KUVEMPU UNIVERSITY

CYCLIC VOLTAMMETRIC INVESTIGATIONS OF CERTAIN ORGANIC COMPOUNDS OF BIOLOGICAL IMPORTANCE AT MODIFIED DIFFERENT ELECTRODES

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

KUVEMPU UNIVERSITY

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Declaration

I hereby declare that, the Ph.D., thesis entitled **"Cyclic Voltammetric Investigations of Certain Organic Compounds of Biological Importance at Modified Different Electrodes"** submitted to the Kuvempu University for the award of degree of **Doctor of Philosophy in Industrial Chemistry,** is the result of the research work carried out by me in the Department of Industrial Chemistry, Kuvempu University, Shankaraghatta, under the guidance of **Dr. B.E. Kumara Swamy,** Assistant Professor, Department of Industrial Chemistry, Kuvempu University, Shankaraghatta - 577451.

I further declare that, the results contained in this thesis have not been previously submitted for any other degree or fellowship of this university or any other higher education institution.

Date : 30/04/2019
Place : Shankaraghatta

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Certificate

This is certify that the thesis entitled **"Cyclic Voltammetric Investigations of Certain Organic Compounds of Biological Importance at Modified Different Electrodes"** submitted here for the award of **Doctor of Philosophy in Industrial Chemistry** to the Faculty of Science, Kuvempu University, Shankaraghatta 577451, Shivamogga, Karnataka, India, is the result of original work completed by **Mr. Sathisha A.,** under my supervision and guidance. To the best of my knowledge and belief, the work embodied in this thesis has not formed early the basis for the award of any degree, associateship/fellowship etc., of any other higher education institution.

Date: 30/4/18
Place: Shankaraghatta

Dr. B.E. KUMARA SWAMY

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……any omission in this short note of acknowledgement does not indicate lack of gratitude.

Sathisha Arehalli

List of Abbreviations

%	-	Per cent
AA	-	Ascorbic Acid
AE	-	Auxiliary Electrode
ASV	-	Anodic Stripping Voltammetry
BARC	-	Bhabha Atomic Research Centre
C0*	-	Concentration
CC	-	Catechol
CE	-	Counter Electrode
CMCPEs	-	Chemically Modified Carbon Paste Electrode
CMEs	-	Chemically Modified Electrodes
CNPs	-	Carbon Nanoparticles
CNS	-	Central Nervous System
CPE	-	Carbon Paste Electrode
CTAB	-	Cetyltrimethyl Ammonium Bromide
CTFE	-	Chlorotrifluoroethylene
CV	-	Cyclic Voltammetry
D0	-	Diffusion Coefficient
DA	-	Dopamine
DCE	-	Dropping Carbon Electrode
DF	-	Diclofenac
DME	-	Dropping Mercury Electrode
DMF	-	Dimethyl Formamide
DMSO	-	Dimethyl Sulphoxide
DPV	-	Differential Pulse Voltammetry
E1/2	-	Half Wave Potential
EBT	-	Eriochrome Black-T
EDS	-	Energy Dispersive X-ray Spectrum
Ef,	-	Final Potential
Ei	-	Initial Applied Potential

Eo	-	Formal Potential
Ep	-	Peak Potential
Epa	-	Anodic Peak Potential
Epc	-	Cathodic Peak Potential
et al.	-	And others (co-authors)
Ev	-	Vertex Potential
FA	-	Folic Acid
FSCV	-	Fast Scan Cyclic Voltammetry
G	-	Gibb's Free Energy
GC	-	Glassy Carbon
GCE	-	Glassy Carbon Electrode
GM	-	Glimepiride
GPE	-	Graphite Pencil Electrode
5-HIAA	-	5- hydroxyl indole acetic acid
5-HT	-	5- hydroxytryptamine (Serotonin)
HOPG	-	Highly Oriented Pyrolytic Graphite
HQ	-	Hydroquinone
Ip	-	Peak Current
ipa	-	Anodic Peak Current
ipc	-	Cathodic Peak Current
IPE	-	Ideally Polarisable Electrode
K0	-	Heterogeneous Rate Constant
KCl	-	Potassium Chloride
KOH	-	Potassium Hydroxide
LOD	-	Limit of Detection
LOQ	-	Limit of Quantification
LSV	-	Linear Sweep Voltammetry
MCPE	-	Modified Carbon Paste Electrode
μM	-	Micromolar
mM	-	Millimolar
mV	-	Millivolt

mVs^{-1}	-	Millivolt Per Second
NaOH	-	Sodium Hydroxide
NPP	-	Normal Pulse Polarographic
NTs	-	Neurotransmitters
O	-	Oxidised Species
PA	-	Paracetamol
PBS	-	Phosphate Buffer Solution
PC	-	Personal Computer
PGB	-	Pregabalin
PMCE	-	Polymer Modified Carbon Paste Electrode
PR	-	Patton-Reeder's
Pt	-	Platinum
R	-	Reduced Species
RE	-	Reference Electrode
S	-	Entropy
SAOS	-	Sodium Alpha Olefin Sulfonate
SCE	-	Saturated Calomel Electrode
SDS	-	Sodium Dodecyl Sulphate
SHE	-	Standard Hydrogen Electrode
SIDS	-	Sudden Infant Death Syndrome
SWV	-	Square Wave Voltammetry
TAA	-	Tetra-Alkyl Ammonium
TBA	-	Tetra-n-Butyl Ammonium
TEA	-	Tetra-Ethyl Ammonium
TOR	-	Torsemide
TX-100	-	Triton X-100
UA	-	Uric Acid
WE	-	Working Electrode
$\nu^{1/2}$	-	Square Root of Scan Rate

List of Papers Published :

- **A. Sathisha** and B.E. Kumara Swamy, Simultaneous Determination of Serotonin and Dopamine at Poly (Patton and Reeder's) Modified Graphite Pencil Electrode: A Cyclic Voltammetric Study, ***Journal of Chemical Engineering and Research, (2014) 2, 137-143.***

- **A. Sathisha** and B.E. Kumara Swamy, Electrosensitive Determination of Paracetamol Using a Poly (glycine) Film Coated Graphite Pencil Electrode: A Cyclic Voltammetric Study, ***J. Anal. Bioanal. Electrochem., (2015) 7, 12-21.***

- **A. Sathisha** and B.E. Kumara Swamy, Simultaneous Determination of Dopamine, Serotonin and Folic Acid at Torasemide Modified Carbon Paste Electrode: A Cyclic Voltammetric Study, ***J. Anal. Bioanal. Electrochem., Vol. 8, No. 5, (2016) 589-603.***

- **A. Sathisha** and B.E. Kumara Swamy, Voltammetric Determination of Dopamine in Presence of Uric Acid at Glimepiride Modified Carbon Paste Electrode, ***Revised and Submitted for Journal of Anal. Bioanal. Tech.***

- **A. Sathisha** and B. E. Kumara Swamy, Voltammetric Determination of Serotonin in Presence of Dopamine at Poly (Eriochrome Black-T) Film-Coated Graphite Pencil Electrode, ***Revised and Submitted for Journal of Chinese Chemical Letters.***

- **A. Sathisha** and B.E. Kumara Swamy, Simultaneous electrochemical determination of Paracetamol, Dopamine and Diclofenac at Diacerein Modified Carbon Paste Electrode: A Voltammetric Study, ***Revised and Submitted for J. Anal. Bioanal. Electrochem.***

- **A. Sathisha** and B.E. Kumara Swamy, Electrochemical Selective Determination of Dopamine in Presence of Paracetamol in Pregabalin Mobilization Glassy Carbon Electrode: A Voltammetric Study, ***Revised and Submitted for J. Anal. Bioanal. Electrochem.***

- **A. Sathisha** and B. E. Kumara Swamy, Simultaneous Determination of Catechol and Hydroquinone at Poly (Sunset yellow) Modified Glassy Carbon Electrode: A Voltammetric Study, ***Revised and Submitted for Journal of Electroanalytical Chemistry.***

- T.V. Sathisha, B.E. Kumara Swamy, K.R. Mahanthesha, **A. Sathisha**, Tushar S. Avnkar and B. Eswarappa, Electrochemical Investigation of Ni(II) ions in Nickel Chloride and Nickel Sulfate at Carbon Paste Electrode: A Cyclic Voltammetric Study, ***J. Anal. Bioanal. Electrochem. (2013) 6, 729-739.***

List of Research Papers Presented at National/International Conferences/ Seminars :

- **A**. **Sathisha** and B.E. Kumara Swamy, Electrosensitive Determination of Paracetamol Using a Poly (glycine) Film Coated Graphite Pencil Electrode: A Cyclic Voltammetric Study, Two days International Conference on **"Recent Advances in Engineering Sciences (ICRAES-2014)"** held on September 4-5, 2014 at M.S. Ramaiah Institute of Technology, Bengaluru.

- **A**. **Sathisha** and B.E. Kumara Swamy, Voltammetric Determination of Dopamine in Presence of Uric Acid at Glimepiride Modified Carbon Paste Electrode, National Conference on **"Current trends in scientific research for engineering applications"** held at the Department of Science, ST Joseph Engineering College, Mangalore on 17th and 18th July, 2014.

- **A**. **Sathisha** and B.E. Kumara Swamy, Simultaneous Determination of Serotonin and Dopamine at Poly (Patton and Reeder's) Modified Graphite Pencil Electrode: A Cyclic Voltammetric Study, One day National Conference on **"Advanced Instrumentation Methods of Chemical Analysis (AIMCA)"** held on 14th February 2015 at A.V. Kamalamma College for Women, Davanagere.

- **A**. **Sathisha** and B.E. Kumara Swamy, Simultaneous Determination of Dopamine, Serotonin and Folic Acid at Torasemide Modified Carbon Paste Electrode: A Cyclic Voltammetric Study, One Day National Level Conference on **"Recent Trends in Novel Carbon Materials"** held on 22nd September 2015 at Field Marshal K.M. Cariappa College, Madikeri.

