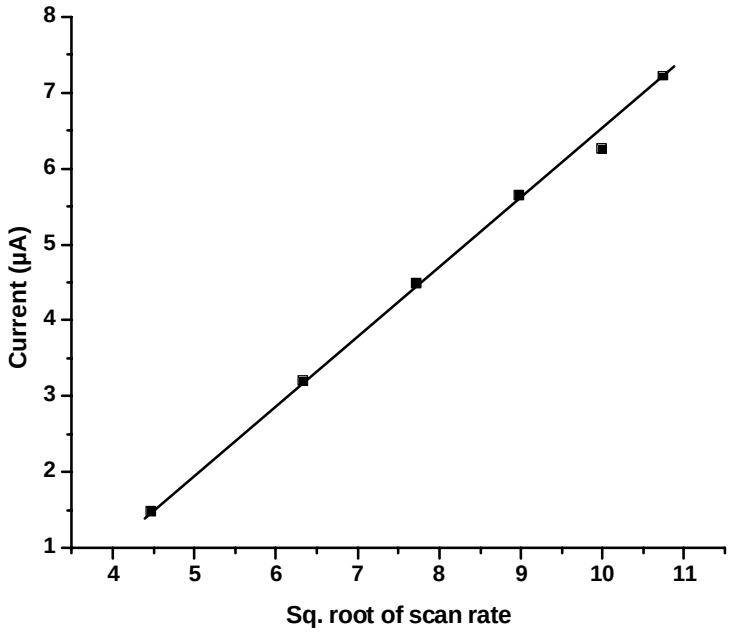


Figure 8.14 Effect of scan rate at PPy modified GE

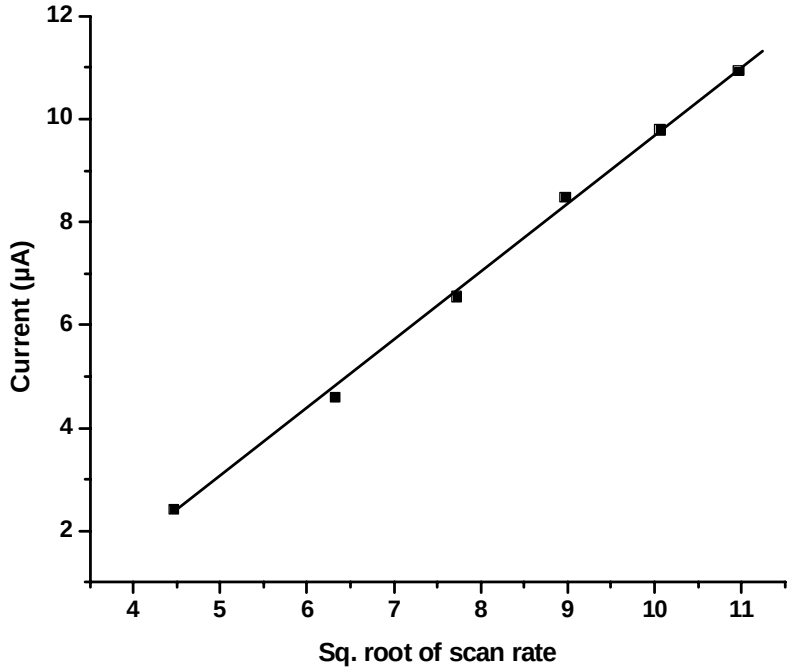


Figure 8.15 Effect of scan rate at PPy/MWCNT modified GE

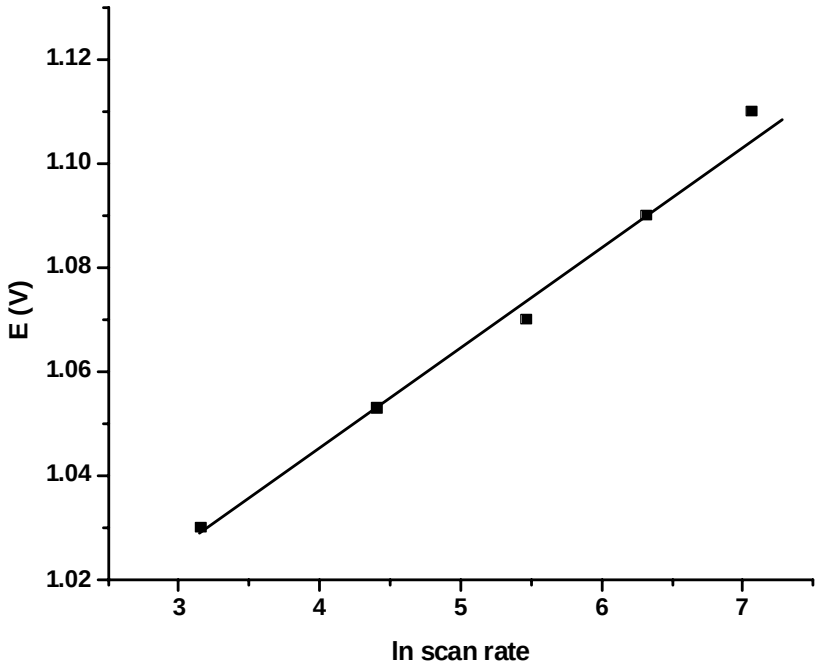


Figure 8.16 Plot of peak potential against ln (scan rate) at PPy modified GE

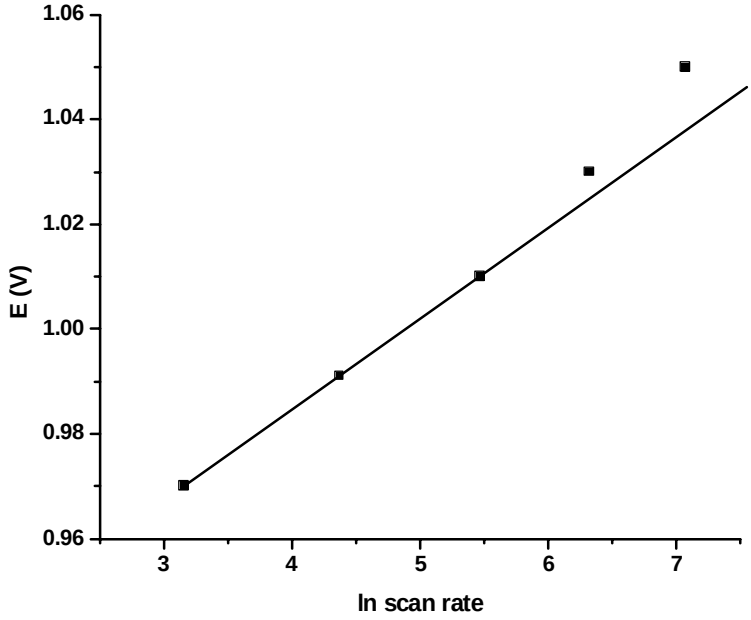


Figure 8.17 Plot of peak potential against ln (scan rate) at PPy/MWCNT modified GE

