KUVEMPU UNIVERSITY

ELECTROCHEMICAL INVESTIGATIONS OF BIOLOGICALLY IMPORTANT DRUGS AT DIFFERENT MODIFIED ELECTRODES: A VOLTAMMETRIC STUDY

Thesis to be Submitted to the Faculty of Science
Kuvempu University

For the Award of the Degree of

Doctor of Philosophy

in

Industrial Chemistry

By

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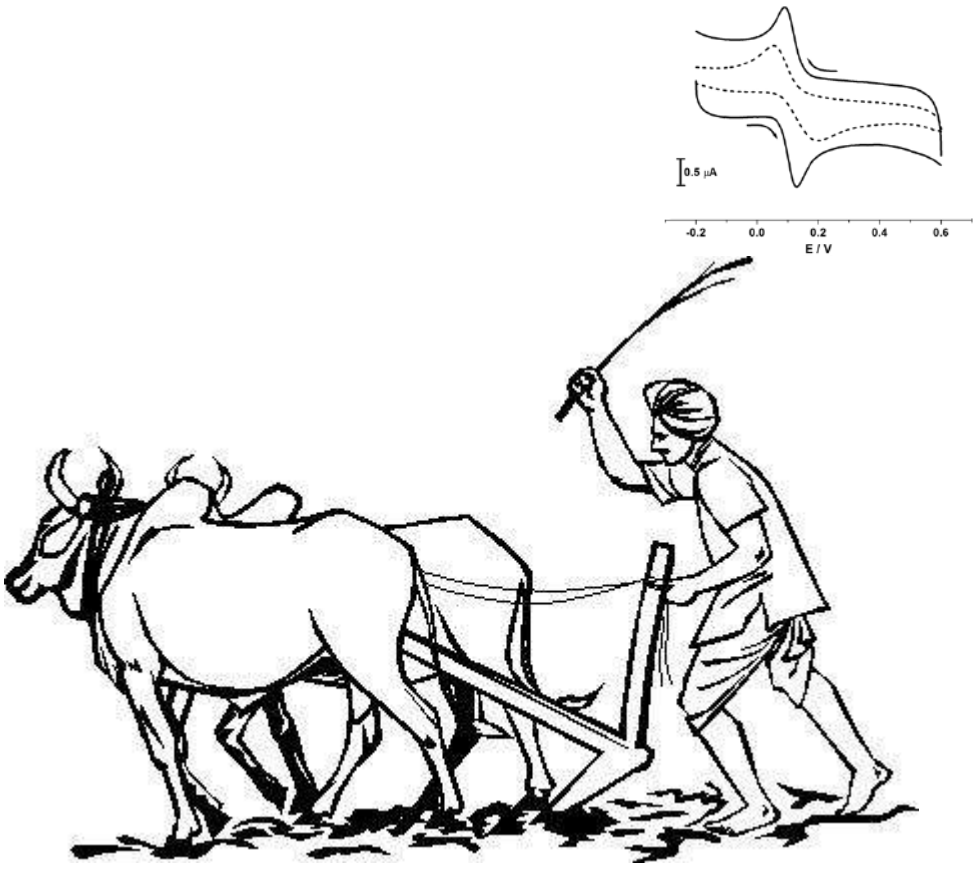
MARCH 2018

Dedicated to

my Beloved Parents



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Declaration

I hereby declare that the Ph.D., thesis entitled "**Electrochemical Investigations of Biologically Important Drugs at Different Modified Electrodes: A Voltammetric Study"** embodies the results of my investigation and this has been composed by me under the Supervision of **Dr. B.E. Kumara Swamy,** Assistant Professor, Department of PG Studies and Research in Industrial Chemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shimoga and the same has not been previously formed the basis for the award of any degree, diploma, associateship, fellowship etc., of any other University or Institution.

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Certificate

This is to certify that the work reported in this thesis entitled "**Electrochemical Investigations of Biologically Important Drugs at Different Modified Electrodes: A Voltammetric Study"** submitted by **Mr. T.S. Sunil Kumar Naik,** to the Faculty of Science, Kuvempu University, for the award of **Doctor of Philosophy in Industrial Chemistry** is a record of the bonafide and original research work carried out by him under my guidance.

I further certify that this or part thereof has not been previously formed the basis of the award of any degree, associateship, fellowship etc., of any other University or Institution.

Date: 9/3/18
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abilities were expressed to me at an early age and instilled within me a desire to achieve greatness. The success that I've achieved until now is largely due to this and to the freedom they've provided me in making my own decisions along the way. Who deserved specially to be mentioned for their inseparable support and prayers.

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Great thanks to all my well wishers……

Mr. T.S. Sunil Kumar Naik,……

Summary of the Thesis

The main attraction of the thesis is to develop the electrochemical sensor by modifying the surface of electrodes (carbon paste electrode, glassy carbon electrode and pencil electrode) by different modification techniques (electropolymerization, electrochemically grinding, surfactant mobilization/immobilization, and electrochemical pre-treatment) to investigate the electrochemical behavior of some biologically important drugs using cyclic voltammetric and differential pulse voltammetric techniques. The electroactive molecules like epinephrine, norepinephrine, dopamine, paracetamol, folic acid, resorcinol, catechol and hydroquinone were chosen for the electrochemical investigations. The following aspects like number of electrons involved in the electrochemical reaction, rate constant, nature of intermediates in the electrode reaction and nature of electrode process were observed. Moreover, the fabricated sensor has been successfully employed for the determination of biomolecules in real samples.

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

Chapter–1

Introduction and overview of cyclic voltammetry

This chapter involves the introduction about voltammetry and its techniques, fundamental principles, theoretical aspects and applications of voltammetry. The solvents, supporting electrolytes and electrode interaction can be seen in this section. A brief review of cyclic voltammetric investigations of certain biologically important compounds has been presented. Importantly, the objectives and scope of present thesis were discussed in this chapter.

Chapter-2

Experimental

This chapter describes the basic experimental setup which is very much essential for voltammetric techniques. Also, procedure for the preparation of bare carbon paste electrode and its modification was explained in detail. In addition, the history of carbon paste electrode was described in this section.

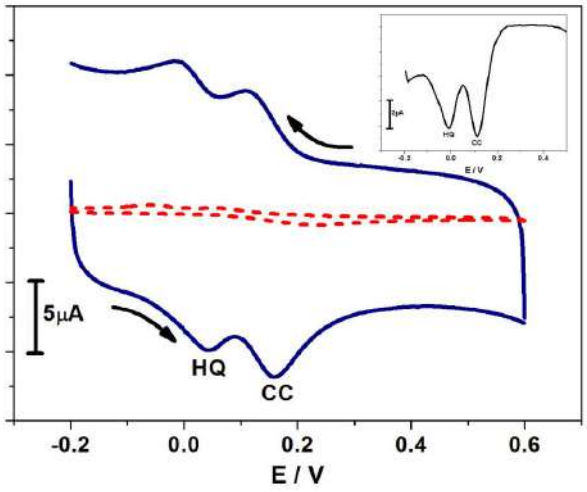
Chapter-3

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

Part-A

Modification of carbon paste electrode by electrochemical polymerization of neutral red and its catalytic capability towards the simultaneous determination of catechol and hydroquinone: A voltammetric study

In this chapter, a new electrochemical sensor was developed and fabricated based upon the electrochemical polymerization of neutral red (NR) on the surface of carbon paste electrode (CPE). The newly developed sensor shows electrocatalytic capability towards the simultaneous determination of catechol (CC) and hydroquinone (HQ) in presence of 0.2M phosphate buffer solution (PBS) at pH 7.4 with the scan rate of 50mVs^{-1}. Electrochemical measurements were performed using both cyclic voltammetry (CV) and differential pulse voltammetric (DPV) methods at poly (neutral red) modified carbon paste electrode (PNR/MCPE). The effects of scan rate, concentration and pH variation for CC and HQ was investigated at PNR/MCPE. The proposed sensor exhibits adsorption-controlled type of electrode process, moreover it show good detection limit (LOD) (CC=6.46 µM and HQ=4.97 µM) and limit of quantification (LOQ) (CC=21.5 µM and HQ=16.5 µM). Hence, the proposed sensor was successfully applied in the simultaneous determination of CC and HQ with satisfied results.



CVs obtained for the simultaneous determination of CC (0.1x10^{-4}M) and HQ (0.1x10^{-4}M) at BCPE (dashed line) and PNR/MCPE (solid line). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the DPVs of CC (0.1x10^{-4}M) and HQ (0.1x10^{-4}M) at PNR/MCPE.

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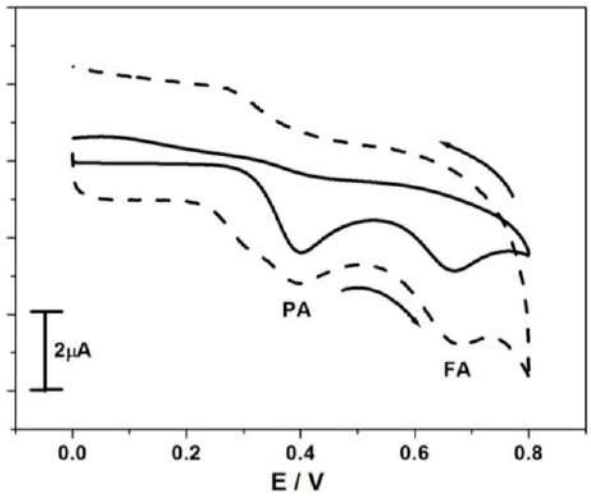
Part-B

Poly (l-leucine) modified carbon paste electrode for the simultaneous electrochemical determination of paracetamol and folic acid: A voltammetric study

In this chapter, bare carbon paste electrode (BCPE) was electropolymerized by L-leucine (LCN) using cyclic voltammetric technique and used as modified carbon paste electrode (MCPE) for the simultaneous electrochemical determination of paracetamol (PA) and folic acid (FA) in 0.2M phosphate buffer solution (PBS) at pH 7.4 with the scan rate of $50mVs^{-1}$. Cyclic voltammetry (CV) and Differential pulse voltammetry (DPV) were used to study the electrochemical behavior of the modified electrode. The effects of scan rate, concentration and real sample analysis at modified carbon paste electrode (MCPE) was studied. The number of electron transfer (n) and the heterogeneous rate constant (k_o) was determined for PA and FA at MCPE. From the scan rate and concentration, the overall electrode process was found to be adsorption-controlled at MCPE. Detection limit of paracetamol and folic acid were found to be 4.41µM and 24.4 µM respectively. The poly (L-leucine) modified carbon paste electrode (PLCN-MCPE) exhibits good electrocatalytic behavior towards the simultaneous determination of paracetamol and folic acid when compared to BCPE. The same method can also be applied for other drug analysis.



Cyclic voltammograms obtained for the mixture of PA ($0.1x10^{-4}$M) and FA ($0.1x10^{-4}$M) at bare (solid line) and PLCN-MCPE (dashed line) using 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}.

Journal of Electroanalytical Chemistry (Revised and Submitted)

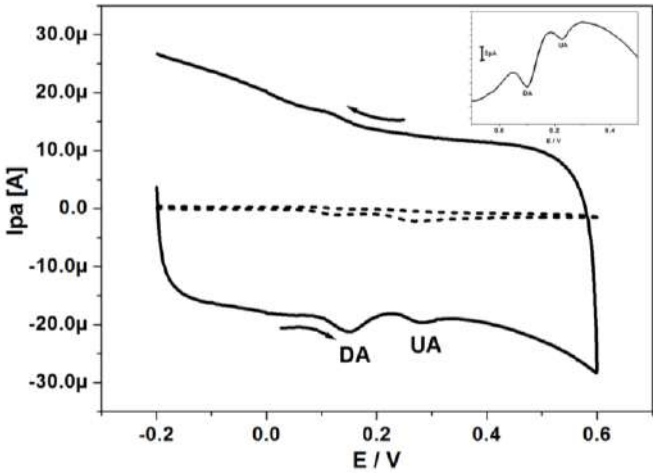
Chapter-4

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

Part-A

Poly (phenosafranine)/SAOS modified sensor for the determination of dopamine and uric acid

The phenosafranine (PS^+) and sodium alpha olefin sulphonate (SAOS) were used as modifiers for the modification of bare carbon paste electrode (BCPE). Electropolymerization and immobilization technique has been employed for the modification of carbon paste electrode to get poly (phenosafranine)/SAOS/MCPE. The electrocatalytic behaviour of the modified electrode was investigated by voltammetric techniques. The modified electrode exhibits excellent electrocatalytic activity towards the determination of dopamine (DA) and uric acid (UA) in presence of 0.2M phosphate buffer solution (PBS) at pH 7.4 with the scan rate of $50mVs^{-1}$. The various parameters like effects of scan rate, concentration and pH variation was studied and the overall electrode process was found to be adsorption-controlled at poly (phenosafranine)/SAOS/MCPE. Both dopamine and uric acid exhibit good detection limits at modified electrode (4.43 µM and 4.18 µM respectively). Hence, the proposed sensor shows good sensitivity towards the determination of DA and UA individually and simultaneously.



Cyclic voltammograms obtained for the mixture of $0.2x10^{-4}$M DA and $0.5x10^{-4}$M UA at BCPE(short dashed line) and PPS/SAOS/MCPE (solid line) using 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}. The inset figure shows the DPVs of the mixture contains DA and UA at PPS/SAOS/MCPE.

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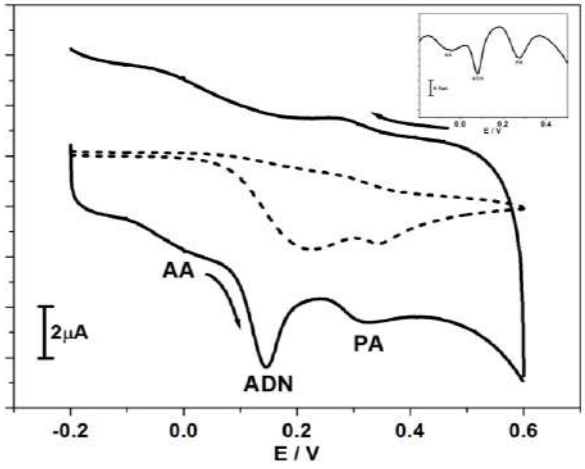
Part-B

Sodium alpha olefin sulphonate modified carbon paste electrode sensor for adrenaline: A voltammetric study

In this chapter, a carbon paste electrode (CPE) modified with sodium alpha olefin sulphonate (SAOS) was used as an electrochemical sensor for the simultaneous determination of adrenaline (ADN) and paracetamol (PA) in presence of 0.2M phosphate buffer solution (PBS) at pH 7.4 with the scan rate of 50mVs^{-1}. The cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques were used to investigate the electrocatalytic behavior of the proposed electrode (SAOS/MCPE). The effects of scan rate, concentration and pH variation were studied at modified carbon paste electrode (MCPE). The electrode process was found to be adsorption-controlled at sodium alpha olefin sulphonate modified carbon paste electrode (SAOS/MCPE). Detection limits (LOD) of adrenaline and paracetamol were found to be 11.3 µM and 3.7 µM respectively. Finally, the modified electrode displayed a strong function for the simultaneous determination of adrenaline and paracetamol.



Cyclic voltammograms obtained for the mixture of 0.1x10^{-4}M ADN, 0.1x10^{-4}M PA and 2.0x10^{-4}M AA at unmodified (dashed line) and SAOS/MCPE (solid line) using 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}. The inset figure shows the DPVs of the mixture containing ADN, PA and AA at SAOS/MCPE.

Sensors and Actuators B: Chemical (Revised and Submitted)

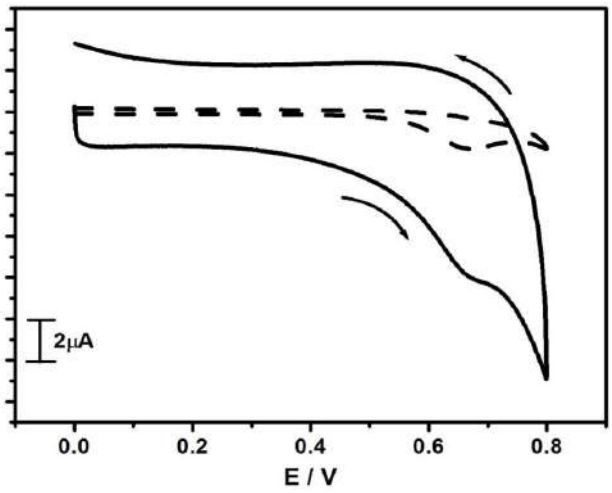
Chapter-5

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

Part-A

Electrochemical Determination of Folic Acid at Sodium Alpha Olefin Sulphonate Modified Carbon Paste Electrode: A Voltammetric Study

In this chapter, sodium alpha olefin sulphonate (SAOS) was used for the modification of carbon paste electrode (CPE) to determine the electrochemical behavior of folic acid (FA) in 0.2M phosphate buffer solution (PBS) at pH 7.4 with the scan rate of 50mVs^{-1}.The effects of scan rate, concentration and simultaneous determination of FA at modified carbon paste electrode (MCPE) were studied. The effect of interference of dopamine was carried out and real sample analysis of FA was studied at MCPE. From the scan rate and concentration shows that, the overall electrode process was found to be diffusion-controlled at SAOSMCPE and detection limit was found to be 28.8µM. The modified electrode (SAOSMCPE) exhibits good electrocatalytic activity towards the determination of folic acid when compared to BCPE. The same method can also be applied for other drug analysis.



Cyclic voltammograms of 0.5x10^{-4}M folic acid at BCPE (dashed line) and SAOSMCPE (solid line) at scan rate 50mVs^{-1} using 0.2M PBS (pH 7.4).

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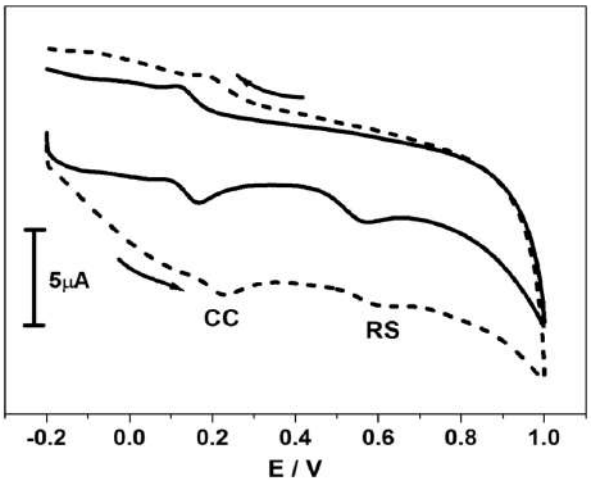
Part-B

Pre-treated Glassy Carbon Electrode Sensor for Catechol: A Voltammetric Study

The surface of glassy carbon electrode (GCE) was modified by electrochemical pre-treatment method and employed for the determination of catechol (CC) in presence of resorcinol (RS) at 0.2M phosphate buffer solution (pH 7.4) with scan rate 50mVs^{-1}. The electrochemical oxidation of catechol (CC) and resorcinol (RS) was investigated by both cyclic voltammetry (CV) and differential pulse voltammetric (DPV) methods at pre-treated glassy carbon electrode (PGCE). The modified electrode (PGCE) showed excellent electrocatalytic activity towards CC and RS determination. The parameters like effect of scan rate, concentration and interference study were investigated at pre-treated glassy carbon electrode (PGCE). The electrode process was found to be adsorption-controlled at pre-treated glassy carbon electrode. Furthermore, the modified electrode exhibited good limit of detection (CC=9.45µM, RS=7.24µM) and limit of quantification (CC=31.5µM, RS=24.1µM) for CC and RS. Hence, the pre-treated glassy carbon electrode shown good electrocatalytic properties and can be applied for the determination of CC and RS individually and simultaneously.



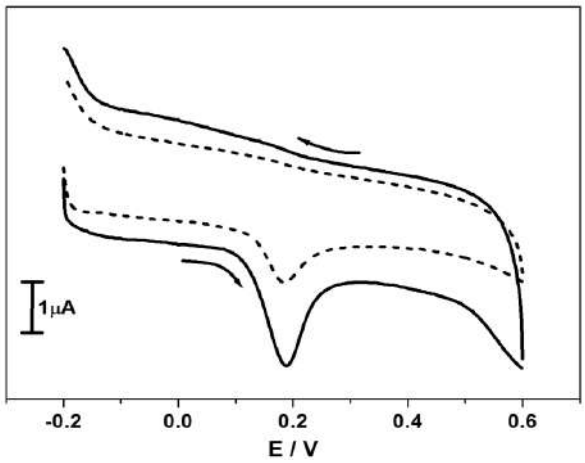
Cyclic voltammograms recorded for the simultaneous determination of CC (0.1x10^{-4}M) and RS (0.2x10^{-4}M) at bare GCE (solid line) and pre-treated GCE (short dashed line). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}.

Journal of Electroanalytical Chemistry (Revised and Submitted)

Chapter-6

Poly (neutral red) modified glassy carbon electrode sensor for epinephrine

A promising and highly sensitive voltammetric technique has been developed for the determination of epinephrine (EP) and nor epinephrine (NEP) based on the electropolymerization of neutral red (NR) on the surface of glassy carbon electrode (GCE). The developed sensor shows excellent stability and sensitivity towards EP and NEP determination. Cyclic voltammetric method (CV) has been employed to scrutinize the electrocatalytic oxidation behaviour of EP and NEP at modified electrode in presence of 0.2M PBS (pH=7). The parameters like effects of scan rate, concentration and pH for EP and NEP were investigated at modified electrode. This poly (neutral red) modified glassy carbon electrode (PNR/MGCE) exhibits diffusion-controlled type of electrode process. In addition to this, the limit of detection (LOD) (EP=4.2 µM and NEP=11.8 µM) and limit of quantification (LOQ) (EP=14 µM and NEP=39.4 µM) were calculated. These excellent properties make the developed sensor suitable for the analysis of EP and NEP.



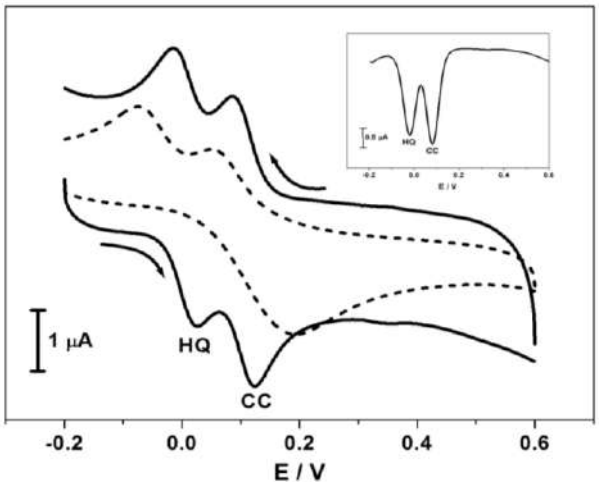
Cyclic voltammograms recorded for EP ($0.1x10^{-4}$M) at bare GCE (dashed line) and PNR/MGCE (solid line) in presence of 0.2M PBS (pH 7) at the scan rate of $50mVs^{-1}$.

Journal of Analytical and Bioanalytical Electrochemistry (Revised and Submitted)

Chapter-7

A simple disposable pencil graphite electrode sensor for catechol and hydroquinone: A voltammetric study

In this chapter, we have carried out one of the simplest method for the modification of pencil graphite electrode (PGE) by using cyclic voltammetric technique. The active surface of the modified electrode showed excellent sensor properties towards the determination of catechol (CC) and hydroquinone (HQ) with good sensitivity and reproducibility in the presence of 0.2M phosphate buffer solution (PBS) at pH 7.4 with the scan rate of 50mVs^{-1}. The different parameters like effects of scan rate, concentration, interference study and pH variation for CC and HQ were carried out at pre-treated pencil graphite electrode (PPGE). From the scan rate study, the electrode process was found to be diffusion-controlled at modified electrode. Furthermore, the modified electrode exhibited lower detection limits for CC and HQ (CC= 5.9µM, HQ= 8.53µM). In addition to this, the practical utility of the proposed electrode has been demonstrated for the determination of CC and HQ in local tap water.



CVs obtained for the simultaneous determination of CC (0.1x10^{-4}M) and HQ (0.1x10^{-4}M) at BPGE (dashed line) and pre-treated PGE (solid line). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the DPVs of CC (0.1x10^{-4}M) and HQ (0.1x10^{-4}M) at modified electrode.

Journal of Electroanalytical Chemistry (Revised and Submitted)

TABLE OF CONTENTS

Page No.

TITLE PAGE

DEDICATION

DECLARATION

CERTIFICATES

ACKNOWLEDGEMENT

SUMMARY OF THESIS

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

LIST OF PUBLICATIONS

Chapter-1: Introduction and Overview of Cyclic Voltammetry **1-28**

1.1 Introduction 1

1.2 Voltammetry 2

1.3 Voltammetric methods 3

1.4 Fundamentals of cyclic voltammetry 6

1.5 Theory 7

1.6 Solvents 9

1.7 Supporting electrolytes 9

1.8 Electrodes 10

1.9 Faradic current and capacitive current 12

1.10 Electrode processes 13

1.11 Electron transfer or charge transfer process 14

1.12 Applications of cyclic voltammetry 17

1.13 A brief literature survey of cyclic voltammetric investigation 18

1.14 Objectives of the thesis 21

1.15 Scope of the present study 21

1.16 References 26

Chapter-2: Experimental **29-49**

2.1 Introduction 29

2.2 Experimental techniques 29

2.3 Instrumentation and basic equipments 30

2.4 pH meter 32

2.5 Electrodes 32

2.6 Binder (pasting liquid) 36

2.7 Polarization characteristics in dependence of the carbon paste composition 37

2.8 Background (residual) signal at CPEs 37

2.9 The highest potential limits attained at CPEs 37

2.10 Preparation and standardization of CPE used in the present study 37

2.11 Construction of carbon paste electrode 38

2.12 Storage of carbon paste electrode 38

2.13 Electrochemical probe system for characterization of CPE in voltammetry 38

2.14 Removal of dissolved oxygen 39

2.15 Glassy carbon electrode used as working electrode 40

2.16 Pencil graphite electrode used as working electrode 40

2.17 References 45

Chapter-3A: Modification of Carbon Paste Electrode by Electrochemical Polymerization of Neutral red and its Catalytic Capability towards the Simultaneous Determination of Catechol and Hydroquinone: A Voltammetric Study **50-74**

3.1 Introduction 50

3.2 Experimental 51

3.3 Results and Discussion 52

3.4 Conclusion 58

3.5 References 71

Chapter-3B: Poly (L-leucine) Modified Carbon Paste Electrode for the Simultaneous Electrochemical Determination of Paracetamol and Folic acid: A Voltammetric Study 75-99

3.6 Introduction 75

3.7 Experimental 76

3.8 Results and Discussion 77

3.9 Conclusion 82

3.10 References 97

Chapter-4A: Poly (phenosafranine)/SAOS Modified Sensor for the Determination of Dopamine and Uric acid 100-120

4.1 Introduction 100

4.2 Experimental 101

4.3 Results and Discussion 102

4.4 Conclusion 107

4.5 References 119

Chapter-4B: Sodium Alpha Olefin Sulphonate Modified Carbon Paste Electrode Sensor for Adrenaline: A Voltammetric Study 121-144

4.6 Introduction 121

4.7 Experimental 122

4.8 Results and Discussion 123

4.9 Conclusion 128

4.10 References 141

Chapter-5A: Electrochemical Determination of Folic Acid at Sodium Alpha Olefin Sulphonate Modified Carbon Paste Electrode: A Voltammetric Study 145-161

5.1 Introduction 145

5.2 Experimental 146

5.3 Results and Discussion 147

5.4 Conclusion 150

5.5 References 159

Chapter-5B: Pre-treated Glassy Carbon Electrode Sensor for Catechol: A Voltammetric Study 162-178

5.6 Introduction 162

5.7 Experimental 163

5.8 Results and Discussion 163

5.9 Conclusion 167

5.10 References 177

Chapter-6: Poly (neutral red) Modified Glassy Carbon Electrode Sensor for Epinephrine 179-195

6.1 Introduction 179

6.2 Experimental 180

6.3 Results and discussion 181

6.4 Conclusion 184

6.5 References 193

Chapter-7: A Simple Disposable Pencil Graphite Electrode Sensor for Catechol and Hydroquinone: A Voltammetric Study 196-213

7.1 Introduction 196

7.2 Experimental 197

7.3 Results and Discussion 198

7.4 Conclusion 202

7.5 References 211

PUBLICATIONS

CONFERENCE CERTIFICATES

LIST OF ABBREVIATION

WE	=	Working electrode
AE	=	Auxiliary electrode
CE	=	Counter electrode
RE	=	Reference electrode
AA	=	Ascorbic acid
ASV	=	Anodic stripping voltammetry
CPE	=	Carbon paste electrode
CMEs	=	Chemically modified electrodes
CMCPEs	=	Chemically modified carbon paste electrodes
CNS	=	Central nervous system
CV	=	Cyclic voltammetry
DA	=	Dopamine
DCE	=	Dropping carbon electrode
DME	=	Dropping mercury electrode
DMF	=	Dimethyl formamide
DMSO	=	Dimethyl sulphoxide
D_0	=	Diffusion coefficient
DPV	=	Differential pulse voltammetry
E_i	=	Initial applied potential
E_v	=	Vertex potential
E_f	=	Final potential
E_{pa}	=	Anodic peak potential
E_{pc}	=	Cathodic peak potential
E^o	=	Formal potential
I_{pa}	=	Anodic peak current
I_{pc}	=	Cathodic peak current
EP	=	Epinephrine
SAOS	=	Sodium alpha olefin sulphonate
FSCV	=	Fast scan cyclic voltammetry

G	=	Gibb's free energy
GCE	=	Glassy carbon electrode
PGE	=	Pencil graphite electrode
KCl	=	Potassium chloride
K_0	=	Heterogeneous rate constant
LSV	=	Linear sweep voltammetry
MCPE	=	Modified carbon paste electrode
NR	=	Neutral red
mM	=	Millimolar
mV	=	Millivolt
mVs^{-1}	=	Millivolt per second
NaOH	=	Sodium hydroxide
NPP	=	Normal pulse polarographic
NTs	=	Neurotransmitters
O	=	Oxidised species
PBS	=	Phosphate buffer solution
PC	=	Personal computer
PME	=	Polymer modified electrode
PVA	=	Polyvinyl alcohol
R	=	Reduced species
S	=	Entropy
SCE	=	Saturated calomel electrode
SEM	=	Scanning electron microscopy
SHE	=	Standard hydrogen electrode
SWV	=	Square wave voltammetry
UA	=	Uric acid
$\upsilon^{1/2}$	=	Square root of scan rate
LCN	=	Leucine
CC	=	Catechol
HQ	=	Hydroquinone
NEP	=	Norepinephrine

Chapter -1

Introduction and overview of cyclic voltammetry

1.1. Introduction

Electrochemistry is a branch of chemistry which deals with the study of transfer of electrons from one substance to another which take place in a solution at the interface of an electron conductor (a metal or a semiconductor) and an ionic conductor (the electrolyte). This creates a current, the magnitude of which can give us clue about the substance. Reduction and oxidation (redox) reactions involve the transfer of electron density from one atom to another. Oxidation describes the loss of electrons or an increase in oxidation state by a molecule, atom or ion. Reduction describes the gain of electrons or a decrease in oxidation state by a molecule, atom or ion.

Electroanalytical technique is a powerful and sensitive tool used for both qualitative and quantitative analysis over a wide range of concentrations. The utility of electrochemical methods stems not only from their sensitivity to trace amounts and the simplicity of the instrumentation, but also because these methods can be used for separation of ionic species in addition to detection. Moreover, electroanalytical measurements offer a number of important potential benefits, which may or may not be realizable in given situations:

- Selectivity and specificity i.e. probing of speciation, resulting from the applied potential.
- Selectivity from the choice of electrode material.
- High sensitivity and low detection limit resulting from the use of complex applied potential programmes.
- Possibility of giving results in real time or close to real time particularly in flow systems for online monitoring.
- Application as miniaturized sensors, in situation where other sensors may not be usable.

Electron transfer plays a fundamental role in governing the path ways of chemical reactions. Measurement of speed of the electron transfer process and the number electrons involved are difficult in traditional experimental methods like spectroscopy. Consequently our knowledge of the driving force for many reactions remains elusive. Electrochemical methods offer the potential to investigate these processes directly by the determination of

number of electrons involved. A series of standard electrochemical methods exist that can be categorized in to three general classes as follows:

Potentiometry is based on the measurement of solution potential in the absence of appreciable current .These methods often employs ion selective electrodes for fast and simple measurement of certain ionic species in solution.

Coulometry measures the current passed through an indicator electrode while it is held at a fixed potential. By appropriate choice of potential for select species, quantitative determinations are achieved by simply integrating the current over time in order to calculate charge passed .The integrated current (charge passed) gives a direct measure of the number of ions that have been oxidized or reduced .Thus in certain cases, no reference solutions are needed to achieve quantitative results.

Voltammetry deals with the effect of the potential of an electrode in an electrolytic cell on the current flow through it with transfer of electrons from one substance to another. This creates a current, the magnitude of which can give us clues about the substance.

1.2 Voltammetry

Voltammetry is a branch of electrochemistry which measures the current response as a function of applied potential or vice-versa. The beginning of voltammetry was facilitated by the discovery of polarography (1922) by Jaroslav Heyrovsky, for which he received the 1959 Nobel Prize in chemistry. The early voltammetric methods experienced a number of difficulties for routine analytical use. However, in the 1960s and 1970's significant advances were made in all areas of voltammetry (theory, methodology and instrumentation), which enhanced the sensitivity and expanded the repertoire of analytical methods. The coincidence of these advances leads to the advent of low-cost operational amplifiers also facilitated the rapid commercial development of relatively inexpensive instrumentation. The common characteristic of all voltammetric techniques is that they involve the application of a potential (E) to an electrode and the monitoring of the resulting current (i) flowing through the electrochemical cell. In many cases the applied potential is varied or the current is monitored over a period of time (t). Thus, all voltammetric techniques can be described as some function of E, i, and t. They are considered as active techniques (as opposed to passive techniques such as potentiometry) because the applied

potential forces a change in the concentration of an electroactive species at the electrode surface by electrochemically reducing or oxidizing it. The analytical advantages of the various voltammetric techniques include excellent sensitivity with a very large useful linear concentration range for both inorganic and organic species (10^{-12} to 10^{-1} M), rapid analysis times (seconds), simultaneous determination of biomolecules of similar structures. 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 UV/Visible, IR and HPLC, they are effective tools for the analysis of complex mixtures.

1.3 Voltammetric methods

The methods used in the voltammetry were distinguished from each other by the function of potential that is applied to the working electrode to drive the electrochemical reaction and by the material used as working electrode. Some of these are explained as follows:

- Linear sweep voltammetry
- Staircase voltammetry
- Squarewave voltammetry
- Anodic stripping voltammetry
- Cathodic stripping voltammetry
- Differential pulse voltammetry
- Normal pulse voltammetry
- Fast scan cyclic voltammetry
- Cyclic voltammetry

Linear sweep voltammetry is a voltammetric method where the current at a working electrode is measured while the potential between the working electrode and reference electrode is swept linearly in time. Oxidation or reduction of species is registered as a peak or trough in the current signal at the potential at which the species begins to be oxidized or reduced.

Staircase voltammetry is a derivative of linear sweep voltammetry. In staircase voltammetry the potential sweep is a series of stair steps. The current is measured at the end of each potential change, right before the next, so that the contribution to the current signal from the capacitive charging current is minimized.

Squarewave voltammetry, a squarewave is superimposed on the potential staircase sweep [1-2]. Oxidation or reduction of species is registered as a peak or trough in the current signal at the potential at which the species begins to be oxidized or reduced. In staircase voltammetry the potential sweep is a series of stair steps. The current is measured at the end of each potential change, right before the next, so that the contribution to the current signal from the capacitive charging current is minimized. The differential current is then plotted as a function of potential, and the reduction or oxidation of species is measured as a peak or trough. In this technique, the peak potential occurs at the $E_{1/2}$ of the redox couple because the current is symmetrical around the potential [3-4]. Due to the lesser contribution of capacitative charging current the detection limits for SWV are on the order of nanomolar concentrations.

Anodic stripping voltammetry is a voltammetric method for quantitative determination of specific ionic species. The analyte of interest is electroplated on the working electrode during a deposition step, and oxidized from the electrode during the stripping step. The current is measured during the stripping step. The oxidation of species is registered as a peak in the current signal at the potential at which the species begins to be oxidized. The stripping step can be either linear, staircase, squarewave, or pulse.

Cathodic stripping voltammetry is a voltammetric method for quantitative determination of specific ionic species. It is similar to the trace analysis method anodic stripping voltammetry, except that for the plating step, the potential is held at an oxidizing potential, and the oxidized species are stripped from the electrode by

sweeping the potential positively. This technique is used for ionic species that form insoluble salts and will deposit on or near the anodic, working electrode during deposition. The stripping step can be either linear, staircase, squarewave, or pulse.

Differential pulse voltammetry (Differential pulse polarography) is often used to make electrochemical measurements. It can be considered as a derivative of linear sweep voltammetry or staircase voltammetry, with a series of regular voltage pulses superimposed on the potential linear sweep or stair steps. The current is measured immediately before each potential change, and the current difference is plotted as a function of potential. By sampling the current just before the potential is changed, the effect of the charging current can be decreased.

Normal pulse voltammetry, the current resulting from a series of ever larger potential pulses is compared with the current at a constant 'baseline' voltage. Another type of pulse voltammetry is squarewave voltammetry, which can be considered a special type of differential pulse voltammetry in which equal time is spent at the potential of the ramped baseline and potential of the superimposed pulse.

Fast scan cyclic voltammetry is a linear sweep voltammetric technique in which the background subtracted voltammogram gives additional information about the electroanlyzed species. The current response over a range of potential is measured, making it a better technique to discern additional current contributions from other electroactive species.

Cyclic voltammetry is generally used to study the electrochemical properties of an analyte in solution. It was first reported in 1938 and described theoretically by Randles [5]. It is 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 the redox processes, on the kinetics of heterogeneous electron transfer process and on couple chemical reactions or adsorption process. Cyclic voltammetry is often 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 [6-10].

1.4. Fundamentals of cyclic voltammetry

1.4.1. Circuit

Voltammetric analysis consists of two 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 versus potential plot is known as voltammogram.

1.4.2. 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.

1.4.3. 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 linear potential scan with a triangular waveform as shown in Fig. 1.1. The excitation signal causes the potential to scan negatively from +0.8V to –0.2V versus 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.

1.4.4. 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 tool 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.

1.4.5. 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.

1.4.6. Characteristic parameters of a cyclic voltammogram

The important parameters of a cyclic voltammogram are peak potential and peak current. 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. Fig.1.2 depicts a typical voltammogram for a reversible process with current (vertical) versus potential. Since the potential varies linearly with time, the horizontal axis can also be thought of as a time axis. More positive potentials will speed up all oxidations and more negative potential will speed up all reductions.

1.5. Theory

In voltammetry, transfer of electrons plays a fundamental role in governing the pathway of chemical reactions; the effects of the applied potential and the behavior of the redox current are described by several well-known laws. The applied potential controls the concentrations of the redox species at the electrode surface (C_O^o and C_R^o) and the rate of the reaction (k^o), as described by the Nernst or Butler-Volmer equations, respectively. In the cases where diffusion plays a controlling part, the current resulting from the redox process (known as the faradic current) is related to the material flux at the electrode-solution interface and is described by Ficks law.

For a reversible electrochemical reaction (that is a reaction so fast that equilibrium is always reestablished as changes are made), which can be described by $O+ne^- \Leftrightarrow R$, the application of potential E forces the respective concentrations of O and R at the surface of the electrode (that is C_O^o and C_R^o) to a ratio in compliance with the Nernst equation.

$$E= E^o- RT/nF \ln C_R^o/C_O^o \quad (1.1)$$

where, R is the molar gas constant (8.3144 J $mol^{-1}k^{-1}$), T is the absolute temperature (k), n is the number of electrons transferred, F= Faraday constant (96,485 C/equivalence) and E^o is the standard reduction potential for the redox couple. If the potential applied to the electrode is changed, the ratio C_R^o/C_O^o at the surface will also change, so as to satisfy equation (1.1). If the potential is made more negative the ratio becomes larger (that is, O is reduced) and conversely, if the potential is made more positive the ratio becomes smaller (that is, R is oxidized).

For some techniques it is useful to use the relationship that links the variables for current, potential and concentration, known as the Butler-Volmer equation.

$$i/nFA= k^o\{C_O^o \exp [-\alpha\theta] – C_R^o \exp \{1-\alpha)\theta]\} \quad (1.2)$$

where, $\theta= nF (E-E^o)/RT$, k^o is the heterogeneous rate constant, α is known as the transfer coefficient, A is the area of the electrode.

This relationship allows us to obtain the values of the two analytical important parameters, i and k^o.

Finally, in most cases the current flow also depends directly on the flux of material to the electrode surface, the increased concentration provides the force for its diffusion toward the bulk of the solution. Likewise, when O or R is transformed, the decreased concentration promotes the diffusion of new material from the bulk solution. The resulting concentration gradient and mass transport is described by the Ficks law, which states that the flux of matter (ϕ) is directly proportional to the concentration gradient.

$$\phi = -AD_o (\partial c_o/\partial x) \quad (1.3)$$

where, D_o is the diffusion coefficient of O and x is the distance from the electrode surface. An analogous equation can be written for R. The flux of O or R at the electrode surface controls the rate of reaction and thus the faradaic current flowing in the cell. In the bulk solution, concentration gradients are generally small and ionic migration carries most of the

current. The current is a quantitative measure of how fast a species is being reduced or oxidized at the electrode surface. The actual value of this current is affected by many additional factors, most importantly the concentration of the redox species, the size, shape and material of the electrode, the solution resistance, the cell volume and the number of electrons transferred [13].

1.6. Solvents

A series of electrochemical properties must be considered while choosing a solvent [14] like being in a liquid state at room temperature, capable of dissolving electro active species of interests, having a large potential window and having required acid-base properties. The dielectric constant is the most important parameter for a solvent.

Water is the cheapest solvent, which possesses many physico-chemical properties. It can dissolve ionic components and form highly conducting solutions. Water, deionized and repeatedly distilled with alkaline $KMnO_4$, is usually considered as pure. The purity is checked by conductivity measurements. The volatile and organic impurities [15] are removed by passing the distilled water vapor through a column containing Pt catalyst at about 800°C over which oxygen also simultaneously passed.

Acetonitrile is perhaps a solvent with inert electrochemical properties. It has +3.0V (versus SCE) anodic and –3.0V cathodic limits. 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 –3.0 V for anion radicals. Hence, this is the solvent of choice for studies on anion radicals and dianinons. In the positive potential regions above +1.0V, the solvent itself decomposes. Cation radicals 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 [16] as well as surface [17-21] processes.

1.7. Supporting electrolytes

An electrolyte solution, whose concentration are not electro active in the range of applied potential being studied and whose ionic strength is usually much larger than the

concentration of an electro active substances to be dissolved in it. All ionic salts or ionizable compounds in a solvent are defined as supporting electrolyte. Supporting electrolyte influences the electrochemical process in number of ways as follows:

- These electrolytes must impart conductivity to the solvent.
- They must remain electro inactive in the potential region of interest.
- They should not get adsorbed on the surface of electrode in which case they can catalyze or inhibit other reactions.
- The electrolyte is added to the solvent to minimize double layer and migration current effects.
- These generally control the acidity of the ionic solution.

For example: KCl, $HClO_4$, HCl, NaOH, EDTA, KOH, $NaClO_4$, KCNS, $LiClO_4^-$, acetates, citrates, phosphates etc.

1.8. Electrodes

The initiation of modern electrochemistry created the need for new electrodes and electrode set-ups. The most familiar arrangement today is the electrochemical cell with three different electrodes.

- Working Electrode (WE)
- Reference Electrode (RE)
- Counter/Auxiliary Electrode (AE)

1.8.1 Working electrode (WE)

The WE is an electrode at which the reaction of interest takes place. The performance of the voltammetric procedure is strongly influenced by the material of the working electrode. The main criterion is the available potential window, which should meet the requirements of the investigations. Usually in the range of positive potentials, platinum, gold and carbon (graphite, glassy carbon) electrodes are used. The surface of these materials are partially oxidized in aqueous solutions at this potential ranges. Thin layers of oxides are formed at gold and platinum and various functional groups, like –C=O and –OH, are attached to the carbon materials. In the negative range of potential, in aqueous solutions solvents, mercury electrodes are superior due to high over potential of the reduction of

hydrogen on the other hand many organic compounds strongly adsorb on mercury, which may complicate the analysis of voltammograms. In aprotic solvents, platinum, gold and carbon electrodes can be used in both +ve and –ve ranges of potentials. Electrodes made of other noble metals, such as iridium and silver are less frequently used.

A solid electrode in comparison to a mercury drop usually requires very careful pretreatment. The electrode surface should be clean and polished on a very wet pad to mirror glass. This can be done using abrasive powders (or their suspensions in water), such as diamond and alumina, of various particle sizes. To obtain well defined voltammograms, it is also important that the electro active part of the electrodes is perfectly sealed into the electrode body. Otherwise the background for the voltammograms is usually excessive and steep.

To obtain reproducible curves, the solid electrode needs an electrochemical activation (modification). This is usually done by cycling the potential in an appropriate range while keeping the electrode in an appropriate solution. There is no universal range of potential and universal solution that can be employed for such activation.

Solid electrodes covered by membranes or modified with polymers, gels and various composite materials cannot be treated by polishing. The only way to make them work reproducibly is to apply an appropriate conditioning potential before the voltammetric experiments.

1.8.2 Reference electrode (RE)

A RE is an electrode which has a stable and well known electrode potential. 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. The selection of a proper reference electrode is equally vital in voltammetry especially when accurate and precise data on the formal potentials of the redox couples under examination are needed. Traditional electrodes based on Hg and Ag (Hg/HgCl, Hg/Hg_2SO_4, Ag/AgCl) can also be used; however, their concentrated electrolytes should be well separated from the analyzed solutions. In other words, everything should be done to prevent a leakage of the solution from the reference electrode to the cell and vice versa, when the experiments are performed with a two-electrode system, the current flows through the reference electrode. Under such

conditions the reference electrode potential may not be stable over a period. The smaller the working electrode the smaller is the risk of affecting the potential of the reference electrode. In the work introducing microelectrodes as the working electrodes, the two-electrode system is often used. If the three-electrode system is used, the reference electrode is charged with a very small current only (in the range of pico ampere). Such small current cannot affect the activities of the species that determine the potential of the reference electrode so, in justified situations, when the voltammetric half-wave potential or peak potential does not have to be known precisely and what really matters is the peak or wave height, the so called quasi-reference electrodes are used. Most often a piece of platinum foil is used as a quasi-reference electrode. Quasi reference electrodes are especially useful when voltammetry at a very low ionic strength solution is performed.

1.8.3 Counter electrode (CE)

In voltammetric studies, the current flows in between working and counter electrode. Platinum electrode is the most widely used counter electrode in aqueous, non aqueous as well as molten salt media. Platinum electrodes in the form of coils or thin foils are normally used. The area of the counter electrode must be sufficiently larger than the working electrode area.

1.9 Faradic current and capacitive current

The electric current flowing through the working electrode has two components:
The first, the faradic current, follows the faraday laws and is due to the discharge of the electro active compound (A_{ox}).

The second, the capacitive current, is produced by the growth of a double electrical layer on the interface between the electrode and the solution. This double layer is due to the high concentration of the supporting electrolyte in the solution and acts as a condenser with high capacity. The total current flowing through the electrode is finally due to the sum of the charging current (capacitive current) of this condenser and the faradic current.

The capacitive current acts as a non specific background interference of the faradic current and sometimes can be higher than the latter, when the depolarizer is present at low concentration in solution. In this case the measure of the faradic current is difficult and

some electronic adjustment has to be used. Therefore, the polarography and voltammetry is growth, as analytical technique, only after the progress in the electronic field: so affirm that the development of this technique is strictly linked to the tentative to electronically overcome problems due to capacitive current.

1.10 Electrode 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 a solution. The electrode reaction rate is governed by the reaction rates such as:

- Mass transfer
- Electron transfer of non-adsorbing species.
- 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.
- Other surface reactions such as adsorption, desorption, crystallisation etc.

The simplest reaction involves only mass transfer of reactant to the electrode, heterogeneous 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 [22-23].

1.10.1 Mass transfer processes

Mass transfer in electrochemistry is the movement of electroactive species from one location to another in solution arising from the differences in electrical or chemical potential. During the charge transfer process, the electroactive material gets depleted at the surface of the electrode and hence 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. The three modes of mass transport (Fig.1.3) generally considered are as follows:

- Diffusion
- Migration
- Convection

Diffusion is the spontaneous movement of those chemical compounds subjected to a concentration gradient that means a situation in which a zone of the solution is poorer than another with the process of diffusion the system tries to destabilize its homogeneity. The diffusion speed is directly proportional to concentration gradient and than to the concentration of the electro active compound in the solution.

Migration is the process of moving due to the attraction force of the electric field generated by the electrode toward every ion having opposite charge and also due to the contemporary repulsion force of every ion having the same charge of the electrode.

Convection is the process independently taking place by the discharge process, a solution is stirred or when in the solution is present a temperature or a density gradient. In this case the molecules of the solvent and the analyte move themselves with a more or less troublesome motion, but that become more laminar in the vicinity of the electrode surface. The layer of solution closer to the electrode surface is practically stationary.

1.11 Electron transfer 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 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 gain or lose electrons from or to the electrode surface respectively with the leak activation energy when a suitable potential is applied and macroscopically, we observe current. This state of 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 of oxidized. This final product after

undergoing suitable reorientation either gets deposited on the electrode surface or move 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

- Reversible process
- Irreversible process
- Quasi-reversible process

1.11.1 Reversible electron transfer process

For a reversible process, oxidation and reduction peak is observed as shown in Fig.1.4. 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

$$E = E^o + RT/ nF \ln C_{ox} / C_{red}$$

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;

$$i_p = (2.69 \times 10^5)\, n^{3/2} A D^{1/2} C_0 \nu^{1/2}$$

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 ν is the scan rate in Vs^{-1}.

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

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

1.11.2 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.1.5**)**. This process is usually due to slow electron exchange or slow chemical reactions at the electrode surface [24]. 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 [25]

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

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

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:

- no reverse peak
- $i_p \; \alpha \; \nu^{1/2}$
- E_p shifts = $30/\alpha n_a$ mV, where α is charge transfer coefficient
- $[E_p - E_{p/2}] = 47.7/\alpha n_a$ mV

1.11.3 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.1.6). The current due to quasi-reversible processes is controlled by both mass transport and charge transfer kinetics [26]. 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_p increases with scan rate, but is not proportional to scan rate.
- $i_{pc}/i_{pa} = 1$, provided $\alpha_c = \alpha_a = 0.5$

- ΔE_p is greater than 59/n mV and its increases with increasing scan rate
- E_{pc} shifts negatively with increasing ν

1.12 Applications of cyclic voltammetry

Cyclic voltammetry (CV) is the most effective and versatile electro analytical technique available for the mechanistic study of redox systems [27-28]. The method enables a wide potential range to be rapidly scanned for reducible or oxidizable species. This capability together with its variable time scale and good sensitivity makes this the most versatile electro analytical technique. It must, however be emphasized that its merits are largely in the realm of qualitative or "diagnostic" experiments. CV has its ability to generate a species during one scan and then probe its fate with subsequent scans. The power of CV results from its ability to rapidly provide information about:

- Reaction mechanism, rate constants, transfer coefficients-diffusion coefficients of redox processes, the kinetics of electron-transfer reactions and detection of chemical reactions coupled to electron transfer or adsorption processes.
- Number of electrons involved in each of the observed redox processes.
- Rapid location of redox potentials of the electroactive species present in new drugs.
- Analysis of the organic compounds, neuroactive compounds in pharmaceutical preparations.
- In vivo analysis of biologically significant molecules.
- "Fingerprint" electrochemical reactions for benchmarking reactions occur.
- Relative surface area and roughness.
- Whether the redox process is kinetic or diffusion controlled ?
- Whether the redox behavior is affected by a change in the concentration of the electroactive species, solvent system, or the electrode surface ?
- Reaction intermediates and their identification in the electrode reaction.
- Products formed in the electrochemical reaction.
- Surface contamination.
- Charge storage capacity.

1.13 A brief literature survey of cyclic voltammetric investigation

A series of work has been reported by many researchers and their group towards the electrochemical investigations of various analytes using cyclic voltammetric methods. Research interests involve the modification of carbon paste electrode, glassy carbon electrode, and pencil graphite electrode by different modification methods like electropolymerization, electrochemical pre-treatment, mobilization and immobilization of surfactants etc. The developed sensor has been utilized for the investigation of some biomolecules like dopamine, uric acid, folic acid, paracetamol, epinephrine, norepinephrine, catechol, and hydroquinone.

P.R. Roy *et. al*., [29] studied the simultaneous electrochemical determination of dopamine and ascorbic acid in 0.2 M phosphate buffer solution as supporting electrolyte using poly (N,N-dimethylaniline) modified glassy carbon electrode. C.R. Raj *et. al*., [30] worked on electroanalytical applications of cationic self-assembled monolayers using square-wave voltammetric determination of dopamine and ascorbate. J.M. Zen *et. al.,* [31] fabricted the poly (4-vinylpyridine)-coated chemically modified electrode for the detection of uric acid in the presence of a high concentration of ascorbic acid. K. Pihel *et. al*., [32] designed the overoxidized polypyrrole-coated carbon fiber microelectrodes for dopamine measurements with fast-scan cyclic voltammetry. L. Zhang *et. al*., [33] explained the covalent modification of glassy carbon electrode with glutamic acid and its application of the simultaneous determination of uric acid and ascorbic acid. B.Venton *et. al*., [34] described the correlating neurochemical changes in the brain with behavior marks the beginning of an exciting new interdisciplinary field, psychoanalytical chemistry. F. Martinello *et. al*., [35] studied the ascorbic acid interference in the measurement of serum biochemical parameters: In vivo and in vitro studies. G.P. Jin *et. al*., [36] prepared the novel choline and acetylcholine modified glassy carbon electrodes for simultaneous determination of dopamine, serotonin and ascorbic acid. W. Ma *et. al*., [37] described the electrochemical properties of dopamine, epinephrine and their simultaneous determination at a poly (l-methionine) modified electrode. Z.-N. Gao *et. al*., [38] made a study of electrocatalytic oxidation of L-cysteine at glassy carbon electrode by (FcM) TMA and its electrochemical kinetics. S. Thiagarajan *et. al*., [39] fabricated an easy modification of

glassy carbon electrode for simultaneous determination of ascorbic acid, dopamine and uric acid. S.A. Kumar *et. al*., [40] studied the electrochemical selective determination of ascorbic acid at redox active polymer modified electrode derived from direct blue. M.Tsunda *et. al*., [41] pointed out the recent advances in methods for the analysis of catecholamines and their metabolites. P. Hernandez *et. al*., [42] designed a carbon fiber ultramicroelectrode for the determination of epinephrine using cyclic voltammetric technique. M.H. Sorouraddin *et. al*., [43] studied the spectrophotometric determination of some catecholamine drugs using sodium bismuthate. A. Tzontcheva *et. al*., [44] studied an analytical interference of drugs on the fluorimetric determination of urinary catecholamines. H.B.He *et. al*., [45] described the determination of catecholamines in sheep plasma by HPLC-ED: Comparison of deoxyepinephrine and 3,4-dihydroxybenzylamine, as internal standard. D.C. Chen *et. al*., [46] studied the determination of urine catecholamines by capillary electrophoresis with dual-electrode amperometric detection. F. Li *et. al*., [47] studied the determination of adrenaline using inhibited Ru(bpy)32+ electrochemiluminescenc. J. Michalowski *et. al* [48] described the flow-injection chemiluminescence determination of epinephrine in pharmaceutical preparations using raw apple juice as enzyme source. Y.Z. Zhou *et. al.*, [49] carried out the work on electroanalysis and simultaneous determination of dopamine and epinephrine at poly (isonicotinic acid)-modified carbon paste electrode in the presence of ascorbic acid. S Shahrokhian *et. al*., [50] studied the application of carbon-paste electrode modified with iron phthalocyanine for voltammetric determination of epinephrine in the presence of ascorbic acid and uric acid. R.C. Matos *et. al*., [51] fabricated the modified microelectrodes and multivariate calibration for flow injection amperometric simultaneous determination of ascorbic acid, dopamine, epinephrine and dipyrone. H. Yaghoubian *et. al*., [52] carried out a work on simultaneous voltammetric determination of norepinephrine, uric acid and folic acid at the surface of modified chloranile carbon nanotube paste electrode. M. Zhu *et. al*., [53] designed the peroxidase-based spectrophotometric methods for the determination of ascorbic acid, norepinephrine, epinephrine, dopamine and levodopa. L.P. Lu *et. al*., [54] done a fabrication of layer-by-layer deposited multilayer films containing DNA and gold nanoparticle for norepinephrine biosensor. Mazloum-Ardakani *et. al*., [55] designed a novel

carbon nanotube paste electrode for simultaneous determination of norepinephrine, uric acid and d- penicillamine. L. Lin *et. al*., [56] studied an electrocatalytic oxidation and determination of norepinephrine in the presence of ascorbic and uric acids at a poly (evans blue)-modified glassy carbon electrode. A.R. Taheri *et. al*., [57] fabricated the modified carbon nanotube paste electrode and applied to the simultaneous voltammetric determination of norepinephrine and folic acid. Y.Y. Su *et. al*., [58] described the determination of epinephrine based on its enhancement for electrochemiluminescence of lucigenin. T. Kamidate *et. al*., [59] studied the effect of mixing modes on chemiluminescent detection of epinephrine with lucigenin by an FIA system fabricated on a microchip. W. Ren *et. al*., [60] studied simultaneous voltammetric measurement of ascorbic acid, epinephrine and uric acid at a glassy carbon electrode modified with caffeic acid. M.C. Buzzo *et. al*., [61] discussed the use of room temperature ionic liquids in gas sensor design. P. He *et. al*., [62] worked on electrochemical deposition of silver in room-temperature ionic liquids and its surface-enhanced raman scattering effect. E. Rozniecka *et. al*., [63] studied the electroactive ceramic carbon electrode modified with ionic liquid. G. Shol *et. al*., [64] described the ion transfer at carbon paste electrode based on ionic liquid. Zhao *et. al*., [65] studied the electrochemical and bioelectrochemistry properties of room-temperature ionic liquids and carbon composite materials. X. Lu *et. al*., [66] studied the composite system based on chitosan and room-temperature ionic liquid: Direct electrochemistry and electrocatalysis of hemoglobin. Q.P. Yang *et. al*., [67] carried out the voltammetric determination of uric acid with a glassy carbon electrode coated by paste of multiwalled carbon nanotubes and ionic liquid. W. Sun *et. al.,* [68] studied the direct electrochemistry and electrocatalysis of hemoglobin in sodium alginate film on a $BMIMPF_6$ modified carbon paste electrode. Xiao-Lin Wen *et. al*., [69] described the studies of micellar effects on the electrochemistry of dopamine and its selective detection in the presence of ascorbic acid. Bhim Bali Prasad *et. Al.*, [70] studied the one monomer doubly imprinted dendrimer nanofilm modified pencil graphite electrode for simultaneous electrochemical determination of norepinephrine and uric acid, Gulcin Bolat *et. Al.,* [71] worked on the highly sensitive electrochemical assay for Bisphenol A detection based on poly (CTAB)/MWCNTs modified pencil graphite electrodes, Nimisha Jadon *et. Al*., [72]

studied electrochemical analysis of amlodipine in some pharmaceutical formulations and biological fluid using disposable pencil graphite electrode.

1.14 Objectives of the thesis

The main focus of this thesis is to study the versatility of modified electrodes (carbon paste electrode, glassy carbon electrode and pencil graphite electrode) in terms of its application to investigate the electrochemical behavior of some electro active drugs. The main objectives are listed as follows:

- Selection of the modifiers to modify the surface of the electrodes.
- Optimization of the modified electrode for the consistent study of the oxidation behavior of the biomolecules.
- Determination of the modified electrode process.
- Simultaneous determination of dopamine/norepinephrine/epinephrine, ascorbic acid, paracetamol, folic acid, catechol, hydroquinone, and uric acid using differential pulse voltammetric and cyclic voltammetric techniques at bare and modified electrodes.
- Reproducibility of the modified carbon paste electrode.
- Application of the modified electrode towards real sample analysis.