- **A. Sathisha,** B.E. Kumara Swamy, Electrocatalytic oxidation and determination of hydroquinone in presence of paracetamol at Poly (Sunset yellow) Modified Glassy Carbon Electrode: A Voltammetric Study, National Conference on **"Recent Advanced of Chemical Biology and Material Science for Industry and Society (RACBMS)"** held at the Department of Industrial Chemistry, Kuvempu University, Shivamogga, India on 09th and 10th February, 2018.

- **A. Sathisha,** B.E. Kumara Swamy, Electrochemical Selective Determination of Dopamine in Presence of Paracetamol in Pregabalin Mobilization Glassy Carbon Electrode: A Voltammetric Study, National Conference on **"Recent Trends in Chemical Biology and Material Sciences (RTCBMS)"** held at the Department of Chemistry, Kuvempu University P.G. Center Kadur, on 26th and 27th February, 2018.

International/National/State Level Seminars/Workshops Attended :

- National Conference on **"Impact of Chemistry and Molecular Nanotechnology for Industry and Society"** held at the Department of Industrial Chemistry, Kuvempu University, Shivamogga, India on 16th and 17th January, 2009.

- State Level Seminar on **"Science, Technology and Environment"** organized by the Department of Chemistry in collaboration with Department of Science, Technology and Environment, Goa in St. Xavier's College of Arts, Science and Commerce, Mapusa, Goa on 24th February, 2014.

- State Level Seminar on **"Recent Trends in Chemistry"** organized by Women Empowerment Cell at Sahyadri Science College, Shivamogga on 24th March, 2015.

- One Day Workshop on **"Prerana and Sadhaneyedege…"** organized by the Department of Chemistry at Sahyadri Science College, Shivamogga on 17th October, 2014.

- State Level Seminar on **"Advanced Analytical Techniques"** organized by the Department of Industrial Chemistry, Sahyadri Science College, Shivamogga on 1st and 2nd April, 2016.

- International Conference on **"Important of Herbal Medicine in the Era of Globalization"** organized by the Department of Chemistry, Sahyadri Science College, Shivamogga on 21st and 23rd December, 2016.

Summary of the Thesis

The focus of the thesis is to use of chemically modified different electrodes for the investigation of organic compounds to get excellent reproducible results by voltammetric techniques. The organic compounds were chosen for electrochemical investigation were dopamine, ascorbic acid, uric acid, serotonin, catechol and hydroquinone. In the real sample these compounds were interfere each other during the investigation by overlapping their voltammetric responses.

The overall work involves the advantages like, high conductivity, wide potential window for analysis, chemically inert, relatively inexpensive, easy modification, easy preparation of paste with organic binder and easily renewal of electrode surface, carbon paste electrode, graphite pencil electrode and glassy carbon electrode were chosen in the investigation of certain organic compounds by using voltammetric technique. The modified different electrodes shows significant improvements of the electrochemical sensor exhibited excellent performances for the determination some neurotransmitters and biological drugs in physiological pH. The proposed method was simple preparation, fast response, high sensitivity and good stability.

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

Chapter-1

Introduction, Review of Voltammetry and Theoretical Considerations

This chapter covers the introduction, voltammetry and voltammetric techniques. Basic and fundamental principles, theoretical aspects and application of voltammetry, solvents, supporting electrolytes and electrode interaction can be seen in this section. A brief review of cyclic voltammetric investigations of certain organic compounds has been presented. Objective and scope of the present thesis were included in this chapter.

Chapter-2

Experimental

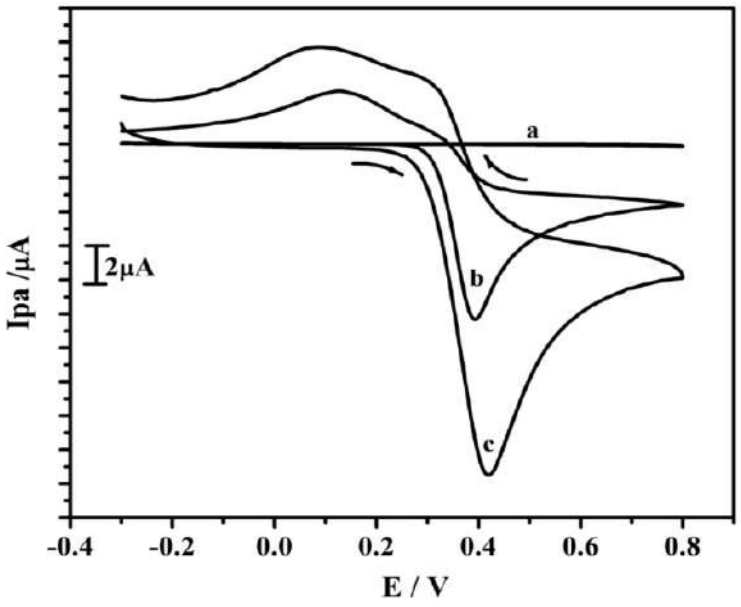
This chapter describes the basic experimental set up which is very much essential for voltammetric technique. The basic equipments like, potentiostat, recording device and electrochemical cell which is composed of three electrodes. The electrode systems with special emphasis on carbon paste electrode,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

This Chapter is divided into two parts. They are,

Chapter-3

Part-A : Electrosensitive Determination of Paracetamol Using a Poly (glycine) Film Coated Graphite Pencil Electrode: A Cyclic Voltammetric Study

The poly (glycine) film was deposited on the surface of graphite pencil electrode (GPE) by cyclic voltammetric technique. The modified film coated graphite pencil electrode exhibits excellent electrocatalytic activity towards the detection of paracetamol at pH 7.0. The scan rate effect was found to be diffusion controlled electrode process. The concentration effect of paracetamol was linear with current. This developed method can also be applied for some neurotransmitters.



Cyclic voltammograms of 2×10^{-4} M paracetamol obtained at the bare GPE (curve b), Poly (glycine) modified GPE (curve c) and in the absence of paracetamol at bare GPE (curve a) in 0.2 M PBS (pH.7.0) at scan rate 50 mVs^{-1}.

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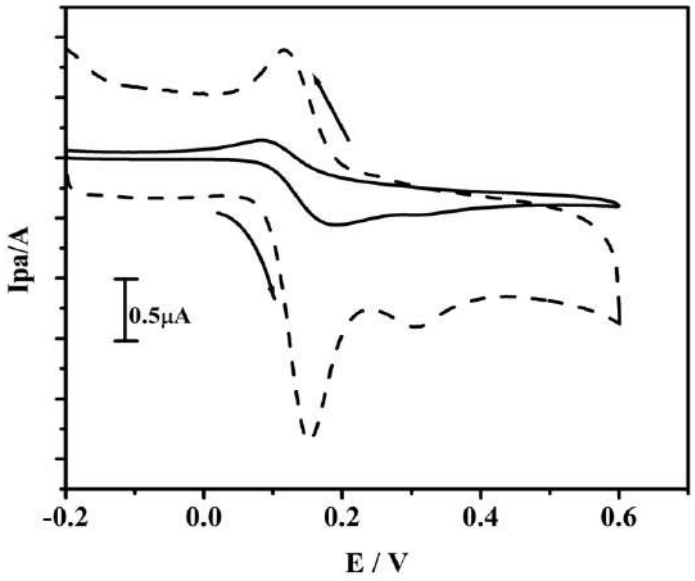
Part-B : Simultaneous Determination of Serotonin and Dopamine at Poly (Patton and Reeder's) Modified Graphite Pencil Electrode: A Cyclic Voltammetric Study

Electrochemical oxidation of serotonin has been studied at Graphite pencil electrode in 0.2M Phosphate buffer using cyclic voltammetric (CV) technique. The poly (Patton and Reeder's) film was synthesized on the surface of graphite pencil electrode in alkaline solution by cyclic voltammetric (CV) technique after pretreatment by H_2SO_4 media. The poly (PR) film coated on Graphite pencil electrode (GPE) exhibited excellent electrocatalytic activity towards the Detection of Serotonin [5-HT] at 7.0 pH. This polymer film coated electrode was very good at simultaneous study of Serotonin in presence of high concentrated DA.



Simultaneous determination of 1×10^{-4} M 5-HT and 1×10^{-3} DA at bare GPE (solid line) and at poly (PR) film coated GPE (dashed line).

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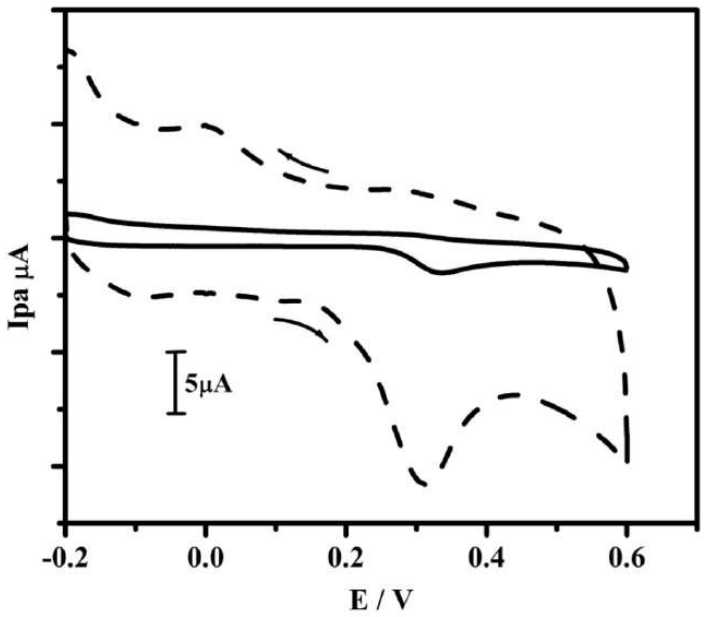
Chapter-4

Voltammetric Determination of Serotonin in Presence of Dopamine at Poly (Eriochrome Black-T) Film-Coated Graphite Pencil Electrode

A Graphite Pencil Electrode (GPE) was modified by electropolymerisation of Eriochrome Black-T (EBT) in alkaline solution by cyclic voltammetric technique (CV) and the electrochemical properties of the polymer film was studied. The poly (EBT) modified electrode was developed for the electrochemical determination of serotonin and it shows an excellent electrocatalytic activity towards the oxidation of serotonin in 0.2M Phosphate buffer solution (pH 7.0). The scan rate and the concentration effects at the modified electrode were found to be a diffusion-controlled electrode processes. The simultaneous study shows excellent result with good potential difference between serotonin and dopamine by using both cyclic voltammetric and differential pulse voltammetric (DPV) techniques.