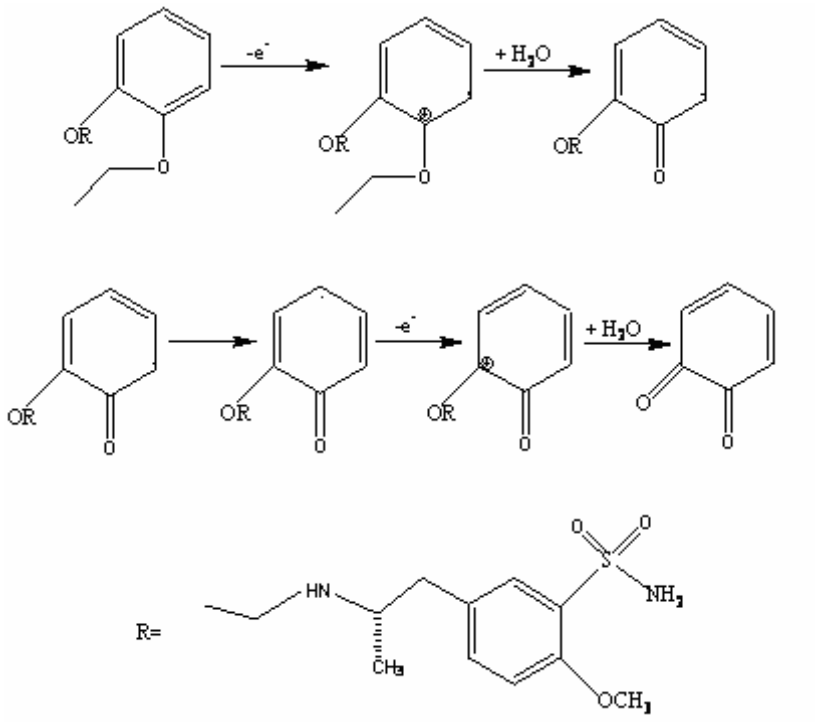


Figure 8.18 Reaction mechanism of oxidation of alkoxybenzene group in TAM to quinone

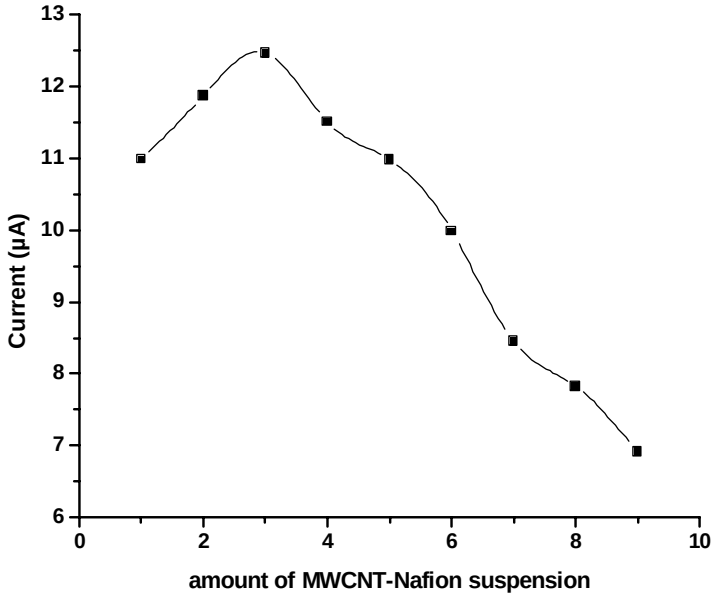


Figure 8.19 Effect of the amount of MWCNT-Nafion suspension at PPy/MWCNT modified GE

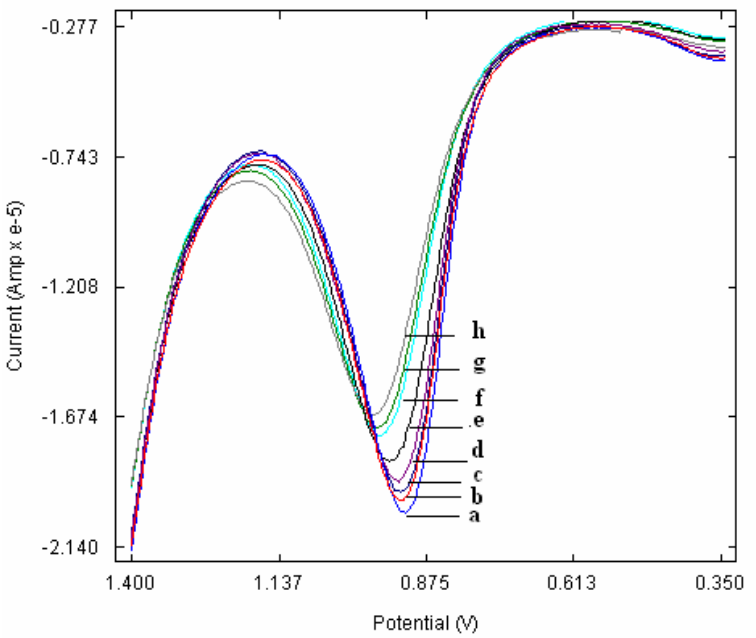


Figure 8.20 Square wave voltammogram of TAM of different concentrations at PPy modified GE (a) 8×10^{-4} M (b) 5×10^{-4} M (c) 2×10^{-4} M (d) 8×10^{-5} M (e) 5×10^{-5} M (f) 2×10^{-5} M (g) 8×10^{-6} M (h) 5×10^{-6} M