1.15 Scope of the present study

The thesis is aimed at development of electrochemical sensors by modifying the surface properties of carbon electrodes (carbon paste electrode, glassy carbon electrode, and pencil graphite electrode) by different methods like electropolymerization, electrochemically grinding, surfactant mobilization/immobilization, and electrochemical pre-treatment. After the modification, the fabricated sensor has been utilized for the electrochemical determination of some electro active biomolecules such as dopamine, epinephrine, norepinephrine, paracetamol, folic acid, uric acid, catechol and hydroquinone individually and simultaneously by cyclic voltammetric and differential pulse voltammetric methods.

The fabrication of the chemically modified electrodes in electroanalysis offers numerous advantages due to their unique electrode surface properties. The modifiers are used for the fabrication of electrode have not shown its redox nature or have not interfered their peaks with the analyte but facilitate in the electron transfer and acts as good electrocatalyst. 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.

Beside 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 preparation, characterization and application to pharmaceutical and real samples. Also, the electrochemical properties, carbon composition and surface roughness of both the surfaces are examined.

Moreover, a much attention has been given to investigate the electrode process near the electrode surface is 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 products formed when compounds are reduced or oxidized electrochemically, electrochemical studies and elucidation of the sequence of electron transfer and chemical reactions that occur at or near the electrode surface etc can be studied.

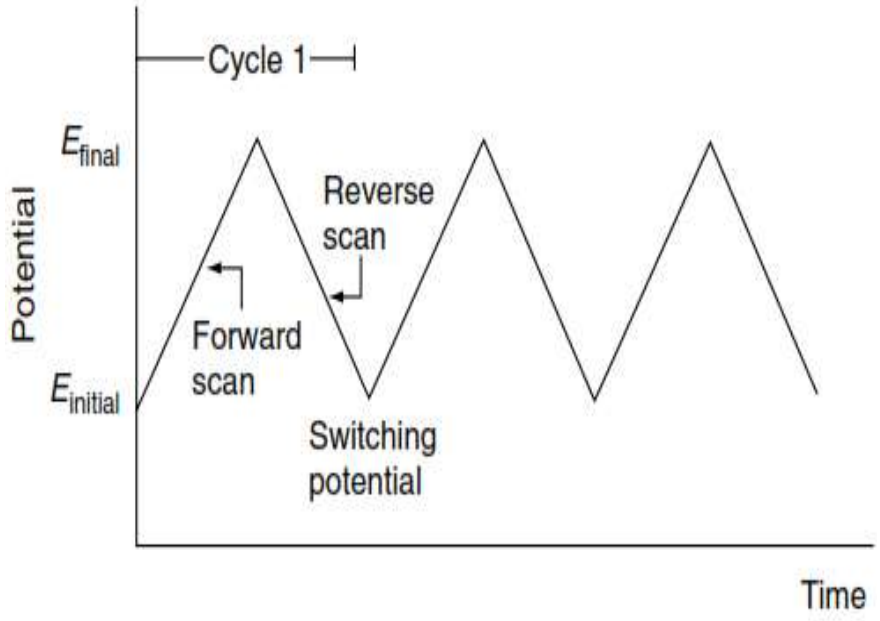


Fig.1.1: Variation of the applied potential as a function of time in a cyclic voltammetry experiment.

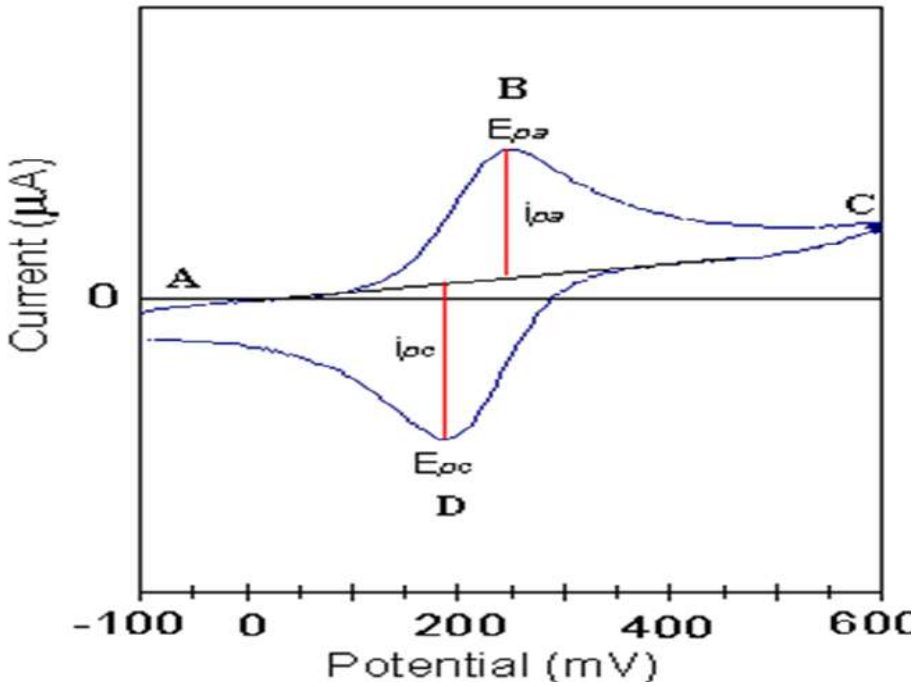


Fig.1.2: Typical cyclic voltammogram

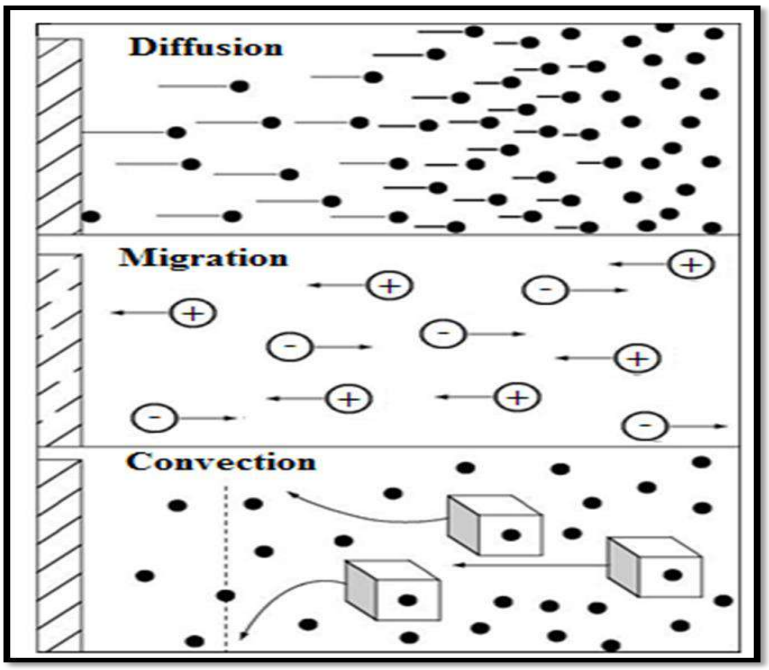


Fig.1.3: Modes of mass transport

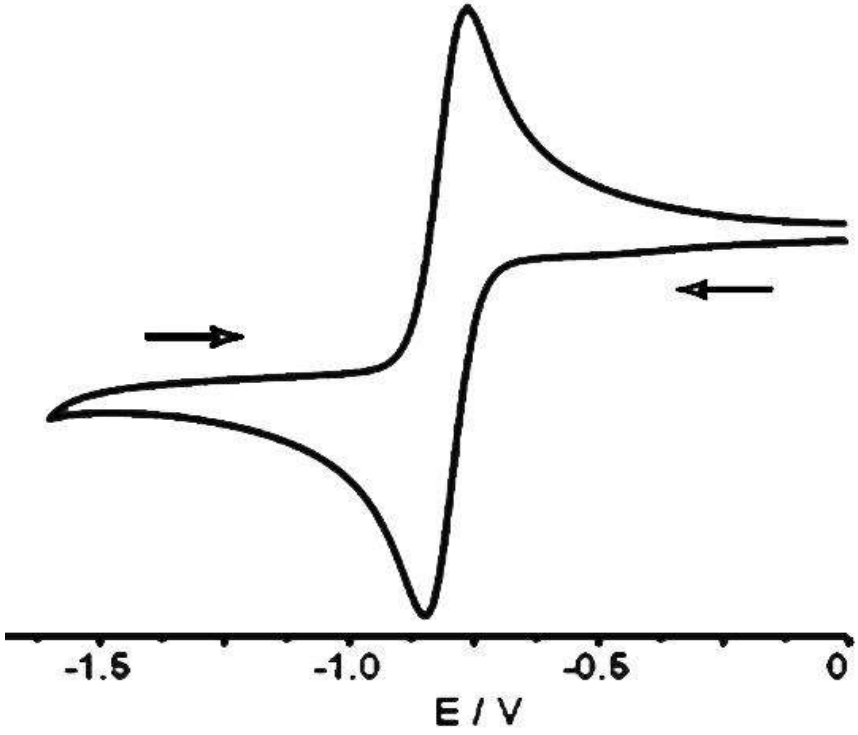


Fig. 1.4: Typical voltammogram for a reversible process

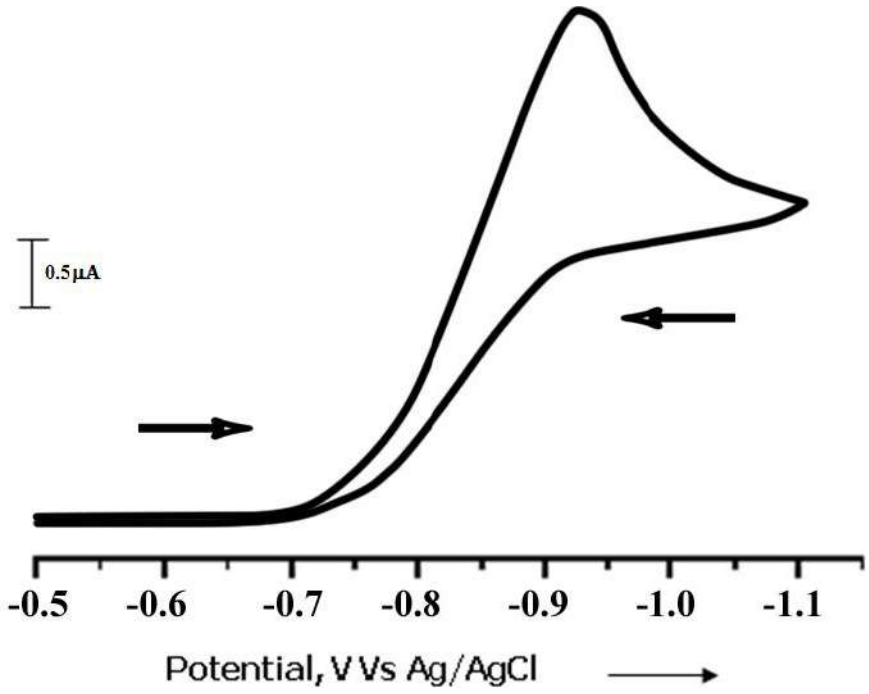


Fig.1.5: Typical voltammogram for an irreversible process

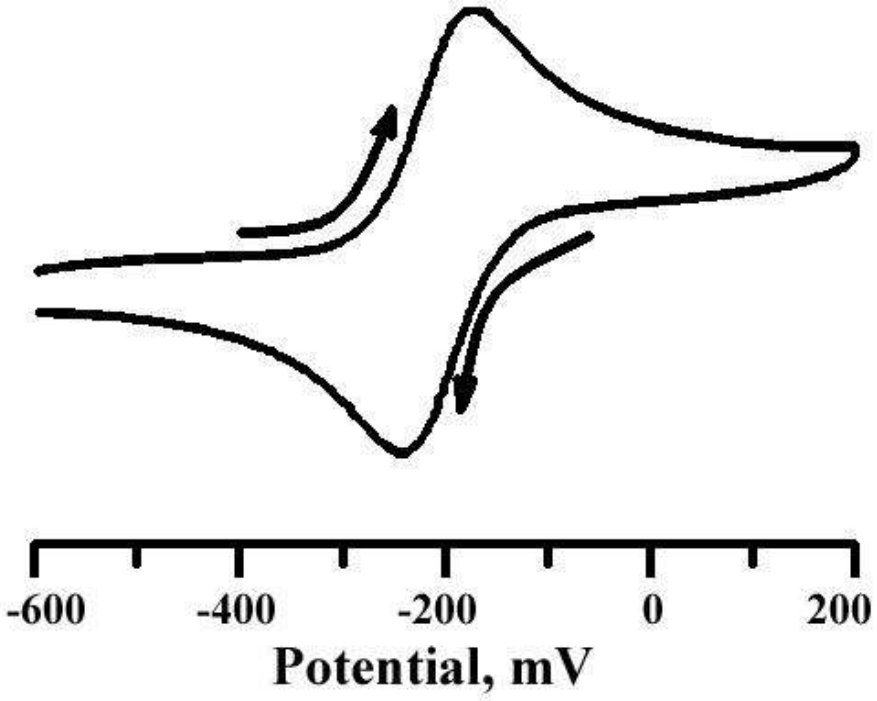


Fig.1.6: Typical voltammogram for a quasi-reversible process

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Chapter -2

Experimental

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 has been discussed. The electrode system with special emphasis on carbon paste electrodes, glassy carbon electrodes and pencil graphite electrodes used in the course of this research is outlined. The preparation and characterization of carbon paste electrodes and modification of the electrodes by different methods like electropolymerization methods, electrochemical pre-treatment methods, immobilization of surfactants and the procedures used in the present work are detailed. In addition, an overview of the theories characterization techniques and related equations are described.

2.2. Experimental techniques

The chief electrochemical / analytical techniques used throughout this study were cyclic voltammetry and differential pulse voltammetry. A brief overview of each technique is given below.

2.2.1. Cyclic voltammetry

Cyclic voltammetry (CV) is often the first experiment performed in an electroanalytical study and can be used to obtain information about simple and complicated electrode reactions. As a result, cyclic voltammetry is one of the most useful and widely applied techniques in electrochemistry. Cyclic voltammetry is a dynamic electrochemical technique wherein 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 in the study of the electrochemical behavior of the polymer film modified carbon paste electrode towards the oxidation and reduction of dopamine (DA). It was also used to investigate the effect of numerous interfering compounds on the DA signal and as an alternative approach to synthesize 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.

2.3. Instrumentation and basic equipments

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 placed in the cell. 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 modern potentiostats 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 WE (s) (Fig. 2.1).

2.3.1.1. The 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 Electrochemical work station (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 instrumentation in 1967 [1] or even earlier. Computer applications in stationary electrode voltammetry [2, 3] and CV [2-6] were reported. Computers can be used to apply potential programmed to the WE through the potentiostat. The initial potential, final potential, sweep rate, the nature of 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 into digital information by an 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 [7, 8] by subtraction of

background current [4]. Voltammetric curves may be differentiated to obtain peak potentials with greater precision [9]. 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 processes and for evaluating the rate parameters.

2.3.3. Electrochemical cell

In its simplest form, the electrochemical cell was glassware capable of holding an appropriate volume of a test solution containing one or more electro active analyte. The cell is then maintained oxygen free by passing nitrogen over the solution through nitrogen inlet. The electrochemical cell consists of three electrodes which are immersed in this solution and are electrically connected to the potentiostat. The RE used is SCE, which is often isolated from the solution by a salt bridge to prevent contamination by leakage from the RE. The AE (platinum foil) and WEs (modified carbon paste electrode, electropolymerised carbon paste electrode and carbon paste electrode) are placed directly into the solution (Fig.2.3). Custom glassware designs include convenient fittings for mounting electrodes, gas inlets and outlets for purging oxygen and temperature jackets. Since the limiting (peak) current in any type of voltammetry is temperature dependent, the cell is thermo stated for the required temperature.

2.4. pH meter

A pH meter, manufactured by Systronic Digital model 335 was used for measuring and adjusting the pH of the solutions making use of a combination of glass and SCE.

2.5. Electrodes

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 or modified carbon paste electrode, glassy carbon electrode and pencil graphite electrode.

2.5.1. 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, the selection of working electrodes depends on the redox behaviour 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 electroanalytic applications. The most popular ones are those involving mercury, carbon, or noble metals (platinum and gold).

2.5.1a. Carbon Electrodes

Solid electrodes based on carbon are currently in widespread use in electroanalysis, primarily because of their broad potential window, low background current, low cost, chemical inertness, and suitability for various sensing and detection applications. In contrast, electron transfer rates observed at carbon surfaces are slower than those observed at metal surfaces. The electron transfer activity is affected by the carbon surface structure. A variety of electrode pre-treatment procedures have been proposed to increase the electron transfer rates. The type of carbon, as well as the pre-treatment method has a profound effect upon the analytical performance. The most popular carbon-electrode materials are glassy carbon, carbon paste, carbon fiber, carbon films, or carbon composites.

2.5.1b. Carbon paste electrodes: Important developments

Carbon paste electrodes (CPE) and related sensors underwent an attractive development. Its inspiring history, illustrating potentialities of electrochemistry as a whole reveal numerous connections with the current trends in electrochemistry. In the initial stage CPE were employed mainly in studying the mechanisms of electrode reaction of various organic compounds [10]. The first modification was done in 1964 in which an organic compound was dissolved with binder [11] and this, which served to study the electrode behavior of the substance itself, was considered as a pioneering step in the field of carbon paste electroactive electrodes. In 1965, CPE was prepared by rubbing a modifier into the

paste had represented its case with which a CPE could be modified [12]. The replacement of non electroactive pasting liquids by electrolyte solution [13] in 1974 opened a new branch of carbon paste electroactive electrodes which at present belong to a special field called solid state electrochemistry [14]. The era of chemically modified electrodes culminated at the beginning of 80's. The modification of carbon paste by impregnating the carbon particles with methanolic solutions of dimethyl glyoxime [15] represents another milestone in the history of CPE.

2.5.2. Carbon paste as electrode material

2.5.2.1. Unmodified carbon paste

Binary mixtures prepared from carbon powder and organic liquid of non-electrolytic character are known as unmodified (virgin or bare) carbon paste [16]. The proper electroactive moiety in carbon paste is still graphite powder with micrometric particles of high purity and distribution uniformity. Such materials are now commonly available on the market as spectroscopic graphite. Non-electrolytic binders such as Nujol [17-19] and Silicone oil [20] are non-polar pasting liquids fulfil all the important criteria; both are chemically inert, insulating, non-volatile, 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 pre-treatment.

2.5.2.2. Modified carbon paste

The modified carbon paste is usually a mixture of graphite powder, non-electrolytic binder and a modifier [16, 18, and 21]. Modifying agent is usually one substance; but, the paste 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 [19] or chemically modified carbon paste electrode (CMCPEs) with a mixture of two modifiers [22]. 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[23] or molecules of an extractant in the bulk [24]. In general, the major cause for

modifying an electrode is to obtain qualitatively new sensor with desired, often pre-defined properties.

In contrast to relatively complicated modifications of solid substrates, the preparation of chemically modified carbon paste electrodes (CMCPEs) is very simple typically by means of various alternative procedures. Modifier can be dissolved directly in the binder [22, 25] or admixed mechanically to the paste during its homogenisation [26, 27]. It is also possible to soak graphite particles with a solution of a modifier, and after evaporating the solvent, use so impregnated carbon powder [28]. Finally, already-prepared paste can be modified in situ [23]. Whereas direct modifications obviously provide special sensors for one-purpose use, considerate in situ approaches offer a possibility to employ the same carbon paste for repetitive modifications with different agents. A number and a diversity of substances used for the preparation of CMCPEs have grown in a geometric order. Among the modifiers recently used, one can find single compounds [26] sophisticated chemical agents [22, 28 and 29] special inorganic materials and matrices [39-54]. Classical analytical reagents like dimethyl-glyoxime [29] 8-hydroxyquinoline [22, 30, 31] or derivatives of 2-naphthol [32, 33] have been used as selective modifiers for adsorptive accumulation of selected ions. Cetyltrimethylammonium bromide (CTAB) served again as a reliable modifier to preconcentrate and detect some less common metal species [34-37], whereas chromatographic packing agent "Amberlite IRC-718" can be recommended for speciation analysis [38]. Zeolites [43, 44] and related materials e.g., montmorrilonite [40, 41, 55] or vermiculite [37, 42] have been shown to exhibit also adsorption and catalytic capabilities. Living Organisms like the use of peat moss [55] or algae [56] or, more recently, bacteria [57] and chitin (horny substance forming insect bodies) [58] have been used.

2.5.3. 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:

- particle size in micrometers

- uniform distribution of the particles
- high chemical purity and
- 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 mm).

2.5.4. Pre-treatment of carbon powder

In order to lower adsorption capabilities of graphite, Lindquist [59] proposed a special treatment of graphite powder where he tried to remove adsorbed oxygen by heating in a vacuum with subsequent stabilization by impregnating with a ceresin wax. Although the method was found effective his recipe did not found many continuators because of rather complicated and time consuming procedure.

2.6. Binder (pasting liquid)

Traditional carbon pastes contain liquids which link mechanically the individual graphite particles. However, beside this main function, the binder as the second main moiety of carbon paste co-determines its properties. Typical parameters required for pasting liquids are as follows:

- chemical inertness and electro inactivity
- high viscosity and low volatility
- minimal solubility in aqueous solutions and
- immiscibility with organic solvents

The most popular binding agents used for the preparation of carbon pastes are mineral (paraffin) oils such as Nujol oil and various silicone oils [60]. Also room-temperature ionic liquids (R ILs or ILs respectively) have soon come into the fore of research interest, which is also reflected in the electrochemistry with carbon pastes [61-64].

2.7. Polarization characteristics in dependence of the carbon paste composition

In the current flow-based experiments, the polarizability of CPEs can be compared to those of related carbonaceous substrates [65-67]. But, in contrast to graphite and other compact electrode materials, both anodic and cathodic potential ranges, as well as the background level, can be controlled via the quality of both main carbon paste components and their ratio; hereby one can postulate more or less specific polarizability of carbon paste electrodes.

2.8. Background (residual) signal at CPEs

In faradic measurements with common types of CPEs and CMCPEs, the background currents are typically below 1 μA; this value being recommended by Adams [66] as the level 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.

2.9. The highest potential limits attained at CPEs

For anodic polarizations, such a priority can be attributed to a value of +1.85 V versus SCE specified for a CPE with impregnated graphite [68]. In cathodic measurements, despite less favorable dispositions of carbon paste for polarization at negative potentials, some special carbon paste 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 whose surface manifested an inhibition effect (against the release of H_2) and could thus be polarized down to -2.0 V versus Ag/AgCl.

2.10. Preparation and standardization of CPE used in the present study

The CPE was prepared by hand mixing graphite powder and silicone oil in an agate mortar to produce a homogeneous mixture. This is advantageous because the analyst can by himself choose the individual components as well as their mutual ratio despite the fact that ready-to-use carbon paste mixtures are commercially available.

Carbon powder and the pasting liquid can simply be mixed and homogenized using a pestle and agate. Porcelain dish is avoided due to the possible contaminations of the paste with porcelain particles originating from the rough rubbing wall. A clean small nickel spatula is used to mix both the components carefully. The powder so mixed in an agate is then rubbed by 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. 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.

2.11. Construction of carbon paste electrode

CPE holders are typically glass or Teflon rods whose end hole can be easily refilled with a new portion of carbon paste. The paste is tamped into a well like depression at one end of the Teflon or glass holder. At this same end, inside the Teflon tube, a graphite rod is placed and the end of this graphite rod is connected to a copper wire which emerges out at the other end of the Teflon tube serves to establish electrical contact with the external circuit (Fig.2.4).

2.12. Storage of carbon paste electrode

The CPE could be placed in a beaker containing distilled water and the tip filled with the paste is completely dipped down to the water level. Such storage prevents the desiccation of carbon paste. The CPE stored in this manner exhibit a very stable behavior.

2.13. Electrochemical probe system for characterization of CPE in voltammetry

2.13.1. Potassium ferrocyanide system

The surface of CPE can be studied by its effect on the rate of electron transfer. This can be judged qualitatively by examining the separation of the peak potentials in a cyclic voltammogram of a molecule whose electron transfer kinetics are known to be sensitive to the state of the surface. To evaluate the overall quality of the paste $[Fe(CN)_6]^{4-}$ / $[Fe(CN)_6]^{3-}$ model system recommended is used [69-70].

2.13.2. Calculation of surface area of the electrode

The surface area of CPE was determined using potassium ferrocyanide (1 mM) system in presence of 1M KCl as supporting electrolyte. The effect of scan rate on the redox peak current of potassium ferrocyanide has been studied from 0.05 - 0.5 Vs^{-1} as shown in Fig.2.5. For a reversible redox couple, the number of electrons transferred in the electrode reaction can be determined by the separation between the peak potentials $\Delta Ep = E_{pa}-E_{pc}/n \approx 0.059$ V. The value found to vary between 0.061 to 0.066 V which correspond to one electron. It is found that the separation of the peak potentials is independent of the scan rate. Also, the ratio of i_{pa}/i_{pc} is found to be close to one (≈ 0.9864) which is a typical behaviour exhibited by a reversible electrochemical charge transfer. On substitution of the diffusion-coefficient value (14.18×10^{-5} cm^2s^{-1}) in the equation (1) [71]

$$i_p = 2.69 \times 10^{5} n^{3/2} A C_o Do^{½} \nu^{½} \quad (1)$$

Where, i_p is the peak current, A is the area of the electrode in cm^2, n is the number of electrons involved in the electrode reaction, C_0 is the concentration in mol cm^{-3}, D_o is the diffusion coefficient in cm^2 s^{-1} and ν is the scan rate in Vs^{-1}. The surface area of the electrode was found to be 0.031 cm^2.

2.14. Removal of dissolved oxygen

Once the sample with supporting electrolyte has been added to the cell, the solution is deoxygenated. Oxygen is capable of dissolving in aqueous solutions at millimolar levels at room temperature and atmosphere pressure. It is often necessary to eliminate dissolved oxygen from the test solution whenever moderate to quite negative potentials are being applied to the working electrode. At these potentials dissolved oxygen can be reduced and the resulting undesired cathodic current may interfere with the measurement of interest. Depending upon the solution pH, dissolved oxygen undergoes reduction in acidic media is in two steps:

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

The potentials of these steps are approximately – 0.05 V and -0.9 V (versus SCE), respectively. These reductions result in an increased background current that obscures the stripping peaks in stripping voltammetry and interferes even in CV experiments. Additionally, oxygen may oxidize the amalgamated metals. In neutral to slightly basic media, hydroxyl ions formed during the reduction of oxygen and can precipitate metals ions at the electrode solution interface.

2.15. Glassy carbon electrode used as working electrode

Glassy carbon electrode is very popular because of its excellent mechanical and electrical properties, wide potential window, chemical inertness (solvent resistance), and relatively reproducible performance. Glassy carbon was first used as an electrode material by Zittel and Miller [72] and many workers continue to use the electrode. The properties of glassy carbon have been described by Yamada and Sato [9].

Before performing the electrochemical experiments, the surface of GCE was polished to a mirror-like surface with 0.05 mm gamma alumina slurry on a polishing cloth and rinsed thoroughly with doubly distilled water between each polishing step. The polished electrode was electrochemically cleaned and characterized by cycling the potential between -1.4 and 1.8 V at 0.1 Vs^{-1} for 10 multiple cycles in 0.1 M H_2SO_4 until a stable cyclic voltammogram for the cleaned GCE dried was obtained.

2.16. Pencil graphite electrode used as working electrode

Pencil graphite electrode (PGE) is a new type of carbon electrode which has been used for the determination of a few varieties of analytes by voltammetric techniques [73-74]. Disposable pencil graphite electrode when compared with others forms of carbon electrodes; “low tech” pencil graphite electrode is extremely inexpensive and provides an attractive alternative to “high tech” carbon electrodes [75]. The experimental procedure for the modification of carbon based electrodes can be simplified if the modifying agent is added to the background electrolyte, which is termed *in situ* modification.

The Pencil graphite electrode has been successfully acting as a biosensor in modern electroanalytical field. A porous composite is consisting of graphite particles, polymeric

binder and other additives such as clay. Due to high electrochemical reactivity, high electrical conductivity, good mechanical rigidity, low cost, low technology, high electrochemical reactivity, ease of modification, renewal, low background current, and miniaturization, the PGE has good application in analysis of neurotransmitter and in the detection of traces of metal ions [76-79]. PGE has a larger active electrode surface area and is therefore able to detect low concentrations and/or volume of the analyt [80]. This type of electrode has been successfully applied to design various biosensors [81–84].

The PGE surface shows high electrical conductivity and serves as a suitable surface on which analyte can easily be adsorbed. The use of pencil graphite electrodes has several advantages, such as avoidance of contamination among samples, ease of use due to lack of need for pre-treatment, constant sensitivity, selectivity and reproducibility.

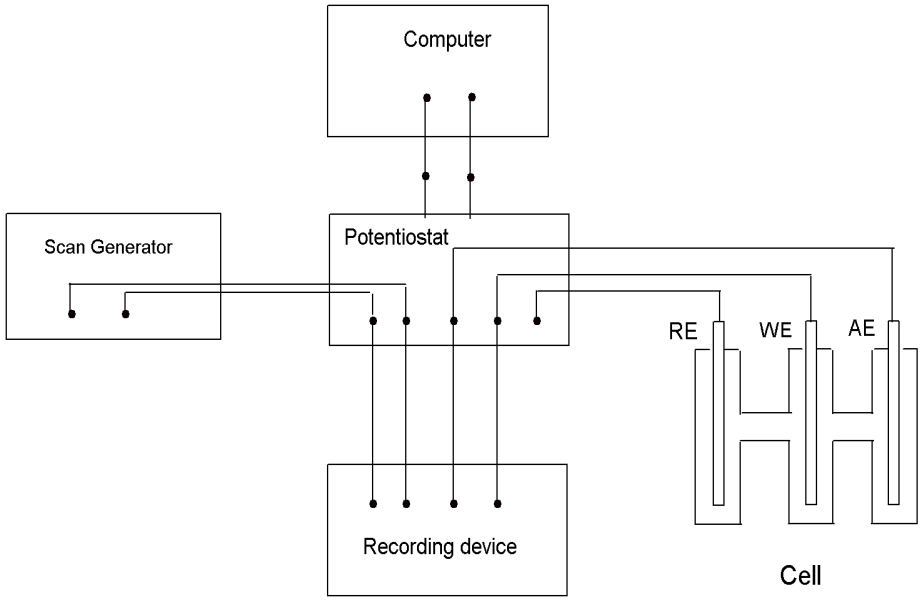

Fig. 2.1: Experimental set up of potentiostat for cyclic voltammetry.

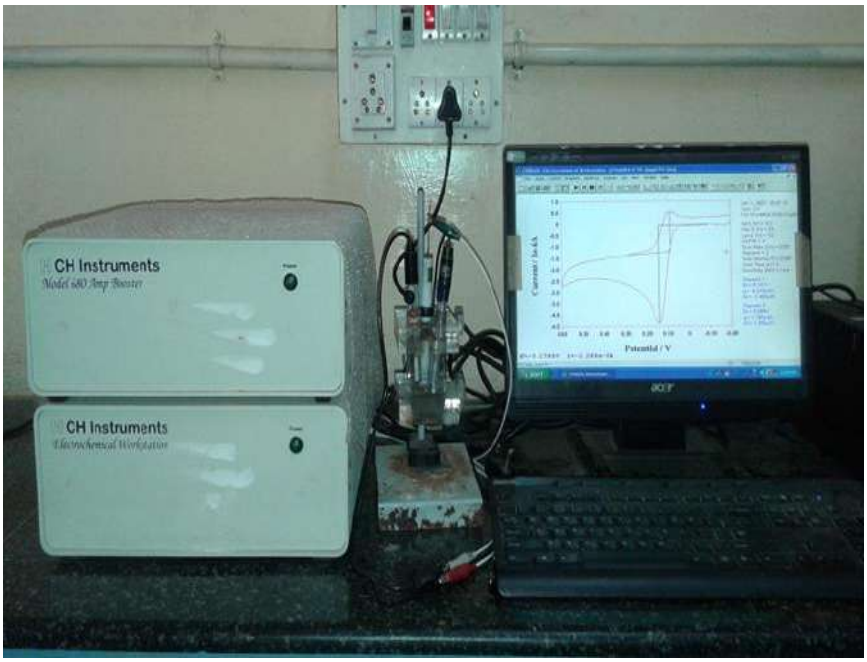

Fig.2.2: Experimental set set-up used to record all electrochemical measurements.

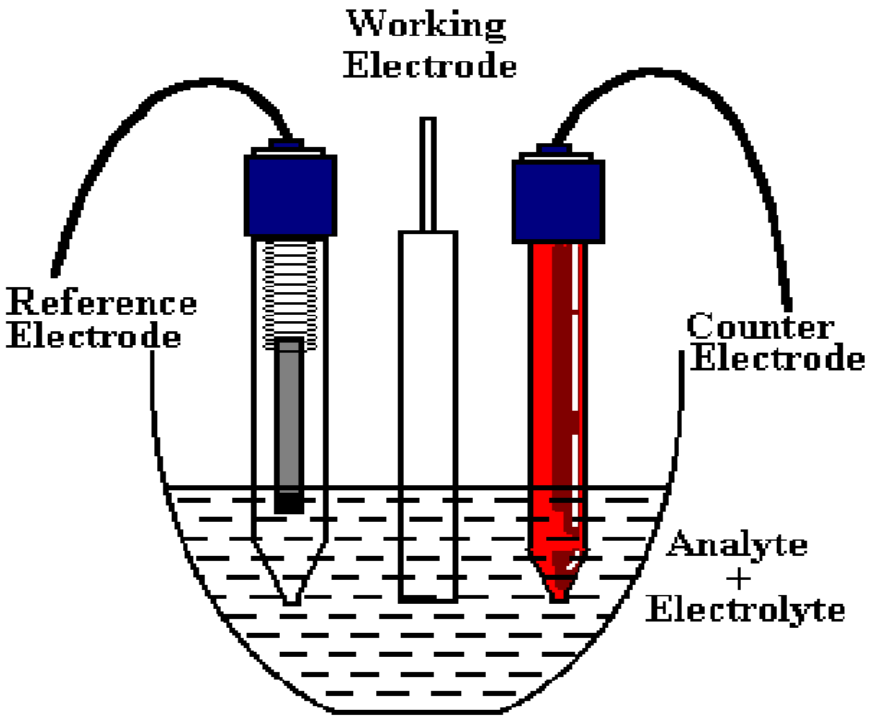

Fig.2.3: Schematic representation of an assembled electrochemical cell containing an electrolyte solution and the three electrodes (WE, RE and CE) for cyclic voltammetric experiments.

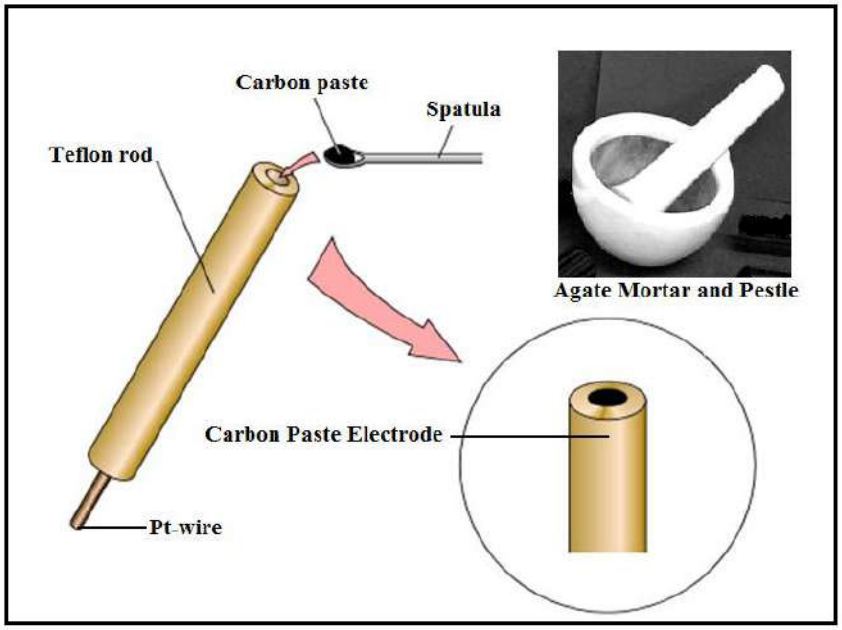

Fig.2.4: Carbon paste electrode preparation and filling

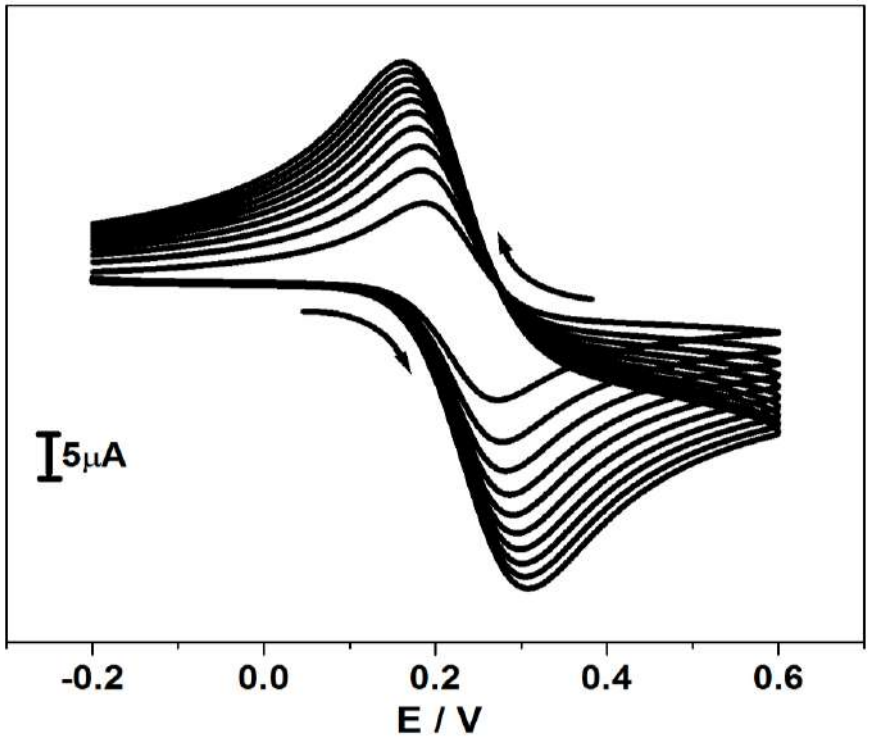

Fig.2.5: Cyclic voltammograms of 1 mM potassium ferrocyanide at BCPE in 1 M KCl at different scan rate (0.05 to 0.5 Vs^{-1})

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Chapter -3

Part-A

Modification of carbon paste electrode by electrochemical polymerization of neutral red and its catalytic capability towards the simultaneous determination of catechol and hydroquinone: A voltammetric study

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3.1 Introduction

In recent years, various methods have been employed for the determination of phenols. Among them electrochemical sensors have attracted more attentions, which are usually prepared by modifying different materials, such as polymers [1–4] and carbon materials [5–6] on supported electrodes. Thin film of conducting polymers is one of the most attractive electrode materials during last decades, which enhance the speed, sensitivity and flexibility of various sensors and biosensors [7–9]. In present study, a thin layer of poly (neutral red) (PNR) was developed which shows good electrocatalytic activity towards the determination of phenols.

Catechol (CC) and Hydroquinone (HQ) are two phenolic compounds commonly exist in nature, which are widely used in cosmetics, pesticides, tanning, medicines, flavouring agents, antioxidant, dye and photography chemicals [10]. Due to their carcinogenicity, genotoxicity and toxicokinetic effect on human and their low degradation in environment, they are considered as environmental pollutants by the US Environmental Protection Agency (EPA) and the European Union (EU) [11]. Catechol and hydroquinone have similar structures and properties; hence the oxidation and reduction of the dihydroxybenzene isomers are always overlapped at the unmodified electrodes which make it difficult for the simultaneous determination. Therefore, it is essential to develop highly sensitive and simple methods for the determination of CC and HQ simultaneously. There are some analytical methods have been established for the determination of catechol and hydroquinone, such as capillary electrochromatography [12], high performance liquid chromatography [13], fluorescence [14], chemiluminescence [15], spectrophotometry [16], gas chromatography/mass spectrometry [17] and electrochemical methods [18-32]. In contrast, electrochemical methods have attracted more attention because of its simplicity, high sensitivity and feasibility [33-46].

Neutral red (NR) (scheme 1) is a planar phenazine dye, utilized for electrochemical investigations of biological system [47]. The structure of neutral red is similar to other planar dyes such as acridine, thiazine and xanthenes [48-49]. Neutral red acts as a redox mediator in enzyme biosensors [50-55] and exhibits outstanding electrocatalytic activities [56-57]. Neutral red can be easily undergoes electropolymerization on the surface of

electrode [58]. In this work, neutral red was electropolymerized on the surface of carbon paste electrode to obtain poly (neutral) red (PNR) modified carbon paste electrode (MCPE).

In this chapter, poly (neutral red) modified carbon paste electrode (PNR/MCPE) was developed and utilized for the simultaneous determination of catechol and hydroquinone in presence of 0.2 M phosphate buffer solution (pH 7.4) with the scan rate of 50 mVs^{-1}. The proposed electrode exhibits good sensitivity, stability and reproducibility towards the determination of catechol and hydroquinone by both cyclic voltammetry (CV) and differential pulse voltammetric (DPV) methods.

3.2 Experimental

3.2.1 Apparatus

All electrochemical measurements, including cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were carried out using a model CHI-660c (CH Instrument-660 electrochemical workstation) with a conventional three-electrode cell. The bare carbon paste electrode (BCPE) and poly (neutral red) modified carbon paste electrode (PNR/MCPE) were used as working electrodes, Platinum electrode as counter electrode and the saturated calomel electrode (SCE) as the reference electrode. All experiments were performed versus saturated calomel electrode.

3.2.2 Reagents

Catechol (CC) and Hydroquinone (HQ) were obtained from Merck chemicals. Disodium hydrogen phosphate (Na_2HPO_4), Sodium dihydrogen orthophosphate (NaH_2PO_4) and Neutral red (NR) were purchased from Himedia chemicals. Spectrally pure graphite powder from Merck and high viscous paraffin oil from Fluka were used for the preparation of carbon paste. All other reagents were of analytical grade and used without further purification.

3.2.3 Electrode modification

Before modification, the bare carbon paste electrode was prepared by hand mixing graphite powder and silicone oil in the ratio 70:30 (w/w) for about 45 minutes in an agate

mortar to attain a carbon paste. The obtained paste was packed into the homemade Teflon cavity and electrical contact was provided at the end of the PVC tube. The poly (neutral red) modified carbon paste electrode (PNR/MCPE) was prepared by the electrochemical polymerization of neutral red on the surface of bare carbon paste electrode using 0.1 M NaOH as supporting electrolyte with the scan rate of 50 mVs^{-1}.

3.3 Results and Discussion

3.3.1 Electropolymerization of neutral red on the surface of carbon paste electrode

The electropolymerization on the surface of carbon paste electrode was carried out using cyclic voltammetry (CV) technique. The poly(neutral red) modified carbon paste electrode (PNR/MCPE) was prepared by cycling CPE for several cycles in 0.1M NaOH aqueous solution containing 1m M neutral red. Electropolymerization was achieved by cyclic sweeping between -0.8V to 1.0 V with the scan rate of 100 mVs^{-1} for 15 cycles (Fig.1a). It can be seen that, in the first scan a broad voltammogram was obtained which goes on decreasing from the second cycle. The gradual decrease of the voltammograms as the number of cycles increases shows that neutral red was deposited on the surface of CPE by electropolymerization. The electrochemical polymerization of NR was published in 1970 by Nikolskii and co-authors [59] and they reported the dependence on pH of the oxidation potential of the system neutral red-leuco- neutral red in the region from pH 0.5 to 11.5 [60]. The effect of polymerization cycle was shown in Fig.1b. Electropolymerization cycle is one of the most important factors which affect the performance of modified electrode towards the redox signal of catechol. Different polymerization cycles were applied (from 5 to 25) on the surface of bare carbon paste electrode and the corresponding electrocatalytic behaviour towards the determination of CC ($0.1x10^{-4}$M) were examined and it can be observed that the current enhancement towards the determination of CC goes on increases up to fifteen cycles, afterwards the nature of voltammogram was not good. Therefore, fifteen polymerization cycles were selected for further electrochemical investigations.

3.3.2 Characteristics of the poly (neutral red) modified carbon paste electrode (PNR/MCPE)

The performance of the prepared poly (neutral red) modified carbon paste electrode was investigated at potassium ferrocyanide [$K_4Fe(CN)_6$] using cyclic voltammetry method. Fig.2 shows the electrochemical behaviour of potassium ferrocyanide at bare (dashed line) and poly (neutral red)/MCPE (solid line) using 1M KCl as supporting electrolyte with the scan rate of 50mVs^{-1}. The bare carbon paste electrode shows a pair of redox peaks, with an anodic peak potential (Epa) at 0.29 V, cathodic peak potential (Epc) at 0.16 V and difference in redox peak potential (ΔEp) was found to be 0.13V. The modified electrode also shows a pair of redox peaks, with an anodic peak potential (Epa) at 0.26 V, cathodic peak potential (Epc) at 0.18 V and difference in redox peak potential (ΔEp) was found to be 0.07V. As ΔEp is a function of the rate of electron transfer, lower the ΔEp, higher is the electron transfer rate. The electrochemical response of potassium ferrocyanide at poly (neutral red) modified carbon paste electrode enhanced to a great extent than bare carbon paste electrode indicating that the surface coverage property of the modified electrode has been changed significantly. Fig.3 (a) and (b) represents the surface morphology in the form of SEM images for both bare and modified carbon paste electrode. The image with respect to the surface of bare carbon paste electrode showed irregular shaped flakes of graphite. After achieving the electropolymerization the electrode surface was covered by a thin polymer layer of neutral red, in this there are numerous uniform aligned valleys on the surface, which lead to increase in the active surface area of the modified electrode. Therefore, these SEM images confirm that the modified electrode can be applied for further electrochemical investigations.

3.3.3 Electrochemical behaviour of poly (neutral red)/MCPE towards the catechol determination

The electrochemical behaviour of PNR/MCPE was investigated on catechol by cyclic voltammetry technology (CV) in 0.2 M PBS (pH=7.4) at scan rate of 50 mVs^{-1}. Fig.4 shows the cyclic voltammograms obtained for 0.1×10^{-4}M catechol at both BCPE (dashed line) and PNR/MCPE (solid line). It can be seen that, the bare carbon paste electrode shows a pair of redox peaks, with an anodic peak potential (Epa) at 0.19 V, cathodic peak

potential (Epc) at 0.08 V and the difference in redox peak potential (ΔEp) was found to be 0.11V. The poly (neutral red) modified carbon paste electrode exhibit a massive enhancement in the redox peak current of catechol than BCPE. The anodic (Epa) and cathodic (Epc) peak potential was observed at 0.15 V and 0.09 V respectively, with a ΔEp of 0.06 V. Therefore, electrochemical behaviour of catechol at PNR/MCPE gives an evidence for the catalytic effect of proposed electrode. The oxidation mechanism of catechol was shown in scheme 2.

3.3.4 Scan rate study of catechol at poly (neutral red)/MCPE

The effect of scan rate on the peak current of catechol at poly (neutral red)/MCPE was investigated using cyclic voltammetry (CV) technique. Fig.5a shows the CVs recorded for $0.1x10^{-4}$ M CC at poly (neutral red)/MCPE with different scan rate in presence of 0.2M phosphate buffer solution (pH 7.4). It can be seen that, the anodic and cathodic peak current of CC goes on increases with increase in the scan rate from 50-500mVs^{-1}. The anodic peak potential (Epa) of CC positively shifted, while cathodic peak potential (Epc) shifted in a negative direction with increasing scan rates. Fig.5b shows the plot of anodic peak current (Ipa) versus scan rate (ν) of CC with good linearity and correlation coefficient value was found to be R^2=0.999. Moreover, the plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) (Fig.5c) was also examined and correlation coefficient value was found to be R^2=0.992. From the above experimental observations, the modified electrode process was found to be adsorption-controlled.

3.3.5 Effect of catechol concentration at poly (neutral red)/MCPE

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques have been employed for the determination of CC at PNR/MCPE with different concentrations. Fig.6a shows the cyclic voltammograms obtained for CC at PNR/MCPE with different concentrations in presence of 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}. The anodic and cathodic peak current goes on increases with increase in the concentration of CC from 0.1 – 1.0 x 10^{-4}M. The Epa of CC slightly shifted towards positive, while Epc shifted towards negative direction with increasing concentration. Fig.6a (Inset) shows the relationship between anodic peak current (Ipa) and concentration of CC with correlation

coefficient value R^2=0.997. Fig.6b shows the differential pulse voltammograms (DPVs) of CC at different concentrations using 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}. The anodic peak current (Ipa) gradually increases with increase in the concentration of CC from 20-120 µM and the relationship between Ipa and CC concentration was shown in Fig.6b (Inset). The PNR/MCPE exhibit good detection limits (LOD) and limit of quantification (LOQ) for CC using the equation (1) and (2) [61-64] and found to be 6.46 µM and 21.5 µM respectively.

LOD=3S/M ..... (1)

LOQ=10S/M.....(2)

where, S is the standard deviation and M is the slope

3.3.6 Effect of pH on electrochemical oxidation of catechol at poly (neutral red)/MCPE

The effect of pH on the oxidation behaviour of catechol at PNR/MCPE was investigated by cyclic voltammetric method. Fig.7 shows the cyclic voltammograms obtained for 0.1 x 10^{-4}M CC at different pH (6.2, 6.6, 7.0, 7.4 and 7.8) in presence of 0.2M phosphate buffer solution with scan rate 50mVs^{-1}. It can be seen that, the anodic and cathodic peak potentials of CC shifted towards positive value with increasing pH. Fig.7 (Inset) shows the relationship between Epa of CC and pH value. The pH 7.4 was chosen as the optimum pH value for the present study.

3.3.7 Electrochemical behaviour of poly (neutral red)/MCPE towards the hydroquinone determination

The electrochemical oxidation behaviour of hydroquinone (HQ) was investigated at PNR/MCPE using cyclic voltammetry method. Fig.8 depicts the cyclic voltammograms obtained for 0.1×10^{-4}M HQ at both BCPE (dashed line) and PNR/MCPE (solid line). From the experimental observations, the BCPE shows a pair of redox peaks, with an anodic peak potential (Epa) at 0.16 V, cathodic peak potential (Epc) at 0.05 V and ΔEp was found to be 0.11V. PNR/MCPE shows enhancement in the redox peak current of HQ than BCPE. The Epa and Epc of HQ was observed at 0.03 V and 0.01 V respectively, with a ΔEp of 0.02 V.

Therefore, modified electrode exhibits well electrocatalytic behaviour towards hydroquinone determination. The oxidation mechanism of HQ was shown in scheme 3.

3.3.8 Scan rate study of hydroquinone at poly (neutral red)/MCPE

Using cyclic voltammetry (CV), the effect of scan rate on the redox peak current of HQ was studied at PNR/MCPE. Fig.9a shows the cyclic voltammograms obtained for $0.1x10^{-4}$ M HQ at poly (neutral red)/MCPE with different scan rate in presence of 0.2M phosphate buffer solution (pH 7.4). The redox peak current of HQ goes on increases with increase in the scan rate from 50-500mVs^{-1}. The plot of anodic peak current (Ipa) versus scan rate (ν) of HQ was shown in Fig.9b, which illustrate good linearity with correlation coefficient value R^2=0.999. Furthermore, the plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) (Fig.9c) was also examined and correlation coefficient value was found to be R^2=0.993. Hence the proposed modified electrode reaction was found to be adsorption-controlled.

3.3.9 Effect of hydroquinone concentration at poly (neutral red)/MCPE

Fig.10a depicts the cyclic voltammograms of HQ at PNR/MCPE with different concentrations in presence of 0.2M phosphate buffer solution (pH 7.4). The experimental result shows that redox peak current goes on increases with increase in the concentration of HQ from 0.2 – 1.2 x 10^{-4}M. The relationship between anodic peak current (Ipa) and concentration of HQ was shown in Fig.10a (Inset). Furthermore, differential pulse voltammetry method also employed for the investigation of HQ at PNR/MCPE with different concentrations. Fig.10b depicts the DPVs of HQ at different concentrations using 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}. The peak current increases gradually with increase in the concentration of HQ from 40-140 µM and the relationship between Ipa and HQ concentration was shown in Fig.10b (Inset). The limit of detection (LOD) and limit of quantification (LOQ) for HQ was calculated using equations (1) and (2), it was found to be 4.9 µM and 16.5 µM respectively.

3.3.10 Capability of poly (neutral red)/MCPE towards the simultaneous determination of catechol and hydroquinone

Under the optimal conditions, the simultaneous determination of CC and HQ at PNR/MCPE was investigated by CV and DPV methods. Fig.11 depicts the CVs obtained for the mixture of $0.1x10^{-4}$M CC and $0.1x10^{-4}$M HQ in presence of 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}. Dashed line shows the CVs obtained for the mixture containing CC and HQ at BCPE, whereas solid line for PNR/MCPE. PNR/MCPE shows enhancement in the redox peak current of CC and HQ than BCPE. Fig.11 (Inset) shows the DPVs obtained for the mixture containing $0.1x10^{-4}$M CC and $0.1x10^{-4}$M HQ at PNR/MCPE. The electrocatalytic performance of the PNR/MCPE towards the determination of CC and HQ was compared with other modified electrodes (Table 1). Hence, the proposed modified electrode proved its electrocatalytic capability towards the simultaneous determination of CC and HQ.

3.3.11 Interference study

Differential pulse voltammetry (DPV) technique was employed for the interference study of CC and HQ at PNR/MCPE in presence of 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}. Fig.12a shows the DPVs of the binary mixture contain CC and HQ, in which the concentration of one species changed (CC), whereas the other species remained constant (HQ). The peak current increases with increase in concentration of CC. The plot of anodic peak current (Ipa) versus concentration of CC was shown in Fig.12a (Inset). In the same way, Fig.12b depicts the DPVs of the binary mixture contain CC and HQ, in which the concentration of one species changed (HQ), whereas the other species remained constant (CC). Fig.12b (Inset) shows the relationship between anodic peak current (Ipa) versus concentration of HQ. Thus, poly (neutral red) modified carbon paste electrode can be used for the simultaneous determination of binary mixture contain CC and HQ.

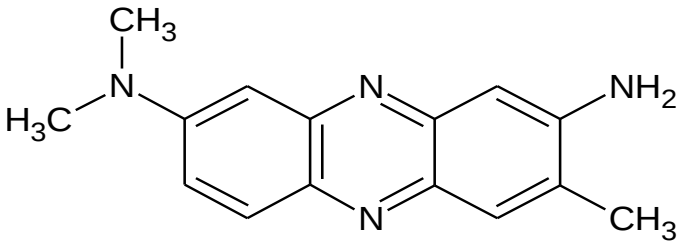
3.3.12 Sample analysis

The performance of the proposed modified electrode in practical analysis was carried out using cyclic voltammetric technique. The local tap water was selected for analysis by the standard addition method [65]. The obtained results were summarized in Table 2.

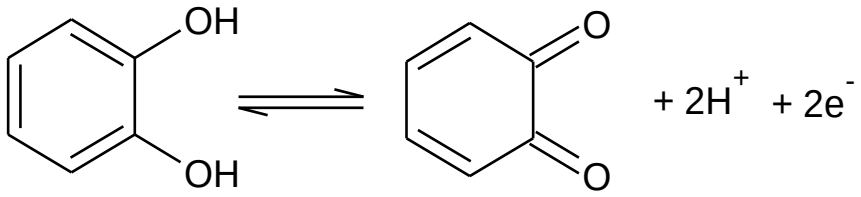
Therefore, the proposed modified electrode could be applied for the real sample analysis with satisfactory results.

3.4 Conclusion

In this chapter, a simple and sensitive electrochemical method for the simultaneous determination of CC and HQ was developed by employing poly (neutral red) modified carbon paste electrode. Due to the excellent eletrocatalytic activity and sensitivity, the PNR/MCPE exhibited increased peak current toward the determination of CC and HQ. The poly (neutral red) modified carbon paste electrode show adsorption-controlled type of electrode process. The limit of detection (LOD) and limit of quantification (LOQ) for CC and HQ was examined at PNR/MCPE. The proposed method will potentially be applied for the simultaneous electrochemical determination of CC and HQ.

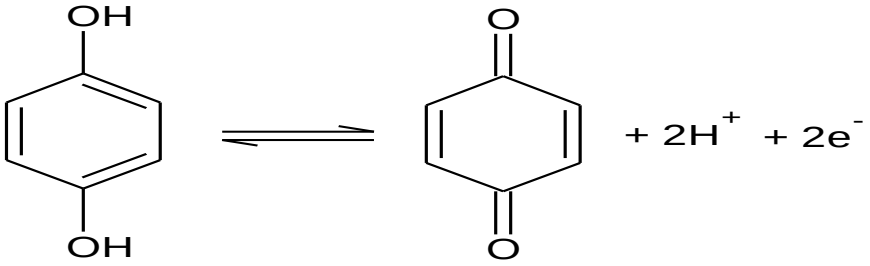


Scheme 1: Structure of neutral red



Catechol 1,2 Benzoquinone

Scheme 2: Oxidation mechanism of catechol



Hydroquinone 1,4 Benzoquinone

Scheme 3: Oxidation mechanism of hydroquinone

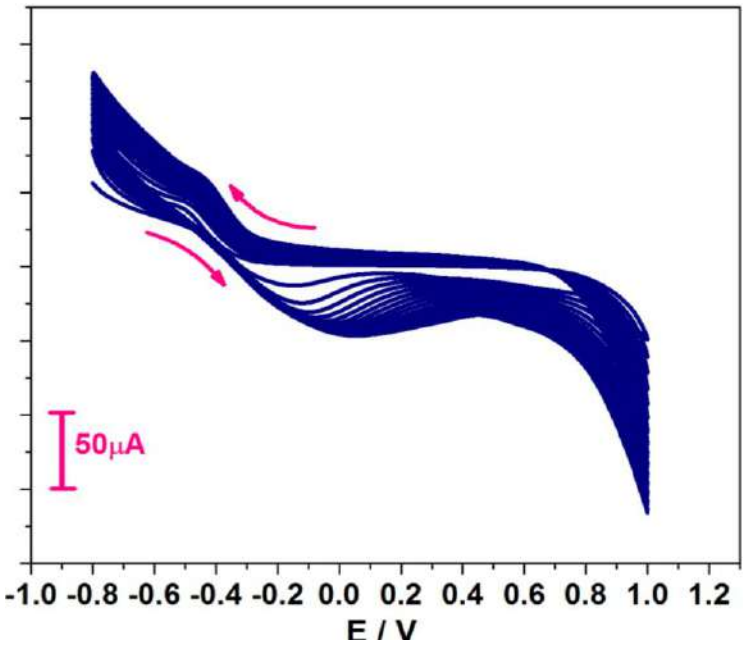


Fig.1a: CVs obtained for the electropolymerisation of 1mM neutral red at fifteen cycles. Supporting electrolyte = 0.1M NaOH, Scan rate = 50 mVs^{-1}.

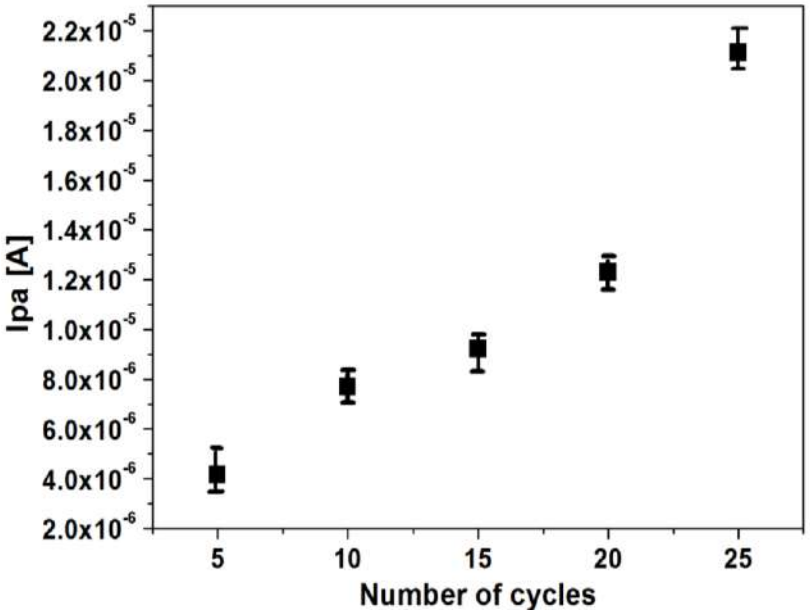


Fig.1b: Plot of anodic peak current (Ipa) versus number of cycles.

