KUVEMPU UNIVERSITY

CYCLIC VOLTAMMETRIC INVESTIGATIONS OF SOME BIOLOGICALLY ACTIVE COMPOUNDS AT MODIFIED CARBON PASTE ELECTRODE

Thesis submitted to the Faculty of Science
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in

INDUSTRIAL CHEMISTRY

By

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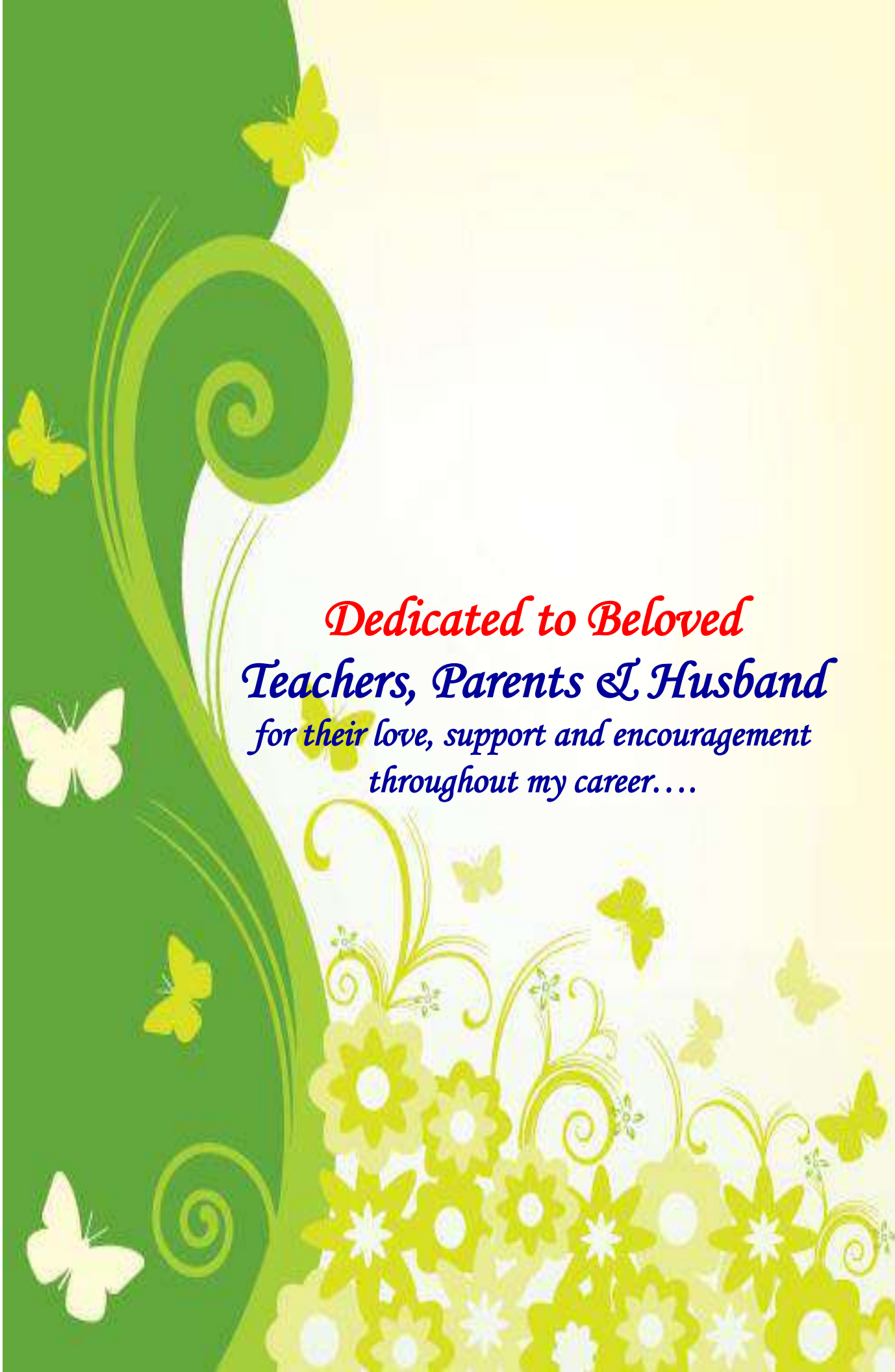
Dedicated to Beloved
Teachers, Parents & Husband
for their love, support and encouragement
throughout my career….

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Declaration

I hereby declare that, the work contained in this thesis has not been previously submitted to meet the requirements for the award of any degree / associateship / fellowship of this University or any other higher education institution. To the best of my knowledge and belief, thesis contains no materials previously published or written by another person except where due reference is made.

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Certificate

This is to certify that the thesis entitled "**Cyclic Voltammetric Investigations of Some Biologically Active Compounds at Modified Carbon Paste Electrode"** which is being submitted here for the award of **Doctor of Philosophy in Industrial Chemistry** under the Faculty of Science, Kuvempu University, Shankaraghatta 577451, Shivamogga, Karnataka, India, is the result of original work completed by **Mrs. Rekha** under my supervision and guidance. To the best of my knowledge and belief the work embodied in this thesis has not formed early the basis for the award of same degree / associateship / fellowship of this University or any other higher education institution.

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Summary of the Thesis

The focus of the thesis is to use of chemically modified electrodes for the investigation of some biologically active compounds to get excellent reproducible results by voltammetric techniques. The biologically active compounds were chosen for electrochemical investigation were Folic acid, Paracetamol, Norepinephrine, Catechol, Hydroquinone, dopamine, ascorbic acid and uric acid. In the real sample these compounds were interfering each other during the investigation by overlapping their voltammetric responses Moreover, the traditional electrodes very often suffer from fouling effect due to the accumulation of oxidized products on the electrode surface which results in rather poor selectivity and sensitivity.

Because of advantages like, high conductivity, wide potential window for analysis, chemically inert, relatively inexpensive, easy modification, easy preparation of paste with organic binder and easily reneaval of electrode surface, the carbon paste electrode was chosen for the investigation. This thesis also discussed on the different types of modifications. In this research work the bioactive organic compounds like dopamine, ascorbic acid and uric acid were investigated at modified carbon paste electrode surface by using voltammetric techniques. The following aspects like, number of electrons involved in the electrochemical reaction, rate constants, nature of intermediates in the electrode reaction and nature of electrode process were observed.

Organization of the Thesis

The work carried out in this thesis is divided and described into seven chapters.

Chapter-1

Introduction, Review of Voltammetry and Theoretical Considerations

This chapter covers the introduction, voltammetry and voltammetric techniques. Basic and fundamental principles, theory theoretical aspects and applications of voltammetry, solvents, supporting electrolytes and electrodes interaction can be seen in this section. A brief review of cyclic voltammetric investigations of certain biologically

active compounds has been presented. Objective and scope of the present thesis were included in this chapter.

Chapter-2

Experimental

This chapter describes the basic experimental setup which is very much essential for voltammetric technique. The basic equipments like, potentiostat, recording device and electrochemical cell which is composed of three electrodes. The electrode systems with special emphasis on carbon paste electrode used in this research work. The procedure of modified, unmodified carbon paste electrode and their characterization were described in detail. In addition, in this chapter the history of carbon paste electrode was described.

Chapter 3

This chapter is divided into two parts such as part-A and Part-B

Part A

Poly(amoxicillin) Modified Carbon Paste Electrode for the Determination of Dopamine: A Cyclic Voltammetric Study

The carbon paste electrode (CPE) was modified by electropolymerisation of amoxicillin in 0.2 M acetate buffer solution (ABS) of pH 5.0 by using cyclic voltammetric (CV) technique. The modified electrode was used for the electrochemical determination of dopamine (DA). The poly(amoxicillin) modified CPE showed excellent electrocatalytic activity towards the oxidation of DA. The study of variation in concentration and scan rate shows that the electrode process was diffusion-controlled. Further the modified electrode was used for the simultaneous determination of DA and ascorbic acid (AA) by CV technique. The oxidation potential of AA was shifted to the negative side leads to the absence of interference in analyzing the DA. Overall the fabricated electrode can be used for the determination of DA in physiological and pharmaceutical samples

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Part B

Poly(lysine) modified carbon paste electrode for the determination of norepinephrine and folic acid: A cyclic voltammetric study

The carbon paste electrode (CPE) was modified by electropolymerisation of lysine in 0.2 M phosphate buffer solution (PBS) of pH 7.4 by cyclic voltammetric(CV) technique. The modified electrode was used for the electrochemical determination of norepinephrine (NE) and folic acid (FA). The fabricated poly(lysine)MCPE showed excellent electrocatalytic activity towards the oxidation of NE. The study of variation in concentration and scan rate reveals the electrode process was diffusion controlled. The sensitive separation was observed for the determination of NE and FA in a binary mixture at physiological pH by CV technique.

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Chapter 4

Multi-walled Carbon Nanotube Modified Carbon Paste Electrode for the Voltammetric Determination of Folic Acid and Uric Acid

The multi-walled carbon nanotube modified carbon paste electrode (MWCNTMCPE) was used for the voltammetric investigation of folic acid (FA) and uric acid (UA) in physiological pH of 7.4. The scan rate and concentration study reveals that the electrode process was controlled by diffusion of the analytes. The sensitive separation for the binary mixture of FA and UA was observed by cyclic voltammetric and differential pulse voltammetric techniques. A simple modification procedure was reported for the determination of FA and UA.The simultaneous study was conducted for the binary mixture of FA and UA, the sensitive separation was observed at the modified electrode by CV and DPV technique. The proposed method can be employed for the some other biological important molecules.

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Chapter 5

Co_3O_4/CuOnano powder modified carbon paste electrode for the determination of folic acid and paracetamol: A Voltammetric study

The mechanochemically prepared Co_3O_4/CuO composite nano powder was used for the modification of carbon paste electrode (CPE), and employed for the determination of folic acid (FA) and paracetamol (PA) by cyclic voltammetric (CV) and differential pulse voltammetric (DPV) techniques. The scan rate effect studies on Co_3O_4/CuO modified CPE (MCPE) for FA and PA was conducted and it shows the diffusion controlled process for both the analytes. The lower limit of detection for FA was 0.99μM in the linear range 0.5 to 16 μM and 0.38μM for PA in the linearity range 0.5 to 5μM by CV technique. The simultaneous determination of FA and PA shows good selectivity and sensitivity. In addition the performance of Co_3O_4/CuO MCPE was tested for pharmaceutical samples and acceptable results are obtained. The Co_3O_4/CuO MCPE shows antifouling property, stability and reproducibility for the determination of FA and PA at physiological pH.

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Chapter 6

This chapter is divided into two parts such as Part-A and Part-B

Part-A

Graphene modified carbon paste electrode for the electroanalysis of norepinephrine

A sensitive and selective electrochemical method was developed for the electroanalysis of norepinephrine by modify electrode with characterised commercially available graphene that has not been chemically treated. As a result of its fabrication, allowing the true electroanalytical applicability of graphene to be properly determined in comparison to the bare carbon past electrode. The graphene modified electrode shows good analytical performance in terms of sensitivity, linearity and observed detection limits towards each of the various analytes such asnorepinephrine, ascorbic acid and uric

acid studiesin phosphate buffer solution of pH 7.4 by voltammetric techniques. The lower limit ofdetection of NE was found to be 0.87µM. The interference studies showed that the modified electrodeexhibits excellent selectivity and the separation of the oxidation peak potentials for NE–AA and NE–UA were found to be 0.141 V and0.247 V respectively. The peak differences were large enough to determine NE, AA and UA individuallyand simultaneously. In summary, a simple electrochemical method for the determination of NE is developed by modifying the CPE with grapheme under the physiological condition. Decrease in oxidation over potential and enhancement in current proved the electrocatalytic activity of CPE modified by graphene. A very minuet quantity of graphene used for the fabrication of electrode make totally inexpensive with is used for the analysis of neurotransmitter. Moreover, by this simple method of fabrication a sensitivity, selectivityand lower detection limit was achieved. This result was more sufficient to analyze NE in presenceof large excess of AA and UA. The prepared modified electrode has potential for the investigation of other neurotransmitters.

Part-B

Graphene modified carbon paste electrode for the determination of paracetamol and folic acid: A Voltammetric study

The Graphenenano powder was used for the modification of carbon paste electrode (CPE), and employed for the determination of folic acid (FA) and paracetamol (PA) by cyclic voltammetric (CV) and differential pulse voltammetric (DPV) techniques. The scan rate effect studies on Graphene modified CPE (MCPE) for FA and PA was conducted and it shows the diffusion controlled process for both the analytes. The lower limit of detection for FA was 0.8015µM in the linear range 0.5 to 5 µM and 4.0199µM for PA in the linearity range 40 to 100µM by CV technique. The simultaneous determination of FA and PA shows good selectivity and sensitivity.

The Graphene MCPE shows antifouling property, stability and reproducibility for the determination of FA and PA at physiological pH.

Chapter 7

This chapter is divided into two parts such as Part-A and Part-B

Part-A

Poly (alcian blue) modified carbon paste electrode for the determination of catechol and hydroquinone:A voltammetric study

The carbon paste electrode (CPE) was modified by electropolymerisation of alcian blue in 0.2 M phosphate buffer solution (PBS) of pH 7.4 by using cyclic voltammetric (CV) technique. The modified electrode was used for the electrochemical determination of Catechol and Hydroquinone. The alcian blue modified electrode shows good analytical performance in terms of sensitivity, linearity and observed detection limits towards each of the various analytes such as Catecholamine and Hydroquinone studiesin phosphate buffer solution of pH 7.4 by voltammetric techniques. The lower limit ofdetection of CC and HQ was found to be 1.0146μM and 1.421μM. The interference studies showed that the modified electrodeexhibits excellent selectivity and the separation of the oxidation peak potentials for CC and HQ were found to be0.1226V. The peak differences were large enough to determine CC and HQ individuallyand simultaneously.

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Part -B

Poly (Toulidineblue) Modified Carbon Paste Electrode for the determination of Catechol and Hydroquinone: A Voltammetric study

The carbon paste electrode (CPE) was modified by electropolymerisation of toluidine blue in 0.2 M phosphate buffer solution (PBS) of pH 7.4 by using cyclic voltammetric (CV) technique. The modified electrode was used for the electrochemical determination of Catechol and Hydroquinone.The toluidine blue modified electrode shows good analytical performance in terms of sensitivity, linearity and observed detection limits towards each of the various analytes such as Catecholamine and

Hydroquinone studiesin phosphate buffer solution of pH 7.4 by voltammetric techniques. The lower limit ofdetection of CC and HQ was found to be 6.658µMand 1.2942µM. The interference studies showed that the modified electrodeexhibits excellent selectivity and the separation of the oxidation peak potentials for CC and HQ were found to be0.1299V. The peak differences were large enough to determine CC and HQ individuallyand simultaneously.

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List of Abbreviations

5-HT	-	5-hydroxytryptamine
AA	-	Ascorbic acid
ABS	-	Acetate buffer solution
ABSA	-	Amino benzene sulphonic acid
AE	-	Auxiliary electrode
ASV	-	Anodic stripping voltammetry
BARC	-	Baba atomic research centre
CE	-	Counter electrode
CMCPEs	-	Chemically modified carbon paste electrode
CMEs	-	Chemically modified electrodes
CNS	-	Central nervous system
CPE	-	Carbon paste electrode
CTFE	-	Chlorotrifluoroethylene
CV	-	Cyclic voltammetry
DA	-	Dopamine
DCE	-	Dropping carbon electrode
DME	-	Dropping mercury electrode
DMF	-	Dimethyl formamide
DMSO	-	Dimethyl sulphoxide
Do	-	Diffusion coefficient
DPV	-	Differential pulse voltammetry
E°	-	Formal potential
$\mathbf{E_{f,}}$	-	Final potential
Ei	-	Initial applied potential
EP	-	Epinephrine
Epa	-	Anodic peak potential
Epc	-	Cathodic peak potential
FSCV	-	Fast scan cyclic voltammetry
G	-	Gibb's free energy
GC	-	Glassy carbon

GI	-	Gastrointestinal
HOPG	-	Highly oriented pyrolytic graphite
Ipa	-	Anodic peak current
Ipc	-	Cathodic peak current
IPE	-	Ideally polarisable electrode
Ko	-	Heterogeneous rate constant
LSV	-	Linear sweep voltammetry
MCPE	-	Modified carbon paste electrode
mM	-	Millimolar
mV	-	Millivolt
$mVs^{1/2}$	-	Millivolt per second
MWCNT	-	Multiwall carbon nanotube
NPP	-	Pulse polarographic techniques
NTs	-	Neurotransmitters
O	-	Oxidised species
PC	-	Personal computer
p-Glu	-	Poly (glutamic acid)
PPF	-	Pyrolysed photoresist film
R	-	Reduced species
RE	-	Reference electrode
S	-	Entropy
SCE	-	Saturated calomel electrode
SEM	-	Scanning electron microscopy
SHE	-	Standard hydrogen electrode
SIDS	-	Sudden infant death syndrome
SWV	-	Square wave voltammetry
TBA	-	Tetra-n-butyl ammonium
TEA	-	Tetra-ethyl ammonium
TPH	-	Tryptophan hydroxylase
UA	-	Uric acid
WE	-	Working electrode
$v^{1/2}$	-	Square root of scan rate

1.1. Introduction

Electrochemistry is a branch of chemistry which deals with the study of chemical reaction take place in a solution at the interface of an electron conductor and an ionic conductor and which involves the electron transfer between the electrode and the electrolyte or species in solution. The science of electrochemistry is concerned with electron transfer at the solution/electrode interface. Electrochemistry is a powerful and sensitive analytical tool used for both qualitative and quantitative analysis over a wide range of concentrations. Electroanalytical techniques are concerned with the interplay between electricity and chemistry, namely, the measurements of electrical quantities, such as current, potential, or charge and their relationship to chemical parameters. Such use of electrical measurements for analytical purposes has found a vast range of applications, including environmental monitoring, industrial quality control or biomedical analysis.

The fundamental process in electrochemical reactions is the transfer of electrons between the electrode surface and molecules in the interfacial region either in solution or immobilized at the electrode surface. The kinetics of this heterogeneous process can be significantly affected by the microstructure and roughness of the electrode surface, the blocking of active sites on the electrode surface by adsorbed materials, and the nature of the functional groups (e.g., oxides) present on the electrode surface [1, 2]. Hence, chemically modified electrodes (CMEs) have evolved as a recently emerging field and much study of interest have been shown by many researchers in this field. In detail, the chemically modified electrodes comprise an approach to electrode system design that finds the use in a wide spectrum of basic electrochemical investigations, including the relationship of heterogeneous electron transfer and chemical reactivity of electrode surface chemistry, electrostatic phenomena at electrode surface, and electron as well as ionic transport phenomena in polymers, and the design of electrochemical devices and systems for applications in chemical sensing, energy conversion and storage, molecular electronics, electrochromic displays and electro-organic synthesis. The applicability of these CMEs is wide-ranging, but one important application is biomolecule sensing.

There has been an increasing interest in the creation of modified electrode surfaces that differ from the corresponding bare surfaces and produce an electrode surface that generates reproducible result, which has a vast application in biological and chemical sensing. The focus of the work covered in this thesis is to controllably alter the properties of carbon surfaces, so that the surfaces can be used for desired sensor applications. Carbon was the chosen surface, as it is highly conducting with a wide potential window, structurally stable, relatively inexpensive and stable layers of modifiers can attach to the surface in a controllable manner. There are many different forms of conducting carbon materials including glassy carbon (GC), highly oriented pyrolytic graphite (HOPG), pyrolysed photoresist film,(PPF), carbon nanotubes, carbon powder, screen printed carbon, carbon fibres, carbon nanocapsules, Fullerene and carbon composites [3].

In this thesis carbon powder was selected and was applied to study the electrochemical properties of the selected neurotransmitters and the process taking place across the interface of the electrode surface and the electrolyte. A species capable of undergoing electron transfer process is called an electroactive species. In order to carry out electron transfer process with the electrode, the electroactive species comes from the bulk solution and approaches the electrode surface. Hence the electron transfer plays a fundamental role in governing the pathways of chemical reactions. Measurement of speed of the electron transfer process and the number of electrons involved are difficult in traditional experimental methods like spectroscopy [4]. Hence the knowledge of the driving force for many reactions remains elusive. Electrochemical methods offer the potential to investigate this process directly by determination of number of electrons involved. Electrochemical studies of biologically active compounds serve to elucidate biological processes and their inter-relationship that are involved in living organisms [5-8]. For any series of interrelated processes within a system, it is normally necessary to study the component part of the system. An attempt has been made to study the electrochemical properties and behaviour of compounds, which plays an important role in biochemical redox reactions in various organisms.

Electrochemical techniques provide efficient tools for surface modifications. Very simple experiments can be performed where species from solution can be physically adsorb, electropolymerised, or covalently attached onto the electrode surface at certain controlled potential. The aim of the work covered in this thesis was to study the effect of modified carbon paste electrodes on the oxidation properties of dopamine, serotonin, norepinephrine, epinephrine, ascorbic acid and uric acid in biological buffer solution. This work searches for alternatives to counter, the major problem associated with the voltammetric detection of dopamine (DA), norepinephrine (NE), folic acid (FA) and catechol (CC) was the coexistence of interfering compounds such as ascorbic acid (AA), uric acid (UA) and hydroquinone (HQ) which results in their overlapped voltammetric response at bare electrode. [9-11]. Moreover, the bare electrodes very often suffer from the fouling effect due to the accumulation of oxidized products on the electrode surface, which results in rather poor selectivity and sensitivity.

Advances since the mid-1980s including the development of ultramicroelectrodes, the design of tailored interfaces and molecular monolayers, the coupling of biological components and electrochemical transducers, the synthesis of ionophores and receptors containing cavities of molecular size, the development of ultratrace voltammetric techniques or of high-resolution scanning probe microscopies, and the microfabrication of molecular devices or efficient flow detectors, have led to a substantial increase in the popularity of electroanalysis and to its expansion into new phases and environments. Indeed, electrochemical probes are receiving a major share of the attention in the development of chemical sensors.

1.2. Voltammetry

Historically, the branch of electrochemistry we now call voltammetry developed from the discovery of polarography in 1922 by the Czech chemist Jaroslav Heyrovsky, for which he received the 1959 Nobel Prize in chemistry. Voltammetry refers to the measurement of current that result from the application of potential. Unlike potentiometric measurements, which employ only two electrodes, voltammetric measurements utilise a three electrode electrochemical cell. The use of three electrodes

(working, auxillary and reference) along with the potentiostat instrument allows accurate application of potential functions and measurement of the resultant current.

Analytical chemists routinely use voltammetric techniques for the quantitative determination of a variety of dissolved inorganic and organic substances. Inorganic, physical, and biological chemists widely use voltammetric techniques for a variety of purposes, including fundamental studies of oxidation and reduction processes in various media, adsorption processes on surfaces, electron transfer and reaction mechanisms, kinetics of electron transfer processes, and transport, speciation, and thermodynamic properties of solvated species. Voltammetric methods are also applied to the determination of compounds of pharmaceutical interest and, when coupled with HPLC, they are effective tools for the analysis of complex mixtures.

1.2.1. Voltammetric Techniques and their Theoretical Aspects

The different voltammetric techniques that are used are distinguished from each other primarily by the potential function that is applied to the working electrode to drive the reaction, and by the material used as the working electrode. These can be described as follows.

1.2.1.1. Linear Sweep Voltammetry (LSV)

Linear sweep voltammetry involves applying a linear potential sweep to the working electrode (the electrode under study) while monitoring simultaneously the current flowing in the circuit. A signal generator produces a voltage sweep from E_i to E_f and a potentiostat applies this potential wave to the electrode under study. The scan direction can be positive or negative and in principle, the sweep rate can possess any constant value:

$$\text{Sweep rate} = dE/dt$$

This method of analysis is commonly employed in polarography whereby under well-defined conditions, the limiting current derived from a redox process in solution during LSV may be used to quantitatively determine the concentration of electroactive species in solution.

1.2.1.2 Square Wave Voltammetry (SWV)

Among the various voltammetric techniques, exceptional versatility is found in a method called square wave voltammetry, which was invented by Ramaley and Krause, and developed extensively by the Osteryoungs and their co-workers [12]. It is a differential technique in which potential waveform composed of a symmetrical square wave of constant amplitude is super imposed on a base staircase potential [13, 14]. It is the plot of the difference in the current measured in forward (i_f) and reverse cycle (i_r), plotted against the average potential of each waveform cycle. In this technique, the peak potential occurs at the $E_{1/2}$ of the redox couple because the current function is symmetrical around the potential [15, 16]. The main advantages of SWV are excellent peak separation and high sensitivity.

1.2.1.3. Anodic Stripping Voltammetry (ASV)

Anodic stripping voltammetry is an electrolytic method in which a mercury electrode is held at a negative potential to reduce metal ions in solution and form an amalgam with the electrode. The solution is stirred to carry as much of the analyte metal to the electrode as possible for concentration into the amalgam. After reducing and accumulating the analyte for some period of time, the potential on the electrode is increased to reoxidize the analyte and generate a current signal. The current produced by anodic stripping depends on the particular type of mercury electrode, but is directly proportional to the concentration of analyte concentrated into the electrode.

1.2.1.4. Normal Pulse Polarography (NPP)

Pulse polarographic techniques are voltammetric measurements which are variants of the polarographic measurement which try to minimise the background capacitive contribution of current by eliminating the continuous varying potential ramp, and replacing it with a series of potential steps of short duration. In normal pulse polarography, each potential step begins at the same value (a potential at which no Faradaic electrochemistry occurs), and the amplitude of each subsequent steps increases in small increments. When the mercury drop is dislodged from the capillary, the potential

is returned to the initial value in preparation for a new step. The polarogram is obtained by plotting the measured current vs. the potential to which the step occurs. As a result the current is not followed during the mercury drop growth, and normal pulse polarogram has the typical shape of a sigmoid. After the initial potential step, the capacitive current decays exponentially while the Faradaic current decays as the square root of time. The diffusion current is measured just before the drop is dislodged, allowing excellent discrimination against the background capacitive current.

1.2.1.5. Differential Pulse Poralography / Voltammetry (DPP/ DPV)

This technique was proposed by Barker and Gardner [17]. DPV can provide greater sensitivity and more efficient resolution and differentiation of various species. This technique differs from NPP because each potential pulse is fixed, of small amplitude (0.01 to 0.1). Current is measured at two points from each pulse, just before the application of the pulse and at the end of the pulse. The difference between the current measurements at these points for each pulse is determined and plotted against the base potential. At potentials around the redox potential, the difference in current reaches a maximum and decreases to zero as the current becomes diffusion controlled. The current response is therefore a symmetric peak.

1.2.1.6. Fast Scan Cyclic Voltammetry

Fast scan cyclic voltammetry (FSCV) is a linear sweep voltammetry technique in which the background subtracted voltammogram gives additional information about the electrolyzed species. The current response over a range of potentials is measured, making it a better technique to discern additional current contributions from other electroactive species. FCV is a relatively fast technique with single scans typically recorded every 100 ms, however, the fast scan rates decrease the signal to noise ratio.

1.2.1.7. Cyclic Voltammetry (CV)

Cyclic voltammetry is a method for investigating the electrochemical behaviour of a system. It was first reported in 1938 and described theoretically by Randles [18]. Cyclic voltammetry is the most widely used technique for acquiring qualitative

information about electrochemical reactions. The power of cyclic voltammetry results from its ability to rapidly provide considerable information on the thermodynamics of redox processes, on the kinetics of heterogeneous electron-transfer reactions, and on coupled chemical reactions or adsorption processes. Cyclic voltammetry is often the first experimental approach performed in an electroanalytical study, since it offers rapid location of redox potentials of the electroactive species and convenient evaluation of the effect of media upon the redox process [17, 19-22].

1.2.1.7a. Basic principles of Cyclic Voltammetry

A cyclic voltammogram is obtained by applying a linear potential sweep (that is, a potential that increases or decreases linearly with time) to the working electrode. As the potential is swept back and forth past the formal potential, E^o, of an analyte, a current flows through the electrode that either oxidizes or reduces the analyte. The magnitude of this current is proportional to the concentration of the analyte in solution, which allows cyclic voltammetry to be used in an analytical determination of concentration.

The equipment required to perform cyclic voltammetry consists of a conventional three-electrode potentiostat connected to three electrodes (working, reference and auxiliary) immersed in a test solution. The potentiostat applies and maintains the potential between the working and reference electrode while at the same time measuring the current at the working electrode. Charge flows between the working electrode and the auxiliary electrode. A recording device (such as a computer or plotter) is used to record the resulting cyclic voltammogram as a graph of current versus potential.

1.2.1.7b. Fundamentals of Cyclic Voltammetry

1.2.1.7b.1 Circuit

Cyclic voltammetry requires two simple operational amplifier circuits one of which is a polarizing circuit that applies the potential to the cell and the other is a measuring circuit that monitors the cell current. The working electrode is potentiostatically controlled. The potential is varied in some systematic manner and resulting current vs. potential plot is known as voltammogram.

1.2.1.7b.2 Scan rate

A simple potential waveform that is used often in electrochemical experiments is the linear waveform i.e., the potential is continuously changed as a linear function of time. The rate of change of potential with time is called scan rate.

1.2.1.7b.3 Switching potentials and the excitation signal

Cyclic voltammetry involves the cycling of potential of an electrode between two designated values called the Switching potentials in an unstirred solution and measuring the resulting current. The controlling potential applied across the working electrode (WE) and the reference electrode (RE) is called the excitation signal which is a Unear potential scan with a triangular waveform as shown in Fig. 1.1. The potential axis is also a time that is related to scan rate [10]. The excitation signal causes the potential to scan negatively from +0.8V to -0.2V vs SCE, at which point the scan direction is reversed causing a positive scan back to the original potential of +0.8V. Single or multiple cycles can be used.

1.2.1.7b.4 Potential control

The potential control of the external point is done using a potentiostat and a three electrode system in which the potential of the WE is controlled relative to the RE, saturated calomel electrode (SCE) or Silver-Silver chloride (Ag/AgCl) electrode. The current passes between WE and the auxiliary electrode (AE). Because of its greater experimental simplicity, CV has become a very popular technique for electrochemical studies of new systems and has proved as a sensitive toll for obtaining information about fairly complicated electrode reactions. CV is a technique, where in a species that undergoes a reduction during a cathodic polarization of the WE in an unstirred solution is reoxidized by applying a reverse (i.e., anodic) scan. The correlation of the cathodic and the anodic peak currents and differences in cathodic and anodic potentials with the voltage scan rates has been studied mathematically for different electrochemical reaction [11, 12]. The sweep rates in the CV can be about the same as in single sweep voltammetry.

1.2.1.7b.5 CV- an active electrochemical method

CV can describe as 'active' electrochemical method because the experiment drives an electrochemical reaction by incorporating the chemistry in to a circuit and then controlling the reaction by circuit parameter such as voltage.

1.2.1.7b.6 Characteristic parameters of a cyclic voltammogram

The cyclic voltammogram is characterized by several important parameters. Four of these observables, the two peak currents and two peak potentials, provide the basis for the diagnostics developed by Nicholson and Shain [13] for analysing the cyclic voltammetric response. There are two peaks associated with the redox reaction and accordingly we have the anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}) and the corresponding current associated are anodic peak current (i_{pa}) and cathodic peak current (i_{pc}) respectively. In cyclic voltammetry, the electrode ramps linearly vs. time. This ramping is known as the experiment's scan rate (V/s). The potential is measured between the reference electrode and working electrode and the current is measured between the working electrode and the counter electrode. This data is then plotted as current (i) vs. potential (E). The basic shape of the current vs. potential response for a cyclic voltammetry experiment is as shown in Fig. 1.2 for a reversible process (a reversible wave is when an analyte is reduced or oxidized on a forward scan and is then reoxidized or re-reduced in a predictable way on the return scan). As the figure shows, the forward scan produces a current peak for any analytes that can be reduced (or oxidized depending on the initial scan direction) through the range of the potential scanned. The scan shown starts at a slightly negative potential, i.e. the initial potential (E_i), there is no net conversion of oxidized species (O) into reduced species (R) (point A). As redox potential is approached, the current is first observed to peak at E_{pa} (with value i_{pa}) (point B) indicating that an oxidation is taking place and then drops due to depletion of the reducing species from the diffusion layer. During the reverse scan i.e. switching potential, E switch, (point C) reduction occurs and a peak current is observed at E_{pc} (with value i_{pc}) (point D). Providing that there is no surface interaction between the electrode and the reagents, and the redox products are stable (atleast in the time frame of the experiment). The situation is very different when the redox reaction is not reversible,

when chemical reactions are couple to the redox process or when adsorption of either reactants or products occurs. In fact, it is these non-ideal situations which are usually of greatest chemical interest and for which the diagnostic properties of cyclic voltammetry are particularly suited. This is helpful in understanding the fundamentals of the technique. As shown in Fig. 1.3, scanning the potential in the negative direction makes the electrode a stronger reductant, whereas scanning the potential in the positive direction makes it a better oxidant.

Voltammetry is a collection of electroanalytical techniques in which information about the analyte is derived from the measurement of current as a function of applied potential. It is widely used by chemists for non-analytical purposes including fundamental studies on redox processes, adsorption processes on surfaces, electron transfer mechanisms and electrode kinetics.

Electrochemistry affords some of the most sensitive and informative analytical techniques. Electroanalytical methods such as CV, stripping voltammetry and differential pulse voltammetry are not only capable of assaying trace concentrations of an electroactive analyte, but supply useful information concerning its physical and chemical properties. Quantities such as oxidation potentials, diffusion coefficients, electron transfer rates and transfer numbers are readly obtained using electroanalytical methods, which are difficult to obtain using other techniques. Moreover, electroanalytical methods can be combined with spectroscopy in situ to provide information concerning molecular structures and reaction mechanisms of transient electroactive species.

CV is a potential sweep technique. It involves sweeping the electrode potential between potential limits E_1 and E_2 at a known sweep rate (also called scan rate). On reaching limit E_2 the sweep is reversed to E_1 to obtain a cyclic scan. The CV scan is a plot of current verses potential and indicates the potential at which redox process occur. The potential axis is also a time axis that is related to scan rate [23]. The excitation signal for CV is a linear potential scan with triangular waveform as shown in Fig.1.1. This triangular potential excitation signal sweeps the potential of an electrode between two values, sometimes called the switching potential.

The current measured during this process is often normalised to the electrode surface area and referred to as the current density. The current density is then plotted against the applied potential, and the result is referred to as a cyclic voltammogram. A peak in the measured current is seen at a potential that is characteristic of any electrode reaction taking place. The peak width and height for a particular process may depend on the sweep rate, electrolyte concentration and the electrode material [24, 25].

To carry out an oxidation process, a positive potential ramp is applied and the electroactive species loses an electron at the electrode giving rise to an anodic peak current (i_{pa}) which usually gives an oxidation peak at a given potential (E_{pa}). Cathodic currents (i_{pc}) are observed when the potential is applied in the negative direction leading to a reduction process, typically giving a reduction peak at a given potential (E_{pc}). The CV is usually initiated at a potential where species are not electroactive.

1.3. Theory

In cyclic voltammetry, a species that undergoes a reduction during a cathodic polarization of working electrode in unstirred solution is reoxidised by applying a reverse (anodic) scan. The correlation of the cathodic and anodic peak currents and differences in cathodic and anodic peak potentials with the voltage scan rates has been studied mathematically for different electrochemical reactions [26, 27]. The sweep rates in cyclic voltammetry can be about the same as in single sweep voltammetry.

Cyclic voltammetry makes possible the elucidation of the kinetics of electrochemical reactions taking place at electrode surfaces [28, 29]. In a typical voltammogram, there can be several peaks. From the sweep-rate dependence of the peak amplitudes, widths and potentials of the peaks observed in the voltammogram, it is possible to investigate the role of adsorption, diffusion, and coupled homogeneous chemical reaction mechanisms [30].

The important parameters of a cyclic voltammogram are the magnitudes of anodic peak current (i_{pa}), the cathodic peak current (i_{pc}), the anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}). The basic shape of the current verses potential response for

a cyclic voltammetry experiment is shown below (Fig. 1.2). At the start of the experiment, the bulk solution contains only the reduced form of the redox couple (R) so that at potentials lower than the redox potential, i.e. the initial potential, there is no net conversion of R into O, the oxidised form (point A). As the redox potential is approached, there is a net anodic current which increases exponentially with potential. As R is converted into O, concentration gradients are set up for both R and O, and diffusion occurs down these concentration gradients. At the anodic peak (point B), the redox potential is sufficiently positive that any R that reaches the electrode surface is instantaneously oxidised to O. At this point, a net reduction of O to R occurs which causes a cathodic current which eventually produces a peak shaped response (point D). If a redox system remains in equilibrium throughout the potential scan, the electrochemical reaction is said to be reversible. In other words, equilibrium requires that the surface concentrations of O and R are maintained at the values required by the Nernst Equation. Under these conditions, the following parameters characterize the cyclic voltammogram of the redox process. The peak potential separation (E_{pa} - E_{pc}) is equal to 57/n mV for all scan rates where n is the number of electron equivalents transferred during the redox process. The situation is very different when the redox reaction is not reversible, when chemical reactions are coupled to the redox process or when adsorption of either reactants or products occurs. In fact, it is these "non-ideal" situations which are usually of greatest chemical interest and for which the diagnostic properties of cyclic voltammetry are particularly suited. This is helpful in understanding the fundermentals of the technique. As shown in Fig.1.3, scanning the potential in the negative direction makes the electrode a stronger reductant, whereas scanning the potential in the positive direction makes it a better oxidant.

1.4. Applications of Cyclic Voltammetry

CV has become a very popular technique for electrochemical studies of new systems, and has proved as a sensitive tool for obtaining information about fairly complicated electrode reactions [31, 32]. CV methods have found to have extensive applications for the evaluation of thermodynamic and kinetic parameters such as number

of electrons change (n), heterogeneous rate constant (k_0), entropy (S), Gibb's free energy (G) and diffusion coefficient (D_0) etc., of a number of redox reactions and associated chemical reactions. These methods are especially useful in both oxidation and reduction process and to study the multiple electron transfer in an electrochemical reaction [33].

The primary objective of developing these CV techniques was analytical, both qualitative as well as quantitative. As the peak current is proportional to concentration, this method can be used for the estimation of a number of inorganic, organic and organolmetallic compounds.

Much intensive research is in progress with the additional regular analytical applications. CV studies in rat brain [34], *in vivo* studies in animals [35], bacteria [36] and even plants [37, 38] are picking up. With the introduction of newer electrode material of very small size [39], these methods of chemical analysis in living systems might grow even faster. CV studies of fused salts and solid electrolyte [40] might prove very useful for trace analysis.

Background-subtracted cyclic voltammetry can be employed for measuring lower concentration. In particular fast-scan (1000 Vs^{-1}) background-subtracted cyclic voltammetry is seeing increased use for the in-vivo monitoring in neurotransmitters (such as dopamine or serotonin) in the brain. Such coupling of digital background substraction and fast voltammetric measurements provides the sub-second temporal resolution necessary for detecting dynamic concentration changes in the micromolar range occurring in the extracellular environment of the brain.

The good temporal and chemical resolution of such in-vivo cyclic voltammetric experiment offers improved understanding of the chemistry of the brain. These repetitive scanning in-vivo experiments generate large quantities of data that are best represented as three-dimensional (potential, current, time) colour contour images. For example, the temporal release of dopamine following electrical stimulation is evidenced from the rapid interferences from adsorption processed and chemical reactions that are coupled to the primary oxidation reaction of catecholamines neurotransmitters.

Voltammetric detectors may also find increasing applications in chromatography [41, 42]. This is an example of situation where an analytical tool of great importance also supplements the applicability of another analytical tool of great scope.

Industrial corrosion processes are being monitored using rotating disk electrodes. Voltammetric amazingly low detection limits are being used to monitor lead levels in the bloodstreams. Electrodes coated with special polymers find use as glucose detectors for diabetics.

Oxidative bursts of reactive oxygen species are used by cells to fight bacteria and viruses, such oxidative bursts produced by human fibroblasts can be followed electrochemically by using a carbon fibre platinized ultra microelectrode place within a few microns of a single living cell, when membrane is punctured with a micrometer sized sealed pipette. In spite of experimental difficulties, it has been possible to obtain electric signals indicating that the oxidative burst of a mixture of cytotoxic chemicals: H_2O_2, NO, ONO_2^- and NO^-. The cell survived the puncture and performs a similar burst after a couple of hours [43].

Coatings for cars that would not change in appearance after years of service might be discovered, along with repulsion systems for electric vehicles and methods to remove toxic materials selectively from streams of H_2O_2. The scientific basis of this has already been demonstrated.

Recent advances in instrumental techniques, however, promise access to molecular-level information about electrochemical systems. This exciting development opens up important new opportunities in fundamental and applied science.

1.5. The Solvent

A number of physicochemical properties must be considered while choosing a solvent for electrochemical work [44]. The solvent used for the electrochemical process must be a liquid at room temperature, it must have sufficient solubility for ionic substances to form conducting electrolyte, it must be able to dissolve the electroactive species of interest, it must have a wide enough potential region for the study of the redox

process of interest, that is, solvent itself must not undergo oxidation or reduction in this potential region and it must possess the required acid-base properties. The dielectric constant is one of the important parameter.

The cheapest solvent is water, which possesses many physico-chemical properties. It can dissolve ionic components and form highly conducting solutions. Many compounds of electrochemical interest dissolve easily in this solvent. It is acid-base properties are well understood. However, the solvent itself gets reduced or oxidized to H_2 and O_2 very easily. Hence, it only possesses region of 2.0 V for the study of other processes. Water also easily forms oxide films on solid electrodes hence affects reactivity and reproducibility. Some organic reactants are less soluble in water. This defect is normally overcome by using alcoholic mixed solvents or alcohol stock solution of reactants.

Acetonitrile is perhaps a solvent with inert electrochemical properties. It has +3.0 V (verses SCE) anodic and -3.0 V cathodic limits. Even these limit are probably set by the supporting electrolyte oxidation and impurity (water) reduction. If impurities are absent, radical ion chemistry may be studied very well. However this solvent has very poor solubility for ionic species. Salts containing organic ions such as tetra alkyl ammonium salts must be employed.

Dimethyl formamide (DMF) is one of the aprotic solvents which has very good dissolving power of ionic species. It has a cathodic limit up to -0.3 V for anion radicals. Hence this is the solvent of choice for studies on anion radicals and dianions. In the positive potential regions above +1.0 V, the solvent it self decomposes. Cation radicals are less stable in this medium.

Dimethyl sulphoxide (DMSO) has electrochemical properties similar to DMF in the cathodic region. It has somewhat a better cathodic potential limits. Since it is not as basic as DMF, cation radicals are somewhat stable in this medium.

Methylene chloride is the solvent of choice for organic oxidation studies. It is stable up to +3.0 V as acetonitrile. Cation radicals and dications are quite stable in this

medium. Electrolytes are easily soluble in methylene chloride when compared with acetonitrile. Even large organic molecules and polymers are soluble in this medium. However, at negative potentials of -1.0 V, the solvent decomposes. The anionic species are less stable in this medium. Even totally non-polar solvents such as benzene and other hydrocarbons may be used to study the solution phase [45] as well as surface [46, 47] processes. A few detailed discussions on the solvents used in electrochemistry [48-50] are available. Most of the solvents with required purity levels for voltammetric studies are commercially available.

Water deionised and repeatedly distilled with alkaline $KMnO_4$ is usually considered as pure. The purity is checked by conductivity measurements. However, this water might still contain some volatile impurities [51]. These may be removed by passing the distilled water vapour through a column containing Pt catalyst at about 800^oC over which oxygen also simultaneously passed. The organic impurities are oxidized completely by this procedure.

The main impurity present in non-aqueous solvents is water. This is usually removed by refluxing with anhydrous copper sulphate, aluminium chloride, P_2O_5 etc and distilling many times and collecting the proper fraction. The distillation is normally conducted at reduced pressure to avoid decomposition of the solvents. Some aprotic solvents may easily absorb moisture. In such cases vacuum lines must be employed during purification, storage and use in voltammetric work [52]. An easier procedure would be to employ a dehydrating agent such as anhydrous alumina as an internal addition [53]. It must, however, be ensured that these materials do not interfere in the voltammetric behaviour in the other ways.

1.6. Supporting Electrolytes

All ionic salts or ionisable compounds in a solvent are defined as supporting electrolytes. It is very important to realise that they can influence the electrochemical processes in a number of ways. These electrolytes impart conductivity to the solvent and hence enable continuous current flow in solution. The salient features of supporting electrolyte are, they must remain electroactive in the potential region of interest, the

concentration of the supporting electrolyte should be very high, in order that they do not form space charges near the surface and hence the space charge potential do not influence the charge transfer kinetics. They should not get adsorbed on the surface, in which case they can catalyse or inhibit other reactions. They should neither form ion pairs with anion radicals formed in the electrode process nor form complexes with the reactants or products.

H_2SO_4, $HClO_4$ and HCl are normally employed for studies in acidic aqueous solutions and NaOH or KOH are employed for alkaline media. In neutral region, if buffering is important, acetate, citrate, pyrophosphate and phosphate buffers are usually employed. B-R buffer is used over a wide pH range. If the redox process does not involve acid-base reactions, no buffer are needed and any electrolyte may be used.

Solubility is the main consideration in selecting supporting electrolyte for aprotic solvent. A number of tetra-ethyl ammonium (TEA) salts show good solubility in aprotic media. Tetra-ethyl ammonium (TEA) salts and more recently tetra-n-butyl ammonium (TBA) salts are widely employed for this purpose. TEA salts are frequently available in the form of halides. The perchlorates or fluroborates may be easily obtained by double decomposition of these salts with the corresponding sodium salts. The precipitated $TAAClO_4$ or $TAABF_4$ may be recrystallised twice or thrice [53]. Hygroscopic electrolytes dehydrated in an oven and stored in desiccators. Care must be exercised in handling explosive salts such as $NaClO_4$. They must neither be overheated nor ground in mortar with force.

1.7. Electrodes

The advent of modern electrochemistry created the need for new electrodes and electrode setups. The most common arrangement today is the electrochemical cell with three different electrodes:

- Working electrode (WE)
- Reference electrode (RE)
- Counter electrode (CE)

1.7.1. Working Electrode

The ideal working electrode is very clean metal surface with a well defined geometry that is in direct contact with an electrochemical test solution. Working electrodes intended for general purpose work are usually made from a metal that is electrochemically inert over a wide range of potentials. The most widely used metals are mercury, platinum, gold, and various forms of carbon. Solid metals are typically fashioned into disks surrounded by a chemically inert shroud made from Teflon, glass, or epoxy. Mercury, being a liquid, tends to be used as a spherical droplet in contact with the solution.

The size and shape of the electrode surface also has an effect on the voltammetric response of the electrode. The overall current observed at an electrode is directly related to its surface area, and disk shaped electrodes with diameters greater than 100 mm, or macroelectrodes, generally produce easily measured currents in the microamp to milliamp range. Electrodes with dimensions less than 100 mm are generally referred to as microelectrodes, and these typically produce currents in the picoamp to nanoamp range. Although the overall currents observed at microelectrodes are small enough to require specialized electrochemical equipment, these electrodes enjoy a greater signal to-background ratio and, being small, find uses in applications where the sample size is quite small.

1.7.1.1. Mercury Electrode

This is the most popular electrode [54] makes use of liquid mercury as a working electrode. In most common incarnation, the dropping mercury electrode, a reservoir of mercury is allowed to slowly drain through a vertical capillary tube immersed in the electrochemical test solution. As the mercury slowly exits from the capillary; it forms a small drop with a nearly spherical shape that is in contact with the test solution. Electroactive analytes in the test solution undergo oxidation or reduction reactions at the surface of the drop.

This electrode configuration enjoys quite a few advantages including a very long history of use and an electrode surface that is easily reproducible. Indeed, in the event that the surface of the mercury drop becomes fouled, the drop is simply allowed to fall into the test solution, and a fresh drop is allowed to form at the capillary tube's exit. In aqueous solutions, the mercury electrode can be used at more negative potentials than other metals without interference from the reduction of hydronium ion. Finally, the mercury electrode plays an important role in stripping voltammetric technique which relies on pre-concentrating one or more analytes into a mercury electrode and then separately electrolyzing (or stripping) each individual analyte out of the electrode.

1.7.1.2. Platinum Electrode

Despite the expense associated with these precious metal, platinum is one of the most widely used materials for fabricating working electrodes. Platinum has the advantage of being an easily machined metal that is electrochemically inert. In aqueous solvent systems, the platinum working electrode is a good choice when working with positive potentials, but at negative potentials, interference from the reduction of hydronium ion is a problem. In rigorously anhydrous organic solvent systems, platinum is the best and most popular choice for the working electrode material due to its wide potential window in both the positive and negative directions.

Large diameter platinum macroelectrodes are generally fabricated by welding a thick platinum disk to the end of a brass rod, machining the platinum disk and brass rod so that they are concentric, and then placing a Teflon shroud around the entire assembly. The platinum surface is then ground to a mirror quality finish using a polishing paste that contains sub-micron alumina particles. As with all solid metal electrodes, the surface must occasionally be repolished to remove surface contaminants picked up during experiments.

Smaller diameter platinum disk electrodes and platinum microelectrodes are usually fabricated by shrouding a short length of platinum wire in soft glass. The diameter of the resulting platinum disk is the same as the diameter of the wire used.

Because of the hardness of the glass shroud, these electrodes are usually polished to a mirror finish using polishing paste that contains sub-micron diamond particles.

Of the solid metal electrodes, it is definitely the most popular due to its applicability to a wide range of electrochemical systems, durable and long lasting. Its primary disadvantage is that it has a limited use at negative potentials in aqueous solutions.

1.7.1.3. Gold Electrodes

Gold working electrodes are designed along the same lines as platinum working electrodes. Gold is usually less expensive than platinum, but it is not as electrochemically inert. The surface of a gold electrode is subject to oxidation at moderately positive potentials, and so it is not as generally useful as platinum.

1.7.1.4. Carbon Electrodes

Various forms of carbon are used as working electrode materials [55, 56]. Carbon electrodes are useful over a fairly wide potential window in both the positive and negative directions, and their principle advantage over platinum electrodes is the ability to work at more negative potentials in aqueous solutions. Solid carbon electrodes are usually made from glassy carbon or pyrolytic graphite, both of which are fairly expensive materials that are more difficult to machine than platinum or gold. The surface of a carbon electrode usually needs to be polished quite frequently, and the surface sometimes has to be “activated” by various empirical methods in order to obtain maximal performance from the electrode.

A less expensive carbon electrode can be fashioned using carbon paste [57]. A cylindrical recess is drilled into a Teflon shroud, and an electrical contact is placed in the back of the recess. Each time the electrode is to be used, the recess is packed with a paste that contains carbon particles, and then the paste is carefully polished to a smooth disk-shaped surface. Working with a carbon paste electrode is technically more demanding because the paste can be gouged inadvertently after being polished.

1.7.1.5. Rotated Electrodes

A special class of electrochemical techniques, known as hydrodynamic methods, actually involve the use of spinning working electrodes. Typically, a specially designed glassy carbon or platinum disk electrode is attached to the end of a rigid shaft, and then this shaft is mounted on a high speed motor. These electrodes are immersed in a test solution and rotated at several thousand rotations per minute. A characteristic "vortex-like" solution flow pattern emerges as a result of the electrode's motion.

Because the solution is constantly stirred, fresh analyte solution is always being conveyed to the region near the surface of the electrode. This steady flow of analyte allows what is known as a "steady-state current" to flow at the rotating electrode as analyte are either oxidized or reduced. Steady-state currents are generally quite easy to measure because they remain constant with respect to time. In most other electrochemical methods, currents tend to decay with time as the supply of analyte near the electrode is depleted.

When using a rotating electrode, it is important that the cell volume be large enough to sustain a rapidly spinning solution flow. Also, the opening at the top of the cell must be large enough for the shaft of the rotating electrode. This means that the cell contents are open to the air, making oxygen removal difficult. A strong flow of inert gas is required to blanket the solution whenever a rotating electrode is being used to study an air-sensitive electrochemical system. Electrical contact to a rotating electrode is usually made with brushes that are in mechanical contact with the rotating shaft.

1.7.2. Reference Electrode

The potential of a working electrode in a voltammetry experiment is always controlled with respect to some standard, and that standard is the reference electrode. While the thermodynamic scale of half-reaction potentials found in most textbooks measures electrode potentials against the "standard hydrogen" reference electrode (SHE), in actual practice the SHE is much too cumbersome to use. For this reason, a number of other reference electrodes have been developed. Experimental measurements of potential

are made against these alternate reference electrodes, and then the potentials are "corrected" by simple addition or subtraction and reported against the SHE.

One of the most generally available reference electrodes for work in aqueous solutions is the saturated calomel electrode (SCE). The half reaction that occurs inside of an SCE reference is given below.

$$Hg_2Cl_2(s) + 2e^- \Longleftrightarrow 2\ Hg\ (l) + 2\ Cl^-(aq)$$

At 25°C, the formal potential for the SCE half reaction lies 0.2415 volts more positive than the SHE reference electrode. A potential measured against using an SCE can be reported versus the SHE simply by adding 0.2415 volts to it.

The SCE electrode must be constructed in an appropriate piece of glassware that can keep a small amount of mercury in direct contact with solid calomel (Hg_2Cl_2) paste while at the same time keeping the paste in contact with a saturated aqueous solution of potassium chloride. The short hand notation for the SCE half cell is as follows:

$$Pt(s) / Hg(l) / Hg_2Cl_2(s) / KCl\ (aq,\ sat'd)\ //$$

Electrical contact is made by immersing a platinum wire into the liquid mercury, and the potassium chloride solution maintains ionic contact with the test solution in the electrochemical cell via a salt bridge or porous glass frit. Such electrodes can be "home made" or purchased from a variety of manufacturers.

Other useful reference electrodes are based on half reactions involving a silver electrode. For work in aqueous systems, the "silver-silver chloride" or "Ag/AgCl" reference is quite popular. The half reaction for this reference electrode is as follows:

$$AgCl(s) + e– <-----> Ag(s) + Cl–(aq)$$

The actual potential assumed by an Ag/AgCl reference depends only on the activity of the chloride anion. (The other two species appearing in the half reaction are solids which always have unit activity.). To serve as a reference, the chloride activity needs to be held constant. To accomplish this, a silver wire (coated with a layer of silver

chloride) is immersed in an internal solution saturated with potassium chloride. The chloride ion concentration remains fixed at the saturation limit. The short hand notation for this reference electrode half cell is given below:

$$Ag(s) / AgCl(s) / KCl\ (aq,\ sat'd),\ AgNO_3\ (aq)\ //$$

Electrical contact is made by direct connection to the silver wire, and the internal electrode solution is placed in ionic contact with the test solution via a salt bridge or porous glass frit.

1.7.3. The Auxiliary Electrode

In traditional two electrode cells that have only a working electrode and a reference electrode, current is necessarily forced to flow through the reference electrode whenever a measurement is made. If enough current flows through a reference electrode, its internal chemical composition may be significantly altered, causing its potential to drift away from the expected standard value. For this and other reasons, it is desirable to make electrochemical measurements without current flowing through the reference electrode. Modern three and four electrode potentiostats use a feedback circuit to prevent this from happening, but this feedback circuit requires that an additional auxiliary electrode be introduced into the electrochemical cell. This auxiliary (or counter) electrode provides an alternate route for the current to follow, so that only a very small current flows through the reference electrode.

The auxiliary electrode can be made from just about any material using any desired electrode geometry. Design choices are usually based on finding a material that is chemically inert in the particular test solution being studied, and it is generally a good idea for the auxiliary electrode to have a large surface area. In most cases, a coil of platinum wire is used, but stainless steel, copper or aluminium wire may work in non-corrosive solutions where metal cation interference is not a concern. If the electrochemical cell is made of metal, then the cell itself might be used as the auxiliary.

Because current flows at the auxiliary electrode, electrochemical processes will also occur there. If the working electrode is reducing something, then the auxiliary

electrode must oxidize something, and vice versa. The products generated at the auxiliary electrode, if allowed to diffuse to the working electrode, may interfere with the experimental measurement. When this is a problem, the auxiliary electrode is placed in a separate compartment containing an electrolyte solution that is in ionic contact with the main test solution via a glass frit. In most cases, however, the auxiliary can be placed right in the test solution along with the reference and working electrodes.

1.8. A Brief Literature Survey of Cyclic Voltammetric Investigations

Research interests involve the study of reactive intermediates that are formed when compound are reduced or oxidized electrochemically. The application of the chemically modified electrodes in electroanalysis offer several advantages due to their unique electrode surface properties. Therefore, there has been an increasing interest in the creation of chemically modified electrode surfaces that differ from the corresponding bare surfaces. These chemically modified electrodes can lower the over potential, increase the reaction rate and improve the selectivity of some bioactive molecules. The literature survey reveals the following prominent references.

Ren *et al.,* [58] investigated the electrocatalytic behaviour of electrodeposited film of caffeic acid on a glassy carbon (GC) electrode under neutral conditions (pH 7.4) in 0.15 M phosphate buffer solution (PBS) using CV. They observed a high electrocatalytic activity of the poly (caffeic acid) modified GC electrode towards the oxidation of epinephrine (EP) a component of neural transmission of nerve impulse.

Jin *et al.,* [59] explored the electrochemical behaviour of a poly rutin modified paraffin impregnated graphite electrode towards the electrocatalytic oxidation of adrenalin, serotonin and ascorbic acid in 0.1 M PBS (pH 7.0) as supporting electrolyte using CV and differential pulse voltammetry (DPV). They observed that as compared with bare electrode the poly rutin modified electrode displayed strong electrocatalytic activity for the oxidation of adrenalin serotonin and ascorbic acid. It resolved the overlapped voltammetric response of adrenalin and AA into two well defined voltammetric peaks of about 172 mV by DPV.

Yongxin and Xiangqin [60] evaluated a poly (vinyl alcohol) modified GC electrode for the selective and sensitive determination of DA, AA and UA and simultaneously in their mixture by using CV and DPV. They observed well separated peaks of the three compounds with potential difference of 140 , 140 and 280 mV in CV and 180, 130 and 310 mV in DPV between DA and AA, DA and UA and AA and UA respectively in 0.1 M PBS (pH. 7.0).

Cheng *et al.,* [61] studied the electropolymerisation of o-Amino benzoic acid as a modifier to fabricate a poly (o-ABA) modified GC electrode towards the oxidation of epinephrine in the presence of ascorbic acid in 0.1 M PBS (pH.7.0). They observed that the polymeric film modified GC electrode has excellent electrocatalytical activity on the oxidation of epinephrine by using CV and DPV.

Aslanoglu *et al.,* [62] performed CV and DPV studies to investigate the electrochemical determination of DA in the presence of AA using a poly (3-Acetythiophene) modified GC electrode in 0.1 M PBS (pH 7.2). They observed that the poly (3-Acetythiophne) modified GC electrode accelerated the rate of electron transfer reaction of DA.

The electrochemical properties of DA, EP and their simultaneous determination at a poly (L-Methionine) modified GC electrode in 0.1 M PBS (pH 7.5) by using CV have been discussed by Ma and Sun [63]. They demonstrated that the poly (L-Methionine) modified GC electrode can be applied for the simultaneous measurement of DA and EP in the presence of high concentration of UA and AA in urine samples.

Zhao *et al.,* [64] developed a poly (sulfosalycyclic acid) modified GC electrode for the electrochemical studies of DA in neutral pH using CV.They observed that the polymer film modified GC electrode exhibited high electrocatalytic activity to DA oxidation.

Roy *et al.,* [65] studied the electroplymerisation of N, N, dimethylaniline (DMA) on the surface of GC electrode in 0.2 M PBS (pH 7.0) by CV. They demonstrated that the poly (N,N,dimethylaniline) film coated GC electrode can simultaneously detect AA and

DA which coexisted in a homogenous solution and the separation of the oxidation peak potentials for AA and DA was about 300 mV which was large enough for the simultaneous determination of these two species in their mixture solution.

Simultaneous determination of DA and AA at a poly (toluidine blue) modified GC electrode in 0.1 M PBS (pH 6.6) has been studied by Chen *et al* [66] They observed that the resulting polymer film modified GC electrode can remarkably catalyse the electrochemical oxidation DA and AA. Zhang *et al* [67] reported the use of poly (styrene sulphonic acid) sodium salt / single wall carbon nanotube film modified GC electrode to fabricate a biosensor for the detection of DA in the presence of AA.