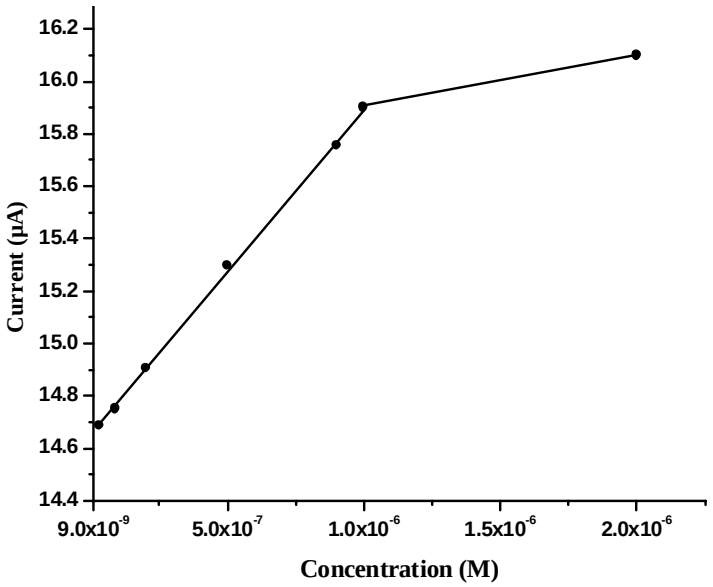


Figure 8.21 Effect of concentration at PPy modified GE

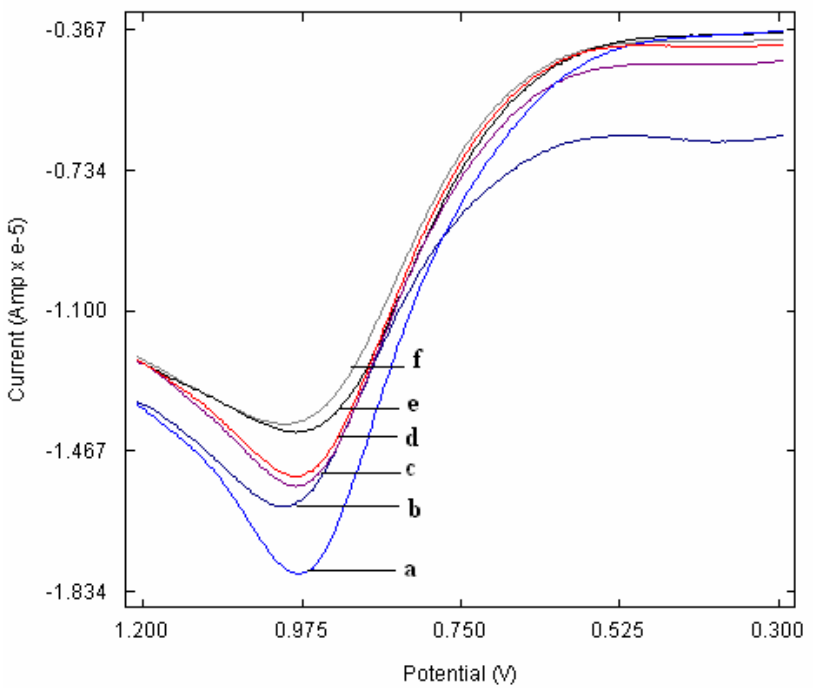


Figure 8.22 Square wave voltammogram of TAM of different concentrations at PPy/MWCNT modified GE (a) 9×10^{-3} M (b) 6×10^{-3} M (c) 4×10^{-3} M (d) 2×10^{-3} M (e) 9×10^{-4} M (f) 6×10^{-4} M

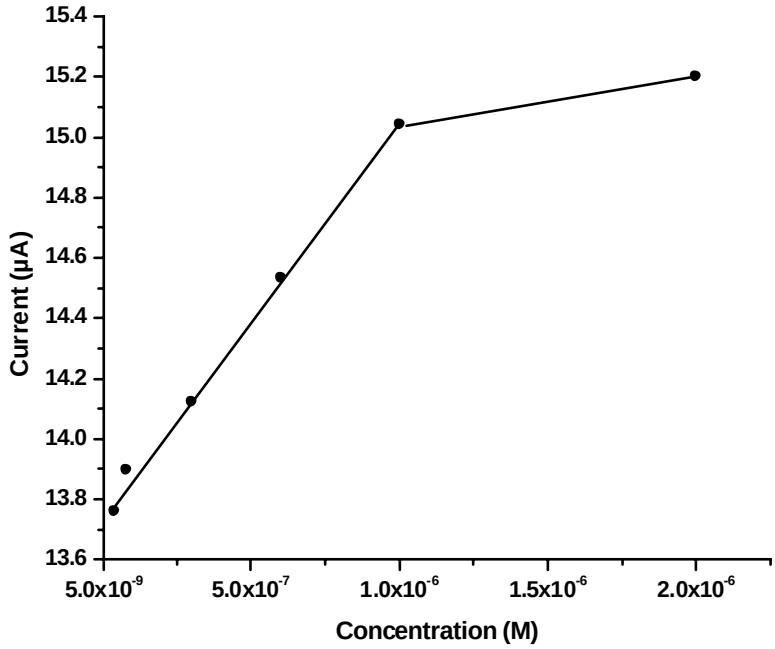


Figure 8.23 Effect of concentration at PPy/MWCNT modified GE

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Chapter 10

Summary and Conclusions

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

The development of electrochemical sensors is currently one of the active areas of research in analytical chemistry. Voltammetric sensors as an important class of electrochemical sensors are extensively used in pharmaceutical applications. In voltammetric analysis, many active compounds in dosage forms, in contrast to excipients, can be readily oxidised or reduced at the electrode surface by applying a potential. Chemically modified electrodes have great significance in the electrochemical determination of pharmaceuticals. The modification of electrode results in efficient determination of electroactive species at very lower potential without any major interferences.

The present study involves the fabrication of 8 voltammmetric sensors for the drugs Metronidazole benzoate, Sulfamethoxazole, Acyclovir, PAM chloride, Trimethoprim, Tamsulosin hydrochloride and Ceftriaxone sodium. Two sensors were developed for the drug tamsulosin hydrochloride while one sensor each was developed for the other drugs.