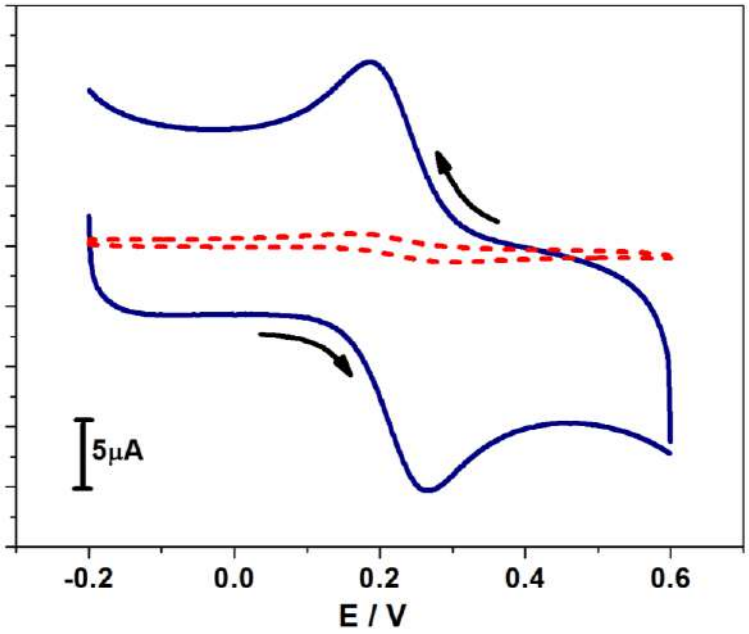


Fig.2: CVs obtained for 1mM $K_4[Fe(CN)_6]$ at BCPE (dashed line) and PNR/MCPE (solid line). Supporting electrolyte = 1M KCl, scan rate = 50 mVs^{-1}.

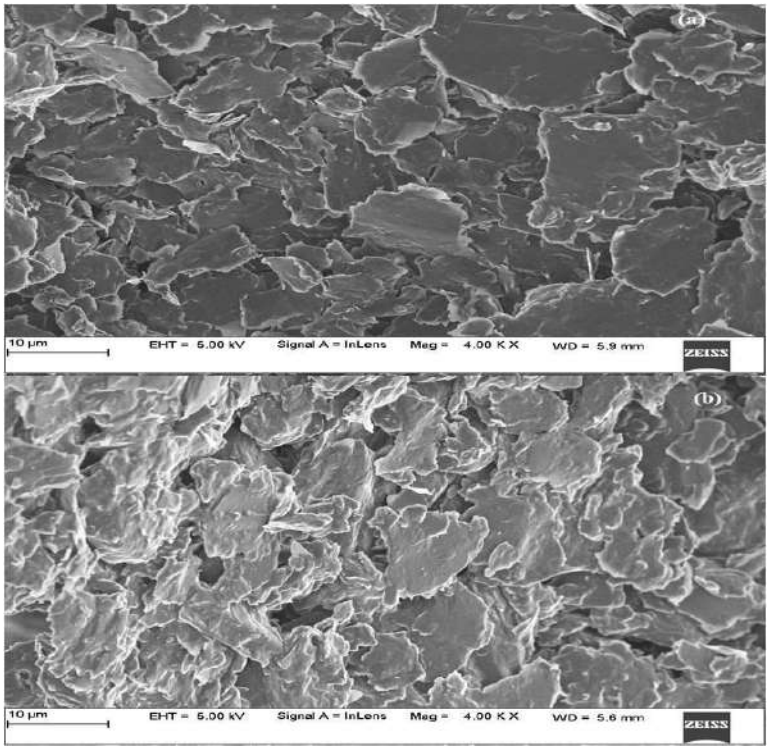


Fig.3: SEM image of (a) bare carbon paste electrode and (b) poly (neutral red)/MCPE.

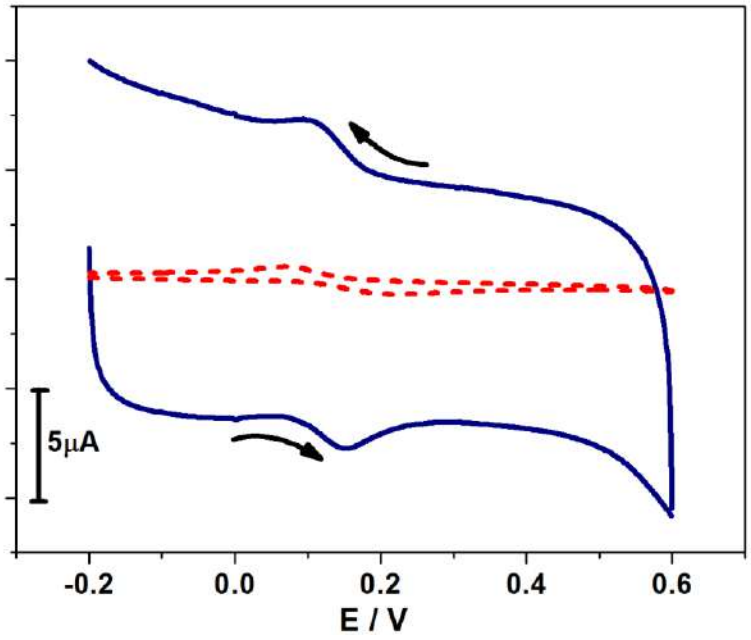


Fig.4: CVs obtained for $0.1x10^{-4}$M CC at BCPE (dashed line) and PNR/MCPE (solid line). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}.

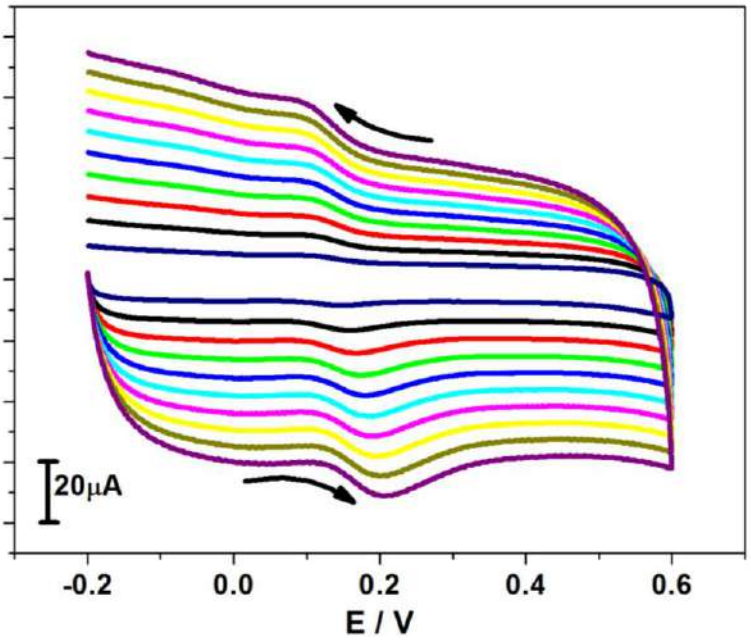


Fig.5a: CVs obtained for $0.1x10^{-4}$M CC with different scan rates at PNR/MCPE. Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50-500 mVs^{-1}.

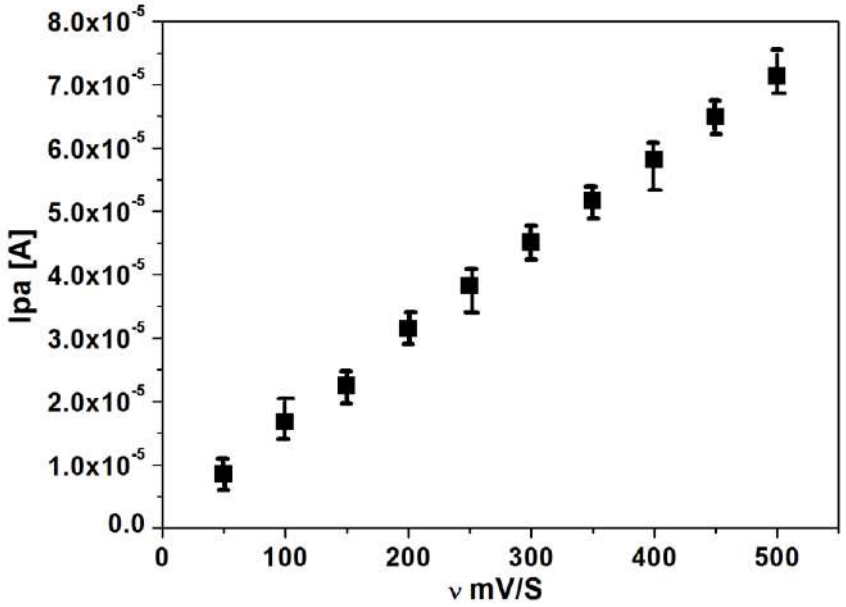


Fig.5b: Plot of anodic peak current (Ipa) versus scan rate (ν) of $0.1x10^{-4}$M CC.

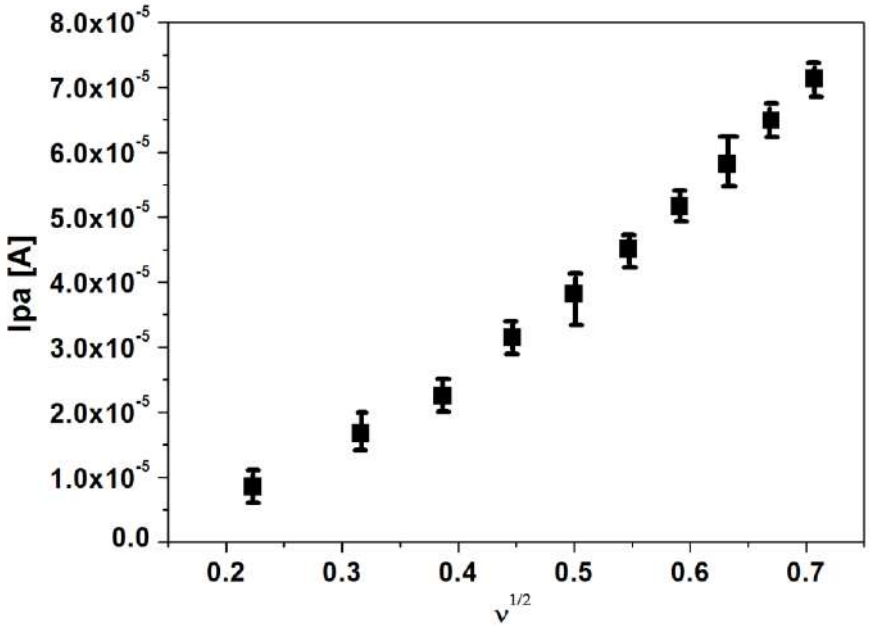


Fig.5c: Plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) of $0.1x10^{-4}$M CC.

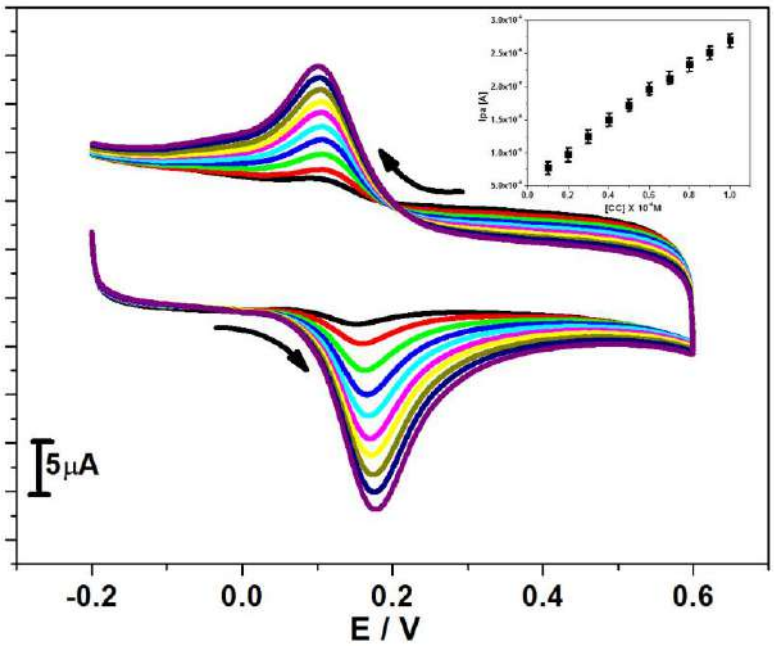


Fig.6a: CVs obtained for CC at different concentrations (0.1 - 1 x 10^{-4}M). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the plot of anodic peak current (Ipa) versus concentration of CC.

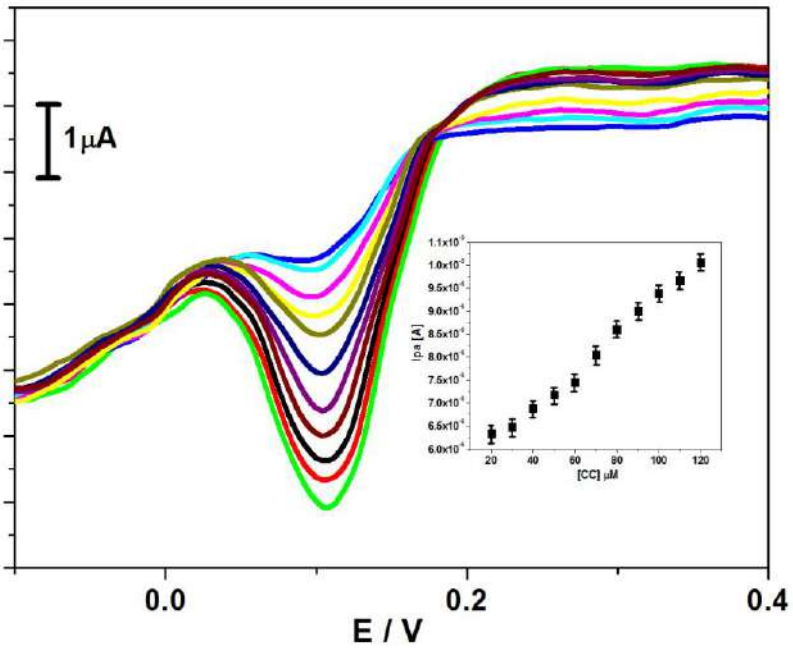


Fig.6b: DPVs obtained for CC at different concentrations (20 - 120 μM). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the plot of anodic peak current (Ipa) versus concentration of CC.

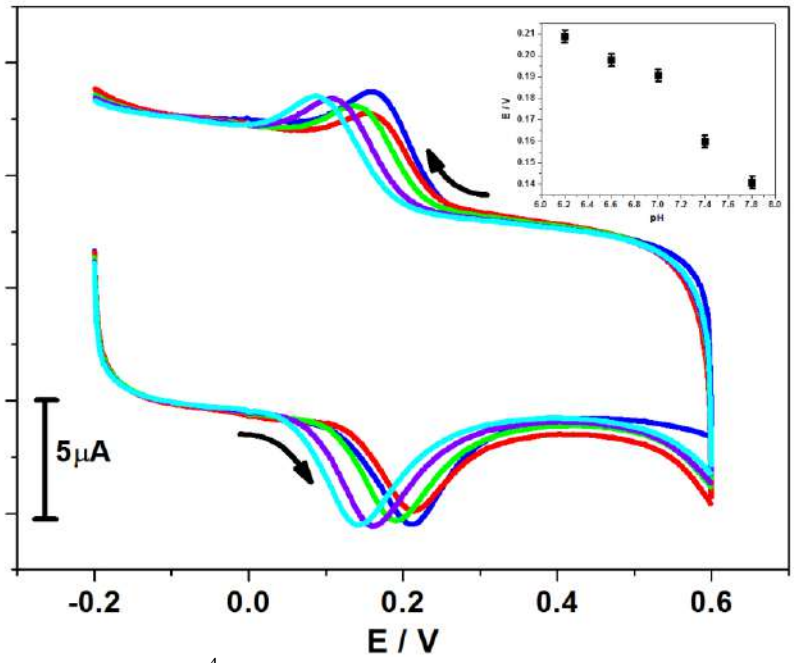


Fig.7: CVs obtained for $0.1x10^{-4}$M CC at different pH (6.2, 6.6, 7.0, 7.4 and 7.8). Supporting electrolyte = 0.2M PBS, scan rate = 50 mVs^{-1}. Inset figure shows the plot of anodic peak potential (Epa) of CC versus pH.

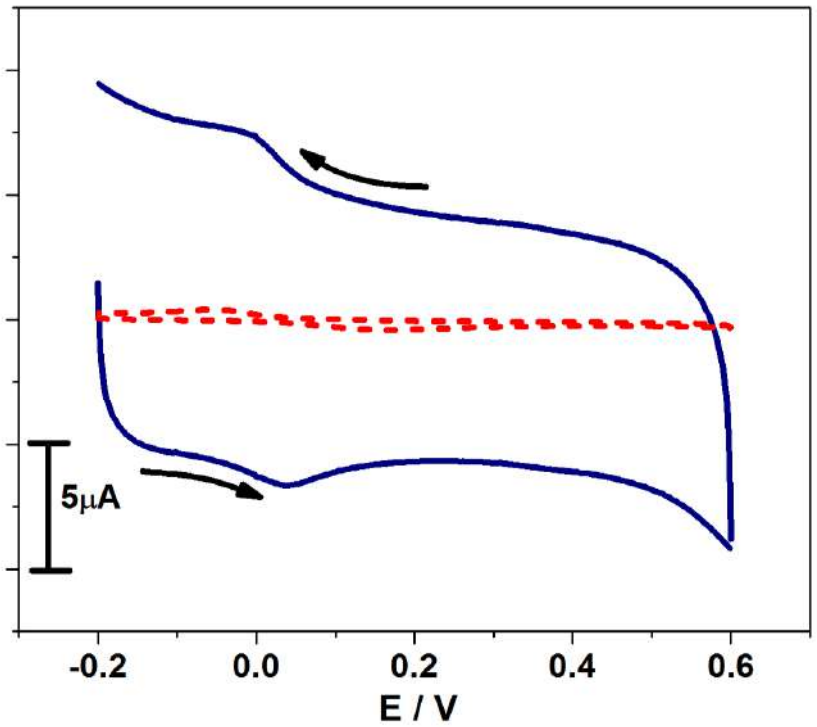


Fig.8: CVs obtained for $0.1x10^{-4}$M HQ at BCPE (dashed line) and PNR/MCPE (solid line). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}.

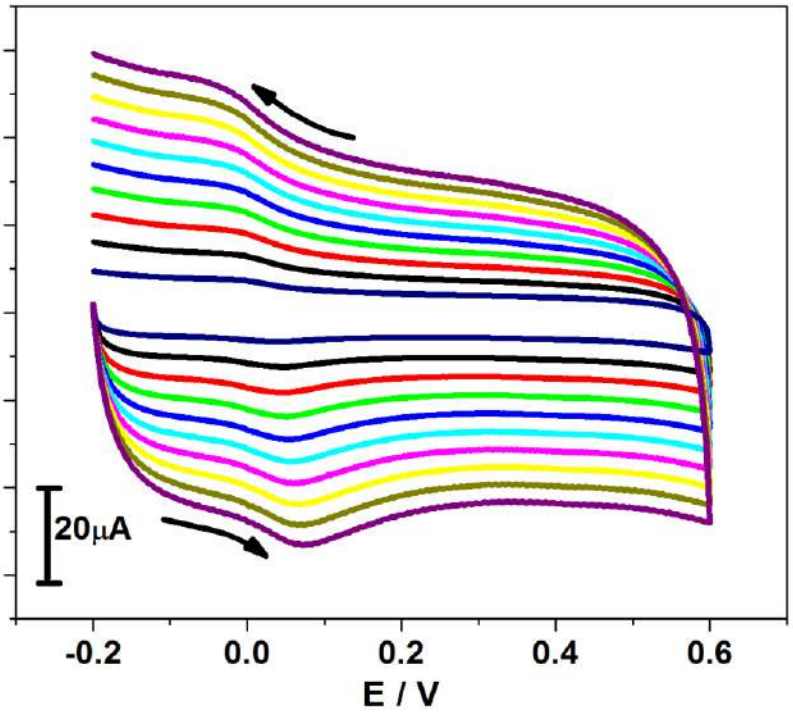


Fig.9a: CVs obtained for $0.1x10^{-4}$M HQ with different scan rates at PNR/MCPE. Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50-500 mVs^{-1}.

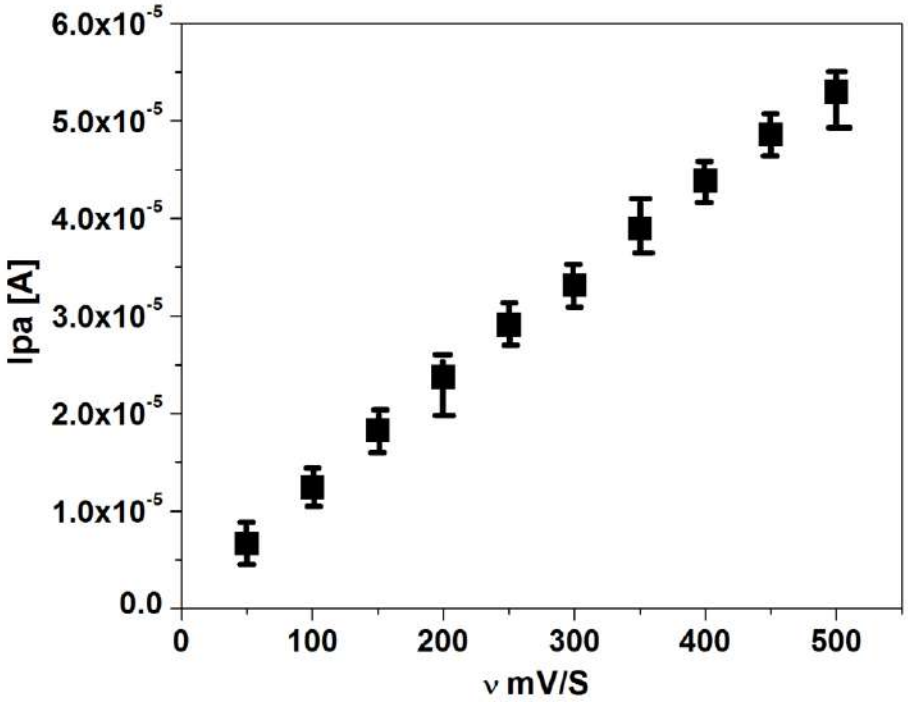


Fig.9b: Plot of anodic peak current (Ipa) versus scan rate (ν) of $0.1x10^{-4}$M HQ.

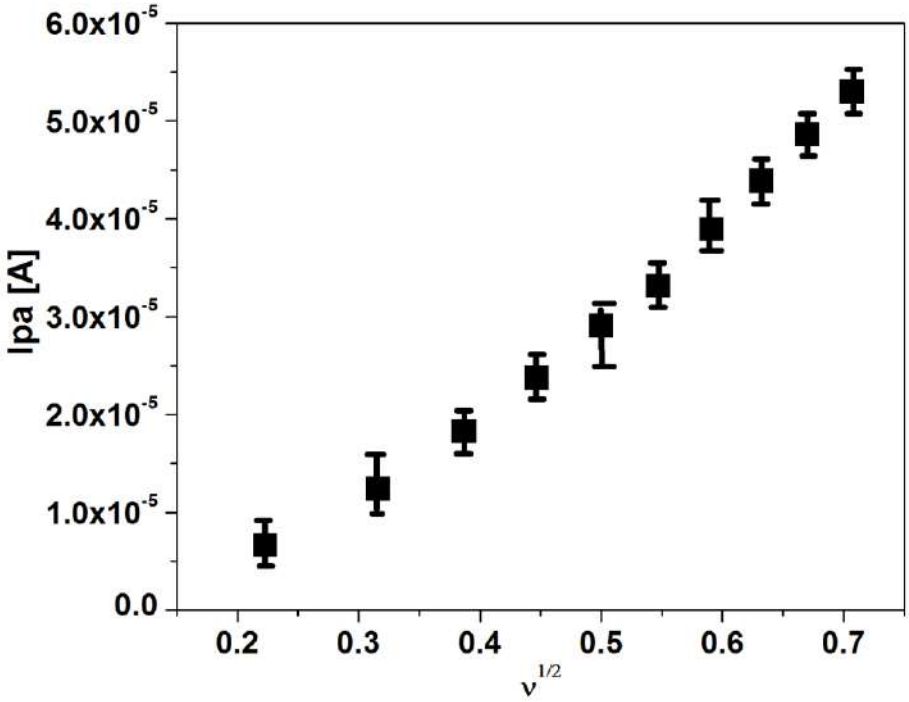


Fig.9c: Plot of anodic peak current (Ipa) versus square root of scan rate ($v^{1/2}$) of $0.1x10^{-4}$M HQ.

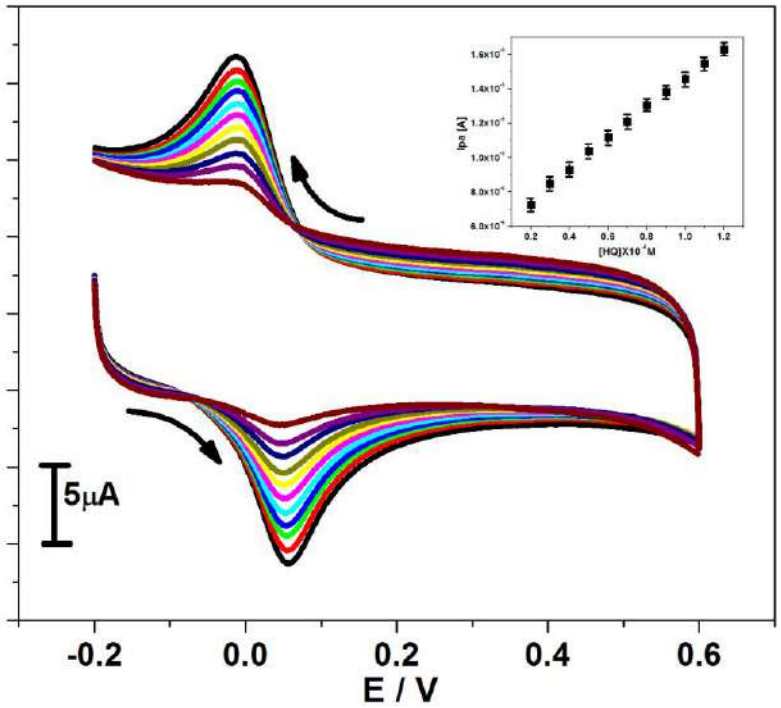


Fig.10a: CVs obtained for HQ at different concentrations (0.2 – 1.2 x 10^{-4}M). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the plot of anodic peak current (Ipa) versus concentration of HQ.

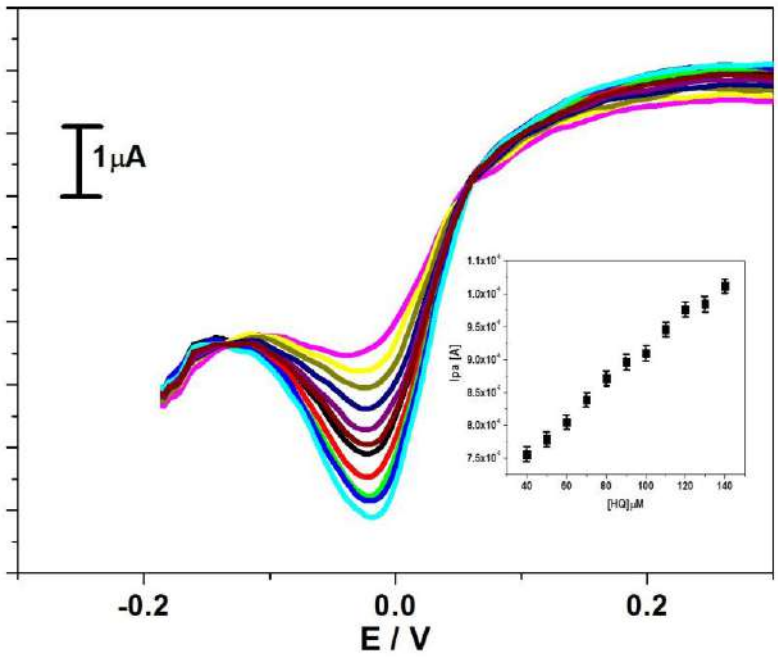


Fig.10b: DPVs obtained for HQ at different concentrations (40 - 140 μM). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the plot of anodic peak current (Ipa) versus concentration of HQ.

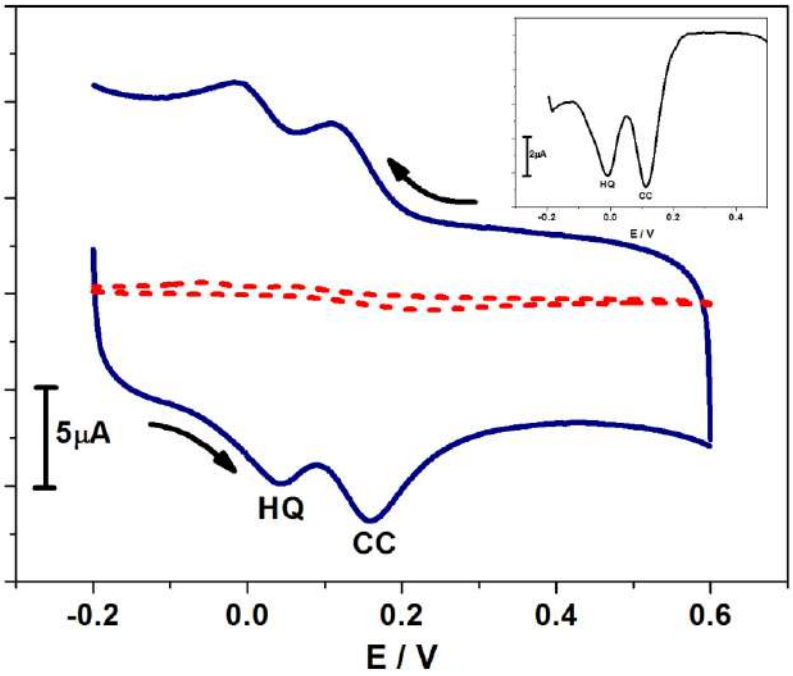


Fig.11: CVs obtained for the simultaneous determination of CC ($0.1x10^{-4}$M) and HQ ($0.1x10^{-4}$M) at BCPE (dashed line) and PNR/MCPE (solid line). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the DPVs of CC ($0.1x10^{-4}$M) and HQ ($0.1x10^{-4}$M) at PNR/MCPE.

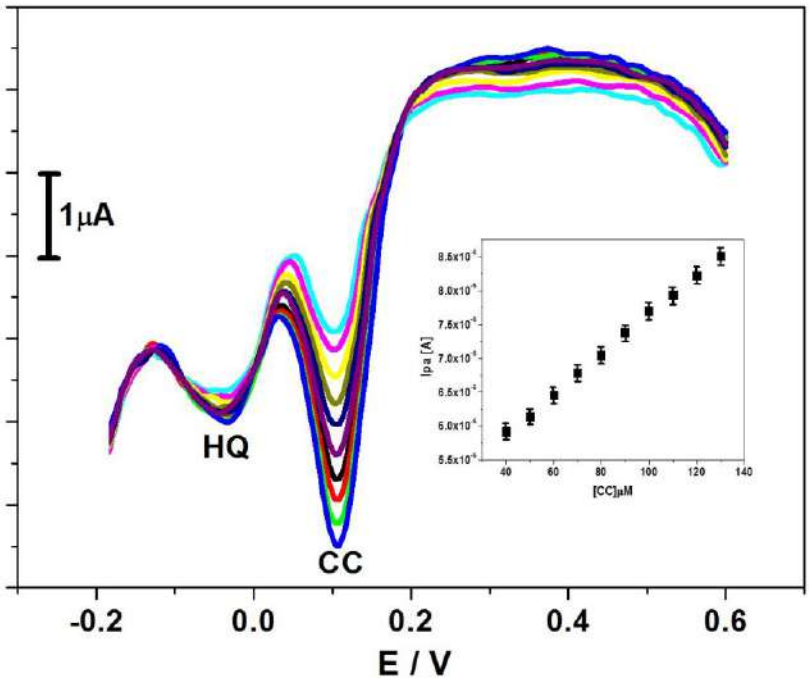


Fig.12a: DPVs obtained for CC at different concentrations (40 - 130 μM) keeping 50 μM HQ constant. Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the plot of anodic peak current (Ipa) versus concentration of CC.

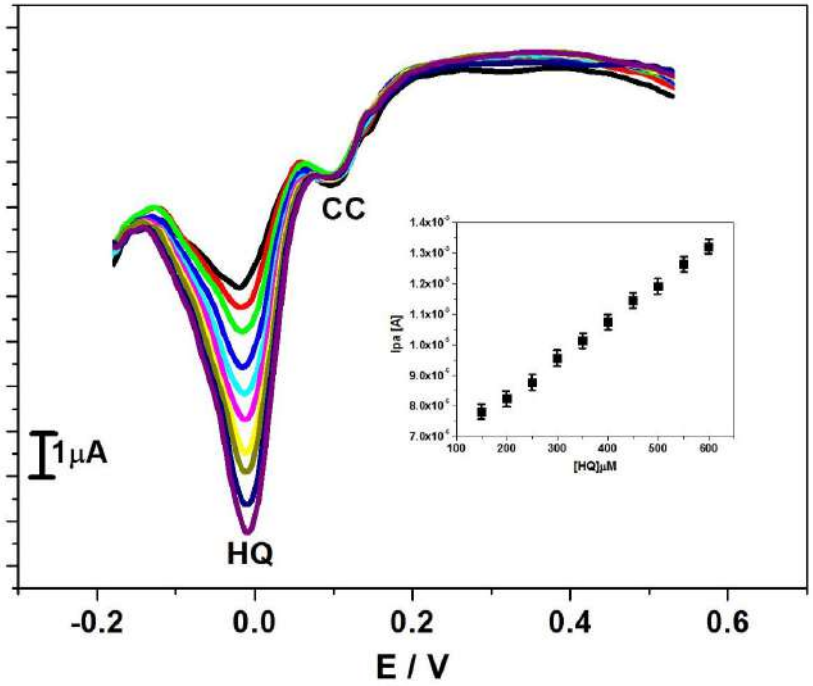


Fig.12b: DPVs obtained for HQ at different concentrations (150 - 600 μM) keeping 50 μM CC constant. Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the plot of anodic peak current (Ipa) versus concentration of HQ.

Table 1: Comparison of the PNR/MCPE electrode with other modified electrodes for CC and HQ determination.

Working electrode	**Linear range (µM)**		**Limit of detection(µM)**		**Reference**
	CC	**HQ**	**CC**	**HQ**	
(CMWNTs-NHCH2CH2NH)6/GCE	5-80	10-120	1.0	2.3	[66]
LDHf/GCE	3-1500	12-800	1.2	9	[67]
RGO-MWCNTs/GC	5.5-5400	8-391	1.8	2.6	[68]
PASA/MWNTs/GCE	6-180	6-100	1	1	[69]
AuNPs/Fe3O4-APTES-GO/GCE	2-145	3-137	0.8	1.1	[70]
MWCNT-PMG/GCE	30-1190	10-480	5.8	1.6	[71]
PNR/MCPE	10-100	20-120	6.4	4.9	This work

Table 2: Recoveries of CC and HQ in tap water sample at PNR/MCPE.

Sample	Compound	Added (µM)	Founded (µM)	Recovery (%)
Tap water	CC	100	99	99
		120	123.6	103
		140	137.6	98.3
	HQ	100	98.8	98.8
		120	117.2	97.7
		140	137.9	98.5

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Chapter -3

Part-B

Poly (L-leucine) modified carbon paste electrode for the simultaneous electrochemical determination of paracetamol and folic acid: A voltammetric study

Journal of Electroanalytical Chemistry (Revised and Submitted)

3.6 Introduction

Treatment, cure, prevention, or diagnosis of disease with the help of chemical substance is known as drugs. A series of drugs are present in the society among them the most commonly and widely used drugs are Paracetamol (N-acetyl-P-aminophenol or Acetaminophen) (scheme 1) and Folic acid (vitamin M or vitamin B_9) (scheme 2). Paracetamol which is analgesic anti-pyretic drug accepted as a very effective for the treatment of fever and relief in pain throughout the world from young to older age group. It is also well known for its replacement for aspirin in patients sensitive to aspirin or those with asthma [1-3]. Normally paracetamol does not show any harmful side-effects, however the over dosage causes kidney damage, liver disorders, skin rashes, inflammation of the pancreas and finally may lead to death [4-8]. Besides this the Folic acid a water soluble vitamin first discovered in Spinach [9] is one of the most important nutrients used for women especially in the pregnancy planning [10]. During the metabolism process FA involves in single electron transfer reactions and it is the antecedent of the active tetrahydrofolic acid coenzyme [11], moreover it plays a significant role in the synthesis of purines and pyrimidines for DNA and in cell replication. FA deficiency leads to the neural tube defects in newborns and also increases the risk of megaloblastic anaemia, cancer, coronary heart disease, etc in children and adults [12]. The fortification of flour with folic acid at 240mg/100g was proposed by department of health in UK, [13]. Further, the Food and Drug Administration of US introduced mandatory reinforcement of cereal-grain products with folic acid at a concentration of 140mg/100g in January 1998 [14]. Therefore, the developments of simple, fast, sensitive and accurate analytical technique are essential for the determination of paracetamol and folic acid. Numerous methods have been reported by authors for the determination of FA and PA; out of these some of the methods are fluorimetry [15], capillary electrophoresis [16-17], high performance liquid chromatography [18-21], spectrophotometry [22-25], titrimetry [26-27] and electrochemical methods [28-34]. These methods have some disadvantages because of their time consumption, expensive and often need the pre-treatment steps. Now day, electrochemical methods have received enormous interest due to its sensitive, less expensiveness besides this they are more convenient and more selective. In recent years,

chemically modified electrodes have fascinated great awareness because of their high electron transfer rate, high sensitivity, selectivity and stability in analyzing the electrochemical behaviour of folic acid [35-37]. In this article we have used L-leucine (scheme 3) as modifier which is one of the important amino acid essential for human beings and can be acquired through diet or supplementation. The polymerized L-leucine i.e. Poly (L-leucine) modified electrode exhibits very good catalytic behaviour and has been reported for some of the electrochemical determination [38-39], but there are no literature supporting the simultaneous determination of paracetamol and folic acid.

This chapter reports the simultaneous determination of paracetamol and folic acid carried out at physiological pH using electropolymerized film of L-leucine modified carbon paste electrode. The analytical application of the proposed modified electrode was successfully demonstrated by the determination of PA and FA in tablet. The modified electrode exhibits an excellent electrocatalytic activity for the simultaneous determination of paracetamol and folic acid.

3.7. Experimental

3.7.1 Reagents and chemicals

In current study, the chemical used was folic acid (FA) from Merck and the stock solution of FA ($25x10^{-4}$M) was prepared in 0.1M NaOH. Paracetamol was purchased from Sigma-Aldrich and stock solution of PA ($25x10^{-4}$M) was prepared in double distilled water. Disodium hydrogen phosphate (Na_2HPO_4), Sodium dihydrogen orthophosphate (NaH_2PO_4) and L-leucine were purchased from Himedia chemicals. Spectrally pure graphite powder (particle size <50 mm) from Merck and high viscous paraffin oil (density=0.88gcm^{-3}) from Fluka were used for the preparation of the carbon paste electrode. The phosphate buffer (0.2M pH 7.4) was used as optimum measurements. All the chemicals used in this experiments were of analytical grade and used without any further purification.

3.7.2 Apparatus

Cyclic voltammetric (CV) measurements were carried out with a model CHI-660c (CH Instrument-660 electrochemical workstation). All electrochemical experiments were performed in a standard three electrode cell. The bare and Poly (L-leucine) modified carbon

paste electrode (PLCN-MCPE) were used as working electrode, platinum electrode as counter electrode and saturated calomel electrode (SCE) as the reference electrode. All the potentials reported are versus the SCE.

3.7.3 Preparation of bare carbon paste electrode (BCPE)

The preparation of bare carbon paste electrode has been explained in chapter-3A section 3.2.3.

3.7.4 Preparation of Poly (L-leucine) modified carbon paste electrode (PLCN-MCPE)

Using cyclic voltammetry technique, electropolymerization was carried out at the surface of bare carbon paste electrode (BCPE) using 1mM L-leucine in 0.1 M NaOH with the scan rate of $50mVs^{-1}$. Electropolymerization was achieved by the repetitive potential cycling between -0.5V to 0.4V for ten cycles. After polymerization, the poly (L-leucine) film was washed with distilled water to remove unreacted L-leucine and the modified electrode was used for further electrochemical investigations.

3.8 Results and Discussion

3.8.1 Electropolymerization of L-leucine on the surface of bare carbon paste electrode (BCPE)

Electropolymerization was carried out at the surface of bare carbon paste electrode by taking 1mM L-leucine in 0.1M NaOH. Electropolymerization was achieved between the potential -0.5V to 0.4V at scan rate $50mVs^{-1}$ for ten cycles as depicted in Fig.1. As increase in the cycle, the peak current goes on increases indicating the growth of polymeric film at the surface of CPE [40]. Fig.2 shows the effect of multiple cycles on polymerization. The CPE was modified by applying different multiple cycles of polymerization (from 5 to 25) and the corresponding electrocatalytic response towards the determination of PA ($0.1x10^{-4}M$) were examined, it can be observed that the current enhancement towards the determination of PA goes on increases. Therefore 10 cycles for polymerization was selected which shows comparatively good peak current enhancement. After polymerization the poly (L-leucine) modified CPE was rinsed in double distilled water and used for further electrochemical investigations.

3.8.2 Electrocatalytic redox behavior of PA at poly (L-leucine) MCPE

The electrocatalytic behavior of paracetamol (PA) at poly (L-leucine) modified carbon paste electrode was studied by cyclic voltammetry method. Fig.3 shows the cyclic voltammograms of 0.1×10^{-4}M PA at BCPE (dotted line) and PLCN-MCPE (solid line) in 0.2M PBS at pH 7.4 with the scan rate of $50mVs^{-1}$. The poor electrochemical response was obtained at BCPE and difference in redox peak potential (ΔEp) was found to be 0.10 V. PLCN-MCPE exhibits great enhancement in redox peak current and difference in redox peak potential (ΔEp) was found to be 0.07 V. As we know that (ΔEp) is a function of rate of electron transfer, lower the ΔEp value higher will be the electron transfer rate [41]. Therefore, an outstanding development in the electrochemical sensitivity towards PA at PLCN-MCPE gives an evidence for the catalytic effect of proposed electrode.

3.8.3 Effect of scan rate towards the redox behaviour of PA at poly (L-leucine) MCPE

Effect of varying scan rate (ν) was studied at PLCN-MCPE in order to investigate the kinetics of electrode reaction. Fig.4a depicts the cyclic voltammograms of $0.1x10^{-4}$ M PA at different scan rate in 0.2M phosphate buffer solution (pH 7.4). The experimental outcome shows that, redox peak current of PA goes on increases with increase in the scan rate (10-$100mVs^{-1}$). To investigate the type of electrode process, plot of anodic peak current (Ipa) versus scan rate (ν) was recorded (Fig.4b) shows good linearity with correlation coefficient value R^2=0.999 and the plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) (Fig.4c) was also examined, correlation coefficient value was found to be R^2=0.993 indicating adsorption-controlled type of electrode reactions. From the above experimental observation paracetamol oxidation is a two-electron two-proton process and the oxidation mechanism of PA was shown in scheme 4 [42]. Number of electron transfer (n) and heterogeneous rate constant (k_0) in the electrochemical oxidation of PA were evaluated using the equation (1) [43] and (2) [44] respectively and tabulated in table 1.

$n = 90.6/ \Delta Ep$..... (1)

$k_o = \Psi [D_o \pi \nu (nF / RT)]^{1/2}$……(2)

Where, Ψ is the degree of reversibility, D_o is the diffusion co-efficient and ν is the scan rate

3.8.4 Effect of paracetamol concentration at poly (L-leucine) MCPE

The redox behaviour of PA at different concentration was investigated using PLCN-MCPE. Fig.5a shows the cyclic voltammograms obtained for paracetamol at different concentration (0.2-0.9x10^{-4}M) in presence of 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}. The redox peak current of PA goes on increases with increase in the concentration. The plot of anodic peak current (Ipa) versus concentration show excellent linearity with the correlation coefficient value R^2=0.999 (Fig. 5b). The limit of detection (LOD) and limit of quantification (LOQ) for PA was found to be 4.41 µM and 14.7 µM respectively. It was calculated by the following equation (3) and (4) [45-48].

LOD=3S/M..... (3)

LOQ=10S/M..... (4)

Where, S is the standard deviation and M is the slope

3.8.5 Electrochemical oxidation behaviour of FA at poly (L-leucine) MCPE

The electrocatalytic oxidation behaviour of folic acid (FA) was investigated using PLCN-MCPE. Fig.6 shows the cyclic voltammograms obtained for 0.1x10^{-4}M FA at BCPE (dotted line) and PLCN-MCPE (solid line) in 0.2M PBS at pH 7.4 with the scan rate of 50mVs^{-1}. The poor electrochemical response was observed at BCPE. PLCN-MCPE exhibited a great enhancement in anodic peak current (Ipa) and the oxidation peak potential (Ep) was observed at 0.67V. Thus, proposed modified electrode exhibited remarkable electrocatalytic behaviour towards the electrochemical sensitivity of folic acid. The oxidation mechanism of FA was shown in scheme 5 [49].

3.8.6 Effect of scan rate towards the oxidation of FA at poly (L-leucine) MCPE

Effect of scan rate (ν) was studied to know the kinetics of electrode reaction and type of electrode process at PLCN-MCPE. Fig.7a shows the cyclic voltammograms obtained for 0.1x10^{-4} M FA at different scan rate in 0.2M phosphate buffer solution (pH 7.4). The anodic peak current (Ipa) of FA gradually increases with increase in the scan rate (20-80 mVs^{-1}). The plot of anodic peak current (Ipa) versus scan rate (ν) was recorded (Fig.7b) shows good linearity with correlation coefficient value R^2=0.999 and the plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) (Fig.7c) was also examined,

correlation coefficient value was found to be R^2=0.991. Hence the electrode reaction was found to be adsorption-controlled. Number of electron transfer (n) and heterogeneous rate constant (k_0) were evaluated for the electrochemical oxidation of FA using the equation (5) [50] and (6) [51] respectively and tabulated in table 2.

$ip = 2.99 \times 10^5 n (\alpha n)^{1/2} A D^{o\,1/2} \nu^{1/2} C_o$ …….…….(5)

$ip = 0.227 n F A C_o k_o \exp \{ -\alpha n_a f (E_p - E_f) \}$ …….(6)

Where, A is the electrode surface area, C_o is the FA concentration and f = F/RT

3.8.7 Effect of FA concentration variation at poly (L-leucine) MCPE

The concentration variation of FA was carried out at PLCN-MCPE. Fig.8a shows the cyclic voltammograms obtained for FA at different concentration (0.2 - 0.9 x 10^{-4}M) in presence of 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}. The peak current increases gradually with increase in the concentration of FA. The plot of anodic peak current (Ipa) versus concentration was recorded, which shows good linearity with correlation coefficient value R^2=0.999 (Fig.8b). By equation (3) and (4), limit of detection (LOD) and limit of quantification (LOQ) for FA was calculated and found to be 24.4 µM and 81.3 µM respectively.

3.8.8 Simultaneous electrochemical determination of PA and FA at poly (L-leucine) MCPE

Using cyclic voltammetry, a mixture of PA and FA was investigated in order to examine the sensitivity and selectivity of PLCN-MCPE. Fig.9 shows the cyclic voltammograms obtained for the mixture of 0.1x10^{-4}M PA and 0.1x10^{-4}M FA in presence of 0.2M PBS (pH 7.4) at the scan rate of 50 mVs^{-1}. The solid line shows cyclic voltammogram obtained for the mixture of PA and FA at BCPE. The dashed line shows the cyclic voltammogram obtained for the mixture of PA and FA at PLCN-MCPE. The enhancement in peak current (Ip) was observed at PLCN-MCPE. The anodic peak potential of PA and FA were found to be 0.417 V and 0.67 V respectively, with a potential difference (PA-FA) of 0.253V. Thus, proposed modified electrode proved its electrocatalytic behaviour towards the simultaneous determination of PA and FA.

3.8.9 Interference study

Differential pulse voltammetry (DPV) exhibits better resolving power and higher sensitivity than CV. Thus it was employed to investigate the interference study of the mixture of samples containing PA and FA at PLCN-MCPE. Fig.10 shows DPVs of PA at different concentration. The peak current gradually increases with increase in the concentration of PA from 20 to 120μM and the resulted calibration curve is shown in the inset figure 10. Similarly, Fig.11 shows the DPVs of FA at different concentrations. The concentration of FA was varied from 100 to 600 μM and the obtained calibration curve is shown in the inset Fig 11.

The mutual interferences of PA and FA were investigated at PLCN-MCPE using 0.2M PBS (pH 7.4) with the scan rate of $50mVs^{-1}$. Fig.12a shows the DPVs of the binary mixture, in which the concentration of PA was varied while keeping the concentration of FA constant. Similarly, peak current increases with increase in concentration of FA while keeping the concentration of PA constant (Fig.12b). Hence PA and FA exhibit two well separated oxidation peaks.

3.8.10 Analytical applications

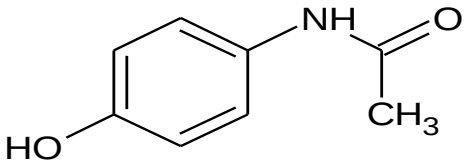
The electrochemical determination of PA and FA in the tablet was investigated to know the capability of the modified electrode. From the voltammetric signals, the recovery test was examined using the standard addition method and obtained results for PA and FA was depicted in table (3) and (4) respectively.

3.8.11 Stability of the modified electrode

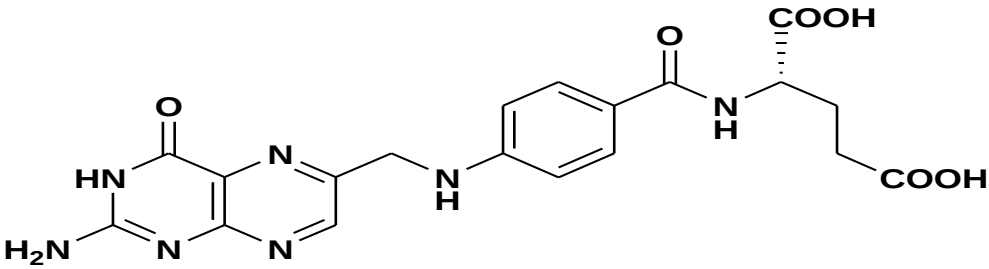
The stability of the modified electrode (PLCN-MCPE) was investigated using cyclic voltammetric method. Fig.13 shows the cyclic voltammograms obtained for 0.1×10^{-4}M PA in presence of 0.2M PBS (pH 7.4) with scan rate of $50mVs^{-1}$ having 10 repetitive cycles. Experimental result shows that, the peak current remains almost constant for multiple cycles indicating the poly (L-leucine) modified carbon paste electrode as stable and the measurements can be repetitive. Hence, the proposed electrode has high stability and was used for the simultaneous electrochemical determination of PA and FA.

3.9 Conclusion

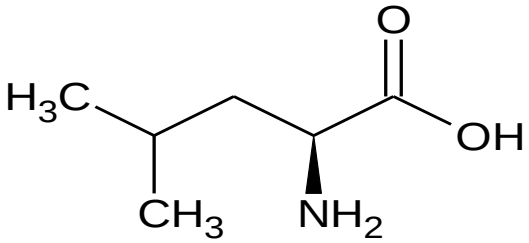
L-leucine was used as modifier and the modified electrode (PLCN-MCPE) was used for the simultaneous electrochemical determination of PA and FA by both CV and DPV techniques. The modified electrode showed high sensitivity and selectivity towards the detection of PA in the presence of FA. Number of electron transfer (n) and the heterogeneous rate constant (k_o) also determined for PA and FA at MCPE. The electrode process of modified electrode was investigated and found to be adsorption-controlled. The detection limits (LOD) of PA and FA at the PLCN-MCPE were evaluated. The PA and FA were determined in the tablet in order to examine the capability of the MCPE. Finally, the proposed electrode (PLCN-MCPE) exhibits incredible electrocatalytic response and can be applied for the simultaneous electrochemical determination of paracetamol and folic acid.



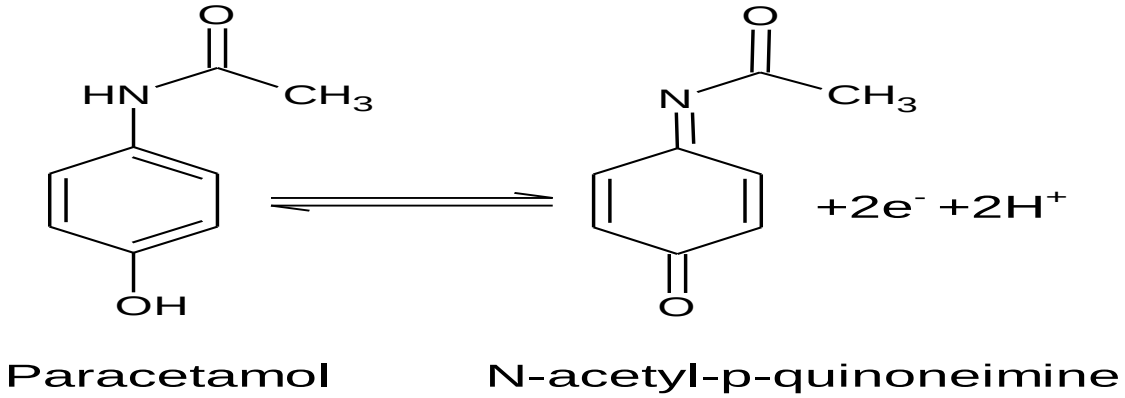
Scheme 1: Structure of Paracetamol



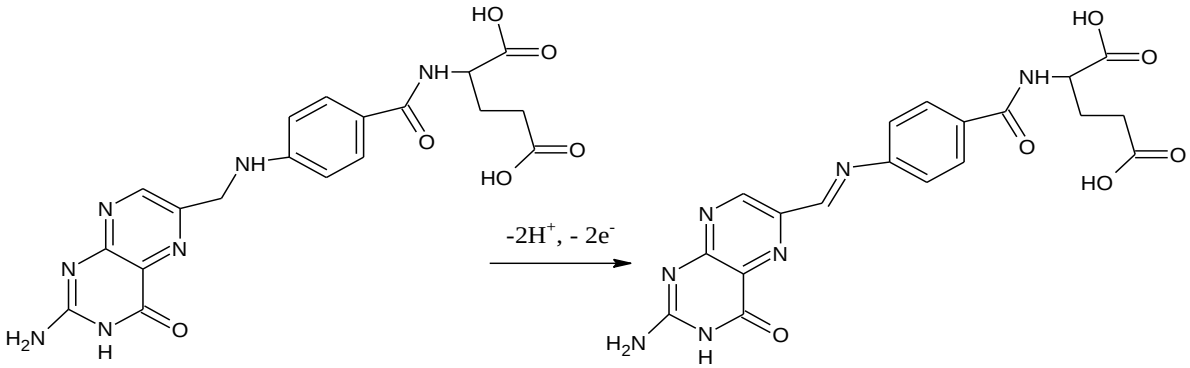
Scheme 2: Structure of Folic acid



Scheme 3: Structure of L-leucine



Scheme 4: Oxidation mechanism of Paracetamol



Scheme 5: Oxidation mechanism of Folic acid

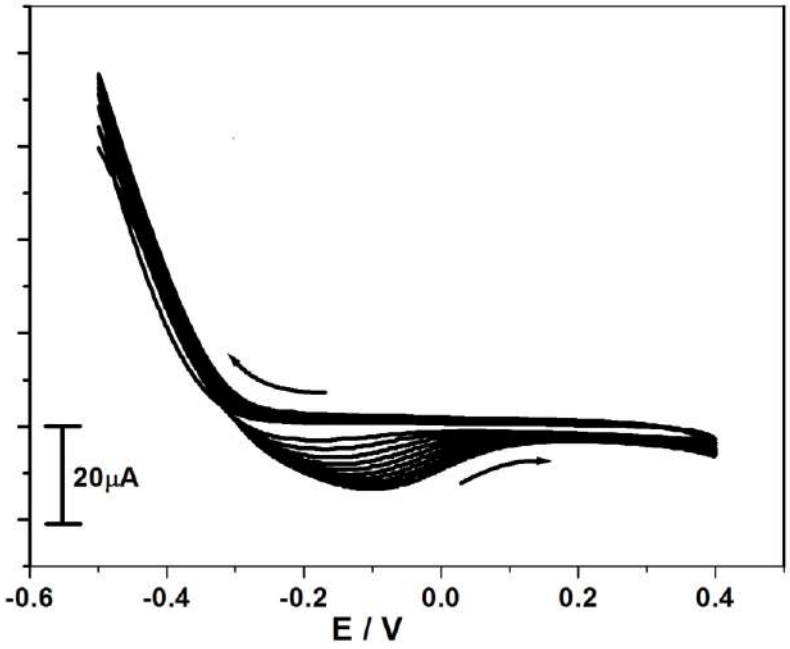


Fig.1: Cyclic voltammogram obtained for the electropolymerisation of 1mM L-leucine in presence of 0.1M NaOH as supporting electrolyte at the scan rate of 50 mVs^{-1} for ten cycles.

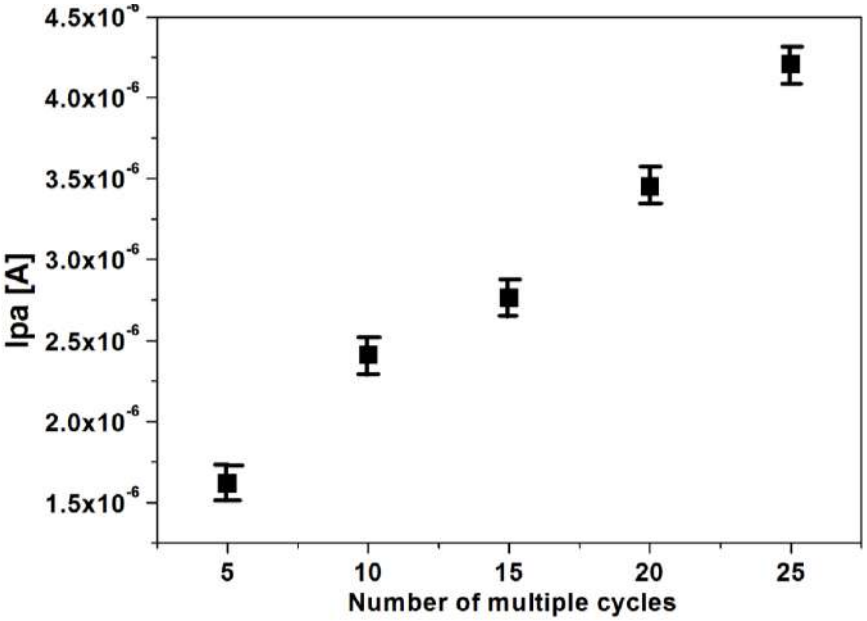


Fig.2: Graph of anodic peak current (Ipa) versus number of multiple cycles.