A poly (3,4-ethylenedioxy) thiophene film modified GC electrode (PEDOT) for the simultaneous determination of various combinations of DA and AA in 0.1 M PBS (pH 7.0) using CV and square wave voltammetry has been developed by Vasantha and Chen [68] They used the PEDOT modified GC electrode to study the electrocatalytic activity of DA and AA. The also studied the mechanism for the electrochemical oxidation of DA and AA using a rotating ring disk electrode method at the PEDOT film modified GC electrode.

Yao *et al.,* [69] studied the electrochemical characterisation of poly (eriochrome black T) and it application to simulataneous determination of DA, AA and UA in 0.05 M PBS (pH 4.0) using CV. They observed that the polymer film modified GC electrode conspicuously enhanced the redox current of DA, AA and UA and could separately determine DA at its low concentration in the presence of higher concentration of AA and UA. Chen *et al* [70] developed a poly (4-(2-pyridyl-azo) resorcinol modified GC electrode and used it for the electrocatalytic oxidation and determination of DA in the presence of AA and UA in PBS (pH 4.0) using CV. They demonstrated that the film modified electrode showed excellent electrocatalytic activity towards the oxidation of DA, AA and UA individually and simultaneously.

Zhang *et al.,* [71] reported a novel polymerised film of acid chrome K on the surface of GC electrode by electropolymerisation method and its application for the simultaneous determination of DA,AA, and UA in 0.05 M PBS (pH 4.0) using CV.

Hou *et al* [72] investigated a polymerised film 3, 5, dihydroxy benzoic acid (DBA) on the surface of GC electrode in neutral solution by using CV and applied it for the electrochemical determination of DA and AA.

Lin *et al.,* [73] developed a nano-composite of DNA/poly (p-aminobenzenesulphonic acid) bilayer modified GC electrode as a biosensor by electrodeposition method in 0.1 M PBS (pH 7.0) using CV. They demonstrated that the fabricated electrode showed a god electrocatalytic activity towards the oxidation of DA, UA and AA and separated the originally overlapped voltammetric signals of DA, UA and AA at bare GC electrode into three well defined peaks with large potential separation in neutral pH.

The electrochemical polymerisation of 4-amino-1-1-azobenzene-3,4-disulphonic acid (acid yellow 9 dye) onto the surface of GC electrode and indium tin oxide coated electrode from acidic solution containing acid yellow 9 dye (AY) monomer using CV has been studied by Kumar *et al.* [74]. They observed that the poly (AY) film coated electrode exhibited excellent electrocatalytic activity towards the oxidation of DA in 0.1 M PBS (pH 7.0). They also demonstrated that the poly (AY) modified electrode was found to be a good sensor for the selective and sensitive determination of DA without any interferences.

Li and Lin [75] developed a gold nanocluster/overoxidized-polypyrrole composite modified glassy carbon electrode by electrodeposition of gold nanoclusters on insulating overoxidized-polypyrrole (PPyox) film modified glassy carbon electrode (GCE). They demonstrated that the nano-Au/PPyox composite-coated exhibited stable and sensitive current responses toward DA and 5-HT oxidation.

Jin *et al.,* [76] fabricated a novel poly rutin (Ru) modified paraffin-impregnated graphite electrode (WGE) by electrochemical method. They observed that Ru/WGE displayed strong catalytic function for the oxidation of adrenalin (EP), serotonin (5-HT), and ascorbic acid (AA) and resolved the overlap voltammetric response of EP and AA into two well-defined voltammetric peaks of about 172 mV with DPV.

Sun *et al.,* [77] developed an electrochemical sensor based on carbon nanotubes (CNTs)-ionic liquid (IL) composite for the simultaneous determination of serotonin (5-HT) and dopamine (DA). They observed that the CNTs-IL composite modified electrode presents excellent selectivity and sensitivity towards 5-HT and DA and eliminates the interference of ascorbic acid.

Goyal *et al.,* [78] reported the simultaneous voltammetric determination of serotonin and 5-hydroxyindole acetic acid using single walled carbon nanotube modified glassy carbon electrode and gold nanoparticles modified indium tin oxide electrode. They demonstrated that this method was fast, simple, accurate with low detection limits for 5-hydroxytryptamine (5-HT) at single-walled carbon nanotube modified glassy carbon electrode and 5-hydroxyindole acetic acid (5-HIAA) at gold nanoparticles modified indium tin oxide electrode.

Wu *et al.,* [79] developed a chemically modified electrode based on the carbon nanotube film-coated glassy carbon electrode (GCE) for the simultaneous determination of dopamine (DA) and serotonin (5-HT). They explored that the multiwall carbon nanotube (MWCNT) film-coated GCE exhibits a marked enhancement effect on the current response of DA and 5-HT and lowers oxidation overpotentials. The responses of DA and 5-HT merge into a large peak at a bare GCE, but they yield two well-defined oxidation peaks at the MWCNT film-coated GCE.

1.9. Residual Current in Voltammetry

On applying a potential sweep, the current that flows through the cell before the charge transfer takes place, is called the residual or background current. It is composed of the following components [80].

1.9.1. Faradaic Current

It is generated from the Faradaic process which is a non-adsorptive process arising from electron transfer across the metal or electrolyte interface. The redox reaction of solution species that takes place is controlled by Faraday's laws [81], that is, the amount of electricity which is passed (charge) is proportional to the number of moles of

reactant converted. Electrode process where Faradaic process takes place are classified as charge transfer electrodes, since the extent of reaction depends on the measured charge passing through the electrode surface.

1.9.2. Non-Faradaic Current

It arises when the adsorption and desorption of ions from the electrode surface results in an electric current due to charging of double layer. The interface between the electrolyte and the working electrode acts like a capacitor. Therefore, a current is required to change the potential applied to the working electrode and this is referred to as non-faradaic current. Since the potential in a CV experiment is constantly changing, there is an approximately constant charging current, which makes a major contribution to the background current. As there is no charge transfer across the double layer, it is not governed by faraday's laws and hence is called non-faradaic current. The charging current is directly proportional to the sweep rate whereas the faradaic current is directly proportional to the square root of scan rate.

1.10. Polarisable and Non-polarisable Interfaces

All electrode-solution interfaces can be classified as polarisable or non-polarisable. An electrode for which an electron can pass easily across the interface is called non-polarisable. In this case, external application of a change of potential may result in more electrons passing rapidly across the interface. Thus, there is a negligible build-up of excess charge on the electrode surface, i.e. the interface does not polarise. Platinum in contact with hydrochloric acid is a non-polarisable interface. In contrast, when the transfer of electrons is difficult, a potential change from outside will induce a substantial build-up of excess charges at the interface, hence, the electrode is termed polarisable. When a potential is applied externally to the electrode, the transfer of electrons through the interface is negligible. That is, a small change in current flow causes a large change in electrode potential. An ideally polarisable interface is one which can allow the passage of current without causing a change in the potential difference across it. In addition, when the current associated with charging the electrode-electrolyte interface arises purely from capacitive effects; such an interface is termed an ideally

polarisable electrode (IPE) [82]. While no real electrode behaves ideally over the entire potential range, some electrode-solution systems, over limited potential ranges, can show behaviour which is approximately, ideal, for instance, a mercury electrode in contact with a de-aerated potassium chloride solution which behaves as an IPE at potentials in excess of 1.5 V.

1.11. Electrodes Processes

The reaction taking place between the electrode surface and species within the solution can proceed through a series of steps that causes the conversion of the dissolved oxidised species (O) to reduced species (R) in solution (Fig. 1.4). The electrode reaction rate is governed by the reaction rates such as.

i. Mass transfer
ii. Electron transfer of non-adsorbing species.
iii. Chemical reactions preceding or following the electron transfer which could be homogeneous such as protonation or dimerization' or heterogeneous ones like catalytic decompositions on the electrode surfaces.
iv. Other surface reactions such as adsorption, desorption, crystallisation etc.

The simplest reaction involves only mass transfer of reactant to the electrode, heterogenous electron transfer involving non adsorbed species and the mass transfer of the product to the bulk solution. More complex reaction sequence involving a series of electron transfer, protonations, branching mechanisms, parallel paths or modifications of the electrode surfaces are quite common. When a steady state current is obtained, the rates of all reactions steps are the same. The magnitude of this current is often limited by the inherent sluggishness of one or more reactions called rate determining steps. The more facile reactions are then held back from maximum rates by the slowness with which such steps disposes of their products or create their participants [83, 84].

1.11.1. Mass Transfer Processes

Whenever an electrochemical charge transfer process takes place at the electrode surface, the electroactive material gets depleted and a concentration gradient is set up.

Under such conditions the reactant diffuses towards the electrode surface and the corresponding product of the electrode reaction diffuses away from the electrode surface. Mass transfer in electrochemistry illustrates the movement of electroactive species from one location in solution to another arising from differences in electrical or chemical potential at the two locations. There are three forms of mass transport which can influence an electrolysis reaction (Fig. 1.5):

i Diffusion

ii Migration

iii Convection

1.11.1a. Diffusion

Diffusion is the movement of a species down a concentration gradient and it must occur whenever there is a chemical change at a surface. Diffusion arises due to the depletion of the electroactive ion near the electrode surface; this depletion is only due to the charge transfer occurring at the electrode surface. The diffusion processes are named after the detailed study of geometry of the electrodes *viz*., planar diffusion (to a plane electrode), spherical diffusion (to a spherical electrode i.e. mercury electrode) and cylindrical diffusion (to a wire electrode). The diffusion is characterized and governed by Flick's laws of diffusion [85].

Fick's Laws of Diffusion

Consider a stationary planar electrode-electrolyte interface. If a species O is reduced to R at the interface, its concentration C_0 at the surface is lowered when compared with its bulk concentration. Hence O from the bulk will move towards the electrode. After a long interval of time, a steady rate of flow of O towards the surface (called the flux, J) would be established. This rate is given by Fick's first law [86].

$$J = - D_{ox} \delta C_{ox}(x,t) / \delta x$$

where, D_{ox} is the diffusion coefficient at the electroactive ion (in cm^2s^{-1}).

The rate of change of concentration with time is given by Fick's second law of diffusion [86]

$$\delta C_{ox}(x,t) / \delta x = D_{ox} \delta^2 C_{ox}(x,t) / \delta x^2$$

In this equations, the concentration is written as $C_{ox}(x,t)$ to specifically indicate that its value depends on both distance (x) from the planar interface and time (t). Similar equations may also be written for $C_{R.}$ These two equations will take different forms depending upon geometry [86, 87] of the electrode. The value of D varies from system to system.

1.11.1b. Migration

Migration involves the movement of charged ions under the influence of electric field. Since migration is nonspecific in nature, migration due to the electroactive ion cannot be distinguished from the migration of other charged species present in the solution. Therefore, it becomes necessary to add a large excess of inert electrolyte to the cell solution, in order to eliminate the migration of the electroactive ions of interest. The inert electrolyte, which is generally called the supporting electrolyte, neutralizes the electrostatic forces of attraction acting between the working electrode and electroactive ion by suppressing the transport number of the reactants. To achieve this inert electrolyte is added to the electrolytic solution (at least 50 to 100 times in excess of the electroactive ions). Inert or indifferent supporting electrolyte contains ions that do not take part in the electrolysis [85]

1.11.1c. Convection

Convection is the movement of ions due to mechanical forces. Natural convection arises due to the difference in density and temperature at different parts of the solution and natural convection cannot be duplicated or reproduced. Sometimes convection is forced by stirring the solution in a known fashion. The forced convection can be reproduced and treated mathematically. The natural convection because of its non-reproducible nature complicates the electrode process. It is imperative to eliminate it. This can be achieved by carrying out the electrolysis in a thermostat in the absence of

stirring or vibrations. Care should be taken to restrict the electrolysis time to a few minutes otherwise the natural convection sets in and reproducible results may not be achieved [85].

1.11.2. Electron Transfer (ET) or Charge Transfer Process

The electron transfer at the interface between the electrode and electrolyte is central to an electrode reaction. Electroactive species having moved from the bulk of the solution by either diffusion or under forced convection enters in the electrical double layer, which is under direct influence of the electrode. On entering the double layer the species undergoes a structural orientation so that it can take up or give up electrons from or to the electrode surface respectively with the least activation energy when a suitable potential is applied and macroscopically, we observe current. This state of the reactant species is known as transition state. Being unstable the species is in transit state converts itself to the final product by release of activation energy and gets reduced or oxidized. This final product after undergoing suitable reorientation either gets deposited on the electrode surface or moves away from the electrode surface into the bulk solution. The transfer of electrons to or from the substrate is an activated process. The electron transfer process can be

i. Reversible process
ii. Irreversible process and
iii. Quasi-reversible process

1.11.2a. Reversible Electron Transfer Process

For a reversible process, oxidation and reduction peak is observed as shown in Fig.1.6. Reversibility can be defined as chemical or electrochemical. In an electrochemically reversible process the electron transfer is not rate limiting. For a chemically reversible process, both forms of redox couple (O for oxidized form and R for reduced form) are stable in the time scale of measurement. The rate of electron transfer is fast compared to the rate of mass transport and does not control the overall rate. In this process the rate of reaction is fast enough to maintain equal concentration of the oxidized

and reduced species at the surface of electrode. The concentration C_{ox} and C_{red} of oxidized and reduced forms of the redox couple respectively follow the Nernst equation

$$E = E^{o} + RT/ nF \ln C_{ox} / C_{red} \qquad \text{------- (1.1)}$$

where, n= no. of electrons transferred, F= Faraday constant, R= Gas constant and T=temperature. If the system is diffusion controlled then the Fick's law of diffusion holds for both oxidation and reduction. Under these conditions, peak current given by Randles Sevcik equation;

$$i_p = (2.69 \times 10^5)\, n^{3/2} A\, D^{1/2} C_0\, v^{½} \qquad \text{------- (1.2)}$$

where, n is the stoichiometric number of electrons involved in the electrode reaction, A is the area of electrode in cm^2, D_o is the diffusion coefficient of the species O in cm^2s^{-1}, C_o is the concentration of the species O in $mol/cm^3$and υ is the scan rate in Vs^{-1}.

Diagnostic tests for cyclic voltammograms of reversible system at 25 ^{o}C

i. $\Delta E_p = E_{pa}-E_{pc} = 59/n$ mV, where n is number of electrons change
ii. $i_{pc}/i_{pc} = 1$
iii. $i_p \ \alpha\ \nu^{1/2}$
iv. E_p is independent of ν

1.11.2b. Irreversible Electron Transfer Process

For an irreversible process, only forward oxidation or reduction peak is observed but at times with a weak reverse peak (Fig. 1.7**)**. This process is usually due to slow electron exchange or slow chemical reactions at the electrode surface [31]. In an irreversible electrode process, the mass transfer step is very fast as compared to the charge transfer step.

For an Irreversible reaction, the peak current is given by [88]

$$i_p = 2.99 \times 10^5\, n\, (\alpha n)^{1/2} A\, D_0^{1/2}\, \nu^{1/2}\, C_o^{*}$$

$$(\alpha n_a) = 47.7/E_p - E_{p/2}$$

The value of E_p, the difference between the cathodic and anodic peak is of the order of 59mV/n is given by equation. The peak separation E_p is a factor determining the reversibility or irreversibility of an electrode reaction. The equation by Nicholson is normally used to calculate electron transfer rate constants.

Diagnostic tests for cyclic voltammograms of irreversible system at 25 ^oC

i. no reverse peak

ii. $i_p \ \alpha \ \nu^{1/2}$

iii. E_p shifts = $30/\alpha n_a$ mV, where α is charge transfer coefficient

iv. $[E_p-E_{p/2}] = 47.7/\alpha n_a$ mV

1.11.2c. Quasi Reversible Electron Transfer Process

This is a class of electrode reactions in which the rates of charge transfer and mass transfer are comparable or competitive. Quasi-reversible process is intermediate between reversible and irreversible systems (Fig. 1.8). The current due to quasi-reversible processes is controlled by both mass transport and charge transfer kinetics [2, 22]. The process occurs when the relative rate of electron transfer with respect to that of mass transport is insufficient to maintain Nernst equilibrium at the electrode surface. In the quasi-reversible region both forward and backward reactions make a contribution to the observed current.

Diagnostic tests for cyclic voltammograms of quasi-reversible system at 25 ^oC

i. i_p increases with scan rate, but is not proportional to scan rate.

ii. $i_{pc}/i_{pa} = 1$, provided $\alpha_c=\alpha_a = 0.5$

iii. ΔE_p is greater than 59/n mV and its increases with increasing scan rate

iv. E_{pc} shifts negatively with increasing ν

1.12. Objectives and Scope

The focus of the work covered in this thesis is to controllably alter the properties of carbon surfaces by electropolymerisation method using cyclic voltammetric technique, so that the surfaces are useful for desired sensor applications.

Alongside the primary goal, the research carried out promotes knowledge at many levels relevant to the interests of the academic community in the field of sensor fabrication, such as the employment of a number of different monomers and their electrosynthesis.

The present work is also aimed at investigating the electrochemical studies and elucidation of the sequence of electron transfer and chemical reactions that occur at or near the electrode surface. Research interests involve the study of reactive intermediates that are formed when compounds are reduced or oxidised electrochemically.

The aspects investigated are reversible/irreversible or coupled nature of electron transfer, number of electrons involved, kinetic and diffusion controlled processes, effect of concentration of electroactive species on the redox pathways, effect of pH, nature of the intermediates formed, nature of the products formed etc.

More emphasis has been given not only to the electrochemical behaviour of dopamine ascorbic acid and uric acid but also the versatility of use of carbon paste. The preparation and characterisation of bare and chemically modified carbon paste electrode surface has been studied. Thorough characterisation of carbon paste electrode before modification has been studied. The electrochemical properties, carbon composition and surface roughness of both the surfaces are examined.

Present work is also aimed at the development of voltammetric sensors for the detection of UA, DA, NE, EP and AA which are neurotransmitters present in the extracellular fluid of the central nervous system. It is generally believed that the direct redox reactions of these species at bare electrodes are irreversible and high over potential are usually required for their amperometric detection. Moreover, the direct redox reaction of these species takes place at very similar potentials and often suffers from pronounced fouling effects, which results in rather poor selectivity and reproducibility. Hence there is need for the development of polymer modified electrodes because of its high selectivity and sensitivity due to the film homogeneity in electrochemical deposition, strong adherence to the electrode surface and chemical stability of the film.

The electrochemical studies of these biologically active species serve to elucidate their biological process and their interrelationship that are involved in living organisms.

In addition to analytical aspects, CV has been used to establish the electrochemical behaviour of the given molecules through mechanistic studies. Electrochemical techniques are most suitable to investigate the redox properties of new drugs.

Because the biological electrons transfer reactions are complicated, though they have many things in common. Both involves essentially heterogeneous electron transfer process, pH and temperature dependent and occur at electrode/electrolyte interface or membrane/solution interface. Hence, explanations based on electrochemistry have played an important role in interpreting and understanding the biological phenomena.

Starting with simple carbon pastes, improving their performances by chemical modification implies it versatility. Exploring the advantage of the modified, an attempt has been made to explore its applications to real life situations.

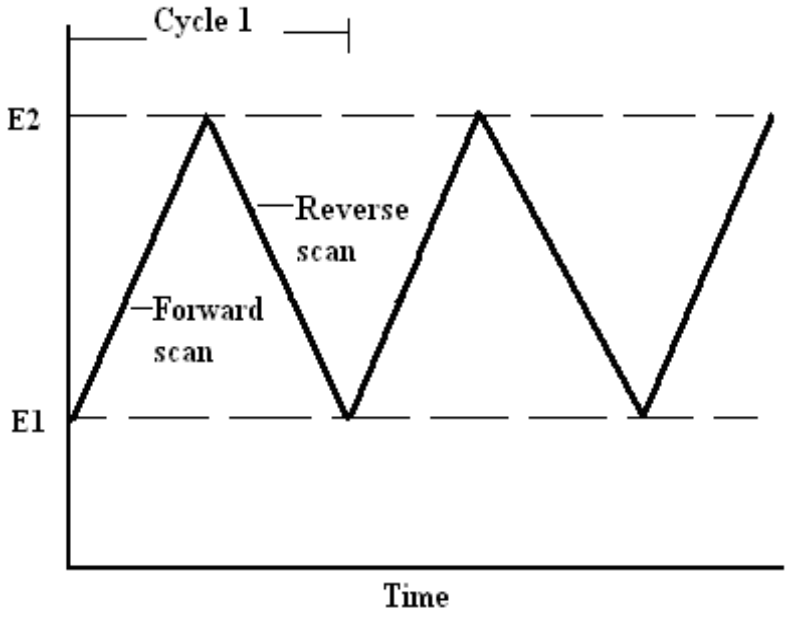

Fig. 1.1. Variation of the applied potential as a function of time in a cyclic voltammetry experiment

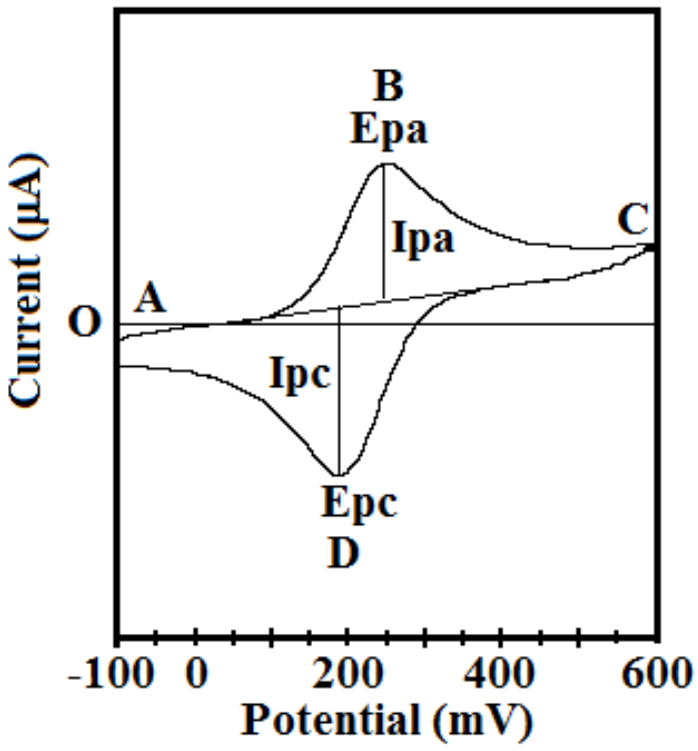

Fig. 1.2. A typical cyclic voltammogram of current verses potential

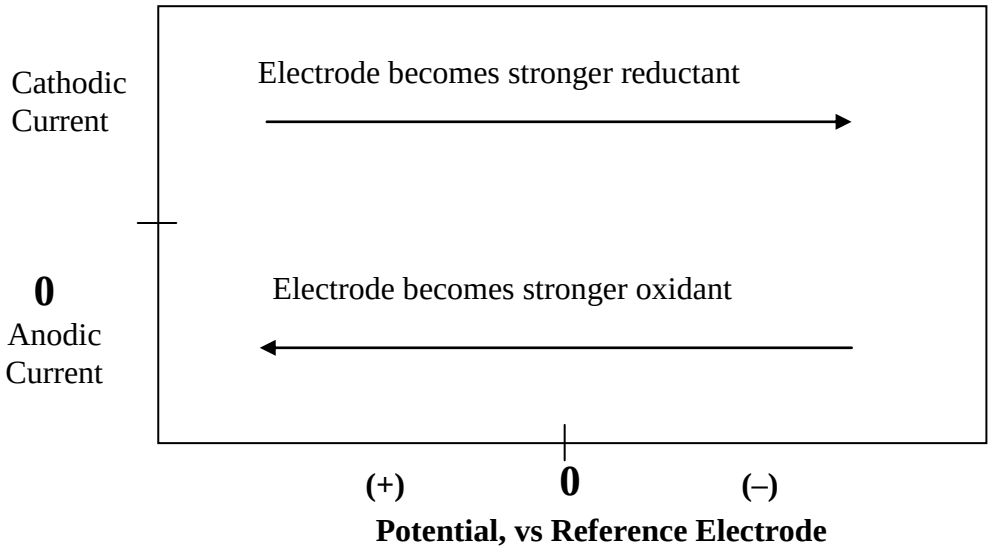

Fig. 1.3. Potential-Current axes for Cyclic Voltammetry

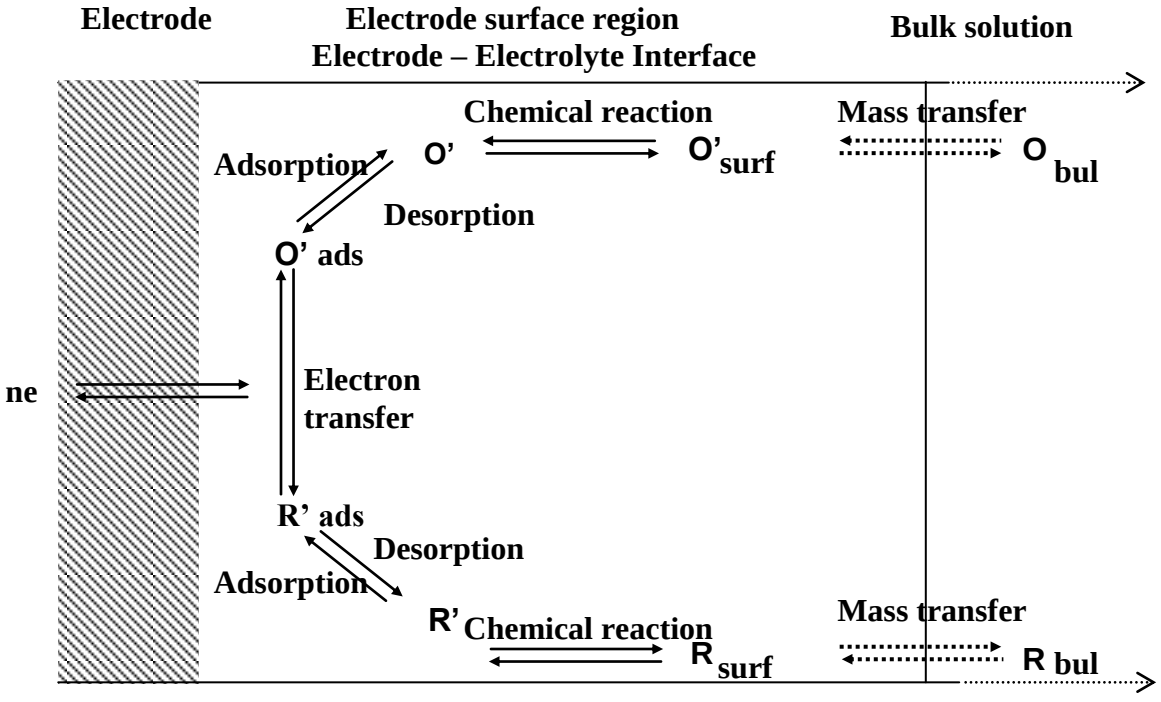

Fig. 1.4. General pathway of electrode-mediated processes of oxidized (O) and reduced (R) electroactive species

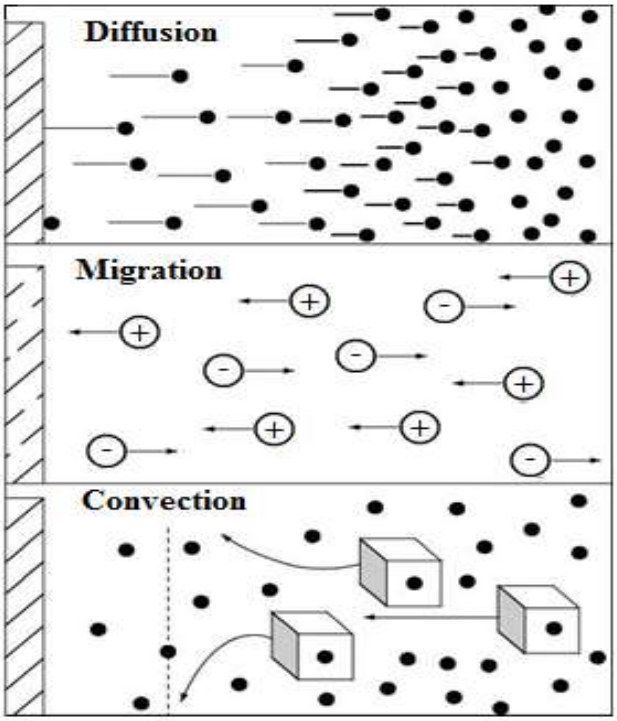

Fig. 1.5. Modes of mass transport

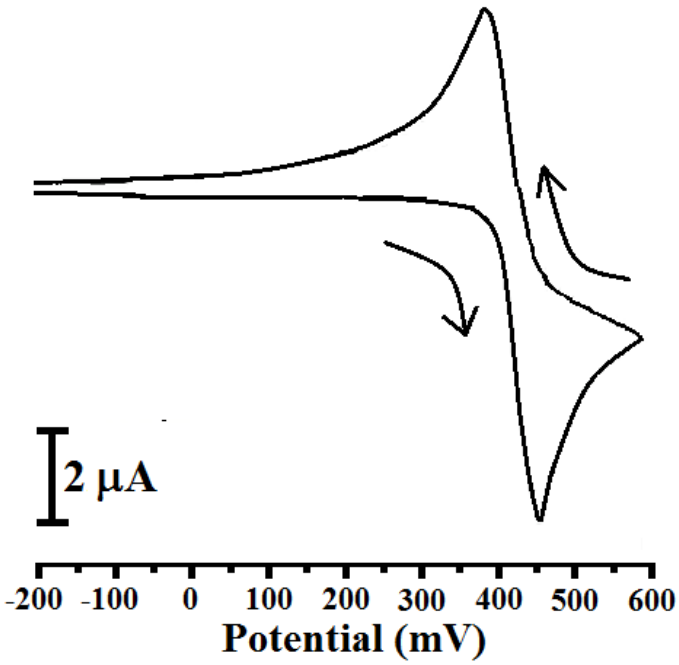

Fig. 1.6. Typical voltammogram for a reversible process

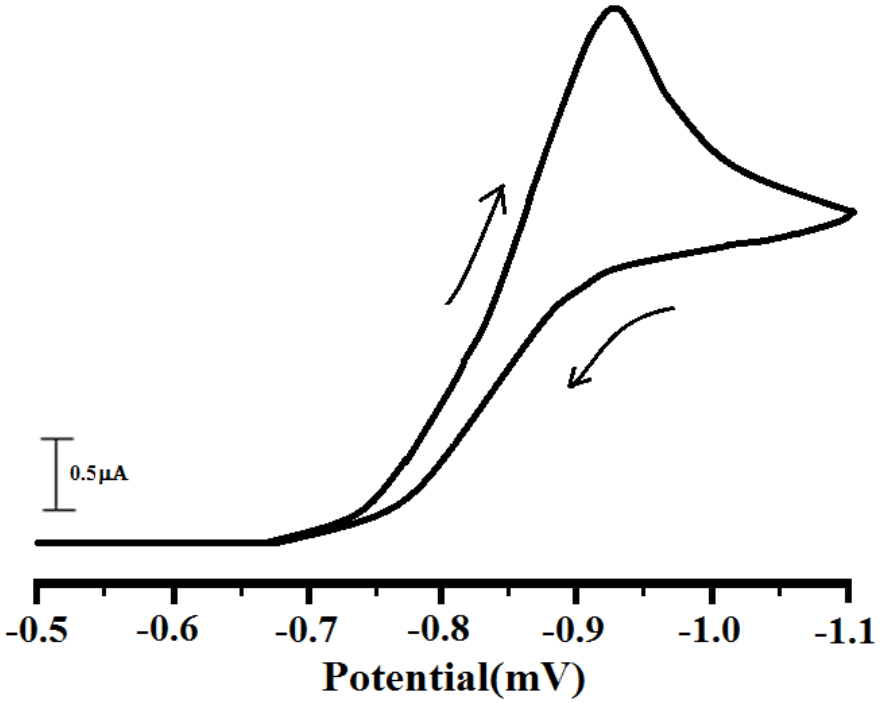

Fig. 1.7. Typical voltammogram for an irreversible process

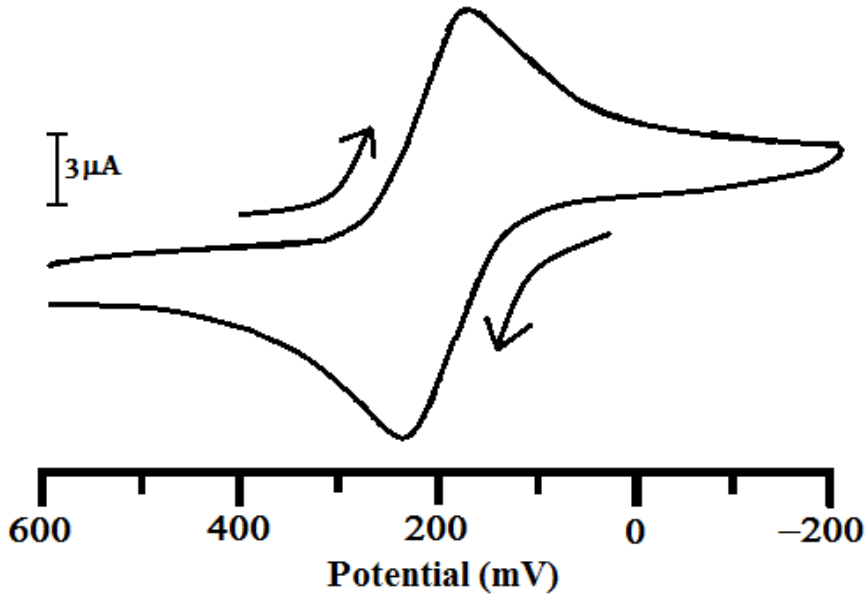

Fig. 1.8. Typical voltammogram for a quasi-reversible process

1.13. References

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2.1. Introduction

In this chapter the experimental techniques, instrumentation, basic equipment needed for electrochemistry like a potentiostat, a recording device and an electrochemical cell. The electrode system with special emphasis on carbon paste electrodes used in the course of this research is outlined. Preparation and characterisation of carbon paste electrodes and polymer film modified carbon paste electrodes surfaces, and procedures used in the present work are detailed. In addition, an overview of the theories and related equations are described.

2.2. Experimental Techniques

The chief electrochemical / analytical techniques used throughout this study were cyclic voltammetry, differential pulse voltammetry and scanning electron microscopy (SEM). A brief overview of each technique is given below

2.2.1. Cyclic Voltammetry

Cyclic Voltammetry is the most versatile electro analytical technique used for both qualitative and quantitative analysis of a substance if the substance is capable of undergoing cathodic reduction and anodic oxidation of a substance. Cyclic voltammetry is a dynamic electrochemical technique where in the applied potential at the working electrode is swept between two chosen potential limits and the change in current is monitored. This is done at a constant rate known as the scan rate. The initial applied potential, E_i, is swept to a vertex potential, E_v, where the scan is reversed and swept back to the final potential, E_f, which usually equals the original potential, E_i. This process creates a cyclic effect and is typically repeated a number of times. Cyclic voltammetry was primarily used to in the study of the electrochemical behavior of the polymer film modified carbon paste electrode towards the oxidation and reduction of dopamine (DA) was also used to investigate the effect of numerous interfering compounds on the DA signal and as an alternative approach to synthesis the polymer film modified carbon paste electrode.

2.2.2. Differential Pulse Voltammetry

Pulse voltammetry was developed to improve the sensitivity of voltammetric measurements. This is achieved by reducing the double layer capacitance to zero so that the current recorded is totally faradaic. There are several types of pulse voltammetry including normal, differential and square wave. In differential pulse voltammetry, the base potential is incremented and increased at a fixed rate. The pulses applied are of the same magnitude each time. The current is measured shortly before the pulse is applied and at the end of the pulse. The difference between these two values is recorded and plotted as a function of the applied potential.

2.2.3. Scanning Electron Microscope (SEM)

The scanning electron microscope (SEM) is a microscope that uses electrons rather than light to form an image. It is routinely used to generate high-resolution images of objects to reveal information about the external morphology and topography. In order to generate such images, the surface of the sample under investigation is bombarded with a high energy beam of electrons (primary electrons). This in turn causes the electrons from the sample to dislodge (secondary electrons). These secondary electrons are attracted and collected by a positive detector and translated into signals which are amplified, analyzed and translated into images. The preparation of samples for SEM analysis is relatively easy since most SEMs only require the sample to be conductive. In this study a model was employed to study the size and structure of the polymer modified carbon paste electrode.

2.3. Instrumentation and Basic Equipment

2.3.1. Potentiostat

The principal function of a potentiostat is to control potential and measure current. The conventional three-electrode potentiostat is connected to the working, reference, and auxiliary electrodes immersed in the test solution. It controls the potential of the working electrode (WE), with respect to the reference electrode (RE) while

simultaneously measuring the current flowing between the WE and the auxiliary electrode (AE). The potentiostat performs the following functions:

- Controls the applied potential, which is potential difference between the WE and RE (the applied potential controls what half reactions occur at the WE);
- Allows to pass current between the WE and AE without passing current through the RE (which would change its potential if current did pass through it) and
- Converts the cell current to a voltage for recording devices.

A potentiostat must be able to bring the potential of the WE (with respect to the RE) to the desired level in a short enough time. The time taken by the potentiostat for controlling the WE potential is called the rise time. The potentiostat's internal feedback circuits prevent all but a very small current from flowing between the WE and RE. Because the very basis of voltammetry is the control of electrode potential, a function generator is required to provide the potential sweep or pulse sequence to be applied to the WE. Most modem potentiostat include a built-in sweep and / or pulse generator and those which are interfaced to a computer usually, rely on the computer to generate the desired waveform. The inputs to the potentiostat are the connections to the electrodes in the cell. The outputs from the potentiostat are signal lines reflecting the current and potential of the WEs (Fig. 2.1).

2.3.1.1. Potentiostat employed in the present work

The electrochemical experiments were carried out using potentiostat provided with the Data Acquisition PC interface Card Model CHI-660c CH instruments, USA, an IBM PC and coupled to a printer (Fig. 2.2). This instrument is capable of performing more than six electro analytical techniques. The instrument incorporates a high speed, high accuracy and an electrolysis mode that consists of high-gain operational amplifier with circuits for controlled potential. The WE current signal is handled a bit differently. This signal line is also presented as a voltage signal, but the voltage level is actually proportional to the current flowing at the WE. The potentiostat has an internal 'current converter' circuit that performs the necessary current-to-voltage conversion automatically. The current converter has a number of ranges and the operator is expected

to choose the range most appropriate for the experiment being performed. Each range is associated with a particular proportionality constant, such as '100 mA/V' or '1 mA/V'.

2.3.2. Recording Device

Computers entered into electroanalytical instrumentations in 1967 [1] or even earlier. Computers applications in stationary electrode voltammetry [2] and CV [3-5] were reported. Computers can be used to apply potential programmes to the working electrode through the potentiostat. The initial potential, final potential, sweep rate, nature of the pulse; current sensitivity etc. may be instructed to the computer in the digital form. Computers can be used very effectively in data acquisition. The applied potential values and the resulting current values may be converted to the digital information by A/D converter and this improves the signal to noise ratio of the experimental cyclic voltammograms. Computers can repeat each experiment under identical conditions. Computers are used for the data analysis. It measures peak current or peak potential very accurately [6,7], by subtracting of background current [3]. Voltammetric curves may be differentiated to obtain peak potentials with greater precision [8]. The information thus obtained such as peak current, peak potential, and peak width at various concentrations may then be correlated with theoretical predictions for establishing the nature of process and for evaluating the rate parameters.

2.3.3. Electrochemical Cell

The electrochemical cell is a single piece of glassware capable of holding an appropriate volume of a test solution (i.e. an electrolyte through which charge transfer can take place by the movement of ions) containing one or more electroactive analytes. Immersed in this solution are three electrodes (working, reference and auxiliary) that are also electrically connected to a potentiostat. An electrode may be considered to be an interface at which the mechanism of charge transfer changes between electronic (movement of electrons) and ionic movement of ions. The electrode in the cell are positioned using an appropriate cell top made of chlorotrifluoroethylene (CTFE) plastic material and appropriate CTFE o-rings. The cell top also provides space for the incorporation of nitrogen line to purge nitrogen gas to remove any dissolved oxygen in

solutions. A standard three-electrode electrochemical cell configuration employed for all electrochemical experiments in the present work is as shown in Figure 2.3. The WE used is a bare carbon paste electrode (CPE) / modified carbon paste electrode The RE was a saturated calomel electrode (SCE) and the AE consisted of a platinum wire. This cell was then connected to a potentiostat and the results were recorded by a computer in the manner shown in Fig. 2.2. In general, the potential is measured between the RE and the WE and the current is measured between the WE and the CE. The electrochemical cell consisted of these three electrodes, unless otherwise stated.

2.3.4. pH Meter

A pH meter, manufactured by Systronics model MK IV was used for measuring and adjusting pH of the solution making use of a combination of glass and saturated calomel electrode.

2.4. Working Electrodes

The performance of the voltammetric procedure is strongly influenced by the working-electrode material. The working electrode should provide high signal to noise characteristics, as well as a reproducible response. Thus, its selection depends primarily on two factors: the redox behavior of the target analyte and the background current over the potential region required for the measurement. Other considerations include the potential window, electrical conductivity, surface reproducibility, mechanical properties, cost, availability, and toxicity. A range of materials have found application as working electrodes for electroanalysis.

In the present work three electrode system is used i.e. WE/AE/REs. The RE used is standard calomel electrode (SCE) which is often isolated from the solution by a salt bridge to prevent contamination by leakage from the RE. The platinum foil as AE and WEs are carbon paste electrode, and modified carbon paste electrode. The performance of the voltammetric procedure is strongly influenced by the working electrode material. The working electrode should provide high signal-to noise characteristics, as well as a reproducible response. Thus, the selection of working electrodes depends on the redox

behavior of the target analyte and the background current over the potential region required for the measurement. Other considerations include the potential window, electrical conductivity, surface reproducibility, mechanical properties, cost, availability and toxicity. A wide range of materials are used as working electrodes for electroanalytical applications. The most popular ones are those involving mercury, carbon, or noble metals (platinum and gold).

2.4.1. Carbon paste as working electrode

2.4.1.1. Unmodifled Carbon Paste

Carbon paste electrodes (CPEs) belong to a special group of heterogeneous carbon electrodes [13-15]. CPEs are represented by carbon paste, i.e., a mixture prepared from carbon powder and a suitable liquid binder packed into a suitably designed electrode body [16, 17].

Binary mixtures prepared from carbon powder and organic liquid of non-electrolytic character are known as unmodified (virgin or bare) carbon pastes [9]. The proper electroactive moiety in carbon pastes is still graphite powder with micrometric particles of high purity and distribution uniformity. Such materials are now commonly available on the market as spectroscopic graphites. Non-electrolytic binders such as Nujol [10-12] and Silicone oil [13] are non-polar pasting liquids fulfill all the important criteria; both are sufficiently chemically inert, insulating, nonvolatile, water-immiscible, and forming paste mixtures of fine consistency. Liquid organophosphate binders have also been used. Though they have attractive property like high ion-pairing ability, they are less stable and a rather atypical signal-to-noise characteristic requires special pretreatments.

2.4.1.2. Modifled Carbon Pastes

In recent times electrochemists have become interested in modifying an electrode by adsorbing, coating or attaching specific molecules to the surface. This deliberate and controlled modification of the electrode surface can produce electrodes with new and interesting properties that may form the basis of new applications and novel devices.

Fundamental studies of such modified electrodes have also provided a better insight into the nature of charge transfer and charge transport processes in thin films. Hence the study of the chemically modified electrodes (CMEs) has evolved as a field of high activity. The base of modified carbon pastes is usually a mixture of powdered graphite and non-electrolytic binder. Another constituent in the mixture is then a modifier itself. Modifying agent is usually one substance; but, the pastes can also be modified with two or even more components, which is the case of carbon paste based biosensors containing enzyme (or its carrier) together with appropriate mediator [12] or chemically modified carbon paste electrode (CMCPEs) with a mixture of two modifiers [19] The amount of modifier in the paste usually varies between 10-30% (w/w), depending on the character of modifying agent and its capability of forming enough active sites in modified paste e.g., functional groups immobilised at the electrode surface [20] or molecules of an extractant in the bulk [21]. In general, the main reason for modifying an electrode is to obtain qualitatively new sensor with desired, often pre-defined properties like:

- To get better catalytic effects
- To reduce the overpotential
- Increase the reaction rate
- For the new applications like chiral induction or other desired groups.
- A better insight into the nature of charge transfer and charge transport processes in thin films
- Improve the selectivity of some bioactive molecules
- Design of electrochemical devices and systems for applications in chemical sensing, energy conversion and storage
- Molecular electronics, electrochromic displays and electro-organic synthesis

In contrast to relatively complicated modifications of solid substrates, the preparation of CMCPEs is very simple, typically, by means of various alternative procedures by a wide variety of adsorbed species, including polymers and other types of films that have received wide attention not only in electrocatalysis, but also in sensor technology and other applications. In the present work various modifiers were adopted like Alcian blue, Toulidine blue, Multi wall carbon nano tube, Surfactant like tween-20,

and CO_3O_4/Cuo nanoparticle and the modified electrodes were prepared by several different techniques like:

- Spontaneous adsorption
- Electropolymerisation
- Grind modification
- Surface modification

Spontaneous adsorption is the affinity of certain species from the bulk solution to attach spontaneously on to the surface of the electrode (the functional groups present on the electrode surface may in some instances are responsible for the adsorption). For ex, organic species such as those containing double bonds are often hydrophobic and strongly adsorb from aqueous solution onto carbon paste electrode surface which acts as a selective electrode.

Macrocyclic-modified electrode surfaces can be prepared by electropolymerization of monomeric species bearing suitable functional groups onto the surface. In recent year's polymer-film modified electrodes has become a preferred method for the construction of chemically modified electrodes (CMEs) because of their good stability, reproducibility, three dimensional distribution of mediators compared to monolayer's and their wide applications in the fields of chemical sensors and biosensors. Polymer layers can be produced by inducing the polymerisation of monomers at the electrode surface by electrochemical means. Several types of polymer electrodes have been studied [22-25]. In some, the polymer itself is electroactive and can undergo redox reactions. In others the polymer acts as polyelectrolyte that is a material which contains ionic groups, which can extract charged ions from the solution and hold them by electrostatic binding.

In **grind modification** the modifier is admixed mechanically to the paste during its homogenisation [26, 27]. The modifier might combine with the substrate in certain forms and strengthen their adsorption on the electrode surface, which facilitated the electron or substance transfer between the electrode and the solution and alter the

properties of the electrode/solution interface and finally influence the electrochemical process of electroactive species [28-31].

Surface modification by Immobilization and mobilization is the simplest method and consists of the adsorption due to electro-static, hydrophobic or dispersive forces on the electrode surface. Surface modification forms an insulating layer which is much more stable. This can be achieved by immersing or immobilizing the electrode with the required molecules in solution. e.g. Surfactants [32-36].

2.4.2. Construction and Design of Carbon Paste Electrodes

The most suitable selection of both carbon powder and pasting liquid along with construction and design of CPE influences the resultant behaviour of CPE [9, 14]. The proper construction and design of CPE is based on short Teflon rod (shaped as a robust plug) with a well drilled in and a Pt-wire which provides electrical contact with the external circuit. New portions of carbon paste can be easily re-filled into the end-hole of Teflon rods each time [23, 28-31, 37]. Various glasses, PVC tubes, simple constructions equipped with a piston for extrusion of the paste [13, 37] are also frequently employed. For common CPEs, the actual diameter of the end-hole forming the proper carbon paste surface is being chosen from 2 to 10 mm, which is convenient for a majority of electrochemical measurements. Both above-mentioned construction variants of CPEs for batch measurements allows to utilize fully one of the most valuable property of carbon pastes easy and quick surface renewal or, in necessary cases, even removal and renewing of a larger portion of the paste. Practically immediate surface renewal can be achieved by wiping some paste off using a wet filter paper. If being performed carefully [13, 19] this procedure provides surface reproducibility nearly comparable to that attained by rather time-consuming circle-like polishing of the electrode surface upon a paper pad [38].

2.4.3. Carbon (Graphite) Powder

Powdered carbon (graphite) as the main carbon paste component ensures the proper function of an electrode or a sensor in electrochemical measurements.

Suitable carbonaceous materials should obey the following criteria:

i) Particle size in micrometers
ii) Uniform distribution of the particles
iii) High chemical purity and
iv) Low adsorption capabilities

Naturally, the type and quality of graphite used, as well as its overall amount in the carbon paste mixture, are reflected in all typical properties of the respective mixture. From the early era of CPEs up until now, the most often selected carbon powder is spectroscopic graphite with particles in the low micrometric scale (typically, 5 - 20 μm).

2.4.4. Binder (Pasting Liquid)

Traditional carbon paste contain organic liquids which link mechanically the individual graphite particles. However beside this main function, the binder as second main moiety of carbon paste co-determines its properties. The binder as the second main purpose i.e. binders give rise to hydrophobic character of the carbon paste surface, which is in principle the main reason for different behaviour of carbon paste electrode compared to carbon solid electrodes [9, 14, 39]. The presence of pasting liquid at the surface decreases the transfer rate (slower kinetics) causing the higher over potential compared to homogeneous electrodes [40]. The increasing lipophility of the pasting liquid enhances the electrode over potential (irreversibility).This is due to the marked hydrophobicity of the liquid which hinders the access of analyte towards the surface. Typical parameters required for pasting liquids are:

i) Chemical inertness and electroinactivity
ii) High viscosity and low volatility
iii) Minimal solubility in aqueous solutions and
iii) Immiscibility with organic solvents.
iv) The most popular binding agents used for preparation of carbon pastes are mineral (paraffin) oils such as Nujol oil and various silicone oils [41].Also room-temperature ionic liquids (R D^s or ILs, respectively) have soon come into the fore of research interest, which is also reflected in the electrochemistry with carbon pastes [41-48].

2.4.5. Preparation of Carbon Paste Electrode

Carbon pastes can be prepared simply by hand mixing the graphite powder with an appropriate amount of mineral oils such as Nujol or various silicone oils. This is advantageous because the analyst can choose himself the individual components as well as their mutual ratio despite the fact that ready-to-use carbon paste mixtures are commercially available. A homogeneous carbon paste is thus prepared by thorough hand mixing in a mortar and pestle by rubbing and intensive pressing with the pestle for effective homogenization. The paste is scrapped off the wall with a spatula and ultimately homogenized again and this step is repeated several times. The paste is kept for 24 hours for self-homogenization. Porcelain dish is avoided due to the possible contaminations of the paste with porcelain particles originating from the rough rubbing of the wall. A clean glass rod is used to mix both the components carefully. The ready prepared paste is then packed into the well (hole) in the electrode body. Its filling is made in small portions when each of them being pressed intimately before adding the next one. The bare carbon-paste electrode in the present study was prepared by mixing graphite powder (70%) with an appropriate amount of silicone oil (30%) and thorough hand mixing in a mortar and pestle to produce a homogenous paste. The portion of the mixture was packed into the end of Teflon rod (i.d.; 3 mm) and then smoothened on a weighing paper. Electrical contact was made by a copper wire provided at the end of the tube (Fig. 2.4). The carbon paste electrode surface was renewed by extrusion of approximately 0.5 mm carbon paste from the cavity of the Teflon rod and replacing it with a new paste. Typically such mechanical renewal of the carbon paste surface was made when starting new series of experiments (e.g. prior to analysis of each sample). An increase in volume of the pasting liquid greatly affected the reversibility of the electrode. This was reflected in the great peak separation (ΔE_p) in the potassium ferrocyanide model system used for the study. The lower volume of the binder affected the stability of the electrode.

2.4.6. Physicochemical and Electrochemical Characteristics of Carbon Pastes

2.4.6.1. Surface Renewal of the Carbon Pastes

Easy and quick surface renewal is one of the key advantages of carbon pastes both in bare and modified forms [12]. Immediate surface renewal is achieved by simple

mechanical removal or wiping of the used carbon paste layer with a soft tissue or wet filter paper and renewing it with a fresh paste. If being performed carefully, this procedure provides surface reproducibility in few seconds nearly comparable to that attained by rather time-consuming circle-like polishing of the electrode surface upon a paper pad and with other solid electrodes [13, 38, 49].

2.4.6.2. Ageing of Carbon Pastes

Bare carbon pastes containing paraffin or silicone oils, are reported to be stable for months or, if stored properly, even some years [50]. Nevertheless, some special mixtures made of more volatile binders (e.g., organic esters [51, 52] may dissociate within a few weeks only.

2.4.6.3. Ohmic Resistance of Carbon Pastes

Dry graphite gives electron transfer rates which give an almost Nemstian response and approach those obtained with platinum. The addition of typically insulating binders such as paraffin and silicone oils or any pasting liquid decreases these rates. But despite the presence of these pasting liquids carbon paste mixtures exhibit a very low ohmic resistance. This phenomenon was first studied in detail by Beilby and Mather [53], in association with examination of CPEs in chronopotentiometric measurements. These carbon pastes made of paraffin oil had the average resistances of 20-50 Ω., whereas some silicon oil-based CPEs were reported to have the resultant values even below 10 Ω. [13]. It seems that minimal resistance of carbon pastes can be due to the tightest systematic arrangement of spherical particles [54].

2.4.6.4. Potential range and background currents of the Carbon Pastes

The polarizability of CPEs for both anodic and cathodic potential ranges, as well as the background level, can be controlled effectively via the quality of both main carbon paste, choice of the binder and their ratio [9, 14, 39]. In faradic measurements with common types of CPEs and CMCPEs, the background currents are typically below 1μA [55] which could be used for definition of both anodic and cathodic limits and of the resultant potential range (window). If so, the operational range is normally between -

1.0 V and 1.0 V vs. SCE, varying in dependence of the actual pH and concentration of the solution chosen.

2.4.6.5. The Highest Potential Limits Attained by carbon pastes

Paraffin and silicone oil-based CPEs are recommended for measurements within the anodic potential range and cathodic polarizations with common CPEs suffer, besides their higher background due to a limited hydrogen over potential, from the unwanted response of oxygen contained in the paste [11-13]. For anodic polarizations, such a priority can be attributed to a value of +1.9 V vs. SCE specified for a CPE with impregnated graphite [56]. In cathodic measurements, despite less favourable dispositions of carbon pastes for polarization at negative potentials, some special carbon pastes could also be polarized at highly negative potentials, yet before the spontaneous hydrogen evolution. Such an extreme and, likely, the most negative potential ever achieved with a CPE, was the case of tricresyl phoshate-based carbon paste [55] whose surface manifested an inhibition effect (against the release of H_2) [13] and could thus be polarized down to -2.0 V vs. Ag/AgCl.

2.4.6.6. Reaction Kinetics at carbon pastes

Carbon pastes have typically hydrophobic surface which, in aqueous solutions, repel hydrophilic species involved in the electrode transformations of numerous redox systems of both inorganic and organic origin. The result is slow reaction kinetics at CPEs which further depends upon quality and composition of both the components concluding that (i) The lesser content of liquid in the paste mixture the more rapid charge transfer (i.e., lesser irreversibility) at the respective CPE (ii) the more lipophilic binder (e.g., via the increased alkyl chain of hydrocarbon like pasting liquid) the lower rate constant and the slower charge transfer; (iii) moderated reaction kinetics at CPEs can be principally changed by electrolytic activation of their surface, resulting in the formation of hydrophilic functional groups (altered surface states; see again) acting either electrocatalytically or as active sites repelling the hydrophobic outer layer of CPE; the latter being proven experimentally [53]. Often undesirable irreversibility (over potential) at CPE scan then be suppressed by means of (i) surface hydrophilization by intensive

electrolysis or chemical treatment [16,54]; (ii) erosion by surfactants in situ [55,56],or using (iii) special modifiers [10, 11]. Regarding the latter, the efficiency of this approach can be demonstrated on an example of CPE with Fe (Il)-phthalocyanine, enabling to lower the over potential for 500 mV [57], which is sufficient to shift the response of interest from a potential range obscured by a high background into the new position with more favourable signal-to-noise characteristics.

2.4.7. Storage of Carbon Paste Electrode

The carbon paste could be stored in a beaker containing distilled water and the tip filled with the paste is completely dipped down to the water level. Suchstorage prevents the desiccation of carbon paste. The CPE stored in this manner exhibits high stability.

2.5. Chemicals and Solutions

The chemicals used throughout this study were purchased from Himedia chemicals (Mangalore, India), Sigma-Aldrich (Bangalore India) and Spectroscopically pure graphite powder was obtained from Loba Chemie. All chemicals were used as supplied. All stock solutions were prepared freshly before each experiment. The electrolytes used were all buffer solutions like Phosphate buffer, B-R buffer and Acetate except HCl. The pH of the solutions was adjusted using Phosphoric acid, CH_3OOH and NaOH. Further details regarding chemicals and reagents used are discussed in the respective working chapters.

2.6. Removal of Dissolved Oxygen

It is often necessary to eliminate the dissolved oxygen from the test solution whenever moderate to quite negative potentials are applied to the working electrode since oxygen is capable of dissolving in aqueous solution in mill molar levels at room temperature and pressure. Therefore the solution in the electrochemical cell was deoxygenated by vigorously bubbling an inert gas such as nitrogen using a purge tube. Bubbling time was adjusted usually for 5-15 min depending upon solution volume. Oxygen can be reduced at negative potential and the resulting undesired cathodic current

may interfere with measurement of interest. The nitrogen should be saturated with the supporting electrolyte prior to purging to prevent evaporative losses from the sample solution. The nitrogen stream is then diverted to pass over the solution, maintaining a nitrogen blanket over the solution. Oxygen undergoes reduction in the potential range approximately between - 0.05 V and -0.9 V as given below in one of the two steps:

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad \text{or} \quad O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

2.7. Procedure used to Record Voltammograms

The solutions were taken into the electrochemical cell. The electrodes were inserted into the cell. The supporting electrolyte was taken without adding the analyte in order to record the voltammogram of the blank. Before the voltammetric measurements the solution was purged with pure (99.9%) nitrogen for 20 minutes to remove the dissolved oxygen. The potentiostat is programmed for sweep rates for measurements. Voltammograms of blank was recorded. A fixed solution of analyte was taken in supporting electrolyte and the same procedure was repeated to record the voltammogram. The electrode surface was renewed by scoping some paste off and filling the new one, and then polishing the electrode on a wet filter paper or transparent polishing paper before conducting any experiments. The experiments were performed in unstirred solution using cyclic voltammetry and differential pulse voltammetric techniques

2.8. Electrochemical Probe System used for surface Characterisation of CPE

2.8.1. Potassium Ferrocyanide System

To investigate interfacial properties of the carbon paste, $[Fe(CN)_6]^{4-}$ / $Fe(CN)_6]^{3-}$ model system was used as the electrochemical redox probe [58, 59]. Usually modification of electrodes with charged species has remarkable effects on the rate of electron transfer. These effects depend on charge of both electrode surface and redox probe. More reversible behavior is observed for the charged probe redox reactions at the modified electrodes with opposite charge and less reversible behavior for the charged probe redox reactions at the modified electrodes with similar charge [60, 61].

2.8.2. Surface Area calculation of the CPE

The surface area of CPE was determined using potassium ferrocyanide system in 1 M KCl. The effect of scan rate on cyclic voltammograms of 1mM solution of ferrocyanide has been studied from 0.05 to 0.1 V/s. For a reversible redox couple, the number of electrons transferred in the electrode reaction can be determined by the separation between the peak potentials AE_p (E_{pa} - E_{pc}) / n ~ 0.059 V. The value found to vary from 0.061 V and 0.065 V which corresponds to one electron transfer. Also the ratio of i_{pa} / i_{pc} was found to be close to one (0.9953) which is a typical behaviour, exhibited by a reversible electrochemical transfer. On substitution of the diffusion-coefficient value (8.33×10^{-5} cm s^{-1}) in the equation 1.2, the surface area of the electrode was found to be 0.031 cm^2.