The different experimental stages involved in the work are

1) Chemical modification of electrode surface

2) Effective surface area calculation as an evidence for modification of electrode surface

3) Study of surface morphology of the electrode surface using SEM technique

4) Fabricaton of different types of sensors

5) Study of the electrochemical response characteristics of the developed sensors such as effect of supporting electrolyte, effect of pH, effect of scan rate, effect of concentration and interference study

6) Analytical applications of the developed sensors

7) Comparison with other reported methods

The salient features of the developed sensors are summarized in the following table

Sensor developed	**Supporting electrolyte used**	**Detection limit (M)**	**Linear concentration range (M)**	**Difference in peak potential between the bare electrode and modified electrode**
TMHPP Zn(II) based carbon paste sensor for MTZB	Phosphate buffer (pH 7)	4.0×10^{-6}	1.0×10^{-3}– 1.0×10^{-5}	Peak potential lowered by 0.117 V
TMHPP Cu(II) based carbon paste sensor for SM	Phosphate buffer (pH 6)	1.0×10^{-9}	1.0×10^{-8} – 1.0×10^{-6}	Peak potential lowered by 0.956 V
MBZ/ TMHPP Cu(II) based gold sensor for ACV	Phosphate buffer (pH 7)	1.0×10^{-9}	6.0×10^{-8}– 1.0×10^{-6}	Peak appears at 0.220 V
DT/MWCNT based gold sensor for PAM Chloride	Phosphate buffer (pH 8)	1.0×10^{-8}	8.0×10^{-7} – 1.0×10^{-3}	Peak potential lowered by 0.184 V
PANI based gold sensor for TMP	Acetate buffer (pH 4)	1.0×10^{-8}	2.0×10^{-7} – 1.0×10^{-3}	Peak appears at 0.192 V
PPy based gold sensor for TAM	Acetate buffer (pH 5)	9.0×10^{-9}	4.0×10^{-8} – 1.0×10^{-6}	Peak potential lowered by 0.126 V
PPy/MWCNT based gold sensor for TAM	Acetate buffer (pH 5)	5.0×10^{-9}	3.0×10^{-8} – 1.0×10^{-6}	Peak potential lowered by 0.230 V
POAP based gold sensor for CFS	Acetate buffer (pH 5)	1.0×10^{-8}	2.0×10^{-7} – 5×10^{-6}	Peak potential lowered by 0.100 V

Voltammetric sensors based on chemically modified electrodes have attracted much attention in the study of pharmaceuticals, due to their simplicity, low cost and relatively short analysis time compared with the other techniques. It is hoped that the developed sensors can find wide applications in the future analytical chemistry.

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References

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

[2] C. M. A. Brett and A. M. O. Brett, *Electroanalysis,* Oxford university press, Oxford (1998).

[3] C. M. A. Brett, *Pure Appl. Chem.*, **73**, 1969 (2001).

[4] http://www.prenhall.com/settle/chapters/ch37.

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

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

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

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

[9] C. C. Liu, *Electrochemical Sensors, The Biomedical Engineering Handbook*, IInd Edn, Ed. Joseph D. Bronzino, Boca Raton: CRC Press LLC (2000).

[10] J. Obirai and T. Nyokong, *Electrochim. Acta,* , **49**, 1417 (2004).

[11] Zilbermann, J. Hayon, T. Katchalski, R. Ydgar, J. Rishpon, A. I. Shames, E. Korin, and A. Bettlelheim, *Inorg. Chim. Acta*, , **305**, 53 (2000).

[12] C. A. Caro, F. Bedioui and J. H. Zagal, *Electrochim. Acta*, **47**, 1489 (2002).

[13] M. J. Cook, *J. Mater. Chem.,* **6**, 677 (1996).

[14] B. M. Hassan, H. Li and N. B. Mckeown, *J. Mater. Chem.*, **10**, 39 (2000).

[15] M. J. Cook, *Pure Appl. Chem.,* **71**, 2145 (1999).

[16] J. P. Collman, X. Zhang, V. J. Lee, U. S. Uffelman and J. I. Brauman, *Science*, **261**, 1404 (1993).

[17] F. Bedioui, J. Devynck and C. B. Charreton, *Acc. Chem. Res.*, **28**, 30 (1995).

[18] H. Ohkita, , T. Ogi, R. Kinoshita, S. Ito and M. Yamamoto, *Polymer,* **43**, 3571 (2002).

[19] G. Li, S. Bhosale, S. Tao, R. Guo, F. Li, Y. Zhang, T. Wang and J. H. Fuhrhop, *Polymer,* **46**, 5299 (2005).

[20] L. Zhu, R. Yang, J. Zhai and C. Tian, *Biosens. Bioelectron*., **23**, 528 (2007).

[21] P. Santhosh, K. M. Manesh, A. Gopalan and K. P. Lee, *Sens. Actuat. B-Chem.,* **125,** 92 (2007).

[22] P. Du, S. Liu, P. Wu and C. Cai, *Electrochim. Acta,* **52**, 6534 (2007).

[23] N. Sato and H. Okuma, *Sens. Actuat. B-Chem.,* **129**, 188 (2008).

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

[25] H. O. Finklea, *Electroanalytical Chemistry*, ed. A. J. Bard and I. Rubinstein, Marcel Dekker, New York, **19,** 109 (1996).

[26] H. O. Finklea, *Encyclopaedia of Analytical Chemistry: Applications Theory and Instrumentations*, ed. R. A. Meyers, Wiley, Chichester, **11,** 10090 (2000).

[27] C. C. Leznoff and A. B. P. Lever (Eds), *Phthalocyanine: Properties and Applications*, VCH Publishers, New York, Vol. **1-4** (1989).

[28] J. J. Gooding, F. Mearns, W. Yang and J. Liu, *Electroanal.*, **15,** 81 (2003).

[29] M. J. Cook, I. Chambrier, *Porphyrin Handbook*, K. M. Kadish, K. M. Smith, R. Guilard (Eds) Academic Press, San Diego, California, Vol. **17** (2003).

[30] G. Che, Z. Li, H. Zhang and C.R. Cabrera, *J. Electroanal. Chem.*, **453,** 9 (1998).

[31] J .J. Gooding, V. Praig and E.A.H. Hall, *Anal. Chim Acta*, **70,** 2396 (1998)

[32] R. G. Compton, C. E. Banks, *Understanding voltammetry*, World scientific publishing Co, Singapore (2007).

[33] D. B. Hibbert, *Introduction to electrochemistry*, Macmillan, London (1993).

[34] L.G. Martinez, P.C. Falco and A.S. Cabeza, *J. Pharm. Biomed. Anal.,* **29,** 405 (2002).

[35] C. Z. jing, J. Zhang , Y. J. Cheng, C. G. Ying, W. X. Jie and S. Y. Guo, *J. Chromatogr. B,* **834,** 63 (2006).