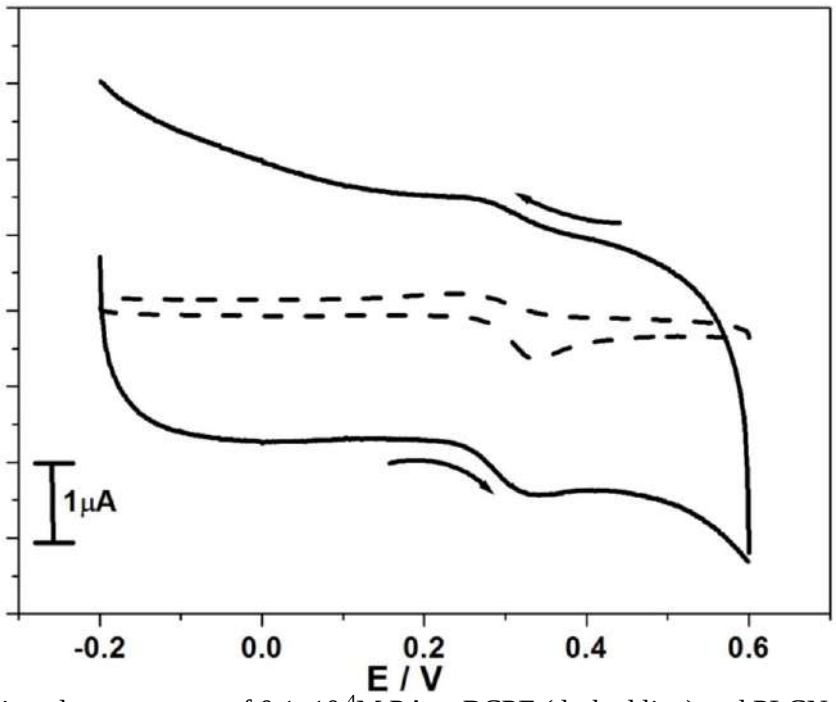


Fig.3: Cyclic voltammograms of $0.1x10^{-4}$M PA at BCPE (dashed line) and PLCN-MCPE (solid line) at scan rate of 50 mVs^{-1} using 0.2M PBS (pH 7.4).

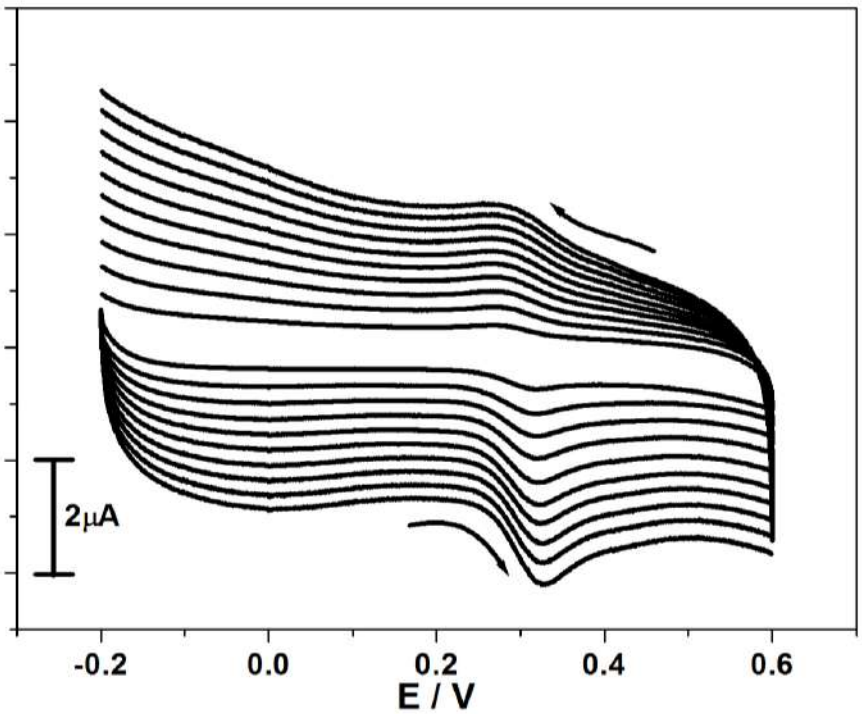


Fig.4a: Cyclic voltammograms of $0.1x10^{-4}$M PA at PLCN-MCPE with different scan rates (10-100mVs^{-1}) using 0.2M PBS (pH 7.4).

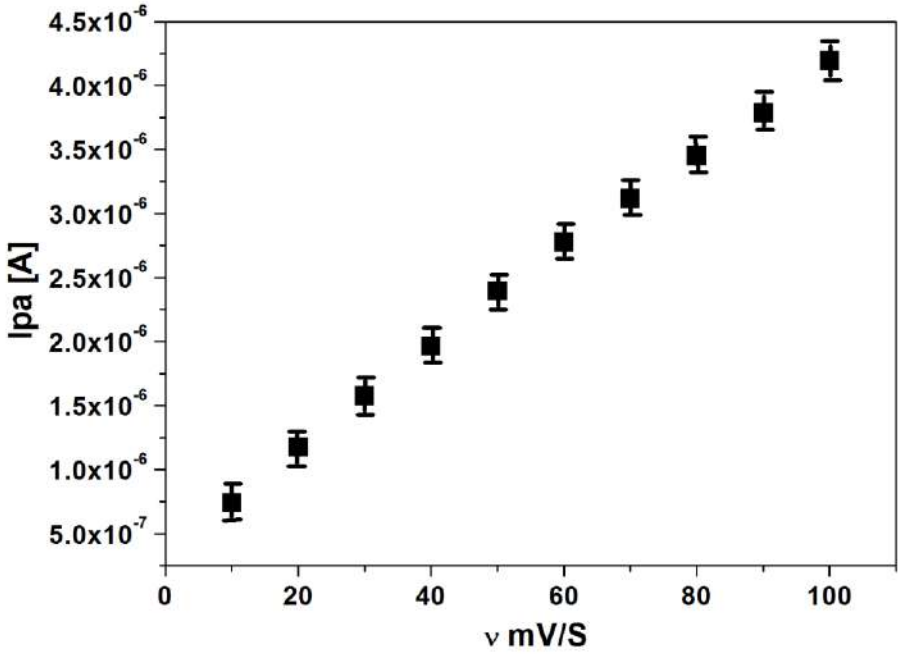


Fig.4b: Graph of anodic peak current (Ipa) versus scan rate (ν) of $0.1x10^{-4}$M PA

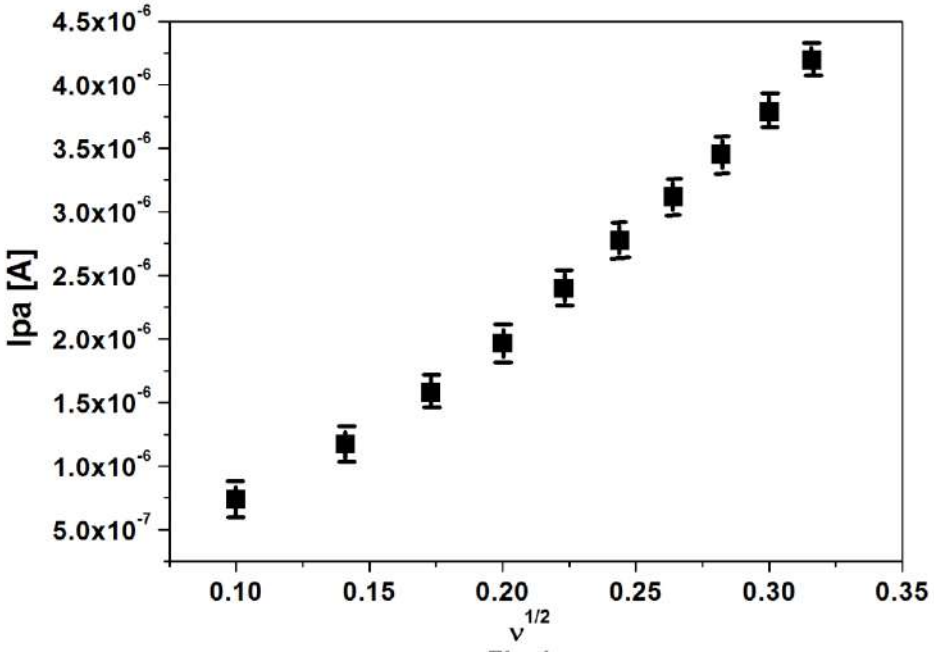


Fig.4c: Graph of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) of $0.1x10^{-4}$M PA

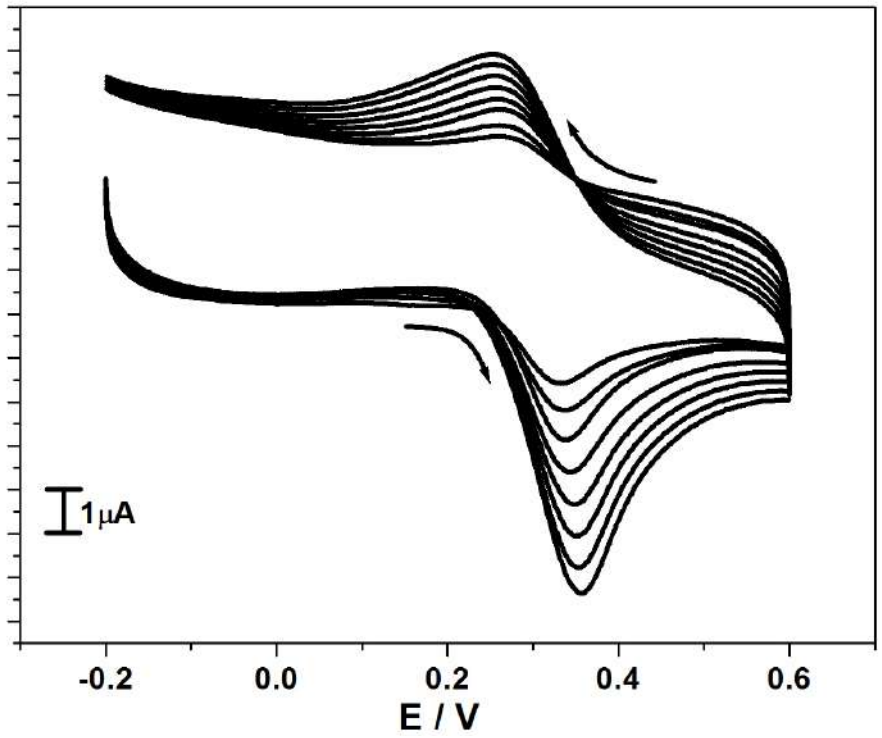


Fig.5a: Cyclic voltammograms obtained for PA at PLCN-MCPE with different concentration (0.2 to 0.9 x 10^{-4}M) using 0.2M PBS (pH 7.4) with the scan rate 50 mVs^{1}.

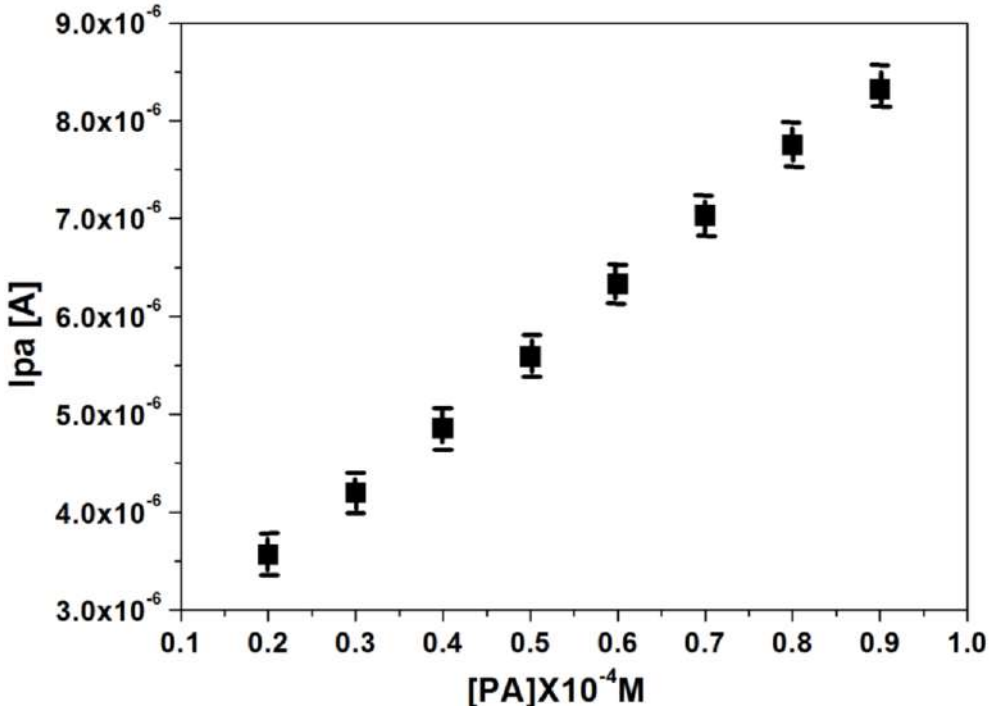


Fig.5b: Graph of anodic peak current (Ipa) versus concentration of PA

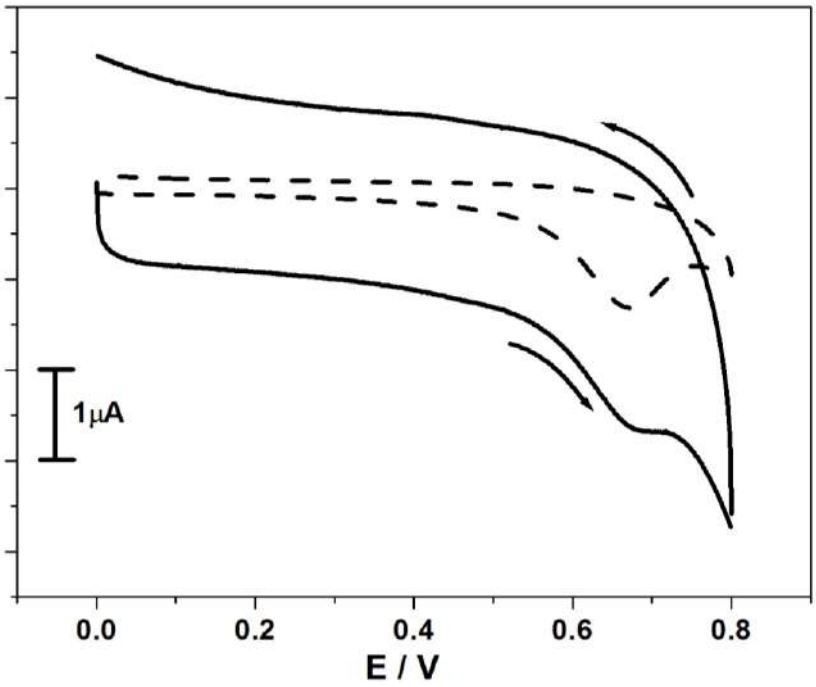


Fig.6: Cyclic voltammograms obtained for $0.1x10^{-4}$M FA at BCPE (dashed line) and PLCN-MCPE (solid line) with the scan rate of 50 mVs^{-1} using 0.2M PBS (pH 7.4).

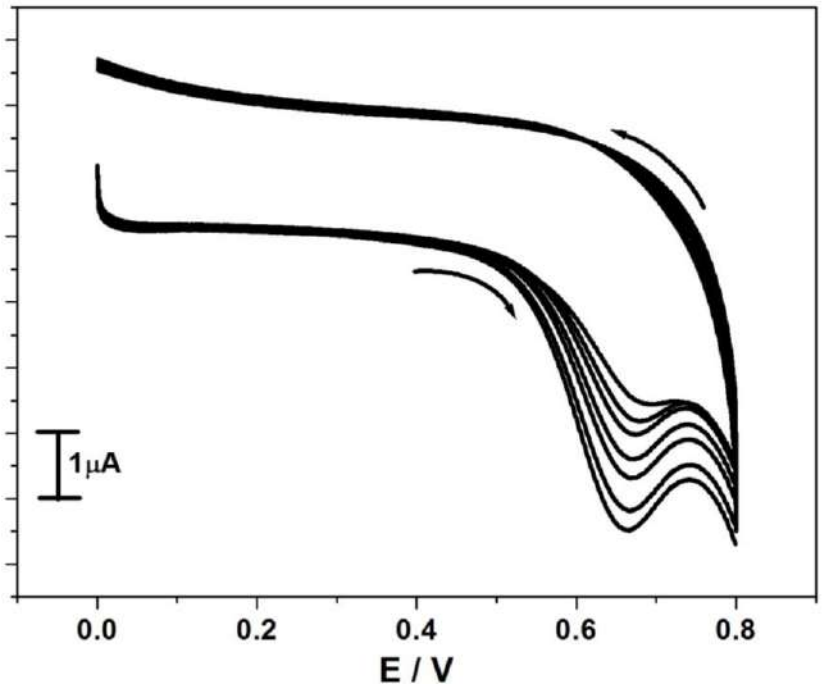


Fig.7a: Cyclic voltammograms obtained for $0.1x10^{-4}$M FA at PLCN-MCPE with different scan rates (20-80 mVs^{-1}) using 0.2M PBS (pH 7.4).

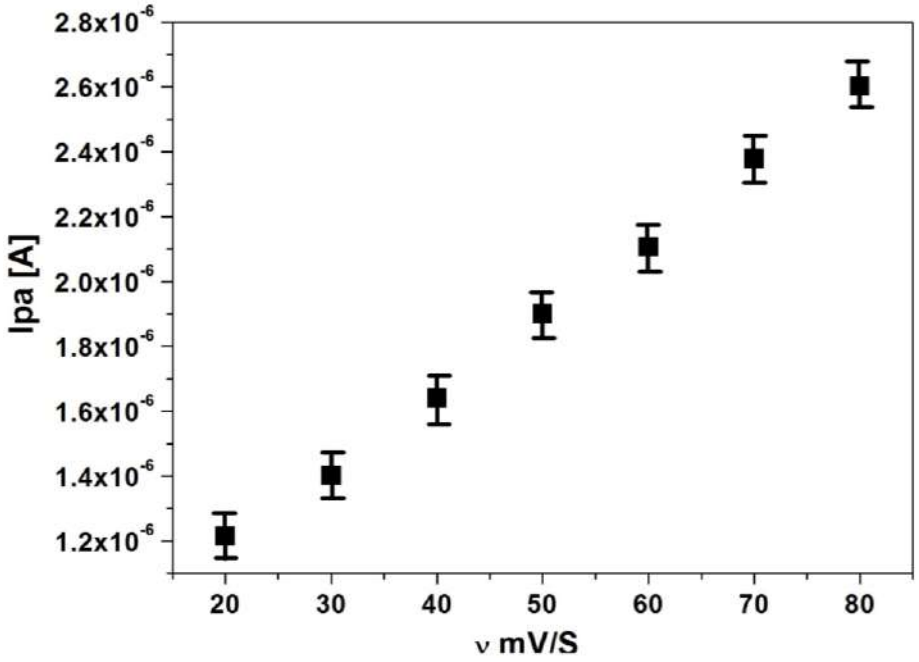


Fig.7b: Graph of anodic peak current (Ipa) versus scan rate (ν) of $0.1x10^{-4}$M FA

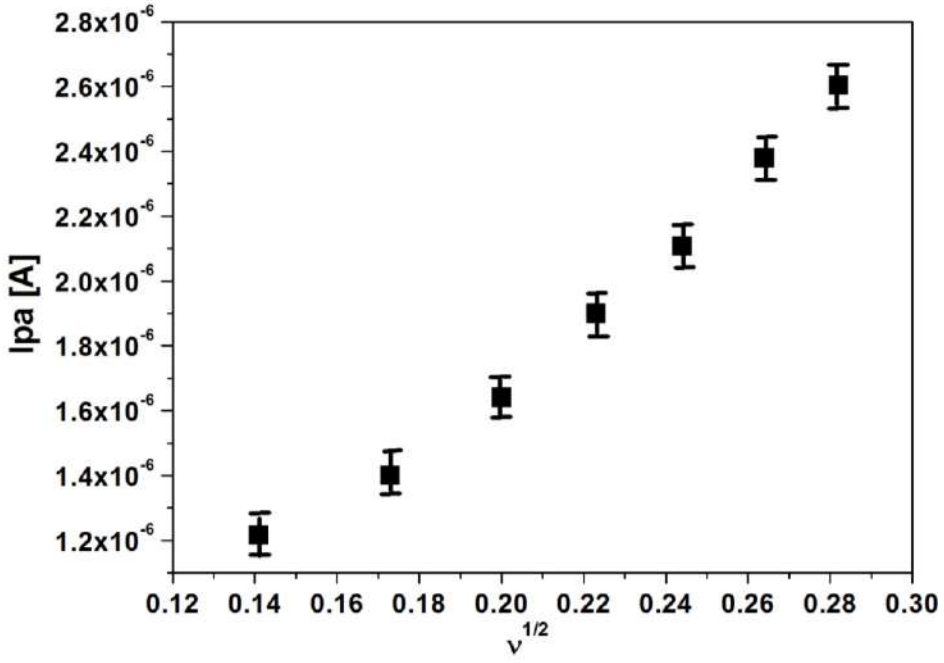


Fig.7c: Graph of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) of $0.1x10^{-4}$M FA

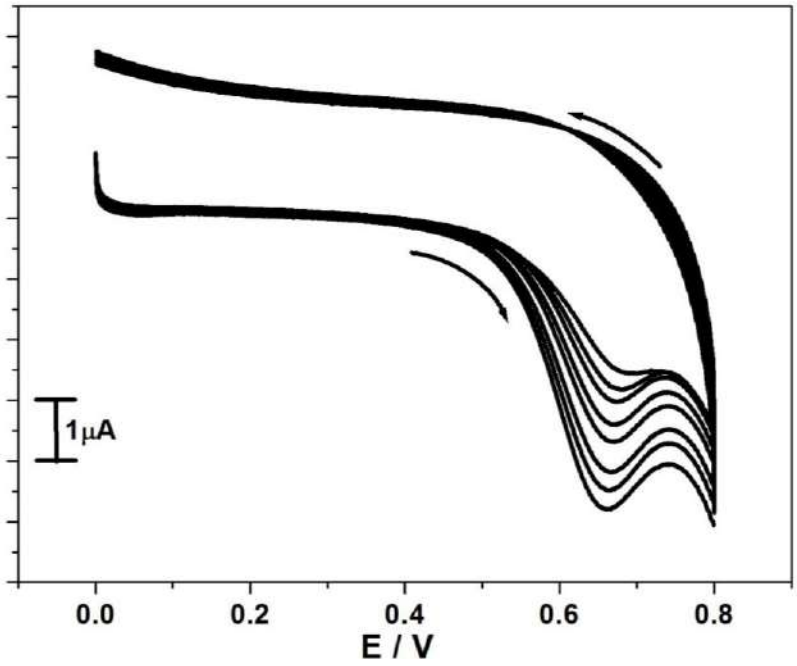


Fig.8a: Cyclic voltammograms obtained for FA at PLCN-MCPE with different concentration (0.2 to 0.9 x 10^{-4}M) using 0.2M PBS (pH 7.4) with the scan rate 50 mVs^{1}.

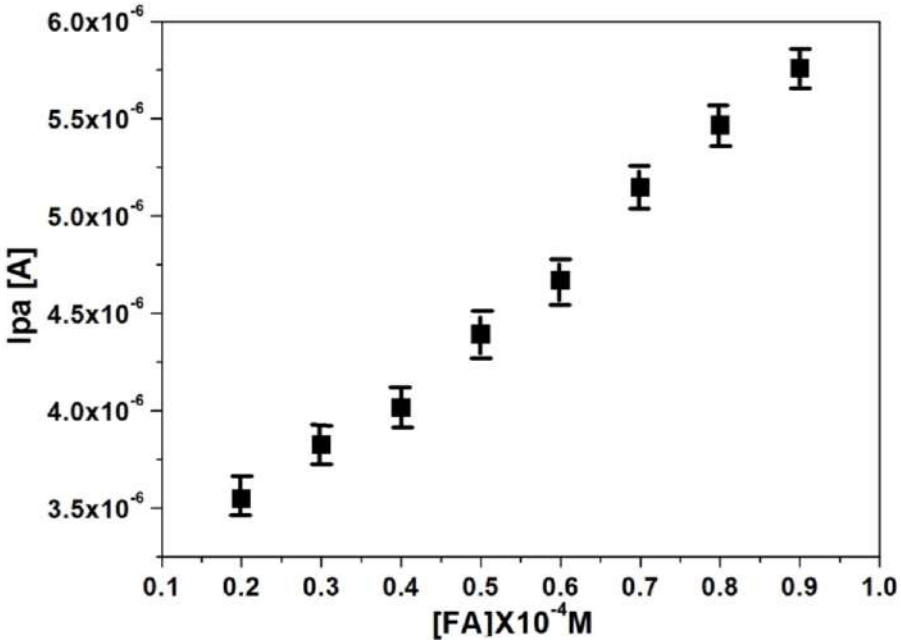


Fig.8b: Graph of anodic peak current (Ipa) versus concentration of FA

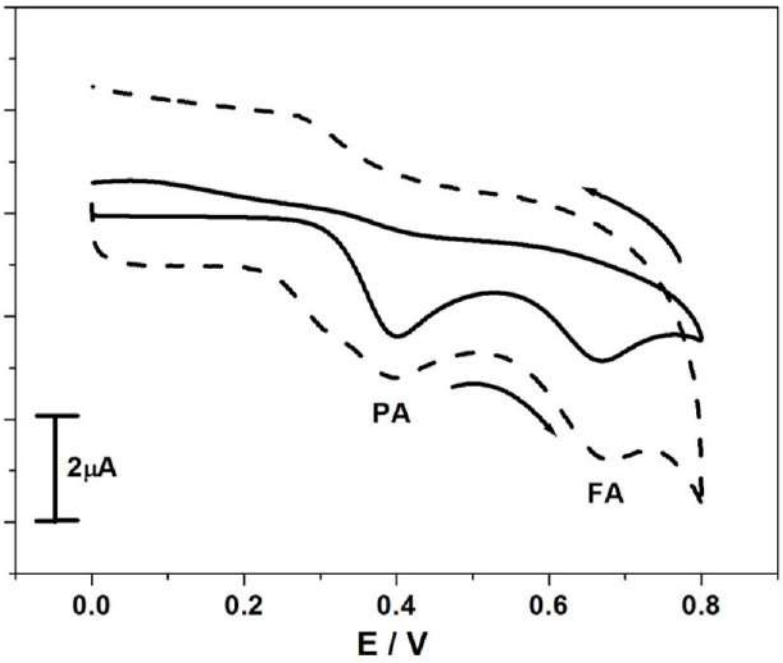


Fig.9: Cyclic voltammograms obtained for the mixture of PA ($0.1x10^{-4}$M) and FA ($0.1x10^{-4}$M) at bare (solid line) and PLCN-MCPE (dashed line) using 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}.

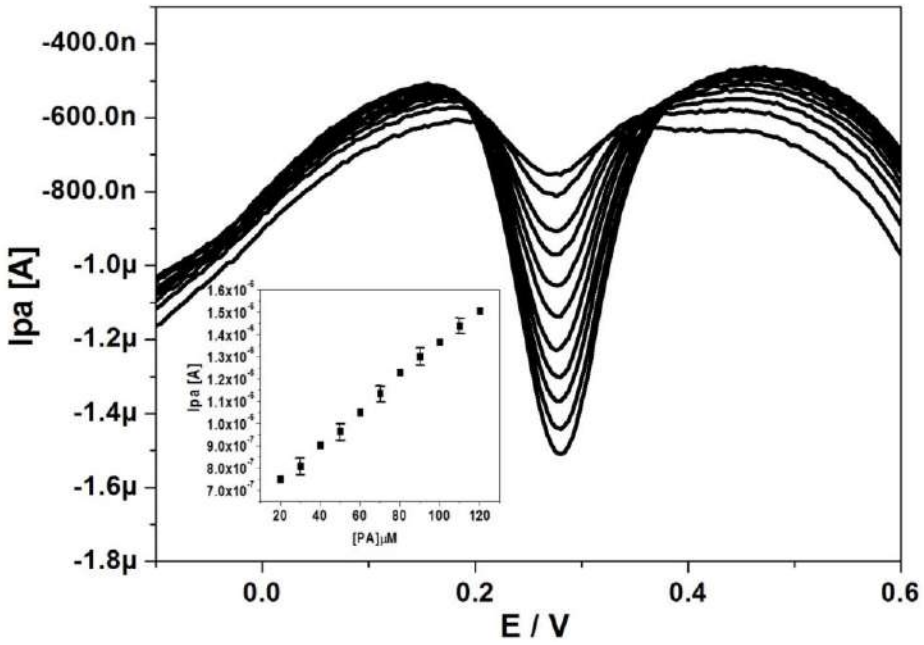


Fig.10: Differential pulse voltammograms of PA at PLCN-MCPE with different concentration (20–120μM) using 0.2M PBS (pH 7.4) with the scan rate $50mVs^{1}$. Inset figure shows the graph of anodic peak current (Ipa) versus concentration of PA.

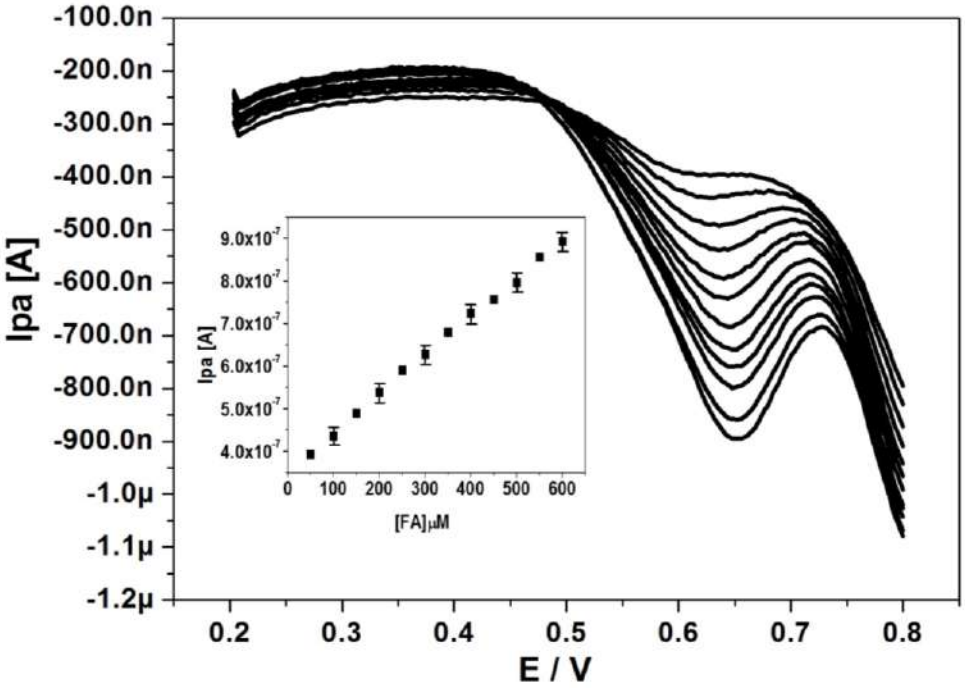


Fig.11: Differential pulse voltammograms of FA at PLCN-MCPE with different concentration (50–600 µM) using 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{1}. Inset figure shows the graph of anodic peak current (Ipa) versus concentration of FA.

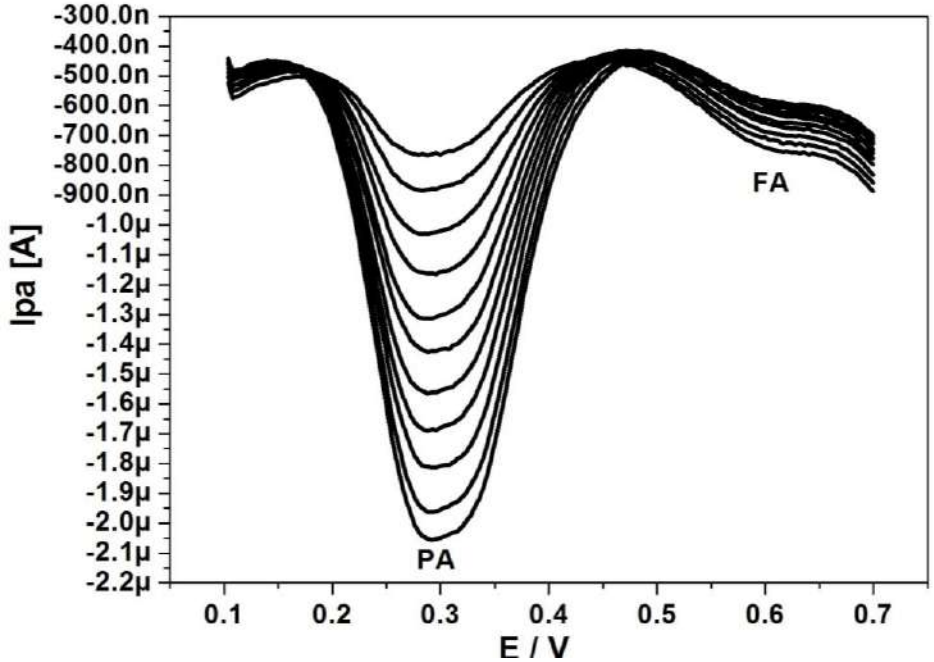


Fig.12a: Differential pulse voltammograms of PA with different concentration (20–120µM) in presence of FA (100 µM) at PLCN-MCPE using 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}.

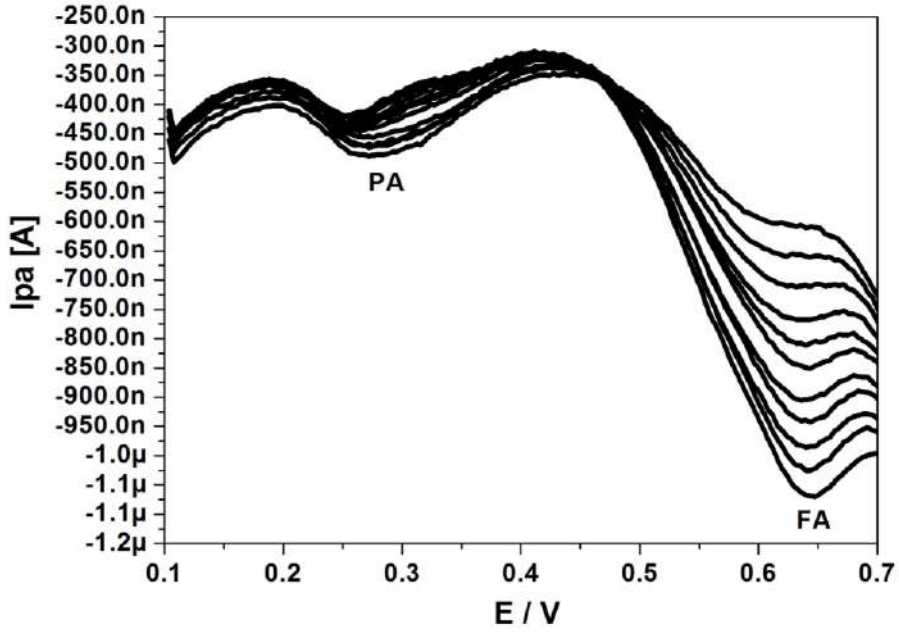


Fig.12b: Differential pulse voltammograms of FA with different concentration (100 – 600µM) in presence of PA (50 µM) at PLCN-MCPE using 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}.

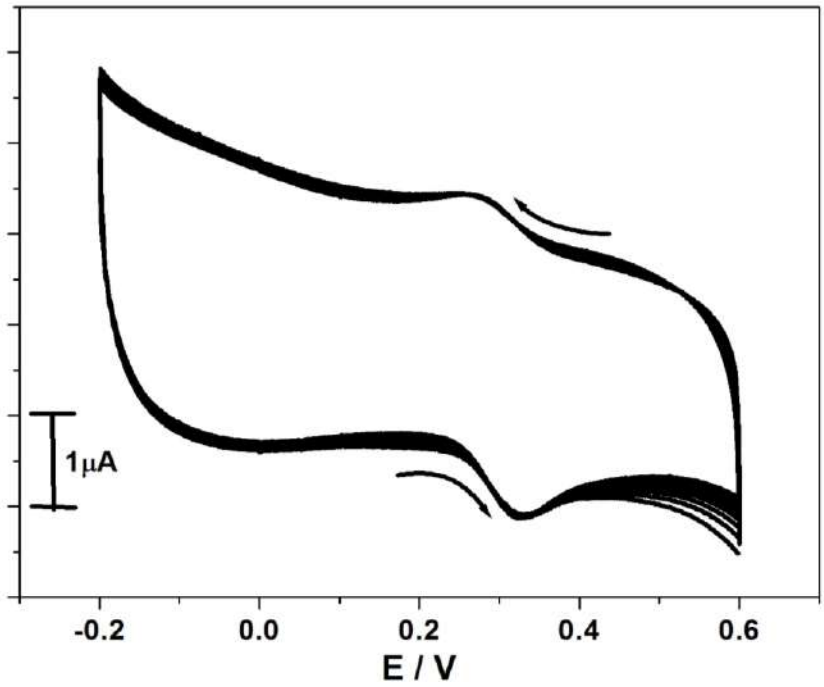


Fig.13: Cyclic voltammogram obtained for 10 multiple cycles of 0.1x10^{-4}M PA at PLCN-MCPE using 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}.

Table 1: Determination of heterogeneous rate constant (k^o) for PA at PLCN-MCPE.

Scan rate (mVs^{-1})	**ΔEp/mV**	**Number of electron transfer (n)**	**Heterogeneous rate constant (k^0s^{-1})**
10	52	1.74	0.018
20	52	1.74	0.026
30	52	1.74	0.032
40	50	1.8	0.038
50	55	1.64	0.04
60	53	1.7	0.045
70	58	1.56	0.046

Table 2: Determination of heterogeneous rate constant (k^o) for FA at PLCN-MCPE.

Scan rate (mVs^{-1})	**Peak potential (Ep)**	**Heterogeneous rate constant (k^0s^{-1})**
20	0.651	0.05
30	0.662	0.06
40	0.672	0.07
50	0.683	0.08
60	0.689	0.08
70	0.691	0.09
80	0.699	0.10

Table 3: Determination of PA in the tablet at PLCN-MCPE

Sample	**Content**	**Added (mL)**	**Found (mL)**	**Recovery**
Tablet	5mg	0.4	0.37	94.6%
Tablet	5mg	0.5	0.48	96%
Tablet	5mg	0.6	0.54	91%

Table 4: Determination of FA in the tablet at PLCN-MCPE

Sample	**Content**	**Added (mL)**	**Found (mL)**	**Recovery**
Tablet	5mg	0.4	0.39	97.8%
Tablet	5mg	0.5	0.47	94.1%
Tablet	5mg	0.6	0.51	86.5%

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Chapter -4

Part-A

Poly (phenosafranine)/SAOS modified sensor for the determination of dopamine and uric acid

Published in Analytical &Bioanalytical Electrochemistry 9 (2017) 424-438

4.1 Introduction

Dopamine (DA) (scheme 1) is one of the crucial catecholamine neurotransmitter molecule widely distributed in the mammalian central nervous system, renal, hormonal and cardiovascular systems for message transfer [1-3]. Neurotransmitters (NTs) are chemical messengers that transmit a message from one neuron to the next. This transmission proceeds by secretion of the NTs from one neuron, followed by their binding to the specific receptor located on the membrane of the target cell [4-5]. Low levels of DA are related to neurological disorders, such as schizophrenia, Parkinson's disease and to HIV infection [6-8]. As a cholinergic drug, DA is widely applied to the treatment of circulatory collapse syndrome caused by myocardial infarction, trauma, renal failure, cardiac surgery, or congestive cardiac failure. Consequently, it has attracted much of interest of electrochemists to develop the detection methods of DA, no matter in route or *in vivo* analysis [9]. One of the most common routes is to use a modified carbon paste electrode which has the ability to eliminate the interfering substances from DA determination. The study of electrochemical determination of DA with different modified electrode was reported [10-11].

Uric acid (UA) (2,6,8-trihydroxypurine) (scheme 2) is the primary product of purine metabolism in the human body. Abnormal concentration levels of UA can lead to several disorders such as gout, Lesch–Nyhan syndrome, hyperuricaemia, cardiovascular disease, and multiple sclerosis [12-14]. DA coexists with UA and AA in body fluids [15-16]. In general, electroactive UA can be irreversibly oxidized in aqueous solution and the major product is allantoin[17-18]. Usually DA and UA can be oxidized at almost the same potential at the traditional electrodes. Thus, the development of modified electrodes for the simultaneous determination of them with sensitivity and selectivity is highly desirable for diagnostic and analytical applications [19].

Phenosafranine (PS^+) (scheme 3), a phenazine dye, is a simple red safranine dye made by oxidation of a 1:2 mixture of para-phenylenediamine and aniline. It is widely used as redox mediator and as sensitizer in photogalvanic cells [20]. Usually, most of the polyazines prepared so far are cationic polyelectrolytes, they provide a simple and convenient method for fixing chelating reagents, enzymes and cofactors on electrode

surfaces. In present work, phenosafranine (PS^+) was electropolymerized on the surface of bare carbon paste electrode to give poly (phenosafranine) modified carbon paste electrode (PPS/MCPE).

The sodium alpha olefin sulphonate (SAOS) (scheme 4) is an anionic surfactant, formed by the direct reaction of olefins with strong sulphonating agents, such as sulphur trioxide. The sodium alpha olefin sulphonate surfactant can be used in the formulation of a variety of skin cleaning products including toilet bars and bubbles bath compositions, a suggested liquid bubble bath formulation containing SAOS. The SAOS surfactant shows excellent foaming and detergency in hard water, also well known for its relative mildness, cleaning efficiency and favourable cost [21-24]. The term surfactant is a compound that contains a hydrophilic (attracted to water) and a hydrophobic (repelled by water) segments, plays an important role in electrochemistry and electroanalytical chemistry for various purposes [25-28]. The surfactants are frequently used in a variety of textile technology and biotechnology. To improve the detection limits of some biomolecules, Hu's group has introduced surfactants to electroanalytical chemistry [29-31]. In present study, the SAOS (anionic surfactant) is used as modifier on the surface of poly (phenosafranine) modified carbon paste electrode by immobilization technique.

The aim of this chapter was to investigate electrochemically modified poly (phenosafranine)/SAOS modified carbon paste electrode (PPS/SAOS/MCPE) towards the determination of dopamine and uric acid at physiological pH.

4.2 Experimental

4.2.1 Reagents and chemicals

The chemicals used were Dopamine (DA) from Merck and Uric acid was from Sigma-Aldrich. The Disodium hydrogen phosphate (Na_2HPO_4), Sodium dihydrogen orthophosphate (NaH_2PO_4), Phenosafranine and sodium alpha olefin sulphonate were purchased from Himedia chemicals. Spectrally pure graphite powder (particle size <50 mm) from Merck and high viscous paraffin oil (density=0.88gcm^{-3}) from Fluka were used for the preparation of the carbon paste electrode. The phosphate buffer (0.2M pH 7.4) was used as optimum measurements. All the chemicals used in this experiments were analytical grade and used without any further purification.

4.2.2 Apparatus

Voltammetric (CV) measurements were performed with a model CHI-660c (CH Instrument-660 electrochemical workstation). All electrochemical measurements were performed in a standard three electrode cell. The bare and Poly (phenosafranine)/SAOS modified carbon paste electrode (PPS/SAOS/MCPE) were used as working electrode, Platinum electrode as counter electrode and saturated Calomel electrode (SCE) as the reference electrode. All the potentials reported versus the SCE.

4.2.3 Preparation of bare carbon paste electrode (BCPE)

The preparation of BCPE has been discussed in chapter-3A at section 3.2.3.

4.2.4 Preparation of Poly (phenosafranine)/SAOS modified carbon paste electrode (PPS/SAOS/MCPE)

The phenosafranine (1mM) was electropolymerized on the surface of the bare carbon paste electrode (BCPE) using 0.2 M PBS (pH 7.4) as the supporting electrolyte with the scan rate 50 mVs^{-1}. The electropolymerization was achieved by the repetitive potential cycling between -0.5V to 1.3V for ten cycles. After polymerization, the poly (phenosafranine) film was washed with distilled water to remove unreacted phenosafranine. The sodium alpha olefin sulphonate surfactant (5µL) was immobilized on the surface of poly (phenosafranine) modified carbon paste electrode to get poly (phenosafranine) / SAOS modified carbon paste electrode. The modified electrode was used for further electrochemical investigations.

4.3 Results and Discussion

4.3.1 Electropolymerization of phenosafranine on the surface of bare carbon paste electrode (BCPE)

Fig.1a shows the cyclic voltammograms of electropolymerization of phenosafranine at the surface of bare carbon paste electrode (BCPE). The electropolymerization was carried out at the surface of bare carbon paste electrode by taking 1mM phenosafranine in presence of 0.2M PBS with the scan rate 50 mVs^{-1}. Electropolymerization was achieved between the potential -0.5V to 1.3V. Increase in peak current with increase in the cycle, shows the growth of polymeric film at the surface of CPE [32]. The effect of multiple

cycles on electropolymerization was shown in Fig.1b. The CPE was modified by applying different multiple cycles of polymerization (from 5 to 25) and the corresponding electrochemical behaviour towards the determination of DA ($0.3x10^{-4}$M) was examined, which can be observed that the current enhancement towards the determination of DA goes on increases. Finally, 10 cycles was selected for electropolymerization which shows comparatively good cyclic voltammograms.

4.3.2 Effect of surfactant concentration (sodium alpha olefin sulphonate) on the surface of poly (phenosafranine) modified carbon paste electrode

Cyclic voltammetry (CV) was employed to study the effect of surfactant on the surface of poly (phenosafranine) modified carbon paste electrode. Different concentrations of surfactant (5, 10, 15, 20 and 25 µL) was immobilized on the surface of poly (phenosafranine) modified carbon paste electrode and their electrochemical response towards $0.3x10^{-4}$M dopamine was studied in presence 0.2M PBS as supporting electrolyte at pH 7.4 with the scan rate of $50mVs^{-1}$. The plot of anodic peak current (Ipa) of 0.3×10^{-4}M DA versus quantity of SAOS in µL was depicted in Fig.2. Finally, 5µL SAOS was selected and immobilized on the surface of poly (phenosafranine) modified CPE to give poly (phenosafranine)/SAOS modified carbon paste electrode (PPS/SAOS/MCPE). The modified electrode was employed for further studies.

4.3.3 Voltammetric behavior of dopamine at PPS/SAOS/MCPE

The voltammetric redox behavior of dopamine (DA) was investigated by cyclic voltammetric technique at poly (phenosafranine)/SAOS modified carbon paste electrode. The cyclic voltammograms of 0.3×10^{-4}M DA at BCPE (dotted line), poly (phenosafranine) modified CPE (dashed line) and poly (phenosafranine)/SAOS/MCPE (solid line) in 0.2M PBS (pH 7.4) with the scan rate of $50mVs^{-1}$ was shown in Fig.3. The BCPE exhibits poor electrochemical response towards the determination of DA and difference in redox peak potential (ΔEp) was found to be 0.045 V. The poly (phenosafranine)/MCPE shows better electrochemical response than BCPE and difference in redox peak potential (ΔEp) was found to be 0.051 V. Comparatively, poly (phenosafranine)/SAOS/MCPE shows great enhancement in the redox peak current and

difference in redox peak potential (ΔEp) was found to be 0.039 V. Since the ΔEp is a function of rate of electron transfer, lower the ΔEp value higher will be the electron transfer rate [33]. Thus, from the above experimental observations the poly (phenosafranine)/SAOS/MCPE proved its electrocatalytic ability towards the determination of dopamine.

4.3.4 Scan rate variation towards the redox behaviour of dopamine at PPS/SAOS/MCPE

Cyclic voltammetry (CV) technique was employed to study the scan rate (ν) variation of dopamine at PPS/SAOS/MCPE. The cyclic voltammograms of $0.3x10^{-4}$ M DA at different scan rate in presence of 0.2M phosphate buffer solution (pH 7.4) was shown in Fig.4a. The redox peak current of DA increases with increase in the scan rate from 10-100mVs^{-1}. Fig.4b shows the plot of anodic peak current (Ipa) versus scan rate (ν) exhibits good linearity and correlation coefficient value was found to be r^2=0.999. Fig.4c shows the plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) also exhibits good linearity with correlation coefficient value r^2=0.992. From the above experimental observation, the electrode process was found to be adsorption-controlled.

4.3.5 Concentration variation of dopamine at PPS/SAOS/MCPE

The effect of dopamine concentration was studied by cyclic voltammetry (CV) technique at PPS/SAOS/MCPE. The cyclic voltammograms of DA at different concentration in presence of 0.2M PBS (pH 7.4) with the scan rate of 50 $mVs^{-1}$was shown in Fig.5a. The redox peak currents of DA increases with increase in concentration from 0.1 - 0.9 x 10^{-4}M. Fig.5b shows the plot of anodic peak current (Ipa) versus concentration of DA having good linearity with correlation coefficient value r^2=0.998. The limit of detection (LOD) and limit of quantification (LOQ) for DA at PPS/SAOS/MCPE was found to be 4.43 µM and 14.7 µM respectively. It can be calculated by the following equation (1) and (2) [34-37].

$LOD=3S/M$ ..... (1)

$LOQ=10S/M$.....(2)

where, S is the standard deviation and M is the slope

4.3.6 Effect of pH variation at PPS/SAOS/MCPE

In order to investigate the electrocatalytic ability of the proposed electrode, the effect of pH variation was studied at PPS/SAOS/MCPE using cyclic voltammetry method. The cyclic voltammograms of 0.3 x 10^{-4}M DA at different pH (6.2, 6.6, 7.0, 7.4, and 7.8) using 0.2M phosphate buffer solution (pH 7.4) with the scan rate of 50mVs^{-1} was shown in Fig.6a. With the increase in the pH, anodic peak potentials of the DA were shifted to negative value. Fig.6b shows the relationship between anodic peak potential of DA and pH. Finally, pH 7.4 was selected as an optimum solution pH for present study.

4.3.7 Voltammetric behavior of uric acid at PPS/SAOS/MCPE

The electrochemical behavior of uric acid (UA) was studied at PPS/SAOS/MCPE using cyclic voltammetry method. Fig.7 shows the cyclic voltammograms obtained for 0.3x10^{-4}M UA at BCPE (short dashed line) and PPS/SAOS/MCPE (solid line) using 0.2M PBS at pH 7.4 with the scan rate of 50 mVs^{-1}. The BCPE exhibits poor electrochemical response towards the determination of UA and the oxidation peak potential (Ep) was observed at 0.28V. Comparatively, PPS/SAOS/MCPE shows a great enhancement in the anodic peak current (Ipa) of UA and the oxidation peak potential (Ep) was observed at 0.27V. Hence, the modified electrode proved its electrocatalytic behavior towards the determination of uric acid.

4.3.8 Scan rate variation of uric acid at PPS/SAOS/MCPE

The type of electrode process was investigated by varying the scan rate of UA at PPS/SAOS/MCPE. The cyclic voltammograms of 0.3x10^{-4} M UA at different scan rate in presence of 0.2M phosphate buffer solution (pH 7.4) was shown in Fig.8a. The oxidation peak current of UA increases with increase in the scan rate from 10 – 70 mVs^{-1}. Fig.8b shows the plot of anodic peak current (Ipa) versus scan rate (ν) with good linearity and correlation coefficient value was found to be r^2=0.999. Fig.8c shows the plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) also exhibits good linearity with correlation coefficient value r^2=0.995. Hence, the modified electrode shows adsorption-controlled type of electrode process.

4.3.9 Concentration variation of uric acid at PPS/SAOS/MCPE

The concentration variation of UA was investigated at PPS/SAOS/MCPE by cyclic voltammetry. Fig.9a shows the cyclic voltammograms obtained for UA at different concentration (0.3 - 1.0 x 10^{-4}M) in presence of 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}. The oxidation peak current of UA increases gradually with increase in the concentration. Fig.9b shows the plot of anodic peak current (Ipa) versus concentration of UA, which shows good linearity with correlation coefficient value r^2=0.998. The limit of detection (LOD) and limit of quantification (LOQ) for UA at PPS/SAOS/MCPE was found to be 4.18 µM and 13.9 µM respectively using the equation (1) and (2).

4.3.10 Simultaneous electrochemical determination of dopamine in presence of uric acid at PPS/SAOS/MCPE

The simultaneous electrochemical determination of DA in presence of UA was investigated by both cyclic voltammetry and differential pulse voltammetry technique at PPS/SAOS/MCPE. Fig.10 shows the cyclic voltammograms obtained for the binary mixture containing 0.2x10^{-4}M DA and 0.5x10^{-4}M UA in the presence of 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}. The short dashed line shows cyclic voltammogram of the binary mixture (DA and UA) at BCPE. The solid line shows cyclic voltammogram obtained for the binary mixture (DA and UA) at PPS/SAOS/MCPE. The above experimental observations illustrate the PPS/SAOS/MCPE exhibits a huge enhancement in peak current (Ip) of the binary mixture (DA and UA) than the BCPE. The differential pulse voltammograms of the binary mixture (0.2x10^{-4}M DA and 0.5x10^{-4}M UA) at PPS/SAOS/MCPE was shown in inset fig.10. Therefore, newly developed sensor has the ability and capability towards the determination of dopamine and uric acid individually and simultaneously.

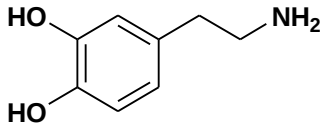
4.3.11 Interference study

Differential pulse voltammetry was employed for the study of interfering substances on the peak current of DA and UA at PPS/SAOS/MCPE. The differential pulse voltammograms of the binary mixture (DA and UA) was shown in Fig.11, in which the concentration of DA was varied (from 200-1000 µM) while keeping the concentrations of

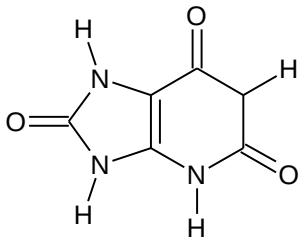
UA constant. With the increase in the concentration of DA, peak current goes on increases. The plot of anodic peak current (Ipa) versus concentration of DA was shown in inset fig.11. Fig.12 shows the DPVs of the binary mixture contain DA and UA, in which the concentration of UA was varied (from 500-1200 µM) while keeping the concentrations of DA constant. The peak current increases with increase in UA concentration. The plot of anodic peak current (Ipa) versus concentration of UA was shown in inset fig.12.

4.4. Conclusion

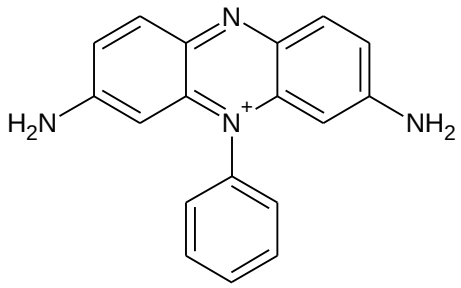
The poly (phenosafranine)/SAOS/MCPE was developed and employed for the simultaneous electroanalysis of dopamine and uric acid by CV and DPV techniques. The newly developed sensor exhibits significant increment in the peak current of DA and UA individually and simultaneously. The modified electrode show adsorption-controlled type of electrode process and good detection limit (LOD) was found for DA and UA. Hence, proposed sensor exhibits good electrocatalytic properties towards the simultaneous determination of DA and UA.



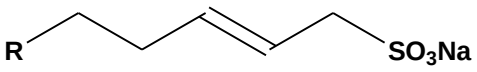
Scheme 1: Structure of Dopamine



Scheme 2: Structure of Uric acid



Scheme 3: Structure of Phenosafranine



Scheme 4: Structure of Sodium Alpha Olefin Sulphonate

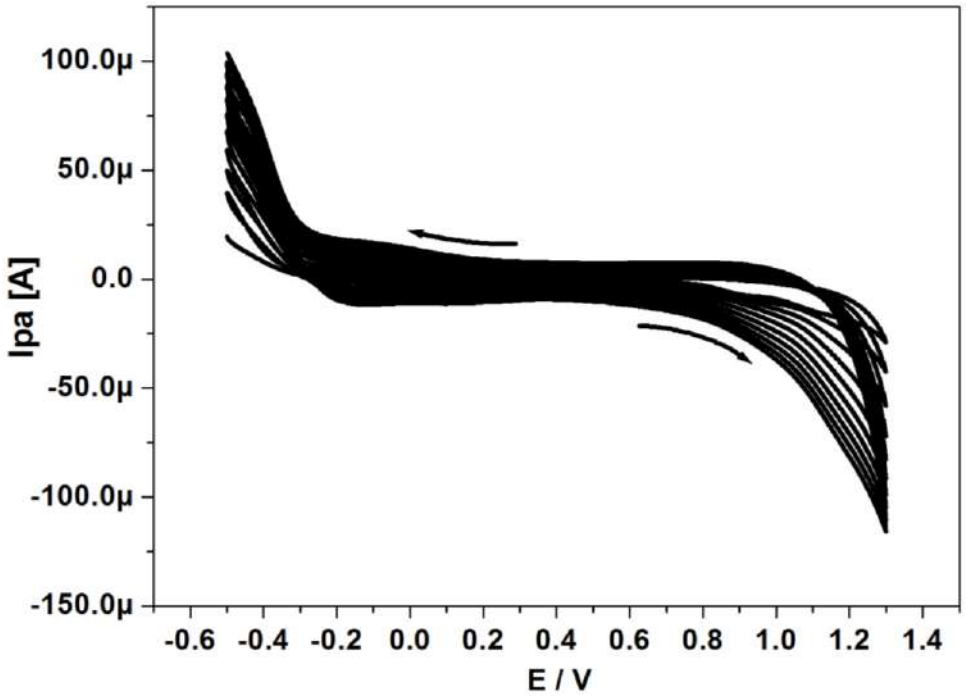


Fig.1a: Repetitive cyclic voltammograms for the electropolymerization of 1 mM phenosafranine on the surface of CPE in presence of 0.2M PBS (pH 7.4) with the scan rate 50mVs^{-1}.

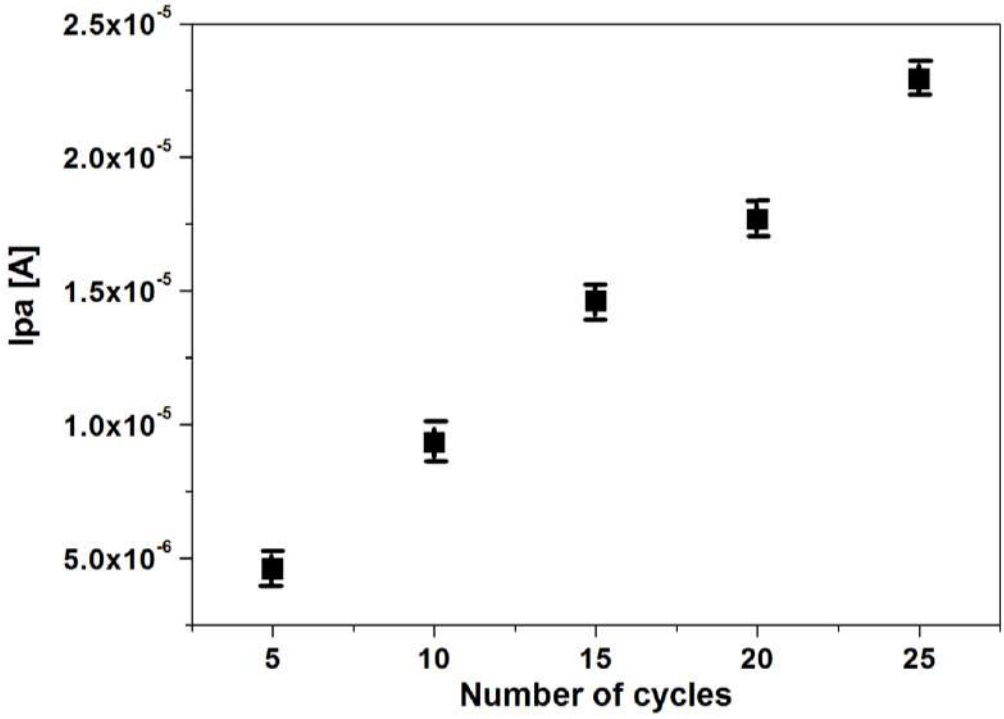


Fig.1b: Effect of polymerization cycle variation on the peak current of 0.3x10^{-4} M DA in presence of 0.2M PBS (pH 7.4) with the scan rate 50mVs^{-1}.