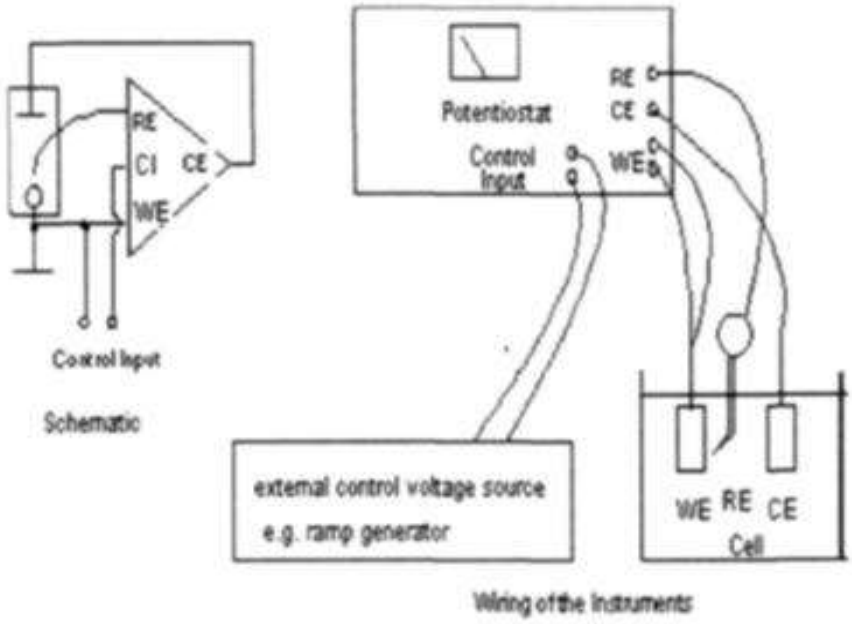

Fig. 2.1. Schematic representation of the experimental setting consisting of an external control voltage source, a potentiostat and the electrochemical cell

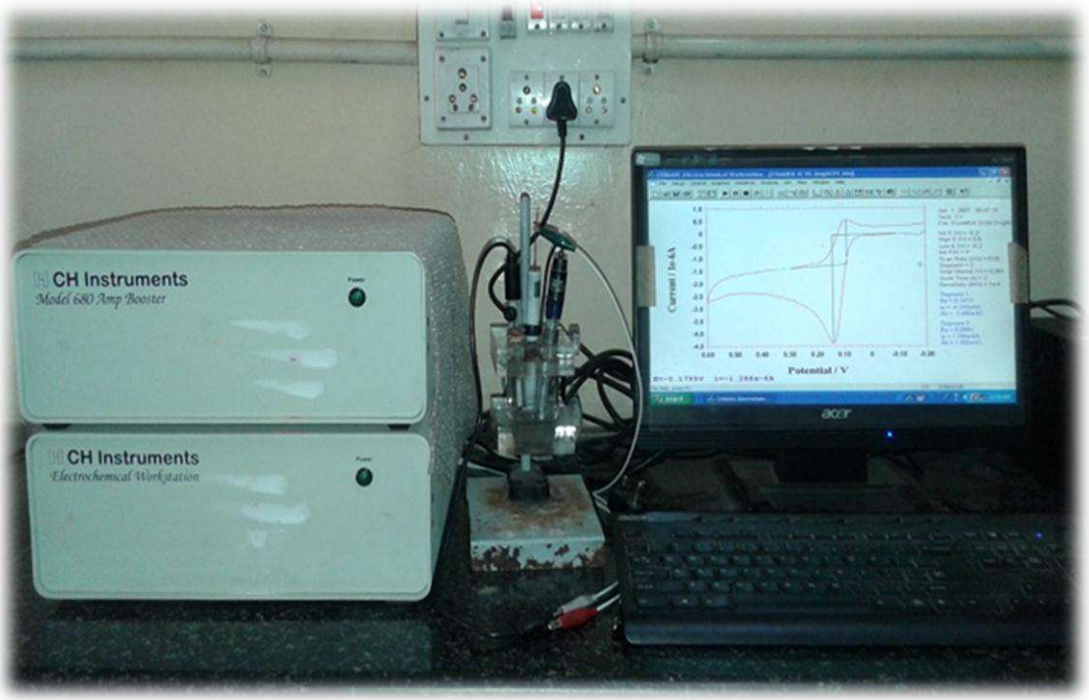

Fig. 2.2. Experimental set set-up used to record all electrochemical measurements

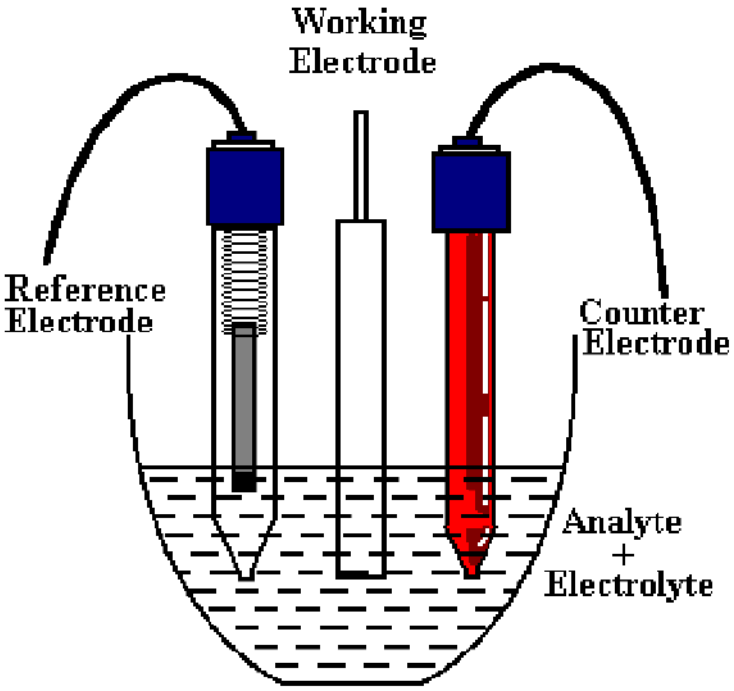

Fig. 2.3. Schematic representation of an assembled electrochemical cell containing an electrolyte solution, sparging device, and the three electrodes (WE, RE, and CE) for cyclic voltammetric experiments

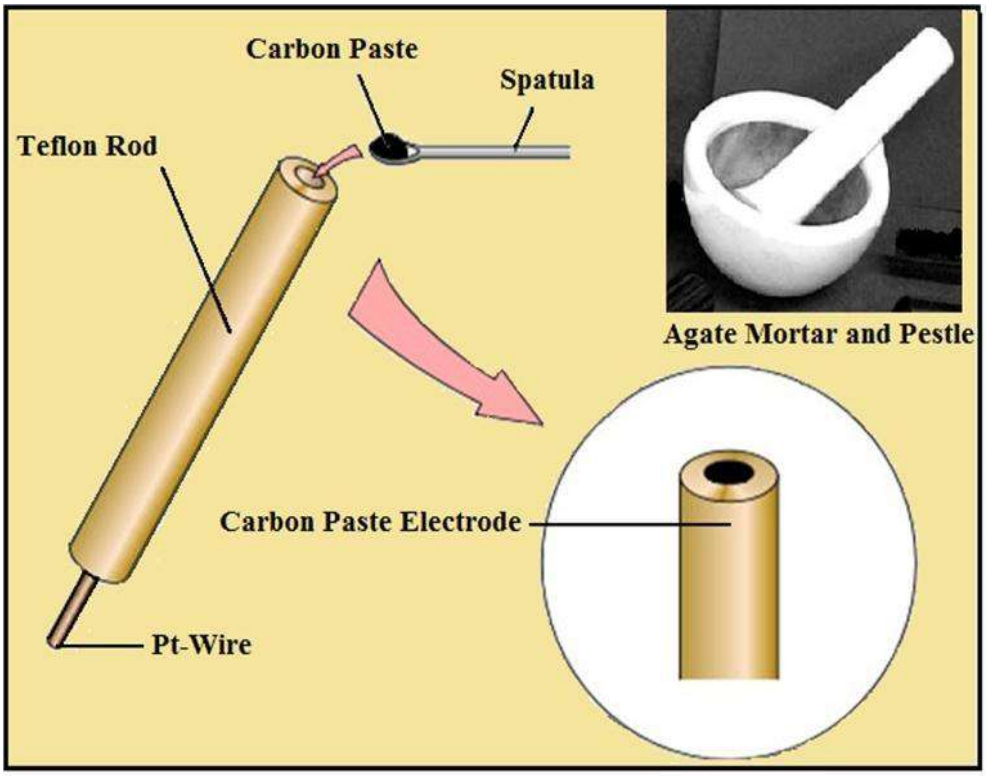

Fig. 2.4. Carbon Paste electrode, Preparation and Filling

2.9. References

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3.1. Introduction

In the present chapter describes the electrochemical response of dopamine at carbon paste electrode in the presence of 0.2 M acetate buffer as supporting electrolyte was investigated by the cyclic voltammetry. The discussion involves the chemistry, biological relevance of Dopamine and its oxidation behavior in 0.2 M acetate buffer solution at the bare and poly(ammoxiciline) modified carbon paste electrode. It showed a well-defined oxidation peak and two sensitive and indiscernible reduction peaks at the bare carbon paste electrode. The effect of concentration and scan rate of Dopamine was studied. The scan rate effect showed that the electrode process is diffusion controlled. The poly(amoxicillin) modified CPE showed excellent electrocatalytic activity towards the oxidation of DA. Further the modified electrode was used for the simultaneous determination of DA and ascorbic acid (AA) by CV technique.

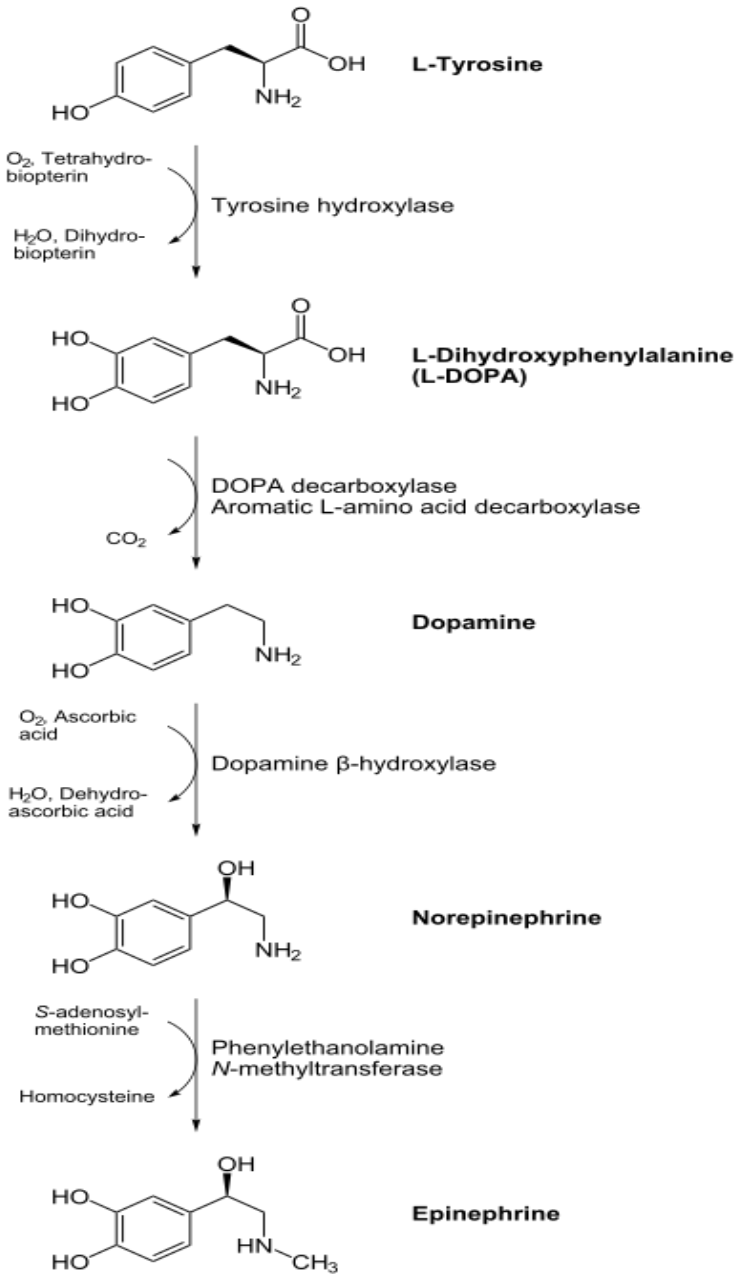
3.2. Chemistry of Dopamine

Dopamine was discovered in the year 1950. Dopamine (DA) is one of the most important neurotransmitters and plays a significant role in the functioning of central nervous, renal and hormonal system as well in drug Addiction and Parkinson's disease [1]. Therefore, it is significant to develop sensitive and simple methods for the determination of Dopamine. DA can be determined with electrochemical methods because it is an electrochemically active compound [2-7]. Serious diseases such as Schizophrenia and Parkinsonism may result by loss of DA containing neurons [8]. Patient with this disease shows a low level of DA. Therefore determination of DA concentration has become important and many methods were introduced to determine DA such as spectroscopy, chromatography and electrochemistry [9]. Since DA is an oxidizable compound it can be easily Detectable by electrochemistry methods based on anodic oxidation [10].

3.2.1. Biosynthesis of Dopamine

Dopamine is also a neurohormone released by the hypothalamus. Its main function as a hormone is to inhibit the release of prolactin from the anterior lobe of the

pituitary. Dopamine can be supplied as a medication that acts on the sympathetic nervous system, producing effects such as increased heart rate and blood pressure. However, since dopamine cannot cross the blood-brain barrier, dopamine given as a drug does not directly affect the central nervous system. To increase the amount of dopamine in the brains of patients with diseases such as Parkinson's disease and Dopa-Responsive Dystonia, a synthetic precursor to dopamine such as L-DOPA (levodopa) can be given, since this will cross the blood-brain barrier.



3.2.2. Biological Relevance of Dopamine

Dopamine is central to the reward system. Dopamine is commonly associated with the pleasure system of the brain, providing feelings of enjoyment and reinforcement to motivate a person proactively to perform certain activities. Dopamine is released by naturally rewarding experiences such as food, sex, use of certain drugs and neutral stimuli that become associated with them. This theory is often discussed in terms of drugs (such as cocaine and amphetamines), which seem to be directly or indirectly related to the increase of dopamine in these areas, and in relation to neurobiological theories of chemical addiction, arguing that these dopamine pathways are pathologically altered in addicted persons. However, cocaine and amphetamine influence separate mechanisms of action. Cocaine is a dopamine transporter blocker that competitively inhibits dopamine uptake to increase the lifetime of dopamine and augments an overabundance of dopamine (an increase of up to 150%) within the parameters of the dopamine neurotransmitters. Like cocaine, amphetamines increase the concentration of dopamine in the synaptic gap, but by a different mechanism. Amphetamines are similar in structure to dopamine, and so can enter the terminal button of the presynaptic neuron via its dopamine transporters as well as by diffusing through the neural membrane directly.

3.2.3. Review of Electrochemistry of Dopamine

In recent years the design, fabrication and application of novel electrochemical sensor has of considerable interest [11]. Particularly the development of voltammetric sensors for the determination of neurotransmitters, such as dopamine (DA) and other catecholamine's has received a lot of interest. Among the families of catecholamine's DA received much interest, because the change in DA levels gives clear information regarding understanding of brain functions, such as learning and memory formation, physiological and pathological process of Parkinson's disease [12]. As a result of this, catecholamine drugs are now widely used in various clinical fields such as, treatment of bronchial asthma, hypertension, Parkinson's disease, myocardial infarction and cardiac surgery etc. There are various techniques have been proposed for the qualitative and quantitative determination of DA in the diagnosis. A major problem was the coexistence

of probable interference AA, which is also present in biological fluids at relatively hundred times higher in concentration compared to DA [13]. It is well known that the direct anodic oxidation of DA and AA is irreversible, requires high over potential, and suffers from fouling effect at bare working electrodes [14-15]. Due to the accumulation of the oxidized products the result obtained was least selective and less sensitive. Therefore, for the determination of DA so many methods are proposed [16-23]. Among these methods, usage of electropolymerised working electrode is the recent trend. In the literature so many methods are proposed for the modification of the bare working electrodes to achieve the simultaneous and sensitive determination of DA and AA. Such as, poly(phenosafranine) [24], N,N-dimethylaniline [25] hippuric acid [26] sulfosalicylic acid [27] styrene sulfonic acid [28] amino benzoic acid [29] aniline [30] cobalt hexacyanoferrate [31] 3,4-ethlenedioxy thiophene [32] eriochrome black-T [33] L-methionine [34] toludine blue [35] DL-alanine [36]. In the present work, amoxicillin was electropolymerised on the surface of bare carbon paste electrode (BCPE) by cyclic voltammetric technique. The amoxicillin is an antibiotic drug used in the treatment of bacterial infections. The chemical structure of amoxicillin contains d-4-hydroxyphenylglycine side chain attached to 6-aminopenicillanic acid moiety. The fabricated poly(amoxicillin) modified carbon paste electrode (MCPE) was used for the determination of DA. The result shows the fabricated electrode can be employed for the analysis of DA in the biological fluids.

3.3. Experimental Part

3.3.1. Apparatus and reagents

Cyclic voltammetric studies were conducted with a model EA-201 Electroanalyzer (Chemilink Systems) connected to a personal computer for control and data storage. All electrochemical experiments were performed in a standard three electrode cell. The BCPE or poly(amoxicillin) MCPE was used as a working electrode, platinum wire as a counter electrode and saturated calomel electrode (SCE) as a reference electrode. All potentials reported were versus the SCE. Dopamine hydrochloride (DA) and ascorbic acid (AA) were obtained from Himedia chemicals and were used as

received. All other chemicals were of analytical grade. An acetate buffer solution (ABS) was prepared by mixing standard stock solutions of 0.2 M CH_3COOH and 0.2 M CH_3COONa and adjusting the pH with 0.1 M NaOH. All the solutions were prepared with double distilled water.

3.3.2. Preparation of bare carbon paste electrode

The BCPE was prepared by hand mixing of 70% graphite powder and 30% silicon oil in an agate mortar for 45 minutes to form a homogenous mixture. The prepared carbon paste was tightly packed into a PVC tube of 3 mm internal diameter and the electrical contact was provided by a copper wire connected to the end of the tube.

3.3.3. Fabrication of poly(amoxicillin) MCPE

The paste packing procedure was same as that at BCPE. Electropolymerisation of amoxicillin at the carbon paste electrode was carried out by using cyclic voltammetric technique as shown in Fig. 3.1. The aqueous solution containing 1 mM amoxicillin in 0.2 M ABS of pH 5.0 was taken in an electrolytic cell. The electropolymerisation was achieved by the formation of a thin film that grew between -0.1 V and +1.5 V at a scan rate of 50 mVs^{-1} for 15 cycles as shown in Fig. 3.2. After the electropolymerisation the electrode was washed thoroughly with double distilled water and used for the electroanalysis. During the process of electropolymerisation, the voltammograms increased at first indicating the growth and formation of polymer film on the surface of carbon paste electrode. After the 15 cycles the increase in the voltammograms tends to be almost constant, suggesting the growth and formation of a polymer film reached the level of saturation [37-38].

3.4. Results and Discussion

3.4.1. Electrochemical investigation of potassium ferrocyanide at poly(amoxicillin) MCPE

The Fig. 3.3 shows the electrochemical response of 1mM solution of potassium ferrocyanide at BCPE (dashed line) and poly(amoxicillin) MCPE (solid line) in 1 M KCl

at the scan rate of 50 mVs-1. The poly(amoxicillin) MCPE shows increase in the redox peak current when compared with the BCPE. The difference between the anodic and cathodic peak potential (ΔEp) is 0.054 at poly(amoxicillin) MCPE, which is attributed to the reversible electrode process, The results obtained greatly improved the voltammetric response of potassium ferrocyanide at poly(amoxicillin) MCPE. This suggests that the surface property of the modified electrode has been significantly changed.

3.4.2. Electrochemical response of dopamine at the poly(amoxicillin) MCPE

Fig. 3.4 shows the cyclic voltammograms obtained for the electrochemical response of 1.0 mM DA at poly(amoxicillin) MCPE (curve c), BCPE (curve a) and in absence of DA (curve b) in 0.2 M ABS of pH 5.0. At BCPE the oxidation of DA was with less sensitivity and oxidation potential was located at 0.265V (Versus SCE). Under the same condition the poly (amoxicillin) MCPE shows significant increment in the peak currents and oxidation potentials was observed at 0.275 V. This remarkable enhancement of peak current confirms the electrocatalytic effect of poly(amoxicillin) MCPE.

3.4.3. Effect of scan rate on the peak current of dopamine

Fig. 3.4 shows the cyclic voltammograms of 1 mM DA at poly(amoxicillin) MCPE at different scan rates. This was carried out in order to investigate the kinetics of the electrode reactions and verify whether diffusion is the only controlling factor for mass transport. The observation shows that with the increased scan rate the redox peak current also increased gradually (Figure 3.5). The relationship between the peak currents with square root of scan rate was shown in the Figure3.6 in the range from 50 to 120 mVs^{-1}. The redox peak currents were proportional to the square root of the scan rate ($\nu^{1/2}$) indicating the electrode process was controlled by the diffusion of the analytes.

3.4.4. Effect of pH

Cyclic voltammetry was used to investigate the effect of solution pH value in the determination of DA at the poly(amoxicillin) MCPE. Figure 3.7 shows the cyclic voltammograms obtained for the oxidation of 0.1 mM DA at a scan rate of 50 mVS^{-1} at the poly(amoxicillin) MCPE in 0.2 M ABS of varying pH. As shown in the Fig. 3.8 the

anodic and cathodic peak potentials of DA were shifted to a less positive potential with the increase of pH values. The plot of E_o versus solution pH gives an almost straight line with a slope of 0.053 V/pH in the pH range from 4 to 9. The obtained slope in a good agreement with the theoretical value of 0.059 V/pH for equal number of proton and electron transfer process [37-39].

3.4.5. Electrochemical behavior of ascorbic acid at poly(amoxicillin) MCPE

Fig. 3.9 shows the cyclic voltammograms obtained for 6.0 mM AA in 0.2 M ABS of pH 6.0 at BCPE and poly (amoxicillin) MCPE at the scan rate of 50 mVS^{-1}. At BCPE the AA shows irreversible behavior nature, the oxidation potential was located at 0.21 V. However, at poly(amoxicillin) MCPE the oxidation was observed at -0.080 V. This improvement of current signal and minimization of over potential confirms the electrocatalytic activity of the poly(amoxicillin) MCPE towards the oxidation of AA. Since the oxidation potential of AA was shifted to the negative side leads to the elimination of interference in the oxidation of DA [37].

3.5. Conclusion

The electropolymerisation of amoxicillin on the carbon paste electrode was achieved by CV technique. The fabricated electrode was used to study the redox behaviors of DA. The poly(amoxicillin) MCPE shows enhanced current response compared to BCPE. The oxidation potential of AA was shifted to the negative side leads to the absence of interference in analyzing the DA. Overall the fabricated electrode can be used for the determination of DA in physiological and pharmaceutical samples.

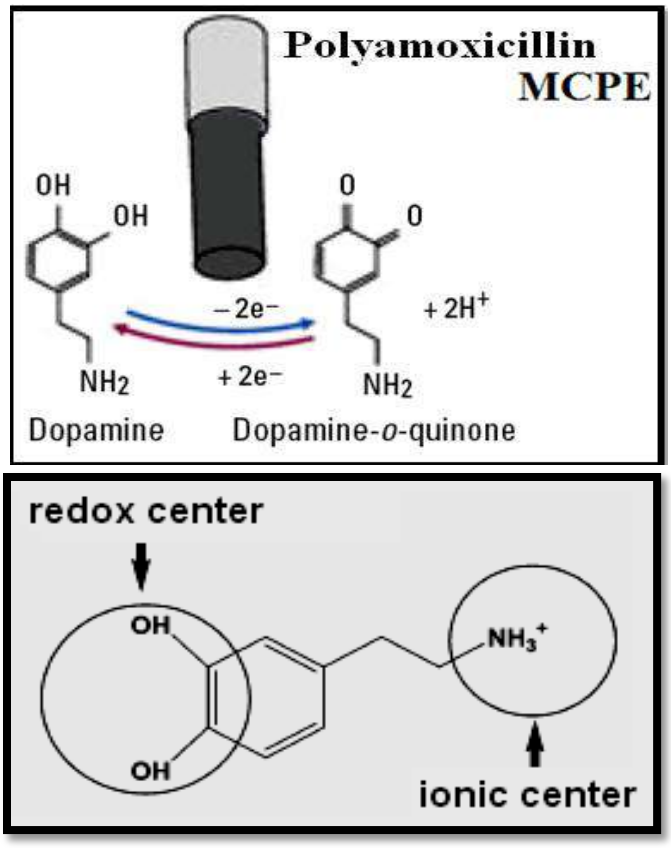


Fig. 3.1. Schematic representation of polyamoxicillin MCPE

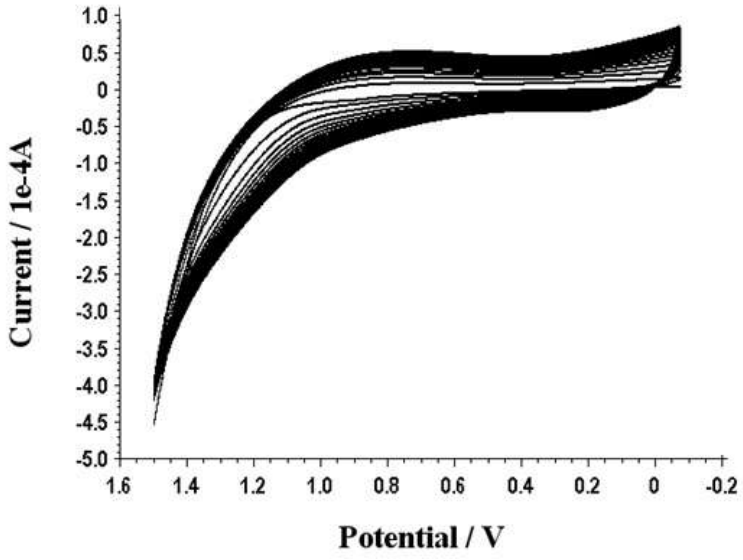


Fig. 3.2. Cyclic voltammograms obtained during the electropolymerisation of amoxicillin on the surface of BCPE

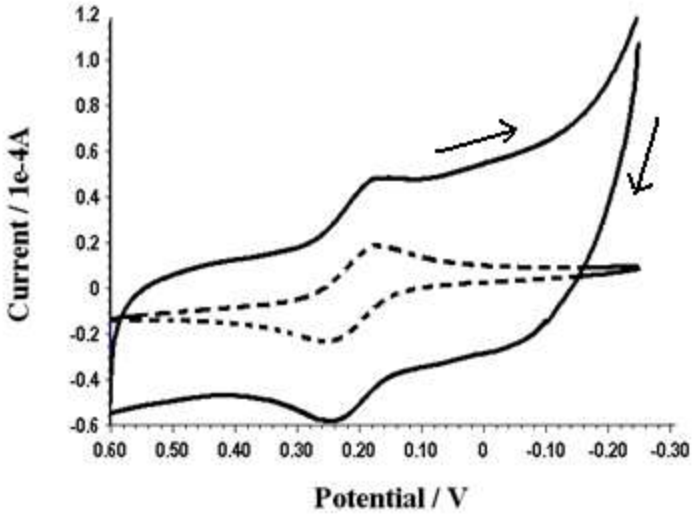


Fig. 3.3. Cyclic voltammograms obtained for the electrochemical response of 1mM $K_4[Fe(CN)_6]$ at BCPE (dotted line) and poly(amoxicillin) MCPE (solid line) in 1 M KCl at scan rate of 50 mVs^{-1}

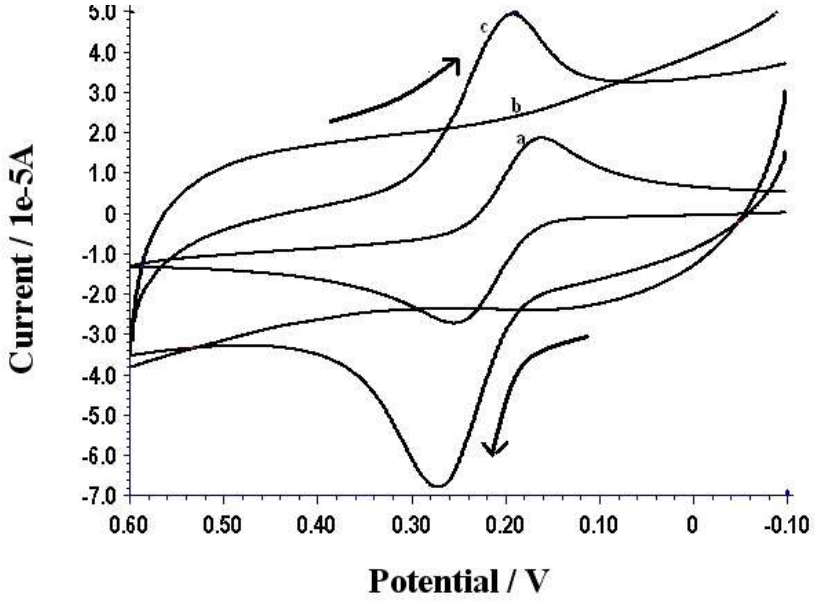


Fig. 3.4. Cyclic voltammograms recorded for the oxidation of 1.0 mM DA at BCPE (curve a) and poly(amoxicillin) MCPE (curve c) in 0.2 M ABS of pH 6.0. In the absence of DA (curve b)

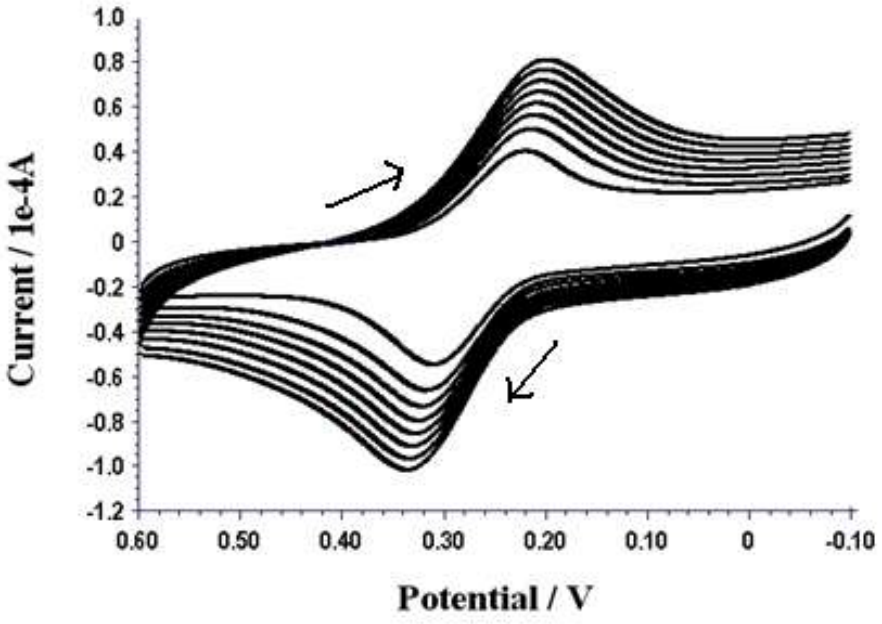


Fig. 3.5. Cyclic voltammograms of 1.0 mM DA at poly(amoxicillin) MCPE at different scan rates (a–h: 50, 60, 70, 80, 90, 100, 110, 120, mVs-1) in 0.2 M ABS of pH 5.0

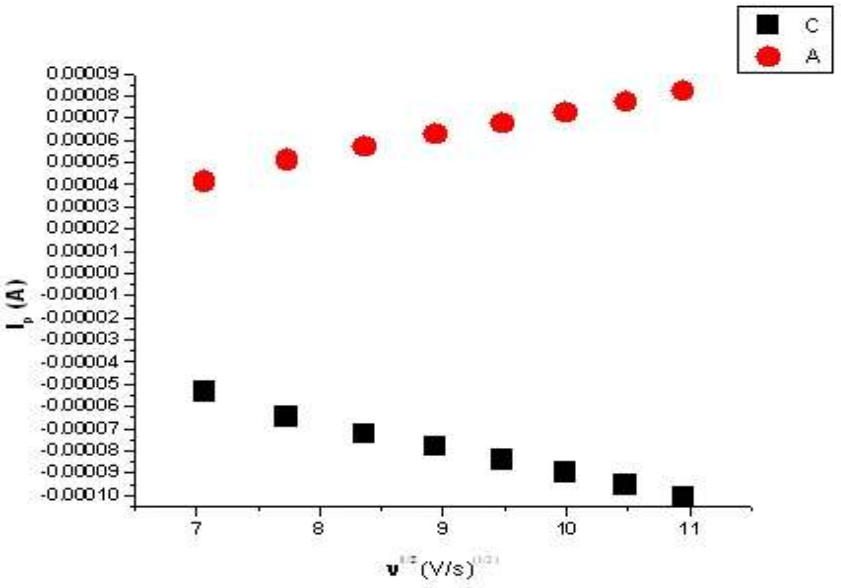


Fig. 3.6. Graph of peak current versus square root of scan rate

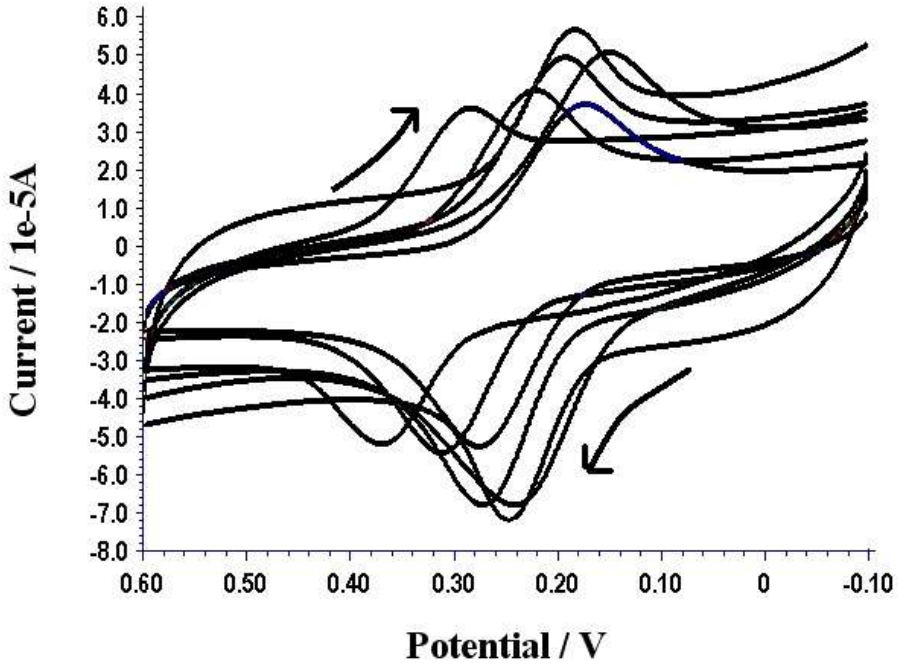


Fig. 3.7. Cyclic voltammograms obtained for the oxidation of 1.0 mM DA at poly(amoxicillin) MCPE in 0.2 M ABS of different pH values with the scan rate of 50 mVs^{-1}

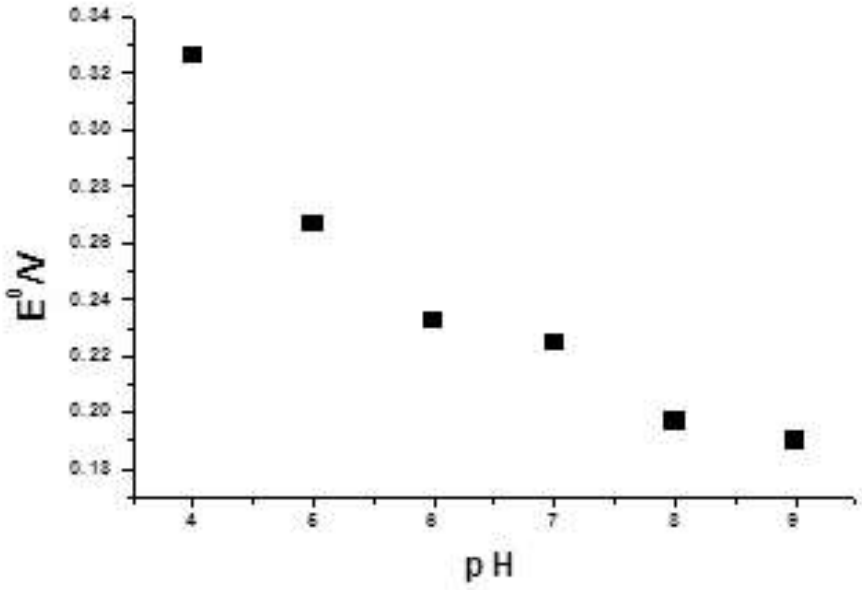


Fig. 3.8. Graph of peak potential of DA versus pH values

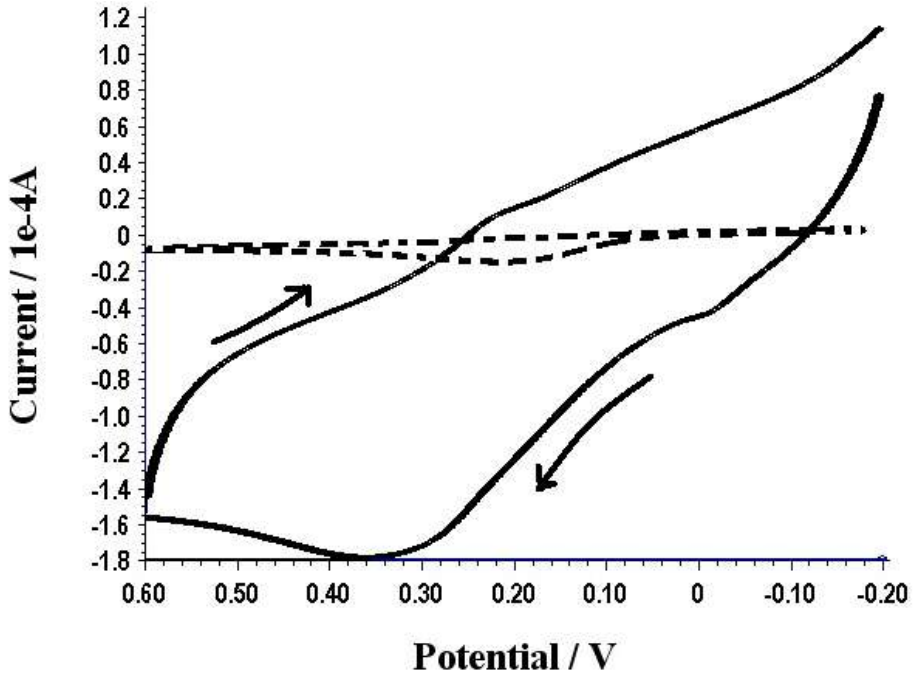


Fig. 3.9. Cyclic voltammograms obtained for the oxidation of 6.0 mM AA at BCPE (dashed line) and poly(amoxicillin) MCPE (solid line) in 0.2 M ABS (pH 6.0) at a scan rate 50 mVs^{-1}

3.6. References

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3.7.1. Introduction

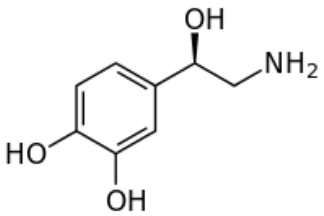
In the present chapter describes the electrochemical response of Norepenphrine and folic acid at carbon paste electrode in the presence of 0.2 M phosphate buffer as supporting electrolyte was investigated by the cyclic voltammetry. The discussion involves the chemistry, biological relevance of Norepenephrine and its oxidation behavior in 0.2 M phosphate buffer solution at the bare and poly(lysine) modified carbon paste electrode. The modified electrode was used for the electrochemical determination of norepinephrine (NE) and folic acid (FA). The fabricated poly(lysine) MCPE showed excellent electrocatalytic activity towards the oxidation of NE. The study of variation in concentration and scan rate reveals the electrode process was diffusion controlled. The sensitive separation was observed for the determination of NE and FA in a binary mixture at physiological pH by CV technique.

3.7.2. Chemistry of Norepinephrine

Norepinephrine (NE), also called noradrenaline (NA) or noradrenalin, is an organic chemical in the catecholamine family that functions in the brain and body as a hormone and neurotransmitter.

Noradrenaline, derived from Latin roots meaning "at/alongside the kidneys," is more commonly used in the United Kingdom in the United States [1] Norepinephrine is also the international nonproprietary name given to the drug [2]. In the brain, norepinephrine is produced in closely packed brain cell neurons or nuclei that are small yet exert powerful effects on other brain areas. The most important of these nuclei is the locus coeruleus, located in the pons. Outside the brain, norepinephrine is used as a neurotransmitter by sympathetic ganglia located near the spinal cord or in the abdomen, and it is also released directly into the bloodstream by the adrenal glands. Regardless of how and where it is released, norepinephrine acts on target cells by binding to and activating noradrenergic receptors located on the cell surface. The general function of norepinephrine is to mobilize the brain and body for action. Norepinephrine release is lowest during sleep, rises during wakefulness, and reaches much higher levels during situations of stress or danger, in the so-called fight-or-flight response. In the brain,

norepinephrine increases arousal and alertness, promotes vigilance, enhances formation and retrieval of memory, and focuses attention; it also increases restlessness and anxiety. In the rest of the body, norepinephrine increases heart rate and blood pressure, triggers the release of glucose from energy stores, increases blood flow to skeletal muscle, reduces blood flow to the gastrointestinal system, and inhibits voiding of the bladder and gastrointestinal motility. Norepinephrine can be used as an injectable drug for the treatment of critically low blood pressure. Beta blockers, which counter some of the effects of norepinephrine, are frequently used to treat glaucoma, migraine, and a range of cardiovascular problems. Alpha blockers, which counter a different set of norepinephrine effects, are used to treat several cardiovascular and psychiatric conditions. Alpha-2 agonists often have a sedating effect, and are commonly used as anesthesia-enhancers in surgery, as well as in treatment of drug or alcohol dependence. Many important psychiatric drugs exert strong effects on norepinephrine systems in the brain



Norepinephrine structure

3.7.3. Biosynthesis of Norepinephrine

Norepinephrine is synthesized from the amino acidtyrosine by a series of enzymatic steps in the adrenal medulla and postganglionic neurons of the sympathetic nervous system. While the conversion of tyrosine to dopamine occurs predominantly in the cytoplasm, the conversion of dopamine to norepinephrine by dopamine β-monooxygenase occurs predominantly inside neurotransmitter vesicles [3]. The metabolic pathway is:

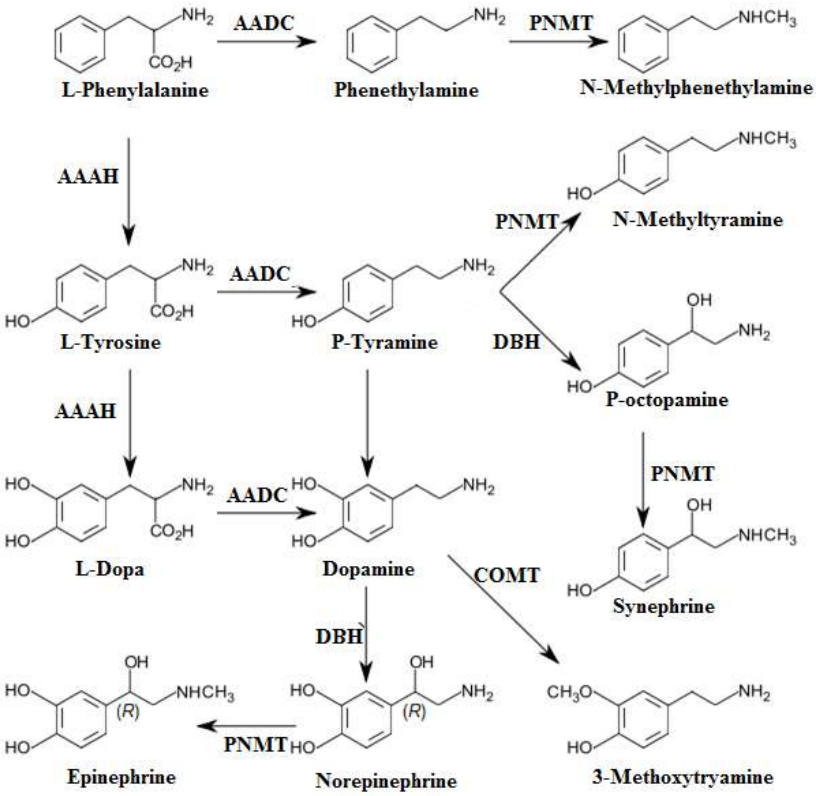
Phenylalanine → Tyrosine → L-DOPA → Dopamine → Norepinephrine

Thus, the direct precursor of norepinephrine is dopamine, which is synthesized indirectly from the essential amino acid phenylalanine or the non-essential amino acid

tyrosine [3]. These amino acids are found in nearly every protein and, as such, are provided by ingestion of protein-containing food, with tyrosine being the most common.

Phenylalanine is converted into tyrosine by the enzyme phenylalanine hydroxylase, with molecular oxygen (O_2) and tetrahydrobiopterin as cofactors. Tyrosine is converted into L-DOPA by the enzyme tyrosine hydroxylase, with tetrahydrobiopterin, O_2, and probably ferrous iron (Fe^{2+}) as cofactors. L-DOPA is converted into dopamine by the enzyme aromatic L-amino acid decarboxylase (also known as DOPA decarboxylase), with pyridoxal phosphate as cofactor [3]. Dopamine is then converted into norepinephrine by the enzyme dopamine β-monooxygenase (formerly known as *dopamine β-hydroxylase*), with O_2 and ascorbic acid as cofactors [3]. Norepinephrine itself can further be converted into epinephrine by the enzyme phenylethanolamine *N*-methyltransferase with *S*-adenosyl-L-methionine as cofactor [3].

Biosynthetic pathways for catecholamines and trace amines in the human brain



Norepinephrine is synthesized from dopamine in the human body by the dopamine β-hydroxylase (DBH) enzyme

3.7.4. Biological Relevance of Norepinephrine

Norepinephrine is the main neurotransmitter used by the sympathetic nervous system, which consists of about two dozen sympathetic chain ganglia located next to the spinal cord, plus a set of prevertebral ganglia located in the chest and abdomen.[4]These sympathetic ganglia are connected to numerous organs, including the eyes, salivary glands, heart, lungs, liver, gallbladder, stomach, intestines, kidneys, urinary bladder, reproductive organs, muscles, skin, and adrenal glands.[13] Sympathetic activation of the adrenal glands causes the part called the adrenal medulla to release norepinephrine into the bloodstream, from which, functioning as a hormone, it gains further access to a wide variety of tissues [4].

Broadly speaking, the effect of norepinephrine on each target organ is to modify its state in a way that makes it more conducive to active body movement, often at a cost of increased energy use and increased wear and tear [5]. This can be contrasted with the acetylcholine-mediated effects of the parasympathetic nervous system, which modifies most of the same organs into a state more conducive to rest, recovery, and digestion of food, and usually less costly in terms of energy expenditure [5].

The sympathetic effects of norepinephrine include:

- In the eyes, an increase in production of tears, making the eyes more moist.[6]and pupil dilation through contraction of the iris dilator.
- In the heart, an increase in the amount of blood pumped [7].
- In brown adipose tissue, an increase in calories burned to generate body heat [8].
- Multiple effects on the immune system. The sympathetic nervous system is the primary path of interaction between the immune system and the brain, and several components receive sympathetic inputs, including the thymus, spleen, and lymph nodes. However the effects are complex, with some immune processes activated while others are inhibited [9].
- In the arteries, constriction of blood vessels, causing an increase in blood pressure [10].

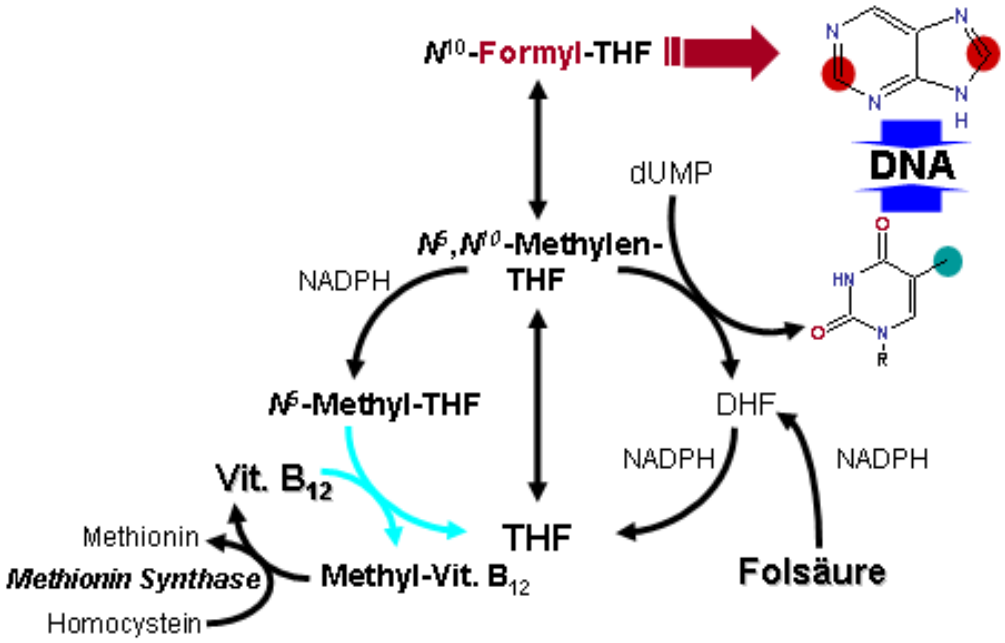
- In the kidneys, release of renin and retention of sodium in the bloodstream [11].
- In the liver, an increase in production of glucose, either by glycogenolysis after a meal or by gluconeogenesis when food has not recently been consumed [11]. Glucose is the body's main energy source in most conditions.
- In the pancreas, increased release of glucagon, a hormone whose main effect is to increase the production of glucose by the liver [11].
- In skeletal muscles, an increase in glucose uptake [11].
- In adipose tissue (i.e., fat cells), an increase in lipolysis, that is, conversion of fat to substances that can be used directly as energy sources by muscles and other tissues [11].
- In the stomach and intestines, a reduction in digestive activity. This results from a generally inhibitory effect of norepinephrine on the enteric nervous system, causing decreases in gastrointestinal mobility, blood flow, and secretion of digestive substances [12].

3.7.5. Chemistry of Folic Acid

Folic acid, is also known as folate, is one of the B vitamins [13]. It is used as a supplement by women to prevent neural tube defects (NTDs) developing during pregnancy. It is also used to treat anemia caused by folic acid deficiency [14]. More than 50 countries require fortification of certain foods with folic acid as a measure to decrease the rate of NTDs in the population [14, 15]. Long term supplementation is also associated with small reductions in the risk of stroke and cardiovascular disease [15]. It may be taken by mouth or by injection [13]. In the 1920s, scientists believed folate deficiency and anemia were the same condition. In 1931, researcher Lucy Wills made a key observation that led to the identification of folate as the nutrient required to prevent anemia during pregnancy. Wills demonstrated that anemia could be reversed with brewer's yeast. In the late 1930s, folate was identified as the corrective substance in brewer's yeast.

3.7.6 Biosynthesis of Folic Acid

The folate derivative, 5,10-methylene-tetrahydrofolate is essential for the synthesis of dTMP from dUMP and it is therefore crucial for DNA replication and cell division. Tetrahydrofolate is an essential substrate in the biosynthesis of amino acid, glycine. Drugs targeting folate biosynthesis pathway has long been prescribed as anti-malarial agents. The two essential precursors of folate biosynthesis are 4-aminobenzoate (a product of shikimate biosynthesis pathway) and GTP. Thymidylate cycle, a part of folate biosynthesis pathway (below) plays important role in the generation of amino acid glycine and dTMP. Dihydrofolate reductase enzyme replenishes tetrahydrofolate from dihydrofolate for the above mentioned biosynthetic processes. The dihydrofolate reductase and thymidylate synthase activities are catalysed by a bifunctional enzyme in both *Plasmodium falciparum* and *Toxoplasma gondii*. In addition to the *de novo* folate biosynthesis pathway, *T. gondii* can salvage folate from host. Massimine *et al.*, demonstrated the uptake of radio-labelled exogenous folic acid and revealed the presence of common folate transporter which has high affinity for folic acid. This transporter is suggested to be bidirectional and concentration-dependent.



3.7.7. Biological Relevance of Folic Acid

Folic acid (vitamin b9) is transformed into a tetra hydro folic acid (THFK, FH4) in the tissues, which can be called coenzyme. This vitamin is involved in the biosynthesis of nitrogen bases, nucleic acids (and thus in the synthesis of DNA), creatine, methionine and serine amino acid.

The studies, which were conducted after the 1970s have shown that pre-cancerous cells can be protected if folic acid is consumed in large quantities. Folic acid has a role (along with vitamin B12) in the protection of various forms of cancer, especially lung cancer. Vitamin B9 is needed for the formation of red blood cells and plays an important role in the prevention of birth defects. The body folic acid is deposited in the liver and is excreted by urine.

3.7.8. Review of Electrochemistry of Norepinephrine and Folic Acid

Norepinephrine (NE) and folic acid (FA) are the important biological compounds which are electrochemically active in voltammetric techniques [16-18]. The qualitative and quantitative determination of these compounds is important not only for diagnostic studies but also for pathological research [19]. There are so many research reports on the determination of these bioactive compounds by electrochemical methods [20-21]. These techniques are widely adopted due to the fast responses with the merits of low detection limit and high accuracy, norepinephrine (NE) belongs to the family of catecholamine secreted in the adrenal medulla and plays important physiological functions in the central nervous system [22-23]. NE is generally used as the drug of choice as a vasoconstrictor, cardiac stimulator and bronchodilator. It exists in protonated form at physiological pH. It is synthesized in the body from L-tyrosine and secreted by the medulla of the adrenal gland [24]. It affects muscle and tissue control, stimulates arteriole contraction, decreases peripheral circulation and activates lipolysis in adipose tissue [25]. It is also critical in mental disease, heart failure; DNA breaks in cardiac myoblast cells, and diabetes [26]. Recent reports have indicated that NE enhances adhesion of human immunodeficiency virus-1 (HIV-1)-infected leukocytes to cardiac microvascular endothelial cells and also accelerates HIV replication via proteinkinase [27]. The level of NE is important for the

diagnosis diseases [28]. NE is an electroactive species and can be detected with electrochemical oxidation at various modified electrodes [29].

Folic acid (FA) is a nutrient of great applications; it is a water-soluble vitamin-B that helps to build up the healthy cells especially for women planning for pregnancy. To reduce the neural tube defects, the supplementation of FA has been recommended [30-31]. The deficiency of FA leads to anaemia and is thought to increase the shells of heart attack and stroke [32]. FA deficiency causes failure to make the purines and thymine required for DNA synthesis and many studies suggests that diminished foliate status is associated with enhanced carcinogenesis [33]. FA is a potential agent in preventing the growth of cancer cells by free radical scavenging and antioxidant property [34].

Over the last few decades there are so many reports have been proposed for the determination of these bioactive molecules either individually or in the presence of probable interferences [35-36]. Among all the proposed methods the polymer modified electrodes have shown to be more significant [37]. Because of the characteristic like uniform film thickness, this can be controlled by adjusting the electrochemical parameter. In the present work L-lysine is used to modify the carbon paste electrode (CPE) and used for the voltammetric investigation of NE and FA at physiological pH. Lysine is a α-amino acid involved in the biosynthesis of proteins. The poly (lysine) modified carbon paste electrode shown sensitivity, stability and reproducibility in the analysis of NE and FA by cyclic voltammetric (CV) technique.

3.7.9. Experimental Part

3.7.9.1. Reagents

Graphite powder of 50µm particle size was purchased from Merck and silicone oil from Himedia was used to prepare carbon paste electrode (CPE). NE and FA were obtained from Himedia. The stock solution 25×10^{-4} M NE and 25×10^{-4} M FA was prepared in 0.1M perchloric acid and 0.1M NaOH solution respectively. Buffer used was

0.2M phosphate buffer solution (PBS) of pH 7.4. All the chemicals mentioned were all of analytical grade used as received without any further purification.

3.7.9.2. Apparatus

The electrochemical experiments were carried out using a model CHI-660C (CH Instrument-660 electrochemical workstation). A conventional three electrode cell was used with a saturated calomel electrode (SCE) as a reference, a platinum wire as a counter electrode, and bare or poly (lysine) modified carbon paste electrode (MCPE) as a working electrode.

3.7.9.3. Preparation of bare CPE

The bare CPE was prepared by hand mixing of 70% graphite powder and 30% silicone oil in an agate mortar for about 45min until a homogeneous paste was obtained. The paste was then packed into a cavity of PVC tube of 3mm internal diameter and smoothened on a tissue paper. The electrical contact was provided by a copper wire connected to the end of the tube.

3.7.9.4. Preparation of poly(lysine) MCPE

The paste packing procedure was same as that at bare CPE. The electropolymerisation of lysine on the surface of CPE was achieved by using cyclic voltammetric technique. The CPE was scanned for 5 multiple cycles in an electrochemical cell containing aqueous solution of 1.0 mM lysine monomer with 0.2M PBS of pH 7.4 in the potential window of -0.2V to +2.0V at a scan rate of $0.1Vs^{-1}$. After this the electrode was rinsed thoroughly with double distilled water.

3.7.10. Results and Discussion

3.7.10.1 Electrochemical Polymerization of Lysine on CPE

The poly (lysine) MCPE was prepared, by placing 1.0mM solution of lysine monomer in 0.2M PBS of pH 7.4 in an electrochemical cell over the potential range of -2.0 V to +2.0V with scan rate $0.1Vs^{-1}$ for 5 successive cycles. It can be seen from the

Fig. 3.10 the anodic peak currents enhanced gradually in the cyclic voltammograms, after the few cycles the increase of this peak current becomes constant and becomes more stable. Which indicates the growth of polymerization was reached the level of saturation [20, 37].

The extent of thickness of the polymer film has a significant contribution on the electrocatalytic activity of the modified electrode. The thickness can be controlled by varying the input parameters. In order to calibrate the thickness of the electroactive layer on the surface of the CPE, the cyclic voltammograms were recorded for the oxidation of 0.2mM NE in 0.2M PBS of pH 7.4 with different cyclised modified electrodes. It is well known the lower value of peak difference (ΔE_p) higher will be the electron transfer rate [38], and it is observed for the 5 repetitive cycles as shown in the Fig. 3.11. Therefore, 5 cycles are used as an optimum to modify the CPE to study all other parameters.

3.7.10.2. Electrocatalytic Oxidation of NE at poly(lysine) MCPE

The Fig. 3.12 shows the cyclic voltammograms of 0.2×10^{-4} M NE at BCPE and poly(lysine) MCPE at scan rate of 0.05 Vs^{-1} with supporting electrolyte 0.2M PBS of pH 7.4. At BCPE the oxidation of NE showed poor sensitivity and the anodic peak potential was located at around 0.238V vs SCE. However, the voltammogram obtained for poly(lysine) MCPE (solid line) in the same condition was with high current signal with slight shifting in the anodic peak potential towards the positive side comparing to BCPE. The anodic peak potential was located at 0.188V vs SCE. This result of maximum enhancement in current signal showed the electrocatalytic activity of poly(lysine) MCPE for the detection of NE.

3.7.10.3. Effect of Scan Rate

The effect of scan rate for 0.2×10^{-4}M NE in 0.2M PBS of pH 7.4 at poly(lysine) MCPE was studied by cyclic voltammetric technique. According to Randles-Sevick's equation the peak current is directly proportional to scan rate. Fig. 3.13 shows the increase of anodic peak currents with increase in scan rate from 0.01–0.08 Vs^{-1}. The graph of logarithm of anodic peak current (log I_{pa}) versus logarithm of scan rate

(log υ) was plotted as shown in Fig. 3.14,the obtained graph was good in linearity with the linear regression equation of log I_{pa} (μA)=0.5816 (log υ Vs^{-1})+3.834 (r^2=0.9968). The obtained slope value of 0.5816 indicates the electrode process diffusion controlled [39].

3.7.10.4. Effect of Concentration on the Peak Currents of NE

The cyclic voltammograms were recorded for the oxidation of NE with varying concentration in 0.2M PBS of pH 7.4 at scan rate 0.05Vs^{-1}. The cyclic voltammogram of different concentration of NE (10×10^{-5} to 19×10^{-5} M) was shown in the Fig. 3.15A, which shows the increase in anodic peak current due to increase in the concentration of NE. The plot shown in the Fig. 3.15B shows the linear relationship between I_{pa} and the concentration of NE in the range 10×10^{-5} to 19×10^{-5} M. The linear regression equation can be expressed as I_{pa} (μA)=1.077×10^{-6}(C_0 10^{-5} M/L) + 5.809×10^{-6}, (r^2=0.9991). The lower detection limit of NE was found to be 0.4116μM.