[36] N. Ozaltin, *J. Pharm. Biomed. Anal.,* **20**, 599 (1999).

[37] A.S.L. Mendez, V. Weisheimer, T. P. Oppe, M. Steppe and E.E.S. Schapoval, *J. Pharm. Biomed. Anal.,* **37,** 649 (2005).

[38] B. Nigovic and B. Simunic, *J. Pharm. Biomed. Anal.,* **31,** 169 (2003).

[39] B.Uslu and S. A.Ozkan, *Electrochim. Acta*, **49**, 4321 (2004).

[40] K. Jain, V. K. Gupta, L. P. Singh and U. Khurana, *Analyst*, **122,** 583 (1997).

[41] H. Abdine and F. Belal, *Talanta*, **56,** 97 (2002).

[42] M. L. S. Silva, M. B. Q. Garcia , J. L. F. C. Lima and E. Barrado, *Anal. Chim. Acta,* **573,** 383 (2006).

[43] H. Beckett and J. B. Stenlake, *Practical Pharmaceutical Chemistry* (Part II), 4th Edn, C. B. S Publishers and Distributors, Darya Ganj, New Delhi.

[44] D. W. Yang, F. C. Gong and Z. Cao, *Sens. Actuat. B,* **114** 152 (2006).

[45] F. C. Gong, Z. D. Xiao, Z. Cao and D. X. Wu, *Talanta*, **72**, 1453 (2007).

[46] S. S. Huang, H. Tang, and B. F. Li, *Mirochim. Acta,* **128**, 37 (1998).

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

[48] R. Cristescu, C. Popescu, A.C. Popescu , S. Grigorescu , I. N. Mihailescu, A. A. Ciucu, S. Iordache, A. Andronie, I. Stamatin, E. F. Cosma and D. B. hrisey, *Appl. Surf. Sci.*,**257**, 5293 (2011).

[49] X. B. Zhang, Z. Z. Li, C. C. Guoa, S. H. Chen, G. L. Shen and R. Q. Yu, *Anal. Chim. Acta,* **439**, 65 (2001).

[50] K. K. Atkowska, D. Shpakovsky, J. Radecki, H. Radeckaa, Z. Jingwei and E. Milaeva, *Talanta,* **78,** 126 (2009).

[51] X. Lu**,** J. Jin**,** J. Kang**,** B. Lv**,** H. Liu and Z. Geng, *Mater. Chem. Phys.*, **77**, 952 (2003).

[52] F. C. Gong, D. X. Wu, Z. Cao and X. C. He, *Biosens. bioelectron.,* **22,** 423 (2006).

[53] K. I. Ozoemena and T. Nyokong, *Electrochim. Acta,* **51**, 5131 (2006).

[54] J. Ren, H. Zhang, Q. Ren, C. Xia, J. Wan and Z. Qin, *J. Electroanal. Chem.,* **504,** 59 (2001).

[55] F. Zhao, F. Huang, Q. Yan and B. Zeng, *Microchim. Acta,* **150**, 179 (2005).

[56] M. Chen, M. Y. Nie and H. L. Li, *J. Colloid Interface Sci.,* **209,** 421 (1999).

[57] B. Saraswathyamma, I. Grzybowska, C. Orlewska, J. Radecki, W. Dehaen, K. Girish Kumar and H. Radecka, *Electroanalysis*, **20**, 2317 (2008).

[58] X. Lu, B. Lv, Z. Xue, M. Zhang, Y. Wang and J. Kang, *Anal. Lett.,* **35**, 1811 (2002).

[59] S. S. Nair, S. A. John and T. Sagara, *Electrochim. Acta,* **54**, 6837 (2009).

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

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

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

[63] Z.Y. hai, Z. Z. ling, Z.Wei and P.D. Wen, *Sens. Actuat. B-Chem.,* **119,** 308 (2006).

[64] **S.**Shahrokhian and L.Fotouhi, *Sens. Actuat. B-Chem.,* **123,** 942 (2007).

[65] R. N. Goyal, V. K. Gupta and S. Chatterjee, *Biosens. bioelectron.*, **24,** 3562 (2009).

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

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

[68] Q. Zhuang, J. Chen and X. Lin, *Sens. Actuat. B-Chem.,* **128,** 500 (2008).

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

[70] L. Lonappan and K. Girish Kumar**,** *Sensor Lett.*,**9**, 541 (2011).

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

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

[73] S. Issac and K.Girish Kumar, *Drug Test. Analysis*, **1**, 350 (2009).

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

[75] G. Guo, F. Zhao, F. Xiao and B. Zeng, *J. Electrochem. Sci.,* **4,** 1365 (2009).

[76] Babaei and M. Babazadeh, *Electroanalysis*, **68**, 144 (2006).

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

[78] S. Komathi, A. I. Gopalan and K. P. Lee, *Analyst*, **135**, 397 (2010).

[79] D. P. Santos, M. F. Bergamini, V. A. F. F. M. Santos, M. Furlan and M.V. B. Zanoni, *Anal. Lett.,* **40**, 3430 (2007).

[80] G. Yang, C.Wang, R. Zhang, C. Wang, Q. Qu and X. Hu, *Bioelectrochem.,* **3,** 37 (2008).

[81] S. Issac and K. Girish Kumar, *Anal. Methods*, **2**, 1484 (2010).

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

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

[84] D. C. Tiwari, R. Jain and S. Sharma, *J. Sci. Ind. Res.,* **66**, 1011 (2007).

[85] S. Mehretie, S. Admassie, M. Tessema and T. Solomon, *Anal. Bioanal. Electrochem.*, **3**, 38 (2011).

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

[87] C. Fang, X. Tang and X. Zhou, *Anal. Sci.,* **15**, 41 (1999).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[102] D. Alder, F. R. Lonyo and J. D. Finarelli, *J. Org. Chem.,* **32**, 476 (1967).

[103] S. C. Tsang, Y. K. Chen, P. J. E. Harris and M. L. H. Green, *Nature,* **372**, 159 (1994).

[104] S. Sarac, M. Ates, B. Kilic, *Int. J. Electrochem., Sci.,* **3**, 777 (2008).

[105] F. Zhao, Y. Peng, X. Ding and B. Zeng, *Electroanalysis,* **13**, 1367 (2001).