Cyclic voltammogram of BGPE (curve B) and poly(EBT) MGPE (curve C) and absence of serotonin at bare GPE (insert curve A) in the presence of 10µM serotonin and 0.2M Phosphate buffer, in pH 7.0, scan rate 100mVs^{-1}.

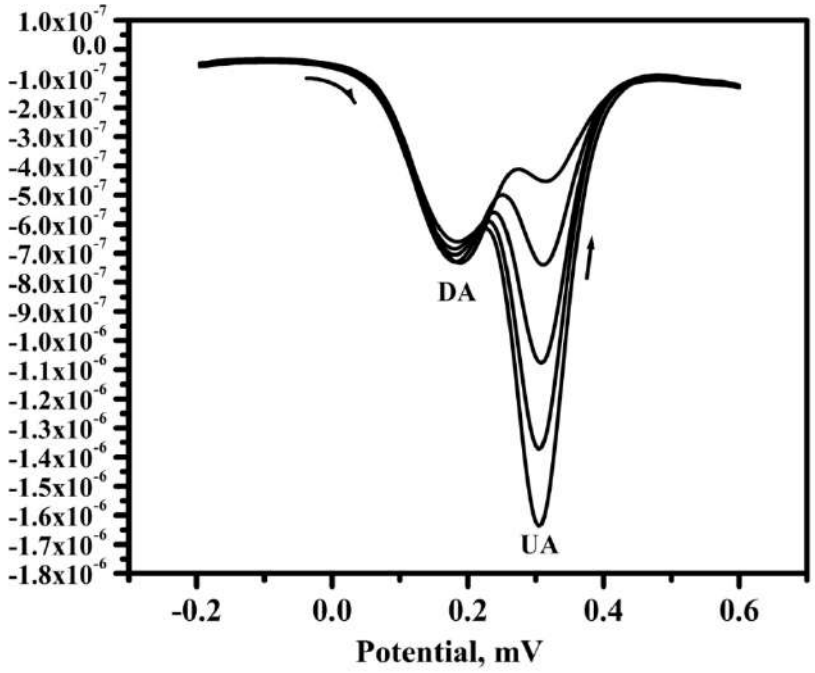
Chinese Chemical Letters (Revised and submitted)

Chapter-5

This Chapter is divided into two parts. They are,

Part-A : Voltammetric Determination of Dopamine in Presence of Uric Acid at Glimepiride Modified Carbon Paste Electrode

The carbon paste electrode was modified with glimepiride [GM] and it was used for simultaneous determination of dopamine (DA), uric acid (UA) in 0.2 M phosphate buffer of pH 7.0. Based on its strong electrocatalytic action towards the oxidation of dopamine, and uric acid. The modified electrode shows is increased in anodic peak currents. The glimepiride modified carbon paste electrode used for the detection of dopamine was stable, reproducible and low detection limit for DA. The effect of scan rate, pH, surfactant and concentration was studied. The effect of interferences was studied by differential pulse voltammetric technique. The modified electrode was used for the analysis of DA and UA in real samples with satisfactory results.



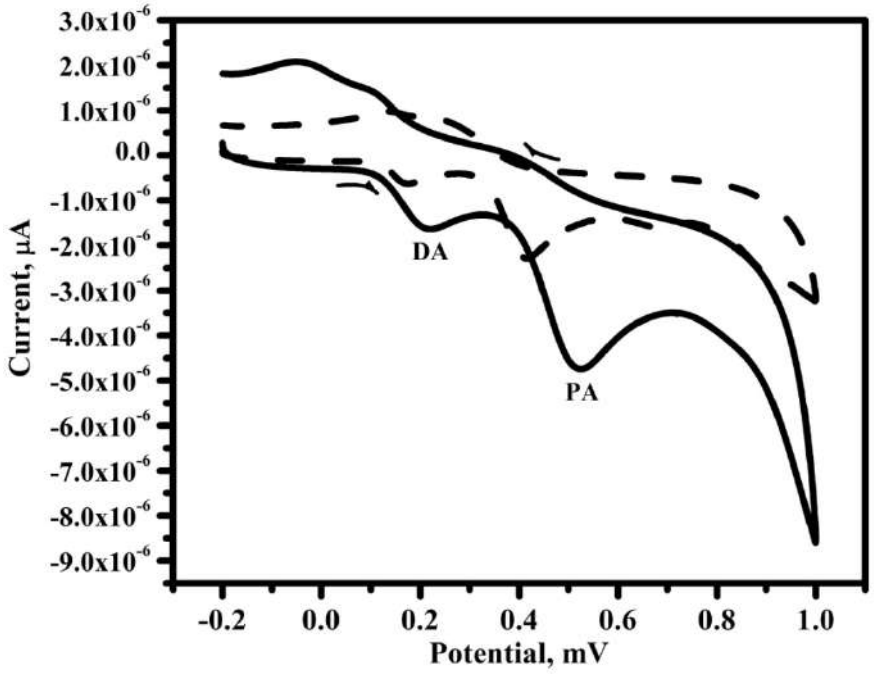
Differential pulse voltammograms of UA (0.1mM, 0.2mM, 0.3mM, 0.4mM, 0.5mM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 20µM DA at GMMCPE with the scan rate of 100 mV/s.

Journal of Analytical and Bioanalytical Techniques (In Press)

Part-B : Simultaneous electrochemical determination of Paracetamol, Dopamine and Diclofenac at Diacerein modified carbon paste electrode: A voltammetric study

Diacerein was used for the modification of carbon paste electrode (CPE) to determine the electrochemical behavior of paracetamol (PA) in 0.2M phosphate buffer solution (PBS) at pH 7. The effect of concentration, scan rate, pH and surfactant was studied for electrochemical studies of paracetamol. The Diacerein modified carbon paste electrode showed an excellent electrocatalytic activity for the selective determination of PA in the presence of DA and DF by using CV and differential pulse voltammetric techniques (DPV) respectively. The catalytic peak current obtained was linearly related to PA concentrations in the ranges of to 0.1 mM to 0.6 mM with correlation co-efficient of 0.9981 which reveals the adsorption controlled process. The detection limit of

paracetamol was found to be 3.8 $\times 10^{-6}$ M. The present technique provides a novel method for the simultaneous determination of PA, DA and DF in their mixture sample.



Cyclic voltammograms recorded containing mixture of PA and DA at bare CPE (dashed line) and Diacerein MCPE (solid line) in0.2M PBS pH7.0 at scan rate 100 mV/s.

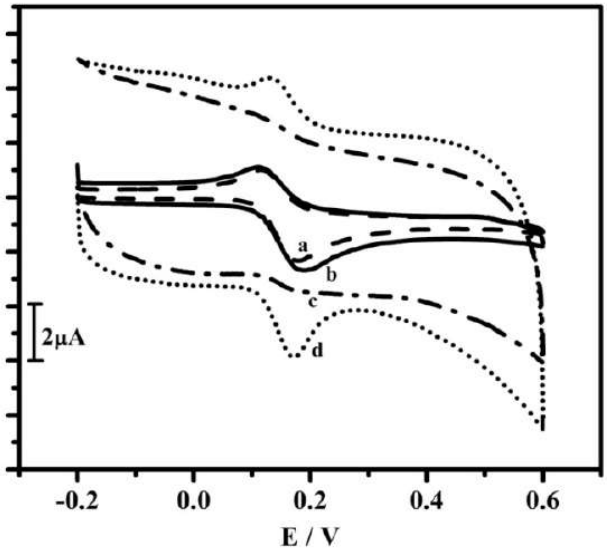
Analytical and Bioanalytical Electrochemisty (Revised and submitted)

Chapter-6

Simultaneous Determination of Dopamine, Serotonin and Folic acid at Torasemide Modified Carbon Paste Electrode: A Cyclic Voltammetric Study

A carbon paste electrode modified by torasemide was used for determination of dopamine (DA). The modified electrode exhibited strong promoting effect and stability towards the electrochemical oxidation of dopamine at pH 7.0.in phosphate buffer solution (PBS). The parameters which influence the electrode response like paste composition; effects of scan rate, concentration, pH, surfactants and interferences have been studied. The linear range of DA 0.9990 and the detection limit for DA was found to be 2.4×10^{-6} M. Anionic surfactant Sodium Dodecyl Sulphate (SDS) showed very good

electrocatalytic effect on the modified carbon paste electrode. The preparation of the modified electrode was easy and renewed by simple polishing gives very good reproducibility, high stability in its voltammetric response and low detection limit for DA.



Cyclic voltammogram of 0.1mM DA at BCPE (dashed line), TORMCPE (solid line), 14μM CTAB (solid dashed line) and 14μM SDS on the modified CPE (dotted line) in 0.2 M phosphate buffer solution pH 7.0, scan rate 100 mV/s.