3.7.10.5. Effect of pH on the Peak Currents of NE

The effect of solution pH has a significant role in the electrochemical oxidations. The Fig. 3.16A shows the dependence of the CV responses of 0.2 mM NE at poly(lysine) MCPE in the pH range of 5.5-8.0. The results show by increasing the pH of PBS the oxidation potential was shifted to more negative side. The anodic peak potential (E_{pa}) versus pH graph clearly indicates that the catalytic oxidation potential depends linearly on the pH with slope of 0.0634 V/pH (r^2= 0.9987) as shown in Fig. 3.16B. This signifies there are an equal number of protons and electrons involved in the redox mechanism. This is consists with the reported in literature [40-41].

3.7.10.6. Electrochemical Oxidation of FA at poly(lysine) MCPE

Fig. 3.17 shows the cyclic voltammograms of 0.2×10^{-4}M FA at BCPE and poly(lysine) MCPE in 0.2M PBS of pH 7.4 with the scan rate 0.05 Vs^{-1}. At BCPE the oxidation of FA shows poor voltammetric response and oxidation occurred at 0.610 V. But, in the same condition poly(lysine) MCPE showed increment in current signal and the oxidation peak potential was observed at 0.6812 V. Therefore the poly(lysine) MCPE can be used as sensor for the determination of FA.

The effect of applied scan rate on the peak current response of 0.2×10^{-4}M FA was studied by CV technique in the range 0.05to 0.14Vs^{-1} as shown in the Fig. 3.18A. A linear relationship was observed for the graph of log I_{pa} versus log υ as shown in Fig. 3.18B, the obtained linear regression equation was log I_{pa} (μA)=0.6887(log υ Vs^{-1})+3.7048, (r^2=0.9985). The obtained slope was 0.6887, which indicates the electrode process was diffusion controlled.

The electrochemical oxidation of FA was carried out by varying its concentration at poly(lysine) MCPE from 10.0 μM to 100.0μM in 0.2 M PBS of pH 7.4 at scan rate 0.05 $Vs^{-1}$by CV technique as shown in Fig. 3.19A. The graph of I_{pa} versus concentration of FA justifies the reason of increase in anodic peak current is due to increase in the concentration of FA as shown in Fig. 3.19B. The expression for linear regression was expressed as I_{pa} (μA) = 3.078×10^{-7}(C_0μM/L) + 3.1026×10^{-5}, (r^2=0.9991).

3.7.11. Simultaneous Determination of NE and FA at poly(lysine) MCPE

The Fig. 3.20 shows cyclic voltammograms obtained for the simultaneous determination of 10μM NE and 0.2×10^{-4} M FA in 0.2M PBS of pH 7.4 at BCPE (dotted line) and poly(lysine) MCPE (solid line). The modified electrode shows relatively good sensitivity as compared to the BCPE. The sensitive and simultaneous separation was observed at poly(lysine) MCPE. Therefore, The poly(lysine) MCPE can be used for the sensitive and simultaneous determination of NE and FA in a physiological pH.

3.8 Conclusion

The electropolymerisation of lysine on the surface of bare carbon paste was carried out by CV technique. The fabricated poly(lysine) MCPE was used for the sensitive determination of NE and FA in physiological pH of 7.4 by CV technique. The simultaneous study was conducted for the binary mixture of NE and FA, the sensitive separation was observed at the modified electrode. A simple modification procedure was reported for the determination of NE and FA. The proposed method can be used for some other electroactive molecules.

Table 3.1. Variation of the voltammetric parameters gathered from the plots shown in Fig. 3.13 as a function of the potential scan rate

ν/Vs^{-1}	ΔE_p / V	k^0/s^{-1}
0.01	0.0578	0.5478
0.02	0.0724	0.5047
0.03	0.0861	0.4801
0.04	0.0704	0.4618
0.05	0.0725	0.4470
0.06	0.0672	0.4345
0.07	0.0766	0.4238
0.08	0.0735	0.4142

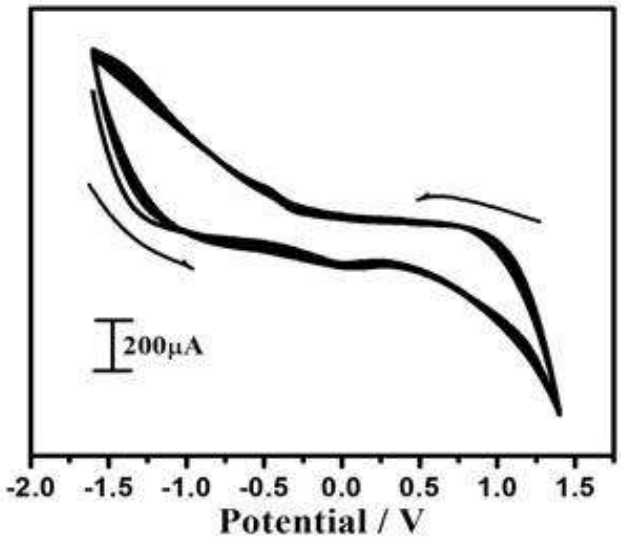


Fig. 3.10. Cyclic voltammograms of preparation of poly(lysine) MCPE. 1 mM lysine solution in 0.2M PBS of pH 7.4 at 5 cycles with scan rate 0.1 Vs^{-1}

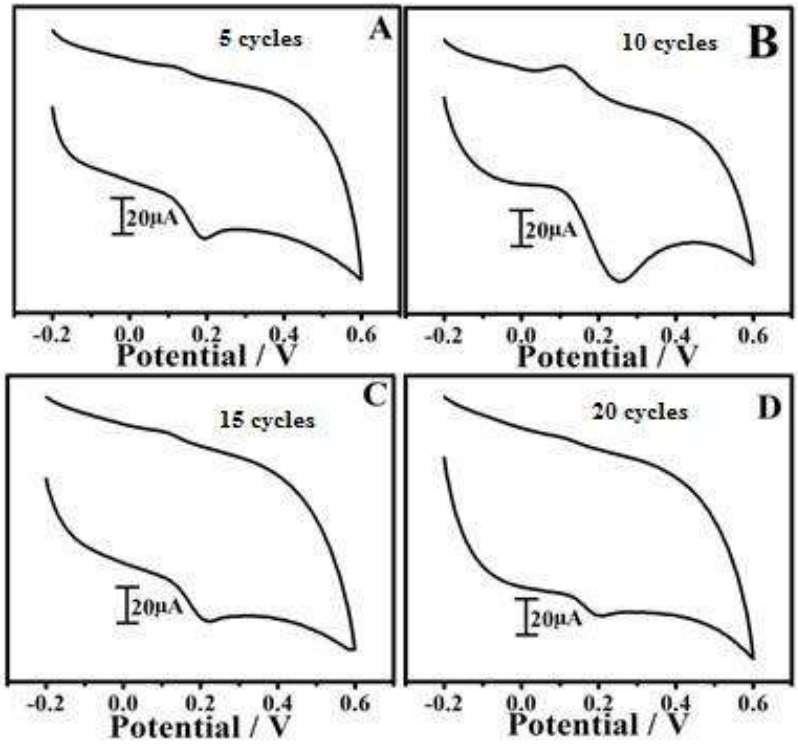


Fig. 3.11. Cyclic voltammograms recorded for the oxidation of 0.2mM NE at poly(lysine) MCPE at scan rate of 0.05 Vs^{-1}. (A-5cycles B-10cycles C-15 cycles D-20 cycles)

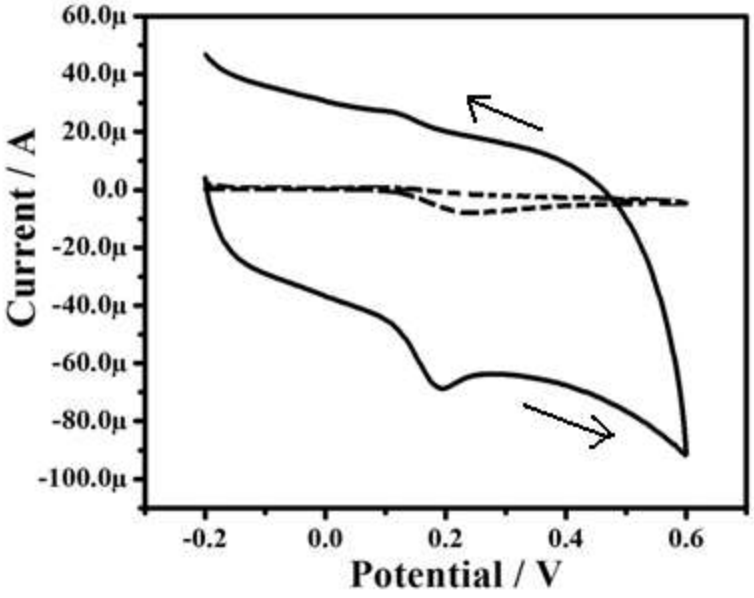


Fig. 3.12. Cyclic voltammograms of 0.2×10^{-4} M NE in 0.2M PBS pH 7.4 at BCPE (dashed line) and poly(lysine) MCPE (solid line) at scan rate of 0.05 Vs^{-1}

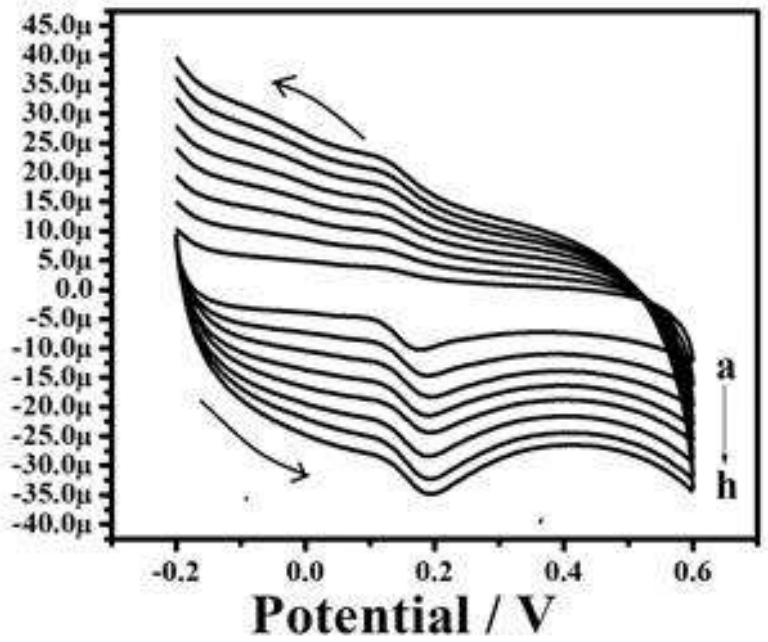


Fig. 3.13. Cyclic voltammograms of 0.2×10^{-4} M NE in 0.2 M PBS solution of pH 7.4 at poly(lysine) MCPE at different scan rate (a–h; 0.01–0.08 Vs^{-1})

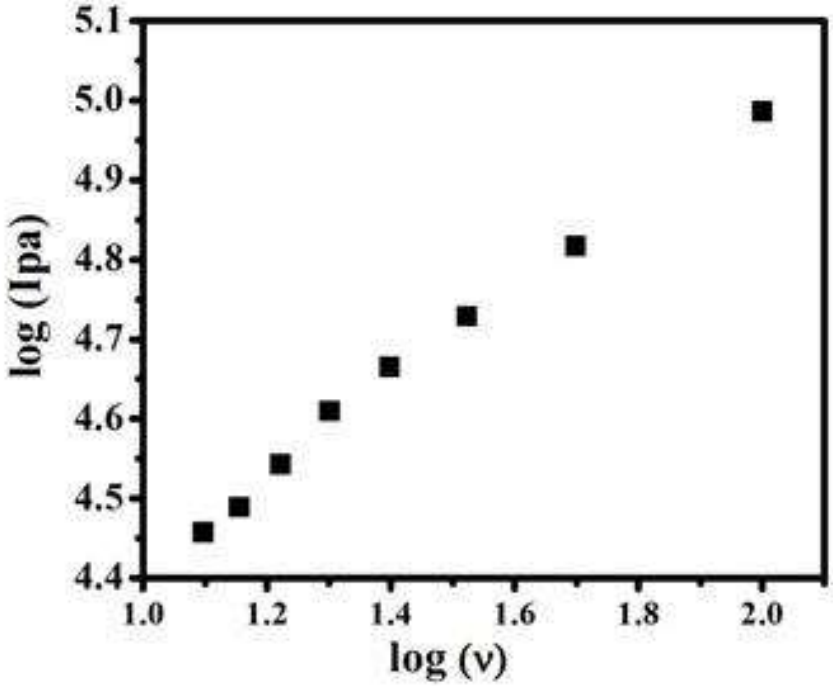


Fig. 3.14. Graph of log I_{pa} versus log υ

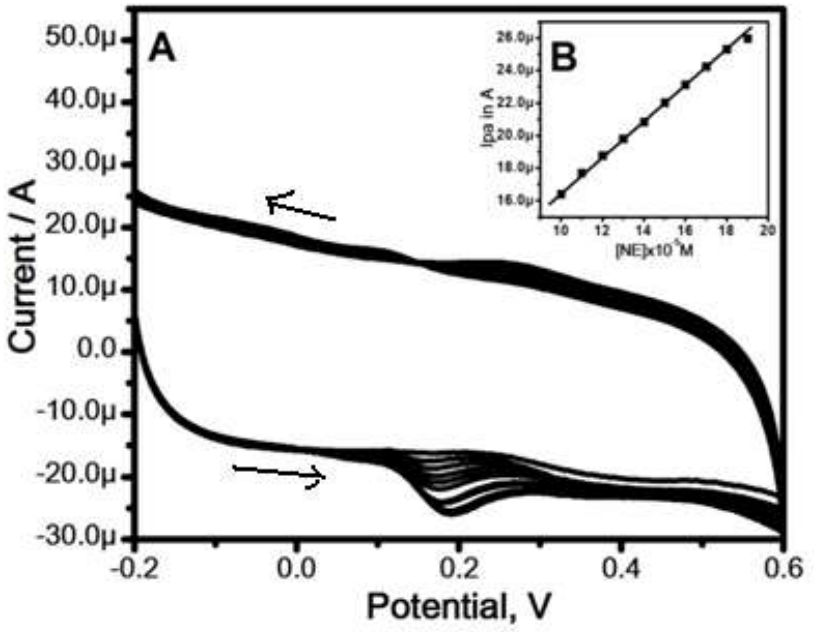


Fig. 3.15. (A) Cyclic voltammograms of NE in 0.2 M PBS solution of pH 7.4 at poly(lysine) MCPE at scan rate of 0.05 Vs^{-1} with different concentration (a–h; 10×10^{-5} to 19×10^{-5} M) and (B) Graph of anodic peak current versus concentration of NE

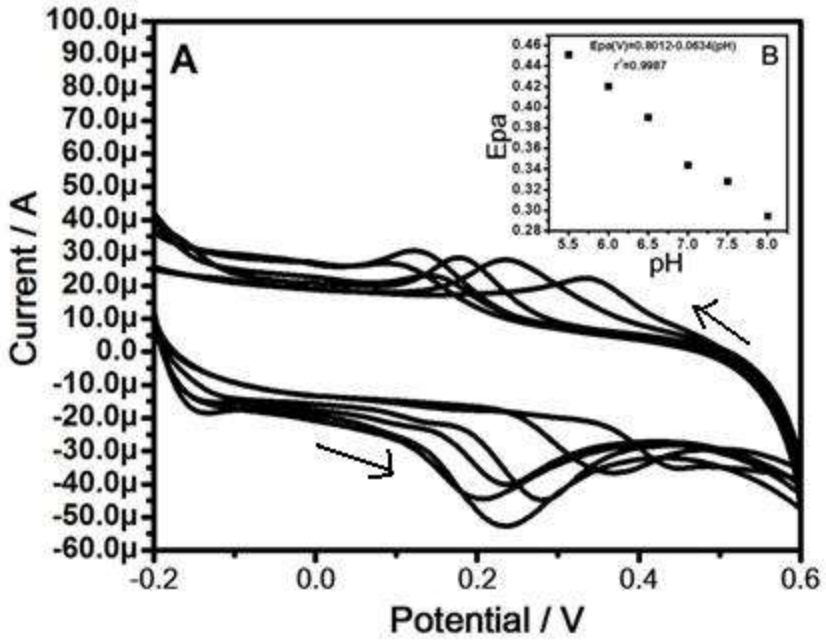


Fig. 3.16. (A) Cyclic voltammograms of the poly(lysine) MCPE in 0.2 M PBS solution at different pH (a–f: 5.5–8.0) at scan rate of 0.05 Vs^{-1}.
(B) Graph of E_{pa} versus pH

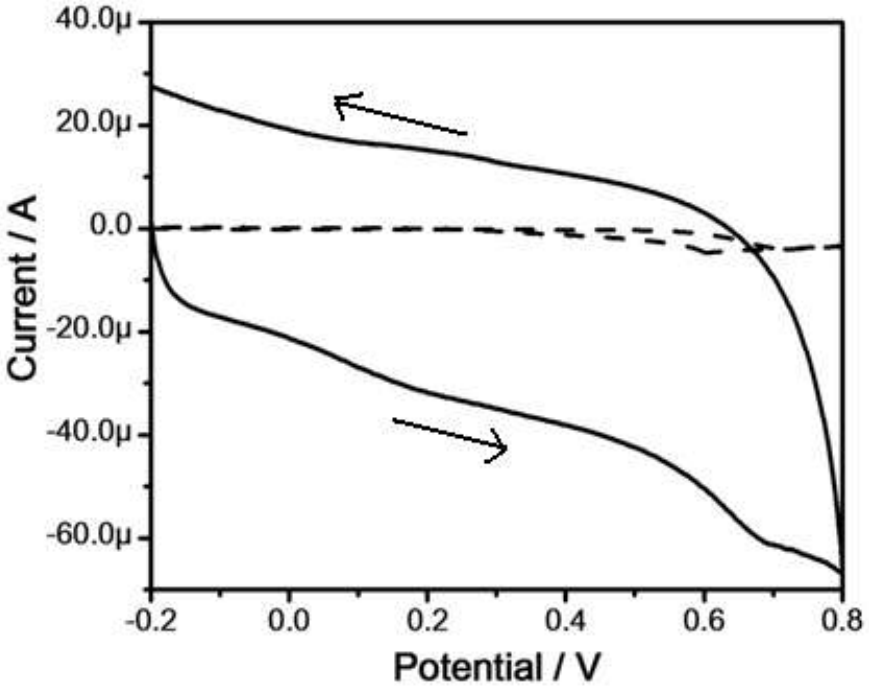


Fig. 3.17. Cyclic voltammograms of 0.2×10^{-4} M FA in 0.2M PBS pH 7.4 at BCPE (dashed line) and poly(lysine) MCPE (solid line) at scan rate of 0.05 Vs^{-1}

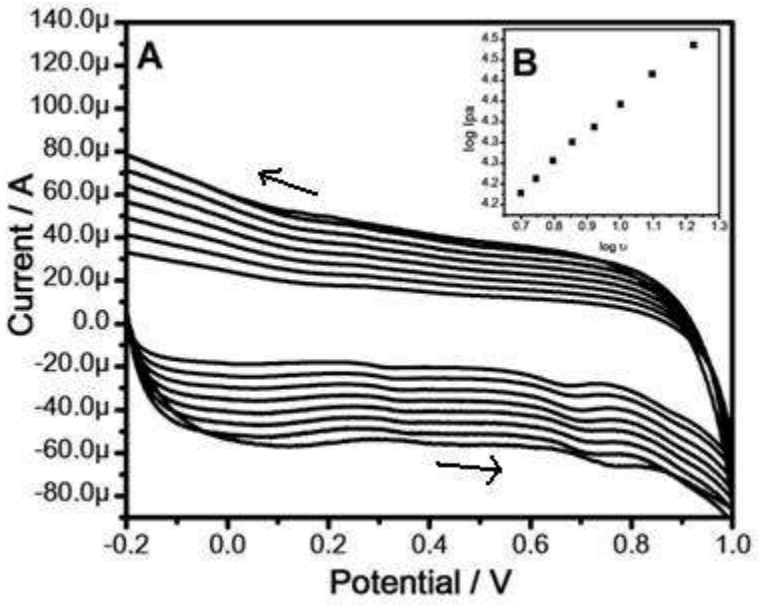


Fig. 3.18. (A) Cyclic voltammograms of 0.2×10^{-4} M FA in 0.2 M PBS solution of pH 7.4 at poly(lysine) MCPE at different scan rate (0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.12 and 0.14 Vs^{-1}). (B) Graph of log I_{pa} versus log ν

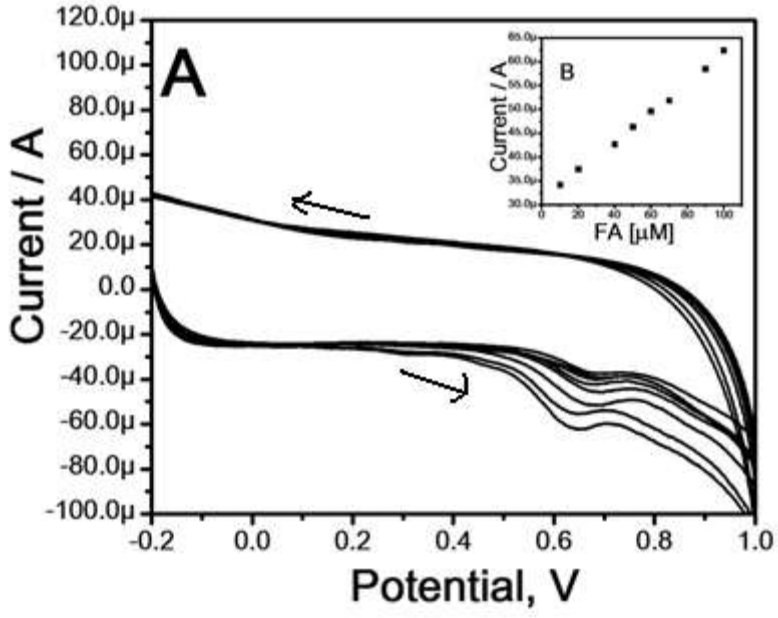


Fig. 3.19. (A) Cyclic voltammograms of FA in 0.2 M PBS solution of pH 7.4 at poly(lysine) MCPE at scan rate of 0.05 Vs^{-1} with different concentration (a-h;10 μM to 100μM). (B) Graph of anodic peak current versus concentration of FA

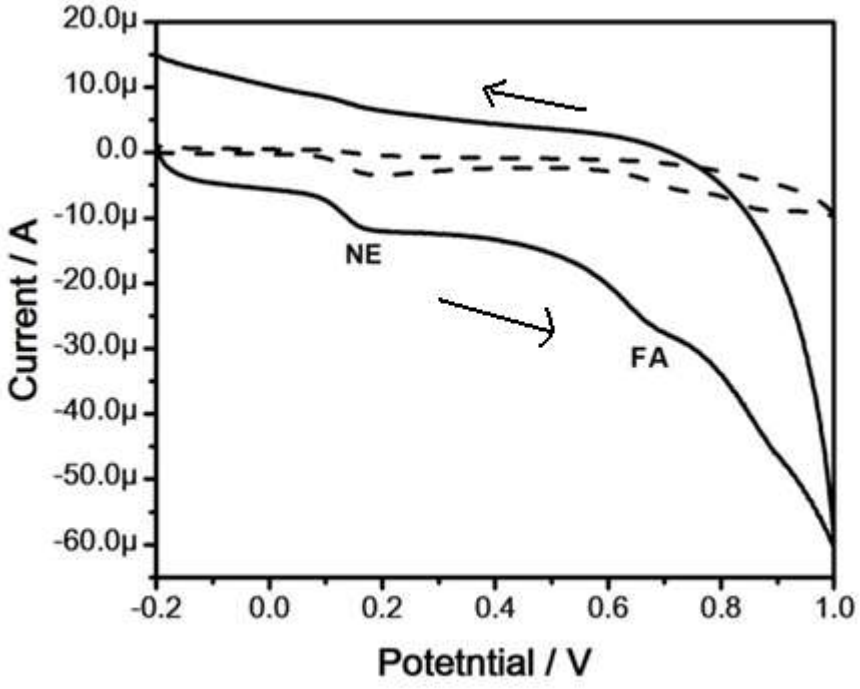


Fig. 3.20. Cyclic voltammograms for simultaneous determination of 0.2×10^{-4} M FA,0.1×10^{-4} M NE at BCPE (dashed line) and poly(lysine) MCPE (solid line) at scan rate of 0.05 Vs^{-1}

3.9. References

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4.1. Introduction

In the present chapter describes the electrochemical response of folic acid and uric acid at carbon paste electrode in the presence of 0.2 M PBS at pH 7.4 as supporting electrolyte was investigated by the cyclic voltammetry. The discussion involves the chemistry, biological relevance of folic acid and uric acid its oxidation behavior in 0.2 M PBS solution at the bare and multiwall carbon nano tube modified carbon paste electrode. It showed a well-defined oxidation peak and two sensitive and indiscernible reduction peaks at the bare carbon paste electrode. The effect of concentration and scan rate of folic acid and uric acid was studied. The scan rate effect showed that the electrode process is adsorption controlled. The bare carbon paste was modified by grinding different quantities of multi walled carbon nanotube. The modified MWCNTMCPE was used for the sensitive determination of FA and UA in physiological pH of 7.4 by CV and DPV techniques. The simultaneous study was conducted for the binary mixture of FA and UA, the sensitive separation was observed at the modified electrode by CV and DPV technique. The proposed method can be employed for the some other biological important molecules.

4.2. Review of Electrochemistry of Carbon Nanotubes

Carbon nanotubes (CNTs) are new kinds of porous nanostructure carbon materials, which are promising as immobilization substances because of their significant mechanical strength, excellent electrical conductivity, high surface area and good chemical stability [1,2]. Therefore, in recent years, CNTs can be used has an electrode materials in electrochemical devices because it shows increase the sensitivity for and promote electron transfer to biomolecules. For example, a cyclic voltammograms for dopamine at a carbon nanotube paste electrode exhibited ideal, reversible behaviour. In addition to the enhanced electrochemical reactivity, CNT-modified electrodes have been shown to alleviate surface fouling effects by biomolecules such as NADH. These properties have led to the idea that CNT modified electrodes are excellent for use as biosensors for the detection of bioactive compounds [3-11].

4.3. Chemistry and Biosynthesis of Folic Acid

The chemistry, biosynthesis and biological relevance of folic acid has been explained in details in chapter 3 sections 3.7.5, 3.7.6 and 3.7.7.

4.4. Chemistry and Biological Relevance of Uric Acid

Uric acid (UA) is a diprotic acid with pKa_1=5.4 and pKa_2=10.3 [12]. Thus in strong alkali at high pH it forms the dually charged full urate ion, but at biological pH or in the presence of carbonic acid or carbonate ions it forms the singly charged hydrogen or acid urate ion as its pK_a^2 is greater than the $pK_a^1$of carbonic acid. As its second ionization is so weak the full urate salts tend to hydrolyse back to hydrogen urate salts and free base at pH values around neutral. It is aromatic because of the purine functional group.

As a bicyclic, heterocyclic purine derivative, uric acid does not protonate in the same manner as do carboxylic acids. X-Ray diffraction studies on the hydrogen urate ion in crystals of ammonium hydrogen urate, formed in vivo as gouty deposits, revealed that the keto-oxygen in the 2 position of a tautomer of the purine structure existed as a hydroxyl group and that the two flanking nitrogen atoms at the 1 and 3 positions shared the ionic charge in the six membered pi-resonance-stabilized ring [13]. Thus, whereas most organic acids are deprotonated by the ionization of a polar hydrogen-to-oxygen bond, usually accompanied by some form of resonance stabilization (resulting in a carboxylate ion), this acid is deprotonated at a nitrogen atom and uses a tautomeric keto/hydroxy group as an electron-withdrawing group to increase the pK_1 value. The five membered ring also possesses a keto group (in the 8 position), flanked by two secondary amino groups (in the 7 and 9 positions), and deprotonation of one of these at high pH could explain the pK_2 and behavior as a diprotic acid. Similar tautomeric rearrangement and pi-resonance stabilization would then give the ion some degree of stability. Generally the solubility of uric acid, its Alkali and Alkali Earth Metal salts in water are rather low and all exhibit greater solubility in hot water than cold allowing for easy recrystallization. The solubility of the acid and its salts in ethanol is very low or negligible. In ethanol water mixtures the solubility are somewhere between the end values for pure ethanol and pure water. Excess serum accumulation of uric acid can lead to a type of arthritis known

as gout. Elevated serum uric acid (hyperuricemia) can result from high intake of purine-rich foods, high fructose intake (regardless of fructose's low glycemic index (GI) value) and/or impaired excretion by the kidneys. Saturation levels of uric acid in blood may result in one form of kidney stones when the urate crystallizes in the kidney. These uric acid stones are radiolucent and so do not appear on an abdominal plain x-ray or CT scan. Their presence must be diagnosed by ultrasound for this reason. Very large stones may be detected on x-ray by their displacement of the surrounding kidney tissues. Some patients with gout eventually get uric kidney stone.

4.5. Chemistry of Folic Acid and Uric Acid

Folic acid (FA) is a form of the water-soluble vitamin B9. Folic acid is a key factor in the making (synthesis) of nucleic acid which is one of a family of large molecules including DNA deoxyribonucleic acid) and RNA (ribonucleic acid). Folic acid often regarded as a part of vitamin B complex, possesses the considerable biological importance for general human health, especially during periods of rapid cell division and growth [14, 15]. It is an essential nutrient, plays a significant role in the synthesis of purines and pyrimidine's for DNA and in cell replication [16]. A lack of folic acid gives rise to gigantocytic anaemia, associated with leukopenia, devolution of mentality, sychosis etc. The determination of FA is often required in pharmaceutical, clinical and food samples. Methods used for it are generally spectrophotometry [17] and chromatography [18,19] and some electrochemical means are also reported for this vitamin [20-23]. Folic acid, N-[p-{[(2-amino-4-hydroxy-6-pteridinyl) methyl] amino} benzoyl]-l-glutamic acid) also known as vitamin M folacin or folate (the anionic form). It is an important component of the haemapoietic system and is the co-enzyme that controls the generation of ferrohaeme. Vegemite or marmite also contains folate, with an average part (5 g) containing 100 μg. Folate is also synthesized in bacteria. FA is important for woman who planning for pregnancy. The Dietary Allowance (RDA) suggested for folate equivalents for pregnant woman is 600-800 μg and 400 μg for women who are not pregnant. Deficient in of FA gives rise to the gigantocytic anemia, associating with leucopoenia, devolation of mentality and psychosis etc. There are many methods for the

detection of FA, including high performance liquid chromatography (HPLC), spectrophotometer, calorimetry, flow injection, microbial method and electrochemical method. Among these methods electrochemical method is an important technique because of its convenience and low cost. Determination FA is often requisite in pharmaceutical, clinical and food samples.

Uric acid (2,6,8-trihydroxypurine, UA), a major nitrogenous compound in urine, is a product of purine metabolism in human body and its higher levels lead to many clinical disorders [24]. High levels of UA in the blood (hyperuricemia or Lesch-Nyhan syndrome) are linked with the body disorders like gout, kidney, and cardiac problems. Many epidemiological studies have suggested that elevated serum UA is also a risk factor for cardiovascular disease [25-30]. In the present work different quantity of multi walled carbon nanotube is grinded with the carbon powder and silicon oil. The modified electrode is used to study the voltammetric response of folic acid and uric acid.

4.6. Experimental Part

4.6.1. Reagents and Chemicals

Disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen orthophosphate (NaH_2PO_4), silicone oil and multi walled carbon nanotube were purchased from Himedia chemicals. The folic acid, uric acid, graphite powder and NaOH produced from Merck chemicals. 25×10^{-4}M FA and 25×10-^{4} M UA were prepared in 0.1 M NaOH solution. Phosphate buffer solution (0.2 M) of pH 7.4 was used. All the stock solutions were prepared with double distilled water.

4.6.2. Apparatus

Cyclic voltammetry (CV) was performed in a model CHI-660c (CH Instrument-660 electrochemical workstation). All experiments were carried out in a conventional electrochemical cell. The electrode system contained a carbon paste working electrode, a platinum wire as counter electrode and saturated calomel as reference electrode.

4.6.3. Preparation of Bare Carbon Paste Electrode

The bare carbon paste electrode (BCPE) was prepared by hand mixing of graphite powder and silicon oil at a ratio of 70:30 (w/w) in an agate mortar until a homogenous paste was obtained. The prepared carbon paste was tightly packed into a PVC tube (3 mm internal diameter) and the electrical contact was provided by a copper wire connected to the paste in the end of the tube.

4.6.4. Preparation of Multi-walled Carbon Nanotube Modified CPE (MWCNTMCPE)

Multi-walled carbon nanotube modified carbon paste electrode (MWCNTMCPE) was prepared by adding different amount of multi-walled carbon nanotube to the graphite powder and silicon oil. By increasing the concentration of MWCNT in the carbon paste, the electrochemical redox peak current goes on increasing for the oxidation of 0.2 mM FA in 0.2 M PBS of pH 7.4. As the quantity of MWCNT increases from 2 mg to 8mg the current signal decreases with increasing quantity. The graph of peak current *vs.* concentration of MWCNT was plotted showed in Fig.4. 1. Maximum enhancement of current signal was observed for the 8 mg MWCNT. So, 8 mg MWCNT was used for the preparation of MWCNTMCPE.

4.7. Results and Discussion

4.7.1. Electrochemical Behavior of Folic Acid at MWCNTMCPE

The electrochemical behaviors of FA at MWCNTMCPE have been investigated by cyclic voltammetric technique. Fig. 4.2 shows the cyclic voltammograms obtained at BCPE (dashed line) and MWCNTMCPE (solid line) for the oxidation of 0.2 mM Folic acid in 0.2 M PBS of pH 7.4 with the applied potential scan rate of 100 mVs^{-1}. At BCPE and MWCNT modified carbon paste electrode the oxidation peak occurs at 0.670 V. However, for the MWCNTMCPE the peak current of FA slightly increased when compared to BCPE. This indicates that the modified electrode acts as a good sensor and which improves the oxidation process of FA.

4.7.2. Effect of Scan Rate on MWCNTMCPE

According to Randles-Sevick's equation increase in the scan rate leads to increases in the peak current. Cyclic voltammogram for the oxidation of 2 mM FA in 0.2 M PBS of pH 7.4 at MWCNTMCPE was shown in Fig. 4.3a. The graph of anodic peak current (I_{pa}) versus scan rate (υ) was plotted and the graph obtained was nearly straight line as shown in Fig. 4.3b in the range from 100 to 500 mVs^{-1}. The anodic peak current was proportional to the scan rate (υ) with correlation coefficient 0.9923. This suggests the electrode transfer reaction is diffusion-controlled.

4.7.3. Effect of Concentration

The anodic peak current was increased as the concentration of FA was increased. The CV curves were recorded for the oxidation of FA with varying concentration in the range 1×10^{-4} M to 4×10^{-4} M as shown in Fig. 4.4a. The plot of anodic peak current versus concentration of FA gives a linear relationship as shown in Fig. 4.4b.

4.7.4. Electrochemical Behaviour of Uric Acid at MWCNT MCPE

The electrochemical behaviors of UA at MWCNTMCPE have been investigated by cyclic voltammetric technique. Fig.4.5 shows the cyclic voltammograms of BCPE (dashed line) and MWCNTMCPE (solid line) electrodes in 0.2 mM UA in 0.2 M PBS of pH 7.4 as a supporting electrolyte with the scan rate 100 mVs^{-1}. At BCPE and MWCNTMCPE the oxidation peak occurs at 0.310 V. In MWCNTMCPE the peak current of UA slightly increased when compared to BCPE.

4.7.5. Effect of Scan Rate MWCNT on MCPE

The CV curves were recorded for the oxidation of 2 mM UA in 0.2 M PBS of pH 7.4 at MWCNTMCPE. The result shows increase in the anodic peak current with increase in scan rate in the range 100 to 500 mVs^{-1} as shown in the Fig. 4.6a. The graph of I_{pa} versus υ was nearly a straight line as shown in Fig. 4.6b with correlation coefficient of 0.9894. This indicates the electrode transfer reaction was diffusion-controlled.

4.7.6. Effect of Concentration

The concentration of uric acid was increased from 0.5×10^{-4}M to 4.5×10^{-4}M as shown in Fig. 4.7a. The CV curve shows increase in the current response due to the increase in the concentration of UA. The graph of I_{pa} versus concentration of uric acid was plotted and it gives a linear relationship between I_{pa} in the range 0.5×10^{-4}M to 3.0×10^{-4}M as shown in Fig. 4.7b. The decrease in the sensitivity in second linear range was due to the kinetic limitation [31].

4.7.7. Simultaneous Determination of FA and UA

The Fig.4.8a shows the cyclic voltammetric response of 2 mM FA and 0.5 mM UA in 0.2 M PBS of pH 7.4 at BCPE (dotted line) and MWCNTMCPE (solid line). The modified electrode shows relatively good sensitivity as compared to the BCPE. The sensitive and simultaneous separation was observed at MWCNTMCPE. The differential pulse voltammetry (DPV) was used due to its high sensitivity and absence of background current. The Fig. 4.8b shows the DPV curve obtained for the mixture of 2 mM FA and 0.5 mM UA in 0.2 M PBS of pH 7.4. So, The MWCNTMCPE shows sensitive and simultaneous separation for the oxidation of FA and UA.

4.8. Conclusion

The bare carbon paste was modified by grinding different quantities of multi walled carbon nanotube. The modified MWCNTMCPE was used for the sensitive determination of FA and UA in physiological pH of 7.4 by CV and DPV techniques. The simultaneous study was conducted for the binary mixture of FA and UA, the sensitive separation was observed at the modified electrode by CV and DPV technique. The proposed method can be employed for the some other biological important molecules.

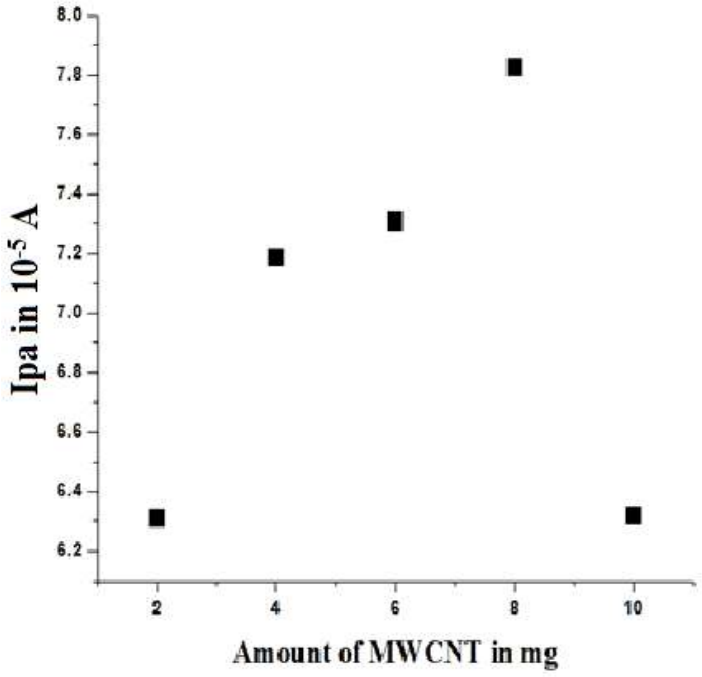


Fig. 4.1. Effect of quantity of MWCNT on anodic peak current oxidation of 0.2 mM FA in 0.2 M PBS of pH 7.4 with scan rate 100 mVs^{-1}

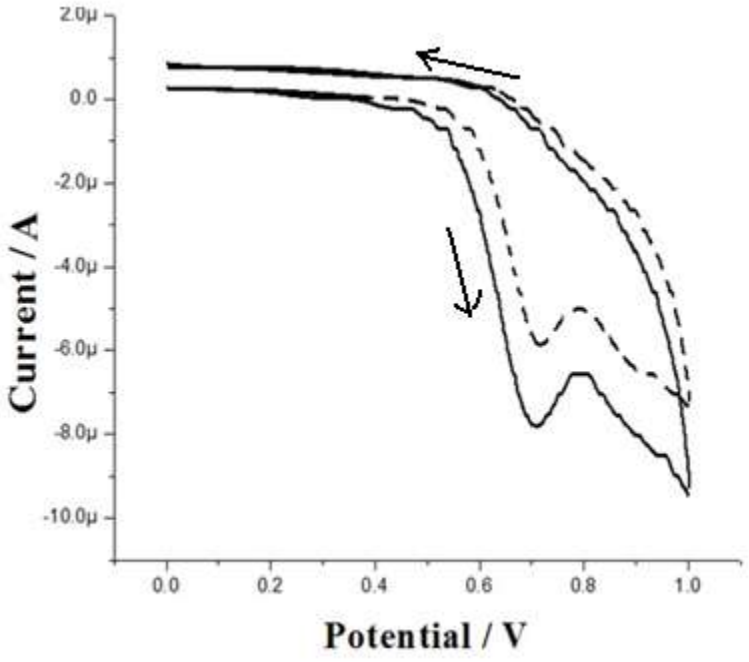


Fig. 4.2. Cyclic voltammograms of BCPE (dotted line) and MWCNTMCPE (Solid line) in the presence of 0.2 mM FA in 0.2 M PBS of pH 7.4 at the scan rate = 100 mVs^{-1}

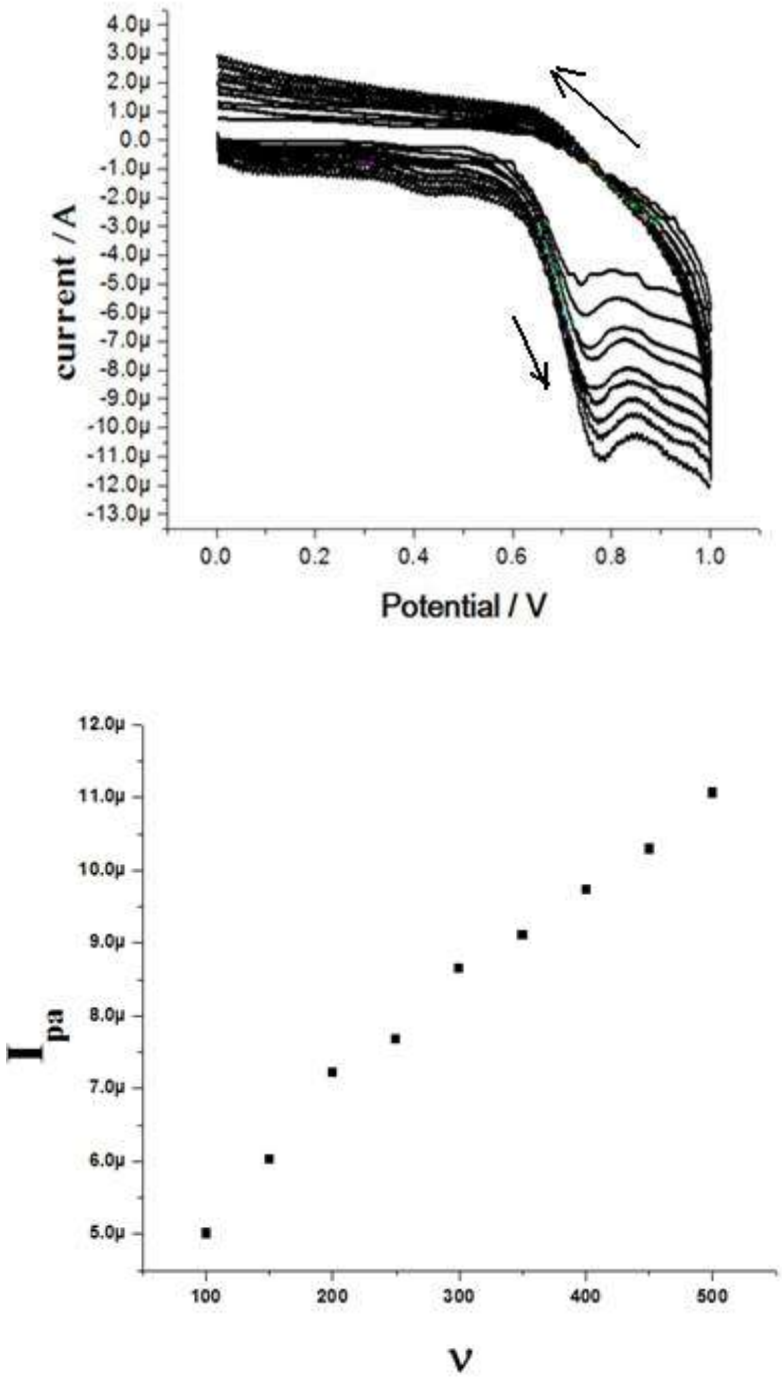


Fig. 4.3. (a) Cyclic voltammograms with different scan rate in the presence of 2 mM folic acid and 0.2 M PBS of pH 7.4, scan rate 100 mVs-1-500 mVs-1;
(b) Graph of anodic peak current versus scan rate of FA

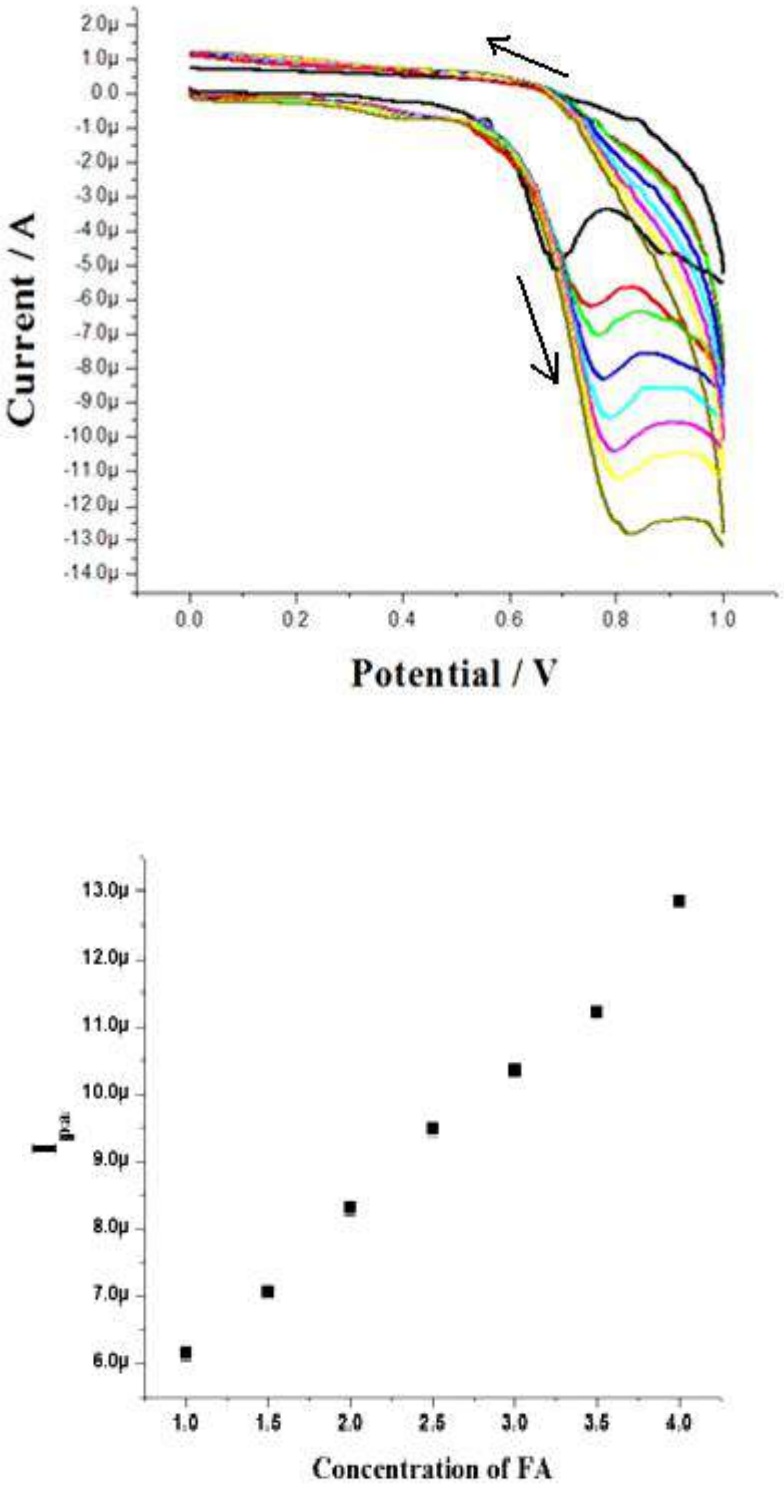


Fig. 4.4. (a) Cyclic voltammogram of variation of concentration of FA from 1×10-4M to 4×10-4 M in presence of PBS of pH 7.4; (b) Effect of variation of concentration of FA versus anodic peak current

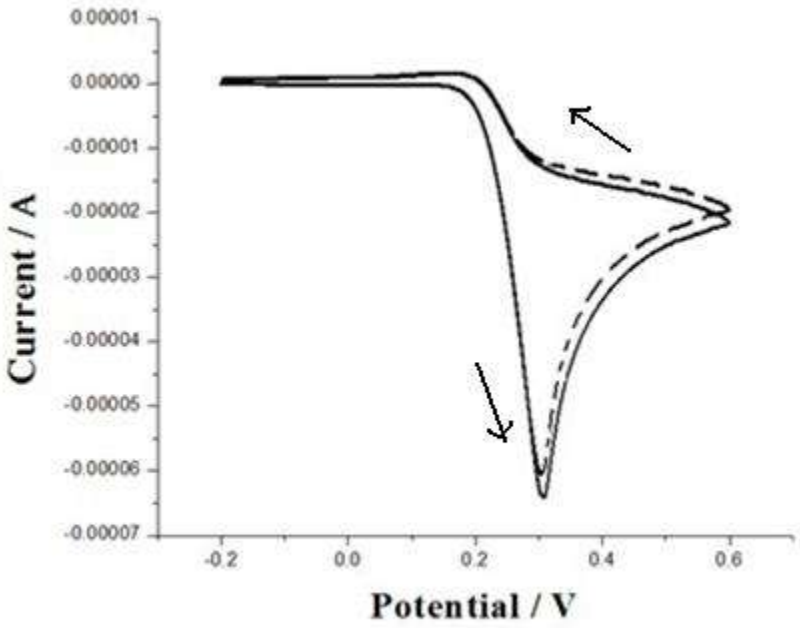


Fig.4. 5. Cyclic Voltammogram of BCPE (dotted line) and MWCNTMCPE (Solid line) in the presence of 0.2 mM UA and 0.2 M PBS, Scan rate=100 mVs^{-1}

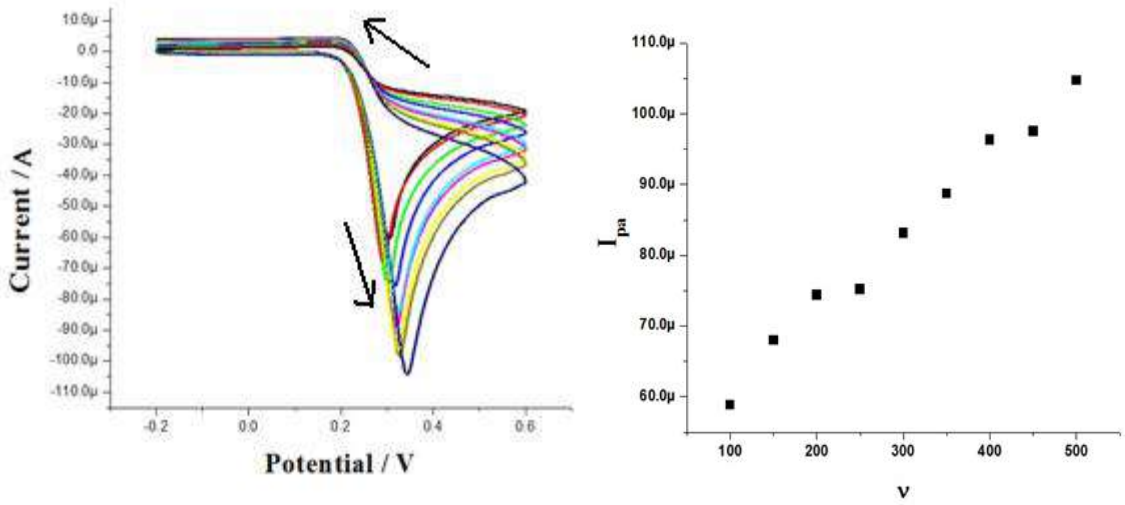
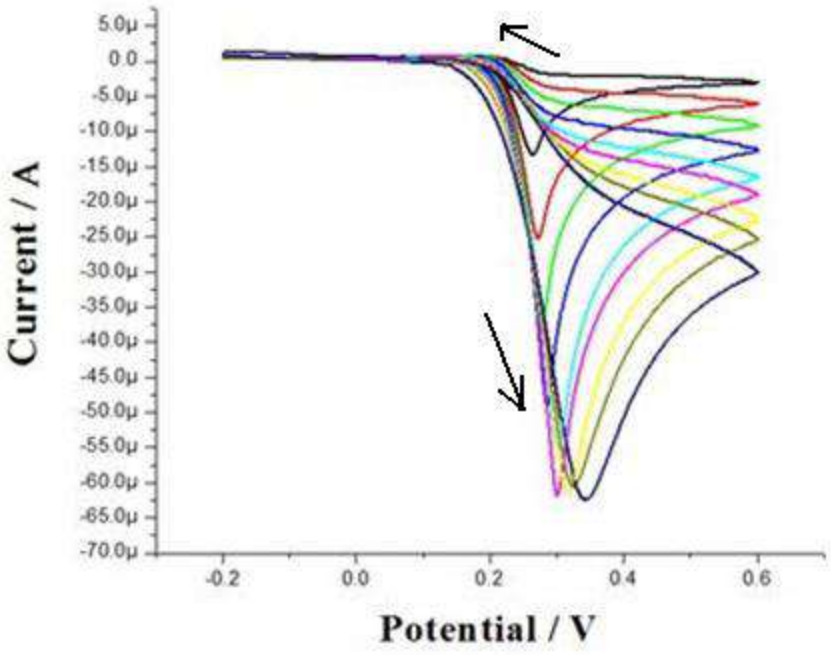
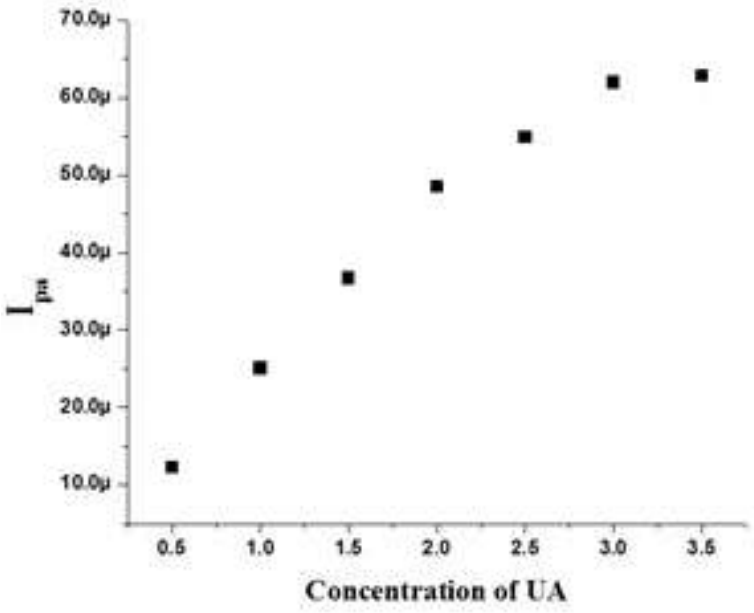


Fig. 4.6. (a) Effect of variation of scan rate on the anodic peak current of 2 mM UA in 0.2 M PBS of pH 7.4; (b) Graph of anodic peak current versus scan rate of UA



(a)



(b)

Fig. 4. 7. (a) Cyclic voltammograms of variation of concentration of UA from 0.5×10^{-4} M to 4.5×10^{-4} M in 0.2 M PBS of pH 7.4; (b) Graph of anodic peak current versus concentration of UA

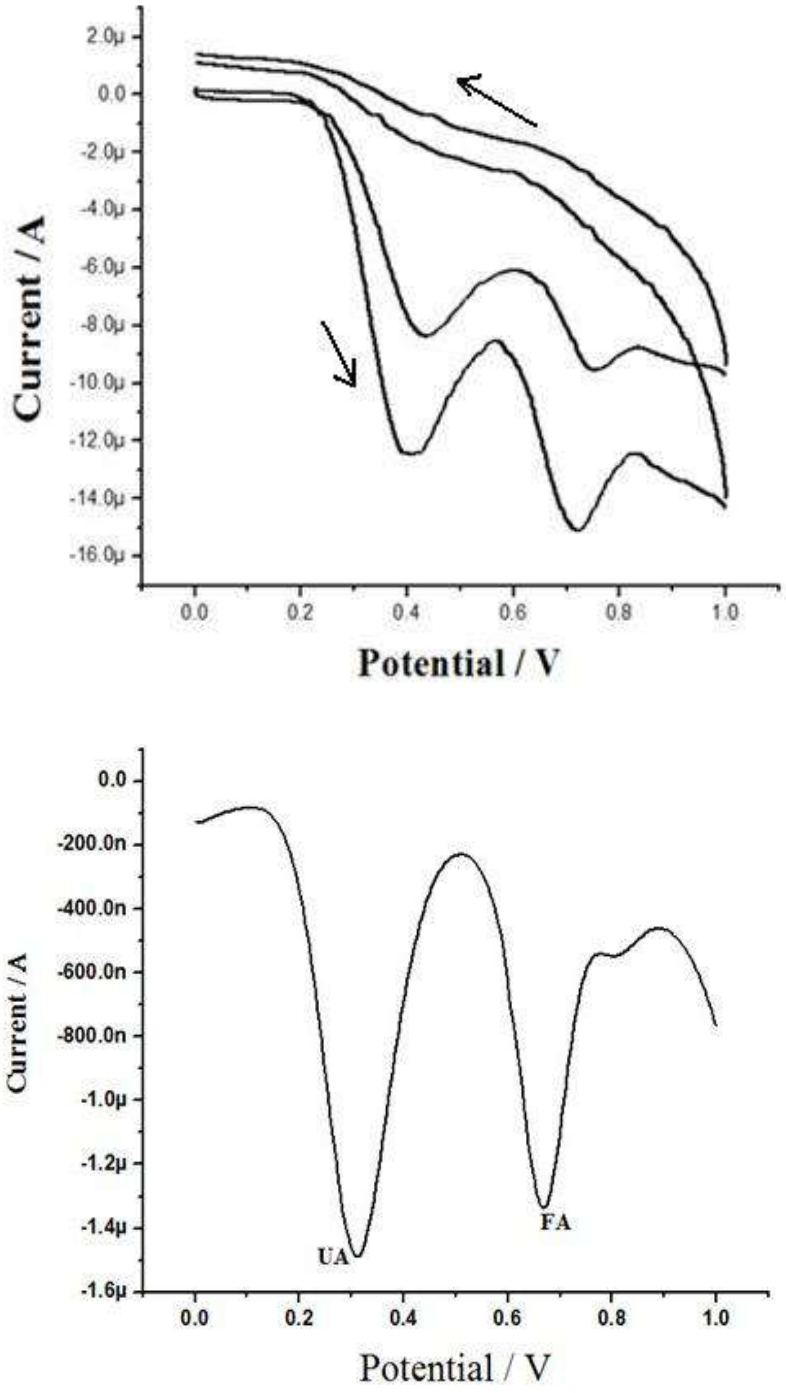


Fig. 4.8. (a) Cyclic voltammogram of 2 mM FA and 0.5 mM UA at BCPE (dotted line) and MWCNTMCPE (solid line) in 0.2 M PBS of pH 7.4 at scan rate 50 mVs^{-1}; (b) Differential pulse voltammogram of 2 mM FA and 0.5 mM UA MWCNTMCPE in 0.2 M PBS of pH 7.4

4.9. References

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5.1. Introduction

The mechanochemically prepared Co_3O_4/CuO composite nanopowder was used for the modification of carbon paste electrode (CPE), and employed for the determination of folic acid (FA) and paracetamol (PA) by cyclic voltammetric (CV) and differential pulse voltammetric (DPV) techniques. The scan rate effect studies on Co_3O_4/CuO modified CPE (MCPE) for FA and PA was conducted and it shows the diffusion controlled process for both the analytes. The lower limit of detection for FA was 0.99µM in the linear range 0.5 to 16µM and 0.38µM for PA in the linearity range 0.5 to 5µM by CV technique. The simultaneous determination of FA and PA shows good selectivity and sensitivity. In addition the performance of Co_3O_4/CuO MCPE was tested for pharmaceutical samples and acceptable results are obtained. The Co_3O_4/CuO MCPE shows antifouling property, stability and reproducibility for the determination of FA and PA at physiological pH.