[106] D. L. Franco, A. S. Afonso, L. F. Ferreira, R. A. Goncalves, J. F.C. Boodts, A. G B. Madurro and J. M. Madurro, *Polymer engineering and science,* **48**, 2043 (2008).

[107] United States Pharmacopoeia, The national formulary, United States Pharmacopoeial Convention Inc, Rockville, 2005, **209.**

[108] Pharmacopoeia of India, 3rd Edition, Controller of Publications, Government of India, Delhi, 1985, **407**.

[109] United States Pharmacopoeia, The national formulary Reissue, Official Monographs, USP 33, 981.

[110] K. S. Lakshmi, *Int. J. Pharm. Sci.,* **1**, 22 (2009).

[111] http://www.medicinenet.com/metronidazole/article.htm

[112] K. Farhadi and S. Bahar, *J. Chin. Chem. Soc.,* **54**, 1521 (2007).

[113] P. Nagarajia, K. R. Sunitha, R. A. Vasath and H. S. Yathirajan, *J. Pharm. Biomed. Anal.,* **28**, 527 (2002).

[114] S. Ashour and N. Kattan, *Int. J. Biomed. Sci.*, **6**, 13 (2010).

[115] D. K. Bempong , R. G. Manning, T. Mirza and L.Bhattacharyya, *J. Pharm. Biomed. Anal.,* **38,** 776 (2005).

[116] S. A. Ozkan, Y. Ozkan and Z. Senturk, *J. Pharm. Biomed. Analysis*, **17,** 299 (1998).

[117] M. K. Amini, J. H. Khorasani, S. S. Khaloo and S. Tangestaninejad, *Anal. Biochem.,* **320**, 32 (2003).

[118] S. Shahrokhian and J. Yazdani, *Electrochim. Acta*, **48**, 4143 (2003).

[119] S. Shahrokhian, A. Souri and H. Khajehsharifi, *J. Electroanal. Chem.,* **56**, 95 (2004).

[120] S. Shahrokhian, A. Hamzehloei, A. Tahgani and S. R. Mousavi, *Electroanalysis*, **16**, 915 (2004).

[121] H. A. O. Hill, *Coord. Chem. Rev.,* **151**, 115 (1996).

[122] S. A. Wring and J. P. Hart, *Analyst*, **117**, 1215 (1992).

[123] B. Ortiz, S. I. Mho, D. Ingersoll, N. Doddapaneni and S. M. Park, *J. Electrochem. Soc.,* **142**, 1436 (1995).

[124] M. A. Ruiz, M. G. Blazquez and J. M Pingarron, *Anal. Chim. Acta,* **305**, 49 (1995).

[125] F. Xu, H. Li, S. J. Cross and T. F. Guarr, *J. Electroanal. Chem.,* **368**, 221 (1994).

[126] D. J. Dobson and S. Saini, *Anal. Chem.,* **69**, 3532 (1997).

[127] S. Griveau and F. Bedioui, *Electroanalysis,* **13**, 253 (2001).

[128] P. Zuman and Z. Fijalek, *J. Electroanal. Chem.,* **296**, 583 (1990).

[129] P. Zuman and Z. Fijaiek, *J. Electroanal. Chem.,* **296**, 589 (1990).

[130] E. I. Jammal, J. C. Vive, G. J. Patriarche, and O. N. Palmeiro, *Electroanalysis,* **4**, 57 (1992).

[131] E. Laviron, *J. Electroanal. Chem.,* **52**, 355 (1974).

[132] http://www.medicinenet.com/sulfamethoxazole/article.htm

[133] P. Nagaraja, S. D. Naik, A. K. Shreshta and A. Shivakumar, *Acta Pharm.,* **57,** 333 (2007).

[134] J. Fan, Y. Chen, S. Feng, C. Ye and J. Wang, *Anal. Sci.,* **19**, 419 (2003**)**.

[135] R. Whelpton, G. Watkins and S. H. Curry, *Clin. Chem.,* **27**, 1911 (1981**)**.

[136] T. You, X. Yang and E. Wang, *Analyst,* **123,** 2357 (1998).

[137] United States Pharmacopoeia, XXIth revision, National formulary XVIth ed., US Pharmacopeial Convention, Rockville, 1985.

[138] K. Girish Kumar and P. Indrasenan, *Analyst,* **113**, 1369 (1988).

[139] B. Chiavarino, M. E. Crestoni, A. D. Marzio and S. Fornarini, *J. Chromatogr. Biomed. Appl.,* **706**, 269 (1998).

[140] G. Knupp, H. Pollmann and D. Jonas, *Chromatographia,* **22**, 1 (1986).

[141] Z. L. Zhang and J. L. Zhou, *J. Chromatogr. A,* **205**, 1154 (2007).

[142] C. D. Souza, O. C. Braga, I. C. Vieira and A. Spinelli, *Sensor Actuat. b Chem.,* **135**, 66 (2008).

[143] T. A. M. Msagati and J. C. Ngila, *Talanta,* **58**, 605 (2002).

[144] M. Kotoucek, J. Skopalova and D. Michalkova, *Anal. Chim. Acta,* **353**, 61 (1997).

[145] T N. Rao, B. V. Sarada, D. A. Tryk and A. Fujishima, *J. Electroanal. Chem*., **491**, 175 (2000).

[146] M. V. Astrid, E. Maria, B. Carrera, V. B. Dietrich and B. Carls, *Anal. Chim. Acta,* **159**, 119 (1984**).**

[147] http://www.medicinenet.com/acyclovir/article.htm.

[148] P. Nebinger and M. Koel, *J. Chromatogr. Biomed. Appl.*, **619**, 342 (1993).

[149] S. M. Tadepalli, R. P. Quinn and D. R. Averett, *Antimicrob. Agents Chemother*., **29**, 93 (1986).

[150] K. M. Skubitz, R. P. Quinn and P. S. Lietman, *Antimicrob. Agents Chemother*., **21**, 352 (1982).

[151] J. D. Ibrahim, S. K. Alaa, F. A. Hassan and M. M. Ramadan, *J. I. Farmaco*, **60**, 555 (2005).

[152] K.Basavaiah and H.C. Prameela, *Indian J. Chem. Techn.,* **11**, 759 (2004).

[153] C.V. Hung, A. H. Paul, T. L. Daniel and L. S. Stephen, *J. Chromatog. B*, **772**, 291 (2002).

[154] J. G. Lv, L. R. Luo and Z. J. Zhang, *Anal. Chim. Acta*, **510**, 35 (2004).