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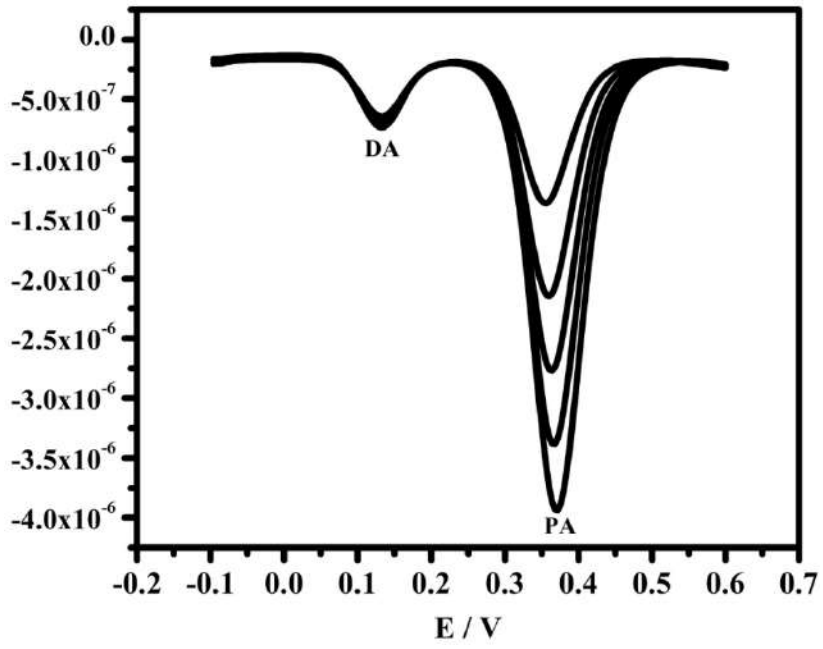
Chapter-7

This Chapter is divided into two parts. They are,

Part-A : Electrochemical Selective Determination of Dopamine in Presence of Paracetamol in Mobilization Pregabalin Glassy Carbon Electrode: A Voltammetric study

Electrochemical sensitive and selective determination of dopamine using a pregabalin mobilization glassy carbon electrode was developed by voltammetric technique. pregabalin mobilization glassy carbon electrode showed excellent

electrocatalytic activity towards the oxidation of dopamine in phosphate buffer solution (pH 7) by cyclic voltammetric technique (CV) and differential pulse voltammetry (DPV). The effect of concentration, scan rate, and pH was studied for dopamine (DA). The detection limit (LOD) and quantification limit (LOQ) were calculated. The interference studies showed that the modified electrode exhibited excellent selectivity in the presence of dopamine and paracetamol (PA). The proposed method was applied for the detection of dopamine in biological samples.



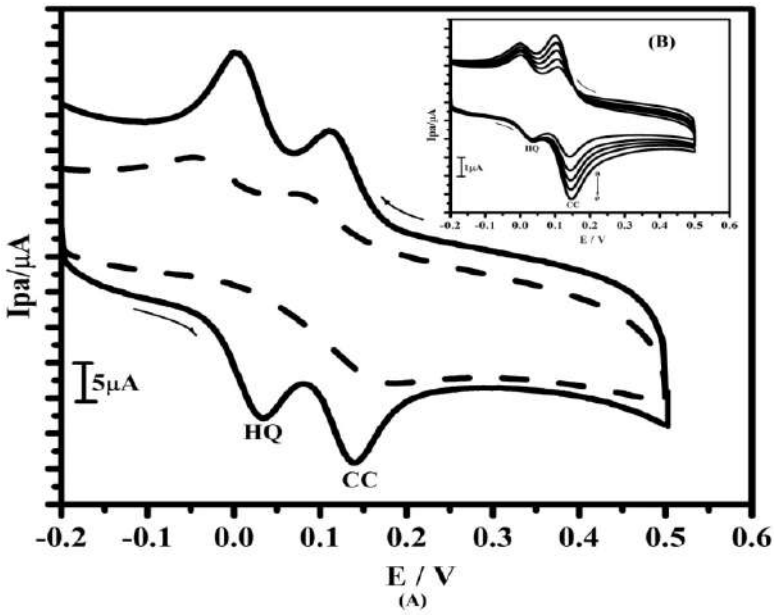
Differential pulse voltammograms of PA (0.1mM, 0.2mM, 0.3mM, 0.4mM, 0.5mM, 0.6mM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.1mM DA at PGBMGCE with the scan rate of 100 mV/s.

Analytical and Bioanalytical Electrochemistry (Revised and submitted)

Part-B : Simultaneous Determination of Catechol and Hydroquinone at Poly(Sunset yellow) Modified Glassy Carbon Electrode: A Voltammetric Study

An electrochemical sensor for the sensitive determination of catechol has been developed by poly (sunset yellow) modified glassy carbon electrode. The electrochemical

oxidation of catechol was studied by using cyclic voltammetric and differential pulse voltammetric techniques. The experimental results show that the poly (sunset yellow) modified glassy carbon electrode shows high electrochemical process towards the oxidation of catechol and hydroquinone. The lower detection limits were found to be 1.45×10^{-6} M and 2.6×10^{-7} M respectively. The peak to peak separation of CC and HQ was more than enough to identify individually by CV technique. This work provides a simple and easy approach for the simultaneous analysis of CC and HQ.



(A) Cyclic voltammograms for simultaneous determination of 0.1mM CC, 0.1mM HQ at BGCE (dashed line) and poly(sunset yellow) MGCE (solid line) at scan rate of 0.1 Vs^{-1}. (B) Cyclic voltammograms of CC (a–e: 0.1mM, 0.2mM, 0.3 mM, 0.4 mM, 0.5mM) in 0.2 M PBS of pH 7.4 in the presence of 0.1mM HQ at poly(sunset yellow) MGCE with the scan rate of 0.1 Vs^{-1}.

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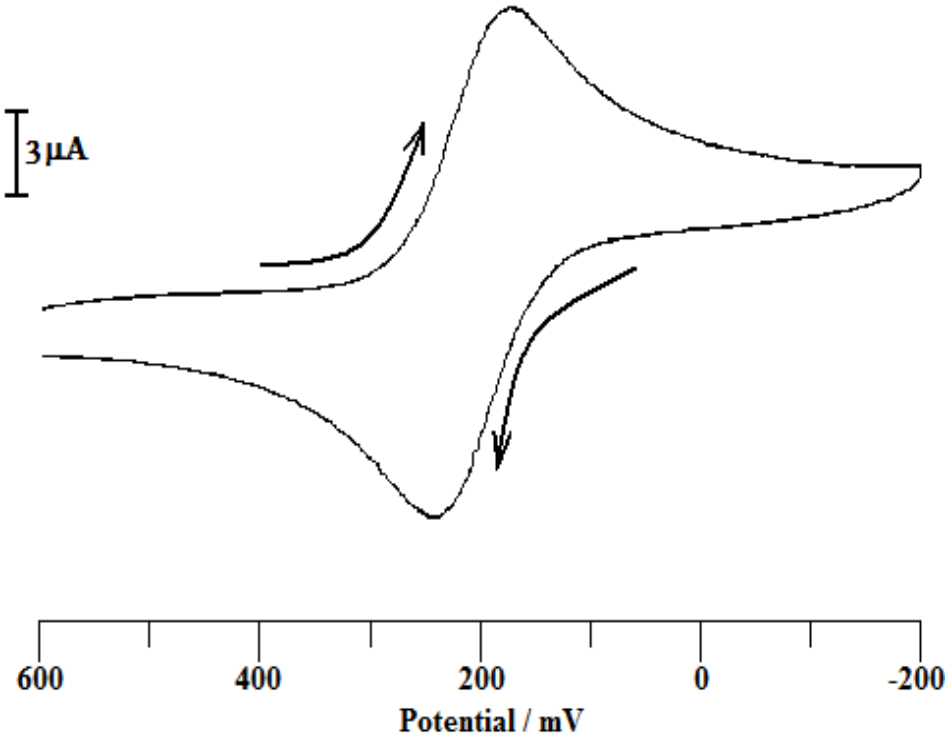
CHAPTER-1

Introduction, Review of Voltammetry and Theoretical Considerations



1.1. Introduction

Electrochemical methods comprise a collection of extremely useful measurement tools for neuroscience. A central feature of these methods is an electrode that provides a surface or interface and molecules in the interfacial region either in solution or immobilised at the electrode surface where some form of a charge-transfer process occurs. This charge-transfer process gives rise to potentials and/or currents that can be measured and related either by theory or by calibration to the concentration of substances in the solution that bathes the electrode. The kinetics of this heterogeneous process can be significantly affected by the microsurface and roughness of the electrode surface, the blocking of active sites on the electrode surface by adsorbed materials and the nature of the functional groups present on the electrode surface [1, 2]. Hence, it is very much essential to use chemically modified electrodes (CMEs) which comprise and approach to electrode design that finds the use in a spectrum in basic electrochemical investigations, including the relationship of heterogeneous electron transfer and chemical reactivity of electrode surface chemistry, electrostatic phenomena at electrode surface, electron as well as ionic transport phenomena in polymer, the design of electrochemical devices and systems for applications in the chemical sensing etc. These are some of the applications of the CMEs. But this thesis shows the application of CMEs as a biomolecules sensing tool.

The focus of the thesis is to use of CMEs for the investigation of bioactive organic molecule to get excellent reproducible results by voltammetric techniques. The bioactive organic molecules were chosen for electrochemical investigation were dopamine (DA), ascorbic acid (AA) and uric acid (UA). In the real sample these compounds were interfering each other during the investigation by overlapping their voltammetric responses [3, 4]. Moreover, the traditional electrodes very often suffer from fouling effect due to the accumulation of oxidized products on the electrode surface which results in rather poor selectivity and sensitivity [5, 6].

1.2. Voltammetry

The polaography in 1922 was invented by the Czechoslovakia chemist Jaroslav Heyrovsky and he received the Noble Prize in 1959. From his invention of polarography, the voltammetry was also emerged and now it become one of the branch in the field of electroanalysis. Electroanalysis can be defined as the application of electrochemistry to solve real-life analytical problems. It has another two branches namely, Conductometry and Potentiometry. The voltammetric technique become most important because of the measurement of current as a function of applied potential where in conductometry, one can measure only current and in potentiaometry, only potential. In voltammetry, three electrodes are used (working electrode (WE), reference electrode (RE) and counter/auxilary electrode (AE)) to monitor both current and potential. Hence, most analytical chemists routinely use voltammetric techniques for the quantitative determination of variety of dissolved organic and inorganic substances. Inorganic, physical and biological chemists widely use voltammetric techniques for a variety of purpose including fundamental studies of oxidation and reduction process in various media, kinetics of electron transfer process and thermodynamics properties of solvated species etc.