5.2. Chemistry, Biosynthesis and Biological relevance of Folic Acid

The chemistry, biosynthesis and biological relevance of folic acid has been explained in details in chapter 3 sections 3.7.5, 3.7.6 and 3.7.7.

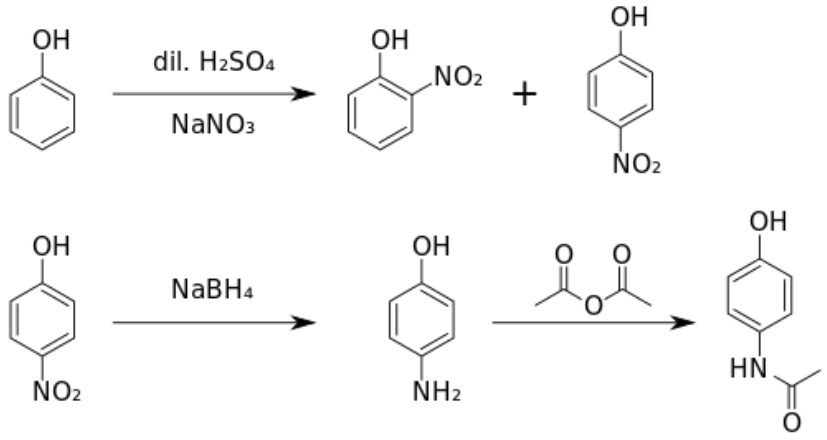
5.3. Chemistry of Paracetamol

Paracetamol was discovered in 1877 [1]. It is the most commonly used medication for pain and fever in both the United States and Europe [2]. Paracetamol consists of a benzene ring core, substituted by one hydroxyl group and the nitrogen atom of an amide group in the *para* (1,4) pattern [3]. The amide group is acetamide ethanamide). It is an extensively conjugated system, as the lone pair on the hydroxyl oxygen, the benzene pi cloud, the nitrogen lone pair, the p orbital on the carbonyl carbon, and the lone pair on the carbonyl oxygen are all conjugated. The presence of two activating groups also make the enzene ring highly reactive toward electrophilic aromatic substitution. As the substituents are *ortho, para*-directing and *para* with respect to each

other, all positions on the ring are more or less equally activated. The conjugation also greatly reduces the basicity of the oxygen and the nitrogen, while making the hydroxyl acidic through delocalisation of charge developed on the phenoxideanion. Paracetamol, also known as acetaminophen or APAP, is a medication used to treat pain and fever. Paracetamol is used for the relief of mild to moderate pain. The use of the intravenous form for pain of sudden onset in people in the emergency department is supported by limited evidence. Preparation of acetaminophen involves treating an amine with an acid anhydride to form an amide. Introduced in the early 1900s, acetaminophen is a coal tar derivative that acts by interfering with the synthesis of prostaglandins and other substances necessary for the transmission of pain impulses.

5.4. Synthesis of Paracetamol

The original method for production involves the nitration of phenol with sodium nitrate gives a mixture of two isomers, from which wanted 4-nitrophenol (bp 279 °C) can easily be separated by steam distillation. In this electrophilic aromatic substitution reaction, phenol's oxygen is strongly activating, thus the reaction requires only mild conditions as compared to nitration of benzene itself. The nitro group is then reduced to an amine, giving 4-aminophenol. Finally, the amine is acetylated with acetic anhydride [4]. Industrially direct hydrogenation is used, but in the laboratory scale sodium borohydride serves [5, 6].



5.5. Review of Electrochemistry of Folic acid and Paracetamol

Folic acid (FA) is a widely distributed water-soluble vitamin and can act as coenzyme in the transfer and utilization of one-carbon groups and in the regeneration of methionine from homocysteine [7]. Deficiency of FA is a common cause of anemia and growth weakness in mammals and it is thought to increase the possibility of heart attack and stroke. Taking 400 μg of FA daily from fortified foods has been suggested. The recommended dietary allowance (RDA) for folate equivalents for pregnant women is 600–800μg, twice the normal RDA for women who are not pregnant. Numerous methods for the determination of FA are available such as capillary electrophoresis [8] and high-performance liquid chromatography (HPLC) [9]. Also some electrochemical methods based on the anodic oxidation have been reported for its qualitative and quantitative determination [10, 11]. Therefore, the determination of FA has drawn significant attention, and a reliable and sensitive detection method is highly expected. In the literature the methods like, spectrophotometry, fluorimetry, high-performance liquid chromatography (HPLC) and flow injection chemiluminescence have been used for the determination of FA. However, these techniques are complex, time-consuming, and require expensive instruments. Electrochemical methods have also been used and attracted enormous interest due to its advantages of simplicity [12-14], fast response [15-17], reproducibility [18-19], good stability [20-22], low cost [23] and low detection limit [24].

Paracetamol (acetaminophen, PA) is a most extensively employed drug as analgesic (pain reliever) and antipyretic (fever reducer). PA is used in the management of cancer or postoperative pain [25]. While generally safe for human use at recommended doses, acute overdoses of paracetamol can cause potentially fatal liver damage and in rare individuals a normal dose can do the same; the risk is heightened by alcohol consumption [26, 27]. Several methods have been used for the determination of PA for quality control analysis in pharmaceutical formulations and for medical control in biological fluids including spectrophotometry [28], flow-injection [29] and chromatographic methods [30]. Electrochemical methods also are used for this purpose [31-35].

The nanomaterials have extensively used in many areas due to their unique optical, electrical, catalytic and magnetic properties contrast with those of bulk materials [36-39]. Metal oxides are largely explored owing towards their potential functions in various fields such as magnetic materials, catalysis, gas sensors, batteries, solar energy and biosensors [40-46]. Cobalt oxide (Co_3O_4) and cupric oxide (CuO) are p-type semiconductors; an essential substance used in the variety of application and has extremely attractive catalytic properties for numerous significant reactions [47, 48]. The mechanochemically prepared Co_3O_4/CuO composite nanopowder were characterized [49] and used as a modifier for the preparation of carbon paste electrode in the determination of folic acid and paracetamol by CV and DPV techniques.

5.6. Experimental Part

5.6.1 Reagents and Chemicals

Disodium hydrogen phosphate monohydrate ($Na_2HPO_4H_2O$), sodium dihydrogen phosphate (NaH_2PO_4) and silicone oil were purchased from Himedia chemicals. The folic acid (FA), paracetamol (PA), graphite powder and NaOH are received from Merck chemicals. The stock solutions of 2.5×10^{-3} M FA and 25×10^{-4}M PA was prepared in 0.1 M NaOH and double distilled water respectively.

5.6.2 Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) was performed in a model CHI-660c (CH Instrument-660 electrochemical workstation). All experiments were carried out in a conventional electrochemical cell containing a bare carbon paste electrode (BCPE) or Co_3O_4/CuO MCPE as a working electrode, the platinum wire as a counter electrode and saturated calomel electrode (SCE) as a reference electrodes.

5.6.3 Preparation of bare carbon paste electrode and Co_3O_4/CuO MCPE

The BCPE was prepared by hand mixing of 70% graphite powder and 30% silicone oil in an agate mortar for about 45min until a homogeneous paste was obtained.

The paste was then packed into a cavity of PVC tube of 3mm internal diameter and smoothened on a tissue paper. The electrical contact was provided by a copper wire connected to the end of the tube [15, 22]. The different quantity of Co_3O_4/CuO nano powder (2, 4, 6, 8 and 10 mg) grinded along with 70% graphite powder and 30% silicone oil in an agate mortar for about 45min to get a homogeneous paste. The paste packing procedure was same as that BCPE.

5.7. Results and Discussion

5.7.1. Effect of quantity ofCo_3O_4/CuO as a modifier towards the detection of FA

The effect of quantity of Co_3O_4/CuO in CPE was characterized by using CV technique. By increasing the amount of Co_3O_4/CuO from 2 mg to 10 mg in the CPE the anodic peak current of 0.2mM FA in 0.2 M PBS of pH 7.4 as supporting electrolyte goes on increasing. Further increase of Co_3O_4/CuO in CPE decreased the current signal of FA. The graph of anodic peak current vs. different quantity of Co_3O_4/CuO in CPE was plotted, Fig. 5.1 shows maximum anodic peak current signal at 6 mg Co_3O_4/CuO MCPE, therefore 6 mg Co_3O_4/CuO MCPE was chosen as optimum for the study of all other parameters. As the quantity of Co_3O_4/CuO in the carbon paste increases the peak current also begins to increase upto 6mg. further increase in the quantity of the modifier resulted in the decrease of peak current due to the insufficient exposure of the active sites present in the carbon paste electrode, and also the reason may be attributed to the slow electron transport phenomenon due to the semiconductor nature of the prepared Co_3O_4/CuO nanopowder [21, 45].

5.7.2. Electrochemical behavior of FA at Co_3O_4/CuO MCPE

The electrochemical behavior of 0.2mM FA at BCPE and Co_3O_4/CuO MCPE in 0.2M PBS of pH 7.4 at scan rate of $0.05Vs^{-1}$ was studied by CV technique. The Figure 5.2 shows at BCPE the oxidation of FA is irreversible and oxidation potential was located at 0.670 V (versus SCE). However, at Co_3O_4/CuO MCPE the oxidation potential was observed at 0.658 V with improved sensitivity. The increase in the peak current response is almost 5 folds for the Co_3O_4/CuO MCPE compared to BCPE. This result shows electrocatalytic activity of the modified electrode.

5.7.3. Effect of scan rate on the peak current of FA

The effect of scan rate on the electrochemical response of 0.2mM FA in 0.2M PBS of pH 7.4 was studied by CV technique at Co_3O_4/CuO MCPE. The Fig. 5.3 shows the CV curves record at different scan rate from 0.05 to 0.5 Vs^{-1}. As a result of increase in the scan rate the oxidation peak current increases gradually along with shifting of the oxidation peak potential towards positive side. Figure 5.4Ashows the graph of anodic peak current versus scan rate with a correlation coefficient of (r^2) 0.9931. The graph of logarithm of anodic peak current versus logarithm scan rate was constructed as shown in the inserted Fig. 5.4B with a linear regression of log I_{pa} (μA) = 4.6934+0.4969 logν (V/s), and gives a slope of 0.4969 which is close to the theoretically predictable value of 0.5 for a diffusion controlled process [50].

5.7.4. Effect of FA Concentration on Co_3O_4/CuO MCPE

Cyclic voltammetric method was employed to study the effect of concentration of FA. The Fig. 5.5A shows the CV curves recorded for the varying concentration of FA in 0.2M PBS of pH 7.4 (0.5μM to16μM) at a scan rate of 0.05Vs^{-1}. It was observed that the oxidation peak current increases gradually with increase in the concentration of FA. The Fig. 5.5B shows the graph of anodic peak current versus concentration of FA, the result shows good linearity with a linear regression equation I_{pa} (μA) = 0.0416×10^{-6} (C_oμM/L) + 0.0338×10^{-6}, (r^2=0.9984). The limit of detection (LOD) was calculated in the lower concentration range by using the equation [9, 49] is found to be 0.99 mM for FA by CV technique. The demonstrated LOD and linearity range was compare to the various modified electrodes and were shown in Table 5.1.

5.7.5. Electrochemical behavior of PA at Co_3O_4/CuO MCPE

Fig. 5.6 shows the CV curves recorded for the oxidation of 0.1mM PA in 0.2M PBS of pH 7.4 at BCPE (dashed line) and Co_3O_4/CuO MCPE (solid line) at the scan rate of 0.05 Vs^{-1}. Compared to BCPE the improved voltammetric response was observed at

Co_3O_4/CuO MCPE with lower ΔE_p values. As ΔE_p is the function of electron transfer rate, Co_3O_4/CuO MCPE can facilitate the oxidation process of PA. This indicates the electrocatalytic activity of the fabricated electrode [58].

The effect of scan rate on the electrochemical response of 0.1mM PA in 0.2M PBS of pH 7.4 was studied by CV technique at Co_3O_4/CuO MCPE as shown in the Figure 5.7. Increase in the scan rate leads to the increases in the redox peak current response of PA. Figure 5.8A shows the graph of I_{pa} versus scan rate with a good linear relationship having the r^2 = 0.9935. The graph of log I_{pa} versus logν gives a slope of 0.4993 is close to the theoretically predictable value of 0.5 for a diffusion controlled process as shown in the insert Figure 5.8B with a linear regression of I_{pa} (μA) = 4.72401 + 0.4993 logν (mV/s), (r^2=0.9985).

5.7.6. Simultaneous Determination of FA and PA

In order to examine the selectivity of Co_3O_4/CuO MCPE, the electrochemical study in the mixture of 0.2mM FA and 0.01mM in 0.2M PBS of pH 7.4 was conducted by CV technique. Fig. 5.9 shows the cyclic voltammograms obtained for the oxidation of FA and PA at BCPE (dotted line) and Co_3O_4/CuO MCPE (solid line). The oxidation of FA and PA at BCPE was less sensitive. However, The Co_3O_4/CuO MCPE shows more sensitivity and selectivity in oxidation process of FA and PA. The DPV technique was performed for the interference study, wherein the concentration of one species maintained constant where other is varied. From the Fig. 5.10, it can be seen that the concentration of FA was increased from 1μM to 8μM by keeping the concentration of PA constant (10μM). The anodic peak current was proportional to concentration of FA and there was no change in the peak current for PA. The LOD for FA in the lower concentration range was 7.83×10^{-7} M for Co_3O_4/CuO MCPE by DPV technique. Similarly, the Fig. 5.11 shows the variation of concentration of PA from 4μM to 11μM by keeping the concentration of FA constant (10μM). The LOD for PA in the lower concentration range was 4.60×10^{-7} M for Co_3O_4/CuO MCPE by DPV technique.

5.7.7. Analytical Application

The practical application of the fabricated Co_3O_4/CuO MCPE was examined by determining the PA and FA in tablet samples. Before using the sample, it was diluted in the distilled water. The experiments were carried at five times for each analyte. The percentage recovery and as in the Table 5.3 were satisfactory and showing that the proposed electrode could be efficiently employing for the detection of FA and PA in commercial pharmaceutical samples.

5.7.8. Stability and Reproducibility of the Co_3O_4/CuO MCPE

To examine the stability and reproducibility of the fabricated Co_3O_4/CuO MCPE it was subjected to the repetitive cyclic records, the anodic peak current is virtually remains constant for the oxidation of PA as shown in the Fig. 5.12. This suggests the fabricated electrode was stable and gives reproducible results [64].

5.7.9. Conclusion

The fabricated Co_3O_4/CuO MCPE was used to study the electrochemical behavior of FA and PA in physiological pH of 7.4 by CV and DPV techniques. The variation in scan rate and concentration study reveals that the electrode process was controlled by diffusion of the analytes. The LOD of FA and PA was 0.99μM and 0.38μM for Co_3O_4/CuO MCPE by CV technique. The simultaneous study of FA and PA shows good selectivity and sensitivity with differentiable peak potential separations. Overall, the Co_3O_4/CuO MCPE can be employed to construct a electrochemical sensor for FA and PA.

Table 5.1. Comparison of detection limits of FA with other modified electrodes

Electrodes	Linear range (µM)	Detection limit (µM)	Techniques	Reference
PAIUCPE	3.0–200.0	0.15	LSV	[56]
PBNBH/CNTPE	15-800	11.0	DPV	[55]
PEDOT/β-CD-SWCNT/GCE	1-1000	0.8	DPV	[53]
Ni/POA/CPE	100-5000	91.0	CV	[54]
ZONPs/CPE	20-2500	9.86	DPV	[34]
Pt:Co/IL/CPE	0.1–500.0	0.04	SWV	[52]
Poly (Alanine) MCPE	10- 40 50-80	A:3.40 B:0.780	CV	[57]
Co_3O_4/CuONPMCP	0.5-16	0.99	CV	Present work

Table 5.2. Comparison of detection limits of PA with other modified electrodes

Electrodes	Detection limit (µM)	Linear Range (µM)	Methods	Reference
MWCNTs/PE	1.1	10-100	SWV	[62]
GCE/PANI-MWCNTs	0.25	1-100	SWV	[61]
GCE/C60	50.0	50-1500	DPV	[60]
f-MWCNTs/GCE	0.6	3-300	DPV	[50]
MWCNTs/poly(Gly)/GCE	0.5	0.5-10	DPV	[58]
Nano Pt-MWCNT/PE	0.17	0.5 -100	DPV	[63]
GCE/Nafion/RuO	1.2	5-250	SWV	[59]
Co_3O_4/CuO MCPE	0.38	0.5-5	CV	Present work

Table 5.3. Determination of FA and PA in real sample

Analyte	Sample (µM)	Spiked (µM)	Found	Recovery (%)
Folic acid (FA)	1	1.0	0.98	98.00
	2	2.0	2.12	106.00
	3	4.0	3.89	97.25
Paracetamol (PA)	1	1.0	1.04	104.00
	2	2.0	1.97	98.50
	3	4.0	4.09	102.20

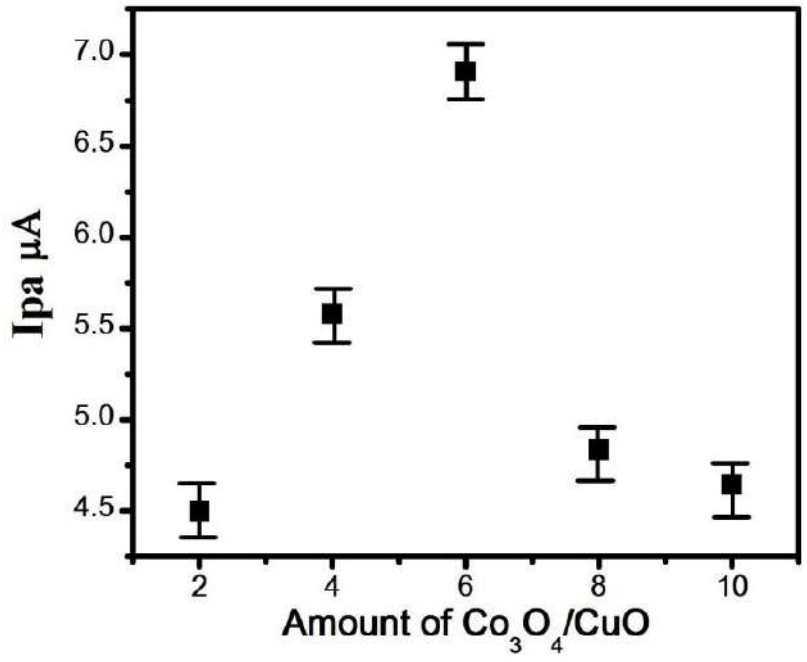


Fig. 5.1. Effect of quantity of Co_3O_4/CuO in the carbon paste electrode

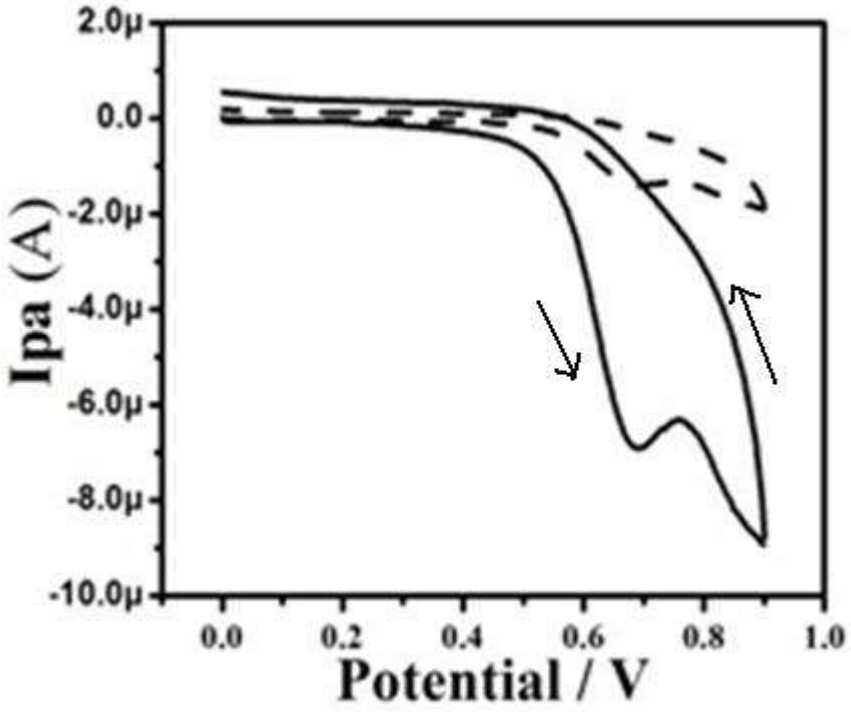


Fig. 5.2. Cyclic Voltammograms for the oxidation of 0.2mM FA at BCPE (dotted line) and Co_3O_4/CuO MCPE (Solid line) in 0.2M PBS of pH 7.4 at scan rate 0.05 Vs^{-1}

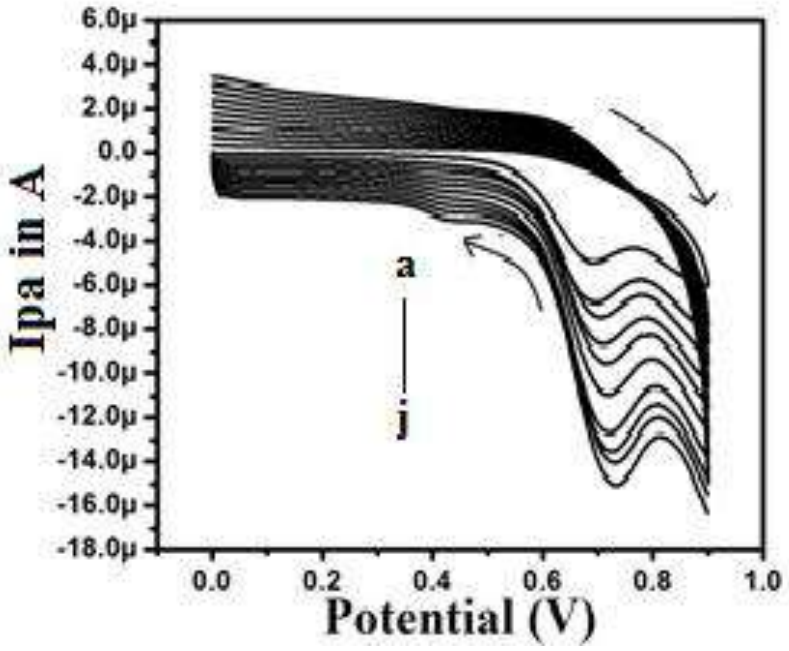


Fig. 5.3. Cyclic voltammograms recorded for the oxidation of 0.2mM FA in 0.2M PBS of pH 7.4 at different scan rate (a:j; 0.05$Vs^{-1}$to 0.5Vs^{-1})

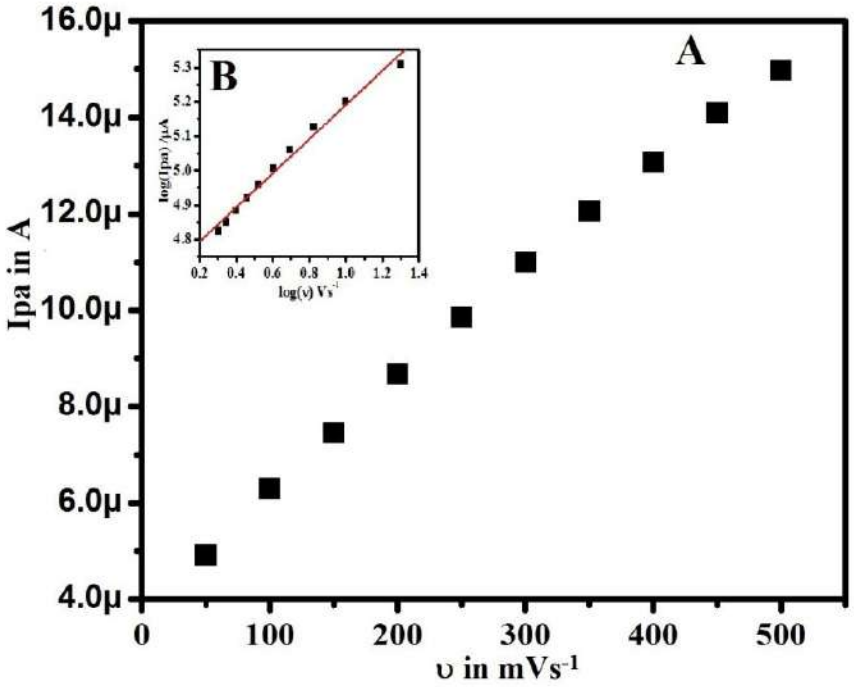


Fig. 5.4. (A) Graph of anodic peak current (I_{pa}) versus scan rate (ν) (B) Graph of log I_{pa} versus the log ν

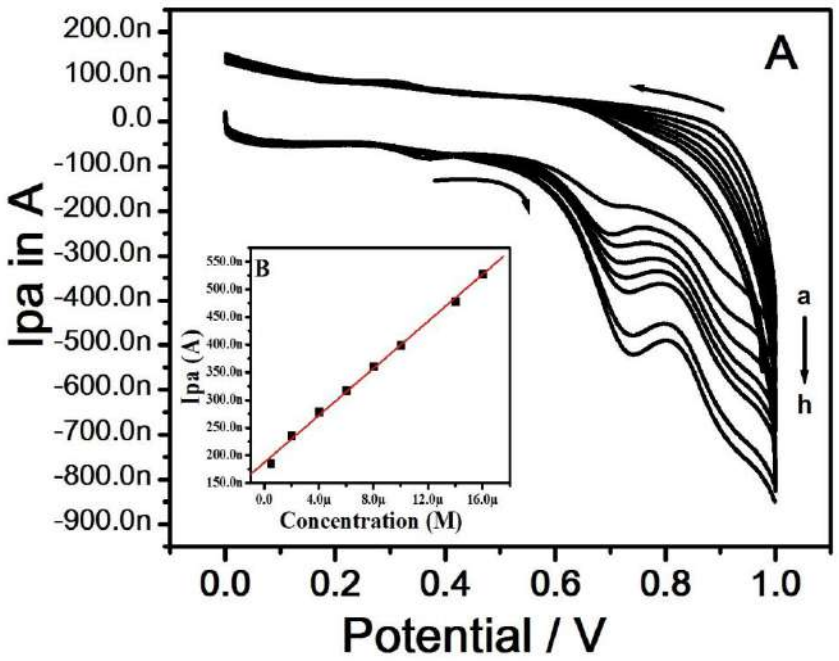


Fig. 5.5. (A) Cyclic voltammograms of FA with different concentration (a-h: 0.5 to16µM) in 0.2M PBS of pH 7.4 at Co_3O_4/CuO MCPE at a scan rate of 0.05Vs^{-1}. (B)Graph of anodic peak current versus concentration of FA

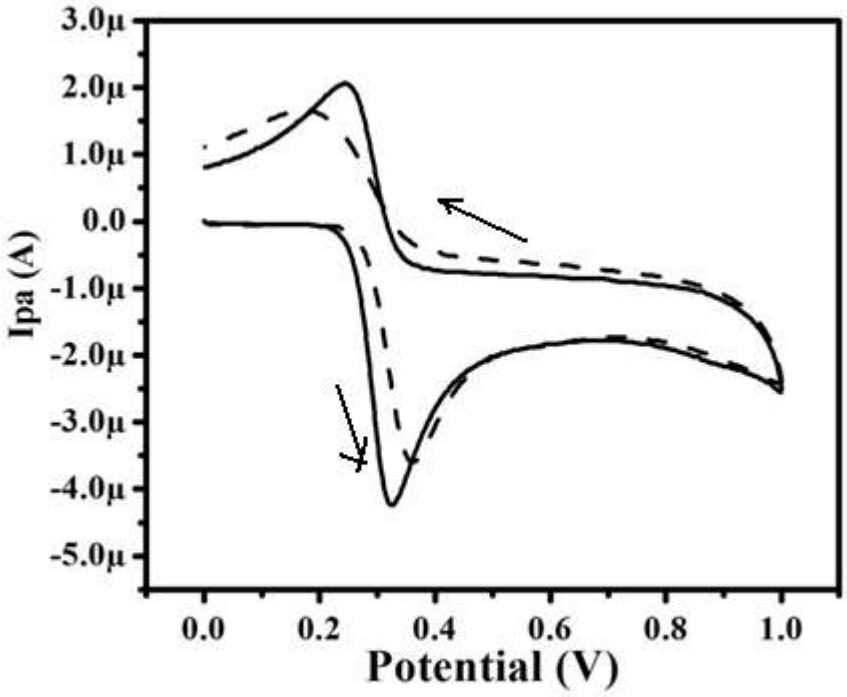


Fig. 5.6. Cyclic Voltammogram for the oxidation of0.2mM PA at BCPE (dotted line) and Co_3O_4/CuO MCPE (Solid line) in 0.2M PBS of pH 7.4 at scan rate 0.05 Vs^{-1}

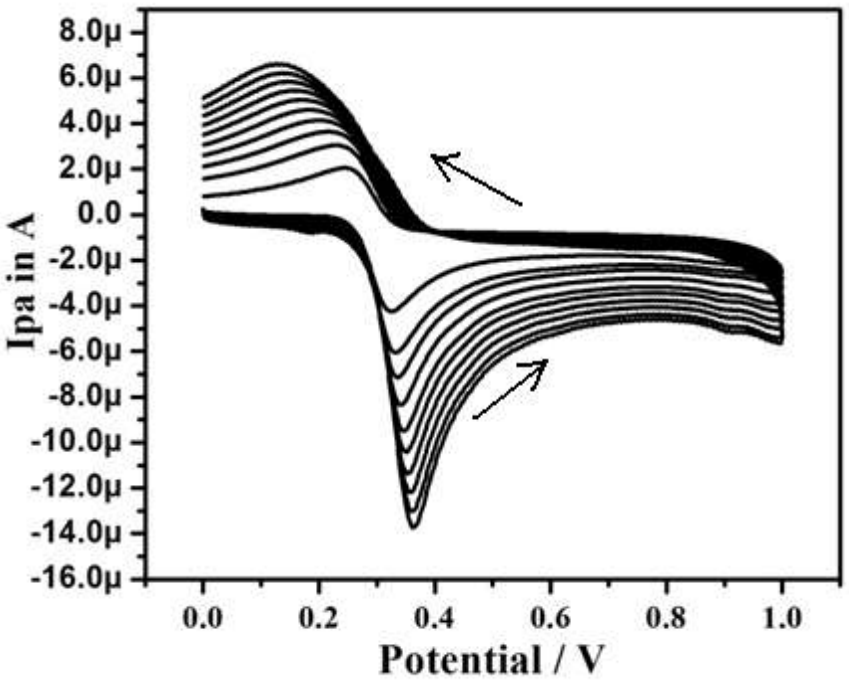


Fig. 5.7. Cyclic voltammograms recorded for the oxidation of 0.1mM PA in 0.2M PBS of pH 7.4 at different scan rates (a:j; 0.05$Vs^{-1}$to 0.5Vs^{-1})

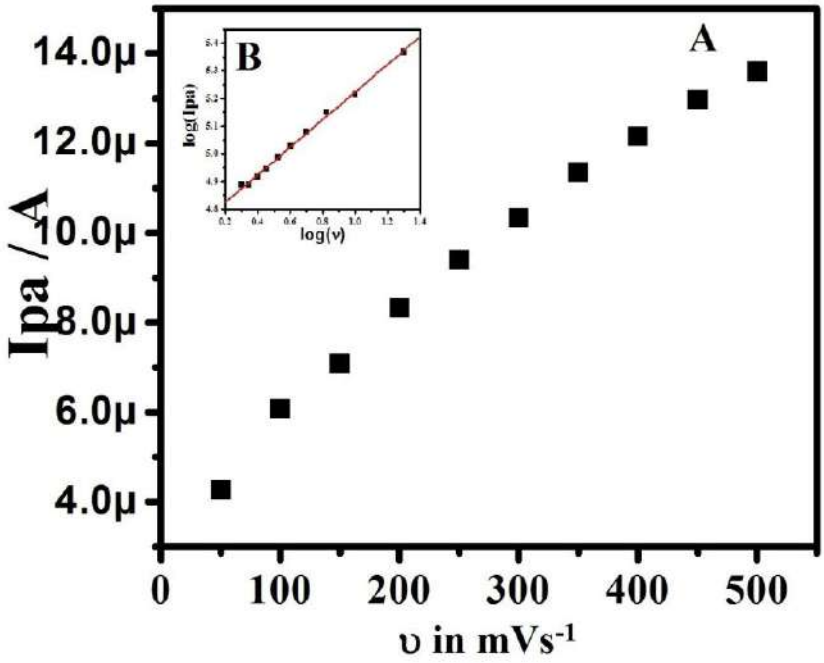


Fig. 5.8. (A) Graph of anodic peak current (I_{pa}) versus the scan rate (ν)
(B) Graph of log I_{pa} versus the log ν

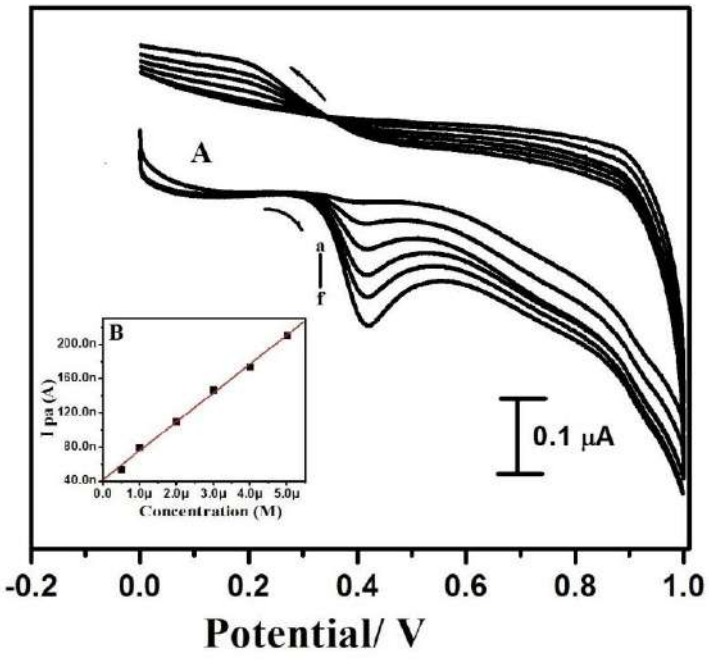


Fig. 5.9. (A) Cyclic voltammograms of PA with varying concentration (a:f; 0.5 to 5µM) in 0.2MPBS of pH 7.4 at scan rate 0.05Vs^{-1}. (B) Graph of I_{pa} versus concentration of PA

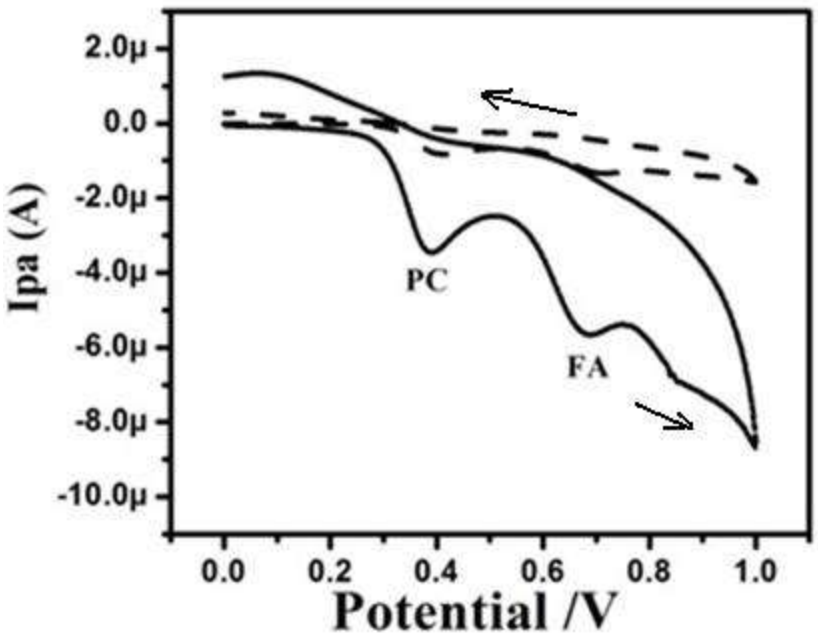


Fig. 5.10. Cyclic voltammograms for 0.2mM FA and 0.01mM PA in 0.2M PBS of pH 7.4 at a scan rate of 0.05Vs^{-1}at BCPE (dotted line) and Co_3O_4/CuO MCPE (solid line)

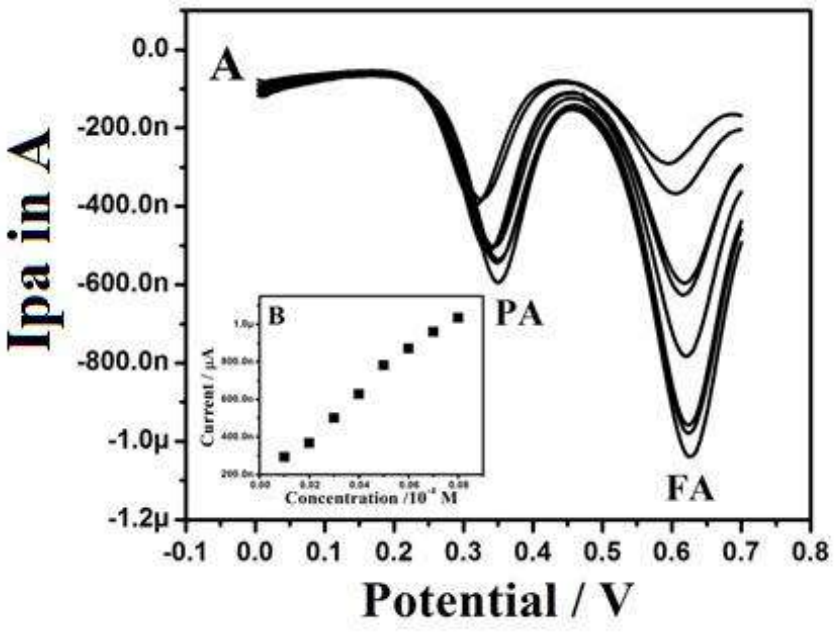


Fig. 5.11. (A) Differential pulse voltammograms obtained for the oxidation of FA (1.0µ to 8.0µM) in presence of 0.2M PBS of pH 7.4 at Co_3O_4/CuO MCPE
(B) Graph of anodic peak current versus the concentration of FA

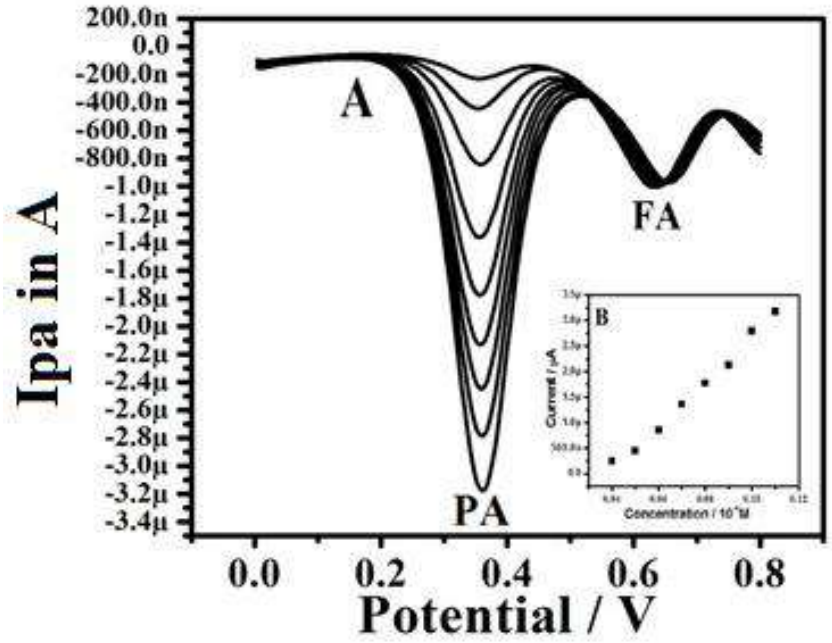


Fig. 5.12. (A) Differential pulse voltammograms obtained for the oxidation of PA(4µM to 11µM) in presence of 0.2M PBS of pH 7.4 at Co_3O_4/CuO MCPE (B) Graph of the anodic peak current versus the concentration of PA

5.8. References

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6.1. Introduction

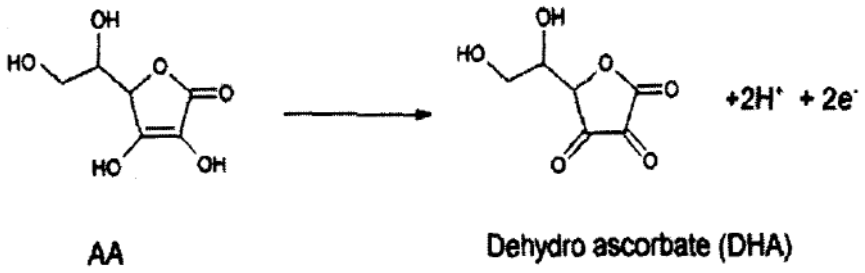
A sensitive and selective electrochemical method was developed for the electroanalysis of norepinephrine by modify electrode with characterised commercially available graphene that has not been chemically treated. As a result of its fabrication, allowing the true electroanalytical applicability of graphene to be properly determined in comparison to the bare carbon paste electrode. The graphene modified electrode shows good analytical performance in terms of sensitivity, linearity and observed detection limits towards each of the various analytes such as norepinephrine, ascorbic acid and uric acid studies in phosphate buffer solution of pH 7.4 by voltammetric techniques. The lower limit of detection of NE was found to be 0.87µM. The interference studies showed that the modified electrode exhibits excellent selectivity and the separation of the oxidation peak potentials for NE–AA and NE–UA were found to be 0.141 V and 0.247 V respectively. The peak differences were large enough to determine NE, AA and UA individually and simultaneously.

6.2. Chemistry, Biosynthesis and Biological relevance of Norepineprine

The chemistry, biosynthesis and biological relevance of norepinephrine has been explained in details in chapter 3 sections 3.7.2, 3.7.3 and 3.7.4.

6.3. Chemistry of Ascorbic Acid

Ascorbic acid (AA) is a sugar of molecular weight 176.13. The molecule, which is partially ionized at physiological pH, contains two acid-ionized groups (pKa 4.04 and 11.34). Though stable to air and light when dry, in aqueous solution it is powerful reducing agent, with redox potential of about 0.05 V at 30°C and pH 7.4. It readily undergoes reversible oxidation to dehydroascorbic acid (Scheme 1).



Scheme 1. Oxidation mechanism of ascorbic acid

6.4. Biosynthesis of Ascorbic Acid

In mammals such as the rat, synthesis of AA occurs through intermediate formation of D-glucuronic acid, L-gulonic acid and L-gulonolactone. As L-gulonolactone oxidase activity is confined to the liver, all AA within the central nervous system ultimately derives from the bloodstream. Primates and guinea-pigs are unusual amongst animals in their inability to synthesise AA and are therefore susceptible to the deficiency disease scurvy.

6.5. Biological Relevance of Ascorbic Acid

This a water-soluble vitamin which is important in forming collagen, a protein that gives structure to bones, cartilage, muscle, and blood vessels. It also helps maintain capillaries, bones, and teeth and aids in the absorption of iron. Ascorbic acid, a reducing agent, is necessary to maintain the enzyme prolyl hydroxylase in an active form, most likely by keeping its iron atom in a reduced state. The precursor molecule to the protein collagen, procollagen, contains an unusual amino acid sequence in that every third amino acid is a glycine and contains a high frequency of two amino acids not found in any other proteins - hydroxyproline and hydroxylysine. These latter two amino acids are converted from proline and lysine, respectively, after the procollagen molecule has been synthesized. The hydroxylation of proline and lysine in procollagen is carried out by the enzyme prolyl hydroxylase using ascorbic acid as a cofactor. The natural form of the vitamin is the L-isomer. Ascorbic acid plays an important role as a component of enzymes involved in the synthesis of collagen and carnitine; however, its most vital role is as a water-soluble vitamin in the human body [7, 8]. Ascorbic acid is a powerful

antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical. As a scavenger of reactive oxygen and nitrogen oxide species, ascorbic acid has been shown to be effective against the superoxide radical ion, hydrogen peroxide, the hydroxyl radical and singlet oxygen [9]. Ascorbic acid protects folic acid reductase, which converts folic acid to folinic acid, and may help release free folic acid from its conjugates in food. Ascorbic acid facilitates the absorption of iron. It helps maintain elasticity of the skin aids the absorption of iron and improves resistance to infection, treatment of scurvy and may prevent the occurrence and development of cancer.

6.6. Chemistry, Biosynthesis and Biological relevance of Uric acid

The chemistry, biosynthesis and biological relevance of norepinephrine has been explained in details in chapter 4 sections 4.4.

6.7. Review of Electrochemistry of Norepinephrine, Ascorbic Acid and Uric Acid

Norepinephrine (NE) is one of the most important biochemical messengers in mammalian central nervous systems, existing in the nervous tissue and biological body fluid. Many diseases and the caducity process are related to changes of its concentration, thus the quantitative determination of trace NE in biological fluids provides important information on its physiological functions provides and the diagnosis of some diseases in clinical medicine [1]. Usually the determination of NE is carried out using high performance liquid chromatography [2], gas chromatography [3], chemiluminescence [4], spectrophotometry [5], and flow injection [6]. Furthermore, NE is an elecroactive molecule, and its electrochemical behavior has been studied extensively. The case of NE oxidation led to development of electrochemical procedures for measuring NE levels at various electrodes and using various electrochemical methods [7-12]. The many chemically modified electrodes include carbon based electrodes [13-16], self-assembled monolayers gold electrodes [17], lead–ruthenium oxide electrodes [18], carbon nanotubes [19], and electropolymerized films [20–25] are used for the determinationof NE. Uric acid (UA) is the primary product of purine metabolism in the human body and a major nitrogenous compound in the urine [26]. Its abnormality in human body leads to many

severe diseases, such as gout, hyperuricemia and Lesch–Nyhan disease [27, 28]. Increased urate level also leads to the pneumonia and leukaemia [29]. Ascorbic acid (AA) is also known as vitamin-C and is a water soluble compound that take part in the maintaining many important life processes. It has been used as a medicine for the treatments of common cold, mental illness and cancer [30]. It can be chemically or electrochemically oxidized to dehydro ascorbic acid [31]. Hence, monitoring the concentration of these biological compounds is very important in clinical diagnosis. There are so many endless reports for the voltammetric determination of these molecules either individually or in presence of probable interferences [32-33].

Nanomaterials have received attention in recent years in numerous fields because of their huge potential. Among them, Graphene have become the topic of intense investigation. Such considerable interest reflects the unique behaviour of, Graphene together with their outstanding electrical, chemical, mechanical and structural properties that make them a very attractive material for a large range of applications [14] Most notably, graphene has been reported to be utilised as an enhanced modified electrode in terms of the improved electroanalytical monitoring of various substances, Owing to its reported advantageous properties, graphene has thus been utilised in the development and exploration of fast, sensitive and reliable detection methods for a range of target analytes, for instance towards the detection of ascorbic acid,9 glucose,10 hydrazine,11hydrogen peroxide 12 and nitric oxide,13 where upon comparison to the performance of the more traditional noble metal and various other fullerene based alternatives.

Electrochemical techniques give an easy, cost less and fast way of analyzing biologically and environmentally molecules. However, the main drawback for the voltammetric detection of NE in the interference studies of the concomitant compounds, such as AA and UA, which usually lead in overlapped voltammetric response due to their very similar oxidation peak potentials. Recently, surfactant modified electrode has been proved to be a successful method to solve this problem, and the applications of surfactants in electroanalytical chemistry have been widely reported [20]. So in the present study is concerned with Graphene effect on electrode behavior and electro-oxidation of NE, AA and UA individually. For the simultaneously studies the Graphene

MCPE is immobilized with a Tween-20 a nonionic surfactant, the outcome result at T-20/G-MCPE is the analytes such as NE, AA and UA is well distinguished peak were obtained at Physiological pH (Scheme 2 and 3).

6.8. Experimental Part

6.8.1. Reagents

Graphene was commercially obtained from Anderlab Technologies Private Limited (> 99% purity) Maharashtra. Norepinephrine (L-Noradrenaline hydrochloride) was purchased from Fluka, uric acid (UA), ascorbic acid (AA) and Tween-80 were purchased from Himedia. The stock solution 25×10^{-4}M NE, 25×10^{-4} M UA and 25×10^{-3}M AA was prepared in 0.1 M perchloric acid, 0.1 M NaOH, and double distilled water respectively. The phosphate buffer solutions with different pH levels were prepared by mixing the same ionic strength (0.2 M) solutions of Na_2HPO_4 and NaH_2PO_4 solutions at different ratios. Physiological pH 7.4 was used as a supporting electrolyte. Graphite powder was purchased from Merck and silicone oil from Himedia was used to prepare carbon paste electrode. All the chemicals mentioned were all of analytical grade used as received without any further purification.

6.8.2. Apparatus

All electrochemical experiments including cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed using a model CHI-660C (CH Instrument-660 electrochemical work station). A conventional three electrode system was used in a electrochemical cell with a saturated calomel electrode (SCE) as a reference, a platinum or counter electrode and bare carbon paste electrode or Graphene modified carbon paste electrode as working electrode.

6.8.3. Preparation of the working electrode

The carbon paste electrode (CPE) was prepared according to the reported literature [43]. Graphene Modified Carbon Paste Electrode was prepared by grinding the different ratios of Graphene with 70% graphite powder and 30% silicon oil in an agate

mortar by hand mixing for about 30 minute to get homogeneous paste. The paste was packed into the home made cavity of CPE of and smoothened on weighing paper it is further modified by immobilizing Tween-20 on the surface of the G-MCPE.

6.9. Results and Discussion

6.9.1 Surface Morphology of Graphene

The morphological surface of the Graphene was investigated with SEM. Fig. 6.1 clearly reveals the SEM image of exfoliated Graphene sheet of single layer structure. The Graphene sheets fold together and make a more accessible surface area. The high surface area of Graphene provides good conductivity for the electrooxidation of NE, AA and UA. Therefore, this typical graphene sheet structure could serve as a good pathway of conductivity in the NE, AA and UA electrocatalysis.

6.9.2 Effect of quantity of Graphene as a Modifier towards the Detection of Potassium Ferrocyanide

The effect of quantity of Graphene in CPE was analyzed by using CV technique. The graph of anodic peak current vs different quantity of Graphene in CPE was plotted, Fig. 6.2 shows maximum anodic peak current signal at 5mg for G-MCPE, therefore 5 mg G-MCPE was chosen as optimum for the study of all other parameters. As the quantity of Graphene in the carbon paste increases from 2 mg to 8 mg the peak current also begins to increase upto 5mg. further increase in the quantity of the modifier resulted in the decrease of peak current due to the insufficient exposure of the active sites present in the carbon paste electrode, and also the reason may be attributed to the slow electron transport phenomenon [44].

6.9.3. Surface Property of CPE and G-MCPE

The freshly prepared stock solutions of 1mM potassium ferrocyanide and 1M KCl as supporting electrolyte were placed in an electrochemical cell. Fig. 6.3A shows the cyclic voltammograms recorded for the 1mM potassium ferrocyanide at both CPE (dashed line) and G-MCPE (solid line) at the scan rate 0.05 Vs^{-1}. The low redox peak

currents response was obtained at CPE but in the same condition G-MCPE exhibited stable enhancement of redox peak currents and also it shows the fast electron transfer rate kinetics. The result obtained greatly improved the voltammetric response of potassium ferrocyanide at G-MCPE. This suggests that the surface property of the modified electrode has been significantly changed and also the result proves that the electrocatalytic activity of the G-MCPE towards the potassium ferrocyanide. The total active surface area available for reaction of species in solution can be estimated by the Randles–Sevcik equation 6.1 [45-48].

$$I_{pa} = 2.69 \times 10^5 n^{3/2} A D^{1/2} C_0 \nu^{1/2} \quad \text{---------- (6.1)}$$

where, I_p is the peak current in A. C_0 is the concentration of the electroactive species (mol cm^{-3}), n is the number of electron exchanged, D is the diffusion coefficient in $cm^2\ s^{-1}$, t is the scan rate(Vs^{-1}) and A is the electroactive surface area (cm^2). For G-MCPE the electroactive surface area is maximum (0.035 cm^2) as compared with CPE (0.027 cm^2).

6.9.4. Electrochemical Response of NE at G-MCPE

Fig. 3B shows the cyclic voltammogram of 0.1mM NE at CPE (dotted line) and G-MCPE (solid line) in 0.2 M PBS of pH 7.4 at scan rate of 0.5 Vs^{-1}. As can be seen, the oxidation peak (E_{pa}) was observed at 0.175 V for CPE, but in case of E-MCPE the E_{pa} shift towards positive direction and moreover shows increase in the peak current and E_{pa} was observed at 0.188 V. The strong improvement and reversible redox peaks were observed at G-MCPE, the E_{pa} were observed around 0.165 V respectively. This is the clear evidence that G- MCPE has better electrocatalytic activity by exposing large surface area for electrochemical oxidation of NE. In addition the π-π interaction between the phenyl structure of NE and in grapheme sp^2 conjugated bond makes the modified carbon paste electrode shows excellent electrocatalytic activity [19], by reducing over potential and also by improving the current signals. The conversion of Norepinephrine (NE) to Norepinephrine quinone (NEQ) is shown in Scheme 1(a).

6.9.5. Effect of Scan Rate on Peak Current of NE

The effect of variation of applied scan rate for 1mM NE in 0.2 M PBS of pH 7.4 was examined by CV technique at G-MCPE as shown in Fig. 6.4A. The experimental results obtained at G- MCPE showed increase in the redox peak currents with increase in the applied scan rate and they are proportional to each other according to Randles–Sevcik equation. The observation shows that there is a shifting of anodic peak potential (E_{pa}) to more positive side and cathodic peak potential (E_{pc}) to the less negative side. In order to confirm the electrode process, the graph of peak current (Ip) versus scan rate (ν) was plotted and the obtained graph is a straight line with good linearity in the range from 0.05 to 0.9 Vs^{-1} as shown in Fig. 6.4B with the correlation coefficient (r^2) 0.9997 and 0.9994. The Ip versus square root scan rate ($\nu^{1/2}$) were plotted as shown in Fig.6.4C with the correlation coefficient (r^2) 0.9873 and 0.9885. This suggests the electrode process was an adsorption-controlled process. The heterogeneous rate constant (k^0) values was determined from the experimental peak potential difference (ΔEp) data's, Eq.(2) was used for such voltammograms whose ΔE_p values are greater than 10 mV [49]

$$\Delta E_p = 201.39 \log(\nu/k^0) - 301.78 \qquad \text{---------- (6.2)}$$

From the experimental ΔE_p values as shown in Table 6.1 and Eq. (2); the values of the k^0 for the NE oxidation was determined. The values of k^0 obtained at different scan rate from 0.05 to 0.9 Vs^{-1} for the G-MCPE.

6.9.6. Effect of NE Concentration

The electrocatalytic oxidation of NE was carried out by vary in its concentration at Graphene MCPE. Fig. 6.5A shows by increasing the concentration of NE from 25µM to 70µM, the I_{pa} and I_{pc} goes on increasing with shifting E_{pa} towards less positive and E_{pc} towards least negative side. The in set graph of I_{pa} versus concentration of NE was plotted and it shows almost straight line with good linearity as shown in Fig. 6.5B. The linear regression equation I_{pa} (µA) = 0.03313 (C_0µM/L) +0.4112, (N = 10, r^2 = 0.9992). The detection limit in the lower concentration range for NE was 1.8778µM for the Graphene MCPE and limit of quantification was 6.259µM was calculated.

6.9.7. Effect of pH Value on the Determination of NE at G-MCPE

The pH of the phosphate buffer solution has a significant contribution on the electrocatalytic oxidation of NE at the G-MCPE by affecting both peak currents and peak potentials. The effect of PBS pH value on the determination of NE at Graphene MCPE was carefully evaluated in a wider pH range of 5.8–7.8. Fig. 6.7A shows cyclic voltammograms recorded for 1mM NE at G-MCPE. The oxidation peak potential shifts to a more negative potential with increasing pH. The E_{pa} versus pH graph clearly indicated that the E_{pa} depends linearly on the pH value in the range of 5.8–7.8 with a slope of 0.062V/pH ($r^2 = 0.995$) with is close to the theoretical value of 59V as shown in inset Fig. 6.7B. This result shows there is an equal number of protons and electrons are involved in the redox mechanism at G-MCPE. This was consistent with that reported in literature [40]. Fig. 6.6 shows the effect of solution pH on the NE current response in the range of pH 5.8 to 7.8. NE showed a well-defined oxidation peak at all pH values. It can be seen that the anodic peak current increases from pH 5.8 to 6.2 but then decreased from pH 6.2 to 6.6. This may be because, as the pH increases beyond 7.0, OH^- ions in the solution also increases and there is a chance of deprotonation reaction accompanied by chemical reactions [55]. The intermediate products obtained by the above reactions may compete for the adsorption sites on the electrode surface and hence led to a reduction in peak current. There is more current enhancement at pH 7.4 in PBS. Therefore pH 7.4 PBS was used for all subsequent determinations of NE [56].

6.9.8. Electrochemical Response of AA and UA at Graphene MCPE

Fig. 6.11 shows the cyclic voltammograms of 1mM AA at the CPE (dotted line) and G-MCPE (solid line) in 0.2 M PBS solution of pH 7.4 with the scan rate 0.05 Vs^{-1}. At the CPE the oxidation peak occurred at around 0.217 V and it was generally irreversible, critically broad and required high over potential due to fouling of the electrode surface by the adsorption of oxidized product of AA. However, at the G-MCPE the oxidation peak potential of AA was obtained at around 0.212 V which shifted to negative potential and showed faster electron transfer kinetics of AA when compared to that of CPE. This result indicated that the G-MCPE lowers the over potential and favors the oxidation process of AA. The conversion of ascorbic acid (AA) to dehydro ascorbate is shown in scheme 6.1A.

Fig. 6.8 shows the cyclic voltammogram recorded for 1mM UA at CPE (a) and G-MCPE (b) in 0.2 M PBS solution of pH 7.4 with the scan rate 0.05 Vs^{-1}. It is noticed that voltammogram obtained at CPE was less sensitive and shows poor electrochemical response for anodic peak current (I_{pa}). However, at G-MCPE showed a significant increment in oxidation peak current and also shows good electrocatalytic activity. The conversion of uric acid (UA) to uric acid 4,5 diol (UDA) is shown in scheme 6.1A.

6.9.9. Effect of Concentration Variation

The DPV plots were recorded at G-MCPE of pH 7.4 was carried out by varying at different concentration of Fig. 6.5A NE, Fig. 6.13A AA and Fig. 6.10A UA from the respective insets the graph of I_{pa} versus concentration was plotted and it shows almost straight line with good linearity, thus all these substances could be electrochemically quantified indivisibly at G-MCPE. The analytic parameters values viz., linearity range, linear regression equation, sensitivity, detection and quantification limits are shown in Table 6.2. In agreement with the results obtained, it can be observed that the G-MCPE is more sensitive toward the NE, as compared with the AA and the UA, and the lower detection limit is also reached for NE. The detection limit (LOD) and quantification limit (LQD) was calculated by using the formulas (1) and (2) written as follows [22, 42]

$$LOD = 3S/M \qquad (1)$$

$$LOQ = 10S/M \qquad (2)$$

where, S is the standard deviation and M is the slope obtained from the calibration plots. The detection limit of various electroanalytical methods proposed for the determination of NE is compared with our analytical data in Table 6.2. The data reveals that a lower limit of detection (LOD) is achieved using the method proposed here.