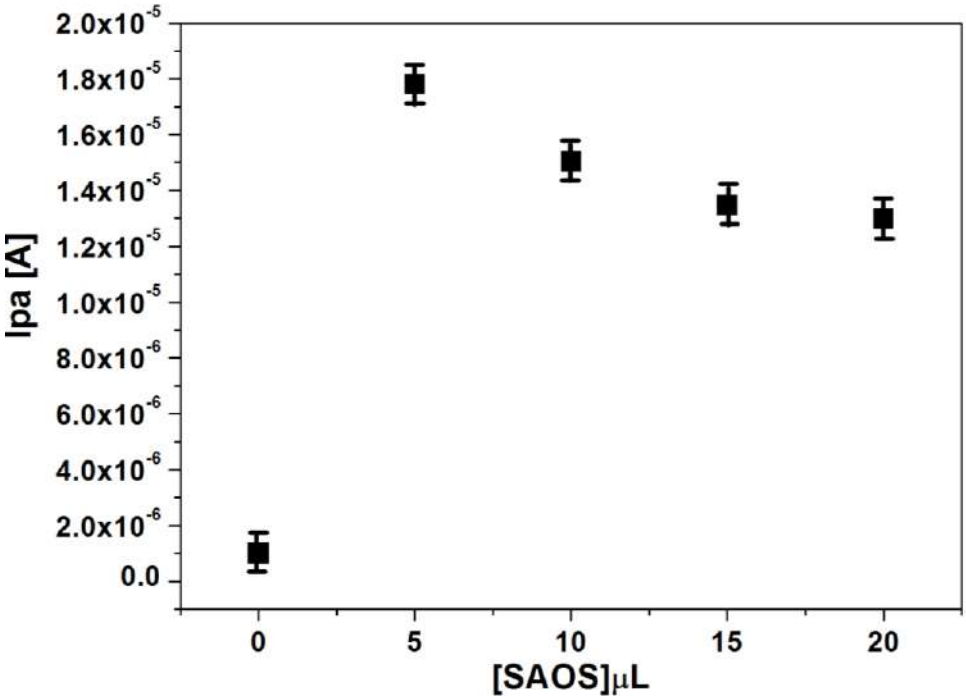


Fig.2: Effect of concentration of SAOS (µL) on the surface of PPS/MCPE towards the oxidation peak current of $0.3x10^{-4}$ M DA in presence of 0.2M PBS (pH 7.4) with the scan rate 50 mVs^{-1}.

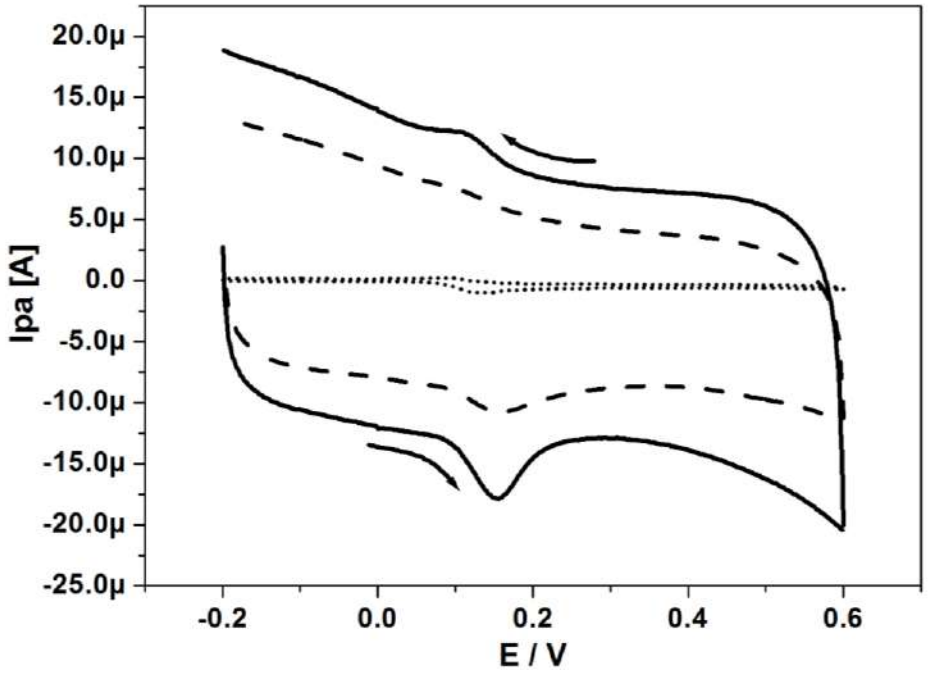


Fig.3: Cyclic voltammograms obtained for $0.3x10^{-4}$M DA at BCPE (dotted line), PPS/MCPE (dashed line) and PPS/SAOS/MCPE (solid line) with the scan rate 50mVs^{-1} using 0.2M PBS (pH 7.4).

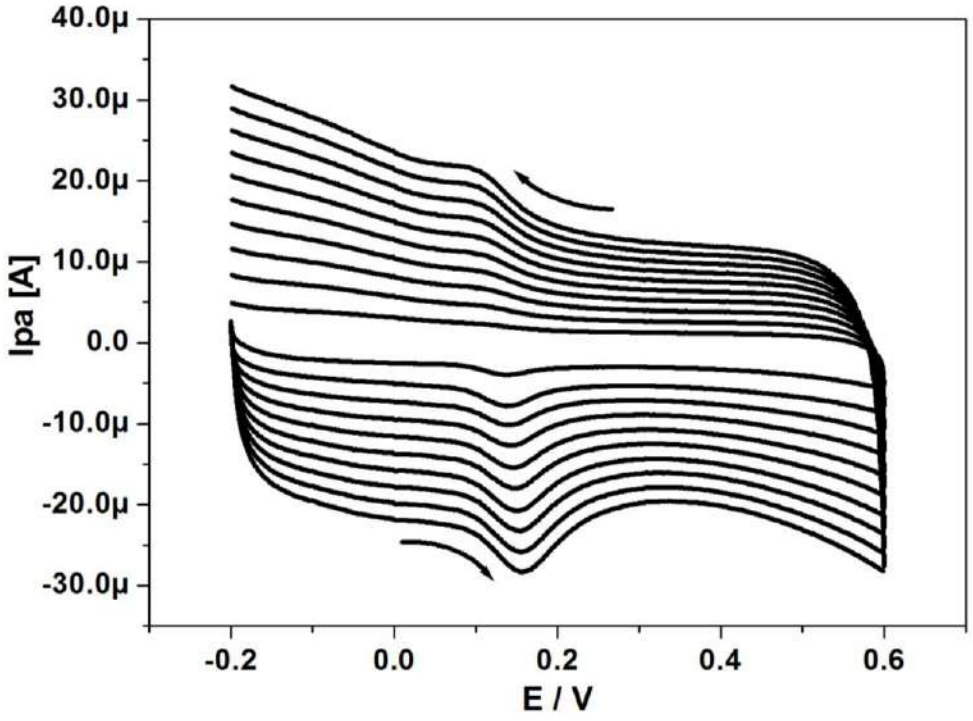


Fig.4a: Cyclic voltammograms obtained for $0.3x10^{-4}$M DA with different scan rates (10-100mVs^{-1}) using 0.2M PBS (pH 7.4) at PPS/SAOS/MCPE.

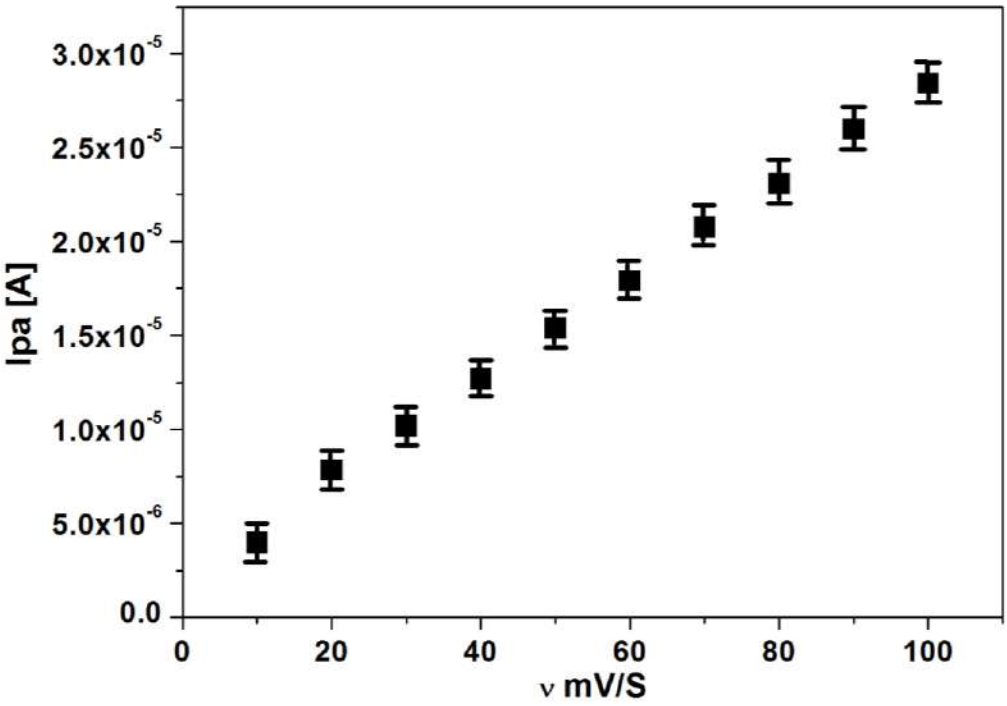


Fig.4b: Plot of anodic peak current (Ipa) versus scan rate (ν)

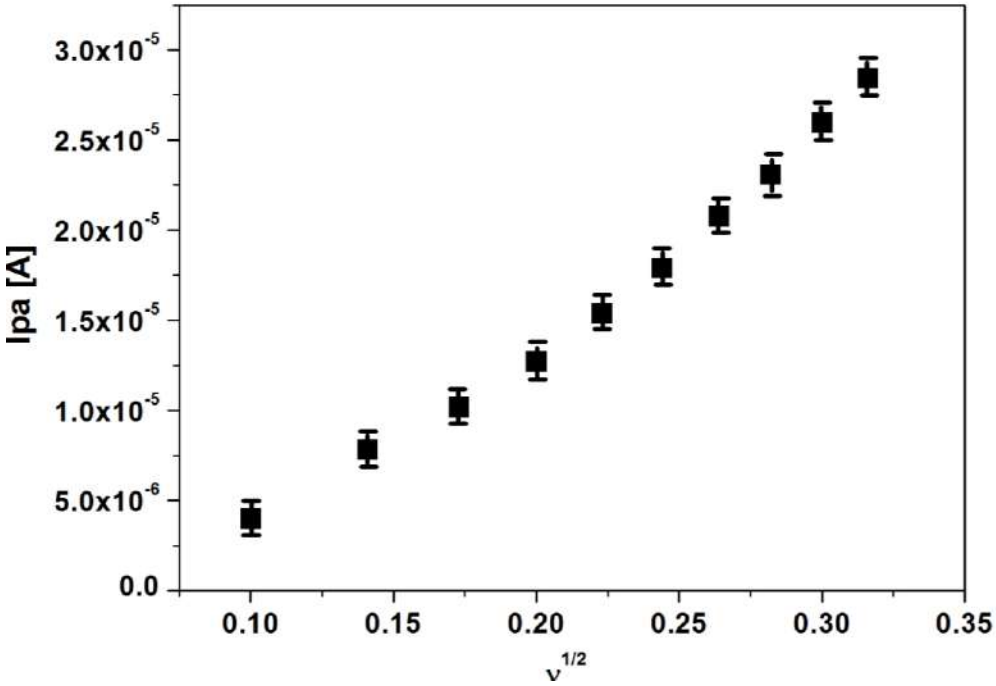


Fig.4c: Plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$)

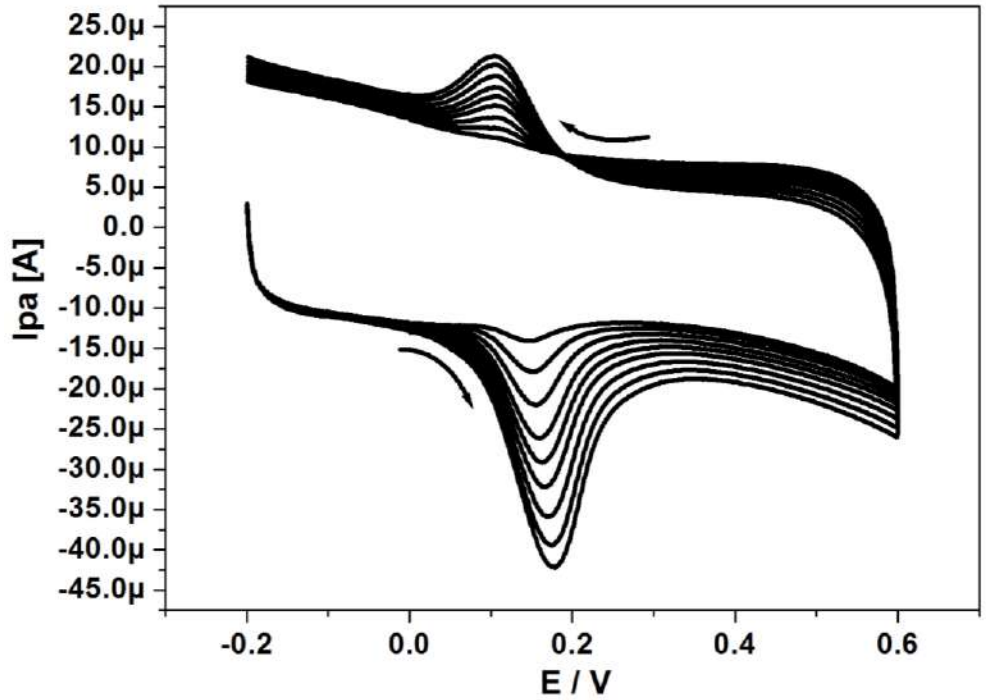


Fig.5a: Cyclic voltammograms of DA at different concentration (0.1 to 0.9 x 10^{-4}M) in presence of 0.2M PBS (pH 7.4) with the scan rate 50 mVs^{1}.

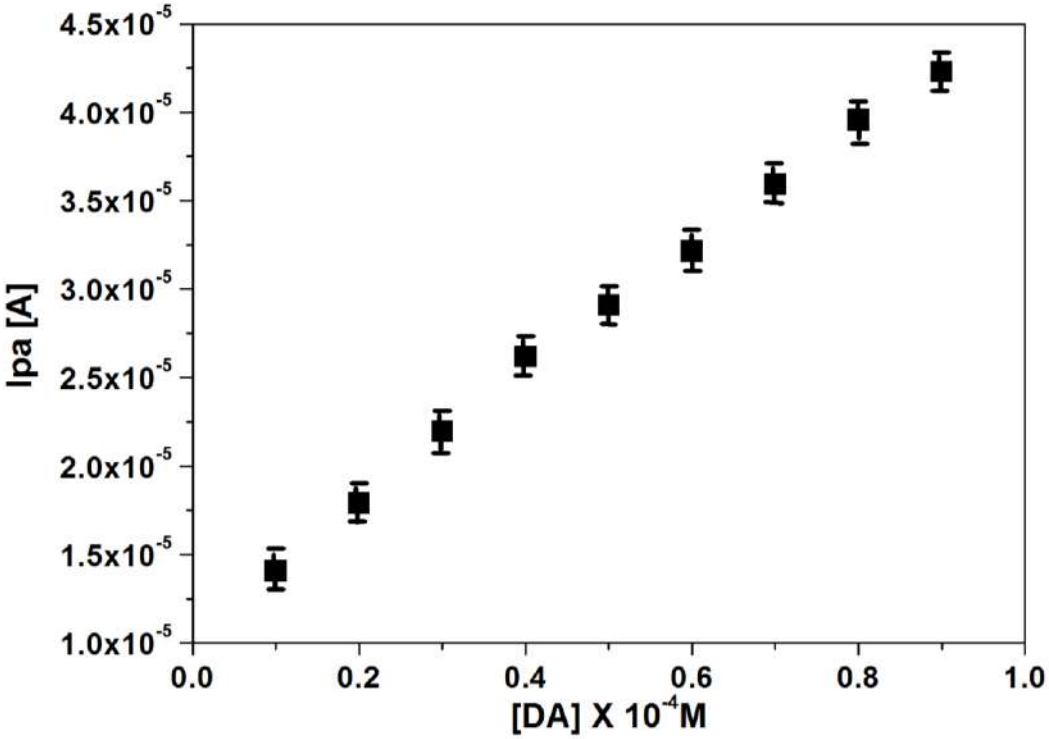


Fig.5b: Plot of anodic peak current (Ipa) versus concentration of DA

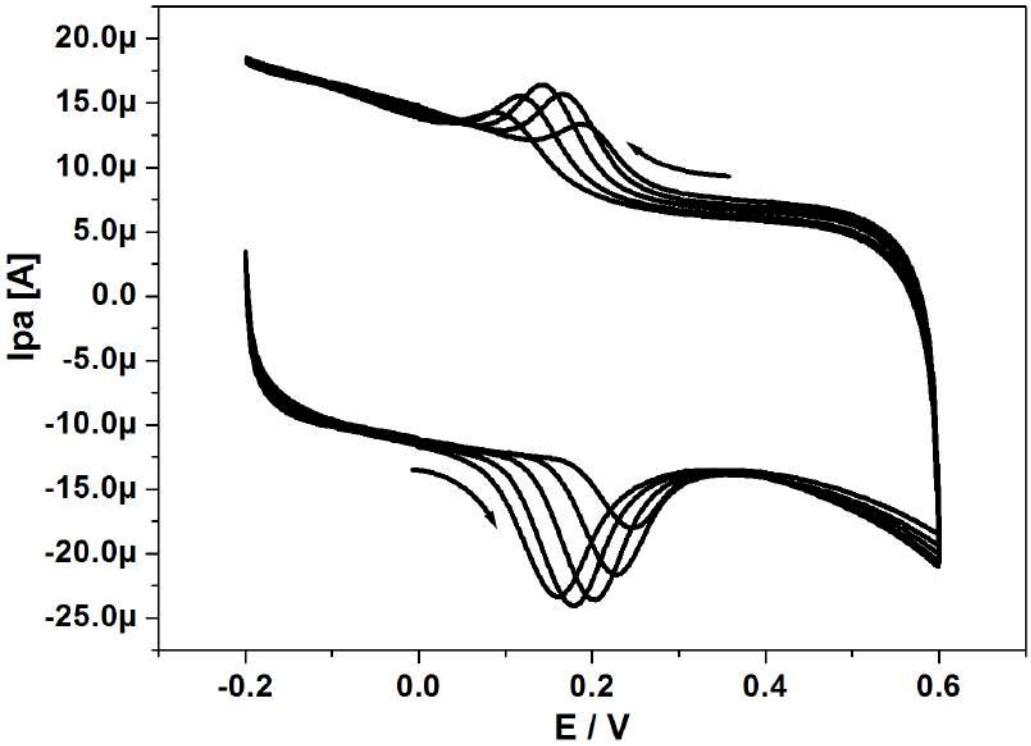


Fig.6a: Cyclic voltammograms obtained for $0.3x10^{-4}$M DA with different pH (6.2, 6.6, 7.0, 7.4 and 7.8) at PPS/SAOS/MCPE.

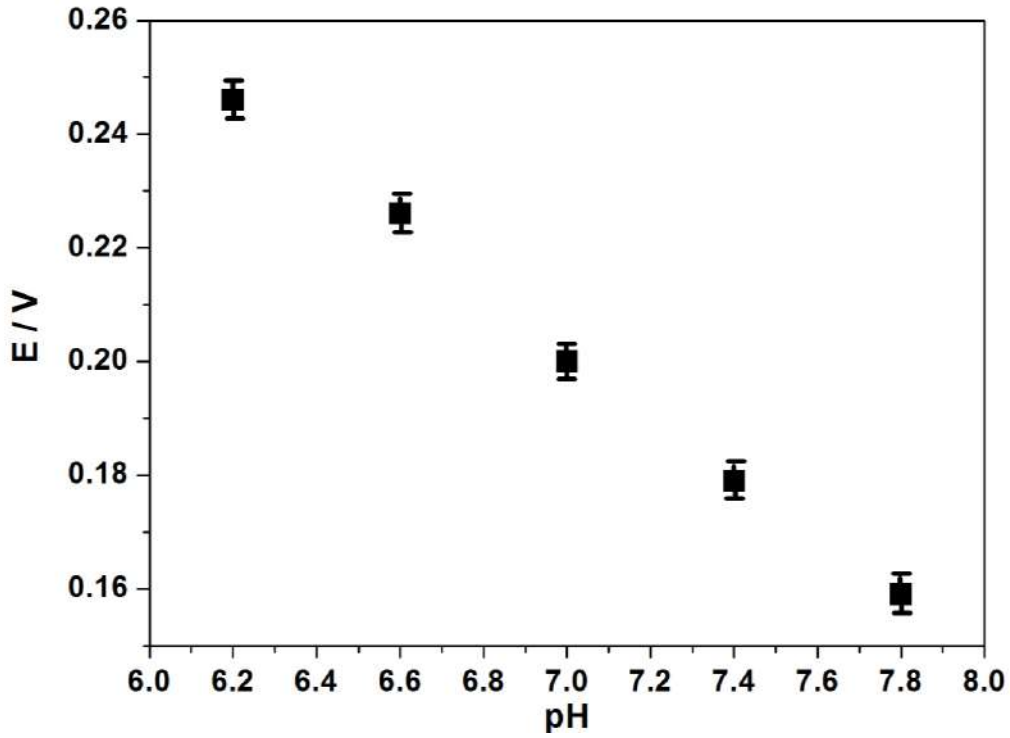


Fig.6b: Plot of peak potential (Ep) versus pH

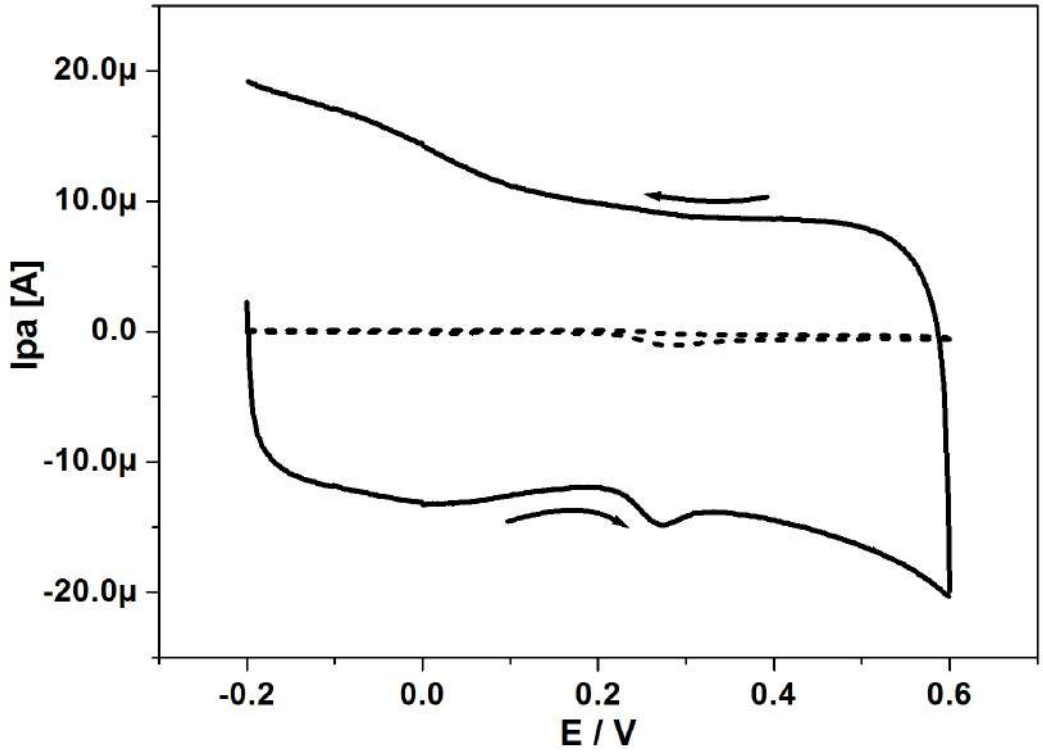


Fig.7: Cyclic voltammograms obtained for $0.3x10^{-4}$M UA at BCPE (short dashed line) and PPS/SAOS/MCPE (solid line) with the scan rate $50mVs^{-1}$ using 0.2M PBS (pH 7.4).

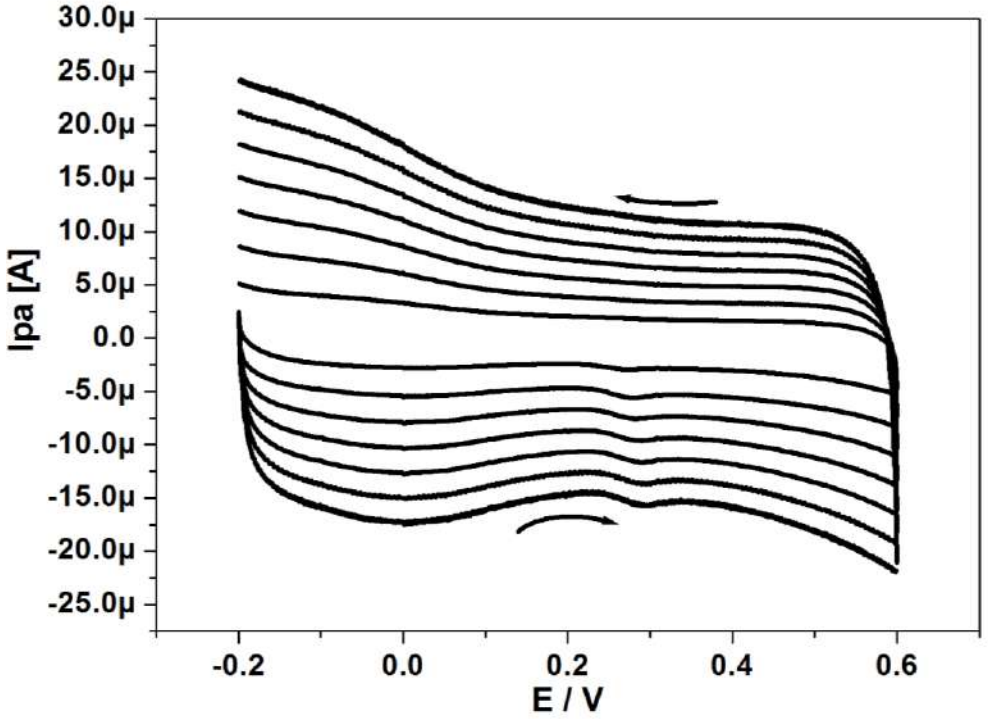


Fig.8a: Cyclic voltammograms obtained for $0.3x10^{-4}$M UA with different scan rates (10-70mVs^{-1}) using 0.2M PBS (pH 7.4) at PPS/SAOS/MCPE.

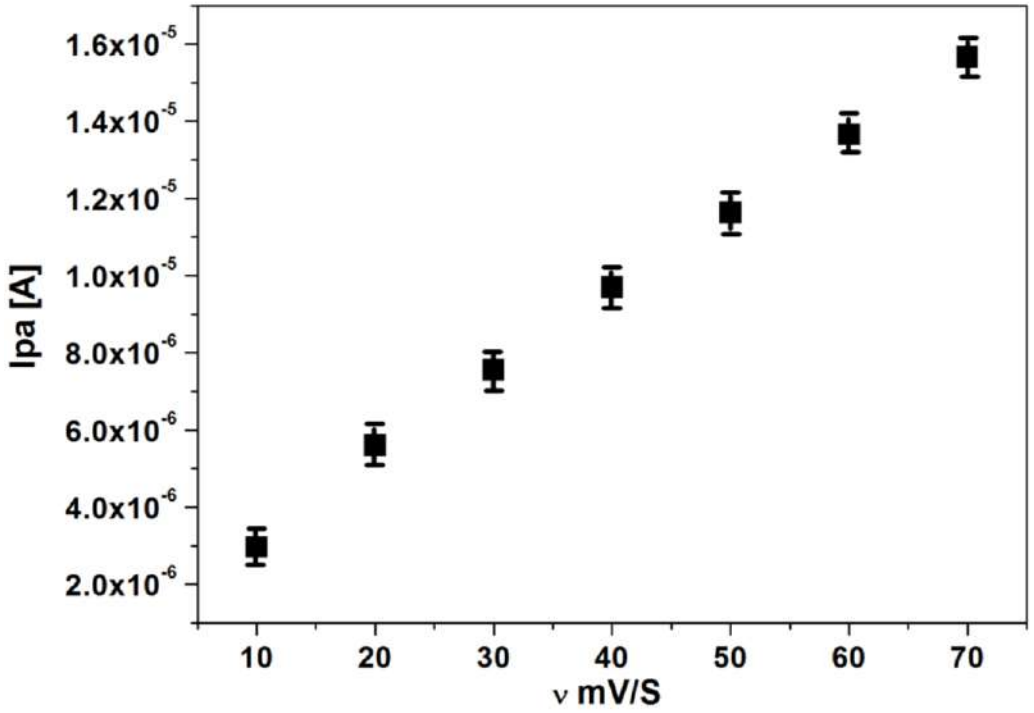


Fig.8b: Plot of anodic peak current (Ipa) versus scan rate (ν)

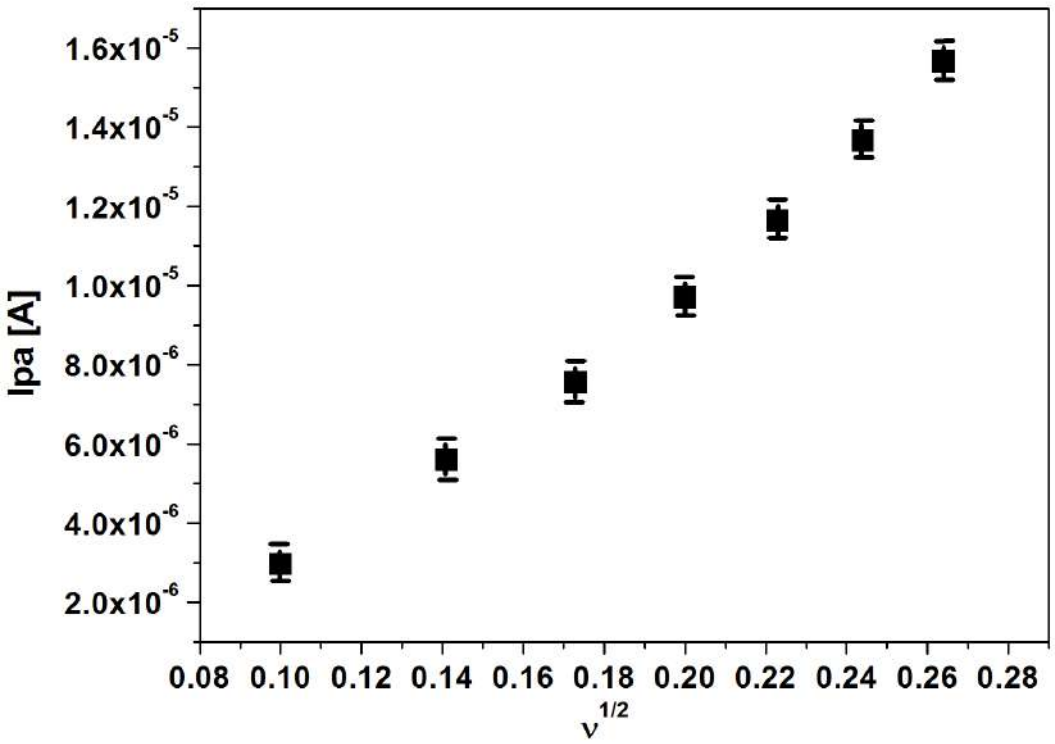


Fig.8c: Plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$)

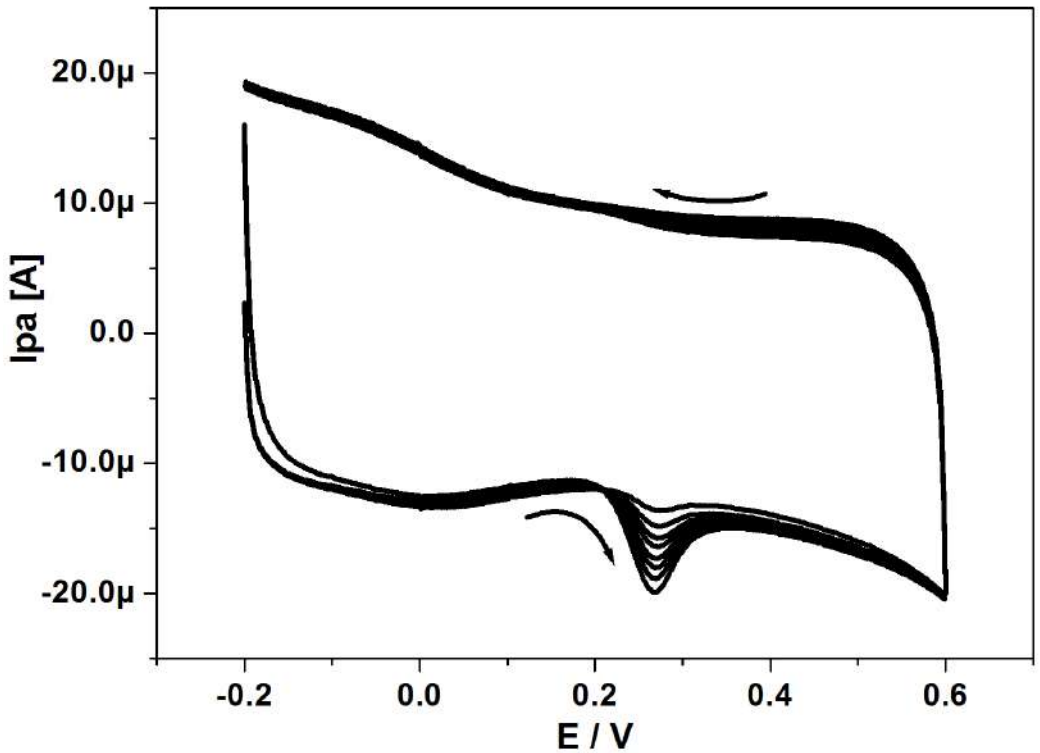


Fig.9a: Cyclic voltammograms of UA at different concentration (0.3 to 1.0 x 10^{-4}M) in presence of 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{1}.

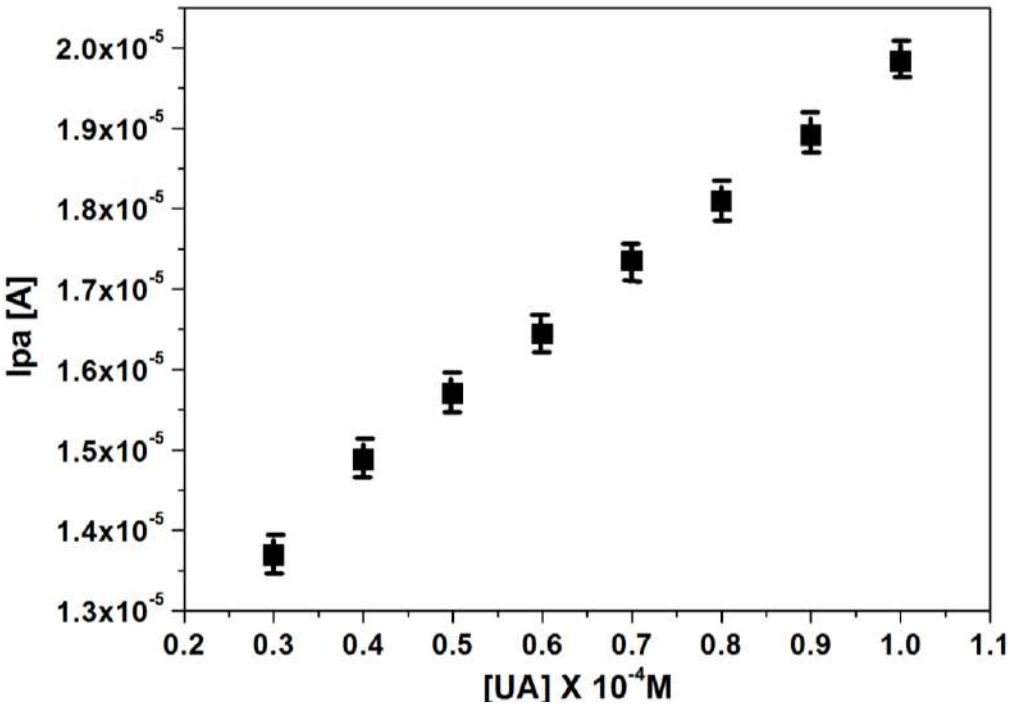


Fig.9b: Plot of anodic peak current (Ipa) versus concentration of UA

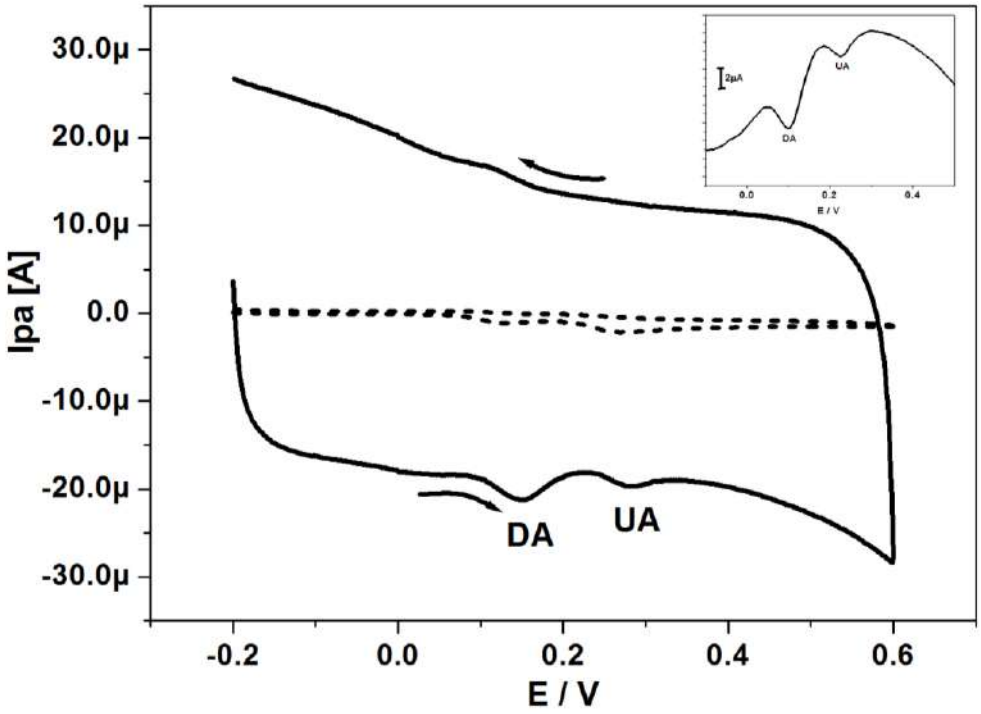


Fig.10: Cyclic voltammograms obtained for the mixture of $0.2x10^{-4}$M DA and $0.5x10^{-4}$M UA at BCPE(short dashed line) and PPS/SAOS/MCPE (solid line) using 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}. The inset figure shows the DPVs of the mixture contains DA and UA at PPS/SAOS/MCPE.

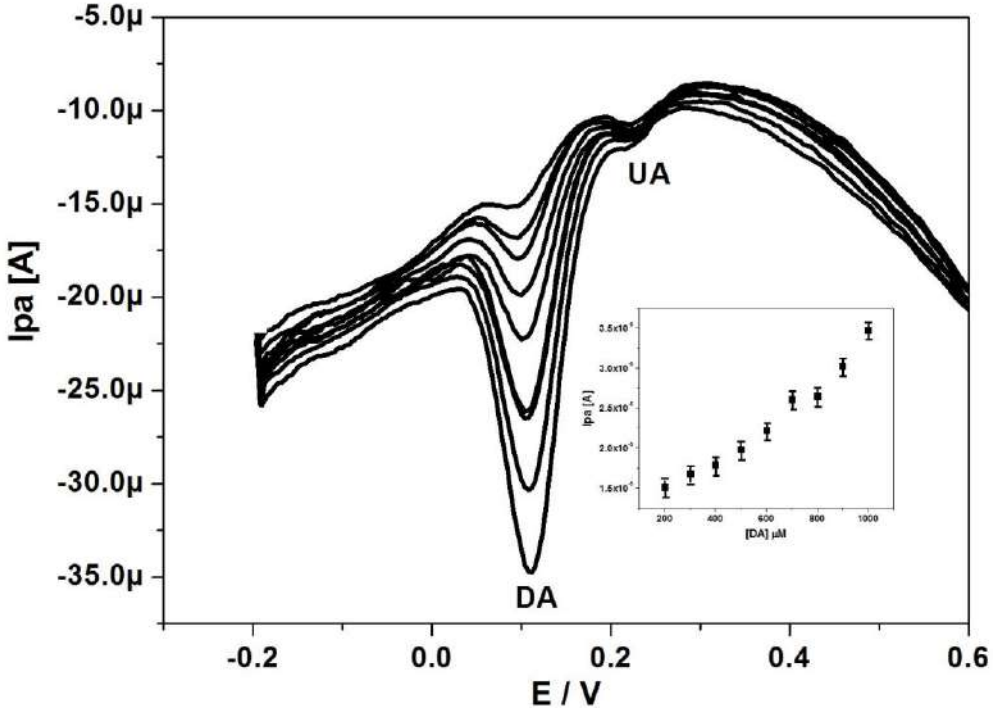


Fig.11: DPVs of DA with different concentration (200–1000µM) in presence of UA (500 µM) at PPS/SAOS/MCPE using 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}. The inset figure shows the plot of anodic peak current (Ipa) versus concentration of DA at PPS/SAOS/MCPE.

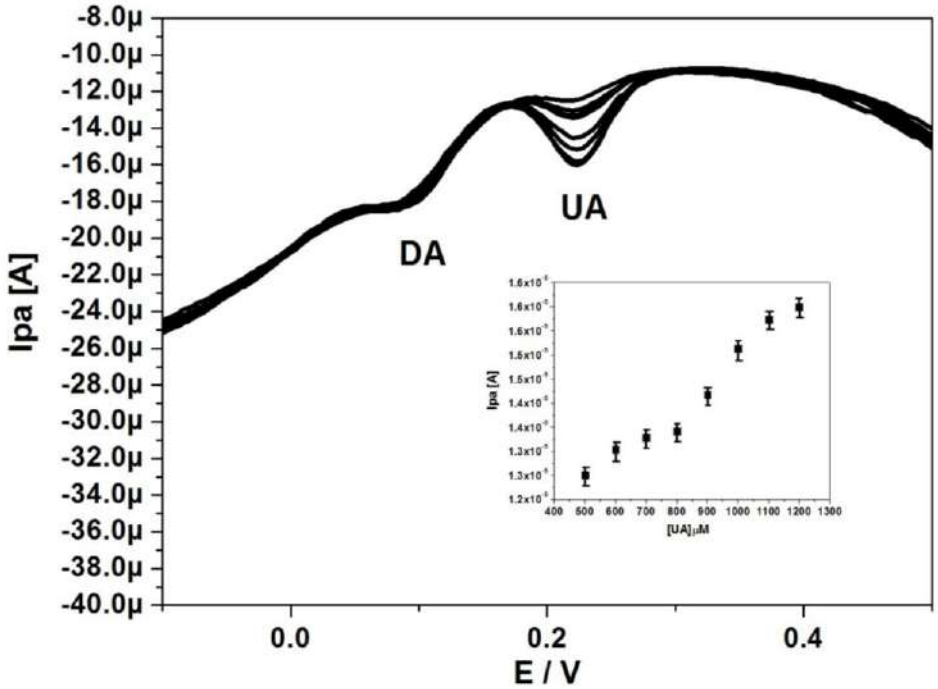


Fig.12: DPVs of UA with different concentration (500–1200µM) in presence of DA (200 µM) at PPS/SAOS/MCPE using 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}. The inset figure shows the plot of anodic peak current (Ipa) versus concentration of UA at PPS/SAOS/MCPE.

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Chapter -4

Part-B

Sodium alpha olefin sulphonate modified carbon paste electrode sensor for adrenaline: A voltammetric study

Sensors and Actuators B: Chemical (Revised and Submitted)

4.6 Introduction

Adrenaline [1-(3, 4-dihydroxyphenyl)-2-methyloaminoethanol] and Paracetamol (N-acetyl-P-aminophenol or Acetaminophen) are the two biochemical compounds which plays an important role in various biological processes. Adrenaline, which is also known as epinephrine (EP) is a hormone secreted by the medulla of adrenal glands [1] and one of the important neurotransmitter present in the mammalian hormonal [2] and central nervous system. The presence of ADN in the body affects the regulation of blood pressure and heart rate, lipolysis, immune system, and glycogen metabolism. Abnormal concentration of ADN may leads to the possibilities of neurological disorders such as HIV infection [3] and Parkinson's disease [4]. Therefore, it is essential to develop a quantitative determination method for ADN in order to investigate its physiological function and the diagnosis of some diseases in clinical medicine [5–9]. A series of analytical methods have been reported for the determination of ADN, such as capillary electrophoresis [10-11], fluorimetry [12], high performance liquid chromatography [13-14], spectrophotometry [15-16], and electrochemistry [17]. Among the above mentioned methods, electrochemical method exhibits higher selectivity and sensitivity. The chemical structure of ADN was shown in scheme 1.

Paracetamol (scheme 2) is one of the most widely used analgesic anti-pyretic drugs, which is well known for its treatment for the relief of pain and fever in adults and children. Paracetamol (PA) is used in the management of cancer or postoperative pain [18]. PA is an effective substitute for aspirin for the patients who are sensitive to aspirin [19-21]. It is available without any prescription; overdoses of paracetamol can lead to the kidney damage, liver disorders, skin rashes, inflammation of the pancreas and finally may lead to death [22-26]. Thus, the development of a simple, precise and accurate procedure for the determination of paracetamol in pharmaceutical products plays an important role. Already many methods have been described for the determination of PA, such as capillary electrophoresis [27-28], fluorimetry [29], high performance liquid chromatography [30-33], spectrophotometry [34-37], titrimetry [38-39], and electrochemical methods [40-46]. The above mentioned methods have some disadvantages related to time consumption and

expensiveness. Therefore, electrochemical methods are being extensively used for the analysis of PA [47].

Surfactants are the molecules, that contains a hydrophilic (attracted to water) and a hydrophobic (repelled by water) segments. Surfactants have the ability to modify and control the surface properties of electrodes *viz*., adsorption at interface and aggregation into supramolecular structures [48-49]. Due to their unique molecular structure, surfactants have been extensively used in the fields of electrochemistry and electroanalytical chemistry [50-53] for various purposes. Hu's group has introduced surfactants to electro analytical chemistry in order to improve the detection limits of some biomolecules. [54-55]. In the present work, sodium alpha olefin sulphonate (anionic surfactant) (scheme 3) was selected as an modifier to modify the surface of carbon paste electrode for the simultaneous determination of ADN and PA. The CPE modified with surfactants have proved as useful sensor for the determination of biological compounds [56].

In this chapter, we report about the application of the sodium alpha olefin sulphonate modified carbon paste electrode (SAOS/MCPE) for the effective determination of ADN and PA using 0.2 M phosphate buffer solution (pH 7.4) with the scan rate of 50 mVs^{-1}. The proposed electrode proved its excellent electrocatalytic activity towards the determination of ADN and PA individually and simultaneously.

4.7 Experimental

4.7.1 Materials

The electrochemistry was performed using a model CHI-660c (CH Instrument-660 electrochemical workstation). The standard conventional three electrode cell contains bare and sodium alpha olefin sulphonate modified carbon paste electrode (SAOS/MCPE) as working electrode, Platinum electrode as counter electrode and the saturated calomel electrode (SCE) as the reference electrode. All potentials reported are versus saturated calomel electrode.

All the chemicals used in this study were of analytical grade and used without any further purification. Adrenaline (ADN) and Paracetamol (PA) were purchased from Merck chemicals. Disodium hydrogen phosphate (Na_2HPO_4), Sodium dihydrogen orthophosphate (NaH_2PO_4) and Sodium alpha olefin sulphonate (SAOS) were procured from Himedia

chemicals. Carbon paste electrode was prepared by using spectrally pure graphite powder (particle size <50 mm) from Merck and high viscous paraffin oil (density=0.88gcm^{-3}) from Fluka. All electrochemical measurements were performed in 0.2 M PBS (pH 7.4).

4.7.2 Preparation of working electrodes.

In present study, the bare and sodium alpha olefin sulphonate modified carbon paste electrodes (SAOS/MCPE) were used as working electrodes. Graphite powder and silicone oil were hand mixed in the ratio 70:30 (w/w) for about 40 minutes in an agate mortar to obtain a bare carbon paste. The paste was then packed into the homemade Teflon cavity. The electrical contact was provided at the end of the PVC tube. In this way, the bare carbon paste electrode has been prepared. Sodium alpha olefin sulphonate was immobilized (10µL) on the surface of BCPE to modify the electrode as sodium alpha olefin sulphonate modified carbon paste electrode (SAOS/MCPE).

4.8 Results and Discussion

4.8.1 The influence of quantity of the surfactant (sodium alpha olefin sulphonate) on the surface of BCPE

The effect of SAOS as modifier on the surface of BCPE was investigated by cyclic voltammetric method. The surfactant (SAOS) was immobilized on the surface of BCPE at different concentrations (5, 10, 15, 20 and 25 µL) and their electrochemical response towards 0.1x10^{-4}M ADN in presence 0.2M PBS as supporting electrolyte at pH 7.4 with the scan rate of 50mVs^{-1} was studied. Fig.1 shows the plot of anodic peak current (Ipa) of 0.1×10^{-4}M ADN versus quantity of SAOS in µL. The obtained experimental result illustrates that, as the quantity of SAOS increases corresponding anodic peak current goes on increases up to 10µL and afterwards the current goes on decreasing with increase in concentration. The probable mechanism is that the SAOS surfactant molecule diffuses into the carbon paste electrode along with the ADN which results in an increase in the current signals [57]. Therefore, 10µL SAOS was selected as the suitable amount for further studies.

4.8.2 Characterization of the modified electrode (SAOS/MCPE)

To investigate the electrochemical properties of the modified electrode (SAOS/MCPE), potassium ferrocyanide [$K_4Fe(CN)_6$] was selected and used as the

electrochemical redox probe. The cyclic voltammograms (Fig.2) of 1mM $K_4[Fe(CN)_6]$ at unmodified electrode (dashed line) shows a pair of redox peaks, with an anodic peak potential (Epa) at 0.28 V and cathodic peak potential (Epc) at 0.17 V in presence of 1M KCl as supporting electrolyte with the scan rate of $50mVs^{-1}$. However, SAOS/MCPE (solid line) shows great enhancement in the redox peak current of 1mM $K_4[Fe(CN)_6]$ than unmodified electrode, the anodic peak potential (Epa) and the cathodic peak potential (Epc) were observed at 0.27 V and 0.18 V respectively. As can be seen from the experimental observations, the enhancement in the peak current proves the excellent electrocatalytic ability of the modified electrode (SAOS/MCPE). Furthermore, the surface morphology of modified electrode was examined by SEM analysis. Fig. 3a shows the SEM image obtained for bare carbon paste electrode, indicating the irregular flake like shapes of graphite and Fig. 3b shows SAOS/MCPE images evidencing the uniformly aligned valleys on the surface, which is important for improving the electrical conductivity [58].

4.8.3 Cyclic voltammetric oxidation behaviour of ADN at the SAOS-modified CPE

Cyclic voltammetry (CV) was applied to examine the electrochemical behaviour of ADN at SAOS/MCPE. Fig.4 shows the cyclic voltammograms of $0.1x10^{-4}$M ADN at the unmodified (dashed line) and SAOS-modified carbon paste electrode (solid line) in 0.2 M phosphate buffer solution (pH 7.4) with the scan rate of $50mVs^{-1}$. Compared with the unmodified electrode, SAOS/MCPE showed great enhancement in the anodic peak current (Ipa) of ADN and the oxidation peak potential (Ep) was observed at 0.13V. Usually ADN undergoes two-electron oxidation with sharing of two protons to form adrenalinquinone [59]. The oxidation mechanism of adrenaline was shown in scheme 4. Based on the above experimental results, we conclude that the modification of CPE with SAOS can be used as an effective electrochemical sensor for the determination of ADN.

4.8.4 Influence of scan rate on the electrocatalytic oxidation behaviour of ADN at SAOS/MCPE

To investigate the kinetics of electrode reaction and type of electrode process, the variation of scan rate was studied at SAOS/MCPE. The cyclic voltammograms of $0.1x10^{-4}$ M ADN at different scan rate (50-500 mVs^{-1}) using 0.2M phosphate buffer solution (pH

7.4) was depicted in Fig.5a. As can be observed from Fig.5a, the anodic peak current (Ipa) of ADN gradually increases with increase in the scan rate. Fig.5b shows the plot of anodic peak current (Ipa) versus scan rate (ν), which shows good linearity and correlation coefficient was found to be R^2=0.997. Also, plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) was constructed (Fig.5c) and the correlation coefficient value was found to be R^2=0.988.

4.8.5 Influence of concentration on the oxidation behaviour of ADN at SAOS/MCPE

The variation of ADN concentration was carried out by both cyclic voltammetry (CV) and differential pulse voltammetric techniques (DPV) at SAOS/MCPE. The cyclic voltammograms of ADN at different concentration (0.1 - 0.8 x 10^{-4}M) in presence of 0.2M PBS at pH 7.4 with the scan rate of 50mVs^{-1} was shown in Fig.6. As we increase the concentration, the oxidation peak current of ADN gradually increases. The inset of Fig.6 shows the plot of anodic peak current (Ipa) versus concentration of ADN, which gave good linearity and correlation coefficient value was found to be R^2=0.993. Fig.7 shows the differential pulse voltammograms (DPVs) of ADN at different concentrations (100-500 µM) using 0.2M PBS at pH 7.4 with the scan rate of 50 mVs^{-1}. The peak current of ADN gradually increases with increase in the concentration. The plot of anodic peak current (Ipa) versus concentration of ADN was shown in the inset of Fig.7. Limit of detection (LOD) and limit of quantification (LOQ) for ADN was calculated by using the equation (1) and (2) [60-63] and found to be 11.3 µM and 37.8 µM respectively.

LOD=3S/M…..... (1)

LOQ=10S/M.....(2)

where, S is the standard deviation and M is the slope

4.8.6 Effect of pH variation on the peak current of ADN at SAOS/MCPE

In determining the performance of proposed electrochemical sensors, pH value of the supporting electrolyte (PBS) plays an important role [64]. The effect of pH variation of the supporting electrolyte (PBS) on the peak current of ADN was investigated by cyclic voltammetry at SAOS/MCPE. Fig.8 shows the cyclic voltammograms of 0.1 x 10^{-4}M ADN at different pH (6.2, 6.6, 7.0, 7.4, and 7.8) using 0.2M phosphate buffer solution with the

scan rate of $50mVs^{-1}$. The anodic peak potentials of the ADN were shifted to positive values with increasing pH. The relationship between anodic peak potential of ADN and pH value was shown in inset of Fig.8. Finally, biological pH value 7.4 was selected as an optimum solution pH for the present investigations.

4.8.7 Voltammetric redox behaviour of PA at SAOS/MCPE

The electrochemical redox behaviour of the PA was investigated by cyclic voltammetric method (CV) at SAOS/MCPE. The cyclic voltammograms of $0.1x10^{-4}$M PA at the unmodified (dashed line) and SAOS-modified CPE (solid line) in 0.2 M phosphate buffer solution (pH 7.4) with the scan rate of $50mVs^{-1}$ was depicted in Fig.9. Compared with the unmodified electrode, SAOS/MCPE shows a great enhancement in the redox peak current of PA and the difference in peak potential (ΔEp) was found to be 0.04V. Normally, paracetamol undergoes two-electron two-proton oxidation process and the oxidation mechanism of PA was shown in scheme 5 [65]. Therefore, SAOS/MCPE exhibits an outstanding development in the electrochemical sensitivity towards PA.

4.8.8 Influence of scan rate on the electrocatalytic redox behaviour of PA at SAOS/MCPE

The effect of scan rate on the electrocatalytic redox behaviour of PA at SAOS/MCPE was investigated by cyclic voltammetric technique. Fig.10a shows the cyclic voltammograms of 0.1×10^{-4}M PA at different scan rate (100-500 mVs^{-1}) using 0.2M phosphate buffer solution (pH 7.4). As can be observed from Fig.10a, the redox peak current of PA gradually increases with increase in scan rate. Fig.10b shows the plot of anodic peak current (Ipa) versus scan rate (ν), which has good linearity and correlation coefficient value was found to be R^2=0.999. Also, plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) was constructed (Fig.10c) and the correlation coefficient value was found to be R^2=0.993.

4.8.9 Influence of concentration on the redox peak current of PA at SAOS/MCPE

The effect of concentration on the electrocatalytic redox behaviour of PA at SAOS/MCPE was studied by both cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. Fig.11 shows the cyclic voltammograms of PA at different

concentration (0.1 - 0.9 x 10^{-4}M) in presence of 0.2M PBS at pH 7.4 with the scan rate of 50mVs^{-1}. With the increase in concentration, the redox peak current of PA gradually increases. The inset of Fig.11 shows the plot of anodic peak current (Ipa) versus concentration of PA, which shows good linearity and correlation coefficient value was found to be R^2=0.999. Fig.12 shows the differential pulse voltammograms (DPVs) of PA at different concentrations (100-500 µM) using 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}. The redox peak current of PA gradually increases with increase in the concentration. The inset of Fig.12 shows the graph of anodic peak current (Ipa) versus concentration of PA. The limit of detection (LOD) and limit of quantification (LOQ) for PA was calculated by the equation (1) and (2) and found to be 3.7 µM and 12.5 µM respectively.

4.8.10 Ability of the proposed electrode (SAOS/MCPE) towards the Simultaneous electrochemical determination of ADN and PA.

In order to examine the sensitivity and selectivity of the SAOS/MCPE, the simultaneous determination of ADN and PA was investigated by both cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods. Fig.13 shows the cyclic voltammograms obtained for the mixture of 0.1x10^{-4}M ADN and 0.1x10^{-4}M PA in presence of 0.2M PBS (pH 7.4) at the scan rate of 50 mVs^{-1}. The dashed line shows cyclic voltammogram obtained for the mixture of ADN and PA at unmodified electrode. The solid line shows cyclic voltammogram obtained for the mixture of ADN and PA at SAOS-modified CPE. From the above experimental observations, the SAOS/MCPE exhibits enhancement in peak current (Ip) of the mixture containing ADN and PA than the unmodified electrode. The inset of Fig.13 shows the differential pulse voltammograms obtained for the mixture containing 0.1x10^{-4}M ADN and 0.1x10^{-4}M PA at SAOS/MCPE. The performance of the modified electrode was compared with other reported results for the determination of ADN and PA (Table 1). Thus, the proposed modified electrode has the electrocatalytic ability towards the simultaneous determination of ADN and PA.

4.8.11 Interference study

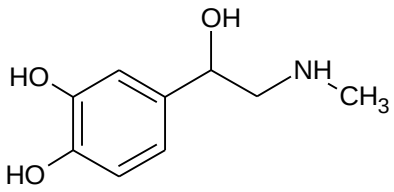
The effect of various substances as potential interferences on the peak current of ADN and PA was investigated at SAOS/MCPE in presence of 0.2M PBS (pH 7.4) with the scan rate of $50mVs^{-1}$ by differential pulse voltammetry. Fig.14 shows the DPVs of the mixture contain ADN and PA, in which the concentration of ADN was varied (from 100-500 µM) while keeping the concentrations of PA constant. The peak current increases with increase in concentration of ADN at SAOS/MCPE. The inset of Fig.14 shows the plot of anodic peak current (Ipa) versus concentration of ADN. Similarly, Fig.15 shows the DPVs of the mixture contain ADN and PA, in which the concentration of PA was varied (from 100-700 µM) while keeping the concentrations of ADN constant. The inset of Fig.15 shows the plot of anodic peak current (Ipa) versus concentration of PA. Thus, SAOS-modified carbon paste electrode has been successfully employed for the simultaneous determination of ADN and PA.

4.8.12 Real sample analysis

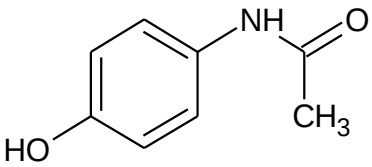
In order to examine the analytical applicability of the proposed sensor, real sample analysis was carried out by taking the injection sample of adrenaline and paracetamol tablet. Standard addition method was employed and recovery values ranged from 97 to 99% (Table 2). The obtained result evidencing that the response of the proposed sensor was reliable and accurate for practical applications to detect ADN and PA.