1.2.1. Voltammetric Techniques

The techniques used in the voltammetry were distinguished from the 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 areas as follows:

- Linear Sweep Voltammetry (LSV)
- Staircase Voltammetry (SV)
- Square Wave Voltammetry (SWV)
- Anodic Stripping Voltammetry (ASV)
- Cathodic Stripping Voltammetry (CSV)
- Normal Pulse Voltammetry (NPV)
- Differential Pulse Voltametry (DPV)
- Fast Scan Cyclic Voltammetry (FSCV)
- Cyclic Voltammetry (CV)

Linear Sweep Voltammetry is a method involves the measurement of current at a working electrode while the potential between the working electrode and a reference electrode is swept linearly in time. Oxidation or reduction of species is registered as a peak or trough in the current signal at the potential at which the species begins to be oxidized or reduced. It involves applying a linear potential sweep to the working electrode (the electrode under study) while monitoring simultaneously the current flowing in the circuit. A signal generator produces a voltage sweep from E_i to E_f and a potentiostat applies this potential wave to the electrode under study. The scan path can be positive or negative and in principle, the sweep rate can possess any constant value:

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

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

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 square wave is superimposed on the potential staircase sweep [7, 8]. 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 [9, 10]. Due to the lesser contribution of capacitive charging current the detection limits for SWV are on the order of nanomolar concentrations. This technique

was invented by Ramaley and Krause and developed extensively by Osteryoungs and their co-workers [11].

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, square wave, 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, square wave 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 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.

By contrast, in 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 square wave 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. It is relatively fast technique with signal scans typically recorded every 100 ms, however, the fast scan rate decrease the signal to noise ratio.

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 [12]. 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 [13-17]. The concentration of analyte is proportional to current, hence the concentration of an unknown solution can be determined by generating a calibration curve of current versus concentration.

1.3. Fundamentals of Cyclic Voltammetry

1.3.1. 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 vs potential plot is known as voltammogram.

1.3.2. 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.

1.3.3. 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 Figure 1.1. The potential axis is also a time that is related to scan rate [18]. The excitation signal causes the potential to scan negatively from +0.8 V to –0.2 V vs SCE, at which point the scan direction is reversed causing a positive scan back to the original potential of +0.8V. Single or multiple cycles can be used.

1.3.4. 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 became a very popular technique for electrochemical studies of new systems and has proved as a sensitive toll for obtaining information about fairly complicated electrode reactions.

CV is a technique, where in a species that undergoes a reduction during a cathodic polarization of the WE in an unstirred solution is reoxidized by applying a reverse (i.e., anodic) scan. The correlation of the cathodic and the anodic peak currents and differences in cathodic and anodic potentials with the voltage scan rates has been studied mathematically for different electrochemical reaction [19, 20]. The sweep rates in the CV can be about the same as in single sweep voltammetry.

1.3.5. 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.

1.3.6. Characteristic Parameters of a Cyclic Voltammogram

The 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 (Epa) and cathodic peak potential (Epc) and the corresponding current associated are anodic peak current (ipa) and cathodic peak current (ipc) respectively. Figure 1.2 depicts a typical voltammogram for a reversible process with current (vertical) vs 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.

1.4. Theory

Electron transfer plays a fundamental role in governing the pathway of chemical reactions. Measurement of speed of electron transfer process and the number of electrons involved are difficult in traditional experimental method like spectroscopy. Consequently our knowledge of the driving force for many reactions remains exclusive.

Cyclic voltammetry is the most widely used technique for acquiring qualitative information about electrochemical research. It is used in the study of electro activity of compounds particularly biological molecules probe coupled chemical reactions particularly to determine mechanisms, rates of oxidation/reduction reactions and also study of electrode surfaces. It is used in all fields of chemistry as a means of studying redox states. The electrode potential at which a drug, a metal ion or complex or some other organic compounds undergoes reduction (addition of electrons) or oxidation (removal of electrons) can be rapidly located by cyclic voltammetry. A very important aspect of cyclic voltammetry is its ability to generate a new redox species during the first potential scan and then probe the species fate on the second and subsequent scans. The power of cyclic voltammetry results from its ability considerable information on the thermodynamics of redox process and the kinetics of heterogeneous electron transfer reactions and on coupled chemical reactions or adsorption process. The correlation of the cathodic and anodic peak currents and difference in cathodic and anodic peak potentials with the voltage scan rate has been studied mathematically for different electrochemical

reaction [21, 22]. The sweep rates in cyclic voltammetry can be about the same as in single sweep voltammetry.

Cyclic voltammetry makes possible the elucidation of the kinetics of electrochemical reactions taking place at the electrode surface [23, 24]. In a typical voltammograms, there can be several peaks. From the sweep-rate dependence of the possible to investigate the role of adsorption, diffusion and coupled homogeneous chemical reaction mechanism [25].

The important parameters of a cyclic voltammogram are the magnitudes of anodic peak current (ipa), the cathodic peak current (ipc), the anodic peak potential (Epa) and cathodic peak potential (Epc). The basic shape of the current verses potential response for a cyclic voltammetry experiment as shown in Figure 1.2. At the start of the experiment, the bulk solution contains only the oxidized form of the redox couple so that at potentials lower than the redox potential, i.e. the initial potential, there is no net conversion of oxidized species (O) into reduced species (R) (point A). As redox potential is approached, there is net cathodic current which increases exponentially with potential. As O is converted to R, concentration gradients are set up for both O and R, and diffusion occurs down these concentration gradients. At the cathodic peak (point B), the redox potential is sufficiently negative that any O that reaches the electrode surface is instantaneously reduced to R. Therefore, the current now depends upon the rate of mass transfer to the electrode surface and so the time dependence is qt resulting in an asymmetric peak shape. Upon reversal of the scan (point C), the current continues to decay with qt until the potential nears the redox potential.

At this point, a net oxidation of R to O occurs which caused a cathodic current which eventually produces a peak shaped response (point D). if a redox system remains in equilibrium throughout the potential scan, the electrochemical reaction is said to be reversible. In other words, equilibrium requires that the surface concentrations of R and O are maintained at the values required by the Nernst Equation. Under these conditions, the following parameters characterize the cyclic by voltammogram of the redox process. The peak potential separation (Epc - Epa) is equal to 57/nmV for all scan rates where n is

the number of electron equivalents transferred during the redox process. The situation is very different when the redox reaction is not reversible, when chemical reactions are couple to the redox process or when adsorption of either reactants or products occurs. In fact, it is these non ideal situations which are usually of greatest chemical interest and for which the diagnostic properties of cyclic voltammetry are particularly suited.

Since the reference electrode has a constant makeup, its potential is fixed. Therefore any changes in the cell are ascribable to the working electrode. the control of potential of working electrode with respect of reference electrode, is equivalent of the controlling of energy of electrons within the working electrode. As shown in Figure 1.3, scanning the potential in the negative direction makes the electrode a stronger reductant, whereas scanning the potential in the positive direction makes it a better oxidant.

1.5. Applications

Cyclic voltammetry (CV) is the most effective and versatile electro analytical technique available for the mechanistic study of redox systems [26-30]. It enables the electrode potential to be rapidly scanned in search of redox couples once located, a couple can then be characterized from the potential of peaks on the cyclic voltammogram and from changes caused by variation of the scan rate. CV method have found to have extensive application for the evaluation of thermodynamic and kinetic parameters such as number of electron change (n), heterogeneous rate constant (k_0), entropy (S), Gibb's free energy (G) and diffusion coefficient (D_0) etc., of a number of redox reaction associated chemical reactions. These methods are especially useful in both oxidation and reduction process and to study the multiple electron transfer in an electrochemical reaction [31].

CV has become increasingly popular in all fields of chemistry as a means of studying redox states. 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 make 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.

1.6. Solvent

A number of physicochemical properties must be considered while choosing a solvent for electrochemical work [32] 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.

The cheapest solvent is water, 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 [33] 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 –3.0 V 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.

Dimethyl sulphoxide has electrochemical properties similar to DMF in the cathodic region. Since it is not as basic as DMF, cation radicals are somewhat stable in this medium.

Methylene chloride is the solvent of choice for organic oxidation studies. It is stable up to +3.0 V as acetonitrile. Cation radicals and dications are quite stable in this medium. Electrolytes are easily soluble in methylene chloride. However, at negative potentials of –1.0 V, the solvent decomposes. The anionic species are less stable in this medium.

Even totally non-polar solvents such as benzene and other hydrocarbons may be used to study the solution phase [34] as well as surface [35, 36-39] processes. Water, deionized and repeatedly distilled with alkaline KmnO4, is usually considered as pure. The purity is checked by conductivity measurements. The volatile and organic impurities are removed by passing the distilled water vapor through a column containing Pt catalyst at about 800°C over which oxygen also simultaneously passed.

The main impurity present in non-aqueous solvents is water. Refluxing with anhydrous copper sulphate, alumina, aluminum chloride, P_2O_5 etc. and distilling under reduced pressure many times and collecting the proper fraction usually remove this. Vacuum lines are employed during purification, storage and dehydrating agent such as anhydrous alumina is added as an internal addition [40].

1.7. Supporting Electrolytes

All ionic salts or ionizable compounds in a solvent are defined as the supporting electrolytes. It is very important to realize that they can influence the electrochemical processes in a number of ways.

i. These electrolytes impart conductivity to the solvent and hence enable the continuous current flow in solution.

ii. They must remain electro-inactive in the potential region of interest, if any useful Voltammetric study is to be conducted.

iii. If the concentration of the supporting electrolyte should be very high, they can form a space charge near the surface and the space charge potential can influence the charge transfer kinetics.

iv. If the ions of the supporting electrolyte are adsorbed on the surface, they can Catalyze or inhibit other reactions.

v. Small cations may form ion pairs with the anion radicals formed in the electrode Process and the properties of the ion pairs can be very different from those of the free anion racial.

vi. Some ions may form complexes with the reactants and products.

vii. The supporting electrolyte generally control the acidity of the ionic solution.

viii. The liquid electrolyte melts and solid electrolyte acts as the medium for the ionic phase.

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

Even today a number of voltammetric results at very positive potentials in KCl media are interpreted without possible influence of Cl^- adsorption. Reductions in Li^+ salt solutions are interpreted without consideration of ion pair effect. One must always consider all possible influence of supporting electrolytes if such pitfalls are to be avoided.

Solubility is the main consideration in selecting supporting electrolyte for aprotic solvent. A number of Tetra-Alkyl Ammonium (TAA) salts show good solubility in aprotic media. Tetra-ethyl ammonium (TEA) salts and more recently Tetra-n-Butyl Ammonium (TBA) salts are widely employed for this purpose.