[155] H. Heli, M. Zarghan, A. Jabbari, A. Parsaei, A. A. M. Movahedi, *J. Solid State Electrochem.,* **14**, 787 (2010).

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

[157] S. Murat, S. Gulsen, Y. Sultan, O. Erdem and Y. Selehattin, *Curr. Anal. Chem.,* **7**, 130 (2011).

[158] R. W. Murray, in: A. J. Bard (Ed.), *Electroanalytical chemistry*, vol.**13**, Marcel Dekker, New York, 191 (1984).

[159] E. Laviron, in: A. J. Bard (Ed.), *Electroanalytical chemistry*, vol.**12**, Marcel Dekker, New York, 53 (1982).

[160] N. K. Chaki and K. Vijayamohanan, *Biosens.Bioelectron.,* **17**, 1 (2002).

[161] S. Wang, D. Du and X. Xu, *J. Appl. Electrochem.,* **34**, 495 (2004).

[162] B. Agboola, P.Westbroek, K. I. Ozoemena and T. Nyokong, *Electrochem. Commun*., **9**, 310 (2007).

[163] B. Agboola and T. Nyokong, *Talanta,* **72**, 691 (2007).

[164] H. Heli, M. Zarghan, A. Jabbari, A. Parsaei, A. And A. M. Movahedi, *J. Solid State Electrochem.*, **14**, 787 (2010).

[165] J. E. B. Randles, *Trans. Faraday. Soc.,* **44**, 322 (1948).

[166] http://en.wikipedia.org/wiki/Pralidoxime

[167] K. Karljikovic Rajic, B. Stankovic and A. Granov, *J.Pharm. Biomed. Anal.,* **8**, 735 (1990).

[168] H. Kalasz, E. Szoko, T.Tabi, G. Petroianu, D. E. Lorke, A. Omar, S. Alafifi, A. Jasem, and K.Tekes, *Medicinal Chemistry,* **5**, 237 (2009).

[169] M. Bodiroga, D. Agbaba, D. Zivanov-Stakic and R. Popovic, *J.Pharm. Biomed. Anal.,* **12**, 127 (1994).

[170] F. S. Felix, C. M. A. Brett and L. Angnes, *J. Pharm. Biomed. Anal*., **43**, 1622 (2007).

[171] S. F. Wang, F. Xie and R. F. Hu, *Sens. Actuators B*, **123**, 495 (2007).

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

[173] F. Wu, G. Zhao and X. Wei, *Electrochem. Commun.,* **4**, 690 (2002).

[174] S. F. Lv, *Anal. Lett.,* **36**, 2597 (2003).

[175] J. Wang, M. Li, Z. Shi, N. Li and Z. Gu, *Microchem. J.,* **73**, 325 (2002).

[176] L. H. Dubois and R. G. Nuzzo, *Annu. Rev. Phys. Chem*., **43**, 437 (1992).

[177] C. D. Bain, E. B. Troughton, Y. T. Tao, J. Eval, G. M. Whitesides and R. G. Nuzzo, *J. Am. Chem. Soc*., **111**, 321 (1989).

[178] Y. M. Lee, O. Y. Kwon, Y. J. Yoon and K. Ryu, *Biotechnol. Lett.*, **28**, 39 (2006).

[179] F. Zhao, F. Huang, Q. Yan and B. Zeng, *Microchim. Acta,* **150,** 179 (2005).

[180] J. W. Bird and D. G. M. Diaper, *Can. J. Chem.,* **47**, 145 (1969).

[181] http://en.wikipedia.org/wiki/Trimethoprim

[182] A.L. E. Ansary, Y.M. Issa and W. Selim, *Anal. Lett.*, **32**, 955 (1999).

[183] M. Polasek and M. Jambor, *Talanta*, **58**, 1253 (2002).

[184] M. A. Brooks, J. A. F. De Silva and L. M. D. Arconte, *Anal. Chem.*, **45**, 263 (1973).

[185] J. M. L. Gallego and J. P. Arroyo *J. Pharm. Biomed. Anal*., **30,** 1255 (2002).

[186] L. S. A. Romeu, C. R. Filho, Q. B. Cass and O. F. Filho, *Electroanalysis,* **21**, 1475 (2009).

[187] T. A. Skotheim (Eds.), *Handbook of Conducting Polymers*, Marcel Dekker, New York, **1/2** (1994).

[188] S. M. Park, in H.S. Nalwa (Eds.), *Handbook of Organic Conductive Molecules and Polymers*, Wiley, Chichester, England, **3** (1997).

[189] S. M. Park and H. J. Lee, *Bull. Korean Chem. Soc.,* **26**, 697 (2005).

[190] D. C. Trivedi, in H.S. Nalwa (Ed.), *Handbook of Organic Conductive Molecules and Polymers,* Wiley, Chichester, England, **2** (1997).

[191] W. Y. Tu, B. S. Xu, S. Y. Dong, H. D. Wang and J. Bin, *J. Mater. Sci.,* **43**, 1102 (2008).

[192] N. Gospodinova and L. Terlemezyan, *Prog. Polym. Sci.,* **23**, 1443 (1998).

[193] [J. Y. Lee, L. H. Ong and G. K. Chuah, *J. Appl. Electrochem.,* **23**, 1031 (1993).

[194] R. N. Goyal and A. Kumar, *Electroanalysis*, **2,** 539 (1990).

[195] R. Foglar, K. Shibata, K. Horie, A. Hirasawa and G. Tsujimoto, *Eur. J. Pharmacol.,* **288,** 201 (1995).

[196] Leonardi, J. P. Hieble, L. Guarneri, D. P. Naselsky, E. Poggesi, G. Sironi, A. C. Sulpizio and R. Testa, *J. Pharmacol. Exp. Ther.,* **281,** 1272 (1997).

[197] J. Macek, J. Klima and P. Ptacek, *J. of Chromatogr. B*, **809**, 307 (2004).

[198] R. N. Rao, M.V.N. K. Talluri, A. N. Raju, D. D. Shinde and G. S. Ramanjaneyulu, *J. of Pharm. and Biomed. Analysis,* **46,** 94 (2008).

[199] L. Ding, L. Li, P. Tao, J. Yang and Z. Zhang *J. Chromatogr. B*, **767,** 75 (2002).

[200] D. B. Patel, N. U. Patel and B. G. Chaudhari, *Der Pharmacia Sinica*, **2**, 172 (2011).