6.9.10. Simultaneous Electroanalysis of NE in Excess Concentration of AA and UA at T-20/G-MCPE

The NE, AA and UA always exist together in the biological environment and the simultaneous analysis of these molecules was difficult at carbon paste electrode. The

concentrations of AA and UA were much higher than that of NE. Moreover, the oxidation potential of both AA and UA was nearly same as that of NE results in an overlapped poor voltammetric response at CPE. Fig. 6.14 shows the cyclic voltammetric response of 0.9×10^{-4}M NE in presence of high concentration of 1.4×10^{-3} M AA and1.2×10^{-4} M UA in 0.2 M PBS of pH 7.4 at the scan rate of 0.05 Vs^{-1} at CPE and T-20/ G-MCPE. The cyclic voltammograms obtained for the mixture of NE and AA at CPE was, less sensible and gives overlapped potential. This leads to their individual identification impossible (dashed line). However, the G-MCPE has an ability to overcome this difficulty and resolved the oxidation potential of all three analytic in the mixture. Three well defined peak potential of NE, AA and UA at different potentials are located at 0.193 V, 0.064 V and0.285 V, respectively (solid line). The peak to peak separation of NE–AA was 0.141 V and that of NE–UA was 0.247 V. This result was more enough to identify and determine NE in the presence of high concentration of UA and AA at T-20/G-MCPE.

Differential pulse voltammetry (DPV) was employed for the analysis of NE, AA and UA at T-20/G-MCPE due to its higher current sensitivity and absence of background current. Fig. 6.15A shows the simultaneous determination of 1×10^{-4} MNE, 2×10^{-3}M AA and 1.8×10^{-4}M UA with oxidation potentials 0.106 V, -0.038 V and 0.219 V, respectively. The peak to peak separation between NE–AA was0.043 V and that of NE–UA was 0.181 V, respectively. Thus,T-20/G-MCPE exhibits good electrocatalytic activity, a considerable oxidation peak separation, enabling the simultaneous detection of NE, AA and UA in physiological pH.

6.9.11. Interference Study

The interference study was performed in the mixture of samples containing NE, AA and UA at the T-20/G-MCPE. The concentration of one species is changed by keeping the concentration of the other two species was constant. From Fig. 6.15B it can be seen that the peak current of NE was increased because of increased concentration from 10×10^{-4}M to 50×10^{-4}M. In the same way Fig. 6.15C AA was increase from

15×10^{-3}M to 40×10^{-3}M at the T-20/G-MCPE at pH 7.4. The experimental results showed that the when the concentration increases only peak current increases but the anodic peak potential of NE and AA doesn't shift towards positive or negative side, even though the higher concentration of other analyte. It could also be noticed from these DPV curves that the responses to NE and AA at T-20/G- MCPE were relatively independent of each other.

6.9.12. Conclusion

In summary, a simple electrochemical method for the determination of NE is developed by modifying the CPE with grapheme under the physiological condition. Decrease in oxidation over potential and enhancement in current proved the electrocatalytic activity of CPE modified by graphene. A very minuet quantity of graphene used for the fabrication of electrode make totally inexpensive with is used for the analysis of neurotransmitter. Moreover, by this simple method of fabrication a sensitivity, selectivity and lower detection limit was achieved. This result was more sufficient to analyze NE in presence of large excess of AA and UA. The prepared modified electrode has potential for the investigation of other neurotransmitters.

Table 6.1. Comparison table of rate constant of different scan rate obtained at Graphene MCPE

νVs^{-1}	ΔEp/ V	K^0/s^{-1}
0.05	0.046	0.447
0.1	0.052	0.397
0.2	0.084	0.341
0.3	0.094	0.305
0.4	0.106	0.278
0.5	0.119	0.255
0.6	0.129	0.235
0.7	0.139	0.218
0.8	0.138	0.203
0.9	0.155	0.188

Table 6.2.Coparison table of limit of detection and limit of quantification obtained at Graphene MCPE

Analyte	Linear Dynamic range (µM)	Regression equation	Correlation coefficient	LOD (µM)	LOQ (µM)
NE	5-70	I_{pa} (µA) = 0.033 (C_0µM/L)+0.411	0.999	0.87	6.25
AA	5-60	I_{pa} (µA) = 0.026 (C_0µM/L)+0.136	0.999	1.91	3.38
UA	10-75	I_{pa} (µA)=0.063(C_0µM/L)+0.118	0.986	1.29	2.38

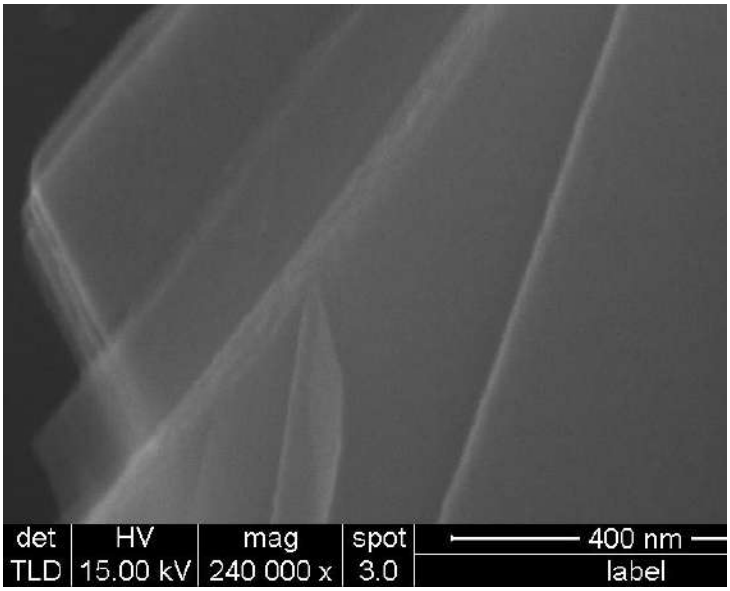
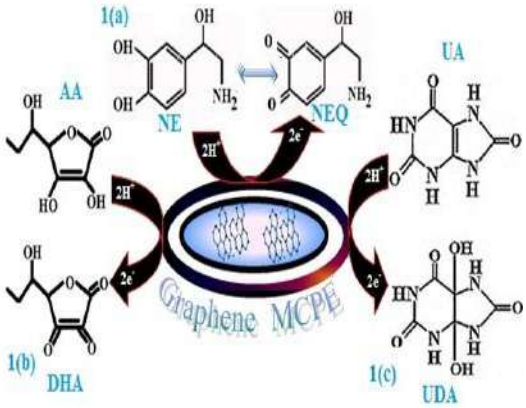
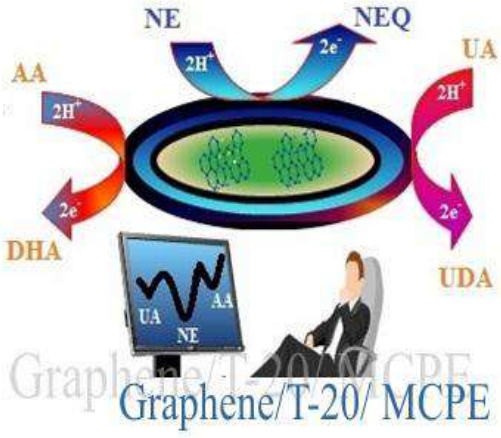


Fig 6.1. SEM image of Graphene



Scheme 2



Scheme 3

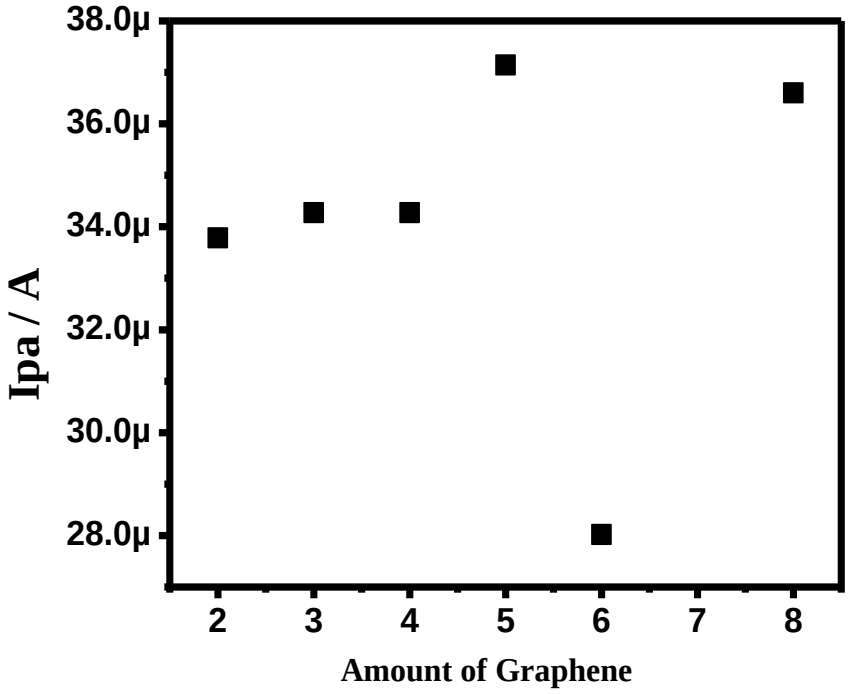


Fig. 6.2. Effect of quantity of Graphene in the carbon paste electrode

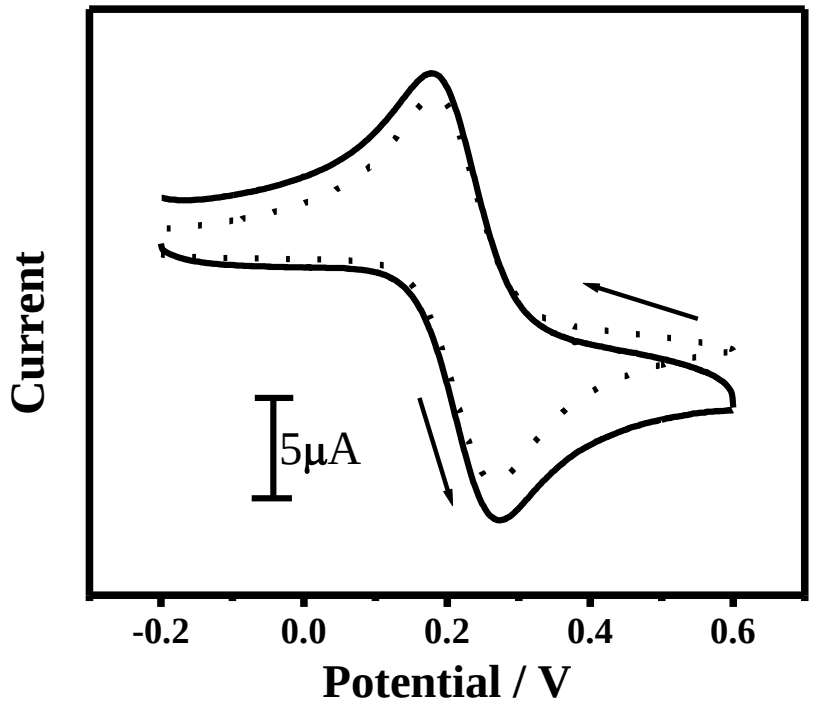


Fig. 6.3A. Cyclic voltammograms of 1 mM potassium ferrocyanide at BCPE (dashed line) and Graphene MCPE (solid line) at scan rate of 0.05 Vs^{-1}

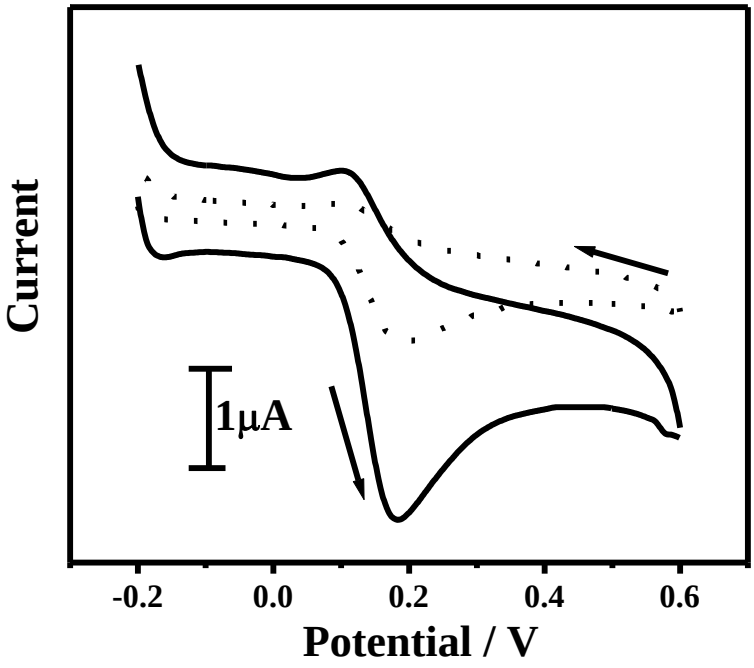


Fig. 6.3B. Cyclic voltammograms of 1mM NE in 0.2 M PBS solution of pH 7.4 at BCPE (dashed line) and Graphene MCPE (solid line) at scan rate of 0.05 Vs^{-1}

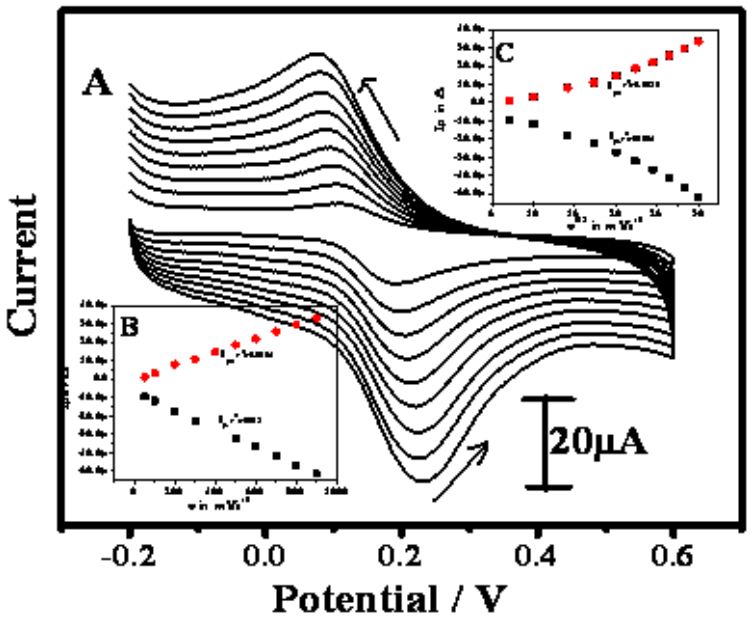


Fig. 6.4. (A) Cyclic voltammograms of 1mM NE in 0.2 M PBS solution of pH 7.4 at Graphene MCPE at different scan rate (a–h; 0.05–0.9 Vs^{-1}). (B) Graph of peak current versus scan rate and (C) Graph of peak current versus square root of scan rate

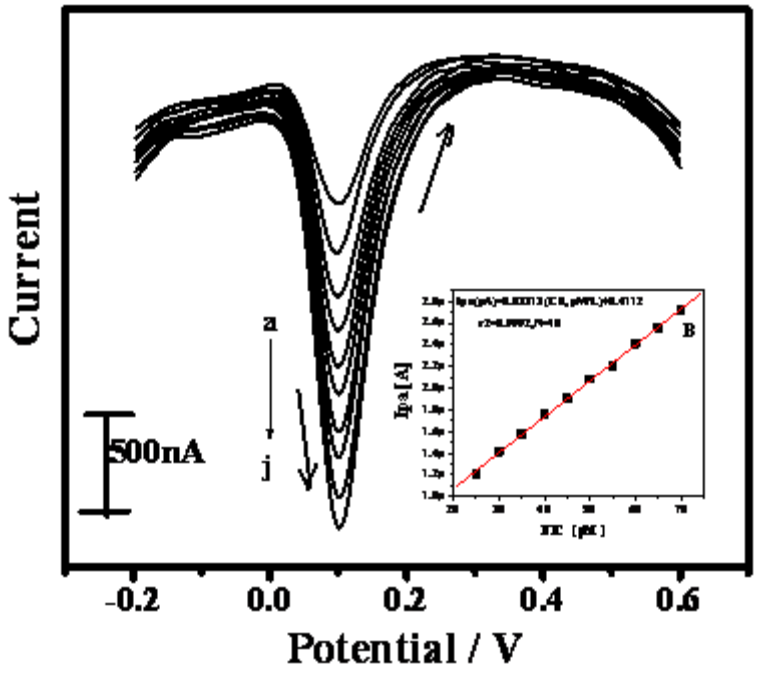


Fig. 6.5. (A) Cyclic voltammograms of NE in 0.2 M PBS solution of pH 7.4 at graphene MCPE at scan rate of 0.05 Vs^{-1} with different concentration (a–j; 25, 30, 35, 40, 45, 50, 55, 60, 65 and 70 µM). (B) Graph of anodic peak current versus concentration of NE

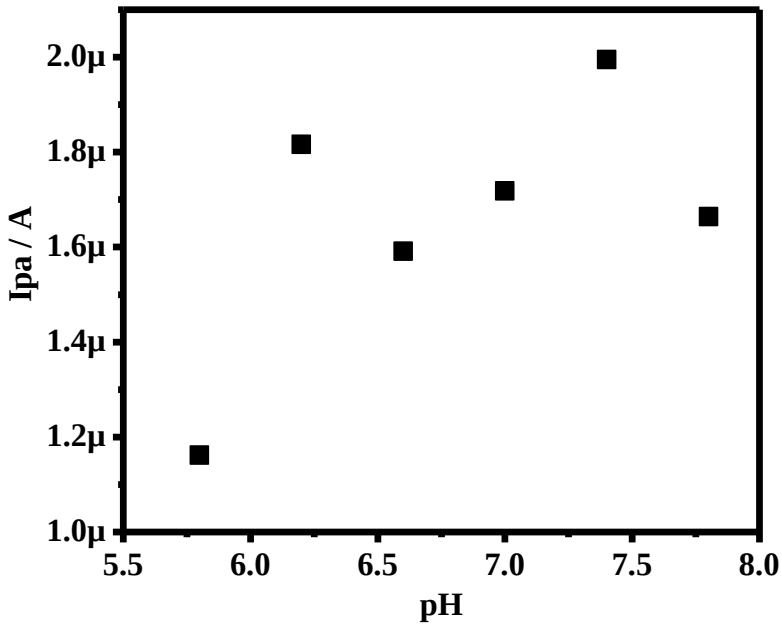


Fig. 6.6. Effect of pH on the anodic peak current of 1mM NE in pH 7.4 0.2M PBS at a sweep rate of 0.05 Vs^{-1}

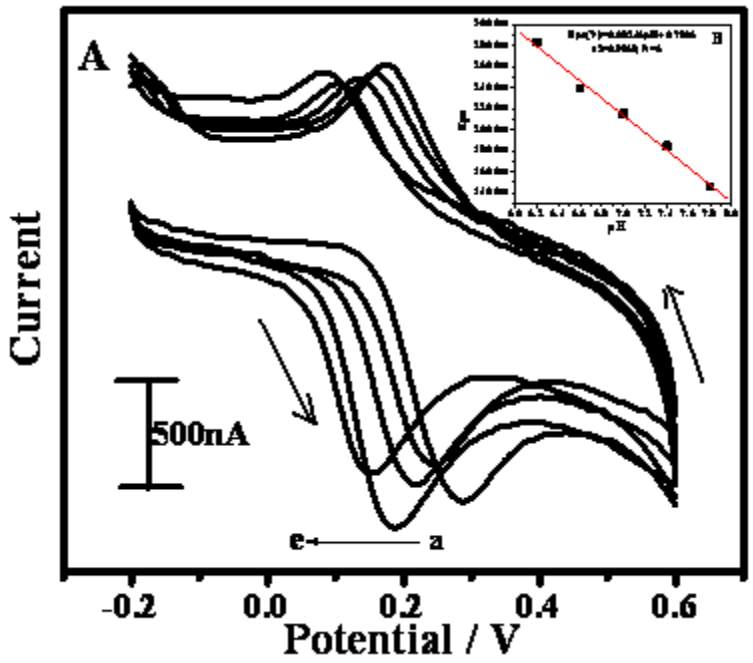


Fig. 6.7. (A) Cyclic voltammograms of the Graphene MCPE in 0.2 M PBS solution at different pH (a–e: 6.2-7.8) at scan rate of 0.05 Vs^{-1}. (B) The effect of pH on the peak potential response of 1×10^{-4} M NE in 0.2 M PBS solution

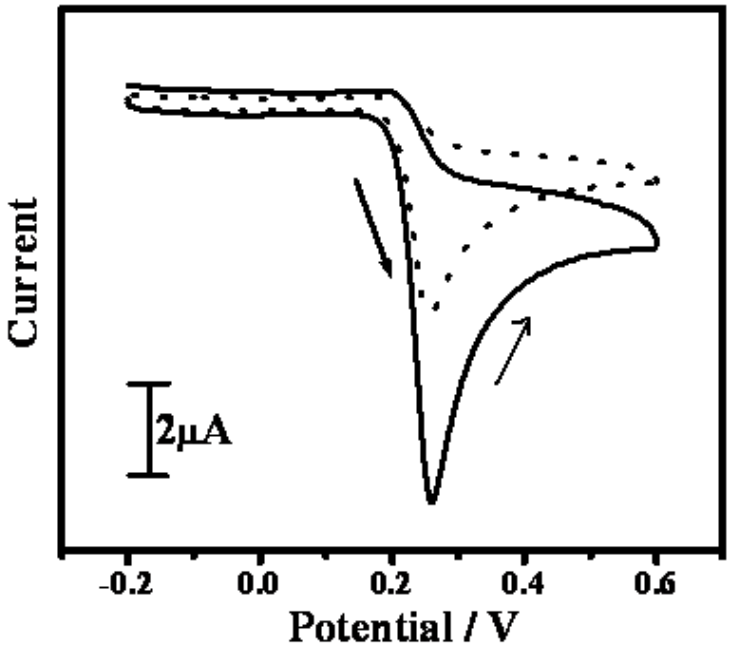


Fig. 6.8. Cyclic voltammograms of 1×10^{-4} M UA in 0.2 M PBS solution of pH 7.4 at BCPE (dashed line) and Graphene MCPE (solid line) at scan rate of 0.05 Vs^{-1}

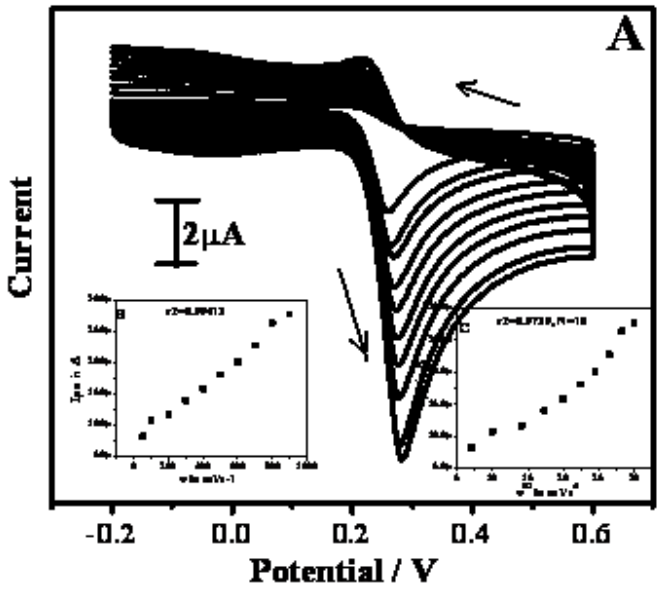


Fig. 6.9. (A) Cyclic voltammograms of 1×10^{-4} M UA in 0.2 M PBS solution of pH 7.4 at Graphene MCPE at different scan rate (a–h; 0.05–0.9 Vs^{-1}). (B) Graph of anodic peak current versus scan rate and (C) Graph of anodic peak current versus square root of scan rate

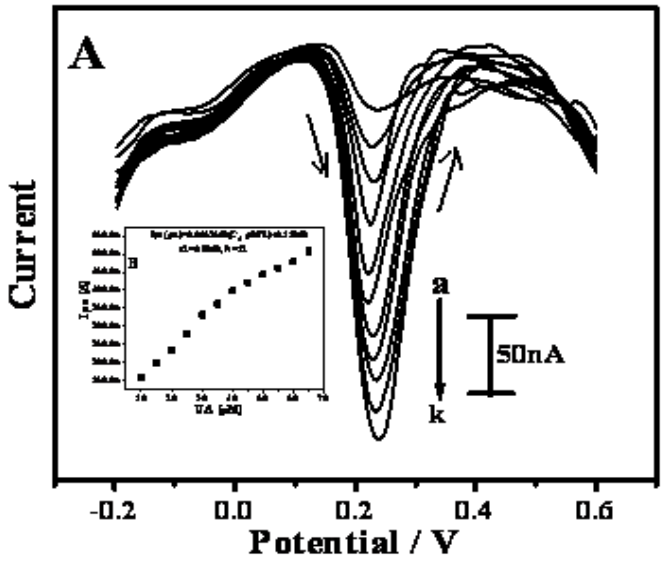


Fig. 6.10. (A) Cyclic voltammograms of UA in 0.2 M PBS solution of pH 7.4 at Graphene MCPE at scan rate of 0.05 Vs^{-1} with different concentration (a-k; 10, 15, 20, 25, 35, 45, 50, 55, 65 and 75 μM. (B) Graph of anodic peak current versus concentration of UA

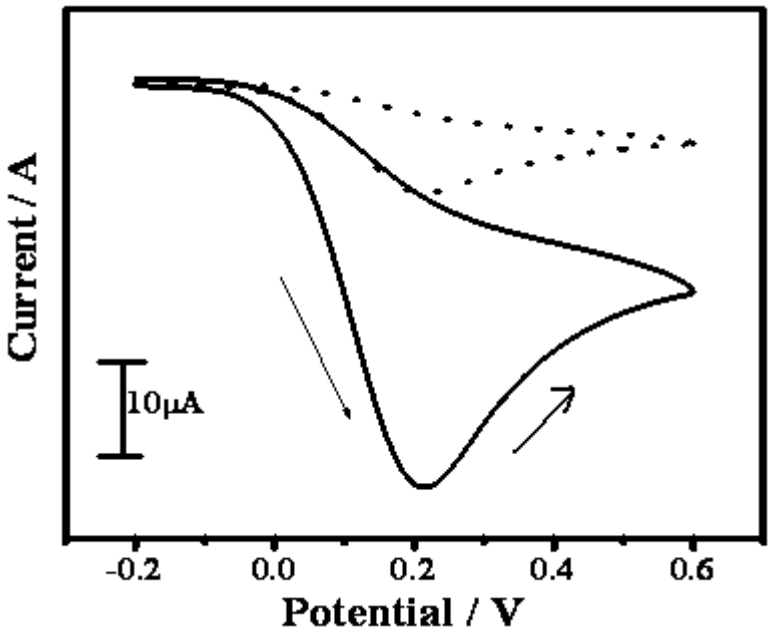


Fig. 6.11. Cyclic voltammograms of 1 mM AA in 0.2 M PBS solution of pH 7.4 at BCPE (dashed line) and Graphene MCPE (solid line) at scan rate of 0.05 Vs^{-1}

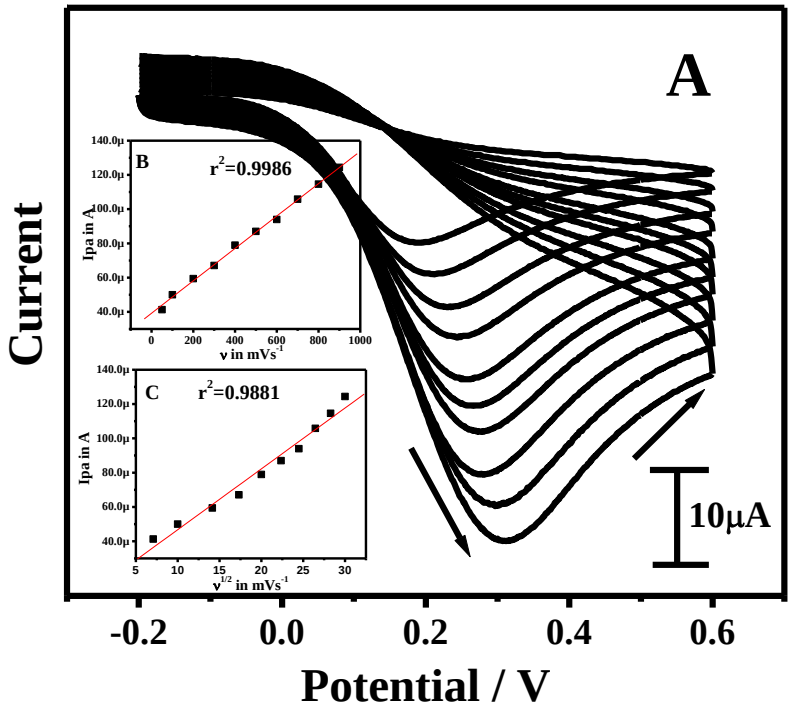


Fig. 6.12. (A) Cyclic voltammograms of 1 mM AA in 0.2 M PBS solution of pH 7.4 at Graphene MCPE at different scan rate (a–h; 0.05–0.9 Vs^{-1}). (B) Graph of anodic peak current versus scan rate. (C) Graph of anodic peak current versus square root of scan rate

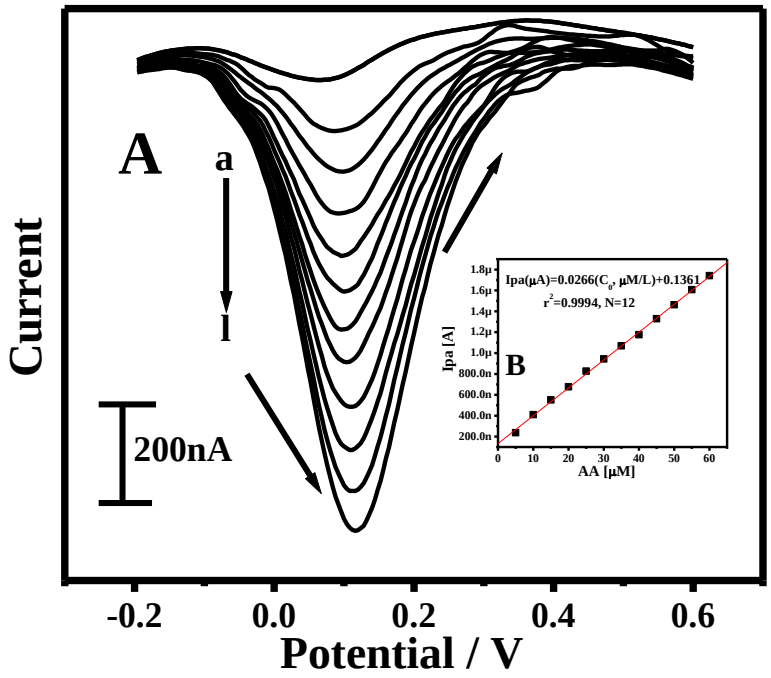


Fig. 6.13. (A) Cyclic voltammograms of AA in 0.2 M PBS solution of pH 7.4 at Graphene MCPE at scan rate of 0.05 Vs^{-1} with different concentration (a– l; 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 µM. (B) Graph of anodic peak current versus concentration of AA

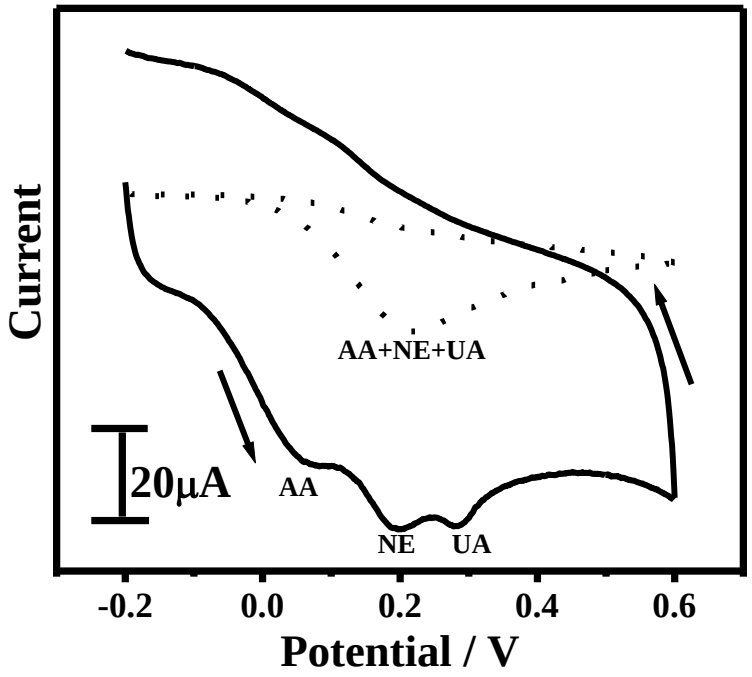


Fig. 6.14. Cyclic voltammograms for simultaneous determination of 0.9×10^{-4} M NE, 1.4×10^{-3} M AA, 1.2×10^{-3} M UA at BCPE (dashed line) and Graphene MCPE (solid line) at scan rate of 0.05 Vs^{-1}

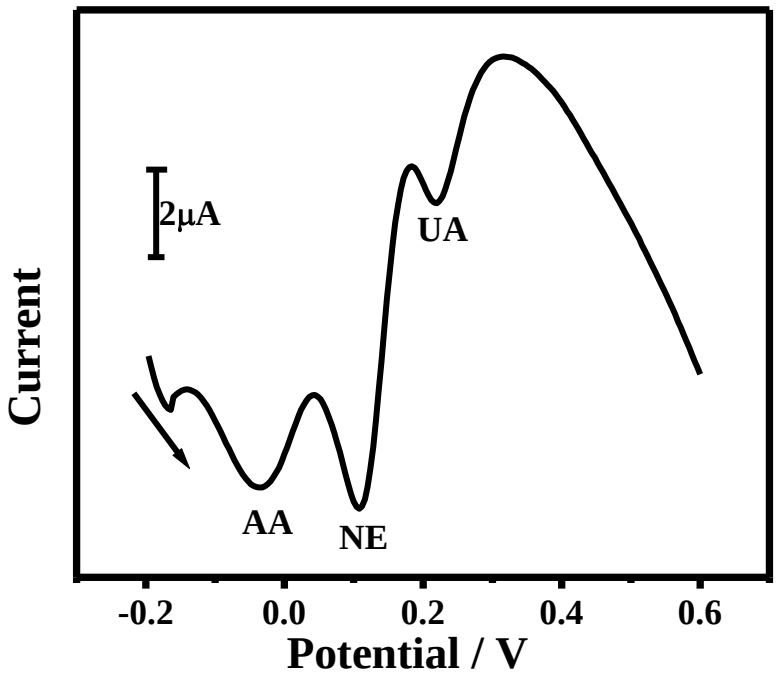


Fig. 6.15A. Differential pulse voltammogram obtained for 1×10^{-4} M NE, 2×10^{-3} M AA, 1.8×10^{-3} M UA in 0.2 M PBS solution of pH 7.4 at Graphene MCPE

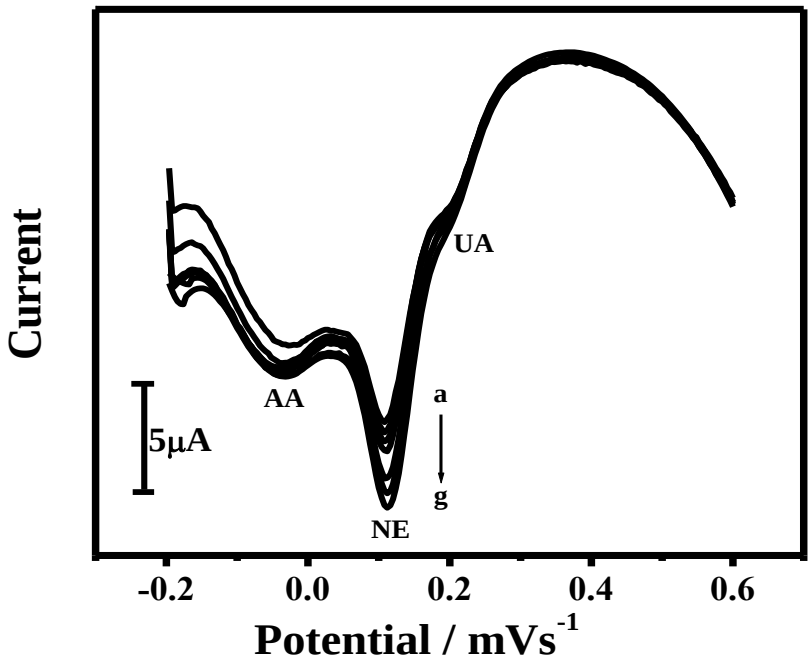


Fig. 6.15B. Differential pulse voltammograms of (a) 1.02×10^{-4} M NE (b) 1.025×10^{-4} M NE (c) 1.03×10^{-4} M NE (d) 1.035×10^{-4} M NE (e) 1.04×10^{-4} M NE (f) 1.045×10^{-4} M NE (g) 1.05×10^{-4} NE in 0.2 M PBS of pH 7.4 in presence of 2×10^{-3} M AA, 1.5×10^{-4} M UA at Graphene MCPE

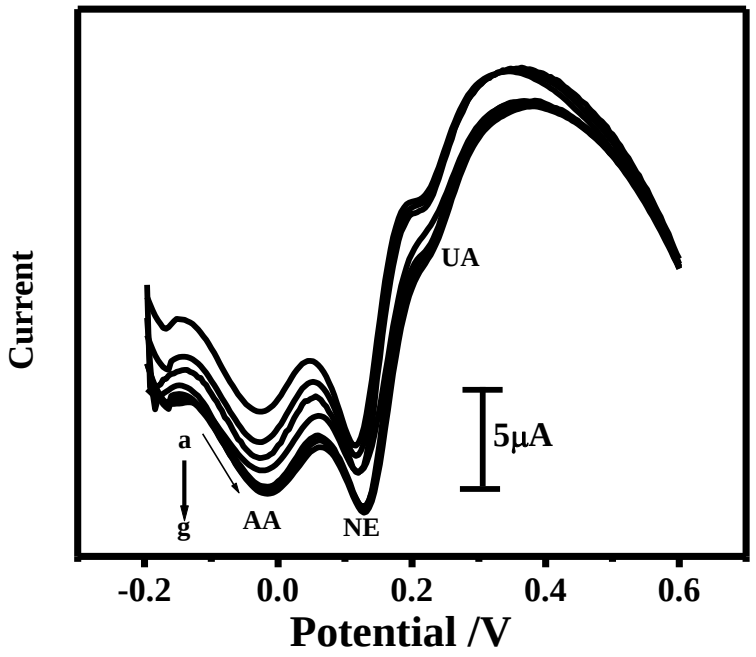


Fig. 6.15C. Differential pulse voltammograms of (a) 2mM+20.2μM (b) 2mM+ 20.4μM (c) 2mM+20.6μM (d) 2mM+20.8μM (e) 2mM+21μM (10^{-3} solution of AA) AA in 0.2 MPBS of pH 7.4 in presence of 1 $\times 10^{-4}$ M NE, 1.8$\times$ 10^{-4} M UA at Graphene MCPE

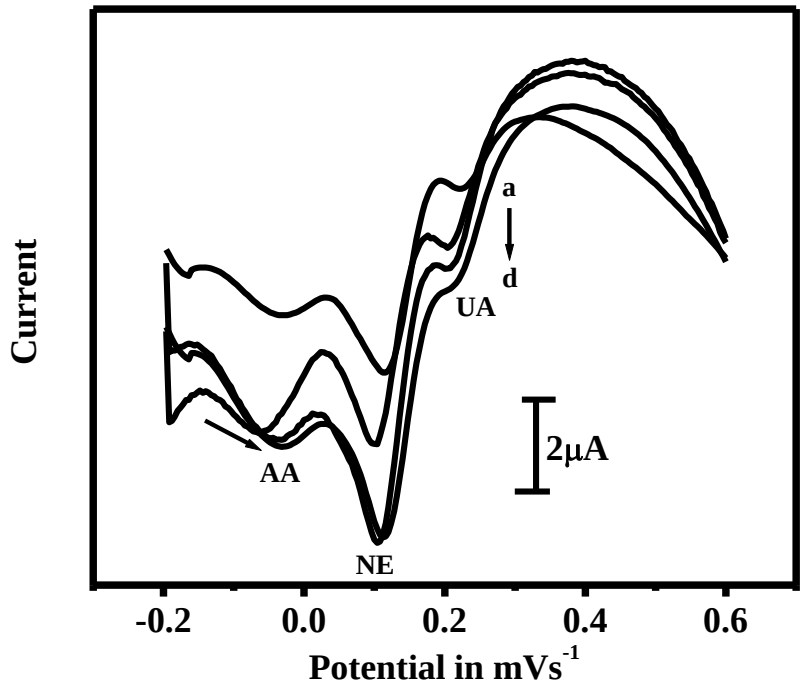


Fig. 6.15D. Differential pulse voltammograms of (a) 1.8×10^{-4} + 0.2μM (b) 1.8×10^{-4} + 0.4μM (c) 1.8×10^{-4} + 0.6μM (d) 1.8×10^{-4} + 0.8μM UA in 0.2 M PBS of pH 7.4 in presence of 2 $\times10^{-3}$ M AA, 1 $\times10^{-4}$ M NE at Graphene MCPE

6.10. References

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6.11. Introduction

The graphene was used for the modification of carbon paste electrode (CPE), and employed for the determination of folic acid (FA) and paracetamol (PA) by cyclic voltammetric (CV) and differential pulse voltammetric (DPV) techniques. The scan rate effect studies on graphene modified CPE (MCPE) for FA and PA was conducted and it shows the diffusion controlled process for both the analytes. The lower limit of detection for FA was 0.80µM in the linear range 0.5 to 5µM and 4.0µM for PA in the linearity range 40 to 100µM by CV technique. The simultaneous determination of FA and PA shows good selectivity and sensitivity. The graphene MCPE shows antifouling property, stability and reproducibility for the determination of FA and PA at physiological pH.

6.12. Chemistry, Biosynthesis and Biological Relevance of Folic Acid

The chemistry, biosynthesis and biological relevance of folic acid has been explained in details in chapter 3 sections 3.7.5, 3.7.6 and 3.7.7.

6.13. Chemistry, Biosynthesis and Biological Relevance of Paracetamol

The chemistry synthesis and biological relevance of Paracetamol has been explained in details in chapter 5 sections 5.4 and 5.5.

6.14. Review of Electrochemistry of Folic acid

Folic acid is a water soluble vitamin, initially identified as an anti-anemia and growth factor. It is produced by plants (green leaves, algae) and micro-organisms (bacteria, yeast). In mammals, folic acid and its derivatives, the folates, serve as acceptors and donors of carbon units and are involved in amino acid and nucleotide biosynthesis [1,2]. Folic acid also prevents neural tube defects such as spina bifida, while its ability to lower blood homocysteine concentration, suggests that it might have a positive influence on cardiovascular disease. A role for this B vitamin in maintaining good health may, in fact, extend beyond these clinical conditions to encompass several others disorders (birth defects, several types of cancer, dementia, affective disorders, Down's syndrome etc). Folate is the generic term to indicate a group of compounds naturally occurring in food

that have vitamin activity similar to folic acid, such as some polyglutamates. The terms folic acid and folates are often used interchangeably, but folic acid is approximately twice as bioavailable as the folates [3].

As result of its importance in biological systems, there is an increasing need for developing methods for the measurement of FA in pharmaceutical, clinical and food samples. There have been several reports on the determination of FA either alone or in combination with other drugs, including the use of enzyme linked immunosorbent assays (ELISAs) [4], chemiluminescnce [5,6] microemulsion electrokinetic chromatography [7], spectrophotometer after coupling reaction with specific compounds [8], forimetry [6,9], high-performance liquid chromatography with ultra-violet, diode-array or electrochemical detection [10-12], liquid chromatography, with tandem mass spectroscopy or with electrospray ionisation mass spectrometry [13,14] capillary electrophoresis [15], or biosensor based determination [16]. Most of the above mentioned method offers very useful information in terms of identification and quantification, excellent resolution and selectivity; however they are prone to many drawbacks, such as expensiveness , complicated and lengthy procedures. Electrochemical methods such as the voltammetric ones, offer certain advantages such as the simplicity, fast response and offering sensitivity and dynamic range comparable to other analytical methods. Various voltammetric techniques have been proposed for analysis of FA individually and simultaneously in combination with other compounds because the molecule is electroactive at several electrodes. Although electrochemical behavior of FA was studied at first on mercury electrodes [17-30].

6.15. Review of Electrochemistry of Paracetamol

Paracetamol is an acylated aromatic amide that was firstly introduced in medicine by Von Mering in 1893 as an antipyretic/analgesic; it has been in use as an analgesic for home medication for over 50 years. Moreover, it is accepted as a very effective drug for the relief of pain and fever in adults and children [31]. Because paracetamol is being increasingly used for therapeutic purposes, its determination and quality control are of vital importance [32]. Paracetamol (N-acetyl-p-aminophenol, acetaminophen) is a

long-established substance, being one of the most extensively employed drugs in the world. It is non-carcinogenic and an effective substitute for aspirin for patients with sensitivity to it [1-4]. Many methods for determining paracetamol have been recently reported, such as chromatographic [33-39], spectrofluorimetric [40–43], chemiluminescent [44,45], spectrophotometric[46-48], and electrochemical techniques [2,5,6,49-59]. Wangfuengkanagul and Chailapakul [60] studied the electrochemistry of paracetamol at a boron-doped diamond thin film electrode using cyclic voltammetry, hydrodynamic voltammetry, and flow injection with amperometric detection. The diamond electrode provided a linear dynamic range from 0.1mmolL^{-1} to 8.0mmolL^{-1} and a detection limit of 10.0µmolL^{-1} for voltammetric measurement. Shang Guan *et al*., [61] studied the electrochemical determination of paracetamol using differential pulse voltammetry (DPV) at a carbon ionic liquid electrode.

6.16. Experimental Part

6.16.1. Reagents and Chemicals

Disodium hydrogen phosphate monohydrate ($Na_2HPO_4H_2O$), sodium dihydrogen phosphate (NaH_2PO_4) and silicone oil were purchased from Himedia chemicals. The folic acid (FA), paracetamol (PA), graphite powder and NaOH are received from Merck chemicals. The stock solutions of 1×10^{-4}M FA and 1×10^{-4}M PA was prepared in 0.1 M NaOH and double distilled water respectively.

6.16.2. Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) was performed in a model CHI-660c (CH Instrument-660 electrochemical workstation). All experiments were carried out in a conventional electrochemical cell containing a bare carbon paste electrode (BCPE) or Graphene MCPE as a working electrode, the platinum wire as a counter electrode and saturated calomel electrode(SCE) as a reference electrodes.

6.16.3. Preparation of Bare Carbon Paste Electrode and Graphene MCPE

The BCPE was prepared by hand mixing of 70% graphite powder and 30% silicone oil in an agate mortar for about 45min until a homogeneous paste was obtained. The paste was then packed into a cavity of PVC tube of 3mm internal diameter and smoothened on a tissue paper. The electrical contact was provided by a copper wire connected to the end of the tube [9, 16]. The different quantity of graphene (2, 4, 6, 8 and 10 mg) was grinded along with 70% graphite powder and 30% silicone oil in an agate mortar for about 45min to get a homogeneous paste. The paste packing procedure was same as that BCPE.

6.17. Results and Discussion

6.17.1. Effect of Quantity of Graphene as a Modifier towards the Detection of PA

The effect of quantity of graphene in CPE was characterized by using CV technique. By increasing the amount of graphene from 2 mg to 6mg in the CPE the anodic peak current of 1mM PA in 0.2 M PBS of pH 7.4 as supporting electrolyte goes on increasing. Further increase of graphene in CPE decreased the current signal of FA. The graph of anodic peak current versus different quantity of graphene in CPE was plotted. Fig. 6.16 shows maximum anodic peak current signal at 5 mg graphene MCPE, therefore 5 mg graphene MCPE was chosen as optimum for the study of all other parameters.

6.17.2. Electrochemical Behavior of FA at Graphene MCPE

The electrochemical behavior of 1mM FA at BCPE and graphene MCPE in 0.2M PBS of pH 7.4 at scan rate of $0.05Vs^{-1}$ was studied by CV technique. The Fig. 6.17 shows at BCPE the oxidation of FA is irreversible and oxidation potential was located at 0.670 V (versus SCE). However, at graphene MCPE the oxidation potential was observed at 0.658 V with improved sensitivity. The increase in the peak current response is almost 5 folds for the graphene MCPE compared to BCPE. This result shows electrocatalytic activity of the modified electrode.

6.17.3. Effect of Scan Rate on the Peak Current of FA

The effect of scan rate on the electrochemical response of 1mM FA in 0.2M PBS of pH 7.4 was studied by CV technique at graphene MCPE. The Fig. 6.18A shows the CV curves record at different scan rates from 0.05 to 0.5 Vs^{-1}. As a result of increase in the scan rate the oxidation peak current increases gradually along with shifting of the oxidation peak potential towards positive side. Fig. 6.18B shows the graph of anodic peak current versus scan rate with a correlation coefficient of (r^2) 0.9997. Fig. 6.18C shows the graph of anodic peak current versus square root of scan rate with a correlation coefficient of (r^2) 0.9889, so electrode process is adsorption controlled [62-63].

6.17.4. Effect of FA Concentration Graphene MCPE

Cyclic voltammetric method was employed to study the effect of concentration of FA. The Fig. 6.19A shows the CV curves recorded for the varying concentration of FA in 0.2M PBS of pH 7.4 (0.5µM to 5µM) at the scan rate of 0.05Vs^{-1}. It was observed that the oxidation peak current increases gradually with increase in the concentration of FA. The Fig. 6.19B shows the graph of anodic peak current versus concentration of FA. The result shows good linearity with a linear regression equation I_{pa}(µA) = 3.91686 (C_oµM/L)+0.128143µM, (r^2=0.98021). The limit of detection (LOD) was calculated in the lower concentration range by using the equation is found to be 0.8015µM for FA by CV technique.

6.17.5. Electrochemical Behavior of PA at Graphene MCPE

Fig. 6.20 shows the CV curves recorded for the oxidation of 1mM PA in 0.2M PBS of pH 7.4 at BCPE (dashed line) and graphene MCPE (solid line) at the scan rate of 0.05 Vs^{-1}. Compared to BCPE the improved voltammetric response was observed at graphene MCPE with lower ΔE_p values. As ΔE_p is the function of electron transfer rate, graphene MCPE can facilitate the oxidation process of PA. This indicates the electrocatalytic activity of the fabricated electrode.

6.17.6 Effect of Scan Rate on the Peak Current of PA

The effect of scan rate on the electrochemical response of 1mM PA in 0.2M PBS of pH 7.4 was studied by CV technique at graphene MCPE. The Fig. 6.21A shows the CV curves record at different scan rates from 0.05 to 0.5 Vs^{-1}. As a result of increase in the scan rate the oxidation peak current increases gradually along with shifting of the oxidation peak potential towards positive side. Fig. 6.21B shows the graph of anodic peak current versus scan rate with a correlation coefficient of (r^2) 0.9901. Fig. 6.21C shows the graph of anodic peak current versus square root of scan rate with a correlation coefficient of (r^2) 0.99301. The graph of logarithm of anodic peak current versus logarithm scan rate was constructed as shown in Fig. 6.21D with a linear regression of log $I_{pa}(\mu A)$ = 3.8636+0.52859logν (V/s), and gives a slope of 0.5285 which is close to the theoretically predictable value of 0.5 for a diffusion controlled process.

6.17.7. Effect of PA Concentration Graphene MCPE

Cyclic voltammetric method was employed to study the effect of concentration of PA. The Fig. 6.22A shows the CV curves recorded for the varying concentration of PA in 0.2M PBS of pH 7.4 (0.5µM to 5µM) at the scan rate of 0.05Vs^{-1}. It was observed that the oxidation peak current increases gradually with increase in the concentration of PA. The Fig. 6.22B shows the graph of anodic peak current versus concentration of PA. The result shows good linearity with a linear regression equation $I_{pa}(\mu A)$=0.1387 ($C_o\mu M/L$)+0.22567µM, (r^2=0.9984). The limit of detection (LOD) was calculated in the lower concentration range by using the equation is found to be 4.0199µM for PA by CV technique.

6.17.8. Simultaneous Determination of FA and PA

The Fig. 6.23 shows the cyclic voltammetric response of 1 mM FA and 0.5 mM PA in 0.2 M PBS of pH 7.4 at BCPE (dotted line) and graphene MCPE (solid line). The

modified electrode shows relatively good sensitivity as compared to the BCPE. The sensitive and simultaneous separation was observed at graphene MCPE.

6.18 Conclusion

The bare carbon paste was modified by grinding different quantities of graphene. The modified graphene MCPE was used for the sensitive determination of FA and PA in physiological pH of 7.4 by CV technique. The simultaneous study was conducted for the binary mixture of FA and PA, the sensitive separation was observed at the modified electrode by CV technique. The proposed method can be employed for the some other biological important molecules.