Most of the inorganic acids, bases or salts are commercially available in the high purity grade. TAA salts are frequently available in the form of halides. The perchlorates or fluroborates may be easily obtained by double decomposition of these salts with the corresponding sodium salts. The precipitated $TAAClO_4$ or $TAABF_4$ may be recrystallized twice or thrice [41].

Some electrolytes may be dehydration may be done in an oven. Dehydrated samples should be stored in desiccators. Care must be exercised in handling explosives salts such as $NaClO_4$. They must neither be overheated nor ground in mortars with force and contact with organics should scrupulously be avoided.

1.8. Electrodes

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

- Working Electrode (WE)
- Reference Electrode (RE)
- Couter/Auxilary Electrode (AE)

1.8.1. Working Electrode (WE)

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

1.8.1.1. Mercury Electrodes

Mercury is a very attractive choice of electrode material because it has a high hydrogen over voltage that greatly extends the cathodic potential window (compared to solid electrode materials) and possesses a highly reproducible, readily renewable, and smooth surface. In electrochemical terms, its roughness factor equals unity (i.e., identical geometrical and actual surface areas). Disadvantages of the use of mercury are its limited anodic range (due to the oxidation of mercury) and its toxicity.

1.8.1.2. Solid Electrodes

The limited anodic potential range of mercury electrodes has precluded their utility for monitoring oxidizable compounds. Accordingly, solid electrodes with extended anodic potential windows have attracted considerable analytical interest. Of the many different solid materials that can be used as working electrodes, the most often used are carbon, graphite, platinum, and gold. Silver, nickel, and copper can also be used for specific applications. A monograph by Adams [42] is highly recommended for a detailed description of solid-electrode electrochemistry. An important factor in using solid electrodes is the dependence of the response on the surface state of the electrode. Accordingly, the use of such electrodes requires precise electrode pretreatment and polishing to obtain reproducible results. The nature of these pretreatment steps depends on the materials involved. Mechanical polishing (to a smooth finish) and potential cycling are commonly used for metal electrodes, while various chemical, electrochemical, or thermal surface procedures are added for activating carbon-based electrodes. Unlike mercury electrodes, solid electrodes present a heterogeneous surface with respect to the electrochemical activity [43]. Such surface heterogeneity leads to deviations from the behavior expected for homogeneous surfaces.

1.8.1.2a. Metal Electrodes

A wide choice of noble metals is available, platinum and gold are the most widely used metallic electrodes. Such electrodes offer very favorable electron-transfer kinetics and a large anodic potential range. In contrast, the low hydrogen over voltage at these electrodes limits the cathodic potential window (to the -0.2V to -0.5V region, depending upon the pH). More problematic are the high background currents associate d with the formation of surface-oxide or adsorbed hydrogen layers. Such films can also strongly alter the kinetics of the electrode reaction, leading to irreproducible data. These difficulties can be address d with a pulse potential (cleaning/reactivation) cycle, as common in flow amperometry [44]. The surface layers problem is less severe in non-aqueous media where noble metals are often an ideal choice. Compared to platinum electrodes, gold ones are more inert, and hence are less prone to the formation of stable

oxide films or surface contamination. Gold electrodes are also widely used as substrates for self-assembled organosulfur monolayers or for stripping measurements of trace metals. Other metals, such as copper, nickel, or silver have been used as electrode materials in connection with specific applications, such as the detection of amino acids or carbohydrates in alkaline medium (copper and nickel) and of cyanide or sulfur compounds (silver). Unlike platinum or gold electrodes, the copper electrode offers a stable response for carbohydrates at constant potential.

1.8.1.2b. Carbon Electrodes

Solid electrodes based on carbon are currently in wide spread use in electroanalysis, primarily because of their broad potential window, low background current, rich surface chemistry, low cost, chemical inertness, and suitability for various sensing and detection applications. In contrast, electrontransfer rates observed at carbon surfaces are often slower than those observed at metal electrodes. The electron-transfer reactivity is strongly affected by the origin and history of the carbon surface [45, 46]. While all common carbon electrode materials share the basic structure of a six-membered aromatic ring and sp^2 bonding, they differ in the relative density of the edge and basal planes at their surfaces. The edge orientation is more reactive than the graphite basal plane toward electron transfer and adsorption. Materials with different edge-to-basal plane ratios thus display different electron-transfer kinetics for a given redox analyte. The edge orientation also displays undesirably high background contributions. A variety of electrode pretreatment procedures have been proposed to increase the electrontransfer rates. The type of carbon, as well as the pretreatment method, thus has a profound effect upon the analytical performance. The most popular carbon electrode materials are those involving glassy carbon, carbon paste, graphite pencil electrode, carbon fiber, screen printed carbon strips, carbon films, or other carbon composites (e.g., graphite epoxy, wax-impregnated graphite, Kelgraf).

1.8.2. Reference Electrode (RE)

The selection of a proper reference electrode is equally vital in voltammetry especially when accurate and precise data on the formal potentials of the red-ox 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/Auxilary Electrode (AE)

The flow of current through the reference electrode which alter the of internal composition causing its potential to drift away from the expected standard value. For this and other reason the electrochemical measurements were made without current flowing through the reference electrode. Modern three electrode potentiostats sue a feedback circuit to prevent this from happening, but this feedback circuit requires that an additional auxilary electrode by introduced into the electrochemical cell. This electrode provides an alternative route for the current to follow, so that only a very small current flows through the reference electrode.

The auxilary electrode can be made from just about any material using any desired electrode geometry. Design choices are usually based on finding a material that is chemically inert in the particular test solution being studied and it is generally a good

idea for the auxilary electrode to have a large surface area. In most cases, a coil of platinum wire is used but stainless steel, copper or aluminium wire may work in non-corrosive solutions where metal cation interference is not a concern. If the electrochemical cell is made of metal, then the cell itself might be used as the auxilary. Because current flow at the auxilary electrode, electrochemical process will also occur here. If the reduction occurs at working electrode, then at auxilary electrode must oxidation occurs and vice versa. The products generated at the auxilary, if allowed to diffuse to the working electrode, may interfere with the experimental measurement. When this is a problem, the auxilary electrode is placed in a separate compartment containing an electrolyte solution that is in ionic contact with the main test solution via a glass frit. In most cases, however, the auxilary can be placed right in the test solution along with the reference and working electrodes.

1.9. A Brief Literature Survey of Cyclic Voltammetric Investigation

Our research interests involve the study of different modified electrodes like modified carbon paste electrode, modified glassy carbon electrode, graphite pencil electrode and the behavior of electrode on the analyte that are taken in the system. The application of the different modified electrodes in electroanalysis offer several advantages due to their unique electrode surface properties. Therefore, there has been an increasing interest in the creation of different modified electrode surfaces that differ from the corresponding bare surfaces.

Lei Zhang *et al.,* studied the separation of anodic peaks of ascorbic acid and dopamine at an α-alanine covalently modified glassy carbon electrode [47]. Hong Zhao *et al.,* determine the dopamine electrochemically using a poly (2-picolinic acid) modified glassy carbon electrode [48]. Selective determination of dopamine in the presence of ascorbic acid at an over-oxidized poly (N-acetylaniline) electrode was done by Longzhen Zheng *et al.,* [49]. Carbon nanotube-modified microelectrodes for simultaneous detection of dopamine and serotonin *in vivo* were carried out [50]. Xiaohua Jiang *et al.,* Immobilized the DNA on carbon fiber microelectrodes by using over oxidized

polypyrrole template for selective detection of dopamine and epinephrine in the presence of high concentrations of ascorbic acid and uric acid [51]. Michael *et al.,* detection the dopamine at overoxidised carbon-fiber microelectrodes [52]. Carbon nanotube-modified electrodes for the simultaneous determination of dopamine and ascorbic acid were done [53]. Levent Ozcan *et al.,* carried out the electrochemical preparation of a molecularly imprinted polypyrrole-modified pencil graphite electrode for determination of ascorbic acid [54]. Suely *et al.,* explored the electrooxidation and determination of dopamine using a nafion-cobalt hexacyanoferrate film modified electrode [55]. Cheng Yin Wang *et al.,* evaluated the voltammetric determination of dopamine in human serum with amphiphilic chitosan modified glassy carbon electrode [56]. Nianhang Chen *et al.,* studied the Voltammetric studies on mechanisms of dopamine efflux in the presence of substrates and cocaine from cells expressing human norepinephrine transporter [57]. Hanwen Sun *et al.,* performed the electrochemical behavior and determination of dopamine and ascorbic acid by cyclic voltammetry using an activated roughened glassy carbon electrode [58].

Chuneya Li [59] worked on voltammetric determination of tyrosine based on chemically electropolymerisation of L-serine. Rui Zhang *et al*., [60] published work on poly (acid chrome blue K) modified glassy carbon electrode by electropolymerisation and achieved selective separation of dopamine, ascorbic acid and uric acid in real sample of human urine. Yong Xin Li *et al*., [61] worked on simultaneous electro analysis of dopamine ascorbic acid by poly (vinyl alcohol) modified. Yuzhong Zhang *et al*., [62] determined dopamine in presence of ascorbic acid by poly (amidosulfonic acid) modified glassy carbon electrode. Xing-Yuan Liu *et al.,* [63] electropolymersied poly (carmine) on glassy carbon electrode for detection of parathion. Tae-Hun *et al*., [64] worked on electrochemical preparation of poly (p-phenyl vinylene) in aceto nitrile. Ai-Min Yu *et al*., [65] worked on catalytic oxidation of uric acid at poly (glycine) modified electrode and its trace determination. Yuzhong Zhang *et al.,* [66] determined dopamine in presence of ascorbic acid using poly (acridine-red) on modified glassy carbon electrode.