4.9 Conclusion

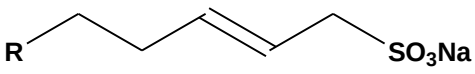
In this chapter, the simultaneous electrochemical determination of ADN and PA was carried out at SAOS-modified CPE by both cyclic voltammetry (CV) and differential pulse voltammetric techniques (DPV). The proposed electrode exhibits high sensitivity and selectivity towards the detection of ADN and PA. The modified electrode exhibits adsorption-controlled process and low detection limits (LOD) for ADN and PA. Finally, the proposed electrode (SAOS/MCPE) showed very good electrocatalytic properties and has the ability to determine the ADN and PA simultaneously. The same method can be used for some other bioactive molecules.



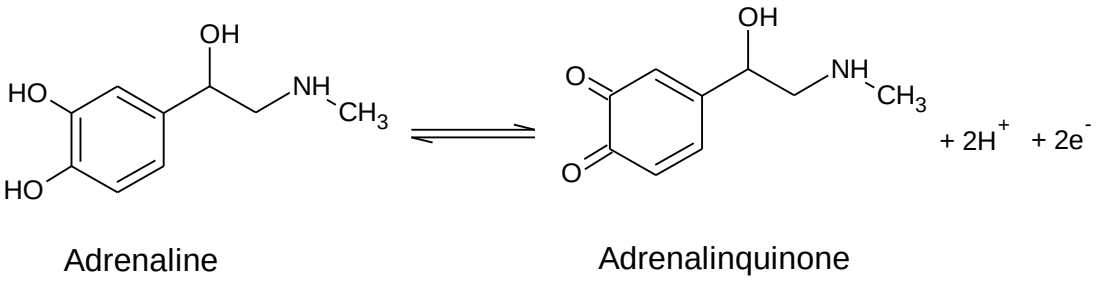
Scheme 1: Structure of Adrenaline



Scheme 2: Structure of Paracetamol

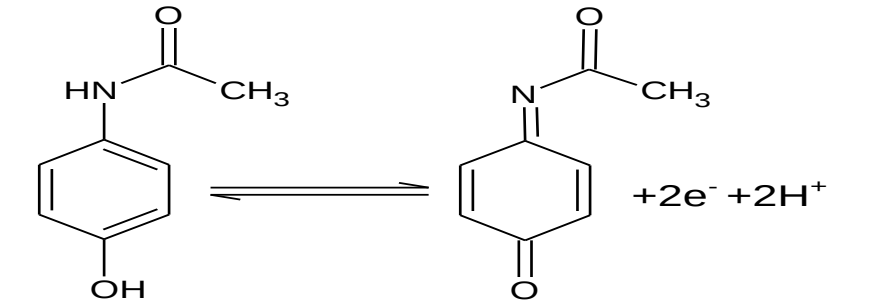


Scheme 3: Structure of Sodium alpha olefin sulphonate



Adrenaline Adrenalinquinone

Scheme 4: Oxidation mechanism of Adrenaline



Paracetamol N-acetyl-p-quinoneimine

Scheme 5: Oxidation mechanism of Paracetamol

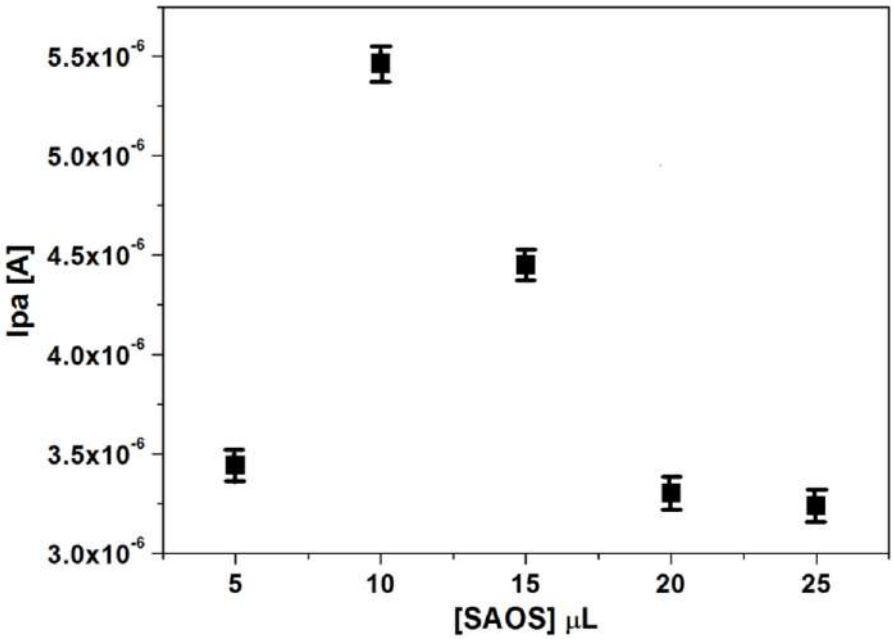


Fig.1: Effect of quantity of SAOS (µL) on the oxidation peak current of $0.1x10^{-4}$ M ADN using 0.2M PBS (pH 7.4) with the scan rate of $50mVs^{-1}$.

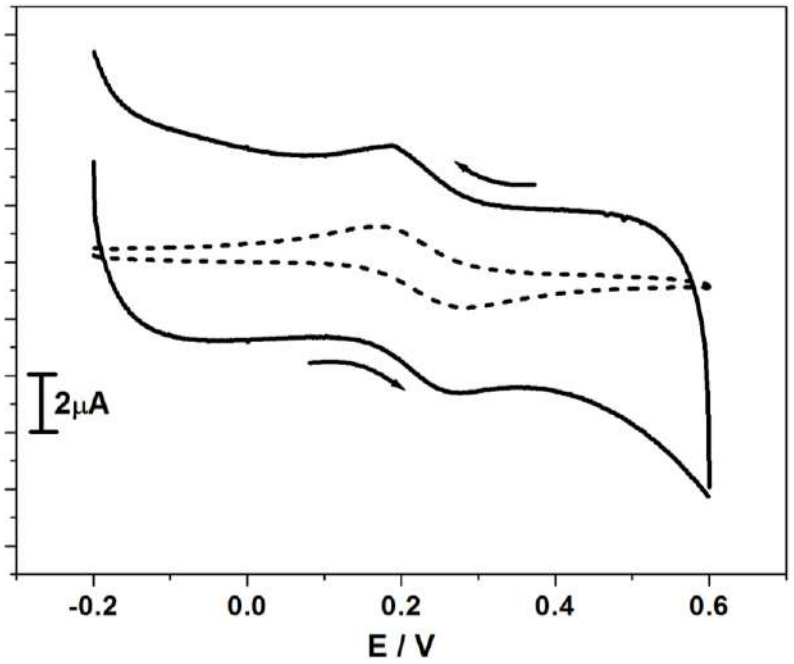


Fig.2: Cyclic voltammograms of 1mM $K_4[Fe(CN)_6]$ at unmodified (dashed line) and SAOS/MCPE (solid line) using 1M KCl as supporting electrolyte with the scan rate of $50mVs^{-1}$.

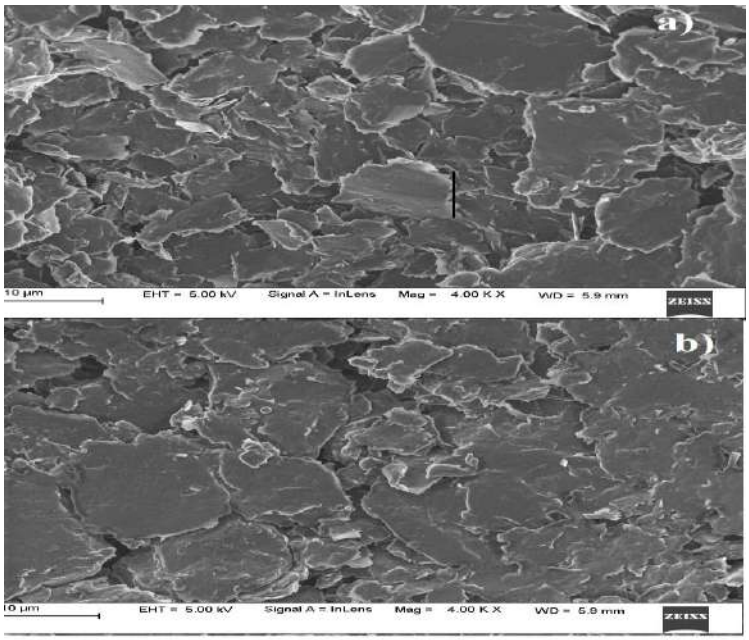


Fig.3: SEM images of bare (a) and SAOS/MCPE (b).

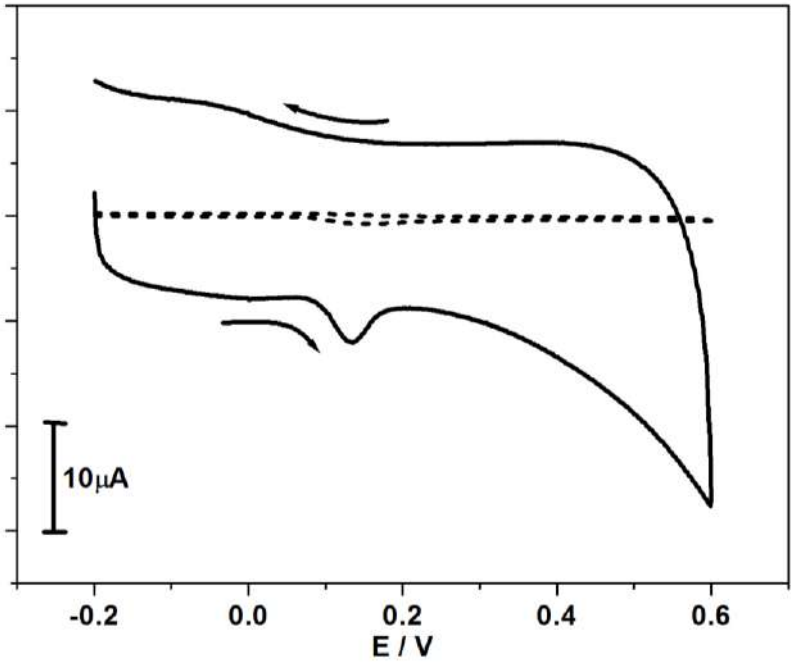


Fig.4: Cyclic voltammograms obtained for $0.1x10^{-4}$M ADN at unmodified (dashed line) and SAOS/MCPE (solid line) with the scan rate $50mVs^{-1}$ using 0.2M PBS (pH 7.4).

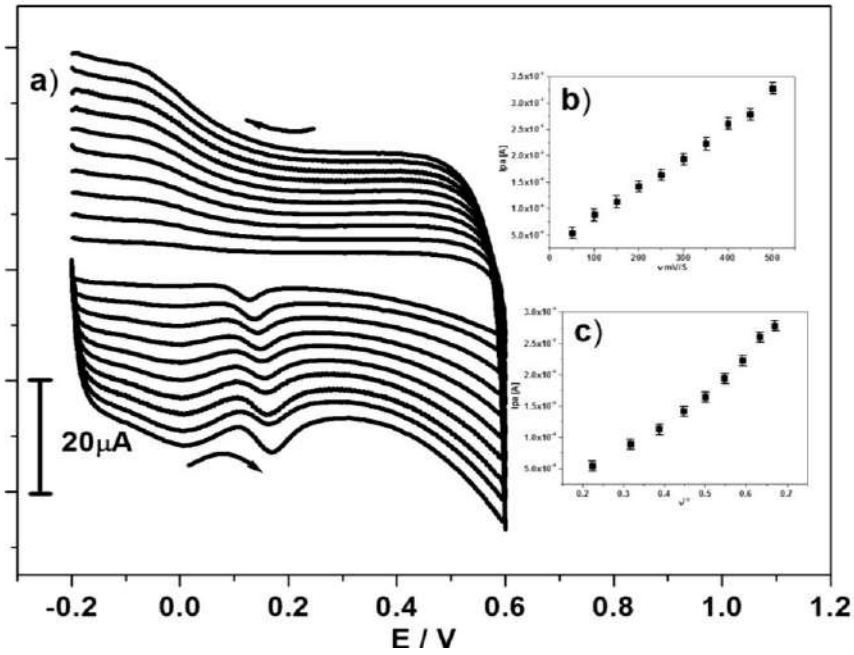


Fig.5: (a) Cyclic voltammograms of $0.1x10^{-4}$M ADN at SAOS/MCPE with different scan rates (50-500mVs^{-1}) using 0.2M PBS (pH 7.4), (b) plot of anodic peak current (Ipa) versus scan rate (v), (c) plot of anodic peak current (Ipa) versus square root of scan rate ($v^{1/2}$)

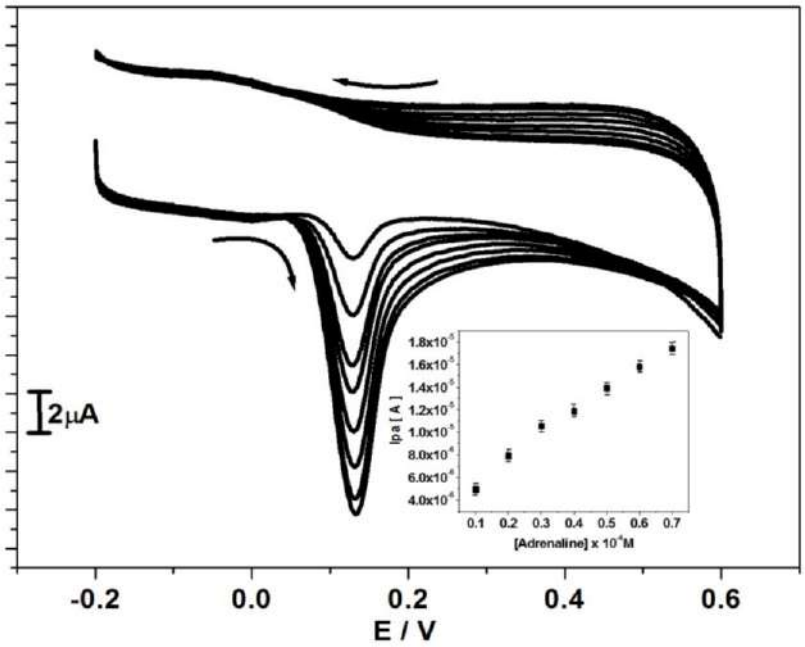


Fig.6: Cyclic voltammograms obtained for ADN at SAOS/MCPE with different concentration (0.1 to 0.8 x 10^{-4}M) using 0.2M PBS (pH 7.4) with the scan rate 50 mVs^{1}. The inset figure shows the plot of anodic peak current (Ipa) versus concentration of ADN .

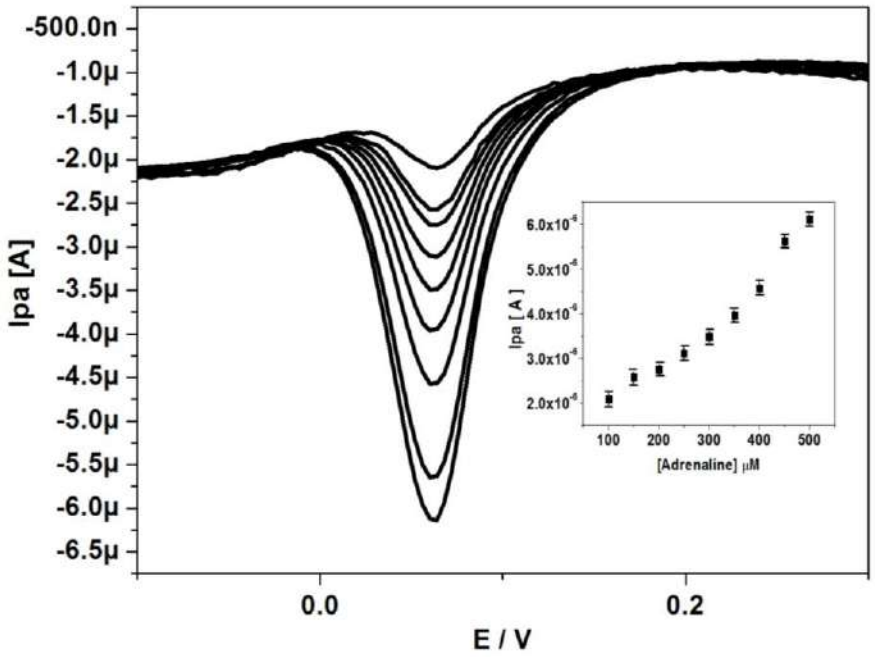


Fig.7: DPVs of ADN with different concentration (100–500 µM) at SAOS/MCPE using 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{1}.The inset figure shows the plot of anodic peak current (Ipa) versus concentration of ADN.

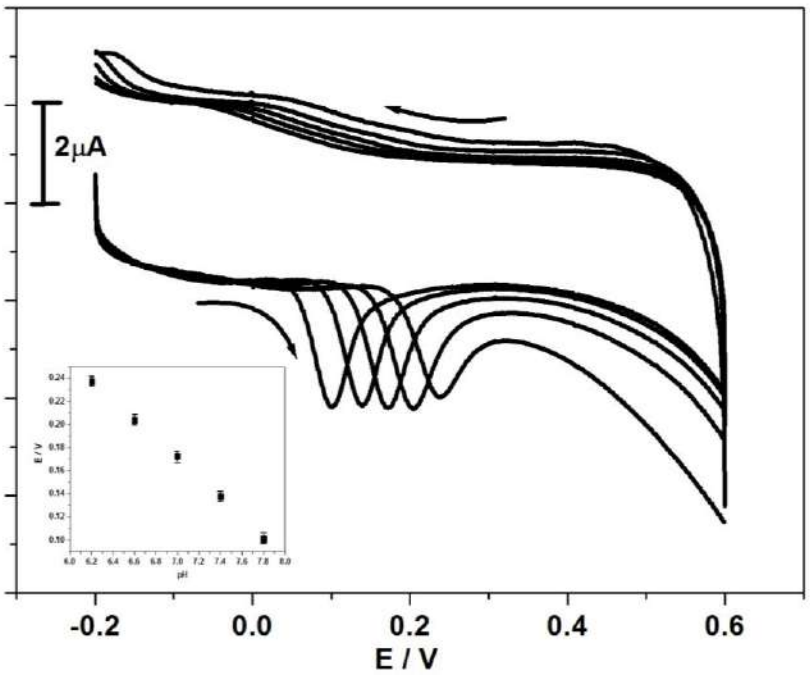


Fig.8: Cyclic voltammograms of $0.1x10^{-4}$M ADN with different pH (6.2, 6.6, 7.0, 7.4 and 7.8) at SAOS/MCPE. The inset figure shows the plot of peak potential (Ep) versus pH

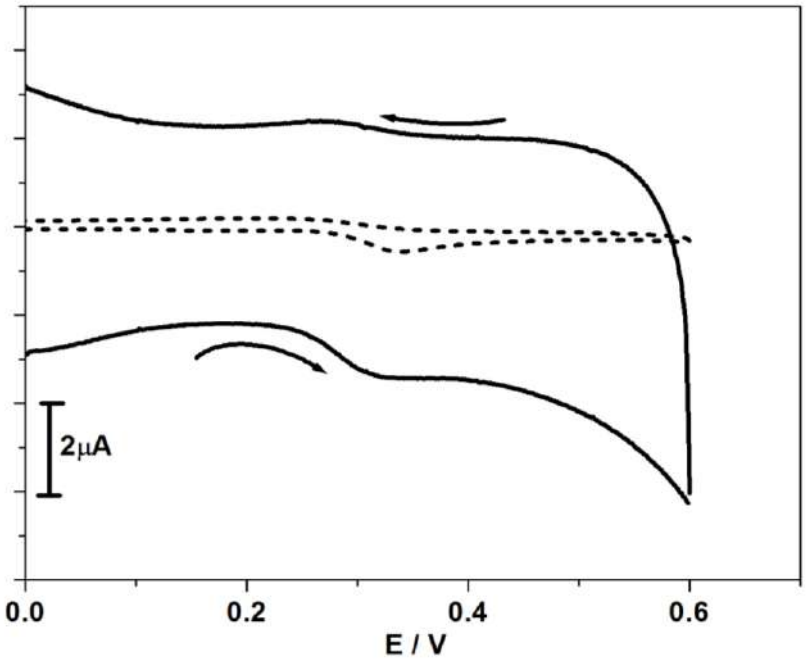


Fig.9: Cyclic voltammograms obtained for $0.1x10^{-4}$M PA at unmodified (dashed line) and SAOS/MCPE (solid line) using 0.2M PBS (pH 7.4) with the scan rate of $50mVs^{-1}$.

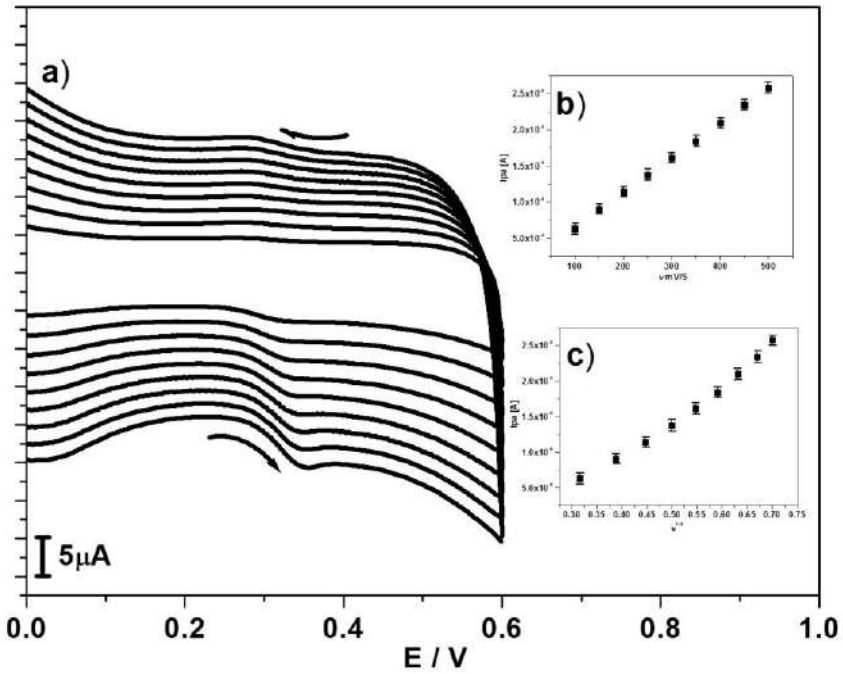


Fig.10: (a) Cyclic voltammograms of $0.1x10^{-4}$M PA at SAOS/MCPE with different scan rates (100-500mVs^{-1}) using 0.2M PBS (pH 7.4), (b) plot of anodic peak current (Ipa) versus scan rate (v), (c) plot of anodic peak current (Ipa) versus square root of scan rate ($v^{1/2}$)

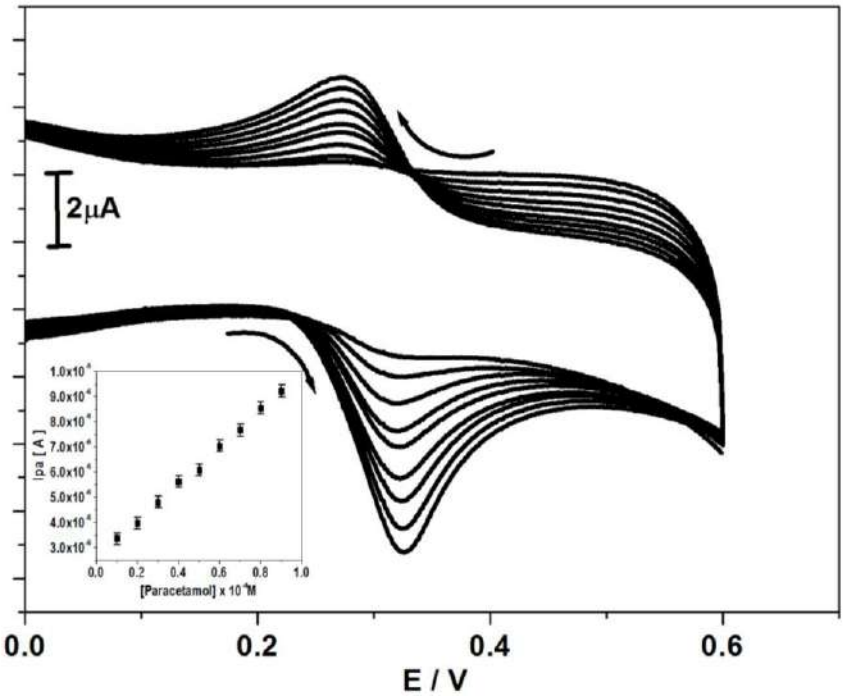


Fig.11: Cyclic voltammograms obtained for PA at SAOS/MCPE with different concentration (0.1 to 0.9 x 10^{-4}M) using 0.2M PBS (pH 7.4) with the scan rate 50 mVs^{1}. The inset figure shows the plot of anodic peak current (Ipa) versus concentration of PA.

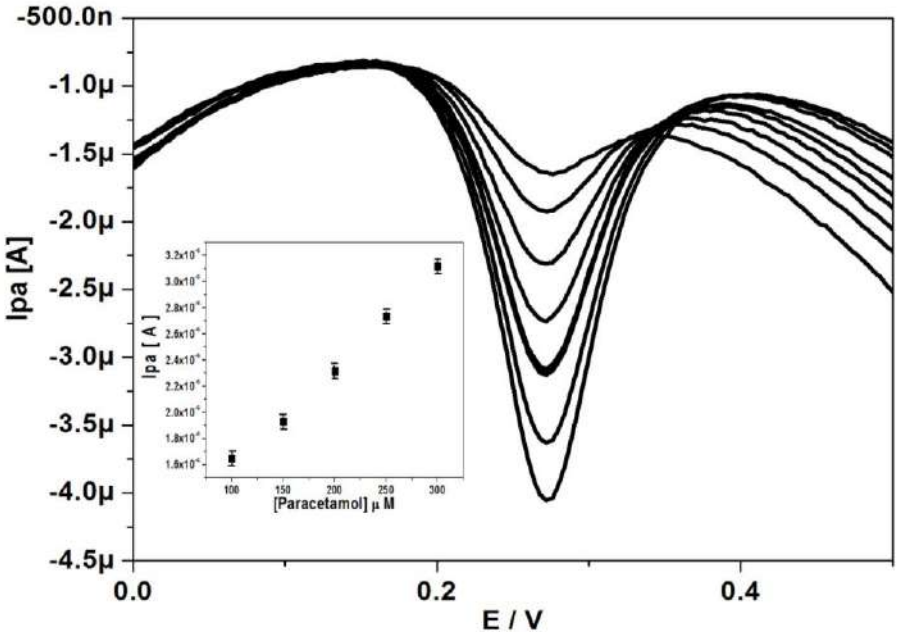


Fig.12: DPVs of PA with different concentration (100–500 μM) at SAOS/MCPE using 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{1}.The inset figure shows the plot of anodic peak current (Ipa) versus concentration of PA

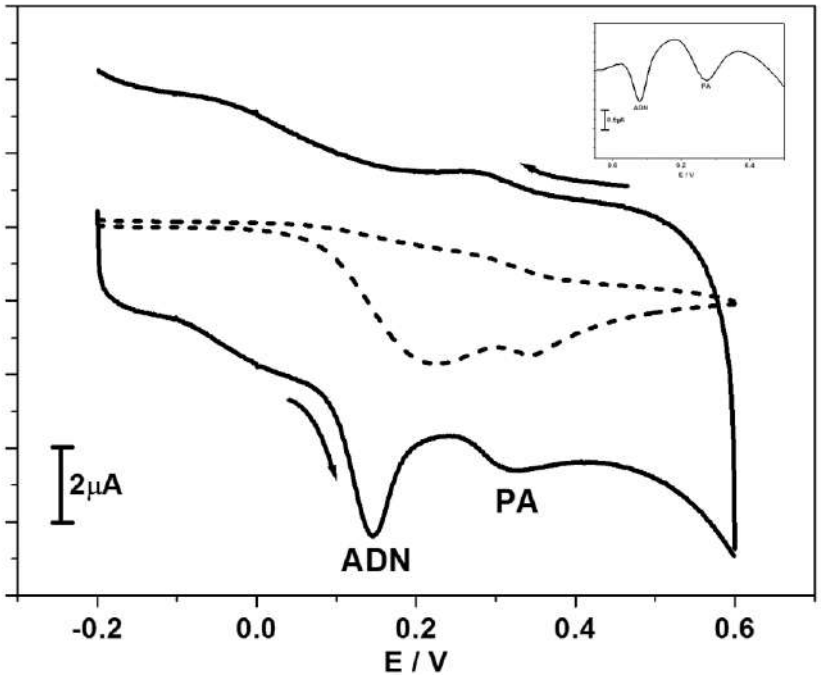


Fig.13: Cyclic voltammograms obtained for the mixture of $0.1x10^{-4}$M ADN and $0.1x10^{-4}$M PA at unmodified (dashed line) and SAOS/MCPE (solid line) using 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}. The inset figure shows the DPVs of the mixture containing ADN and PA.

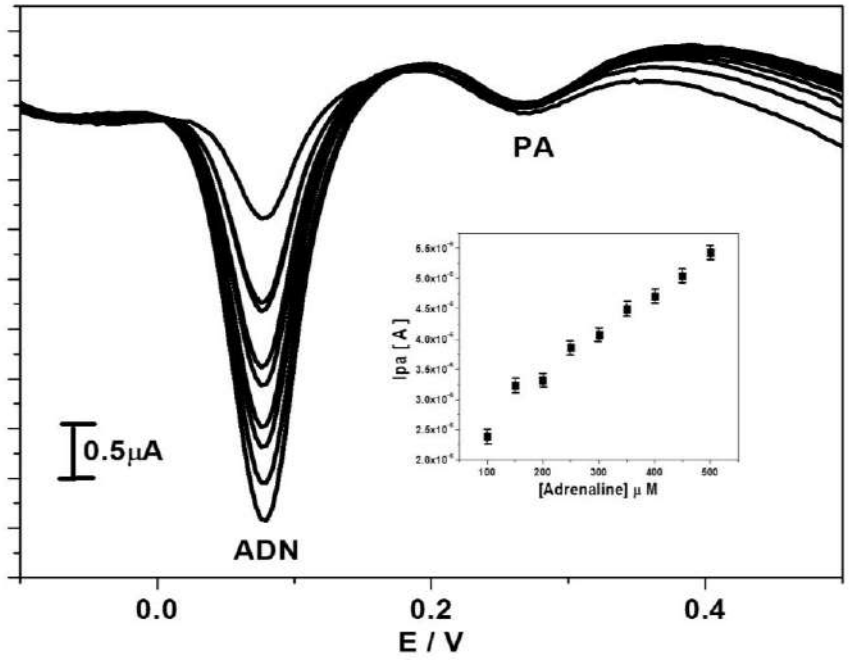


Fig.14: DPVs of ADN with different concentrations (100–500μM) in presence of PA (100 μM) at SAOS/MCPE using 0.2M PBS (pH 7.4) with the scan rate of $50mVs^{-1}$. The inset figure shows the plot of anodic peak current (Ipa) versus concentration of ADN

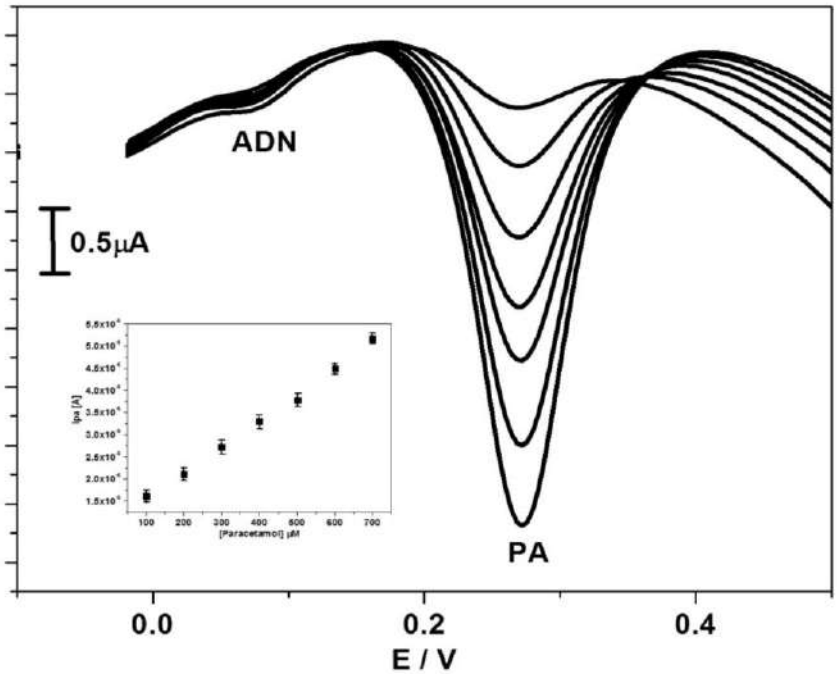


Fig.15: DPVs of PA with different concentrations (100–700μM) in presence of ADN (10 μM) at SAOS/MCPE using 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}. The inset figure shows the plot of anodic peak current (Ipa) versus concentration of PA

Table 1: Comparison of the proposed sensor (SAOS/MCPE) with other reported results for ADN and PA determination

Analyte	**Working electrode**	**Linear range (µM)**	**Limit of detection (µM)**	**Reference**
ADN	Poly(isonicotinic acid)-modified CPE	5-100	1.0	[66]
	GC/nano-TiO2/polymer	12-120	2.0	[67]
	Caffeic acid/GCE	2-80	20	[68]
	GCE/MWCNT/poly-FA sensor	73.0-1406	22.2	[69]
	SAOS/MCPE	10-70	11.3	This work
PA	BDD electrode	100-8000	10	[70]
	Aluminium electrode modified by Thin layer of palladium	100-5000	5.0	[71]
	Graphite electrode	6.10-66.10	2.2	[72]
	Nafion /ROP/GCE	50-2500	1.2	[73]
	SAOS/MCPE	10-90	3.7	This work

Table 2: Determination of ADN and PA in real samples

Sample	**Compound**	**Added (mL)**	**Found (mL)**	**Recovery (%)**
Injection sample	ADN	03	2.94	98
		04	3.9	97.5
		05	4.8	96
Tablet	PA	02	1.98	99
		03	2.94	98
		04	3.88	97

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Chapter -5

Part-A

Electrochemical determination of folic acid at sodium alpha olefin sulphonate modified carbon paste electrode: A voltammetric study

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5.1 Introduction

Vitamins are a group of organic compounds, essential in small amount for the normal functioning of the body and regulate the metabolic activity [1]. Deficiency of vitamins may result in often painful and potentially harmful diseases. As a result vitamins play an important role in our body. Folic acid (FA) (vitamin BC, vitamin M or vitamin B_9) is a water soluble vitamin and was first discovered in Spinach [2]. During metabolism FA involved in single carbon transfer reactions and it is the precursor of the active tetrahydrofolic acid coenzyme [3]. FA is a nutrient of great importance, especially for women planning for pregnancy. To reduce significantly the incidence and reoccurrence of neural tube defects periconceptual supplementation of folic acid has been demonstrated [4]. Moreover FA is usually employed in the treatment or prevention of megaloblastic anemia during pregnancy [5].The US Food and Drug Administration introduced mandatory reinforcement of cereal-grain products with folic acid at a concentration of 140mg/100g in January 1998 [6]. The Department of Health in the UK proposed fortification of flour with folic acid at 240mg/100g [7]. Deficiency of FA gives rise to the macrocytic anemia [8]. Hence FA determination is often required in pharmaceutical, clinical and food samples. There are several methods were reported for the determination of FA including liquid chromatography / tandem mass spectrometry (LC/MS/MS) [9], High Performance Liquid Chromatography (HPLC) [10], Capillary Electrophoresis [11], Microemulsion Electrokinetic Chromatography (MEEKC) [12] and Enzyme linked Immunosorbent assay (ELISAs) [13] and electrochemical methods [14]. Among these techniques electrochemical method is significant one because of its convenience and low cost[15] . FA (Scheme 1) is one of the electroactive species and accordingly we employed electrochemical method for the determination and some of the electrochemical methods for other electroactive species have been reported [16-20].

Dopamine (Scheme 2) is one of the most significant catecholamine, functioning as a neurotransmitter in the central nervous system and deficiency of DA leads to the Parkinson's disease [21-22]. Changes in DA concentration in biological sample indicate the possibility of abnormalities or diseases in human being. Therefore, the determination of DA plays an important role. DA possesses high electrochemical activity and has been widely

studied by electroanalytical techniques to significantly advantage towards biosciences [23-25]. Dopamine has been determined using various electrochemical methods [26]. One of the most significant method is the determination of analytes by using modified carbon paste electrode through the voltammetic technique, which has the ability to eliminate the interfering substances from DA. The modification can be done by adding different types of modifiers [27-32].

In this study, the surfactant (sodium alpha olefin sulphonate (Scheme 3)) is used as a modifier for the electrochemical determination of FA and DA. The term surfactant is derived from surface active agent and is a compound that contains a hydrophilic (attracted to water) and a hydrophobic (repelled by water) segments. Because of their unique molecular structure, surfactant has been extensively used in the fields of electrochemistry and electroanalytical chemistry [33-36] for various purposes. To improve the detection limits of some biomolecules Hu's group [37-39] has introduced surfactants to electroanalytical chemistry.

In this chapter, a simple and sensitive voltammetric method was presented for the electrochemical determination of folic acid at SAOS modified carbon paste electrode. The modified electrode showed an excellent electrocatalytic activity for the oxidation of FA in presence of dopamine.

5.2 Experimental

5.2.1 Reagents and chemicals

Chemicals used in present work were Folic acid (FA) from Merck chemicals. Stock solution of FA was prepared in 0.1M NaOH. Disodium hydrogen phosphate (Na_2HPO_4), Sodium dihydrogen orthophosphate (NaH_2PO_4) and Sodium alpha olefin sulphonate were purchased from Himedia chemicals. Spectrally pure graphite powder (particle size <50 mm) from Merck and high viscous paraffin oil (density=0.88gcm^{-3}) from Fluka were used for the preparation of the carbon paste electrode. Phosphate buffer (0.2M pH 7.4) was used as optimum measurements. All chemicals used in this experiments were analytical grade and used without any further purification.

5.2.2 Apparatus

Cyclic voltammetric (CV) measurements were performed with a model CHI-660c (CH Instrument-660 electrochemical workstation). All electrochemical experiments were performed in a standard three electrode cell. The bare and sodium alpha olefin sulphonate modified carbon paste electrode (SAOSMCPE) was used as working electrode, Platinum electrode as counter electrode and saturated Calomel electrode (SCE) as the reference electrode. All potentials reported were versus the SCE.

5.2.3 Preparation of bare and modified carbon paste electrode (SAOSMCPE)

The bare CPE was prepared by hand mixing graphite powder and silicone oil in the ratio 70:30 (w/w) for about 30 minutes in an agate mortar to produce a homogeneous mixture. The paste was then packed into the homemade cavity. Sodium alpha olefin sulphonate modified carbon paste electrode (SAOSMCPE) was prepared by immobilizing 15µL of sodium alpha olefin sulphonate (SAOS) on to the surface of bare carbon paste electrode for 5 minutes.

5.3 Results and Discussion

5.3.1 Effect of sodium alpha olefin sulphonate (SAOS) on the surface of CPE

In optimization of modified CPE towards the $K_4[Fe(CN)_6]$, the bare CPE was immobilized on to the surface of the carbon paste electrode in the different concentrations (5, 10, 15, 20, 25 and 30 µL) of SAOS and their electrochemical response towards 1mM $K_4[Fe(CN)_6]$ in presence of 1MKCl as supporting electrolyte was studied. The obtained result illustrates the increase in the quantity of SAOS the corresponding anodic peak current increases upto 15µL and afterwards the current goes on decreasing (Fig.1), as a result the 15µL of SAOS immobilized modified CPE was preferred for further electrochemical investigation.

5.3.2 Electrocatalytic behavior of SAOSMCPE at potassium ferrocyanide

In order to standardize the fabricated SAOSMCPE, potassium ferrocyanide was used for the electrochemical parameters. Fig.2 shows the cyclic voltammograms of 1mM $K_4[Fe(CN)_6]$ at bare (dotted line) and SAOSMCPE (solid line) in presence of 1M KCl as supporting electrolyte with a scan rate of $50mVs^{-1}$.Comparatively SAOSMCPE shows

increase in redox peak currents of 1mM $K_4[Fe(CN)_6]$ than BCPE. The probable mechanism is the SAOS surfactant molecules diffuses into the carbon paste electrode along with the potassium ferrocyanide and causes increase in the redox peak signals [40]. The difference in redox peak potential (ΔEp) for BCPE was found to be 0.114V and 0.084V for SAOS modified carbon paste electrode. The ΔEp is a function of the rate of electron transfer, hence lower the ΔEp value higher will be the electron transfer rate [41]. The proposed modified electrode shows lower ΔEp value than the BCPE and exhibits great enhancement in the redox peak current. Fig.3a shows the cyclic voltammograms of potassium ferrocyanide at different scan rates. The redox peak current of potassium ferrocyanide goes on increases with increase in scan rate. The plot of Ipa versus scan rate shows good linearity with correlation coefficient value R^2=0.99958 as shown in Fig.3b. The plot of Ipa versus square root of scan rate also shows good linearity with the correlation coefficient value R^2 =0.9965 as shown in Fig.3c. From the scan rate study the electrode process was found to be both adsorption and diffusion controlled. Based on the above observation SAOSMCPE shows favorable electrocatalytic behavior and it might be used as a chemically modified electrode to explore electroanalytical application.

5.3.3 Electrochemical response of folic acid at SAOSMCPE

The electrochemical response of folic acid (FA) was studied at SAOS modified carbon paste electrode. The Fig.4 shows the cyclic voltammograms of $0.5x10^{-4}$M folic acid at BCPE (dotted line) and SAOSMCPE (solid line) in 0.2M PBS at pH 7.4 with the scan rate of $50mVs^{-1}$. Comparatively SAOSMCPE shows great enhancement in the anodic peak current (Ipa) than BCPE. Thus remarkable improvement in electrochemical sensitivity towards folic acid at SAOSMCPE gives an evidence for the catalytic effect of proposed electrode.

5.3.4 Effect of scan rate on SAOSMCPE

In order to investigate the kinetics of electrode reaction, effect of scan rate (ν) was studied at SAOSMCPE. Fig.5a shows the cyclic voltammograms of $0.5x10^{-4}$M folic acid at different scan rate in 0.2M phosphate buffer solution (pH 7.4). The result shows that, with the increase in the scan rate (10-100mVs^{-1}) the anodic peak current (Ipa) of folic acid goes

on increases. The plot of anodic peak current (Ipa) versus scan rate (ν) shows good linearity with correlation coefficient value R^2=0.99916 as shown in Fig.5b. Along with the plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) was also studied and correlation coefficient value was found to be R^2=0.9846 indicating the modified electrode process was both adsorption and diffusion controlled electrode reactions (Fig.5c).

5.3.5 Effect of folic acid concentration at SAOSMCPE

The electrochemical oxidation of folic acid at different concentration using SAOSMCPE was studied. Fig.6a shows the cyclic voltammograms obtained for the folic acid at different concentration in presence of 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}. With the increase in the concentration (1.0-3.5x10^{-4}M) the anodic peak current (Ipa) of folic acid goes on increasing. The plot of anodic peak current (Ipa) versus concentration (Fig.6b) shows good linearity with the correlation coefficient value R^2=0.9958. The limit of detection (LOD) was calculated by using the formula (1) [42-45] and it was found to be 2.88x10^{-5}M and the limit of quantification (LOQ) was calculated by using the formula (2) and it was found to be 9.6x10^{-5}M.

LOD=3S/M…..... (1)

LOQ=10S/M.....(2)

where, S is the standard deviation and M is the slope

5.3.6 Simultaneous electrochemical determination of FA and DA at SAOSMCPE

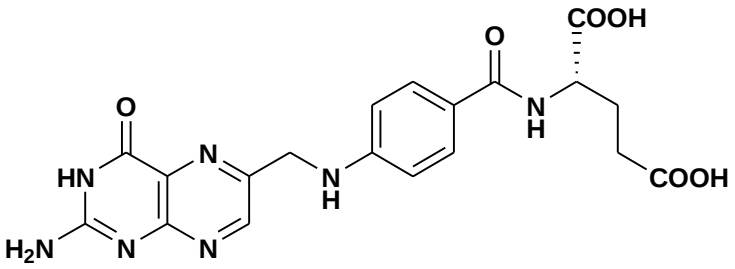
The cyclic voltammetry was employed for the simultaneous electrochemical determination of FA and DA. The Fig.7 shows the cyclic voltammograms obtained for the mixture of 0.5x10^{-4}M FA and 0.5x10^{-4}M DA in the presence of 0.2M PBS (pH 7.4) at the scan rate of 50mVs^{-1}. The Dotted line shows cyclic voltammograms obtained for the mixture of FA and DA at BCPE. The solid line shows cyclic voltammogram obtained for the mixture of FA and DA at SAOSMCPE. The modified electrode shows great enhancement in peak current (Ip) than BCPE and showing good voltammetric separation for both DA and FA and proved its electrocatalytic behaviour in simultaneous determination. Hence the proposed modified electrode (SAOSMCPE) has the ability for the simultaneous determination of FA and DA.

5.3.7 Determination of folic acid in tablet

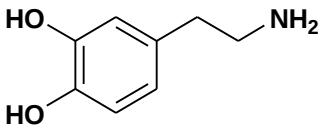
Real sample analysis was carried out to investigate the capability of the modified electrode towards the determination of the folic acid in the tablet. By using standard addition method, the recovery test was obtained for the voltammetric signals and the observed results were shown in Table 1.

5.4 Conclusion

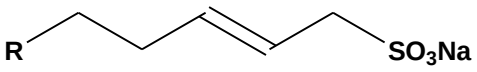
For the electrochemical determination of folic acid, an anionic surfactant sodium alpha olefin sulphonate was used as the modifier. The modified electrode exhibits good electrocatalytic response towards the determination of folic acid. The electrode process of modified electrode was investigated and found to be both adsorption and diffusion controlled and it shows good detection limit. To know the capability of the modified electrode folic acid was determined in the tablet. Finally, it concludes that the proposed electrode (SAOSMCPE) shows very good electrocatalytic properties and can be applied to the electrochemical determination of folic acid.



Scheme 1: Structure of Folic acid



Scheme 2: Structure of Dopamine



Scheme 3: Structure of Sodium alpha olefin sulphonate

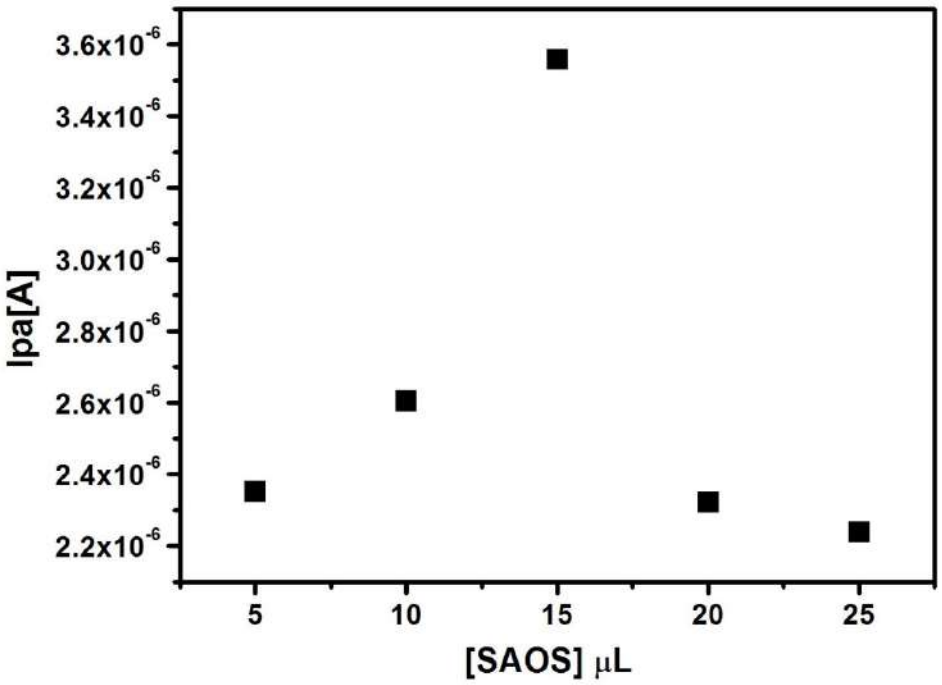


Fig.1: The effect of quantity of SAOS (μL) on the peak current of 1mM $K_4[Fe(CN)_6]$ at the scan rate of 50 mVs^{-1}in presence of 1M KCl as supporting electrolyte.

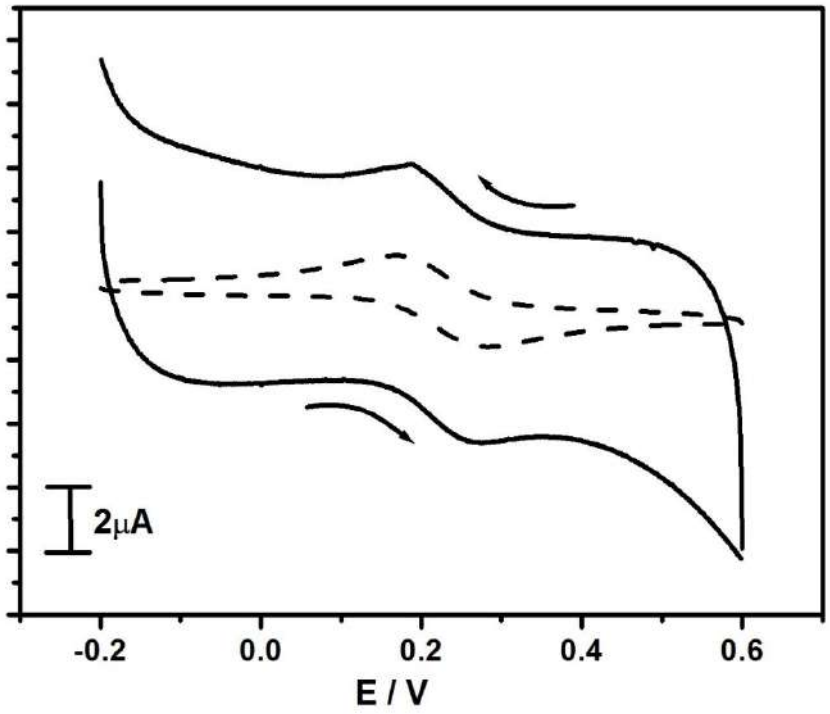


Fig.2: Cyclic voltammograms of 1mM $K_4[Fe(CN)_6]$ at bare (dashed line) and SAOS/MCPE (solid line) in 1M KCl at the scan rate of 50mVs^{-1}.

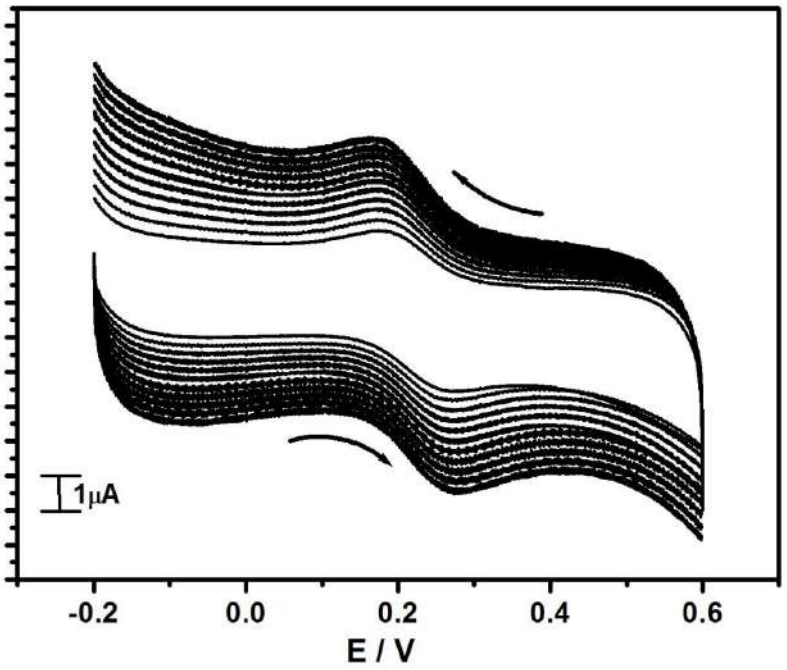


Fig.3a: Cyclic voltammograms obtained for 1mM $K_4[Fe(CN)_6]$ at different scan rates (50 to 150mVs^{-1}) containing 1M KCl.

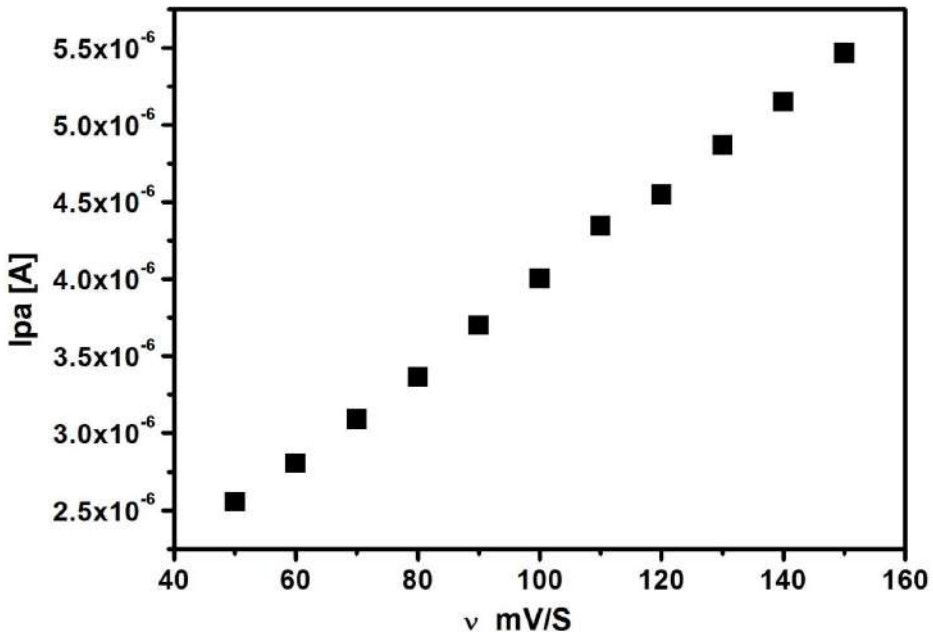


Fig.3b: Graph of anodic peak current (Ipa) versus scan rate (ν)

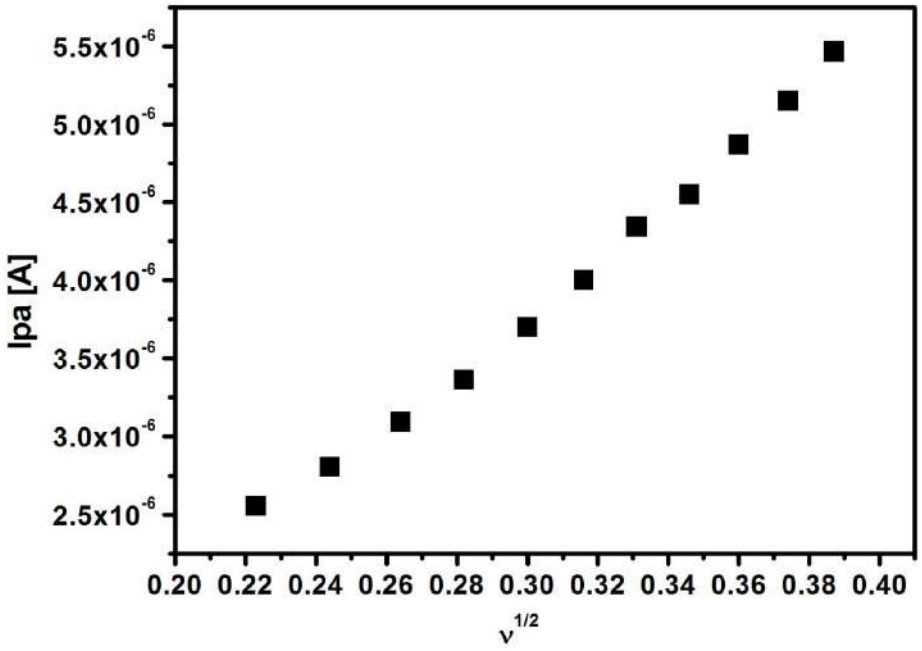


Fig.3c: Graph of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$)

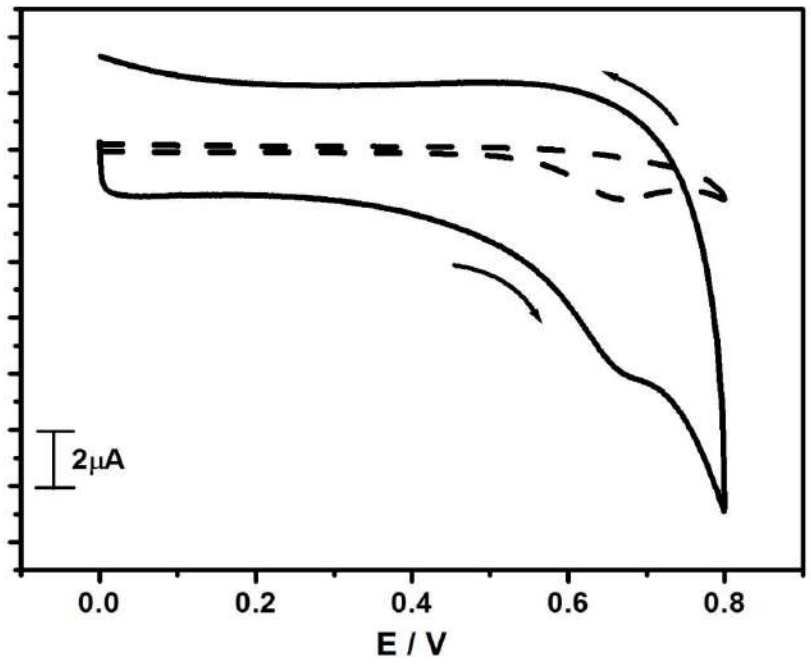


Fig.4: Cyclic voltammograms of $0.5x10^{-4}$M FA at BCPE (dashed line) and SAOS/MCPE (solid line) at scan rate $50mVs^{-1}$ using 0.2M PBS (pH 7.4).

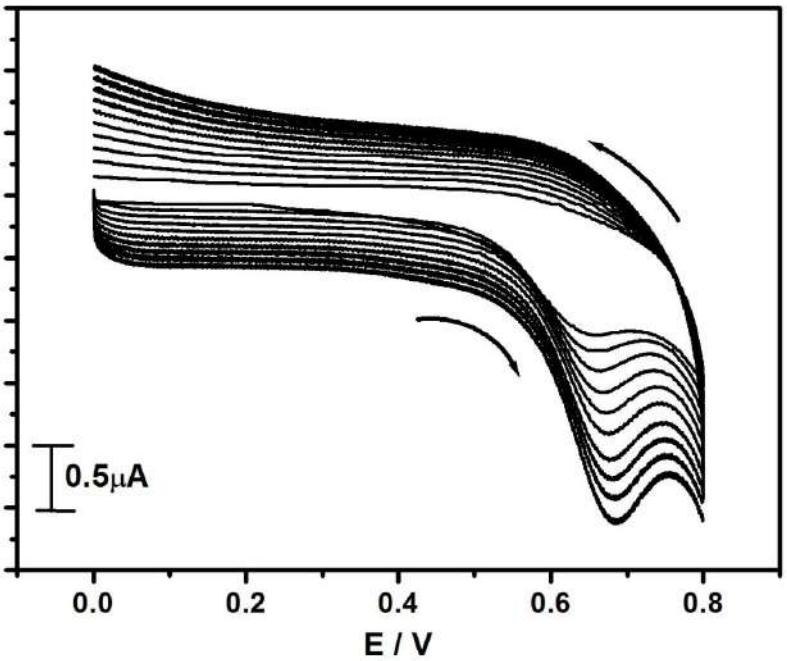


Fig.5a: Cyclic voltammograms of $0.5x10^{-4}$M FA at SAOS/MCPE with different scan rates (10-100mVs^{-1}) using 0.2M PBS (pH 7.4).