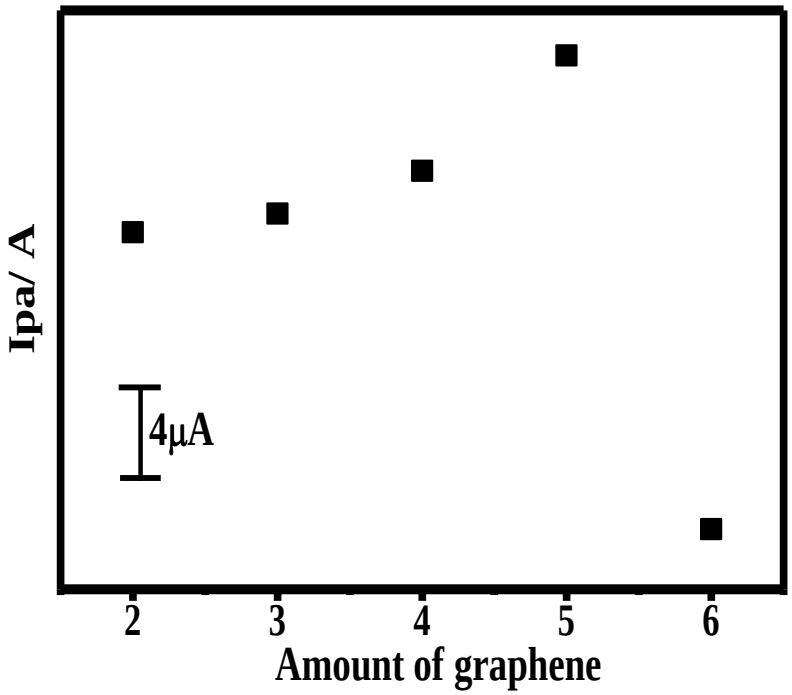

Fig. 6.16. Effect of quantity of graphene in the carbon paste electrode

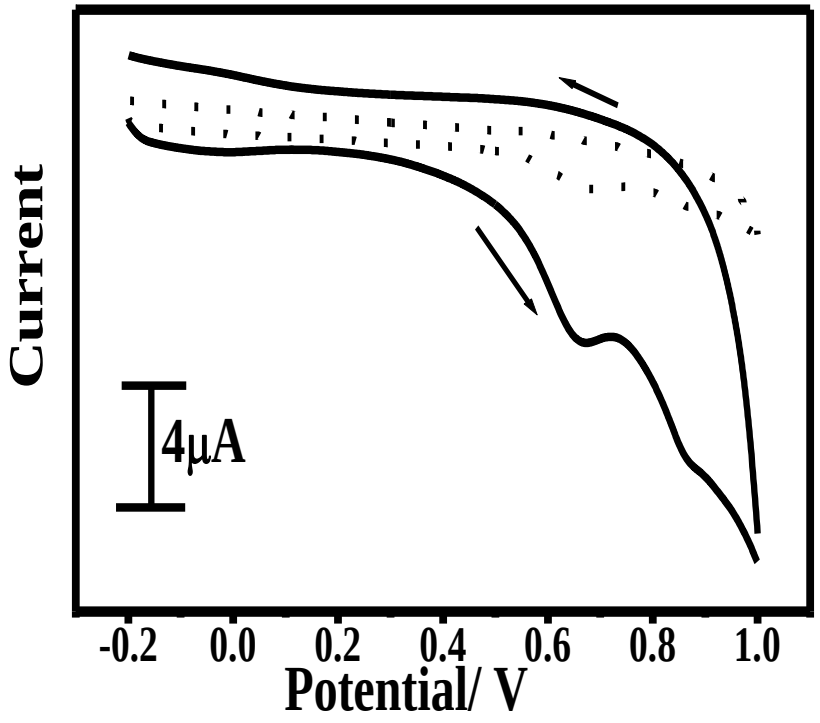

Fig. 6.17. Cyclic voltammograms of 0.1 mM folic acid at BCPE (dashed line) and Graphene MCPE (solid line) at scan rate of 0.05 Vs^{-1}

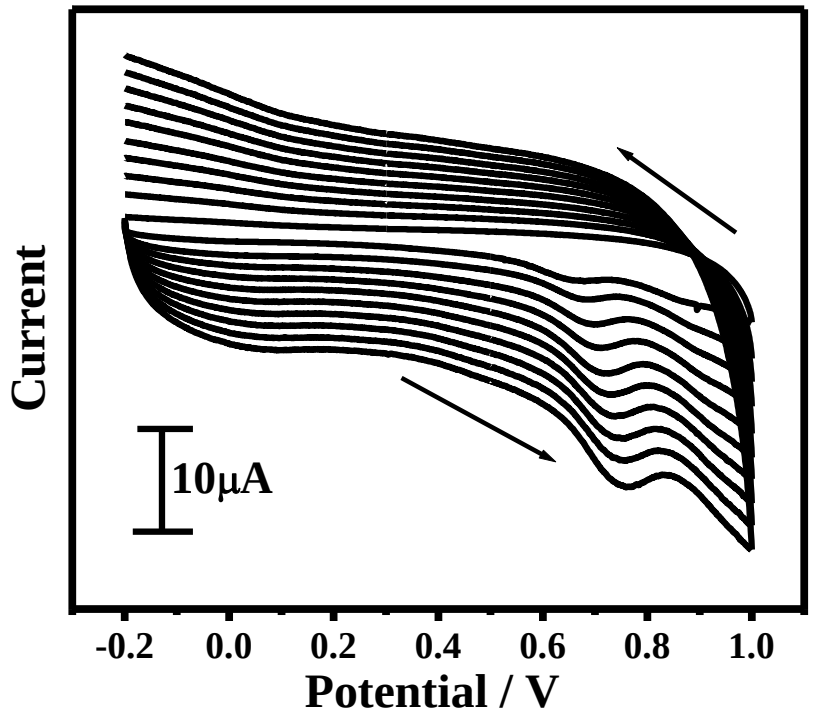

Fig. 6.18A. Cyclic voltammograms recorded for the oxidation of 0.1mM FA in 0.2M PBS of pH 7.4 at different scan rate (a:j; 0.05Vs^{-1}to 0.5Vs^{-1})

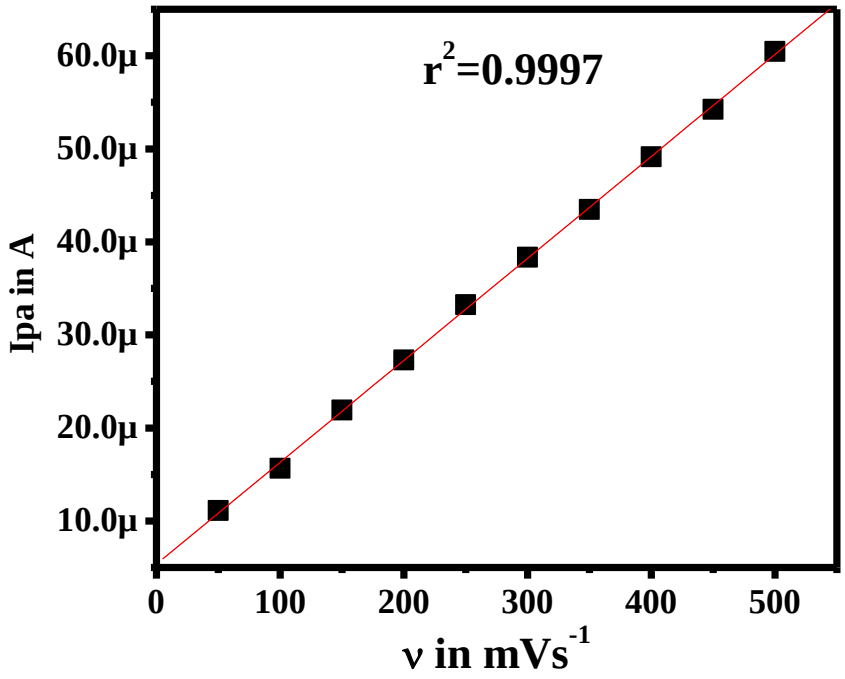

Fig. 6.18B. Graph of anodic peak current (I_{pa}) versus scan rate (ν)

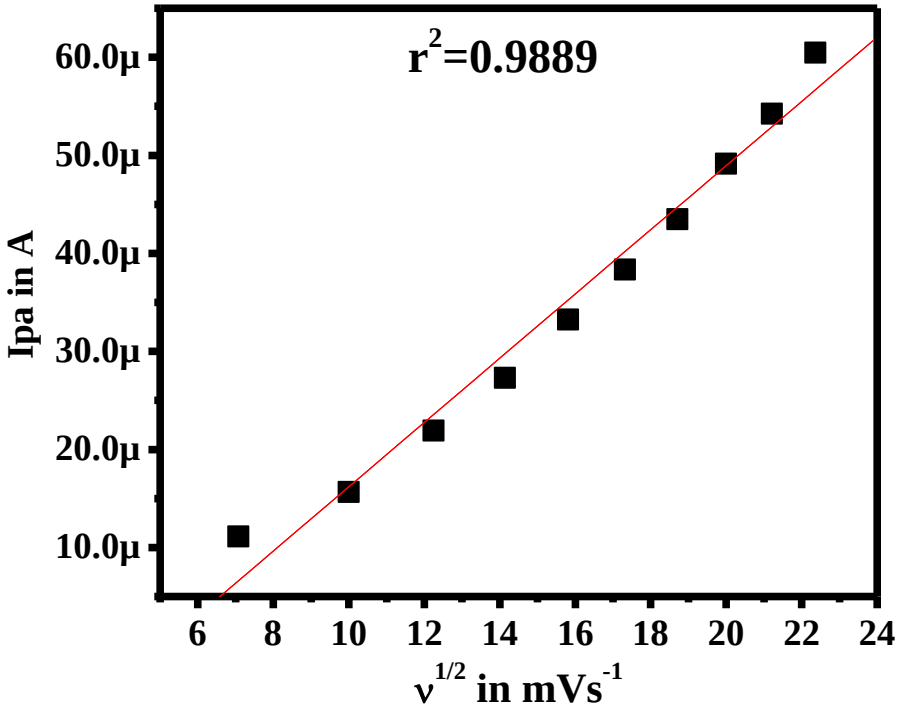

Fig. 6.18C. Graph of anodic peak current (I_{pa}) versus square root of scan rate ($\nu^{1/2}$)

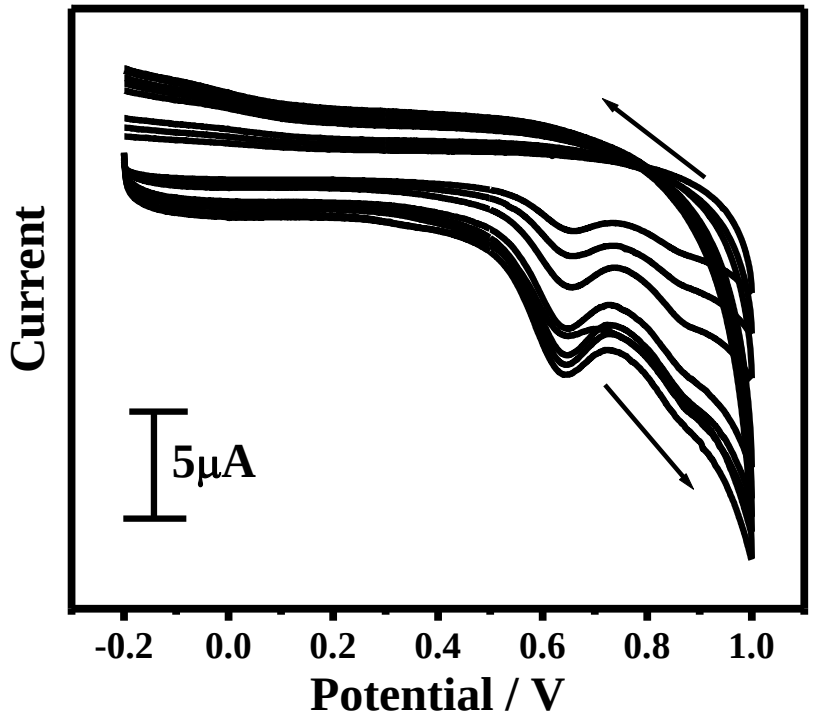

Fig. 6.19A. Cyclic voltammograms of FA with different concentration (a-h: 0.5 to16µM) in 0.2M PBS of pH 7.4 at Graphene MCPE at a scan rate of 0.05 Vs^{-1}

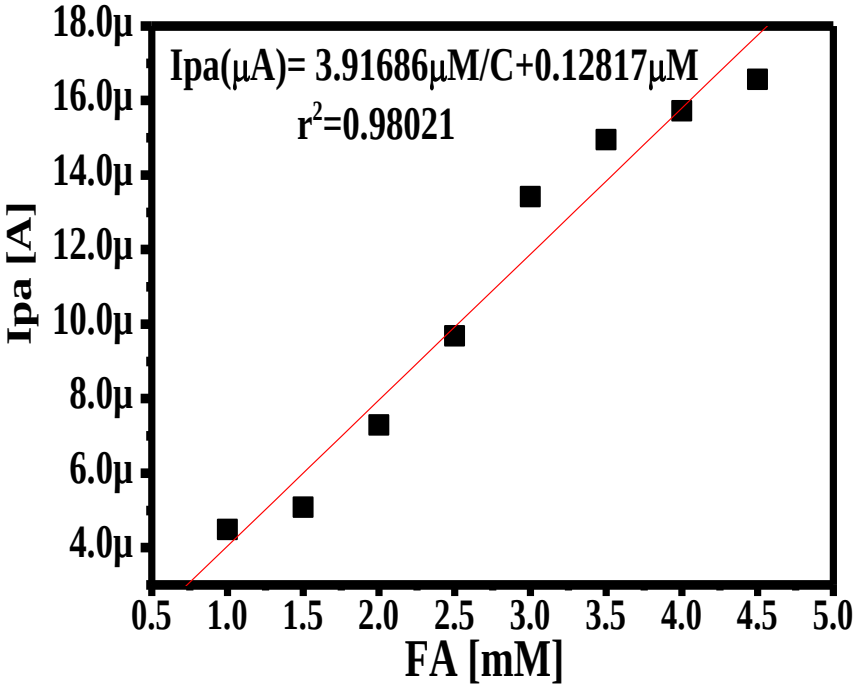

Fig. 6.19B. Graph of anodic peak current versus concentration of FA

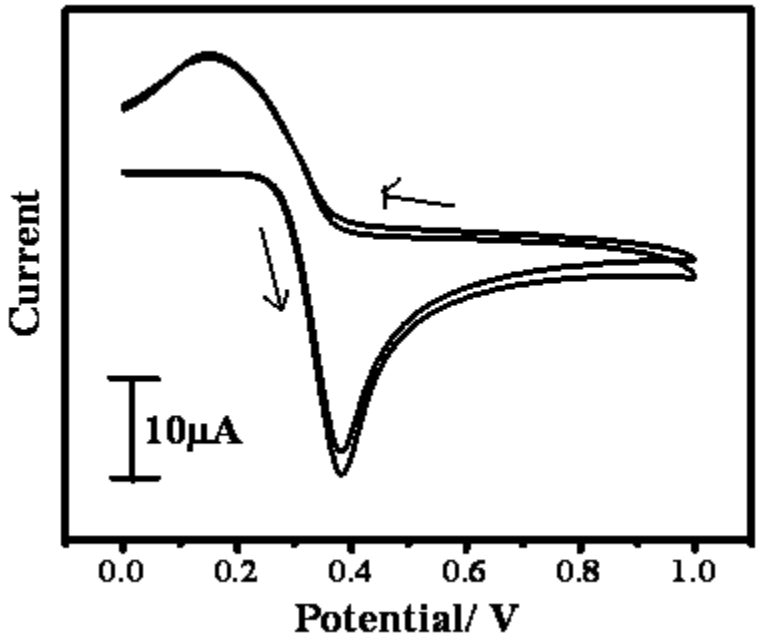

Fig. 6.20. Cyclic voltammograms of 0.1 mM paracetamol at BCPE (dashed line) and Graphene MCPE (solid line) at scan rate of 0.05 Vs^{-1}

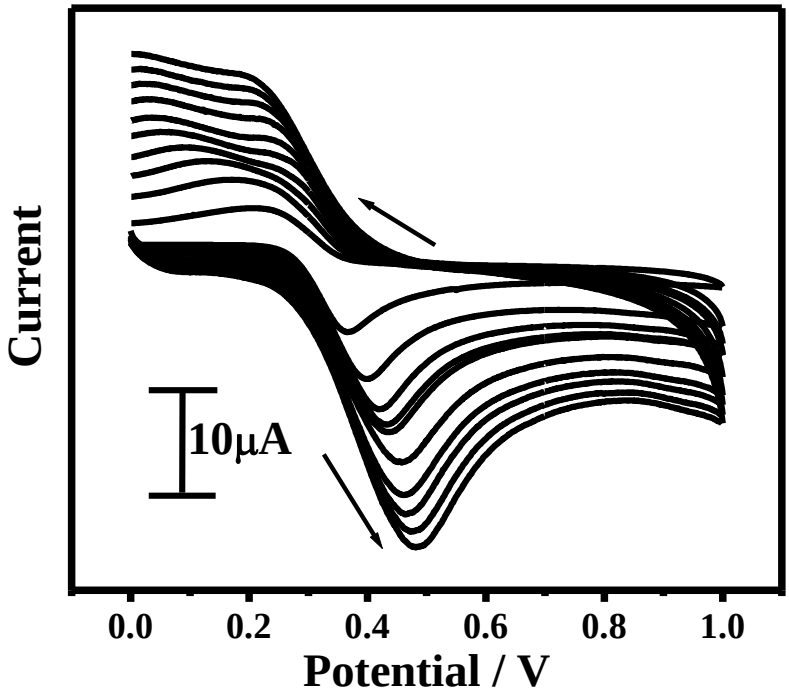

Fig. 6.21A. Cyclic voltammograms recorded for the oxidation of 0.1mM PA in 0.2M PBS of pH 7.4 at different scan rate (a:j; 0.05$Vs^{-1}$to 0.5 Vs^{-1})

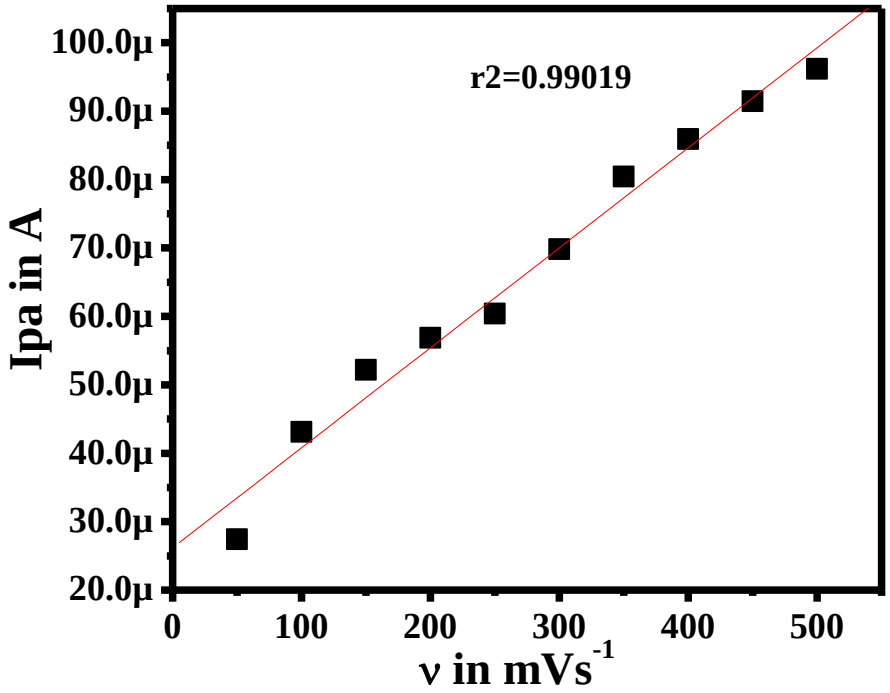

Fig. 6.21B. Graph of anodic peak current (I_{pa}) versus scan rate (ν)

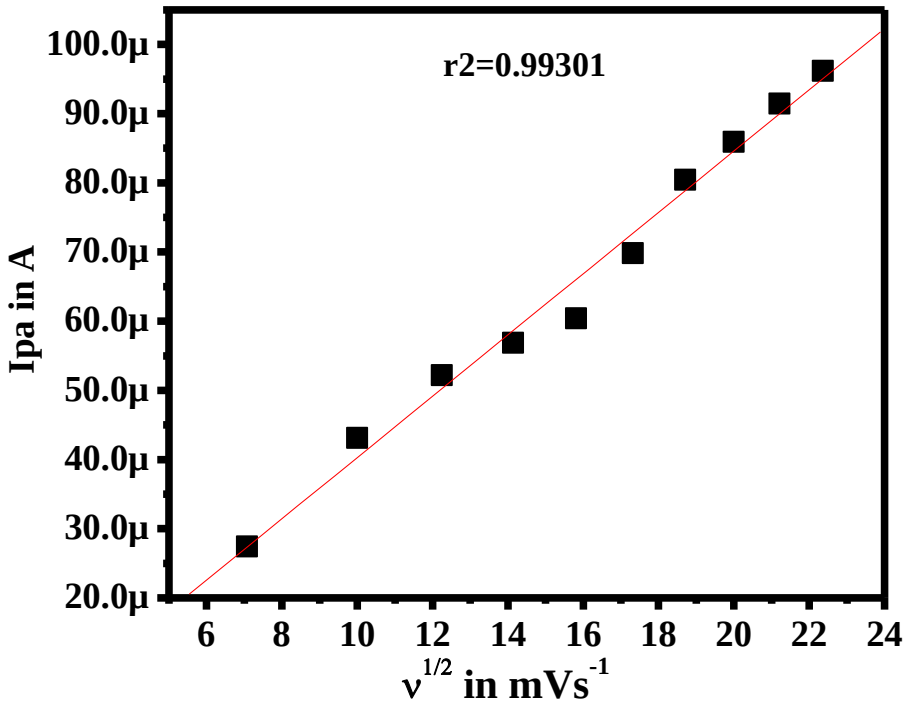

Fig. 6.21C. Graph of anodic peak current (I_{pa}) versus square root of scan rate ($\nu^{1/2}$)

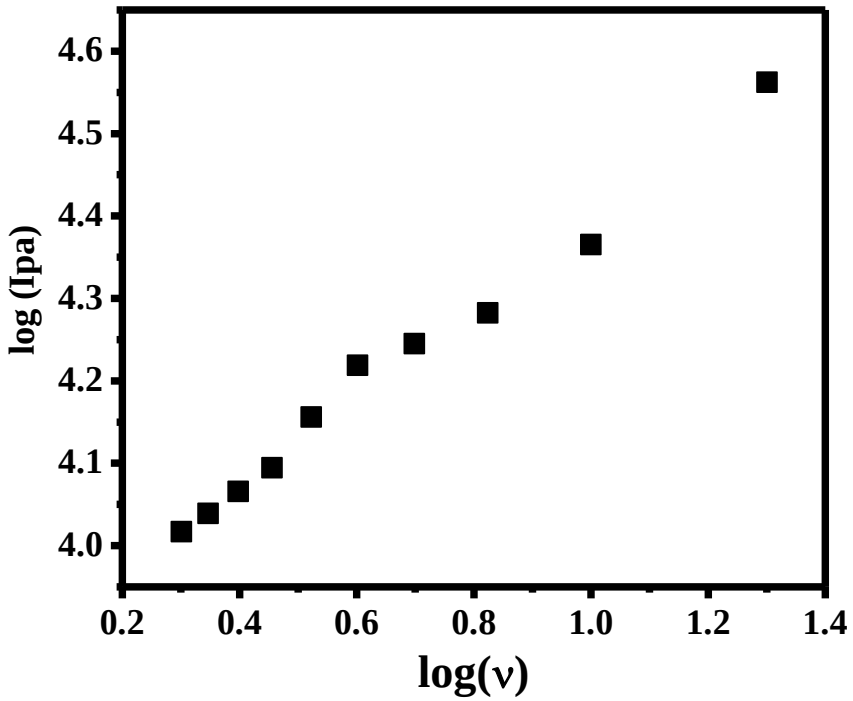

Fig. 6.21D. Graph of log(I_{pa}) versus log (ν)

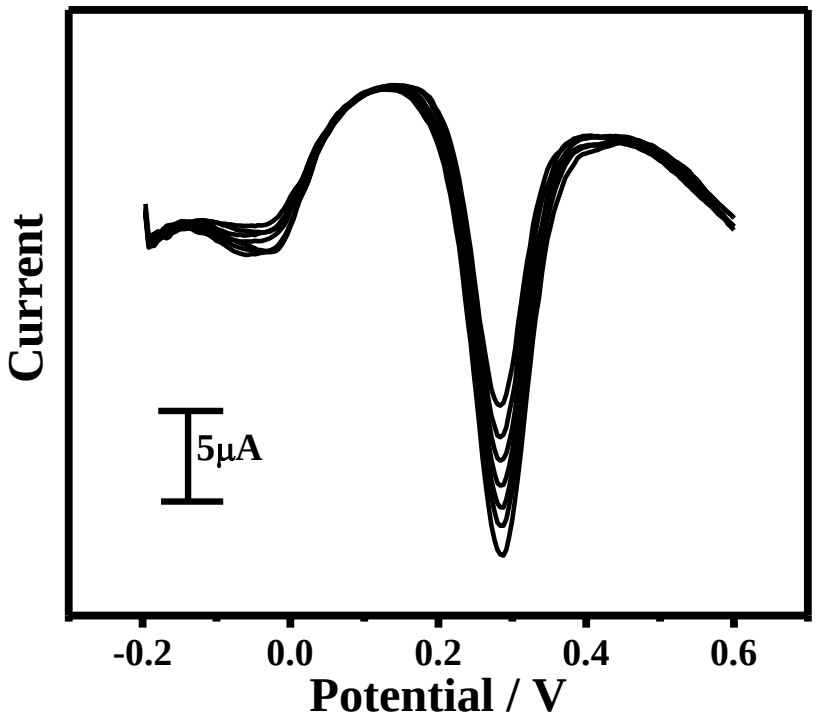

Fig. 6.22A. Differential pulse voltammograms of PA with different concentration (a-h: 40 to100µM) in 0.2M PBS of pH 7.4 at Graphene MCPE at a scan rate of 0.05 Vs^{-1}

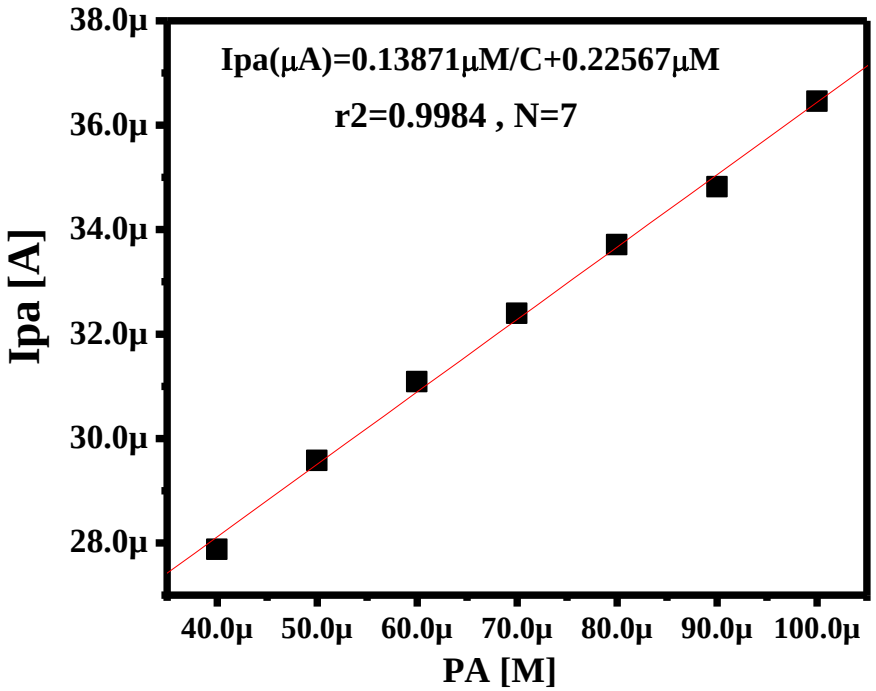

Fig. 6.22B. Graph of anodic peak current versus concentration of PA

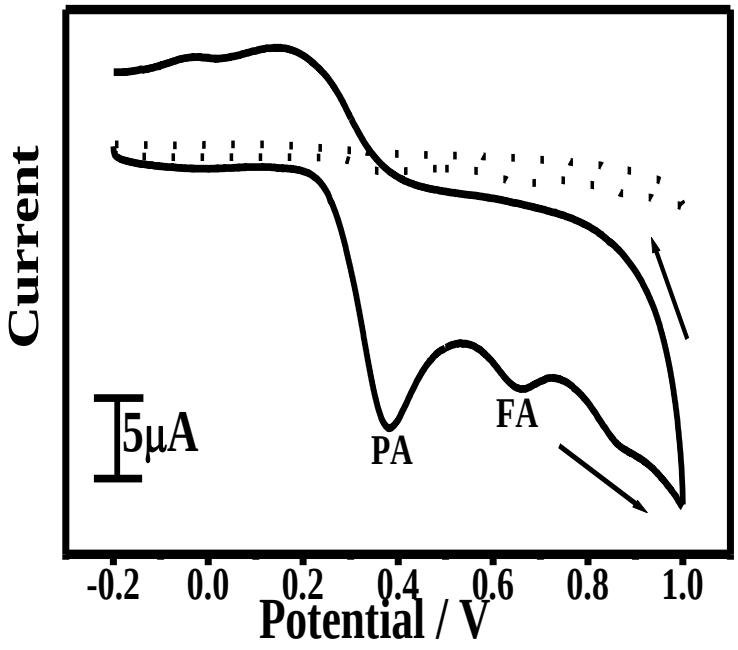

Fig. 6.23. Cyclic voltammograms for 0.2mM FA and 0.01mM PA in 0.2M PBS of pH 7.4 at a scan rate of $0.05Vs^{-1}$at BCPE (dotted line) and Graphene MCPE (solid line)

6.19. References

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7.1. Introduction

In the present chapter the electrochemical response of catechol and hydroquinone at carbon paste electrode in the presence of 0.2 M phosphate buffer solution as supporting electrolyte was investigated by the cyclic voltammetry. The discussion involves the chemistry, biological relevance of Catechol and hydroquinone and its oxidation behavior in 0.2 M phosphate buffer solution at the bare and poly(alcian blue) modified carbon paste electrode. It showed a well-defined oxidation peak and two sensitive and indiscernible reduction peaks at the bare carbon paste electrode. The effect of concentration and scan rate of catechol and hydroquinone was studied. The scan rate effect showed that the electrode process is adsorption controlled. The poly (alcian blue) modified CPE showed excellent electrocatalytic activity towards the oxidation of catechol and hydroquinone. The carbon paste electrode (CPE) was modified by electropolymerisation of alcian blue in 0.2 M phosphate buffer solution (PBS) of pH 7.4 by using cyclic voltammetric (CV) technique. The fabricated electrode was employed for the electrochemical analysis of catechol (CC) and hydroquinone (HQ). The effect of scan rate suggests the adsorption controlled electrode process. A good analytical performance was observed in terms of sensitivity, selectivity, linearity and observed detection limits. The lower limit of detection of CC and HQ was found to be 0.104μM and 0.142μMby Differential pulse voltammetric technique. Because of the catalytic capability of the fabricated electrode the simultaneous separation was observed in a binary mixture containing CC and HQ.

7.2. Chemistry of Catechol and Hydroquinone

Phenolic compounds are formed during the biological degradation processes; they are widely used as raw materials in the production of dyes, photostabilizer, plasticizers, cosmetics, pesticides and some pharmaceuticals [1, 2]. Catechol (CC) and hydroquinone (HQ) are two simple electroactive molecules, belongs to the class of dihydroxybenzene isomers. Since their wide spread applications in day to day life, they are the major cause for environmental pollutions [3, 4]. HQ and CC are highly toxic and even at very low concentrations can be harmful to animals and plants [5]. Therefore, sensitive and

selective analytical methods are necessary for their determination. Several methods are proposed [6-10] including chromatography [11, 12], spectrophotometry [13], pH based flow injection analysis [14], synchronous fluorescence [15], and voltammetry [16-21]. Among the various fabrication methodologies in getting the new chemically modified electrodes, electropolymerisation is a conveniently simple and powerful method by easy modification of different types of electrodes with desired matrices. The easy synthesis and deposition of desired electroactive polymers onto the conductive surface from monomer solutions and the formation rate and thickness can be precisely controlled by electrochemical input parameters. These electroactive polymers have useful properties such as electronic conductivity and ionic conductivity [3, 22-25]. Most of all the bare carbon paste electrodes suffer from a fouling effect due to surface accumulation of the oxidized products, and because of the similar structure and properties the oxidation of dihydroxy benzene isomers at bare electrodes was undistinguishable. Swamy *et al.* developed an electrochemical sensor for the electroanalysis of CC and HQ by electropolymerising brilliant blue on the surface of carbon paste electrode (CPE) [26]. J. He *et al*., reported the modification of the glassy carbon electrode by electropolymerised film of eosin Y and used for the voltammetric separation of CC and HQ [27]. Wang *et al*., studied the application of simple amino acids in the fabrication of a new modified glassy carbon electrode for the qualitative determination of CC and HQ in a binary mixture [3, 22, 23].

Alcian Blue is a phthalocyanine dye contains copper metal and used in the cationic form. The alcian blue has many positive charges on the molecule and it is thought to work by forming reversible electrostatic bonds between the cationic dye and the negative sites on the polysaccharide. Alcian Blue is a group of polyvalent basic water soluble compound which imparts blue color in the solution form due to the presence of copper ion in the molecule [28]. We proposed a modification of CPE by weight ratio method using alcian blue as a modifier for the determination of dopamine [29]. The present work gives a detailed work for the modification of carbon paste electrode by electropolymerising alcian blue followed by its characterization for the voltammetric determination of catechol and hydroquinone by cyclic voltammetric and differential pulse

voltammetric techniques. The fabricated poly(alcian blue) modified carbon paste electrode shows catalytic capability in the oxidation of both CC and HQ. The sensitivity, selectivity, stability and reproducibility in the result were obtained at the fabricated electrode. The structure of alcian blue was shown in the Scheme 1.

7.3. Experimental Part

7.3.1. Reagents and Apparatus

Hydroquinone (HQ), catechol (CC) and alcian blue were purchased from Himedia. The stock solutions of 25×10^{-4} M HQ, 25×10^{-4} M CC and 25×10^{-3} M alcian blue was prepared in double distilled water. Phosphate buffer solution (PBS) of same ionic strength was maintained (0.2 M) and the desired pH was obtained by mixing appropriate ratio of $NaH_2PO_4 \cdot H_2O$ and Na_2HPO_4. Graphite powder of 50 μM particle size was purchased from Merck and silicone oil from Himedia was used to prepare the carbon paste electrode (CPE). All the chemicals are of analytical grade used as received without any further purification.

All electrochemical experiments were performed using a modelCHI-660c (CH Instrument-660 electrochemical work station). A conventional three electrode system was used in a electrochemical cell with a saturated calomel electrode (SCE) as a reference, a platinum wire counter electrode and bare carbon paste electrode (BCPE)or poly(alcian blue) modified carbon paste electrode as a working electrode. Oxidation potentials of all the analytes were recorded versus SCE at an ambient temperature of 25±0.5°C.

7.3.2. Preparation of the Bare Carbon Paste Electrode

The BCPE was prepared by homogeneously mixing graphite powder and silicone oil in a ratio of 70:30% for about 45 minutes. The paste was then packed into a PVC tube with homemade cavity of 3 mm internal diameter and the surface was smoothened on a weighing paper. Unless otherwise stated, the paste was carefully removed prior to pressing anew portion into the electrode after every measurement. The electrical contact was provided by a copper wire connected to the end of the tube.

7.4. Results and Discussion

7.4.1. Electropolymerisation of Alcian Blue on BCPE

The poly(alcian blue) MCPE was fabricated by cyclic voltammetric technique by potential sweeping the bare carbon paste electrode between the potential window of -0.8 V to + 1.8 V with scan rate 0.1 Vs^{-1} for 5 multiple cycles in 0.2 M PBS of pH 7.4 until a stable cyclic voltammogram was observed. During this process of recording the multiple cycles the voltammogram was slowly increased with increasing in cyclic times as shown in Fig. 7.1A and later becomes constant [30,31]. This indicates that a thin polymer layer of alcian blue was formed and deposited on BCPE. As the extent of thickness can vary the electrocatalytic performance of the fabricated electrode, it was subjected for the oxidation of 0.2 mM CC in 0.2 M PBS of pH 7.4 with the scan rate of 0.05 Vs^{-1} as shown in the Fig. 7.1B. A better catalytic performance was observed for the electrode which was modified by five consecutive cycle scans. Therefore, the poly(alcian blue) MCPE was fabricated by considering all the above mentioned parameters.

7.4.2. Characterization of BCPE and poly(Alcian Blue) MCPE

The convenient redox probe of $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ was used for the electrochemical characterization of the fabricated electrodes. The freshly prepared stock solutions of 1 mM potassium ferrocyanide in 1M KCl as a supporting electrolyte were placed in an electrochemical cell. Fig. 7.2 shows the cyclic voltammograms obtained for the redox behavior of $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ at both BCPE (solid line) and Poly(alcian blue) MCPE (dotted line) at the scan rate 0.05 Vs^{-1}. A poor catalytic performance was observed at BCPE with low redox current response. On the other hand, poly(alcian blue) MCPE has shown the capability of enhancing the redox peak currents with improved electrocatalytic effect. The electrochemical results obtained at poly(alcian blue) MCPE suggests that the surface property was significantly changed. This remarkable change in the surface of the poly(alcian blue) MCPE is a platform for the analysis of the targeted

molecule. The total active surface area available for reaction of species in solution can be estimated by the Randles-Sevcik equation (1) [26, 31].

$$I_p = 2.69\times 105\ n^{3/2}\ A\ D.\ C^0\ \upsilon^{1/2} \qquad \text{----------} (1)$$

where, I_p is the peak current in A. C^0 is the concentration of the electroactive species (mol cm^{-3}), n is the number of electron exchanged, D is the diffusion coefficient in $cm^2\ s^{-1}$, is the scan rate (Vs^{-1}) and A is the electroactive surface area (cm^2). As calculated, the electroactive surface is maximum for poly(alcian blue) MCPE (0.0358 cm^2) as compared with BCPE (0.0279 cm^2). Scanning electron microscopy (SEM) images of BCPE and Poly(alcian blue) MCPE are shown in the Fig. 7.3, which reveals that the surface of BCPE is of irregular shape (A) with less available surface area. After the electropolymerisation the electrode surface is covered by a thin polymer layer of alcian blue (B), with uniform aligned surface. This morphological future is entirely different from BCPE.

7.4.3. Electrochemical Response of CC at Poly(Alcian Blue) MCPE

The Fig. 7.4 shows the cyclic voltammograms recorded for the oxidation of 0.1 mM CC in 0.2 M PBS of pH 7.4 with the scan rate of 0.05 Vs^{-1} at BCPE and poly(alcian blue) MCPE. The CC shows quasi-reversible behaviour at BCPE with a broad voltammetric response and the oxidation peak potential was located at around 0.230 V versus SCE. On the other hand, an improved current response was obtained at poly(alcian blue) MCPE (solid line) with a slight shifting in the anodic peak potential towards the positive side comparing to BCPE. The anodic peak potential was observed at 0.176 V versus SCE. This refinement in the voltammetric response shows the catalytic capability of fabricated poly(alcian blue) MCPE towards the oxidation of CC. The oxidation mechanism of CC and HQ was shown in Scheme 2.

7.4.4. Effect of Scan Rate on Peak Current of CC

The effect of variation of applied scan rate for 0.1 mM CC in 0.2 M PBS of pH 7.4 was examined by cyclic voltammetric technique at poly(alcian blue)MCPE as shown in Fig. 7.6A. The experimental results obtained at poly(alcian blue) MCPE suggests the peak current and scan rate are proportional to each. It can be observed that, there is a slight positive shift of anodic peak potential (E_{pa}) and negative shift in the cathodic peak potential (E_{pc}). In order to evaluate the electrode phenomenon, the graph of peak current (Ip) versus scan rate (ν) was plotted and the obtained graph is a straight line with good linearity in the range from 0.01 to 0.14 Vs^{-1} as shown in Fig. 7.6B with the correlation coefficient of (r^2) 0.9973 and 0.9992. On other hand, the I_p versus square roots can rate ($\nu^{1/2}$) was plotted as shown in Fig. 7.6C with the correlation coefficient (r^2) 0.9911 and 0.9949. This suggests the electrode phenomenon was an adsorption-controlled [32]. The heterogeneous rate constant (k^0) values was determined from the experimental peak potential difference (ΔE_p) data's, Eq. (2) was used for the voltammograms whose ΔE_p values are greater than 10 mV [26, 31].

$$\Delta E_p = 201.39 \log (\nu/k^0)-301.78$$

From the experimental ΔE_p values and corresponding k^0 values for the oxidation of catechol and hydroquinone was tabulated in Table 7.1 (Fig. 7.4 and Table 7.1).

7.4.5. Effect of CC Concentration

Differential pulse voltammetry (DPV) shows better defined peaks at lower concentrations than those obtained by cyclic voltammetry. The variation of concentration of CC at poly(alcian blue) MCPE was conducted by DPV technique. The Fig. 7.6A shows by increasing the concentration of CC from 10 to 60 μM the I_{pa} and I_{pc} goes on increasing with a small shifting in the peak potentials. The graph of I_{pa} versus concentration of CC was plotted as shown in the Fig. 7.6B and it shows a straight line with good linearity. The linear regression equation is I_{pa} (μA) = 0.1172(C^0μM/L)+0.4275,

(r^2=0.9872). The limit of detection was calculated as previously reported [24, 26]. The poly(alcian blue) MCPE shows a limit of detection of 0.104μM for CC by DPV technique, which is relatively lower compared to other reported as shown in Table 7.2 [8,33-39].

7.4.6. Influence of Solution pH on the Determination of CC at Poly(Alcian Blue) MCPE

The pH of the phosphate buffer solution plays a key role in the oxidation of the electroactive molecules, and it was evaluated at poly(alcian blue) MCPE by cyclic voltammetric technique. The effect of phosphate buffer pH value at poly(alcian blue) MCPE for the determination of CC was evaluated in a pH range of 5.5-8.0. Fig. 7.7A shows cyclic voltammograms recorded for 0.1 mM CC at poly(alcian blue) MCPE. It is clear that as the pH increases the oxidation peak potential shifts to a negative potential. The linear relationship was observed between anodic peak potential (E_{pa}) versus pH with a slope of 0.0588 V/pH as shown in inset Fig. 7.7B. The result suggests the involvement of equal number of electrons followed by equal number of protons in the redox mechanism [24, 26].

7.4.7. Electrocatalytic Oxidation of HQ at poly(alcian blue) MCPE

The Fig. 7.8 shows the oxidation of 0.1 mM HQ at BCPE (dotted line) and poly(alcian blue) MCPE (solid line) in 0.2 M PBS of pH 7.4 at the scan rate of 0.05 Vs^{-1}. From the figure it is observed that the oxidation potential of HQ at BCPE was broad and poor in sensitivity, the anodic peak potential was located at around 0.126 V. However, at poly(alcian blue) MCPE the oxidation peak potential was shifted towards negative side by minimizing the over potential with enhancement in redox peak current, the anodic peak potential was situated at -0.0585 V. Therefore, by this it came to know that the fabricated poly(alcian blue) MCPE can catalyze the favorable oxidation process of HQ. The effect of applied potential scan rate for 0.1 mM HQ in 0.2 M PBS of pH 7.4 was examined by CV technique at poly(alcian blue) MCPE as shown in Fig. 7.9A. The

applied scan rate was proportional to the redox peak current, with a small shift in the anodic peak potential (E_{pa}) towards more positive side and cathodic peak potential (E_{pc}) to the less negative side. The graph I_p versus ν and I_p versus $\nu^{1/2}$ was plotted in the range from 0.01 to 0.14 Vs^{-1} as shown in Fig. 7.9B and 7.9C. As the obtained correlation coefficient is more linear with I_p versus ν, suggesting electrode phenomenon was an adsorption-controlled [40].The electrocatalytic oxidation of HQ was conducted by varying its concentration in the range 10 to 60μM at Poly(alcian blue) MCPE as shown in the Fig. 7.10A. A linear relationship was established between the I_{pa} and concentration of HQ as shown in the Fig. 7.10B, with a linear regression equation of $I_{pa}(\mu A) = 0.3563 (C_0\mu M/L)+0.55407$, ($r^2=0.9753$). The limit of detection for HQ at poly(alcian blue) MCPE was calculated in the lower concentration range was found to be 0.142 μM by DPV technique. The effect of phosphate buffer pH value on the cyclic voltammetric determination of 0.1 mM HQ at poly(alcian blue) MCPE was shown in the Fig. 7.11A. The oxidation peak potential shifts to a more negative potential with increasing pH. The E_{pa} versus pH (Fig. 7.11B) graph clearly indicated the depends of E_{pa} with pH in the range of 5.5–8.0 with a slope of 0.0634V/pH ($r^2=0.9939$). This was consistent with the reported literature [24, 26].

7.4.8. Simultaneous Determination of CC and HQ

The cyclic voltammetry was employed for the simultaneous electrochemical determination of CC and HQ. The Fig. 7.12 shows the cyclic voltammograms obtained for the equimolar (0.1 mM) mixture of CC and HQ in 0.2 M PBS of pH 7.4 at the scan rate of 0.05 Vs^{-1}. The dotted line shows cyclic voltammogram obtained at BCPE, on the other hand the solid line for poly(alcian blue)MCPE and the oxidation potential of CC and HQ were observed at the same potential as determined individually, this voltammetric resolution of CC and HQ at poly(alcian blue) MCPE has great significance in the simultaneous analysis. Differential pulse voltammetry has some advantages like higher current sensitivity and absence of background current, The Fig. 7.13 shows the DPV curves recorded for the equimolar mixture of CC and HQ at BCPE and poly(alcian

blue) MCPE. Therefore, the discrimination of oxidation potentials of CC and HQ was achieved at poly(alcian blue) MCPE (Fig. 7.13A and 7.13B).

7.5. Conclusion

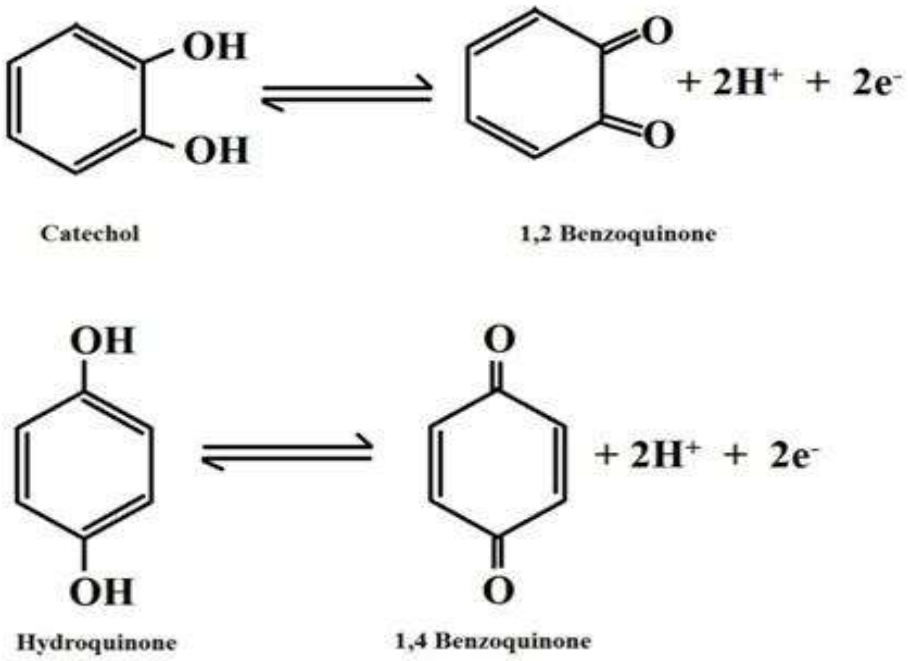
A simple and convenient method for the modification of bare carbon paste electrode was proposed by the electropolymerisation of alcian blue followed by its electrochemical and scanning electron microscopic characterization. The strong electrocatalysis in the oxidation of catechol and hydroquinone has made the fabricated electrode as a promising analytical sensor in the simultaneous analysis of both the isomers by cyclic voltammetric and differential pulse voltammetric techniques. Overall, the sensitivity, selectivity, antifouling property, reproducibility, stability was shown by the electrode.

Table 7.1. Voltammetric parameters gathered from Figures 7.6 and 7.10

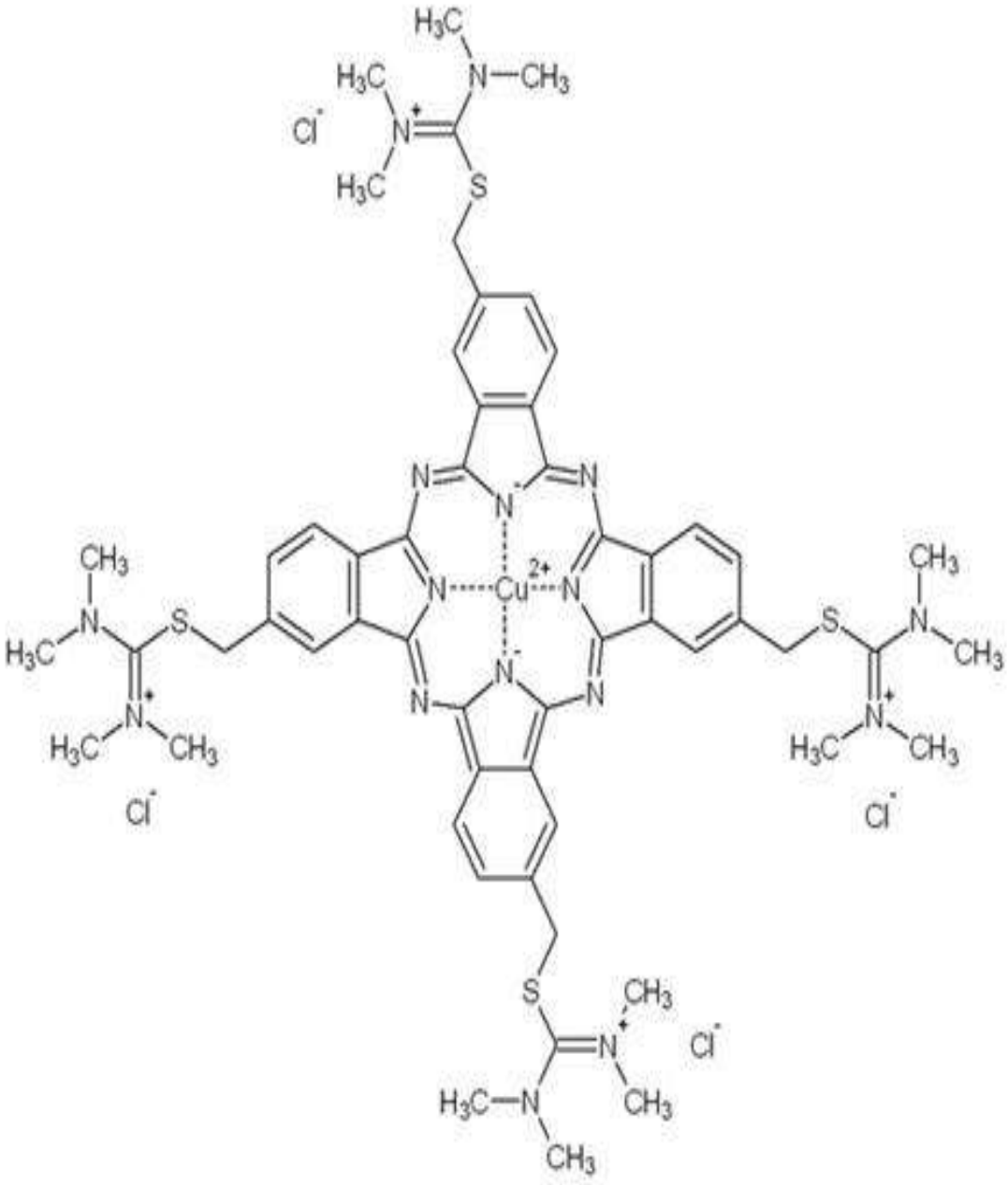
υ/ mVs^{-1}	ΔEp/mV		k^o/s	
	Catechol (CC)	**Hydroquinone (HQ)**	**Catechol (CC)**	**Hydroquinone (HQ)**
10	0.0388	0.0403	0.3172	0.3280
20	0.0393	0.0425	0.6344	0.6212
30	0.0425	0.0445	0.9515	0.9008
40	0.0434	0.0458	1.2680	1.2242
50	0.0426	0.0462	1.5852	1.4828
60	0.0398	0.0466	1.9028	2.0012
70	0.0424	0.0468	2.2202	2.1098
80	0.0412	0.0462	2.5368	2.4880
90	0.0422	0.0458	2.8516	2.7102
100	0.0430	0.0478	3.1717	3.1882
110	0.0436	0.0460	3.8053	3.6022
120	0.0446	0.0468	4.4401	4.2202

Table 7.2. Comparison table of limit of detection obtained at poly(alcian blue) MCPE with other electrodes

Working electrode	Limit of detection in µM		Technique	Reference
	CC	HQ		
Zn/Al Layered Double Hydroxide Film MGCE	1.2	9	DPV	[33]
Glassy carbon electrode in CPB and SDBS	3	8	DPV	[34]
Silsesquioxane-MCPE	10	10	DPV	[8]
Graphene oxide and multiwall carbon nanotubes	1.8	2.6	DPV	[35]
[Cu(Sal-β-Ala)(3,5-MPz)2]/SWCNTs/GCE	3.5	1.46	DPV	[36]
Poly(calmagite) MCPE	2.55	1.70	DPV	[37]
PEDOT/GO modified Electrode	1.6	1.6	DPV	[38]
PASA/MWNTs/GCE	1.0	1.0	DPV	[39]
Poly(alcian blue)MCPE	0.104	0.142	DPV	This work



Scheme 7.1. Oxidation mechanism of catechol and hydroquinone



Scheme 7.2. Structure of alcian blue

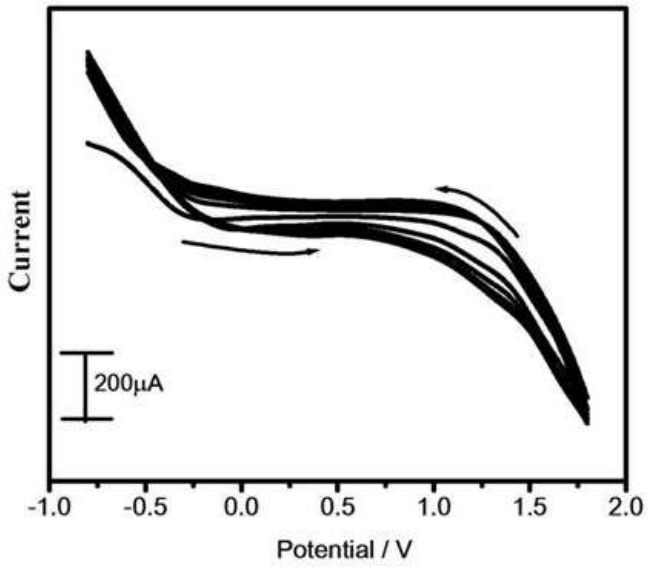


Fig. 7.1A. Cyclic voltammograms of preparation of poly(alcian blue) MCPE. 1mM solution in 0.2M PBS of pH 7.4 at 5 cycles with scan rate 0.1 Vs^{-1}

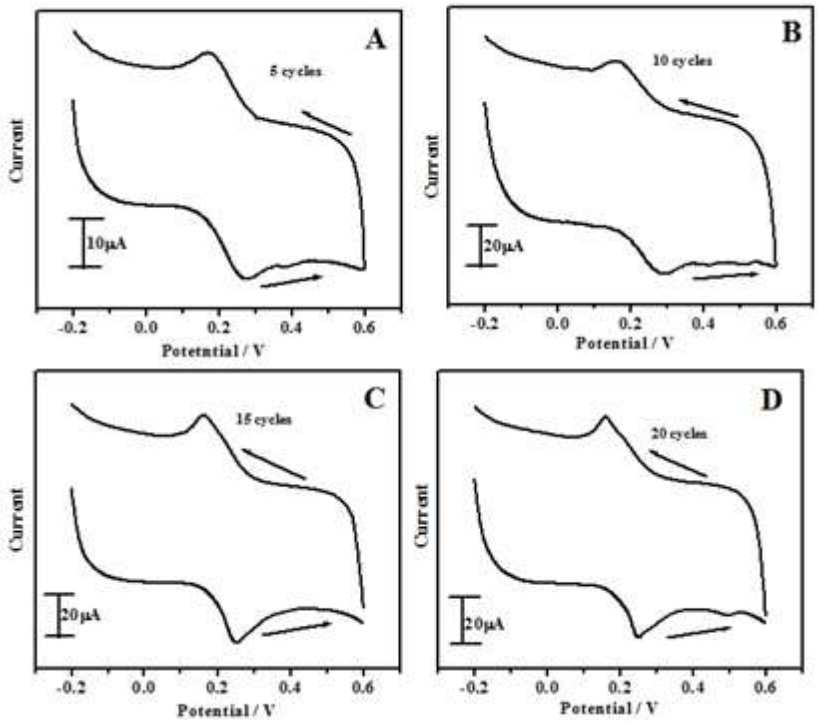


Fig. 7.1B. Cyclic voltammograms recorded for the oxidation of 0.2 mM catechol in 0.2 M PBS of pH 7.4 at poly (alcian blue) MCPE at scan rate of 0.05Vs-1. (A-5cycles B-10 cycles C-15 cycles D-20 cycles)

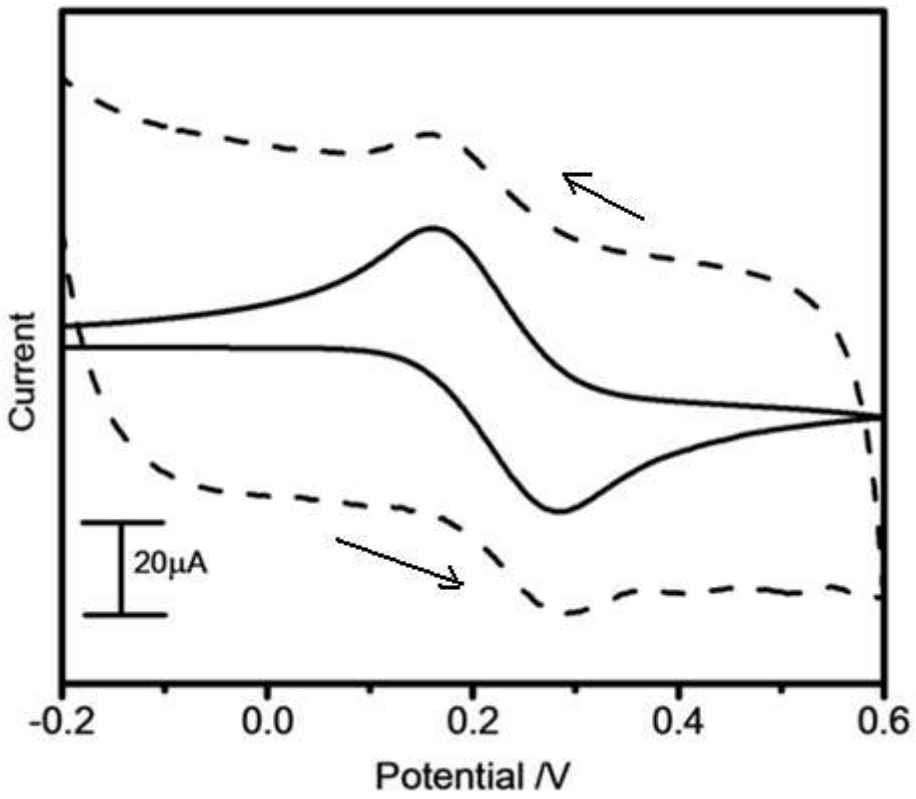


Fig. 7.2. Cyclic voltammograms of 1 mM potassium ferrocyanide in 1M KCl at BCPE (solid line) and poly(alcian blue) MCPE (dashed line) at scan rate of0.05 Vs^{-1}

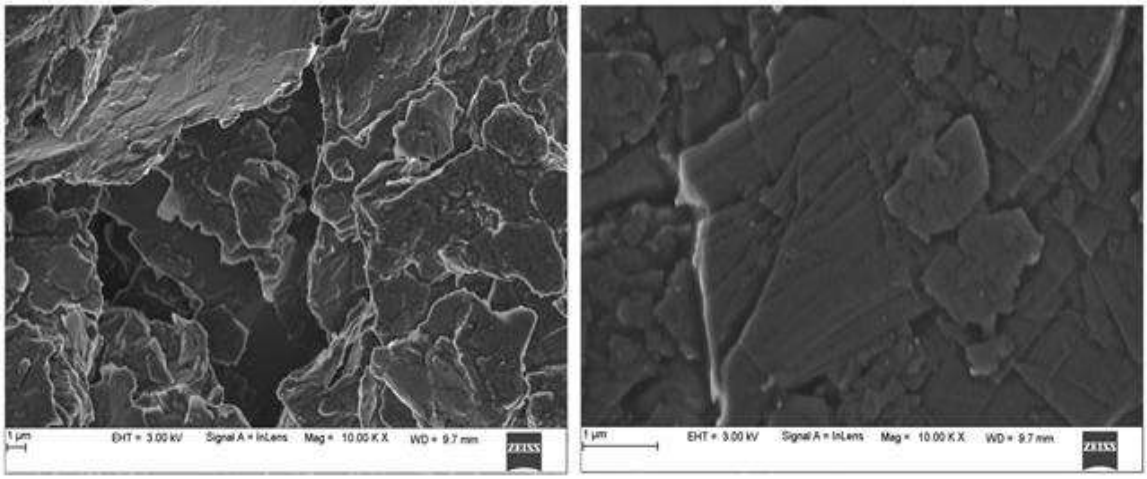


Fig. 7.3. SEM Images of BCPE (A) and poly(alcian blue) MCPE (B).