Lei Zhang *et al*., studied for simultaneous determination of uric acid and ascorbic acid with modified poly (glutamic) acid. Selvaraju *et al*., [68] worked on simultaneous detection of dopamine and serotonin in presence of ascorbic acid and uric acid at poly (o-phenyldiamine) electrode. Xiangqin Lin *et al*., developed DNA/Poly(p-aminobenzensulfonic acid) composite bi-layer modified glassy carbon electrode for determination of dopamine and uric acid under coexistence of ascorbic acid was investigated using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV) [69]. Hossnia Mohran *et al.,* worked on An Electrochemical Investigation of the Redox Properties of Murexide in Aqueous and Non-Aqueous Media has been studied [70]. Jill Venton *et al.,* published Psychoanalytical electrochemistry: Dopamine and behavior was constructed [71]. Mo *et al.,* reported simultaneous measurement of dopamine and ascorbate at their physiological levels using voltammetric microprobe based on over oxidized poly (1,2-phenylenediamine)-coated carbon fiber was found [72].

Zahra Nasri *et al.,* [73] worked on Application of silica gel as an effective modifier for the voltammetric determination of dopamine in the presence of ascorbic acid and uric acid. Simultaneous determination of dopamine and serotonin on a glassy carbon electrode coated with a film of carbon nanotubes has been studied [74]. Yanyi Sun *et al.,* [75] was analysed the simultaneous electrochemical determination of xanthine and uric acid at a nanoparticle film electrode. Umesh Chandra *et al.,* was reported that determination of dopamine in presence of uric acid at poly (eriochrome black T) film modified graphite pencil electrode [76]. Colin-Orozco *et al.,* [77] published work on the electrochemical oxidation of dopamine, ascorbic acid and uric acid onto a bare carbon paste electrode from a 0.1 M NaCl aqueous solution at pH 7. Zhihong Zhu *et al.,* was published electrochemical detection of dopamine on a Ni/Al layered double hydroxide modified carbon ionic liquid electrode was studied [78]. Electrochemical determination of dopamine using banana-MWCNTs modified carbon paste electrode has been reported [79]. Corona-Avendano *et al.,* influence of CTAB on the electrochemical behavior of dopamine and on its analytic determination in the presence of ascorbic acid was developed [80].

1.10. Faradaic and Non-Faradaic Process

Two types of process occur are electrode. One kind comprises reactions involves the electron transfer across the metal-solution interface. Electron transfer causes oxidation or reduction or occur. Since the reactions are governed by Faraday's law (i.e., the amount of chemical reaction caused by the flow of current is proportional to the amount of electricity passed), they are called Faradaic Process. Electrode at which faradic processes occur are sometimes called charge transfer electrodes. Under some conditions, a given electrode-solution interface will show a range of potentials where no charge-transfer reaction occur because such reactions are thermodynamically of kinetically unfavorable. However, processes such as adsorption and desorption can occur and the structure of the electrode-solution interface can change with changing potential or solution composition. These processes are called Non-faradic Processes. Although charge does not across the interface, external currents can flow (at least transiently) when the potential, electrode area or solution composition changes, both faradic and non-faradic process occurs when electrode reaction takes place. Although faradic process are usually of primary interest in the investigation of an electrode reaction (except in studies of the nature of the electrode-solution interface itself), the effects of the non-faradic processes must be taken into account in using electrochemical data of obtain information by discussing about the charge transfer and associated reactions.

1.11. Polarisable and Non-Polarisable Interface

All electrode-solution interfaces can be classified as polarisable or non-polarisable. An electrode for which an electron can pass easily across the interface is called non-polarisable. In this case, external application of a change of potential may result in more electrons passing rapidly across the interface. Thus, there is a negligible build-up of excess charge in the electrode surface, i.e., the interface does not polarise. Platinum in contact with hydrochloric acid is a non-polarizable interface. In contrast when the transfer of electrons is difficult, a potential change from outside will induce a substantial build-up of excess charges are the interface, hence, the electrodes is termed polarisable. When a potential is applied externally to the electrode, the transfer of electrons through is negligible. That is, a small change in current flow causes a large

change in electrode potential. An ideally polarisable interface is one which can allow the passage of current without causing a change in the potential difference across it. In addition, when the current associated with charging the electrode-electrolyte interface arises purely from capacitive effect; such an interface is termed an ideally polarisable electrode. while no real electrode behaves ideally over the entire potential rage, some electrode-solution system, over limited potential ranges, can show behaviour which is approximately, ideal for instance, a mercury electrode in contact with a de-aerated potassium chloride solution which behaves as an ideal polarisable electrode at potential in excess of 1.5V.

1.12. Electrodes Processes

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

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

The simplest reaction involves only mass transfer of reactant to the electrode, 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 [81, 82].

1.12.1. Mass Transfer Processes

Whenever an electrochemical charge transfer process takes place at the electrode surface, the electroactive material gets depleted and a concentration gradient is set up. Under such conditions the reactant diffuse towards the electrode surface and the corresponding product of the electrode reaction diffuses away from the electrode surface. Mass transfer in electrochemistry illustrates the movement of electroactive species from differences in electrical or chemical potential at the two locations. There are three forms of mass transport namely, *convection*, *migration* and *diffusion* which influence and electrolysis reaction (Fig. 1.5).

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.

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.

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.

1.13. 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 n 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

1.13.1. Reversible Electron Transfer Process

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

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

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 °C

i. ΔEp = Epa-Epc = 59/n mV, where n is number of electrons change

ii. ipc/ipc = 1

iii. ip α ν1/2

iv. Ep is independent of ν

1.13.2. 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. 1.7). This process is usually due to slow electron exchange or slow chemical reactions at the electrode surface [83]. 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 [84]

$$ip = 2.99 \times 105\ n\ (\alpha n)1/2\ A\ D_0 1/2\ \nu 1/2\ Co^*$$

$$(\alpha na) = 47.7/Ep - Ep/2$$

The value of Ep, the difference between the cathodic and anodic peak is of the order of 59 mV/n is given by equation. The peak separation Ep 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 °C

i. no reverse peak

ii. ip α ν1/2

iii. Ep shifts = 30/αna mV, where α is charge transfer coefficient

iv. [Ep-Ep/2] = 47.7/αna mV

1.13.3. 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. 1.8). The current due to quasi-reversible processes is controlled by both mass transport and charge transfer kinetics [85]. 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 ^{o}C

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

ii. ipc/ipa = 1, provided $\alpha c = \alpha a = 0.5$

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

iv. Epc shifts negatively with increasing ν

1.14. Objectives and Scope of the Thesis

The focus of the work covered in this thesis is to controllably alter the properties of carbon surfaces by chemically grinding modification method, surfactant mobilization and immobilization method, Electropolymerization of monomer, pretreatment of carbon paste electrode, activation of glassy carbon electrode and graphite pencil electrode was used in cyclic voltammetric and differential voltammetric techniques leads to the surfaces are useful for desired sensor applications.

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

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

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

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

Present work is aimed at the development of voltammetric sensors for the detection of UA, DA, AA, PA, FA, DF, 5-HT, CC, HQ, which are neurotransmitters present in the extracellular fluid of the central nervous system. It is generally believed that the direct redox reactions of these species at bare electrodes are irreversible and high over potential are usually required for their amperometric detection. Moreover, the direct redox reaction of these species takes place at very similar potentials and often suffers from pronounced fouling effects, which results in rather poor selectivity and reproducibility. Hence there is need for the development of polymer modified electrodes because of its high selectivity and sensitivity due to the film homogeneity in electrochemical deposition, strong adherence to the electrode surface and chemical stability of the film.

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

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

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

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

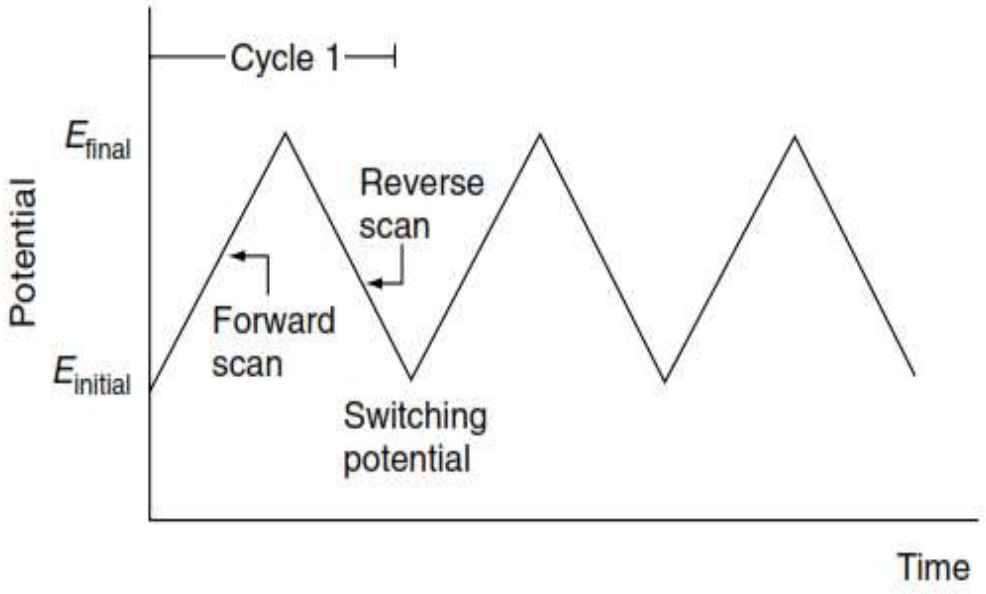


Fig. 1.1. Variation of the applied potential as a function of time in a cyclic voltammetry experiment