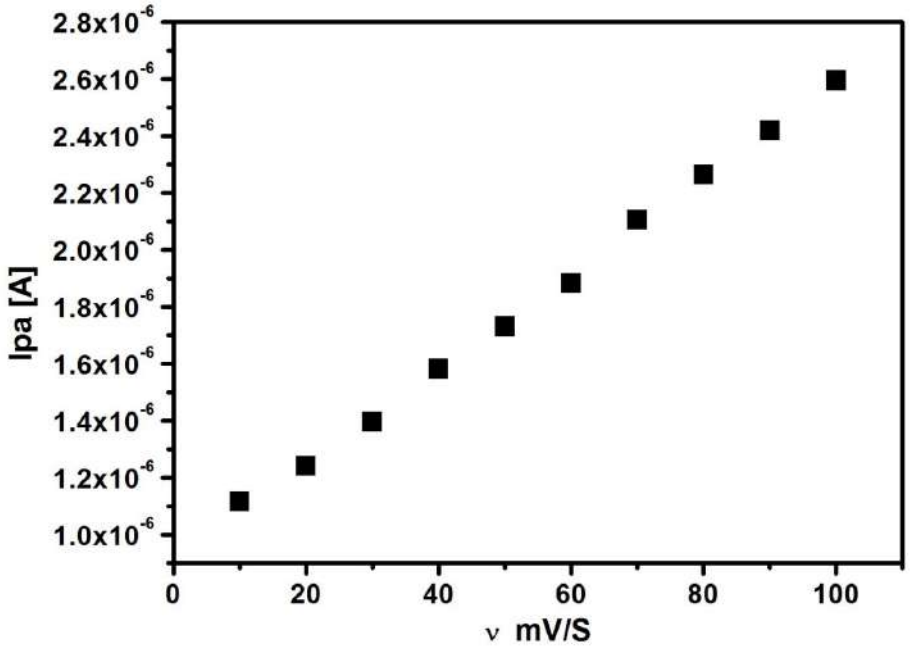


Fig.5b: Graph of anodic peak current (Ipa) versus scan rate (ν)

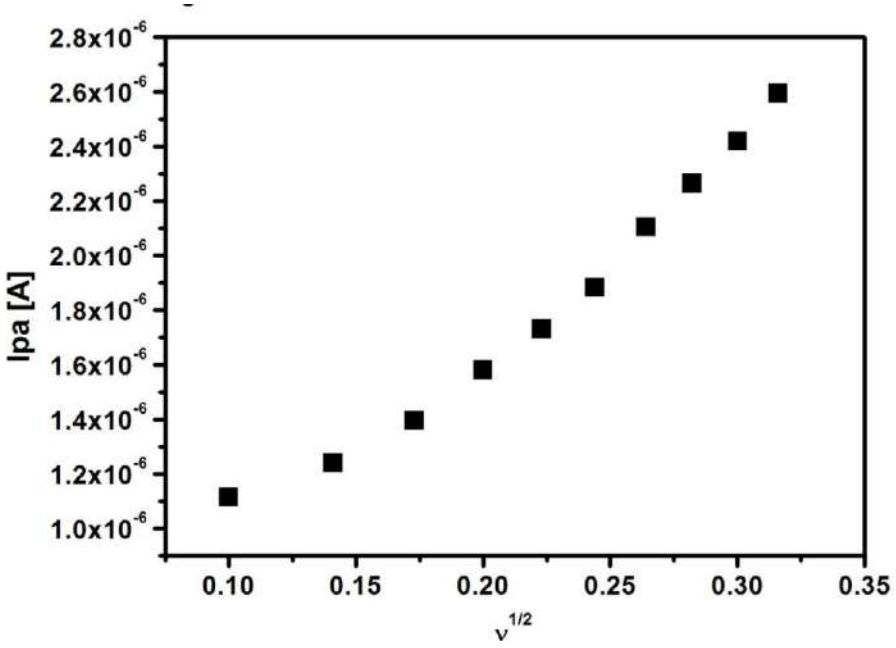


Fig.5c: Graph of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$)

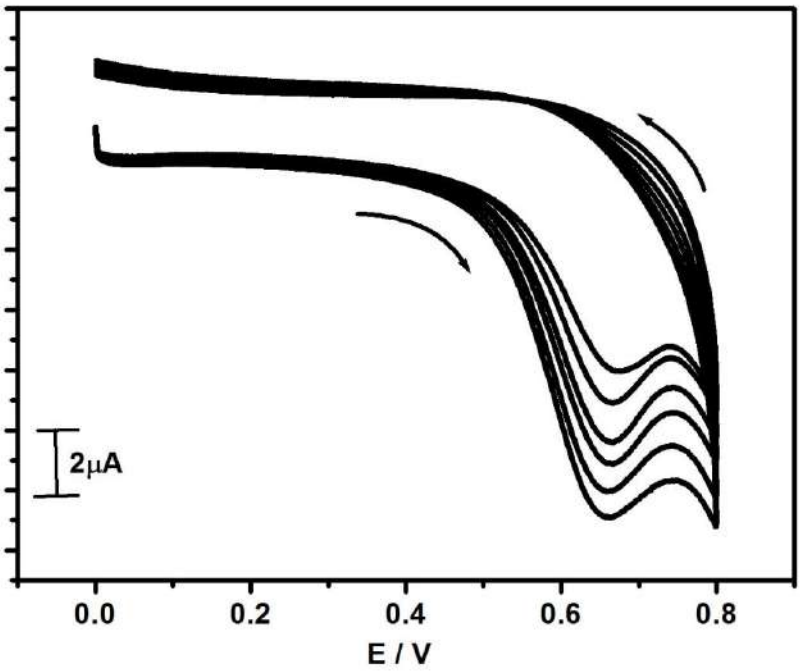


Fig.6a: Cyclic voltammograms of FA at SAOS/MCPE with different concentrations (1.0, 1.5, 2.0, 2.5, 3.0 and $3.5x10^{-4}$M) using 0.2M PBS (pH 7.4) at scan rate $50mVs^{1}$.

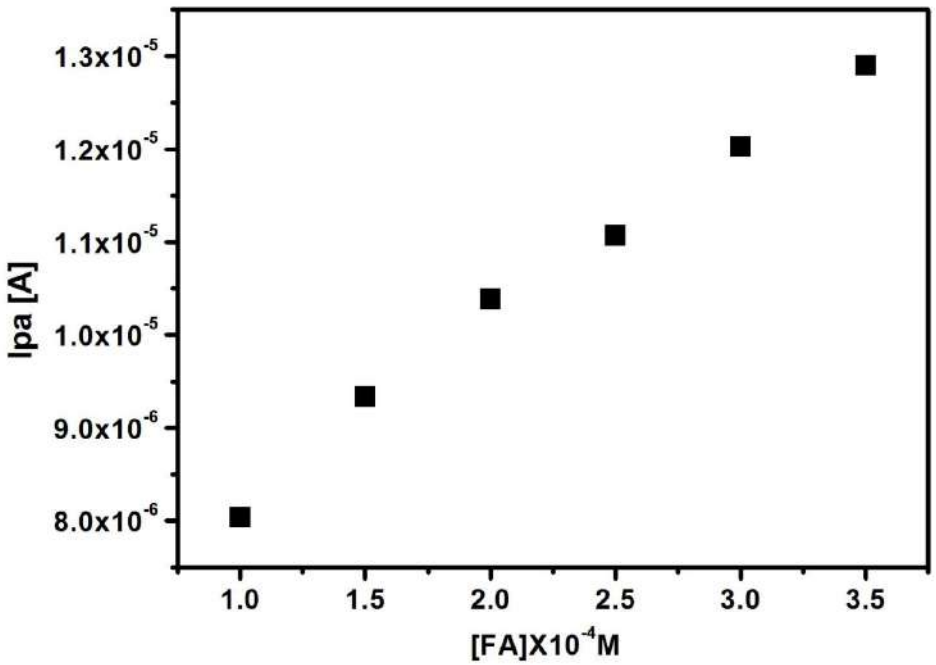


Fig.6b: Graph of anodic peak current (Ipa) versus concentration of FA

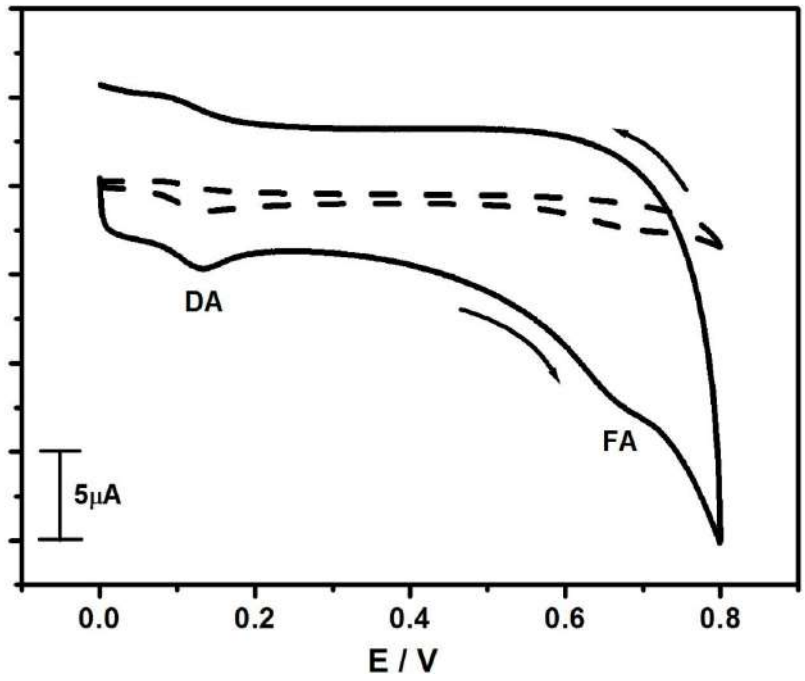


Fig.7: Cyclic voltammograms obtained for the mixture of FA and DA at bare (dashed line) and SAOS/MCPE (solid line) using 0.2M PBS (pH 7.4) at scan rate 50mVs^{-1}.

Table 1: Determination of FA in tablet at modified electrode

Sample	Content	Added (mL)	Found (mL)	Recovery
Tablet	5mg	3.5	3.22	92.2%
Tablet	5mg	4.0	3.96	99.2%

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Chapter -5

Part-B

Pre-treated glassy carbon electrode sensor for catechol: A voltammetric study

Journal of Electroanalytical Chemistry (Revised and Submitted)

5.6 Introduction

In recent years, carbon electrodes are frequently used for electrochemical investigations of oxidizable compounds [1]. The modification of electrode surface is one of the important parameter for the catalytic activity of a carbon electrode. There are several methods for surface modification which improves electrocatalytic activity of an electrode. Among them electrochemical pre-treatment method is one which increases the rate of the electron transfer at the electrode surface in order to overcome slow kinetics of electrode processes. Several authors employed pre-treated glassy carbon electrode (PGCE) for electrochemical investigations has been reported [2-4]. The present study describes the electrochemical pre-treatment of glassy carbon electrode for electrochemical investigations of some oxidizable compounds (catechol and resorcinol).

Catechol (CC) and resorcinol (RS) (phenolic compounds) are highly toxic environmental pollutants and seriously make threats human's physical condition. Phenolic compounds are widely used in pharmaceutical, antioxidant, photographic and cosmetics industries [5-6]. Some of the phenolic compounds have been listed as control targets by the US Environmental Protection Agency (EPA) and the European Union (EU) due to their high toxicity and low degradability in the ecological environment [7]. The simultaneous determination of catechol and resorcinol is an interesting subject in electroanalysis because they have similar structures and properties and generally coexist in environmental samples as pollutants. Therefore, it is necessary to develop a simple and rapid analytical method for the simultaneous determination of catechol and resorcinol. Till now, numerous methods have been established for the determination of phenolic compounds, such as chemiluminescence [8], high performance liquid chromatography [9], spectrophotometry [10] and electrochemical methods [11-25]. Among them, the electrochemical technique attracted more contemplation due to its advantages of cheap instrumentation, simple operation and high sensitivity [26-27].

Now-a-days, the development of reagentless biosensor plays an important role in the research field. Therefore, in the present chapter we activated the glassy carbon electrode in 1M H_2SO_4, which shows good sensitivity and electrochemical properties towards the simultaneous determination of catechol and resorcinol. The response of the CC and RS was

tested before and after the surface activation of glassy carbon electrode. The activated electrode not only exhibits sensitivity but also showed lower detection limit when compared to traditional electrodes. Therefore, the pre-treated glassy carbon electrode has been employed for further electrochemical investigations.

5.7 Experimental

All reagents used in this experiment were of analytical grade and used without further purification. The chemicals like Sodium dihydrogen orthophosphate, disodium hydrogen phosphate and sulphuric acid were obtained from Himedia chemicals. Catechol and resorcinol were purchased from Merck chemicals. The stock solution of catechol and resorcinol (25×10^{-4}M) were prepared using distilled water.

The electrochemical experiments were carried out using CH Instrument-660 with a conventional three-electrode cell. The electrode system contain bare glassy carbon electrode (BGCE) and pre-treated glassy carbon electrode (PGCE) were used as working electrodes, Platinum electrode as counter electrode and the saturated calomel electrode (SCE) as the reference electrode.

The electrochemical pre-treatment of glassy carbon electrode was carried out using cyclic voltammetry method. A glassy carbon electrode was successively polished with 0.05μm alumina slurry and rinsed with distilled water. The polished glassy carbon electrode (GCE) was oxidised by sweeping the potential between -1.2 V to +1.5 V in 1M H_2SO_4 at the scan rate of 100 mVs^{-1} to get pre-treated glassy carbon electrode (PGCE). The activation of GCE surface occurs when the potential is cycled between moderately negative potential (i.e. about -0.5 V) and more positive potentials (i.e. about +0.9 to +1.5 V) [28-31]. Hence, the pre-treated glassy carbon electrode exhibits excellent electrocatalytic activity towards the determination of oxidizable compounds.

5.8 Results and Discussion

5.8.1 Characterization of pre-treated GCE at potassium ferrocyanide system

The electrocatalytic behaviour of pre-treated glassy carbon electrode was investigated at potassium ferrocyanide system using cyclic voltammetry method. Fig.1 shows the redox behaviour of potassium ferrocyanide at bare GCE (solid line) and pre-treated GCE (short

dashed line) in presence of 1M KCl as supporting electrolyte with the scan rate of 100mVs^{-1}. The cyclic voltammograms exhibit peaks in both forward scan as well as reverse scan corresponding to the oxidation and reduction of potassium ferrocyanide respectively. The bare GCE exhibits a couple of redox peaks with peak separation value (ΔEp) of 0.08 V. The pre-treated GCE also exhibit a couple of redox peak with enhancement in peak current than bare GCE and peak separation value (ΔEp) was found to be 0.07 V. As we know that ΔEp is a function of rate of electron transfer, lower the ΔEp value, higher is the electron transfer rate. Hence, the pre-treated GCE exhibited lower peak separation value (ΔEp) than bare GCE. Therefore, the modified electrode proved its catalytic capability and can be applied for further electrochemical investigations.

5.8.2 Electrochemical behaviour of catechol at pre-treated glassy carbon electrode (PGCE)

The pre-treated GCE was employed for the electrochemical investigations of catechol using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. Cyclic voltammograms (CVs) of 0.1×10^{-4}M CC on the glassy carbon electrode in phosphate buffer of pH 7.4 at the scan rate of 50mVs^{-1} is demonstrated in Fig.2. The solid line shows the CVs obtained for CC at bare GCE and short dashed line shows the CVs obtained at pre-treated GCE. As it can be found, catechol exhibits a couple of redox peaks at bare GCE, with an anodic peak potential (Epa) at 0.16 V and cathodic peak potential (Epc) at 0.13 V. After the pre-treatment, catechol shows oxidation and reduction peaks at pre-treated GCE. The anodic and cathodic peak potential (Ep) was found at 0.17 V and 0.13 V respectively. The redox peak current response of CC at pre-treated GCE is more, while the response is very poor at bare GCE. The oxidation mechanism of catechol was shown in scheme 1.

In order to ensure the kinetics of electrode process, the scan rate variation on the redox peak current of catechol at pre-treated GCE was carried out using cyclic voltammetry method in presence of phosphate buffer solution (pH 7.4). Fig.3a demonstrates the cyclic voltammograms obtained for $0.1\text{x}10^{-4}$ M CC at pre-treated GCE with different scan rates. As seen from Fig.3a, CC's peak current increased by increase in the scan rate from 50-500mVs^{-1}. The plot of anodic peak current (Ipa) versus scan rate (ν) was plotted (Fig.3b),

which shows good linearity and correlation coefficient value was found to be R^2=0.998. On the other hand, the relation of anodic peak current (Ipa) versus square root of scan rate ($v^{1/2}$) was plotted and shown in Fig.3c. The plot obtained was linearly straight with correlation coefficient value R^2=0.995. Hence, from the scan rate study the electrode process was found to be adsorption-controlled.

The concentration variation of CC at pre-treated GCE was carried out using CV and DPV techniques. Fig.4a reveals the CVs of catechol at pre-treated GCE with different concentrations in presence of phosphate buffer (pH 7.4) with the scan rate of 50mVs^{-1}. The peak current corresponding to the oxidation and reduction of CC increases with increase in the concentration from 0.2 – 1.0 x 10^{-4}M. The plot of anodic peak current (Ipa) versus CC concentration was depicted in Fig.4a (Inset) and correlation coefficient value was found to be R^2=0.994. In the same way, concentration variation of CC at pre-treated GCE was also carried out using DPV technique. Fig.4b shows the DPVs obtained for CC at pre-treated GCE with different concentrations. The current response corresponding to the oxidation of CC increases with increase in the concentration from 20-120 µM. The relation of anodic peak current (Ipa) versus CC concentration was plotted and depicted in Fig.4b (Inset) having good linearity. The main aim of this parameter is to determine the LOD (limit of detection) and LOQ (limit of quantification) of catechol at pre-treated GCE. The LOD and LOQ were found to be 9.45 µM and 31.5 µM respectively using the equation (1) and (2) [32-36].

LOD=3S/M ..... (1)

LOQ=10S/M.....(2)

where, S is the standard deviation and M is the slope

5.8.3 Electrochemical behaviour of resorcinol at pre-treated glassy carbon electrode (PGCE)

The electrochemical behaviour of resorcinol (0.2×10^{-4} M) at bare GCE (solid line) and pre-treated GCE (short dashed line) was investigated using cyclic voltammetry method in presence of phosphate buffer (pH 7.4) at the scan rate of 50mVs^{-1} (Fig.5). It can be seen that, there is a low peak current at 0.57 V corresponding to the oxidation of resorcinol (RS) was observed at bare GCE. However, for the pre-treated GCE, the oxidation peak current

of RS increases remarkably resulted from the excellent electrochemical properties. Hence, the pre-treated GCE exhibited excellent catalytic activity towards RS determination. The oxidation mechanism of RS was shown in scheme 2.

Fig.6a depicts the effect of scan rate on the cyclic voltammetric response of pre-treated GCE for the detection of 0.2×10^{-4}M resorcinol in PBS (pH 7.4) using cyclic voltammetry method. It was found that, the anodic peak current linearly increases with the scan rate (from 50-500mVs^{-1}). The relation of anodic peak current (Ipa) versus scan rate (ν) was found to be linear with correlation coefficient value R^2=0.998 (Fig.6b). Also, the relation of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) (R^2=0.993) was depicted in Fig.6c, which shows adsorption-controlled electrode process.

Fig.7a depicts the cyclic voltammograms of resorcinol at pre-treated GCE with different concentrations in presence of phosphate buffer (pH 7.4) with the scan rate of 50mVs^{-1}. It can be seen that, the anodic peak current linearly increases with the concentration of RS (from 0.2 – 1.4 x 10^{-4}M). The relation of anodic peak current (Ipa) versus RS concentration was presented in Fig.7a (Inset) and correlation coefficient value was found to be R^2=0.998. The effect of RS concentration at pre-treated GCE was also conducted by DPV method under the optimized conditions. Fig.7b shows the DPVs obtained for RS at pre-treated GCE with different concentrations. The anodic peak current corresponding to the oxidation of RS increases with increase in the concentration from 100-600 µM. The relation of anodic peak current (Ipa) versus RS concentration was presented in Fig.7b (Inset). The LOD (limit of detection) and LOQ (limit of quantification) of RS was found to be 7.24 µM and 24.1 µM respectively at pre-treated GCE using the equation (1) and (2).

5.8.4 Simultaneous electrochemical determination of catechol and resorcinol at pre-treated glassy carbon electrode (PGCE)

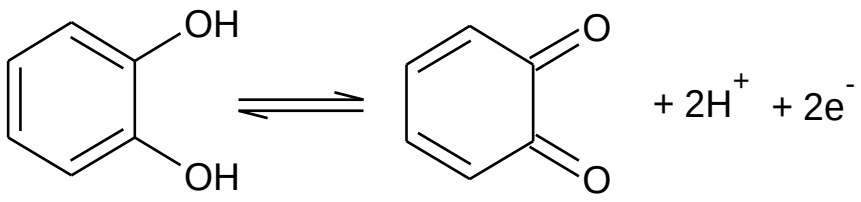
Cyclic voltammetry method was employed for the simultaneous determination of CC and RS at pre-treated GCE. The Fig.8 demonstrates the CVs obtained for the mixture contain $0.1x10^{-4}$M CC and $0.2x10^{-4}$M RS in presence of phosphate buffer (pH 7.4) with the scan rate of 50mVs^{-1}. As can be seen from Fig.8, the poor current response towards simultaneous determination of CC and RS was observed at bare GCE (solid line).

However, the current response at pre-treated GCE (short dashed line) is approximately larger than that at the bare GCE. Hence, it proved that the electrochemical activity of pre-treated GCE is superior to the bare GCE.

The interference study was carried out at pre-treated GCE using DPV method. Fig.9a depicts the DPVs obtained for the binary mixture (CC and RS) in presence of phosphate buffer (pH 7.4) with the scan rate of $50mVs^{-1}$. It can be seen that, the current response corresponding to oxidation of CC increases with concentration (from 50-400 µM) by keeping the concentration of RS constant (100 µM). The relation of anodic peak current (Ipa) versus concentration of CC was presented in Fig.9a (Inset). In the same way, the concentration of RS varied (from 300-1000 µM) by keeping the concentration of CC constant (100 µM). The peak current increases with increase in the concentration of RS (Fig.9b). The relation of anodic peak current (Ipa) versus concentration of RS was shown in Fig.9b (Inset). This result shows the electrochemical properties of pre-treated GCE towards the simultaneous determination of CC and RS.

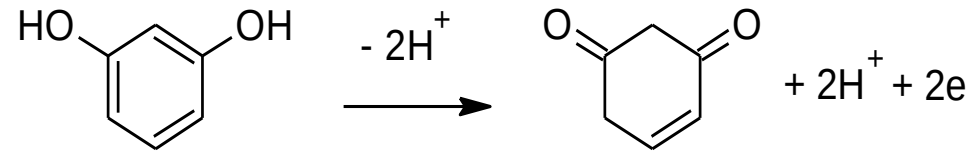
5.9 Conclusion

In conclusion, the pre-treated glassy carbon electrode (PGCE) was simply prepared by electrochemical pre-treatment method. The pre-treated GCE showed well-defined electrochemical properties with enhanced oxidation current response towards the determination of CC and RS in presence of phosphate buffer (pH 7.4) with the scan rate of $50mVs^{-1}$. The electrode process of modified electrode was investigated and found to be adsorption-controlled. The LOD and LOQ of CC and RS were investigated at pre-treated GCE. Hence, the pre-treated GCE was used for the determination of CC and RS with satisfactory results.



Catechol 1,2 Benzoquinone

Scheme 1: Oxidation mechanism of Catechol



benzene-1,3-diol cyclohex-4-ene-1,3-dione

Scheme 2: Oxidation mechanism of Resorcinol

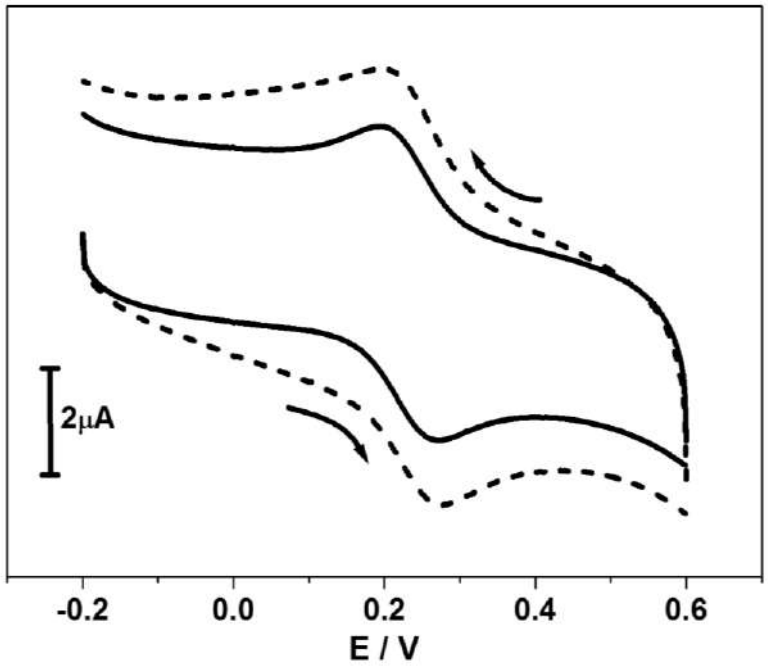


Fig.1: Cyclic voltammograms recorded for 1mM $K_4[Fe(CN)_6]$ at bare GCE (solid line) and pre-treated GCE (short dashed line). Supporting electrolyte = 1M KCl, scan rate = 50 mVs^{-1}.

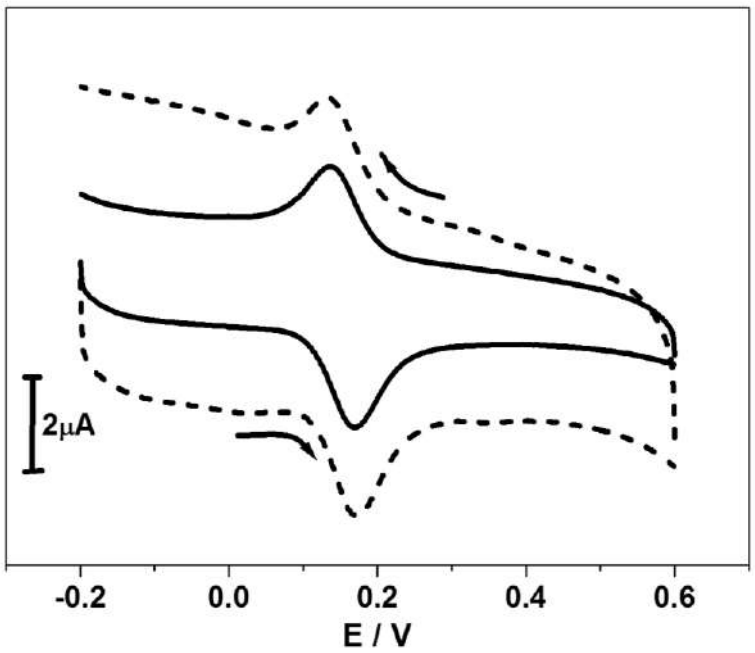


Fig.2: Cyclic voltammograms recorded for $0.1x10^{-4}$M CC at bare GCE (solid line) and pre-treated GCE (short dashed line). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}.

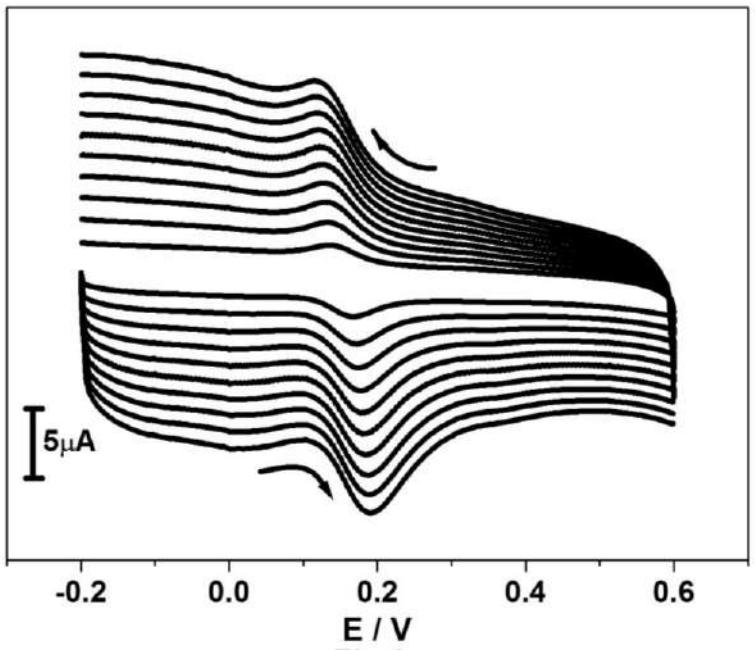


Fig.3a: Cyclic voltammograms recorded for $0.1x10^{-4}$M CC at pre-treated GCE with different scan rates. Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50-500 mVs^{-1}.

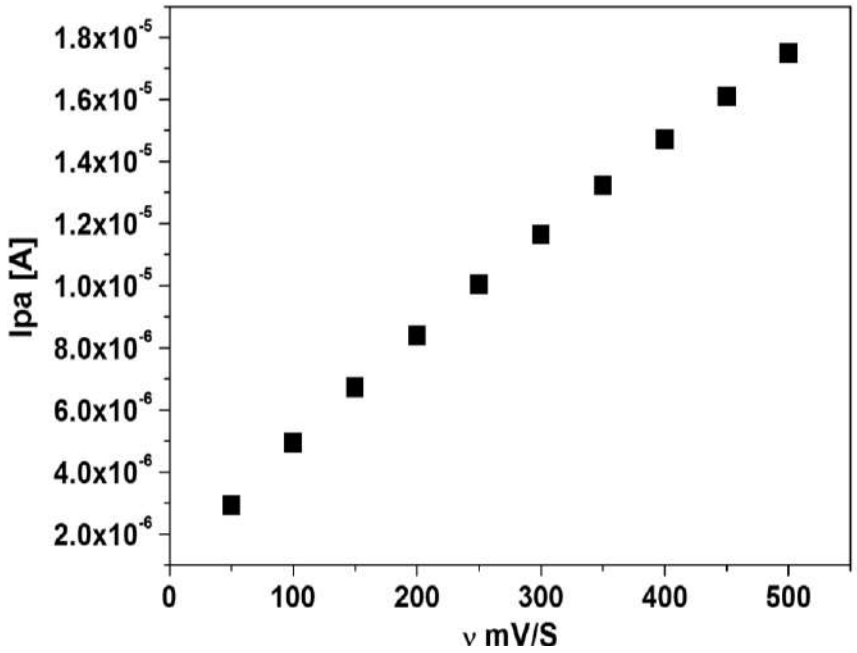


Fig.3b: The relation of anodic peak current (Ipa) versus scan rate (ν) of CC.

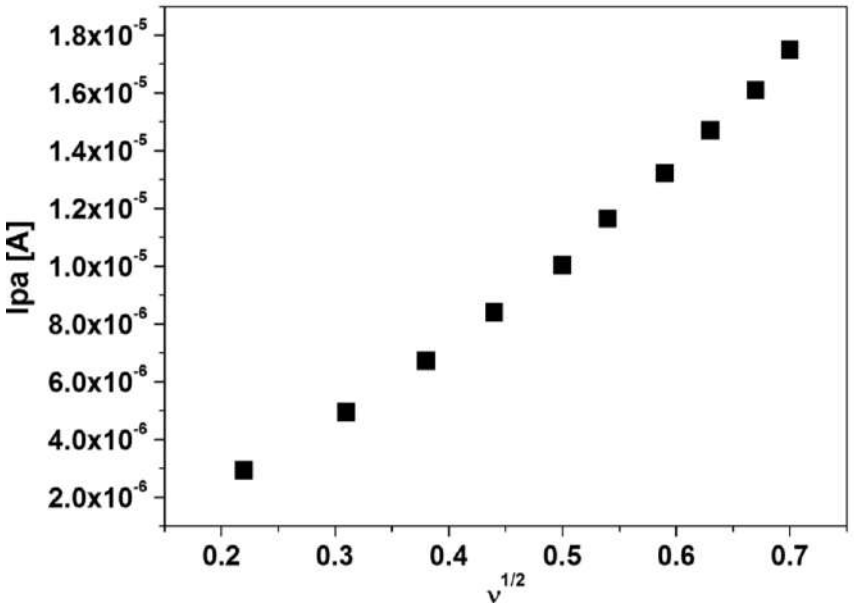


Fig.3c: The relation of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$)

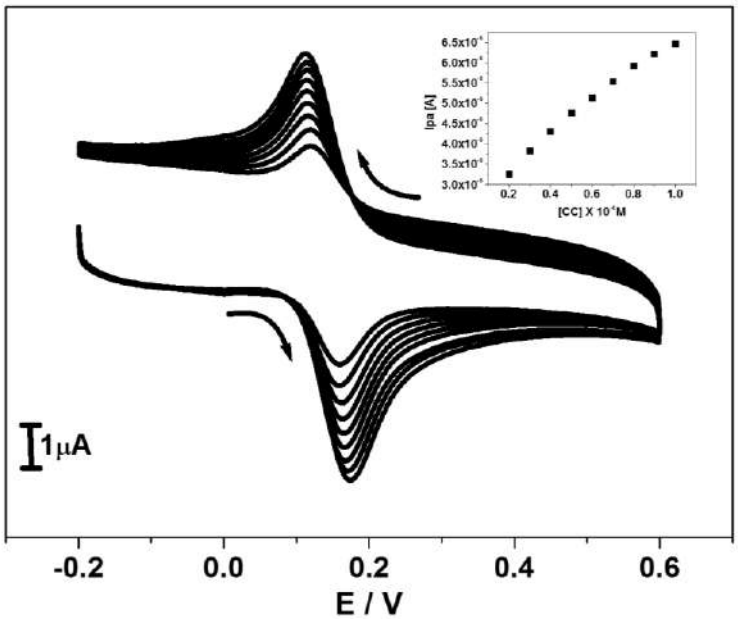


Fig.4a: Cyclic voltammograms recorded for CC at pre-treated GCE with different concentrations (0.2 - 1 x 10^{-4}M). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the relation of anodic peak current (Ipa) versus concentration of CC.

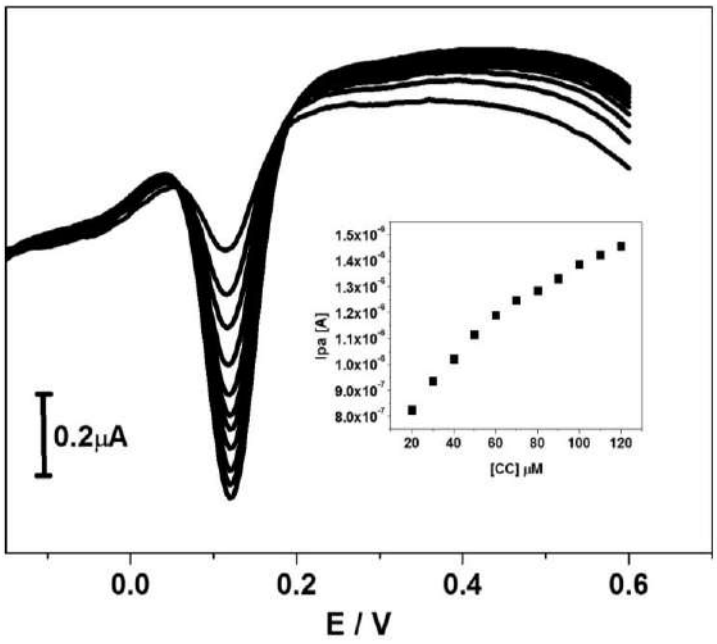


Fig.4b: Differential pulse voltammograms recorded for CC at pre-treated GCE with different concentrations (20 - 120 μM). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the relation of anodic peak current (Ipa) versus concentration of CC.

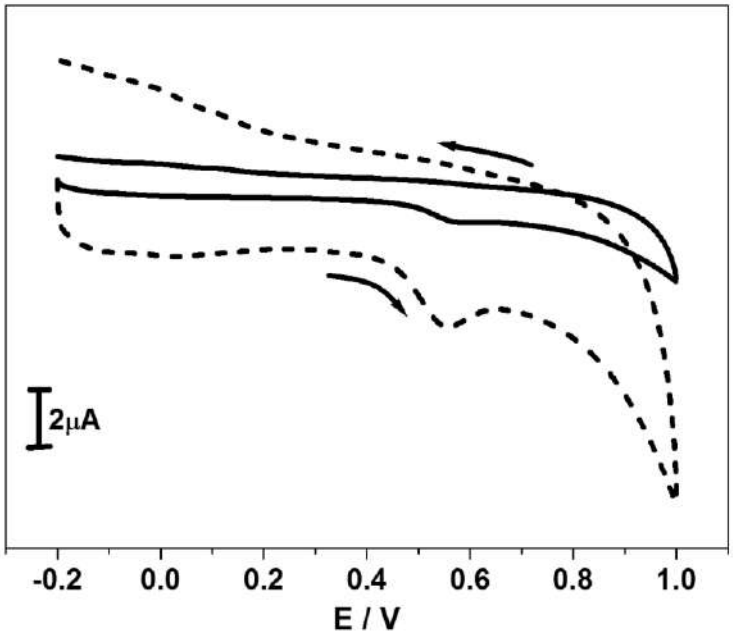


Fig.5: Cyclic voltammograms recorded for $0.2x10^{-4}$M RS at bare GCE (solid line) and pre-treated GCE (short dashed line). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}.

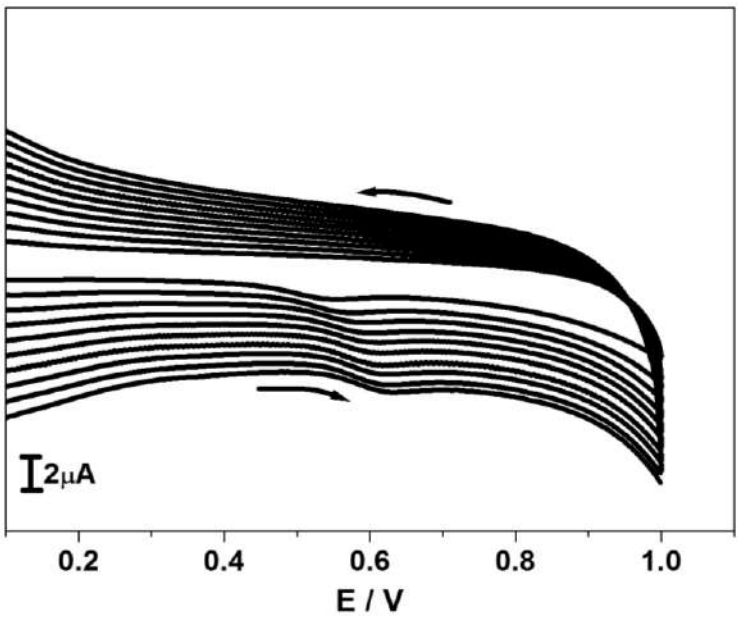


Fig.6a: Cyclic voltammograms recorded for $0.2x10^{-4}$M RS at pre-treated GCE with different scan rates. Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50-500 mVs^{-1}.

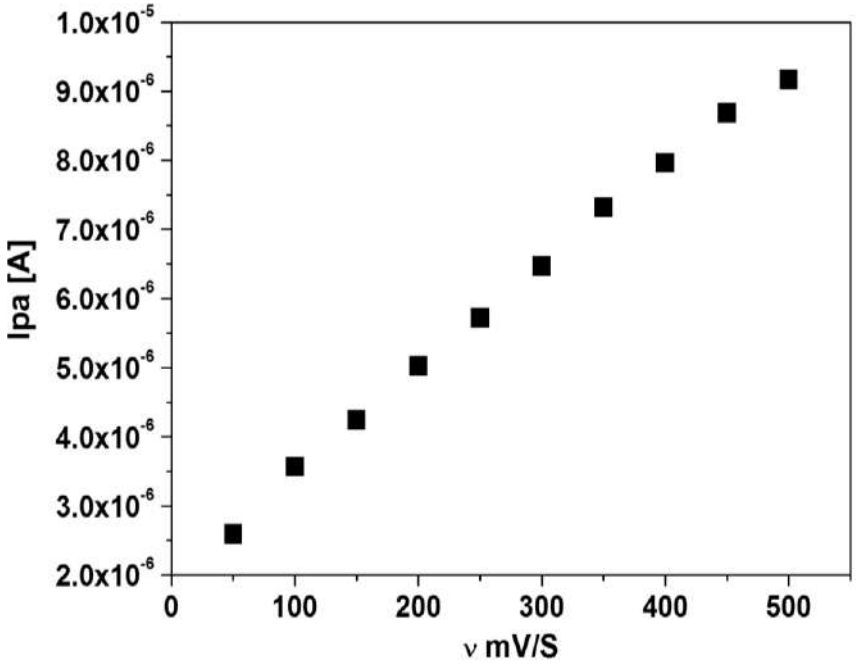


Fig.6b: The relation of anodic peak current (Ipa) versus scan rate (ν)

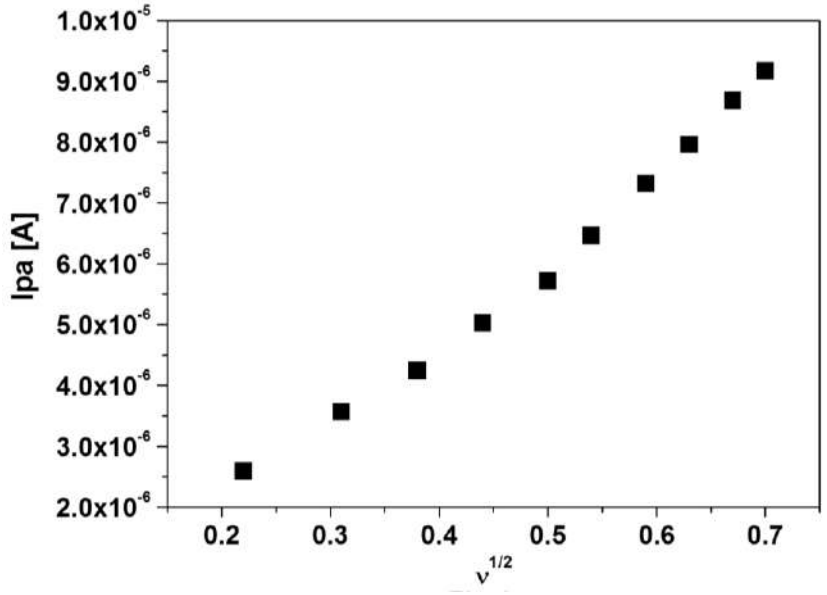


Fig.6c: The relation of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$)

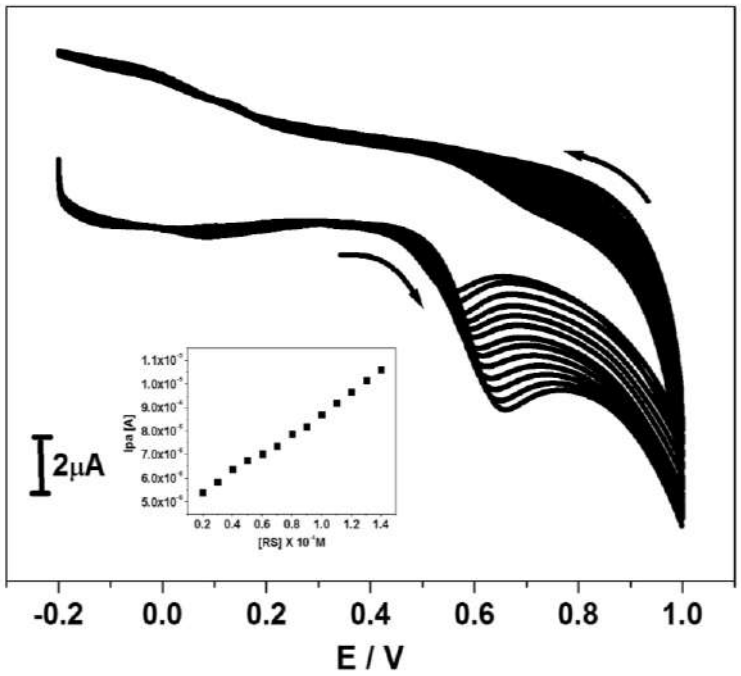


Fig.7a: Cyclic voltammograms recorded for RS at pre-treated GCE with different concentrations ($0.2 – 1.4 \times 10^{-4}$M). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the relation of anodic peak current (Ipa) versus concentration of RS.

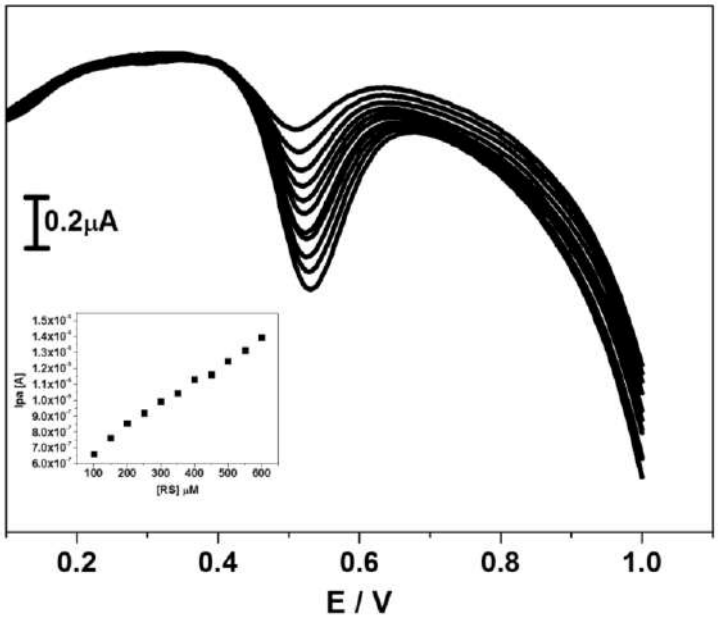


Fig.7b: Differential pulse voltammograms recorded for RS at pre-treated GCE with different concentrations (100 - 600 μM). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the relation of anodic peak current (Ipa) versus concentration of RS.

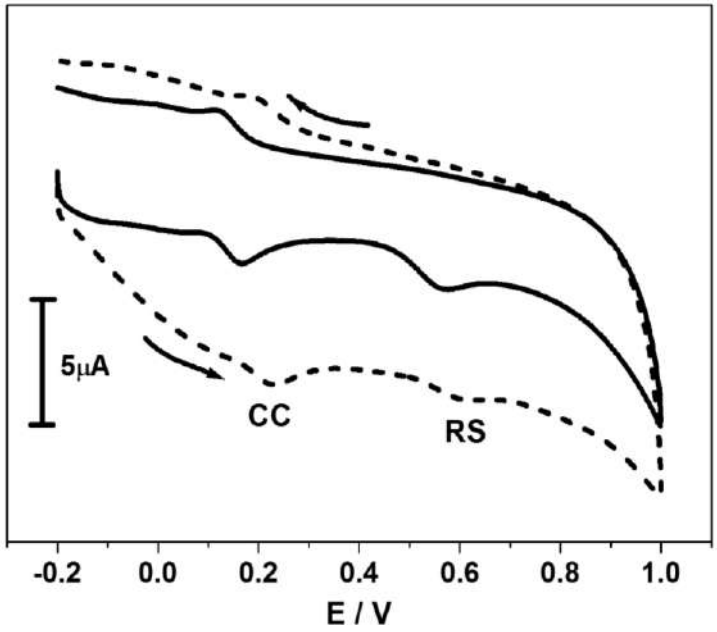


Fig.8: Cyclic voltammograms recorded for the simultaneous determination of CC ($0.1x10^{-4}$M) and RS ($0.2x10^{-4}$M) at bare GCE (solid line) and pre-treated GCE (short dashed line). Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}.

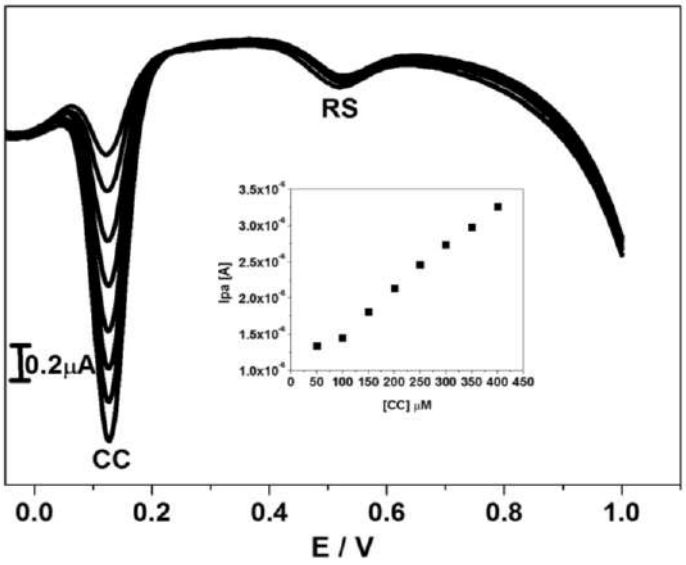


Fig.9a: Differential pulse voltammograms recorded for CC at different concentrations (50 - 400 μM) keeping 100 μM RS constant. Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the relation of anodic peak current (Ipa) versus concentration of CC.

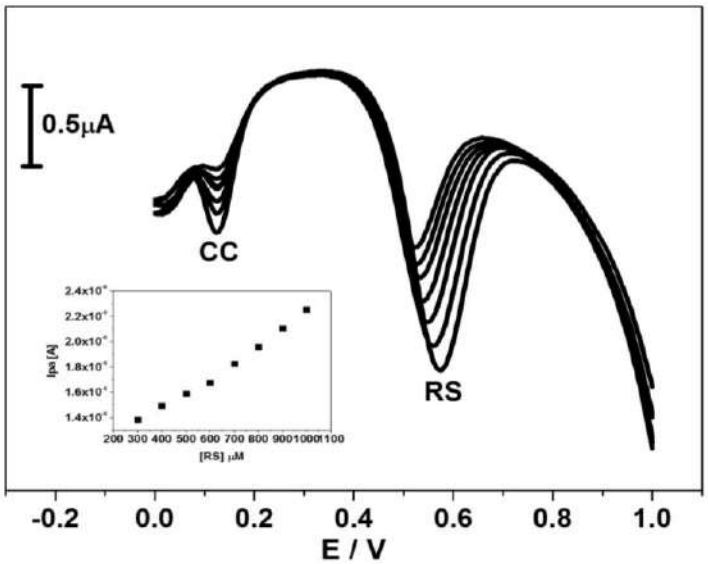


Fig.9b: Differential pulse voltammograms recorded for RS at different concentrations (300 - 1000 μM) keeping 100 μM CC constant. Supporting electrolyte = 0.2M PBS (pH 7.4), scan rate = 50 mVs^{-1}. Inset figure shows the relation of anodic peak current (Ipa) versus concentration of RS.

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Chapter -6

Poly (neutral red) modified glassy carbon electrode sensor for epinephrine

Analytical and Bioanalytical Electrochemistry (Revised and Submitted)

6.1 Introduction

In recent years, the approach for the modification of electrodes especially, the chemically modified electrodes (CMEs) has received extensive consideration. These modifications of electrodes are the promising way to achieve a desired result with respect to their high sensitivity and selectivity, which makes them an ideal sensor. Modification of electrodes can be achieved by covalent attachment, physical coating and adsorption [1]. Besides these methods eletrodeposition and electropolymerization have also been discussed by many research groups in the recent decades. [2-9]. Polymer modification of electrodes have attracted many researchers due to their increased number of binding sites in comparison to the above mentioned methods and have good stability with easy way of engineering. Due to the simplicity, strong adherence on the electrode surface, broad potential window, chemical stability and ability to provide larger surface area makes electropolymerization method the most attractive among the methods of electrode modification with polymer [10-11]. A series of studies have been reported by many research groups with respect to electropolymerization of numerous molecules on different electrodes for the selective detection of neurotransmitter [12-17]. Attracted by these results we have chosen neutral red as a modifier for electropolymerization. Neutral red (NR) (scheme 1), which is a planar phenazine dye can be electrochemically polymerized and utilized in biological system for electrochemical investigation [18]. Among the family of azines the neutral red (NR) is an acid base indicator which can be easily electropolymerized and has reported as a redox mediator in enzyme electrodes with outstanding electrocatalytic activities [19-22]. These properties have made us to choose neutral red as modifier for electropolymerization.

Epinephrine (EP) (scheme 2) and norepinephrine (NEP) (scheme 3) are the important class of catecholamine neurotransmitter in the mammalian central nervous system. The concentration of EP in the blood stream is related to many life phenomena [23]. Besides this person with Parkinson's disease carry low levels of EP. In specific NEP are the derivatives of catecholamine, whose concentration level affects the muscle and tissue control and is also critical in mental disease, heart failure and so on [24]. Consequently, these results make researcher to develop a simple and brisk analytical methods for the

qualitative and the quantitative estimation of EP and NEP. In support of these, numerous methods have been proposed and reported for their determination, such as spectrophotometry [25-28], fluorimetry [29], liquid chromatography [30-32], capillary electrophoresis [33-35], chemiluminescence [36-37] and amperometry [38-39]. But these methods cannot be performed at every level due to their high cost equipment and sophisticated techniques. Among these reported methods the attractive and simple technique in the recent times is consider to be the electrochemical technique due to its advantages of inexpensive instrumentation, trouble-free operation and high sensitivity [40-41].

In this chapter we have proposed a simple method for the electrochemical determination of epinephrine and norepinephrine using cyclic voltammetric (CV) method. The poly modified glassy carbon electrode was obtained by modifying the surface of glassy carbon electrode by neutral red through electropolymerization technique. The newly tailored sensor exhibits excellent electrocatalytic activity towards EP and NEP with high selectivity, sensitivity and good detection limit. These factors suggest that the poly (neutral red) modified glassy carbon electrode (PNR/MGCE) open ups a new era for the determination of EP and NEP.

6.2 Experimental

6.2.1 Materials and Reagents

The voltammetric experiment was performed on model CHI-660c (CH Instrument-660 electrochemical workstation) through a conventional three-electrode cell. The glassy carbon electrode (GCE) and poly (neutral red) modified glassy carbon electrode (PNR/MGCE) were used as working electrodes and Platinum electrode as counter electrode, whereas the saturated calomel electrode (SCE) as reference electrode. The reagents like epinephrine (EP) and norepinephrine (NEP) were obtain from Merck chemicals and the stock solution of $25x10^{-4}$M was prepared in 0.05M perchloric acid. Phosphate buffer solution (PBS) (0.2M) was prepared using disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen orthophosphate (NaH_2PO_4) (both from Himedia chemicals). Neutral red (NR) was purchased from Sigma-Aldrich and all other reagents were of analytical grade and used as received.

6.2.2 Fabrication of poly (neutral red) modified glassy carbon electrode (PNR/MGCE)

Prior to fabrication of poly modified electrode, the glassy carbon electrode (GCE) surface was manually polished with alumina powder (0.05μm) and rinsed with distilled water. After cleaning the electrode, the surface was electropolymerized by 1mM neutral red in presence of 0.1M NaOH as supporting electrolyte at the scan rate of 100mVs^{-1} to obtain poly (neutral red) modified glassy carbon electrode (PNR/MGCE).

6.3 Results and discussion

6.3.1 Electropolymerization of neutral red on the surface of glassy carbon electrode

The electroactive species neutral red (NR) was electropolymerized on the surface of GCE in presence of 0.1M NaOH at the scan rate of 100 mVs^{-1} as depicted in Fig 1a. Electropolymerization was achieved in the potential range of -0.8V to 1.0 V for 15 cycles. The peak current increased with further scanning indicating the continuous growth of the polymeric film on the surface of GCE. The effect of the polymer film thickness on the anodic current of EP (0.1×10^{-4}M) was studied by varying the polymerization cycles from 5 to 25. The anodic peak current of EP continuously increased upon increasing the number of polymerization cycles (Fig 1b). Finally, fifteen polymerization cycles were chosen to fabricate the poly (neutral red) modified glassy carbon electrode (PNR/MGCE).

6.3.2 Electrochemical measurements of epinephrine at poly (neutral red)/MGCE

We have employed cyclic voltammetric technique (CV) to establish the electrocatalytic oxidation behaviour of EP at poly (neutral red) modified glassy carbon electrode (PNR/MGCE). Cyclic voltammograms obtained for 0.1×10^{-4}M EP at both bare (dashed line) and modified (solid line) electrode in presence of 0.2 M PBS (pH 7) with the scan rate of 50mVs^{-1} can be seen in Fig 2. The result from Fig 2 suggests that the oxidation of EP was very weak at bare GCE showing an anodic peak potential (Epa) at 0.184 V. However, a significant increase in the peak current of EP (Epa= 0.188 V) was observed at PNR/MGCE confirms that modified electrode acts as an efficient electron promoter to enhance the rate of electrochemical reaction. To understand the kinetics of the electrode reaction, scan rate study was carried out in the range of 20-100 mVs^{-1}. The effect of scan rate on the peak currents of EP (0.1×10^{-4}M) was depicted in Fig 3a. The voltammograms

displays that the oxidation peak current goes on increasing with respect to scan rate. Fig 3b depicts the plot of anodic peak current (Ipa) versus scan rate (ν) of EP, which was linearly associated with corresponding correlation coefficient value R^2=0.99. Besides this the plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) was depicted in Fig 3c with correlation coefficient value R^2=0.958. These results suggest that the electrode process was found to be diffusion-controlled at PNR/MGCE. To estimate the limit of detection (LOD) and limit of quantification (LOQ) for EP, we have carried out the concentration variation parameter at modified electrode. Fig.4 displays the effect of concentration on the peak current of EP in presence of 0.2M PBS (pH 7) at the scan rate of 50mVs^{-1}. It can be seen that the peak current goes on increasing with respect to concentration of EP (from 0.2 to 0.8×10^{-4}M). The correlation coefficient value (R^2=0.998) was obtained by plotting anodic peak current (Ipa) versus concentration of EP (Inset of Fig 4). The LOD and LOQ were calculated using the equation (1) and (2) [42-48] and were found to be 4.2 µM and 14 µM, respectively.

LOD=3S/M..... (1)

LOQ=10S/M..... (2)

Where, S is the standard deviation and M is the slope

6.3.3 Electrochemical measurements of norepinephrine at poly (neutral red)/MGCE

The redox behaviour of norepinephrine (NEP) at PNR/MGCE was carried out using cyclic voltammetric technique (CV). Fig.5 depicts the cyclic voltammetric response of NEP (0.1×10^{-4}M) in 0.2 M PBS (pH 7.0) at the surface of a bare GCE (dashed line) and PNR/MGCE (solid line). At the surface of bare GCE, the NEP undergoes oxidation at a potential of 0.203 V and reduction at 0.162 V. On the other hand, redox peak current of NEP was significantly increased at PNR/MGCE, which confirms the electrocatalytic behaviour of modified electrode. The anodic and cathodic peak potentials were observed at 0.192 V and 0.173 V, respectively. The influence of scan rate on the electrochemical behaviour of NEP at PNR/MGCE was also studied. Fig.6a depicts the cyclic voltammetric response of NEP (0.1×10^{-4}M) at different scan rates. As can be seen in Fig. 6a, both oxidation and reduction peak current increases gradually with increase in the scan rate from 50-500mVs^{-1}. Fig. 6b depicts the relationship between anodic peak current (Ipa) versus

scan rate (ν) of NEP and correlation coefficient value was found to be R^2=0.999. In specific, Fig.6c shows the relationship between anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) having correlation coefficient value R^2=0.973. Based on these results from i.e. the scan rate study, indicates that the electrode process was found to be diffusion-controlled. The variation of NEP concentration at modified electrode was also carried out using 0.2 M PBS (pH 7.0) at the scan rate of 50mVs^{-1}. Fig.7 represents the cyclic voltammetric response of NEP at different concentrations. The anodic and cathodic peak current of NEP significantly increased with increase in the concentration from 0.1 to 0.9×10^{-4}M. Inset of Fig.7 shows the plot of anodic peak current (Ipa) versus concentration of NEP, which exhibits correlation coefficient value R^2=0.991. Based on these results, the LOD and LOQ were calculated to be 11.8 µM and 39.4 µM, respectively. Therefore, the modified electrode exhibited excellent electrocatalytic activities towards NEP determination.