[201] Shrivastava**,** P. Saxena and V. B. Gupta**,** *Pharm. Methods,* **2**, 53 (2011).

[202] S. A. Ozkan, B. Uslu and H. Y. A. Enein, *Talanta*, **61,** 147 (2003).

[203] D. L. Wise, G. E. Winek, D. J. Trantolo, T. M. Cooper and J. D. Gresser, *In Electrical and Optical Polymer Systems*, Marcel Dekker, Inc., NewYork, **17** (1998).

[204] [204] J. Rodriguez, H. J. Grande and T. F. Otero, *In Handbook of Organic Conductive Molecules and Polymers*, ed. H. S. Nalwa, John Wiley and Sons, New York (1997) 415.

[205] J. Simonet and J. R. Berthelot, *Prog. Solid State Chem.*, **21,** 1(1991).

[206] J. Wang, M. Li, Z. Shi, N. Li and Z. Gu, *Electrochim. Acta,* **47,** 651 (2001).

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

[208] J. Wang, M. Li, Z. Shi, N. Li and Z. Gu, *Anal. Chem.,* **74,** 1993 (2002).

[209] B. T. Demircigil, S. A. Ozkan, O. Coruh and S. Yilmaz, *Electroanalysis,* **14,** 122 (2002).

[210] E. Bermejo, A. Zapardiel, J. A. P. Lopez, M. Chicharro, A. Sanchez and L. Hernandez, *J. Electroanal. Chem.,* **481,** 52 (2000).

[211] http://en.wikipedia.org/wiki/Ceftriaxone

[212] M. A. Omar, O. H. Abdelmageed and T. Z. Attia, *Talanta*, **77**,1394 (2009).

[213] W. Zhao, Zhang and Q. Li, *Clin. Chim. Acta*, **391**, 80 (2008).

[214] D. Zhang, Y. Ma, M. Zhou, L. Li and H. Chen, *Anal. Sci.,* **22**, 183 (2006)

[215] G. A. Saleh, S. R. E. Shaboury, F. A. Mohamed and A. H. Rageh, *Spectrochim. Acta*, **73**, 946 (2009).

[216] S. Majdi, A. Jabbari, H. Heli, H. Yadegari, A. A. Moosavi-Movahedi and S. Haghgoo, *J. Solid State Electrochem.,* **13**, 407 (2009).

[217] A.G. B. Madurro, L. F. Ferreira, S.N. Vieira, R.G. Ariza, L.R.G. Filho and J.M. Madurro, *J. Mater. Sci.,* **42**, 3238 (2007).

[218] S. N. Vieira, L. F. Ferreira, D. L. Franco, A. S. Afonso, R. A. Goncalves, A.G. B. Madurro and J. M. Madurro, *Macromol. Symp.,* **245**, 236 (2006).

[219] R. Andreozzi, M.S. Lo Casale, R. Martota, G. Pinto and A. Pollio, *Water Res.,* **34**, 4419 (2000).

[220] C. Barbero, J.J. Silber and L. Sereno, *Macromolecules*, **29**, 81 (1990).

[221] C. Barbero, J.J. Silber and L. Sereno, *J. Electroanal.Chem.,* **263**, 333 (1989).

[222] H. Yang and A.J. Bard, *J. Electroanal. Chem.,* **339**, 423 (1992).

[223] N. Yamada, K. Teshima, N. Kobayashi and R. Hirohashi, *J. Electroanal. Chem.,* **394**, 71 (1995).

[224] J. Chiang and A.G. M. Diarmid, *Synth. Met.,* **13**, 193 (1986).

[225] R. Lapuente, F. Cases, P. Garces, E. Morall and J. L. Vazquez, *J. Electroanal. Chem.,* **451**, 163 (1998).

[226] M. Gattrell and D.W. Kirk, *J. Electrochem. Soc.,* **139**, 2736 (1992).

[227] S. A. Ozkana, B. Uslu and P. Zuman, *Anal. Chim. Acta,* **457,** 265 (2002).

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RESEARCH PAPERS PUBLISHED

International Journals

1. Renjini Joseph and K. Girish Kumar, Electrochemical reduction and voltammetric determination of metronidazole benzoate at modified carbon paste electrode, *Anal. Lett.,* **2009**, 42, 2309.

2. Renjini Joseph and K. Girish Kumar, Differential pulse voltammetric determination and catalytic oxidation of sulfamethoxazole using [5,10,15,20- tetrakis (3-methoxy-4-hydroxy phenyl) porphyrinato] Cu(II) modified carbon paste sensor, *Drug Test. Analysis,* **2010**, 2, 278 .

3. Renjini Joseph and K. Girish Kumar, Electrochemical sensor for the determination of acyclovir based on 2-mercaptobenzothiazole-[5,10,15,20-tetrakis(3-methoxy-4-hydroxyphenyl)porphyrinato] copper(II) modified gold electrode, *Anal. Sci.,* **2011**, 27, 67.

4. Laina Lonappan, Sindhu Issac, Renjini Joseph, Divya Thomas and K. Girish Kumar, Electrochemical studies of TAM using multiwalled carbon nanotube modified glassy carbon sensor, *Micro & Nano letters*, **2011**, 6, 867.

Conference papers

1. "Voltammetric sensor for the determination of tamsulosin hydrochloride at polypyrrole and multiwalled carbon nanotube modified gold electrode" (National seminar "New horizons of physics", St.Pauls College Kalamassery, Kochi, August 2011).

2. "Development of a polyaniline modified gold electrode sensor for the voltammetric determination of trimethoprim" (1st Kerala Women's Science Congress, St.Teresa's College Ernakulam, August 2010).

3. "Electrochemical sensor for the determination of acyclovir based on 2-mercaptobenzothiazole-[5,10,15,20-tetrakis(3-methoxy-4-hydroxy phenyl)porphyrinato]copper(II) modified gold electrode" ("National Convention of Electrochemists – 15", VIT University, Vellore, February 2010).

4. "Voltammetric sensor for the determination of Pyridine-2-aldoxime methochloride at 2-mercaptobenzothiazol and multiwalled carbon nanotube modified gold electrode" ("International Conference on Materials for the Millenium", Cochin University of Science & Technology, January 2010).

5. "Metalloporphyrin based voltammetric sensor for the determination of metronidazole benzoate" ("International Conference on Frontiers in Chemical research", Mangalore University, December 2008).

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