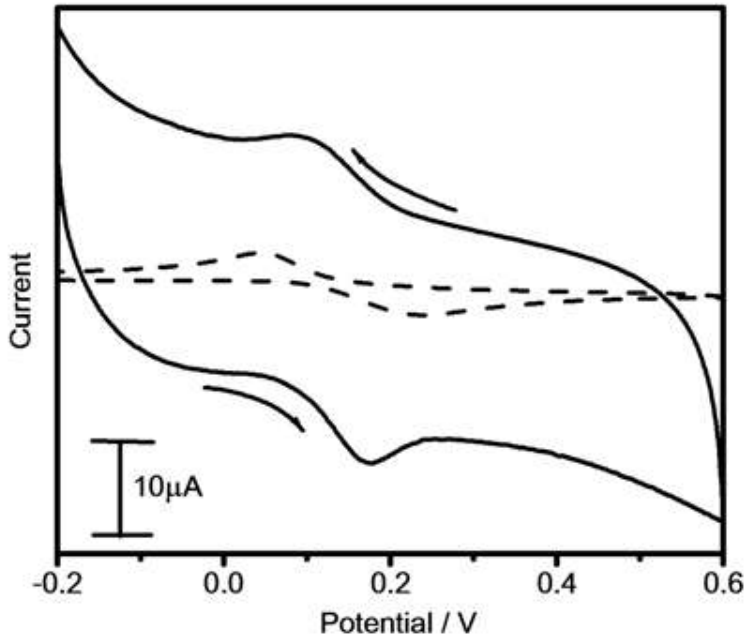


Fig. 7.4. Cyclic voltammograms of 0.1 mM CC in 0.2M PBS pH 7.4at BCPE (dashed line) and poly(alcian blue) MCPE (solid line) at scan rate of 0.05 Vs^{-1}

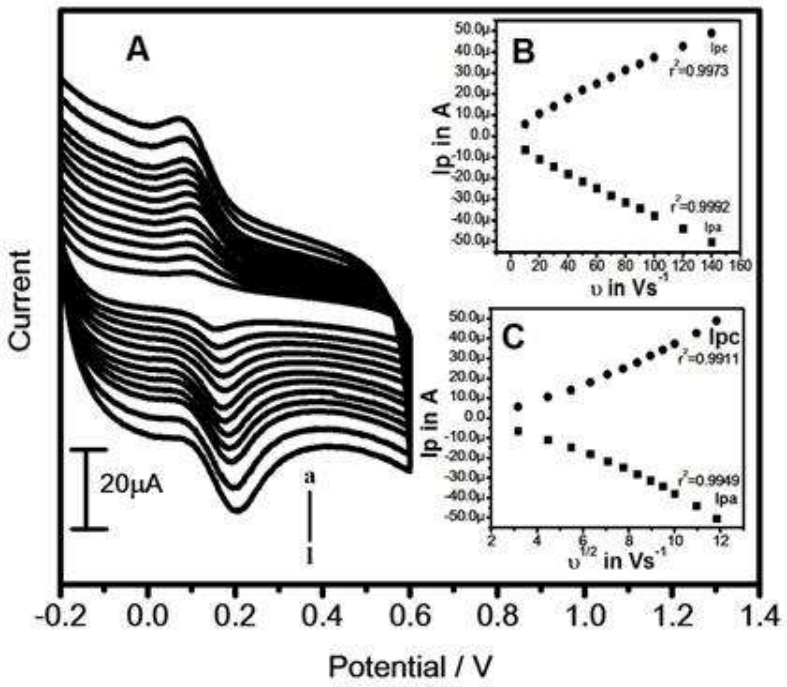


Fig. 7.5. (A) Cyclic voltammograms of 0.1 mM CC in 0.2 M PBS pH 7.4 at different scan rate (a-l: 0.01 to 0.14 Vs-1) (B) Graph of peak current versus scan rate. (C) Graph of peak current versus square root of scan rate

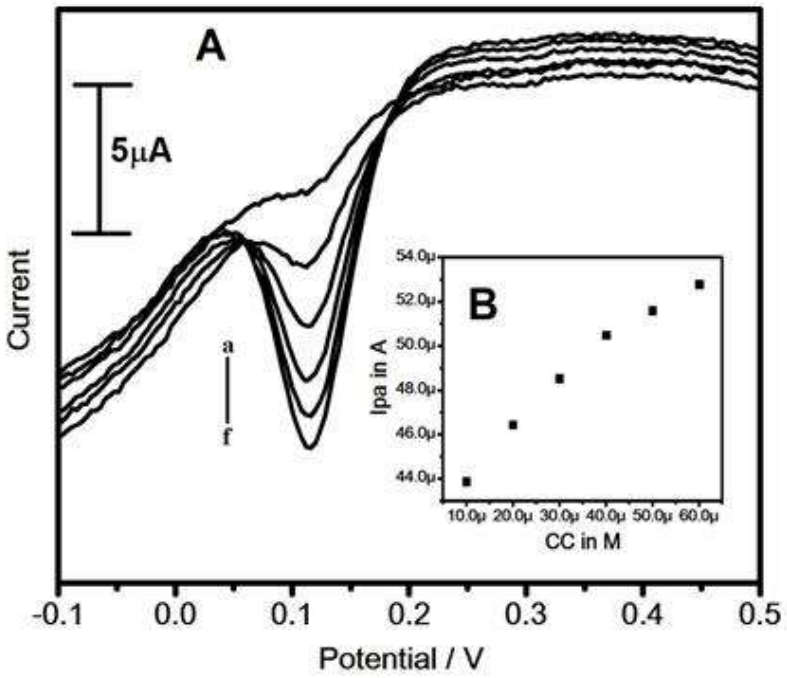


Fig. 7.6. (A) Differential pulse voltammograms of CC in 0.2 M PBS solution of pH 7.4 at poly(alcian blue) MCPE with different concentrations (a–f: 10-60μM).
(B) Graph of anodic peak current versus concentration

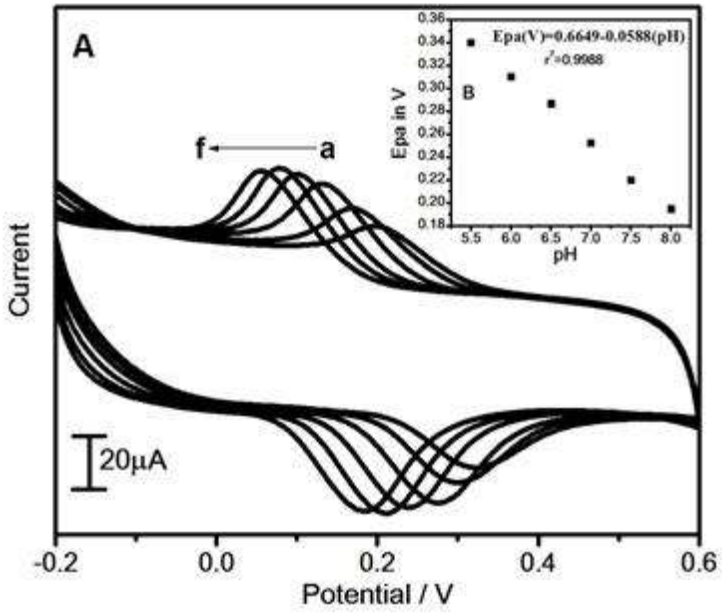


Fig. 7.7. (A) Cyclic voltammograms of 0.1 mM CC at poly(alcian blue)MCPE in 0.2 M PBS solution with different pH values (a–f: 5.5 to 8.0) at scan rate of 0.05 Vs^{-1}.
(B) Graph of anodic peak potential versus pH

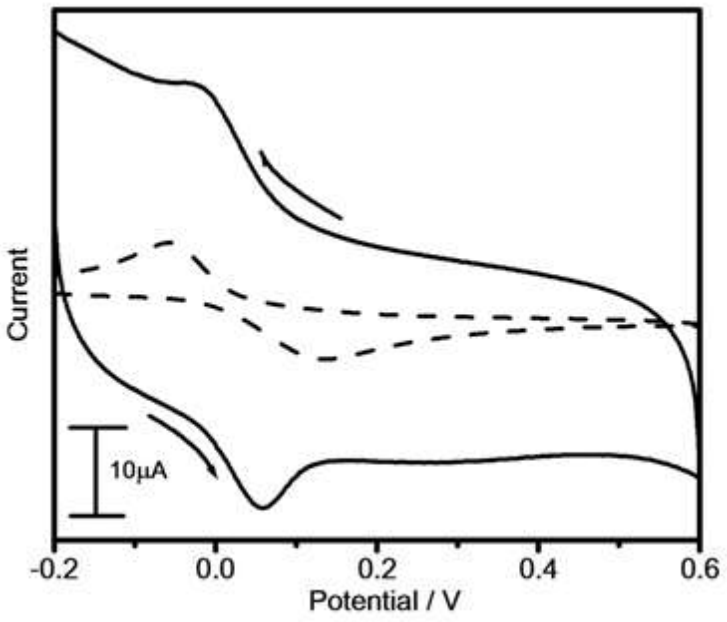


Fig. 7.8. Cyclic voltammograms of 0.1 mM HQ in 0.2M PBS pH 7.4 at BCPE (dashed line) and poly(alcian blue) MCPE (solid line) at scan rate of 0.05 Vs^{-1}

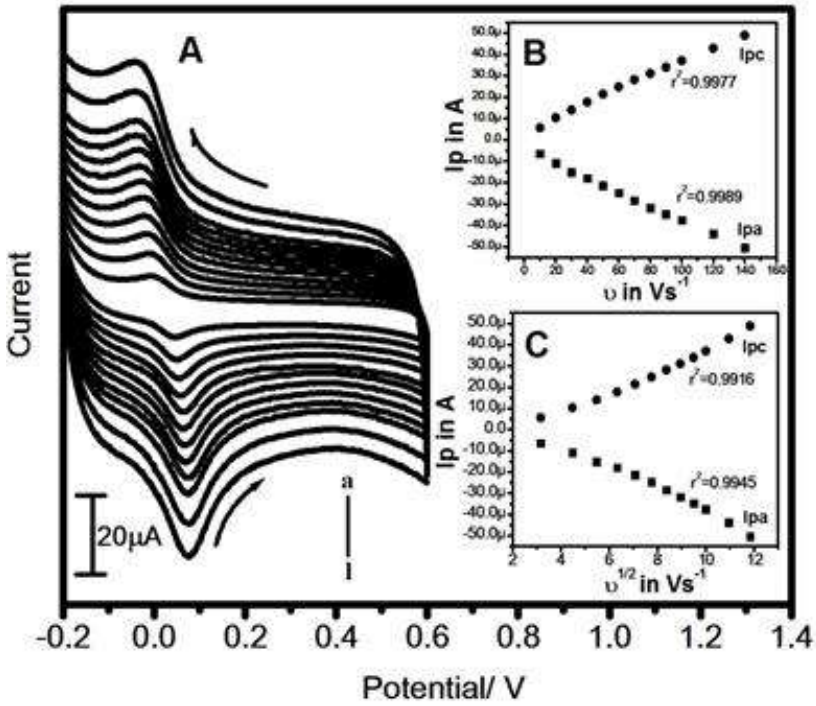


Fig. 7.9. (A) Cyclic voltammograms of 0.1 mM HQ in 0.2 M PBS solution of pH 7.4 at different scan rate (a-l: 0.01 to 0.14Vs-1) (B) Graph of peak current versus scan rate. (C) Graph of peak current versus square root of scan rate

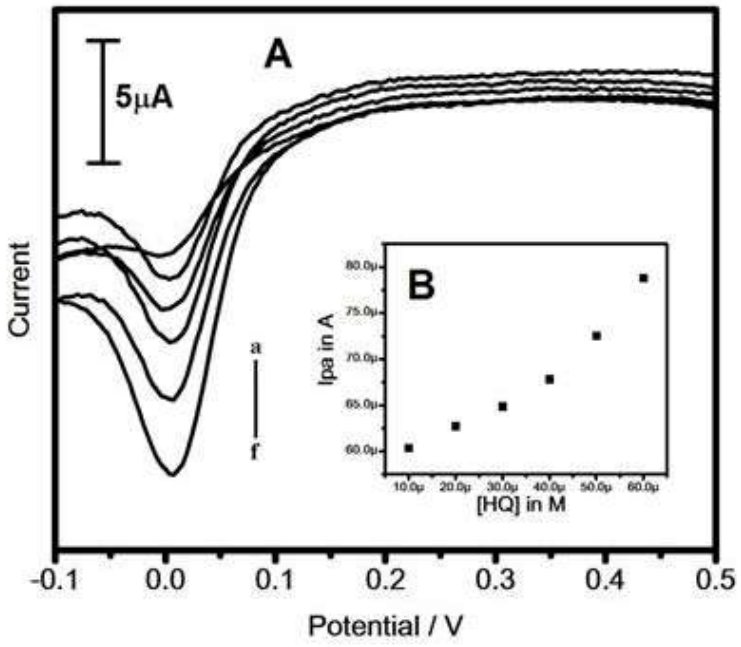


Fig. 7. 10. (A) Cyclic voltammograms of 0.1 mM HQ at poly(alcian blue) MCPE in 0.2 M PBS solution with different pH values (a–f: 5.5 to 8.0) at scan rate of 0.05 Vs^{-1}
(B) Graph of anodic peak potential versus pH

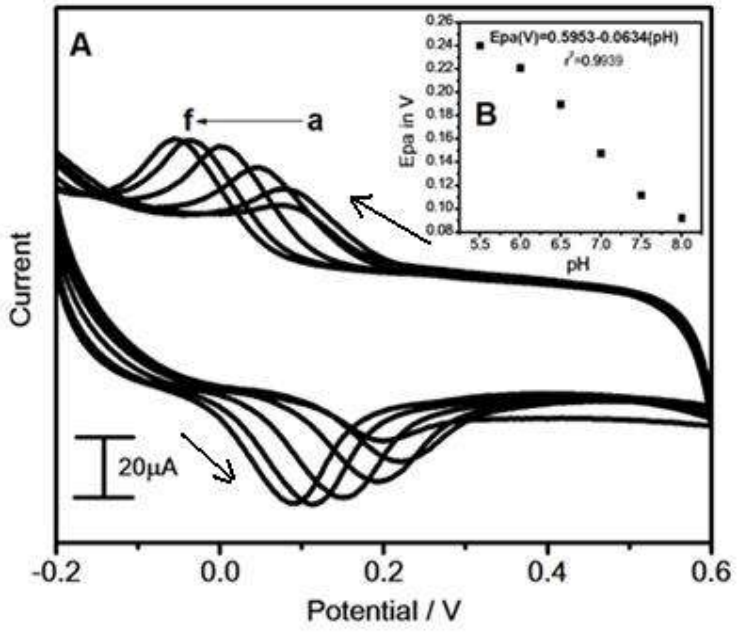


Fig. 7.11. (A) Cyclic voltammograms of 0.1 mM HQ at poly(alcian blue)MCPE in 0.2 M PBS solution with different pH values (a–f: 5.5 to 8.0) at scan rate of 0.05 Vs^{-1}.
(B) Graph of anodic peak potential versus pH

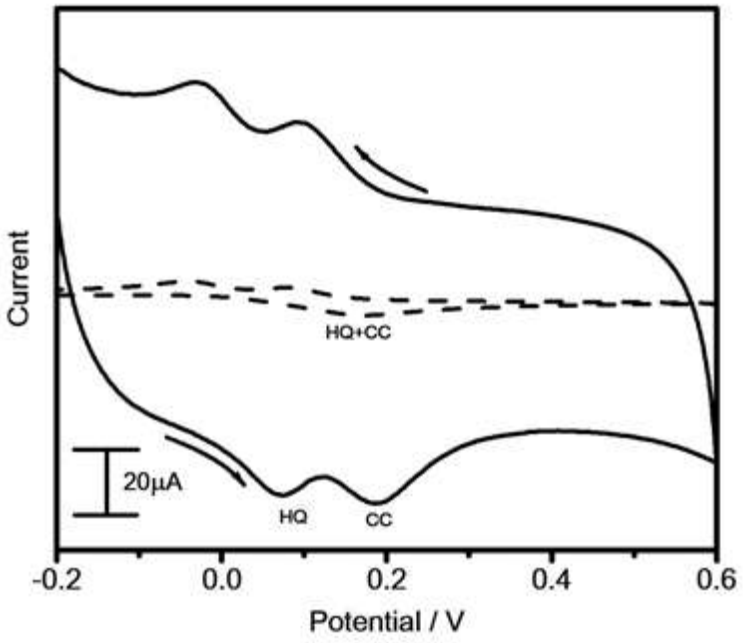


Fig. 7.12. Cyclic voltammograms for simultaneous determination of 0.1 mMCC and 0.1mM HQ at BCPE (dashed line) and poly(alcian blue) MCPE (solidline) at scan rate of 0.05 Vs^{-1}

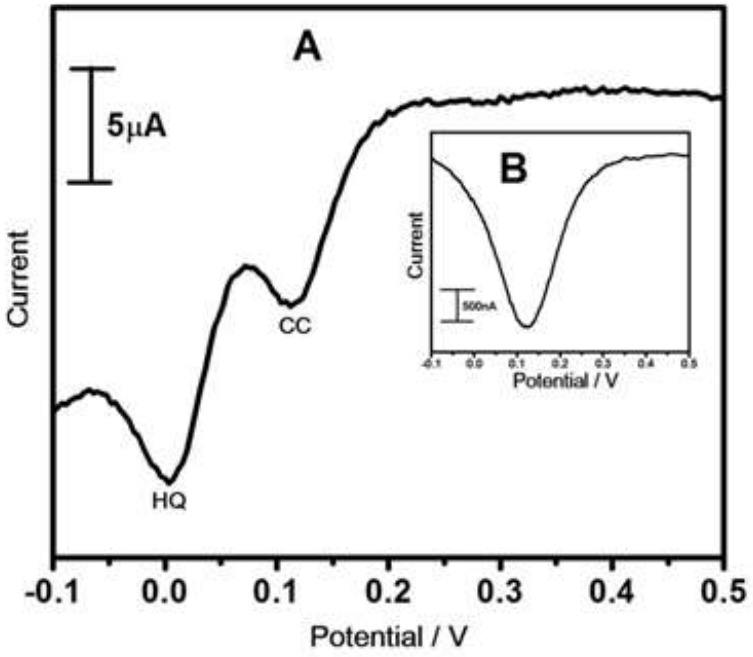


Fig. 7.13. Differential pulse voltammogram obtained for 0.1 mM CC and 0.1mM HQ in 0.2M PBS solution of pH 7.4 at poly(alcian blue) MCPE (A) and BCPE (B)

7.6. References

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7.7. Introduction

There are several reasons for studying the redox behavior in Quinone/ hydroquinone not the least of which is their biological importance [1]. Catecholamine neurotransmitters, Quinone based metabolic cofactor and the fundamental electron-transfer properties of Quinones have stimulated extensive examinations of Quinone redox chemistry in both aqueous and non-aqueous environments [2-9]. Two dihydroxybenzene isomers like Catechol (CT) and Hydroquinone (HQ) are widely used in industrial applications such as cosmetics, pesticides, flavoring agents antioxidant, dyes and pharmaceutics [10-13]. Usually there are different types of chemically modified carbon paste electrodes, in that electroppolymerization is a simple but powerful method in the role of selective modification of different types of electrode with desired dyes. The important advantages of electropolymerization are the easy synthesis and deposition of desired electroactive polymers onto the conductive surface from monomer solutions and the precise electrochemical control of their formation rate and thickness. These electroactive polymers have useful properties such as electronic conductivity and ionic conductivity [14].

Toluidine blue is an example of a partially orthochromatic dye, as it stains nucleic acids by its orthochromatic color is blue. Toluidine blue is a basic thiazine metachromatic dye with high affinity for acidic tissue components [15]. Azines group like phenazines, phenothazines and phenoxazines are comes under conjugated polymers have been widely used in bioelectrochemistry as redox indicators and mediators [16]. In acidic medium presence of electropolymeriztion of azine group compounds is usually undergo anodic oxidation [17, 18]. Similar to these azine dyes, malachite green (MG) and alcian blue is also a dye compound that has an open but ionized structure; hence, the resulting polymer is promising in exhibiting interesting features such as fast rate of charge transfer and ion transport and good catalytic ability toward small biomolecules [19, 20]. The chemical structures of TB are shown in Scheme 1 and 2.

7.8. Experimental Part

7.8.1. Reagents

Hydroquinone (HQ), catechol (CC) and toluidine blue were purchased from Himedia. The stock solutions of 25×10^{-4} M HQ, 25×10^{-4} M CC and 25×10^{-3} M Toluidine blue was prepared in double distilled water. Phosphate buffer solution (PBS) of same ionic strength was maintained (0.2 M) and the desired pH was obtained by mixing appropriate ratio of $NaH_2PO_4 \cdot H_2O$ and Na_2HPO_4. Graphite fine powder of 50μM particle size was purchased from Merck and silicone oil from Himedia was used to prepare the carbon paste electrode (CPE). All the chemicals are of analytical grade used as received without any further purification.

7.8.2. Apparatus

All electrochemical experiments including cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed using a model CHI-660c (CH Instrument -660 electrochemical work station). A conventional three electrode system was used in a electrochemical cell with a saturated calomel electrode (SCE) as a reference, a platinum or counter electrode and bare carbon paste electrode or Toluidine blue modified carbon paste electrode as working electrode.

7.8.3. Preparation of the Working Electrode

The carbon paste electrode (CPE) was prepared according to the reported literature [20]. Electrochemical polymerization of Toluidine blue at the BCPE was carried out using cyclic voltammetric method in aqueous solution containing 1mM Toluidine blue monomer in 0.2 M PBS of pH 7.4. The electropolymerization was achieved by the formation of film that grew between -0.6 V and +1.6 V at the scan rate of 0.1 V for 10 successive cycles. After that the electrode was rinsed thoroughly with double distilled water.

7.8.4. Results and Discussion

7.8.5. Electrochemical Polymerization of Toluidine Blue on BCPE

In an electrochemical cell over the potential range of -0.6 V to +1.6 V with scan rate $0.1Vs^{-1}$, the poly (Toluidine blue) MCPE was prepared, by using 1mM solution of toluidine blue monomer in 0.2M PBS for 10 successive cycles. It can be seen from the Fig. 7.14, the anodic peak currents enhanced gradually in the cyclic voltammograms, after the few cycles the increase of this peak current becomes constant and becomes more stable, which indicates the growth of polymerization was reached the level of saturation [21, 22].

7.8.6. Surface Property of CPE and Toluidine Blue MCPE

Electrochemical cell contain the freshly prepared stock solutions of 1 mM potassium ferrocyanide and 1M KCl as supporting electrolyte. Fig. 7.15 shows the cyclic voltammograms recorded for the 1 mM potassium ferrocyanide at both CPE (dotted line) and toluidine blue MCPE (solid line) at the scan rate 0.05 Vs^{-1}. The low redox peak currents response was obtained at CPE but in the same condition toluidine blue MCPE exhibited stable enhancement of redox peak currents and also it shows the fast electron transfer rate kinetics. The result obtained greatly improved the voltammetric response of potassium ferrocyanide at poly (Toluidine blue) MCPE. This suggests that the surface behavior of the modified electrode has been significantly changed and also the result proves that the electrocatalytic activity of the toluidine blue MCPE towards the potassium ferrocyanide.

A broad voltammogram was obtained at BCPE due to the slow electron transfer phenomenon. However, in the same identical condition the poly (toluidine blue) MCPE not only exhibited increment in redox peak currents, it also improved the electron transfer kinetics. The result suggests that the surface property of the modified electrode was been

changed. The surface area available for reaction of species in solution can be calculated by the Randles-Sevcik equation (1).

$$I_p = 2.69\times10^5\, n^{3/2} A\, D^{1/2}\, C_0 \upsilon^{1/2} \qquad \text{---------- (1)}$$

where, I_p is the peak current in A. C_0 is the concentration of the electroactive species (mol cm^{-3}), n is the number of electrons involved, D is the diffusion coefficient in cm^2S^{-1}, υ is the scan rate (Vs^{-1}) and A is the electroactive surface area (cm^2). For poly (toluidine blue) MCPE the electroactive surface area is maximum (0.0398 cm^2) as compared with BCPE (0.0278 cm^2).

An approximate surface coverage of the poly(toluidine blue) layer formed on the surface of carbon paste electrode (CPE) was calculated by the following equation (2)

$$I_p = n^2F^2A\Gamma\upsilon/4RT \qquad \text{---------- (2)}$$

where, Γ (M/cm^2) represents the surface coverage concentration which is proportional to the peak current (I_p), υ is the scan rate, A is the geometric surface area of the electrode, n is the number of electrons involved in the reaction and R, F, T have their usual significance. The surface coverage of poly(toluidine blue) adhered on the surface of CPE was determined to be 0.0366×10^{-10} M/cm^2.

To characterize the surface morphology of the working electrodes scanning electron microscopy (SEM) images were compared. The SEM images for BCPE and poly (toluidine blue) MCPE was showed in Fig. 7.16. The surface of BCPE is of irregular shape (A). After the electropolymerisation (B), toluidine blue forms a uniform film with number of aligned ridges and valleys on the surface, which is entirely different from the BCPE. This morphological feature is having more advantages due to the exposure of large surface area. This will enable as an active platform for the electroanalysis of the targeted molecule.

7.8.7. Electrochemical Response of CC at Poly (Toluidine Blue) MCPE

The Fig. 7.17 shows the cyclic voltammograms of 1×10^{-4} M CC at BCPE and poly (Toluidine blue) MCPE at scan rate of 0.05 Vs^{-1} with supporting electrolyte 0.2M PBS of pH 7.4. At BCPE the oxidation of CC showed poor sensitivity and the anodic peak potential was located at around 0.230 V vs SCE. However, the voltammogram obtained for poly (toluidine blue) MCPE (solid line) in the same condition was with high sensitivity signal with slight shifting in the anodic peak potential towards the positive side comparing to BCPE. The anodic peak potential was located at 0.176 V vs SCE. This result of maximum enhancement in current signal showed the electrocatalytic activity of poly (toluidine blue) MCPE for the detection of CC.

7.8.8. Effect of Scan Rate on Peak Current of CC

If you increase the scan rate current also increases because the rate of change of potential with respect to time is scan rate. The effect of variation of applied scan rate for 1mM CC in 0.2 M PBS of pH 7.4 was examined by CV technique at poly (toluidine blue) MCPE as shown in Fig. 7.18A. The experimental results obtained at poly (toluidine blue) MCPE showed increase in the redox peak currents with increase in the applied scan rate and they are proportional to each other according to Randles-Sevcik equation. The observation shows that there is a shifting of anodic peak potential (E_{pa}) to more positive side and cathodic peak potential (E_{pc}) to the less negative side. In order to confirm the electrode process, the graph of peak current (I_p) versus scan rate (ν) was plotted and the obtained graph is a straight line with good linearity in the range from 0.01 to 0.14 Vs^{-1} as shown in Fig. 7.18B with the correlation coefficient (r^2) 0.9954 and 0.9950. The I_p versus square root scan rate ($\nu^{1/2}$) were plotted as shown in Fig. 7.18C with the correlation coefficient (r^2) 0.9932 and 0.9929. This suggests the electrode process was an adsorption-controlled process [23]. The heterogeneous rate constant (k^0) values was determined from the experimental peak potential difference (ΔE_p) data's, eq. (3) was used for such voltammograms whose ΔE_p values are greater than 10 mV [24,25].

$$\Delta E_p = 201.39 \log(\nu/k^0)-301.78 \qquad \text{---------} (3)$$

From the experimental ΔE_p values as shown in Table 7.3 and Eq. (2); the values of the k^0 for the CC oxidation was determined. The values of k^0 obtained at different scan rate from 0.01 to 0.14 Vs^{-1} for the poly (toluidine blue) MCPE.

7.8.9. Effect of pH on the Determination of CC at Poly(toluidine blue) MCPE

Significant contribution on the electrocatalytic oxidation of CC at the poly(toluidine blue) MCPE by affecting both peak currents and peak potentials at the pH of the phosphate buffer solution. The effect of PBS pH value on the determination of CC at poly(toluidine blue) MCPE was carefully evaluated in a wider pH range of 5.5-8.0. Fig. 7.19A shows cyclic voltammograms recorded for 1mM CC at poly(toluidine blue) MCPE. The oxidation peak potential shifts to a more negative potential with increasing pH. The E_{pa} versus pH graph clearly indicated that the E_{pa} depends linearly on the pH value in the range of 5.5-8.0 with a slope of 0.075 V/pH ($r^2 = 0.9925$) with is close to the theoretical value of 59 V as shown in inset Fig. 7.19B. This result shows there is an equal number of protons and electrons are involved in the redox mechanism at poly (toluidine blue) MCPE. This was consistent with that reported in literature [26]. Fig. 7.19C shows the effect of solution pH on the CC current response in the range of pH 5.5 to 8.0 CC showed a well-defined oxidation peak at all pH values. It can be seen that the anodic peak current increases from pH 5.5 to 7.5 but then decreased from pH 7.5 to 8.0. This may be because, as the pH increases beyond 7.0, OH^- ions in the solution also increases and there is a chance of deprotonation reaction accompanied by chemical reactions [27]. The intermediate products obtained by the above reactions may compete for the adsorption sites on the electrode surface and hence lead to a reduction in peak current. There is more current enhancement at pH 7.4 in PBS. Therefore pH 7.4 PBS was used for all subsequent determinations of CC [28].

7.8.10. Effect of CC Concentration

Differential pulse voltammetry (DPV) was used to determine the CC at the poly(toluidine blue) MCPE, since DPV shows sharper and better defined peaks at lower concentration than those obtained by cyclic voltammetric technique. The electrocatalytic oxidation of CC was carried out by varying its concentration at poly(toluidine blue)

MCPE. The Fig. 7.20A shows by increasing the concentration of CC from 10µM to 60µM the I_{pa} and I_{pc} goes on increasing with a small shifting in the oxidation potentials. The graph of Ipa versus concentration of CC was plotted as shown in the Fig. 7.20B and it shows a straight line with good linearity. The linear regression equation is I_{pa} (µ A) = 0.2093 (C_0µM/L) + 0.46466, (r2 = 0.9944, N = 6). The limit of detection was calculated and the detection limit in the lower concentration range for CC was 6.658 µM for the poly(toluidine blue) MCPE and limit of quantification was 22.1933 µM. The analytical parameters obtained in the present work were compared with other reported electrodes as shown in Table 7.4 [29-37]. These data show that the obtained analytical parameters of the proposed method are either superior or comparable to the previously reported results.

7.8.11. Electrochemical Response of HQ at Poly (toluidine Blue) MCPE

The Fig. 7.21 shows the cyclic voltammograms of 1×10^{-4} M HQ at BCPE and poly (toluidine blue) MCPE at scan rate of 0.05 Vs^{-1} with supporting electrolyte 0.2M PBS of pH 7.4. At BCPE the oxidation of HQ showed poor sensitivity and the anodic peak potential was located at around 0.230V vs SCE. However, the voltammogram obtained for poly (toluidine blue) MCPE (solid line) in the same condition was with high current signal with slight shifting in the anodic peak potential towards the positive side comparing to BCPE. The anodic peak potential was located at 0.176V vs SCE. This result of maximum enhancement in current signal showed the electrocatalytic activity of poly (toluidine blue) MCPE for the detection of HQ.

7.8.12. Effect of Scan Rate on Peak Current of HQ

The effect of variation of applied scan rate for 0.1 mM HQ in 0.2 M PBS of pH 7.4 was examined by cyclic voltammetric technique at poly (toluidine blue) MCPE as shown in Fig. 7.22A. The experimental results obtained at poly (toluidine blue) MCPE suggests the peak current and scan rate are proportional to each. It can be observed that, there is a slight positive shift of anodic peak potential (E_{pa}) and negative shift in the

cathodic peak potential (E_{pc}). In order to evaluate the electrode phenomenon, the graph of peak current (I_p) versus scan rate (ν) was plotted and the obtained graph is a straight line with good linearity in the range from 0.01 to 0.14 Vs^{-1} as shown in Fig. 7.22B with the correlation coefficient of (r^2) 0.9962 and 0.9946. On other hand, the I_p versus square roots can rate ($\nu^{1/2}$) was plotted as shown in Fig. 7.22C with the correlation coefficient (r^2) 0.9959 and 0.9970. This suggests the electrode phenomenon was an adsorption-controlled [32]. The heterogeneous rate constant (k^0) values was determined from the experimental peak potential difference (ΔE_p) data's, eq. (3) was used for the voltammograms whose ΔE_p values are greater than 10 mV [26, 31].

From the experimental ΔE_p values as shown in Table 1 and Eq. (2); the values of the k^0 for the HQ oxidation was determined. The values of k^0 obtained at different scan rate from 0.01 to 0.14 Vs^{-1} for the poly (toluidine blue) MCPE.

7.8.13. Effect of pH Value on the Determination of HQ at poly(toluidine blue) MCPE

Significant contribution on the electrocatalytic oxidation of HQ at the poly(toluidine blue) MCPE by affecting both peak currents and peak potentials at the pH of the phosphate buffer solution. The effect of PBS pH value on the determination of HQ at poly(toluidine blue) MCPE was carefully evaluated in a wider pH range of 5.5-8.0 Fig. 7.23A shows cyclic voltammograms recorded for 1mM HQ at poly(toluidine blue) MCPE. The oxidation peak potential shifts to a more negative potential with increasing pH. The E_{pa} versus pH graph clearly indicated that the E_{pa} depends linearly on the pH value in the range of 5.5-8.0 with a slope of0.072V/pH (r^2 = 0.9927) with is close to the theoretical value of 59V as shown in inset Fig. 7.23B. This result shows there is an equal number of protons and electrons are involved in the redox mechanism at poly (toluidine blue) MCPE.

7.8.14. Effect of HQ Concentration

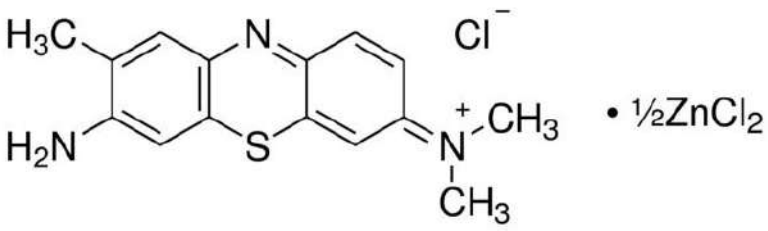
Differential pulse voltammetry (DPV) was used to determine the HQ at the poly(toluidine blue) MCPE, since DPV shows sharper and better defined peaks at lower concentration than those obtained by cyclic voltammetric technique. The electrocatalytic oxidation of HQ was carried out by varying its concentration at poly(toluidine blue) MCPE. The Fig. 7.24A shows by increasing the concentration of HQ from 10µM to 70µM the I_{pa} and I_{pc} goes on increasing with a small shifting in the oxidation potentials. The graph of I_{pa} versus concentration of HQ was plotted as shown in the Fig. 7.24B and it shows a straight line with good linearity. The linear regression equation is I_{pa} (µ A) = 0.1765 (C_0µM/L) + 0.19282, (r^2 = 0.9837, N = 7). The limit of detection was calculated and the detection limit in the lower concentration range for HQ was 1.2942µM for the poly(toluidine blue) MCPE and limit of quantification was 4.3140µM.

7.8.15. Simultaneous Determination of CC and HQ

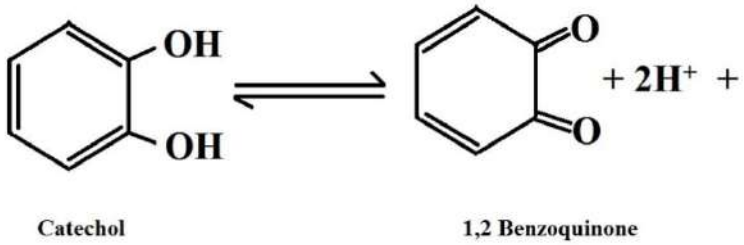
In order to examine the selectivity of poly(toluidine blue) MCPE, the electrochemical study in the mixture of equimolar concentration of CC and HQ in 0.2M PBS of pH 7.4 was conducted by CV technique. Fig. 7.25 shows the cyclic voltammograms obtained for the oxidation of CC and HQ at BCPE (dotted line) and poly(toluidine blue) MCPE (solid line). The oxidation of CC and HQ at BCPE was less sensitive. However, The poly(toluidine blue) MCPE shows more sensitivity and selectivity in oxidation process of CC and HQ. The DPV technique was performed for the interference study, wherein the concentration of one species maintained constant where other is varied. From the Fig. 7.26 it can be seen that the concentration of CC was increased from 0µM to 200µM by keeping the concentration of HQ constant (1mM). The anodic peak current was proportional to concentration of CC and there was no change in the peak current for HQ. Similarly, the Fig. 7.27 shows the variation of concentration of HQ from 20µM to 200µM by keeping the concentration of CC constant (1mM). Therefore, the separation of oxidation potentials of CC and HQ was achieved at Poly(toluidine blue) MCPE.

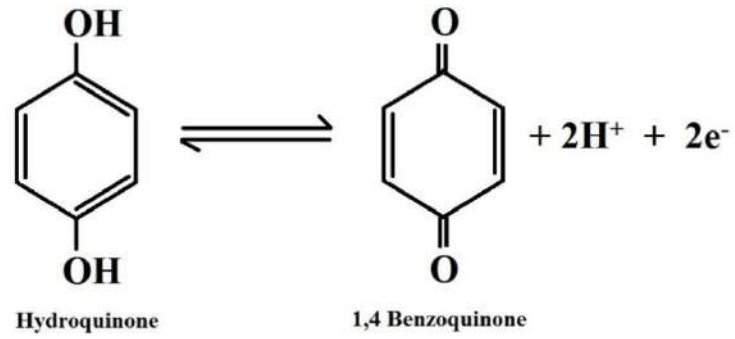
7.9. Conclusion

The fabricated poly(toluidine blue) MCPE was used to study the electrochemical behavior of CC and HQ in physiological pH of 7.4 by CV and DPV techniques. The variation in scan rate and concentration study reveals that the electrode process was controlled by adsorption of the analytes. The LOD of HQ and CC was 1.29μM and 6.65μM for poly(toluidine blue) MCPE by DPV technique. The simultaneous study of CC and HQ shows good selectivity and sensitivity with differentiable peak potential separations. Overall, the poly(toluidine blue)MCPE can be employed to construct a electrochemical sensor for CC and HQ. A simple and convenient method for the modification of bare carbon paste electrode was proposed by the electropolymerisation of toluidine blue followed by its electrochemical and scanning electron microscopic characterization. The strong electrocatalysis in the oxidation of catechol and hydroquinone has made the fabricated electrode as a promising analytical sensor in the simultaneous analysis of both the isomers by cyclic voltammetric and differential pulse voltammetric techniques. Overall, the sensitivity, selectivity, antifouling property, reproducibility, stability was shown by the electrode.



Scheme 1





Scheme 2

Table 7.3. Voltammetric parameters gathered from Figures 7.18 and 7.22

υ/ mVs^{-1}	ΔE_p/mV		k^o / s^{-1}	
	Catechol	**Hydroquinone**	**Catechol**	**Hydroquinone**
10	0.1123	0.0015	0.3018	0.3024
20	0.0693	0.0007	0.7037	0.7046
30	0.0735	0.0089	1.6625	0.9858
40	0.0798	0.0235	0.9866	0.6983
50	0.105	0.0172	0.6994	0.1660
60	0.1249	0.0329	0.5545	0.5537
70	0.126	0.0245	0.4611	0.4605
80	0.1175	0.0309	0.3937	0.3932
90	0.1186	0.0308	0.3418	0.3415
100	0.1259	0.0047	0.3003	0.2998
120	0.1338	0.0384	0.2366	0.2362
140	0.1533	0.031	0.1872	0.1868

Table 7.4. Comparison table of limit of detection obtained at poly(toluidine blue) MCPE with other electrodes

Working electrode	Limit of detection in µM		Technique	Reference
	CC	HQ		
Silsesquioxane-MCPE	10	10	DPV	[29]
Zn/Al Layered Double Hydroxide Film MGCE	1.2	9	DPV	[30]
Glassy carbon electrode in CPB and SDBS	3	8	DPV	[31]
Graphene oxide and multiwall carbon nanotubes	1.8	2.6	DPV	[32]
PASA/MWNTs/GCE	1.0	1.0	DPV	[33]
[Cu(Sal-β-Ala)(3,5-MPz)2] /SWCNTs/GCE	3.5	1.46	DPV	[34]
Poly(calmagite) MCPE	2.55	1.70	DPV	[35]
PEDOT/GO modified electrode	1.6	1.6	DPV	[36]
Poly(alcian blue)MCPE	0.104	0.142	DPV	[37]
Poly(toluidine blue)MCPE	6.658	1.294	DPV	Present work

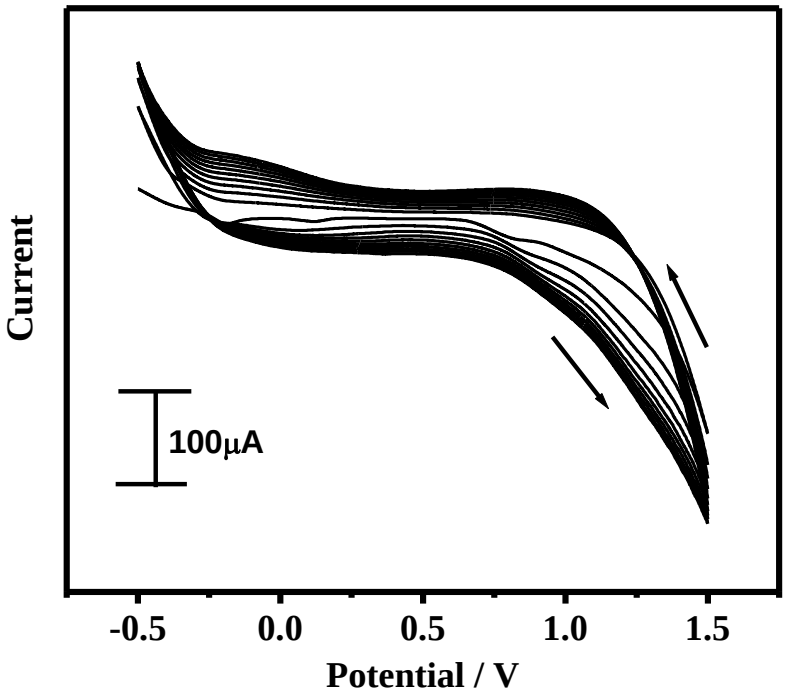


Fig. 7.14. Cyclic voltammograms of preparation of poly(toulidine blue) MCPE. 1mM solution in 0.2M PBS of pH 7.4 at 5 cycles with scan rate 0.1 Vs^{-1}

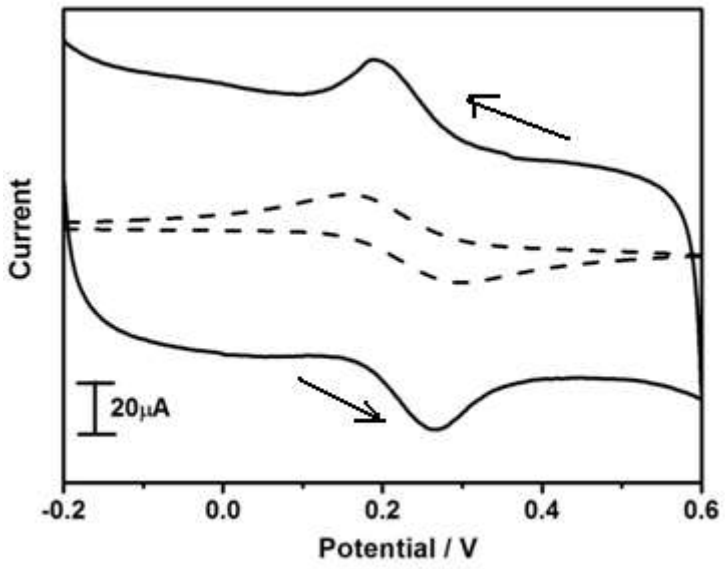


Fig. 7.15. Cyclic voltammograms of 1mM potassium ferrocyanide in 1M KCl at BCPE (solid line) and poly(toluidine blue) MCPE (dashed line) at scan rate of 0.05Vs^{-1}

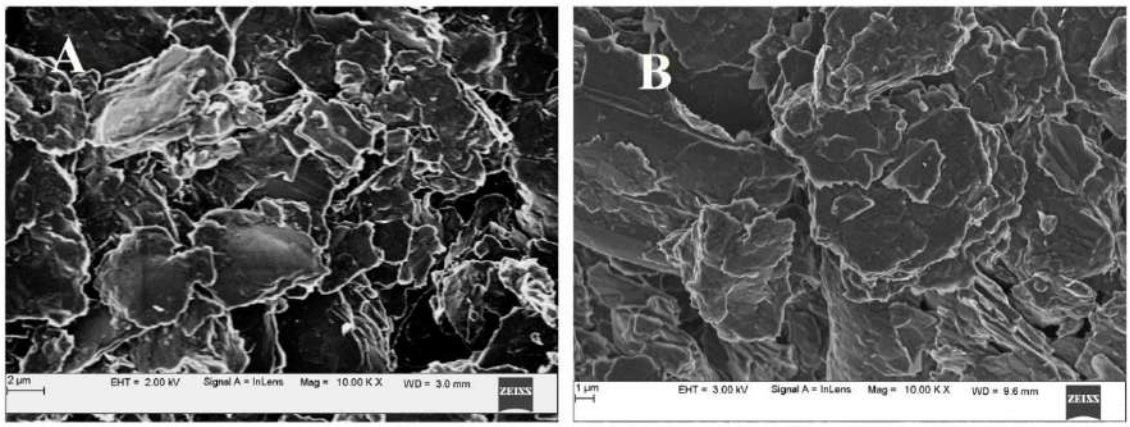


Fig. 7.16. SEM Images of BCPE (A) and poly (toluidine blue) MCPE (B)

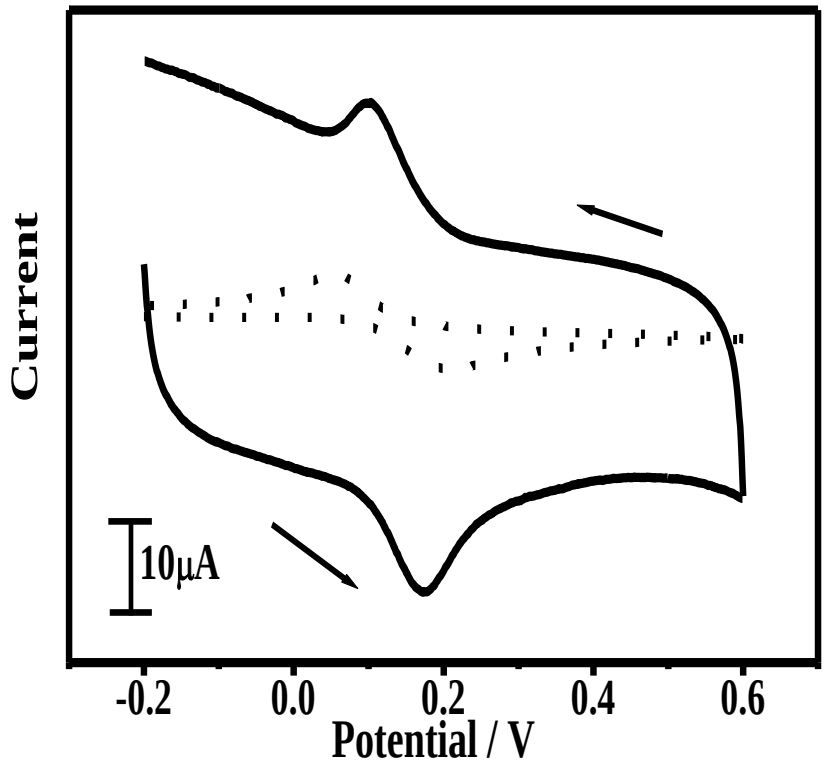


Fig. 7.17. Cyclic voltammograms of 1 mM potassium ferrocyanide in 1M KCl at BCPE (solid line) and poly(toulidine blue) MCPE (dashed line) at scan rate of 0.05 Vs^{-1}

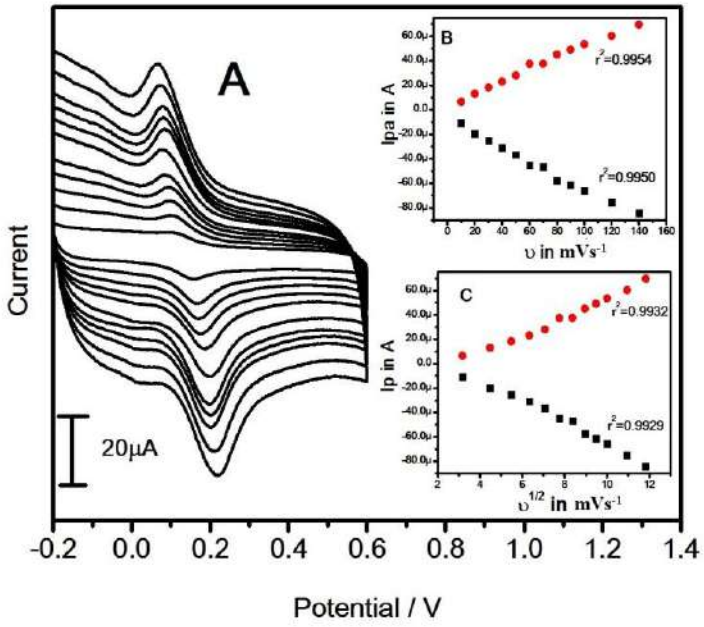


Fig. 7.18. (A) Cyclic voltammograms of 0.1mM CC in 0.2 M PBS solution of pH 7.4 at different scan rate in the range 10-140 mVs^{-1} (B) Graph of peak current versus scan rate. (C) Graph of peak current versus square root of scan rate

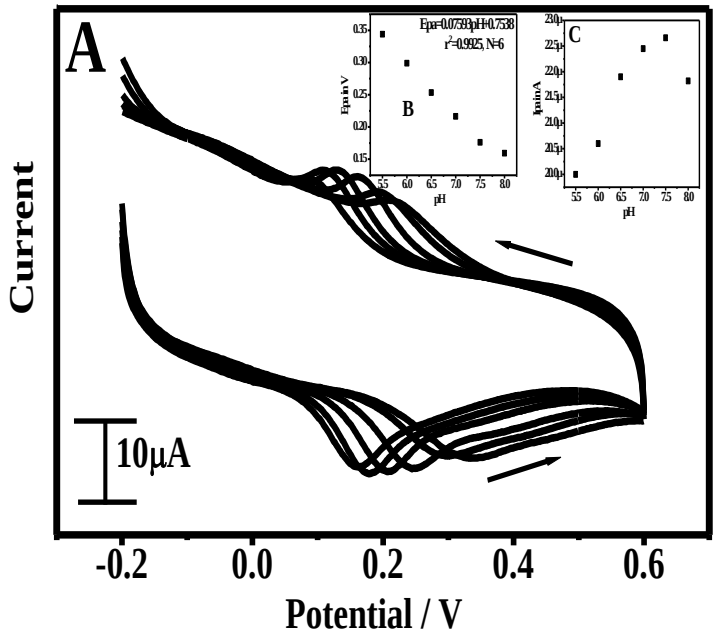


Fig. 7.19. (A) Cyclic voltammograms of 0.1 mM CC at poly(toulidine blue) MCPE in 0.2 M PBS solution with different pH values (a–f: 5.5 to 8.0) at scan rate of 0.05 Vs^{-1}. (B) Graph of anodic peak potential versus pH. (C) Graph of anodic peak current versus pH

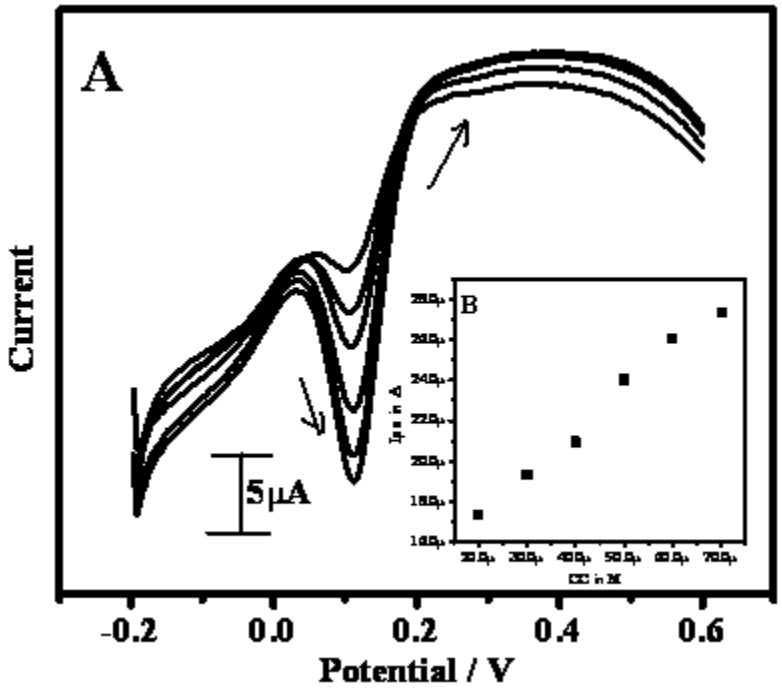


Fig. 7.20. (A) Differential pulse voltammograms of CC in 0.2 M PBS solution of pH 7.4 at poly(toulidine blue) MCPE with different concentrations (a–f: 10-60μM).
(B) Graph of anodic peak current versus concentration

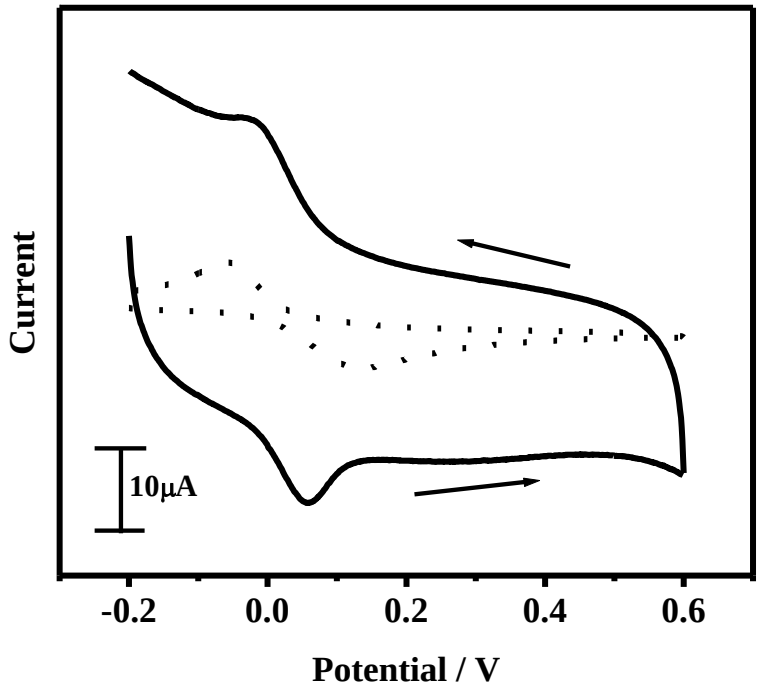


Fig. 7.21. Cyclic voltammograms of 0.1 mM HQ in 0.2M PBS pH 7.4 at BCPE (dashed line) and poly(toulidine blue) MCPE (solid line) at scan rate of 0.05 Vs^{-1}

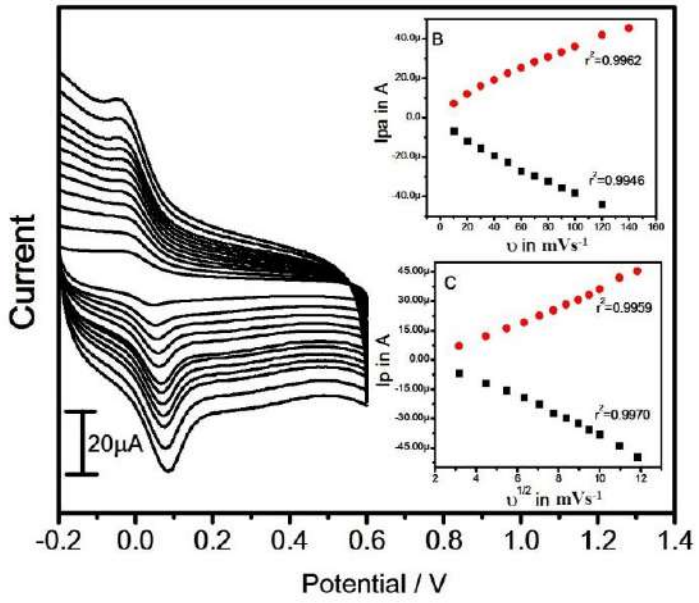


Fig. 7.22. (A) Cyclic voltammograms of 0.1mM HQ in 0.2 M PBS solution of pH 7.4 at different scan rate in the range 10-140 mVs^{-1} (B) Graph of peak current versus scan rate. (C) Graph of peak current versus square root of scan rate

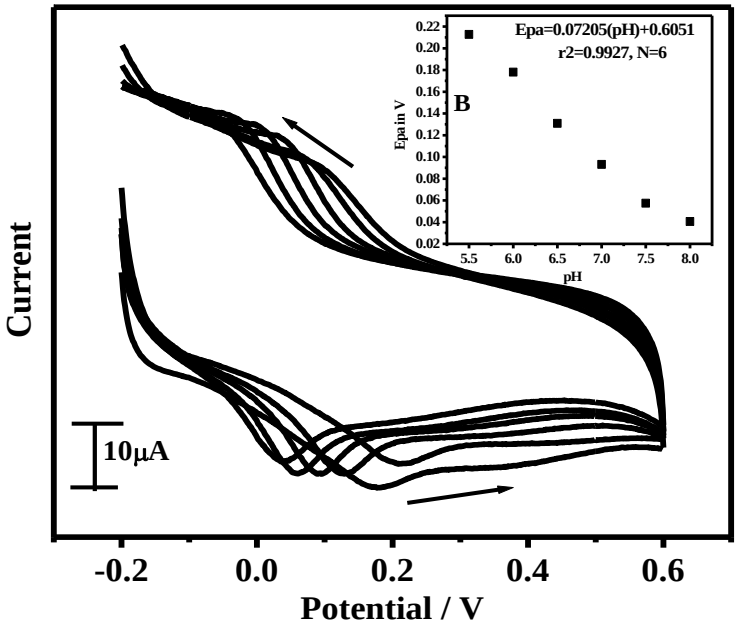


Fig. 7.23. (A) Cyclic voltammograms of 0.1 mM HQ at poly(toulidine blue) MCPE in 0.2 M PBS solution with different pH values (a–f: 5.5 to 8.0) at scan rate of 0.05 V s^{-1} (B) Graph of anodic peak potential versus pH. (C) Graph of anodic peak current versus pH

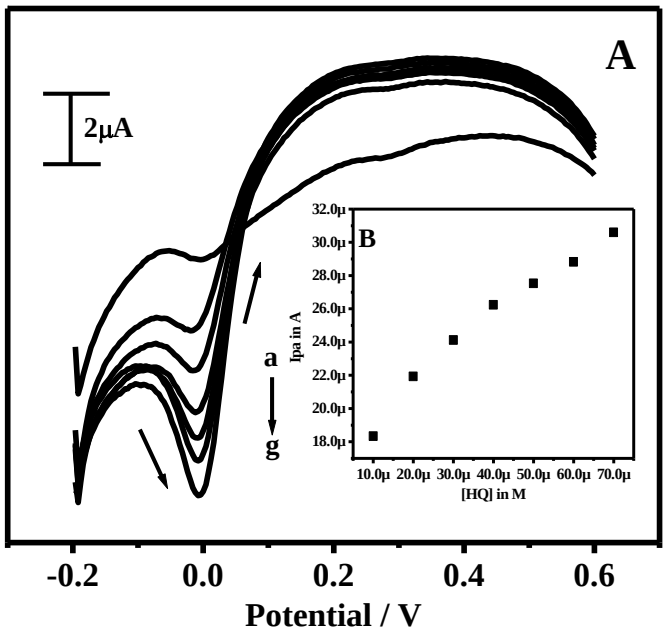


Fig. 7.24. (A) Differential pulse voltammograms of HQ in 0.2 M PBS solution of pH 7.4 at poly(toulidine blue) MCPE with different concentrations (a–f: 10-60μM).
(B) Graph of anodic peak current versus concentration

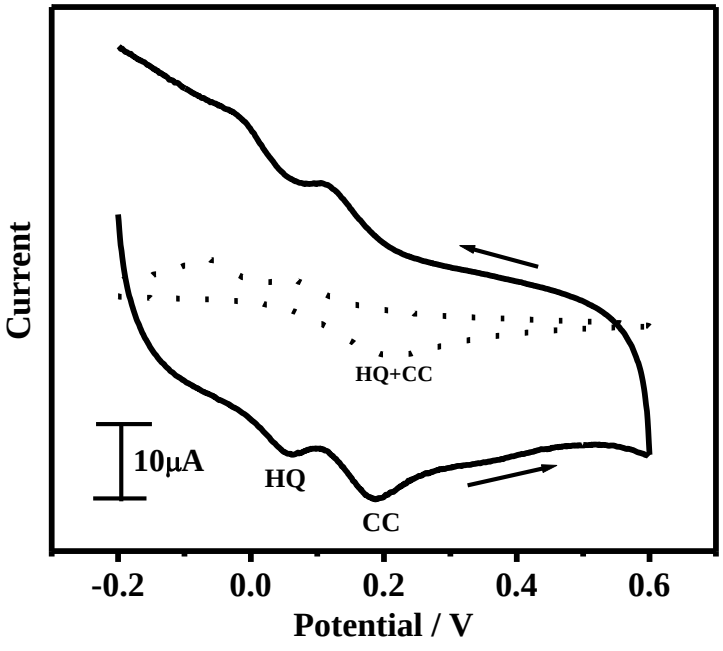


Fig. 7.25. Cyclic voltammograms for simultaneous determination of 0.1 mMCC and 0.1mM HQ at BCPE (dashed line) and poly(toulidine blue) MCPE (solid line) at scan rate of 0.05 Vs^{-1}

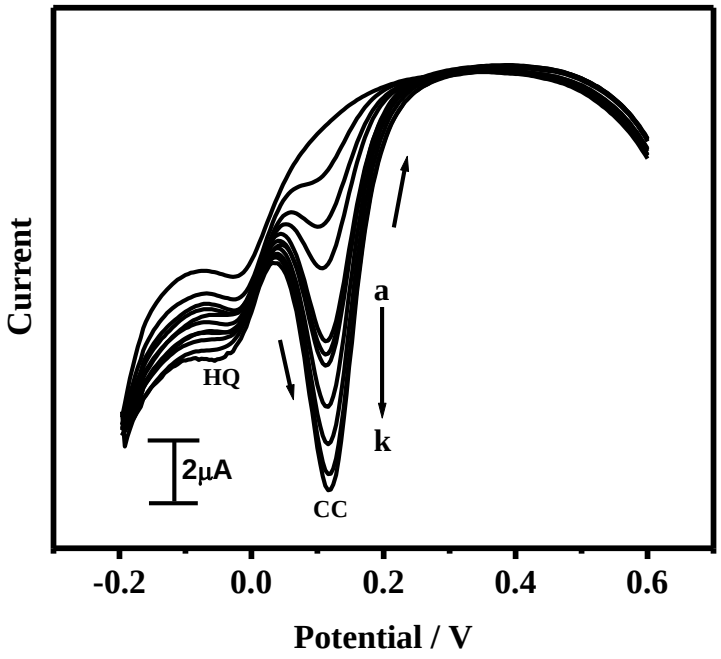


Fig. 7.26. Differential pulse voltammograms obtained for the oxidation of CC (0, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200μM) and 1mM HQ constant in 0.2M PBS of pH 7.4 at poly(toulidine blue) MCPE

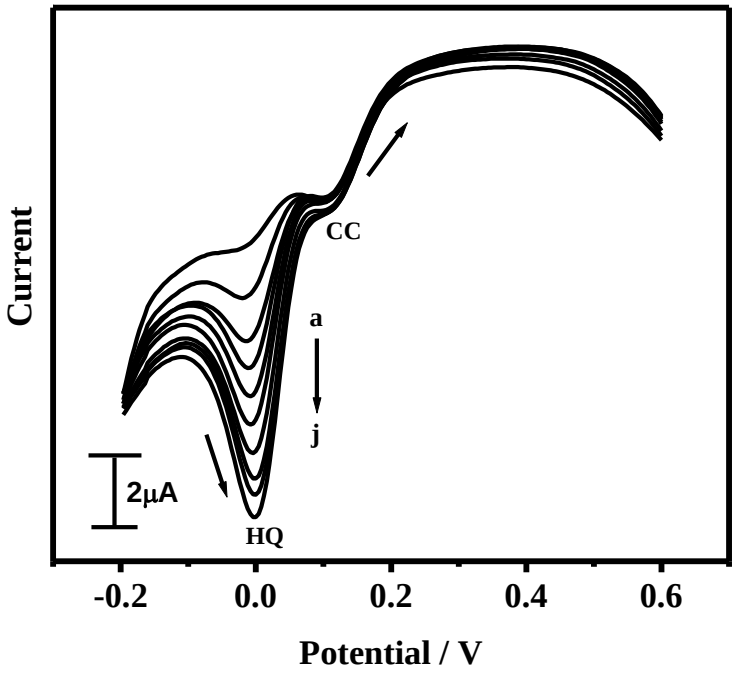


Fig. 7.27. Differential pulse voltammograms obtained for the oxidation of HQ (20, 40, 60, 80, 100, 120, 140, 160, 180 and 200μM) and 1mM CC constant in 0.2M PBS of pH 7.4 at poly(toulidine blue) MCPE

7.10. References

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