6.3.4 Effect of pH

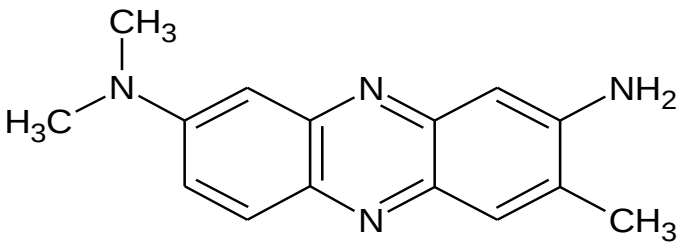
The influence of pH on the electrochemical behavior of NEP at PNR/MGCE was carried out using cyclic voltammetric method (CV). Fig.8 displays the cyclic voltammetric response of 0.1×10^{-4}M NEP at different pH in presence of 0.2M PBS with the scan rate of 50mVs^{-1}. The obtained voltammograms suggests that the anodic and cathodic peak potential of NEP shifted towards positive value with increasing pH (6.0, 6.5, 7.0, 7.5 and 8.0). Inset Fig.8 depicts the plot of anodic peak current of NEP versus pH value, it can be seen that the anodic peak potential (Epa) of NEP decreased with increase in pH indicating an interruption of the protons in the oxidation of NEP.

6.3.5 Sample analysis

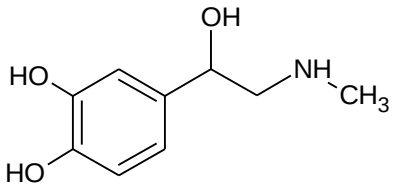
To determine the capability of the proposed modified electrode, real sample analysis was carried out by taking the injection samples of epinephrine and norepinephrine. Standard addition method was employed for the analysis of injection samples at PNR/MGCE and the obtained results were demonstrated in the Table 1. These results suggest that the proposed method can be efficiently applied to the determination of EP and NEP in real samples.

6.4 Conclusion

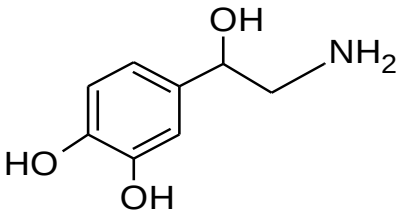
In this chapter we have shown a novel and simple method for the fabrication of poly (neutral red) modified glassy carbon electrode based on the electropolymerization method, which serves as an electrochemical sensor for epinephrine and norepinephrine determination. Due to the excellent eletrocatalytic activity, the PNR/MGCE exhibited increased peak current toward the determination of EP and NEP. The scan rate study of EP and NEP at modified electrode reveals that the electrode process was controlled by diffusion. In addition, lowest LOD and LOQ were exhibited for EP and NEP at modified electrode. Thus, the poly (neutral red)/MGCE exhibited higher stability, sensitivity and shown promising electrocatalytic activity towards the electrochemical determination of EP and NEP.



Scheme 1: Structure of Neutral red



Scheme 2: Structure of Epinephrine



Scheme 3: Structure of Norepinephrine

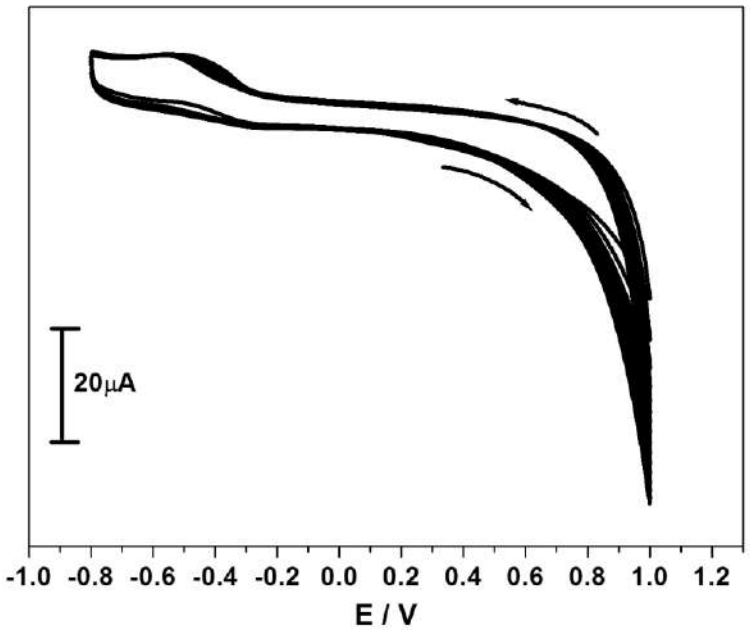


Fig.1a: Successive cyclic voltammograms of NR on the surface of GCE at 15 cycles. Experimental conditions: [NR] = 1mM in 0.1M NaOH, pH 7 and υ= 100 mVs^{-1}.

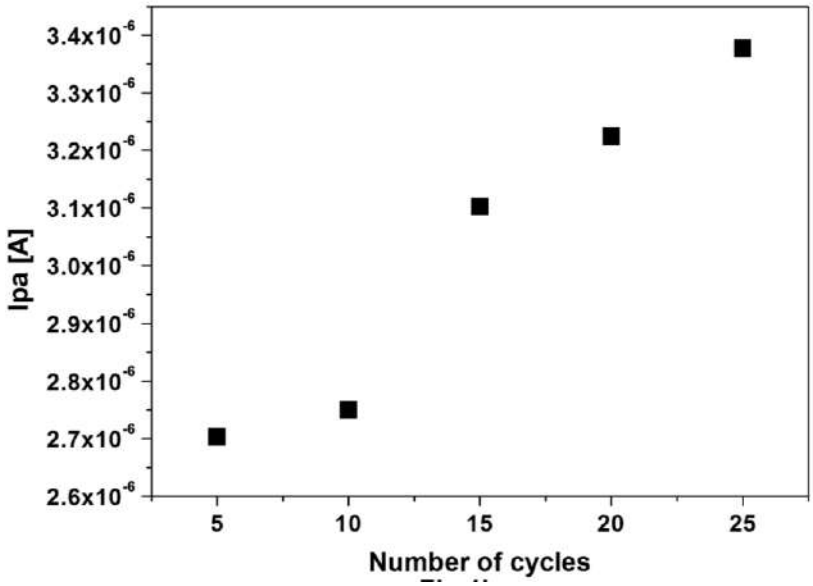


Fig.1b: Plot of anodic peak current (Ipa) versus number of cycles.

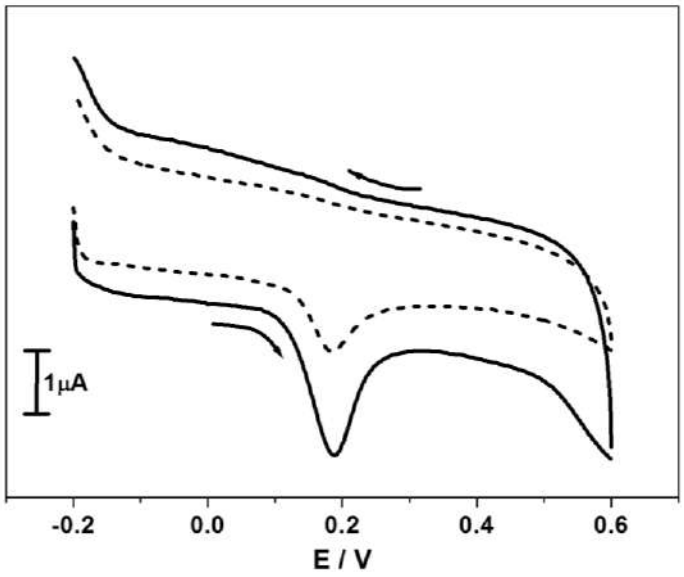


Fig.2: A comparison of cyclic voltammograms recorded for EP ($0.1x10^{-4}$M) at bare GCE (dashed line) and PNR/MGCE (solid line) in presence of 0.2M PBS (pH 7) at the scan rate of $50mVs^{-1}$.

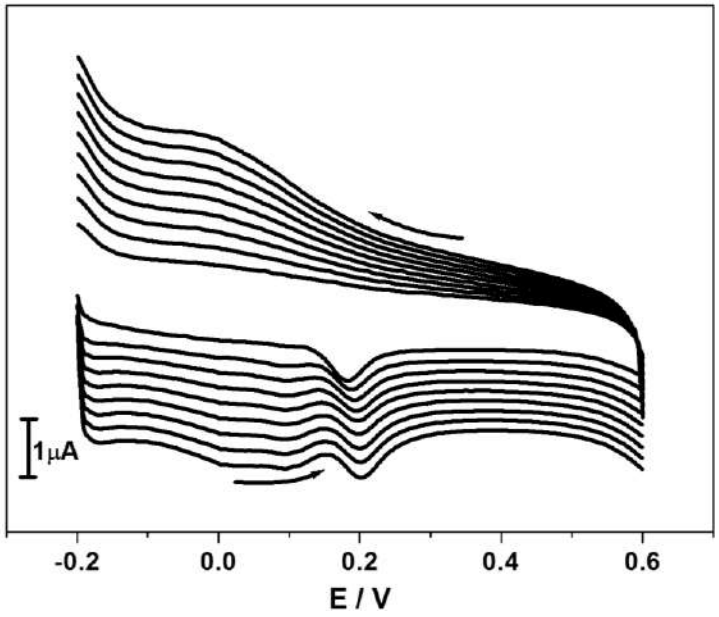


Fig.3a: Cyclic voltammograms obtained for EP ($0.1x10^{-4}$M) at modified electrode with increasing scan rate from 20 to $100mVs^{-1}$ in presence of 0.2M PBS (pH 7).

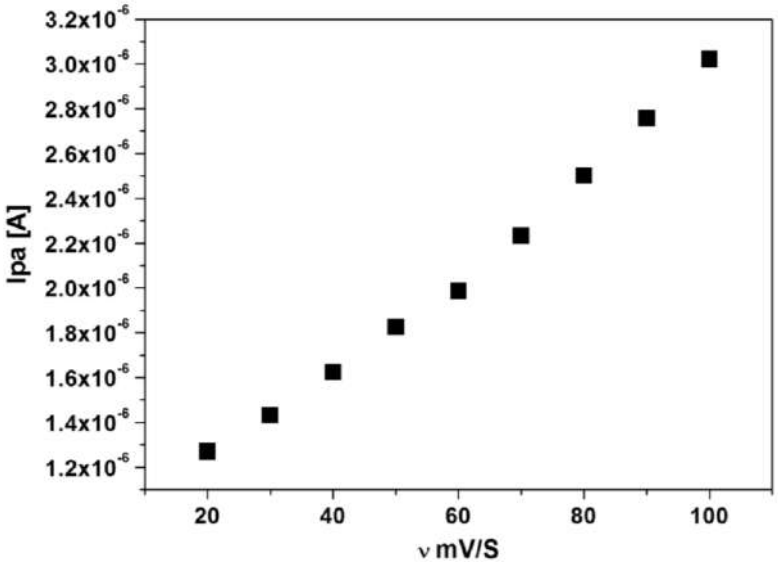


Fig.3b: Plot of anodic peak current (Ipa) versus scan rate (ν)

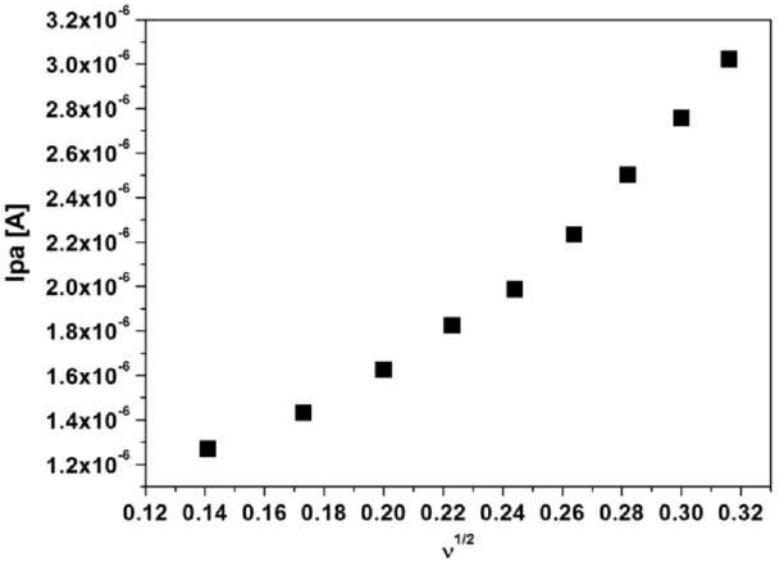


Fig.3c: Plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$)

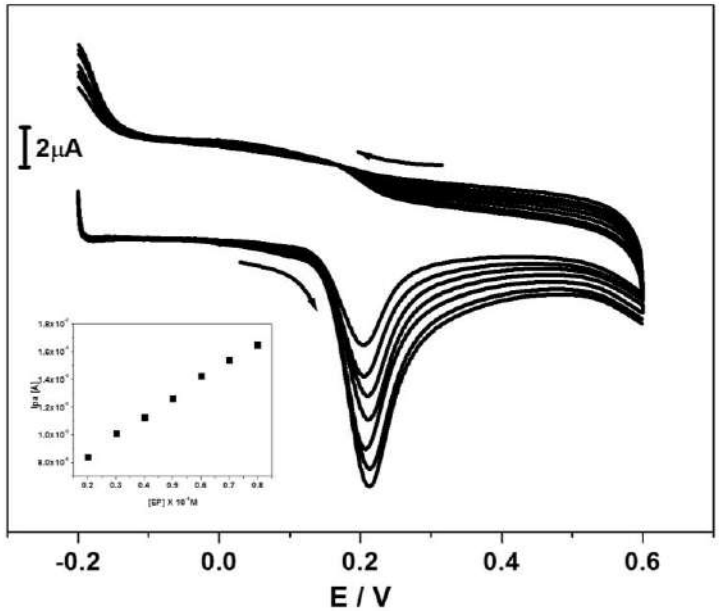


Fig.4: Cyclic voltammograms obtained for EP at modified electrode with increasing concentration from 0.2 to 0.8 x 10^{-4}M in presence of 0.2M PBS (pH 7) at the scan rate of 50mVs^{-1}. Inset figure shows the plot of anodic peak current (Ipa) versus concentration of EP.

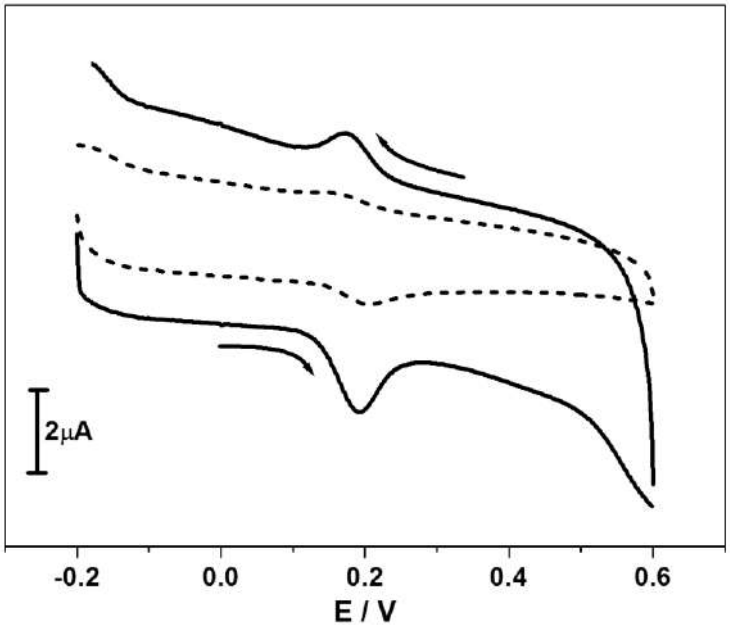


Fig.5: A comparison of cyclic voltammograms recorded for NEP ($0.1x10^{-4}$M) at bare GCE (dashed line) and PNR/MGCE (solid line) in presence of 0.2M PBS (pH 7) at the scan rate of 50 mVs^{-1}.

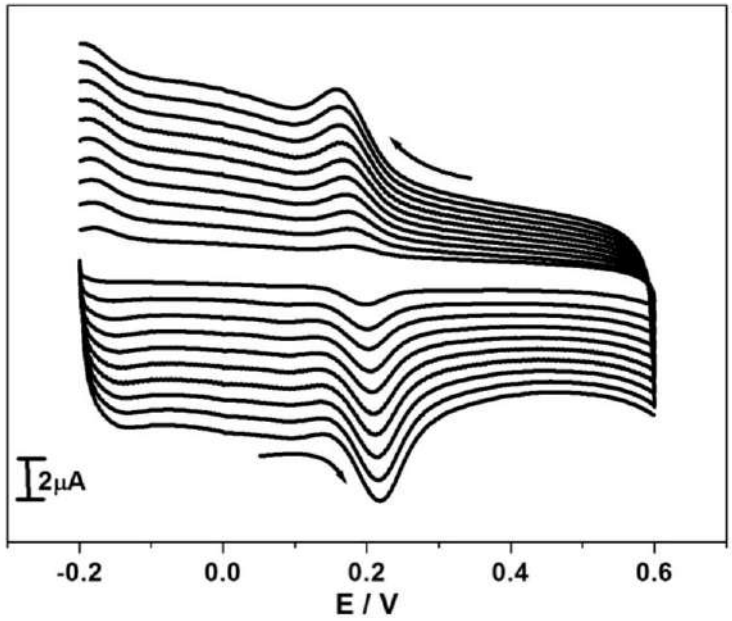


Fig.6a: Cyclic voltammograms obtained for NEP ($0.1x10^{-4}$M) at modified electrode with increasing scan rate from 50 to 500mVs^{-1} in presence of 0.2M PBS (pH 7).

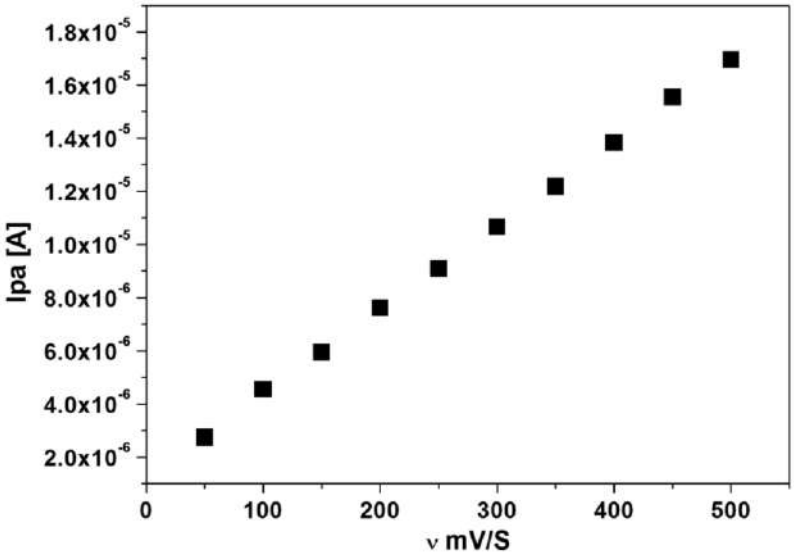


Fig.6b: Plot of anodic peak current (Ipa) versus scan rate (ν)

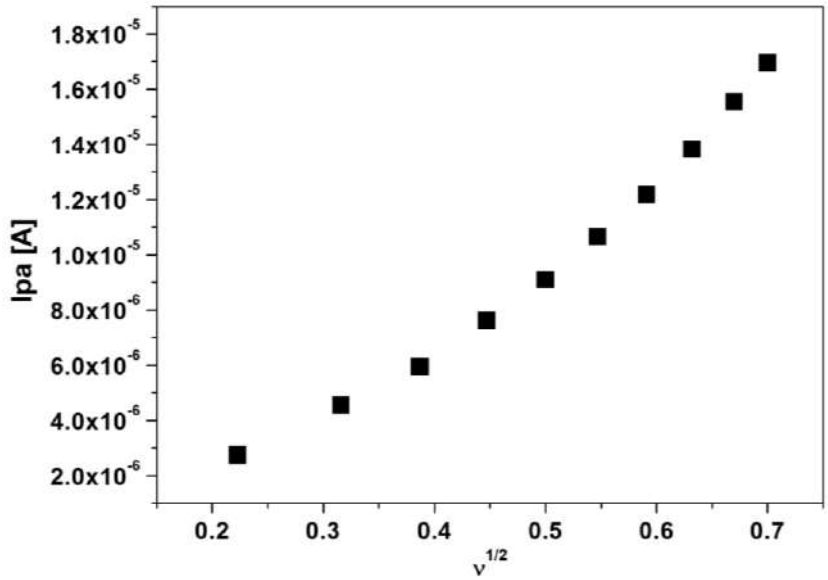


Fig.6c: Plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$)

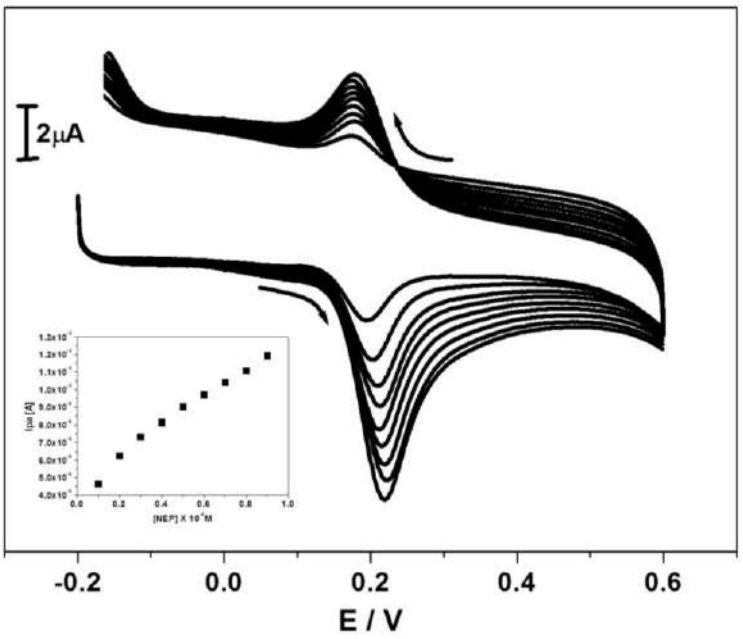


Fig.7: Cyclic voltammograms obtained for NEP at modified electrode with increasing concentration from 0.1 to 0.9 x 10^{-4}M in presence of 0.2M PBS (pH 7) at the scan rate of 50mVs^{-1}. Inset figure shows the plot of anodic peak current (Ipa) versus concentration of NEP.

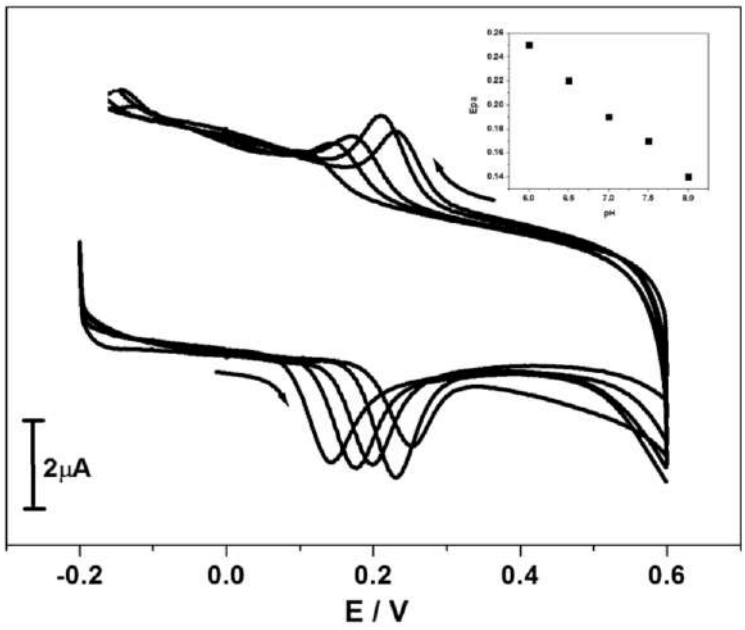


Fig.8: Cyclic voltammograms obtained for $0.1x10^{-4}$M NEP at different pH (6, 6.5, 7, 7.5 and 8) in presence of 0.2M PBS at the scan rate of $50mVs^{-1}$. Inset figure shows the plot of anodic peak potential (Epa) versus pH.

Table 1: Determination of EP and NEP in injection samples at PNR/MGCE.

Injection sample	Added (mL)	Found (mL)	Recovery (%)
EP	06	5.7	96.2
	08	7.9	99.7
NEP	04	3.8	97.2
	08	7.9	99.6

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Chapter -7

A simple disposable pencil graphite electrode sensor for catechol and hydroquinone: A voltammetric study

Journal of Electroanalytical Chemistry (Revised and Submitted)

7.1 Introduction

In recent years, researchers are focusing on the development of sensors which are inexpensive with more sensitivity, stability and reproducibility. The carbon based working electrodes have been widely used in electrochemical analysis due to its high electrical conductivity, abundant porosity and large specific surface area. It also ensures rapid electron transfer and fast mass transport in electrochemical applications. Pencil graphite electrode (PGE) is a type of carbon electrodes comprises of three main components i.e. graphite, plumbum and clay have some advantages such as cost effectiveness, commercial availability, disposability, low technology and easy modification [1-5]. The renewable surface property of the PGE which is simpler and faster than polishing procedures results in high degree of reproducibility for the individual surfaces. Because of these excellent properties PGE have been employed for numerous electroanalytical applications [6-11]. But in a few cases PGE show poor sensitivity towards the determination of some diverse electroactive molecules [12-13]. Therefore modification of PGE is essential to develop sensitive electrochemical sensors. There are some reports in literature for chemically modified PGEs [14-22]. Among the different modifications, electrochemical pre-treatment method seems to be simpler, less time consuming and more applicable. Therefore, in this article a simple method was employed pre-treated pencil graphite electrode (PPGE) for the electrochemical determination of some phenolic compounds.

The biological and environmental importance of catechol (1, 2-dihydroxybenzene, CC) and hydroquinone (1, 4-dihydroxybenzene, HQ) the two important isomers of phenolic compounds, which are aromatic molecules have conceivable applications in the chemical and pharmaceutical industries i.e. in cosmetics, antioxidants, plastic, leather, paint and steel [23-25]. The toxic and non-bio-degradable nature of these compounds especially in their isomer forms are the serious concern in terms of environmental pollution, which shows a huge impact on living being. Therefore, development of efficient technique for the determination of these toxic compounds is highly desirable. A series of stabilized methods have been reported by many researcher for the determination of these toxic isomers such as; high performance liquid chromatography (HPLC) [26-27], spectrophotometry [28], electrochemiluminescence [29], pH based-flow injection analysis [30] and synchronous

fluorescence [31]. But in the case of all the above mentioned method the number of electron involved in the redox mechanism was not predicted and they are also time consuming. Some of the electroanalytical techniques when compared to these methods have more advantages in terms of rapid and reliable response, reproducible results and easy operating procedures [32-35]. Besides, electroanalysis is more interesting subject because it helps the researcher in simultaneous and interference free determination of isomers [36-38].

Herein, facile approach for the development of pre-treated pencil graphite electrode (PPGE) sensor for the electrochemical analysis of CC and HQ in presence of 0.2 M phosphate buffer solution (pH 7.4) with the scan rate of $50mVs^{-1}$. The proposed sensor exhibited good sensitivity, stability and reproducibility towards the analysis of CC and HQ using cyclic voltammetric method.

7.2 Experimental

7.2.1 Chemicals and apparatus

The chemicals used in this work are catechol, hydroquinone, sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4), which are purchased from Himedia chemicals. The graphite powder and silicon oil were procured from Merck chemicals for the preparation of carbon paste. The stock solution of CC (25×10^{-4} M) and HQ (25×10^{-4} M) were prepared in double distilled water. All other chemicals were of analytical grade and used without further purification. All the voltammetric measurements were carried out with a model CHI-660c (CH Instrument-660 electrochemical workstation). The conventional three-electrode cell assembly was used which contains working electrode (PGE and pre-treated PGE), reference electrode (saturated calomel electrode) and auxiliary electrode (platinum electrode).

7.2.2 Preparation and pre-treatment of pencil graphite electrode

The readily available HB pencil lead (diameter=0.5mm, length=60mm) was purchased from local bookstore, in which one end of the pencil lead was connected to the copper wire in order to provide electrical contact. The electrochemical pre-treatment of PGE was carried out by cyclic voltammetric technique. The surface of PGE was pre-treated by applying a potential between -0.4V and 1.4V in 0.1M NaOH for 15 cycles with the scan

rate of 100mVs^{-1}. The electrochemical pre-treatment consequences increase in the hydrophilic properties of the electrode surface [39].

7.3 Results and Discussion

7.3.1 Characterization of pre-treated PGE at potassium ferrocyanide system.

The surface conductivity of the modified electrode was investigated at potassium ferrocyanide [$K_4Fe(CN)_6$] system using cyclic voltammetric technique. Fig.1 shows the CVs of [$K_4Fe(CN)_6$] at PGE (dashed line) and pre-treated PGE (solid line) in presence of 1M KCl with the scan rate of 50mVs^{-1}. A poor current response was obtained for [$K_4Fe(CN)_6$] at PGE due to the slow electron transport phenomena, however pre-treated PGE shows stagnant enhancement in the redox peak current of [$K_4Fe(CN)_6$] indicating the better electrical conductivity with fast rate of electron transfer with decrease in over potential. Therefore, the obtained result reveals that the surface property of the modified electrode has been changed drastically.

7.3.2 Voltammetric response of CC at pre-treated PGE

The electrochemical oxidation and reduction behaviour of CC at pre-treated PGE was examined using a method called cyclic voltammetry in presence of 0.2 M phosphate buffer solution (pH=7.4). Fig.2 demonstrates the CVs obtained for 0.1×10^{-4}M CC at PGE (dashed line) and pre-treated PGE (solid line) with the scan rate of 50mVs^{-1}. As can be seen from Fig.2, CC shows a low redox peak current response at PGE, on the other hand, CC show substantial enhancement in the redox peak current at pre-treated PGE. This result confirms the sensor behaviour of the modified electrode towards CC determination. The oxidation mechanism of CC was depicted in scheme 1. In addition to this, the effect of scan rate on the redox peak current of CC was investigated at modified electrode in order to find out the kinetics of electrode reaction. Fig.3a depicts the CVs obtained for 0.1×10^{-4}M CC at different scan rates (50 - 500 mVs^{-1}). It can be seen that, the oxidation and reduction peaks of CC gradually increases with increase in the scan rate, which indicates the proportionality of scan rate with respect to time. Fig.3b shows the plot of anodic peak current (Ipa) versus scan rate (ν) having the linear correlation coefficient value R^2=0.997. Fig.3c depicts the plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) and linear correlation

coefficient value was found to be R^2=0.996. Therefore, the electrode process was found to be diffusion-controlled. The heterogeneous rate constant (k^o) for CC was also determined at modified electrode by using the equation (1) [40] and the obtained results were tabulated in Table 1.

$\Delta Ep = 201.39 \log (\upsilon / k^0) - 301.78$…… (1)

Moreover, the concentration variation of CC was carried out at pre-treated PGE to evaluate the limit of detection (LOD) and limit of quantification (LOQ). Fig.4a shows the CVs obtained for CC at different concentrations with the scan rate of 50mVs^{-1}. The observed result from Fig.4a reveals that the redox peak current response of CC increases by increasing the concentration from 0.1 – 0.7 x 10^{-4}M. Fig.4b shows the relationship between anodic peak current (Ipa) and concentration of CC and linear regression coefficient value was found to be R^2=0.996. From this experimental data, the LOD and LOQ for CC were found to be 5.9 µM and 19.6 µM respectively, which were calculated by the equation (2) and (3) [41-44].

LOD=3S/M…….. (2)

LOQ=10S/M…… (3)

where, S is the standard deviation and M is the slope

7.3.3 Voltammetric response of HQ at pre-treated PGE

In this case also, the electrochemical response of HQ was investigated at pre-treated PGE using cyclic voltammetric method in presence of 0.2 M phosphate buffer solution (pH=7.4). In Fig.5, the redox behaviour of 0.1×10^{-4}M HQ was recorded at PGE (dashed line) and pre-treated PGE (solid line) with the scan rate of 50mVs^{-1}. HQ undergoes oxidation and reduction at PGE with low current response, however in the same identical condition the pre-treated PGE results enhancement in the peak current of HQ. The enhancement in peak current gave an evidence for the sensor behavior of the modified electrode towards HQ determination. The oxidation mechanism of HQ was shown in scheme 2 and the variation of scan rate on the redox peak current of HQ was carried out at modified electrode. Fig.6a shows the CVs recorded for 0.1×10^{-4}M HQ at different scan rates (50 - 500 mVs^{-1}). The peak current response of HQ gradually increases with increase in the scan rate. Fig.6b depicts the plot of anodic peak current (Ipa) versus scan rate (ν)

having the linear correlation coefficient value R^2=0.996. Fig.6c demonstrates the plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$) which show good linearity and correlation coefficient value was found to be R^2=0.997. The obtained result reveals that the electrode process was found to be diffusion-controlled. The heterogeneous rate constant (k^o) for HQ (Table 1) was also determined at pre-treated PGE by using the equation (1). In addition to this, the effect of HQ concentration variation at modified electrode was also studied. Fig.7a shows the CVs recorded for redox peak current response of HQ at different concentrations with the scan rate of 50mVs^{-1}. It can be seen that the redox peak current response of HQ increases by increasing the concentration from 0.4 – 1.2 x 10^{-4}M. The Fig.7b shows the plot of anodic peak current (Ipa) versus concentration of HQ and linear regression coefficient value was found to be R^2=0.995. From the obtained experimental data, the LOD and LOQ for HQ were calculated by using the equation (2) and (3) was found to be 8.53 µM and 28.4 µM respectively. The performance of the pre-treated PGE electrode was compared with other reported modified electrodes towards the determination of CC and HQ (Table 2).

7.3.4 Simultaneous electroanalysis of CC and HQ at pre-treated PGE

The CC and HQ have similar structures and properties, because of this reason they are always overlap at the unmodified electrodes. Therefore it is essential to separate CC and HQ simultaneously using modified electrode. Fig.8 shows the CVs recorded for the binary mixture contain 0.1x10^{-4}M CC and 0.1x10^{-4}M HQ at PGE (dashed line) and pre-treated PGE (solid line) in presence of 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}. PGE fails to separate CC and HQ and a broad overlapped oxidation peak was appears at 0.193 V. Under the same optimized condition, pre-treated PGE resolved the CC and HQ with well defined oxidation peaks at 0.12 V and 0.023 V respectively. Moreover, the modified electrode result enhancement in the peak current response of CC and HQ than PGE. On the other hand, the equimolar mixture (0.1x10^{-4}M) of CC and HQ was also distinguished and resolved using differential pulse voltammetric technique (Inset of Fig.8). Therefore, pre-treated PGE confirms the sensor behaviour towards the simultaneous determination of CC and HQ.

7.3.5 Interference study

Interference study was carried out to illustrate the accurate and interference free determination of CC and HQ at pre-treated PGE using differential pulse voltammetry (DPV) technique in presence of 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}. Fig.9a demonstrates the DPVs recorded for the binary mixture contain CC and HQ, in which the concentration of one analyte was varied (CC), whereas the concentration of other analyte kept constant (HQ). It can be seen that the peak current response of CC increases by increase in its concentration. Fig.9b shows the plot of anodic peak current (Ipa) versus concentration of CC. Similarly, the concentration of HQ was varied by keeping the concentration of CC constant, as a result peak current of HQ goes on increases due to its increase in concentration was depicted in Fig.10a. The relationship between anodic peak current (Ipa) versus concentration of HQ was depicted in Fig.10b.

7.3.6 Effect of pH on the redox behaviour of CC and HQ

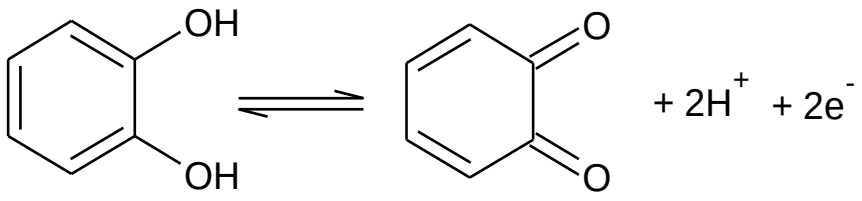
The influence of pH of the supporting electrolyte on the redox behaviour of CC and HQ was studied at pre-treated PGE in 0.2M PBS using cyclic voltammetric method. Fig.11a depicts the CVs recorded for the equimolar mixture ($0.1x10^{-4}$M) of CC and HQ at modified electrode with the scan rate of 50mVs^{-1}. The observed results from Fig.11a suggest that the anodic peak potentials of both CC and HQ shifted to more negative side due to increase in the solution pH (6.2, 6.6, 7.0 and 7.4). The slope value was determined by plotting the Epa of CC versus pH (Fig.11b) and found to be 0.051 reveals the transfer of equal number of electrons and protons in the redox reaction [45].

7.3.7 Sample analysis

In order to investigate the applicability of the proposed method, the pre-treated PGE was employed for the determination of CC and HQ in local tap water sample. The standard addition method was applied for the analysis and obtained results were tabulated in Table 3, which show the recoveries in the range of 97.8 to 99.7%. Therefore, the obtained result suggests the feasibility of the modified electrode towards the determination of CC and HQ in tap water sample with satisfactory results.

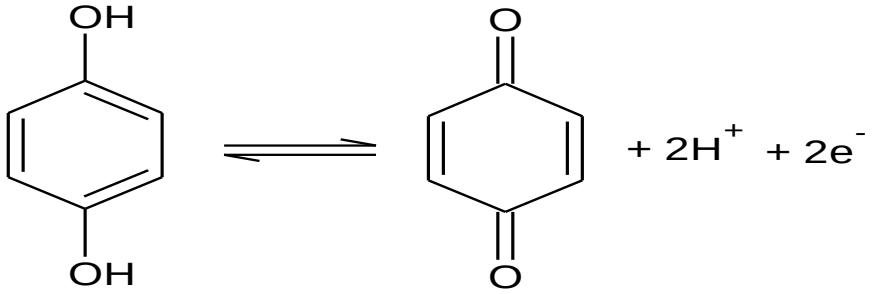
7.4 Conclusion

Electrochemical pre-treatment method was employed for the modification of PGE using cyclic voltammetric method. The developed sensor (PPGE) showed excellent sensitivity toward the determination of phenolic compounds (CC and HQ). The modified electrode showed diffusion-controlled type of electrode process and exhibited LOD and LOQ in low range for CC and HQ. Moreover, the modified electrode was utilized for the determination of CC and HQ in real samples. These results demonstrate the excellent sensor behaviour of the pre-treated PGE towards the electrochemical determination of phenolic compounds and can be applied for various analytes.



Catechol 1,2 Benzoquinone

Scheme 1: Oxidation mechanism of CC



Hydroquinone 1,4 Benzoquinone

Scheme 2: Oxidation mechanism of HQ

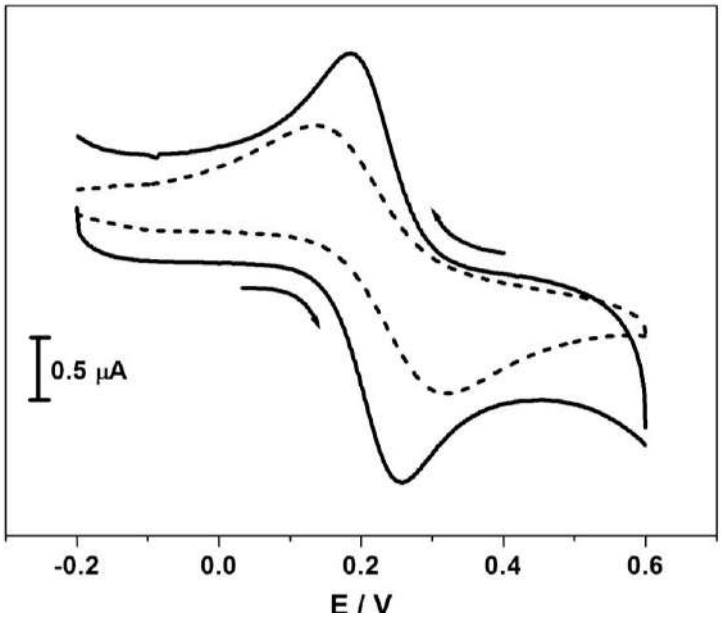


Fig.1: CVs recorded for 1mM $K_4[Fe(CN)_6]$ at PGE (dashed line) and PPGE (solid line) in presence of 0.2M PBS (pH 7.4) with the scan rate of $50mVs^{-1}$.

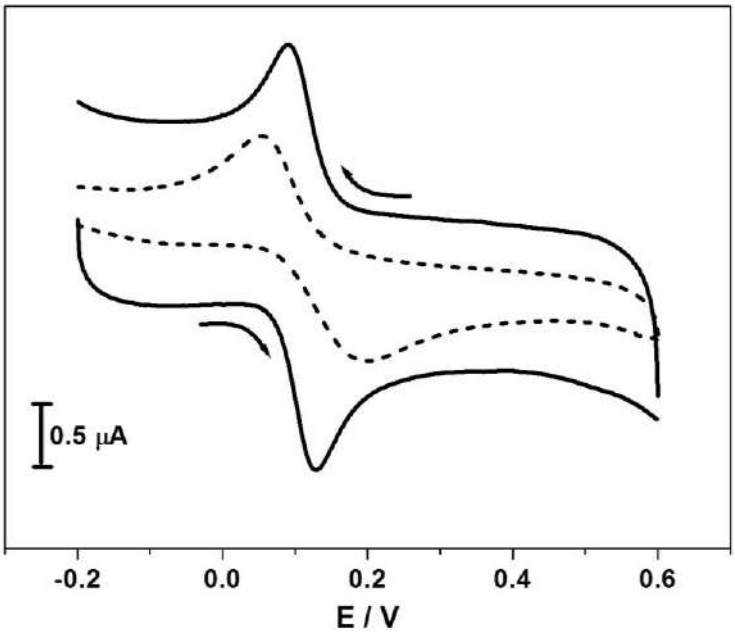


Fig.2: CVs recorded for $0.1x10^{-4}$M CC at PGE (dashed line) and PPGE (solid line) in 0.2M PBS (pH 7.4) with the scan rate of $50mVs^{-1}$.

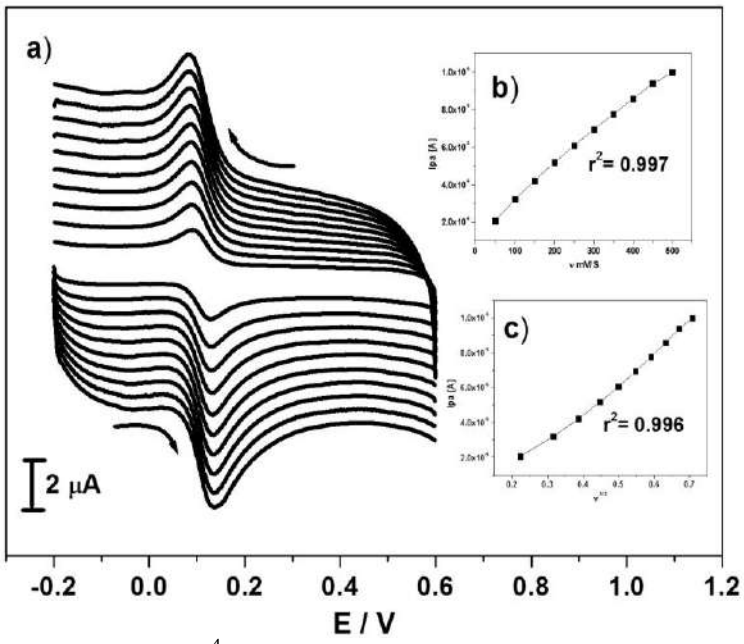


Fig.3: (a) CVs recorded for $0.1x10^{-4}$M CC with different scan rates (50-500 mVs^{-1}) at PPGE in 0.2M PBS (pH 7.4), (b) Plot of anodic peak current (Ipa) versus scan rate (ν), (c) Plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$).

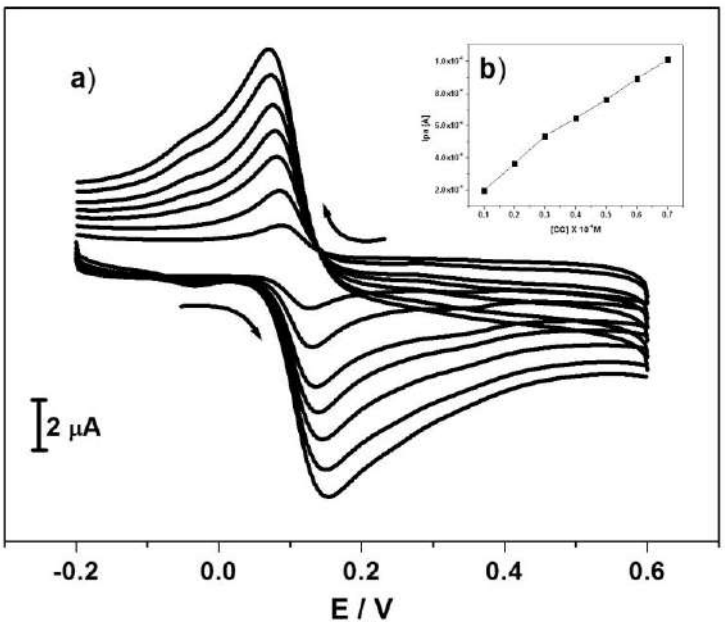


Fig.4: (a) CVs recorded for CC at different concentrations (0.1 – 0.7 x 10^{-4}M) at PPGE in 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}, (b) plot of anodic peak current (Ipa) versus concentration of CC.

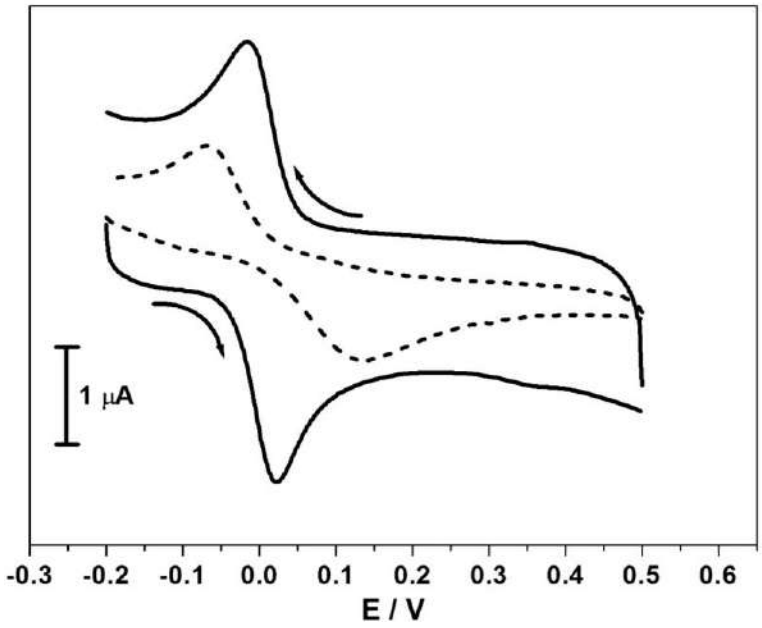


Fig.5: CVs recorded for $0.1x10^{-4}$M HQ at PGE (dashed line) and PPGE (solid line) in 0.2M PBS (pH 7.4) with the scan rate of $50mVs^{-1}$.

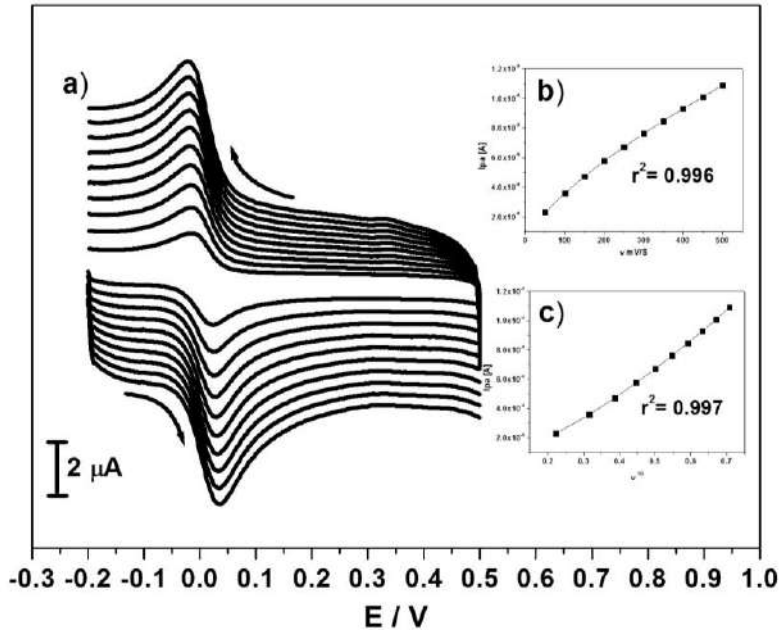


Fig.6: (a) CVs recorded for $0.1x10^{-4}$M HQ with different scan rates (50-500 mVs^{-1}) at PPGE in presence of 0.2M PBS (pH 7.4), (b) Plot of anodic peak current (Ipa) versus scan rate (ν), (c) Plot of anodic peak current (Ipa) versus square root of scan rate ($\nu^{1/2}$).

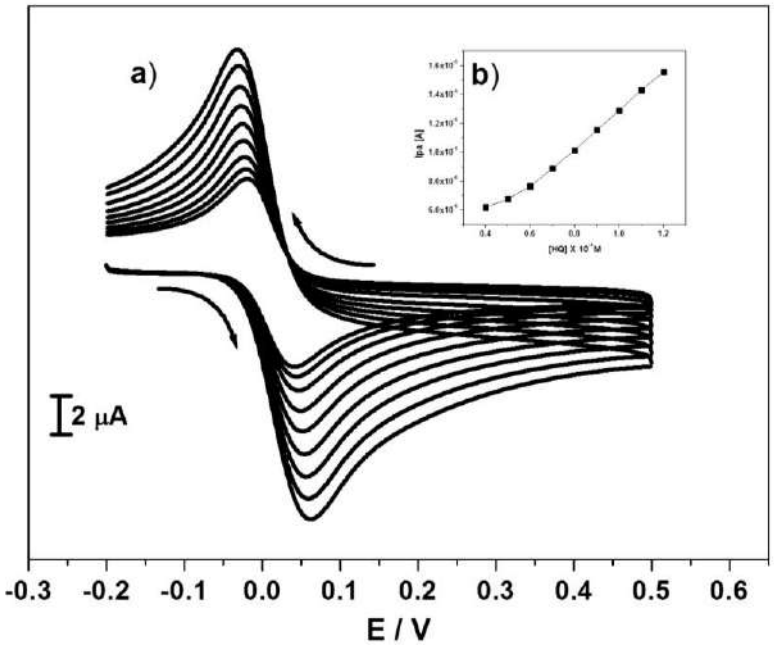


Fig.7: (a) CVs recorded for HQ at different concentrations (0.4 – 1.2 x 10^{-4}M) at PPGE in 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}, (b) plot of anodic peak current (Ipa) versus concentration of HQ.

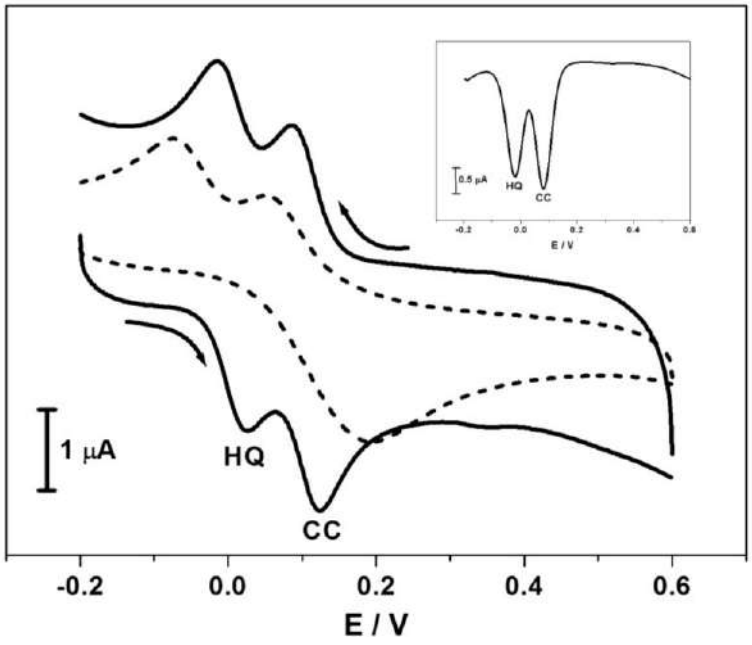


Fig.8: CVs recorded for the equimolar mixture (0.1x10^{-4}M) of CC and HQ at PGE (dashed line) and PPGE (solid line) in presence of 0.2M PBS (pH 7.4) with the scan rate of 50mVs^{-1}. Inset figure shows the DPVs of CC (0.1x10^{-4}M) and HQ (0.1x10^{-4}M) at PPGE.

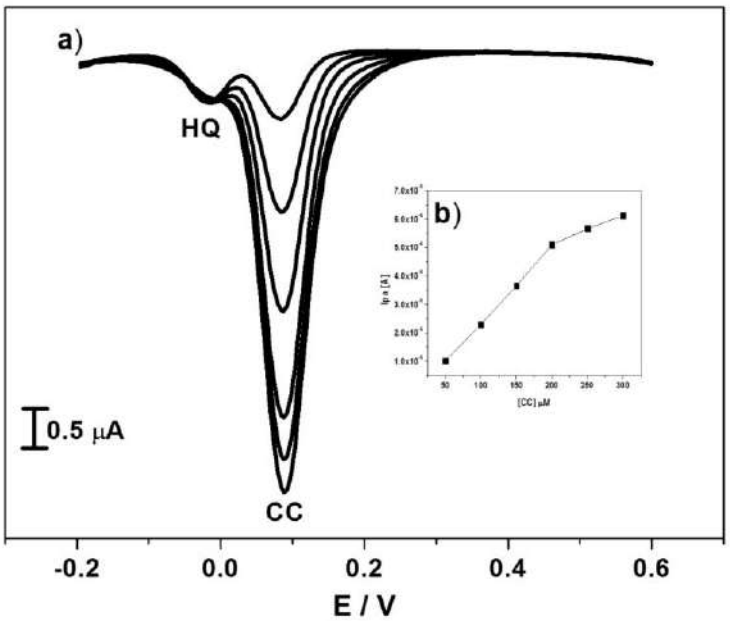


Fig.9: (a) DPVs recorded for CC at different concentrations (50 - 300 μM) keeping 50 μM HQ constant in presence of 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}, (b) plot of anodic peak current (Ipa) versus concentration of CC.

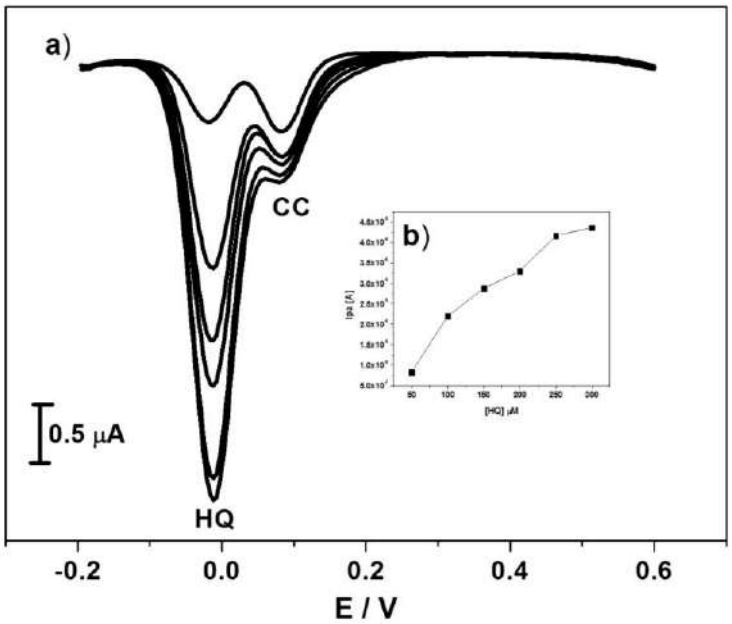


Fig.10: (a) DPVs recorded for HQ at different concentrations (50 - 300 μM) keeping 50 μM CC constant in presence of 0.2M PBS (pH 7.4) with the scan rate of 50 mVs^{-1}, (b) plot of anodic peak current (Ipa) versus concentration of HQ.

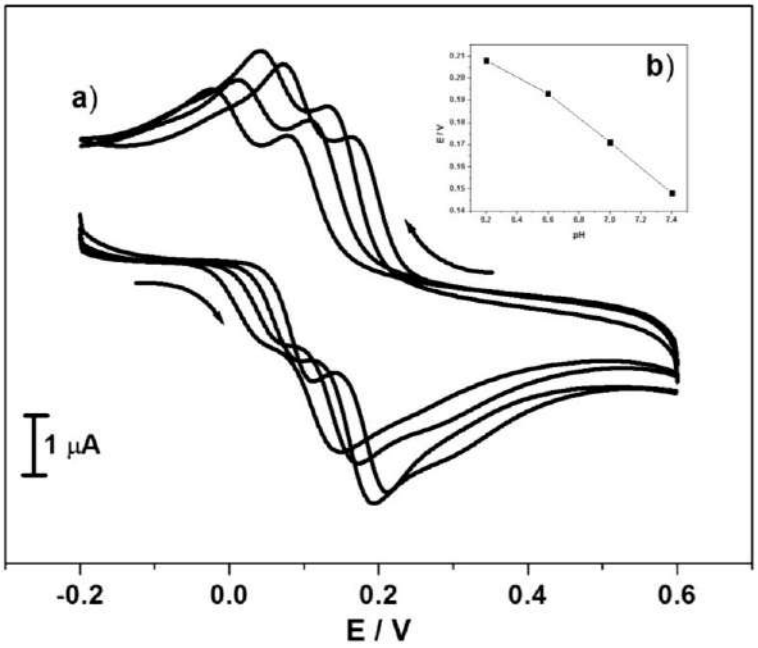


Fig.11: (a) CVs recorded for the equimolar mixture ($0.1x10^{-4}$M) of CC and HQ at different pH (6.2, 6.6, 7.0 and 7.4) in presence of 0.2M PBS with the scan rate of $50mVs^{-1}$, (b) plot of anodic peak potential (Epa) of CC versus pH.

Table 1: Determination of heterogeneous rate constant (k^o) for CC and HQ at pre-treated PGE.

ν (mVs^{-1})	**ΔEp (V)**		**k^o (s^{-1})**	
	CC	**HQ**	**CC**	**HQ**
50	0.036	0.007	1.58	1.58
100	0.04	0.007	3.16	3.23
150	0.04	0.01	4.78	4.78
200	0.043	0.01	6.45	6.45
250	0.043	0.011	7.94	7.79
300	0.049	0.009	9.54	9.54
350	0.051	0.01	11.2	11.2
400	0.049	0.012	12.8	12.8
450	0.05	0.014	14.4	14.4
500	0.053	0.013	15.8	15.8

Table 2: Comparison of the pre-treated PGE with other modified electrodes towards the determination of CC and HQ.

Working electrode	Linear range (µM)		Limit of detection(µM)		Reference
	CC	HQ	CC	HQ	
RGO-MWCNTs/GC	5.5-5400	8-391	1.8	2.6	[46]
MWCNT-PMG/GCE	30-1190	10-480	5.8	1.6	[47]
LDHf/GCE	3-1500	12-800	1.2	9	[48]
Silsesquioxane/CPE	10-300	10-450	10	10	[49]
Pre-treated PGE	10-70	40-102	5.9	8.5	This work

Table 3: Recoveries of CC and HQ in local tap water sample at pre-treated PGE.

Sample	Compound	Added (µM)	Found (µM)	Recovery (%)
Tap water	CC	50	49.6	99.2
		60	58.8	98
		70	68.4	97.8
	HQ	50	49.8	99.7
		60	59.4	99.1
		70	68.8	98.4

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