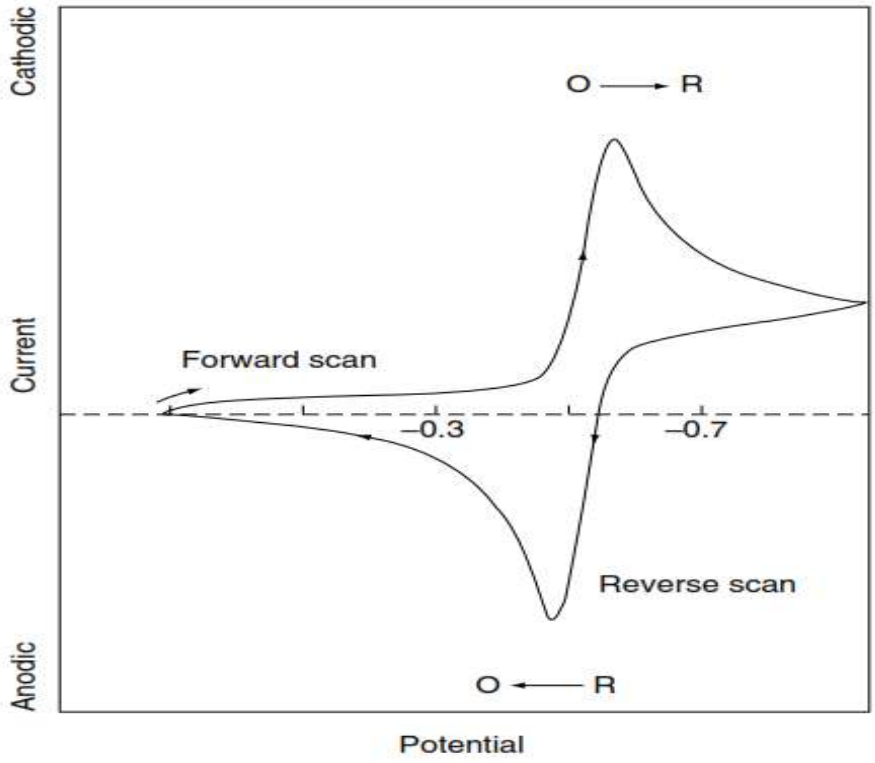


Fig. 1.2. A typical cyclic voltammogram of current verses potential

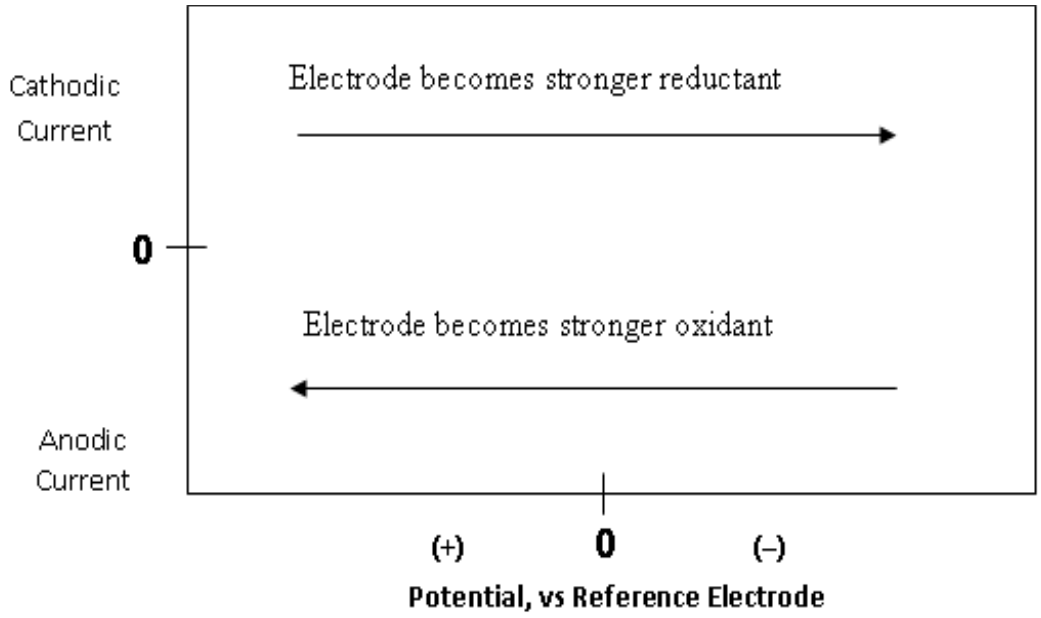


Fig. 1.3. Potential-Current axes for Cyclic Voltammetry

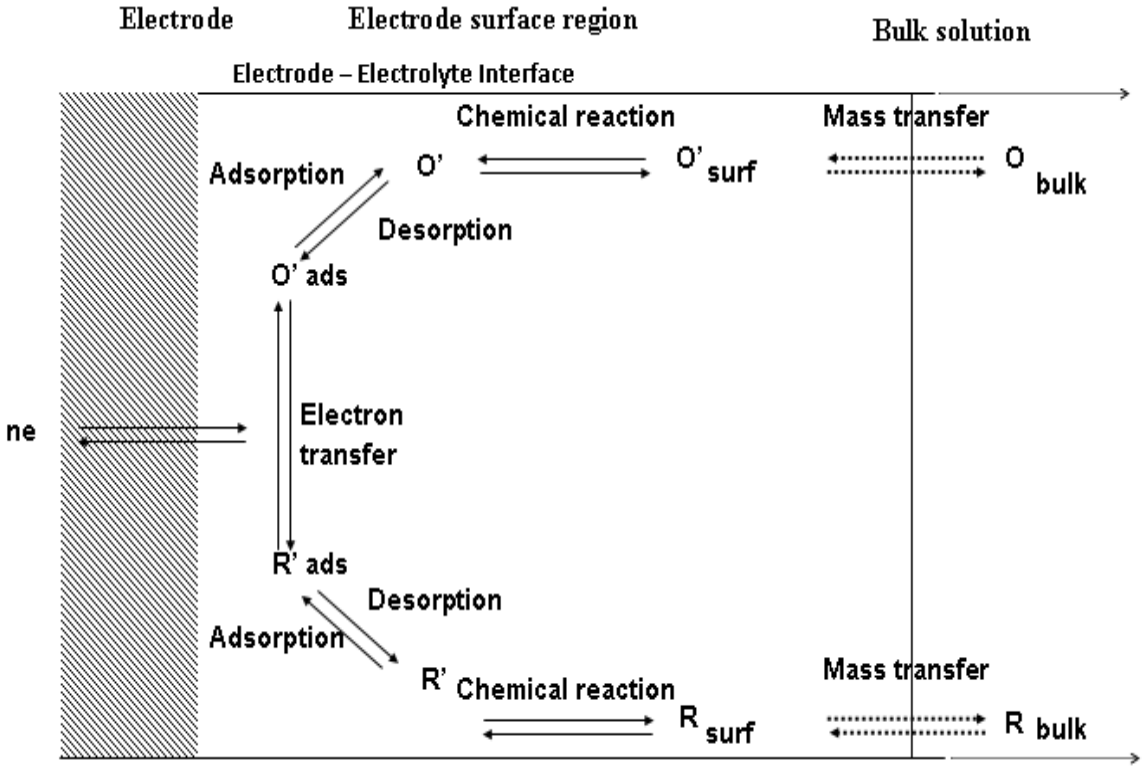


Fig. 1.4. General pathway of electrode-mediated processes of oxidized (O) and reduced (R) electroactive species

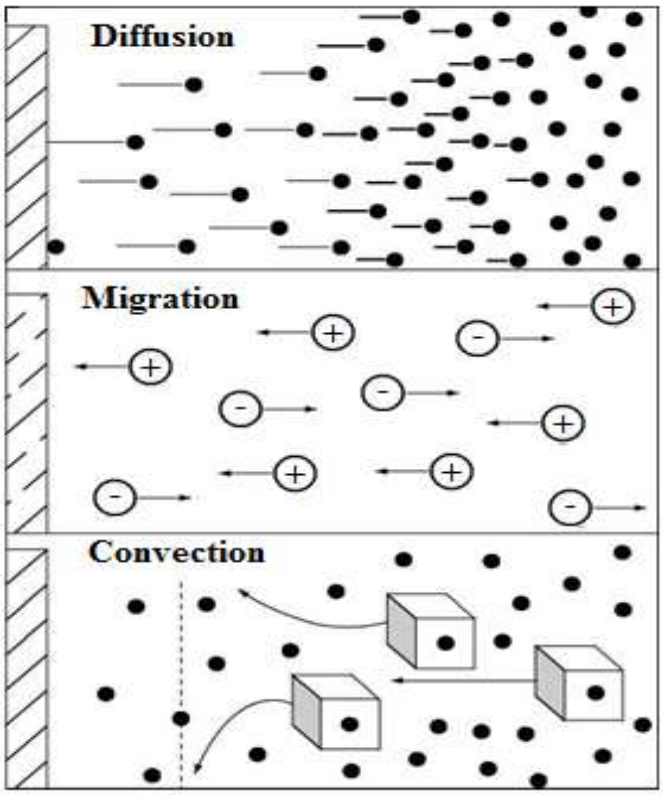


Fig. 1.5. Modes of mass transport

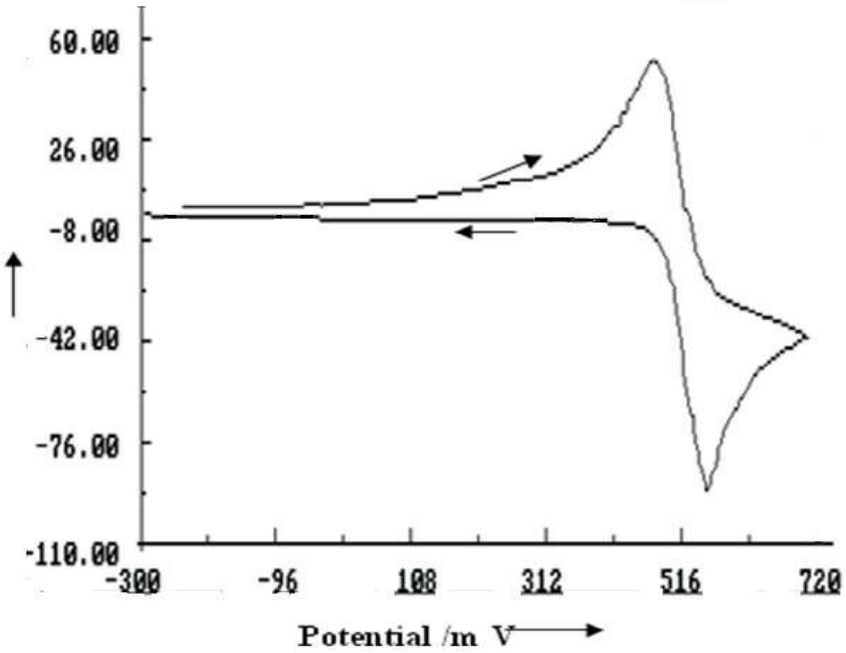


Fig. 1.6. Typical voltammogram for a reversible process

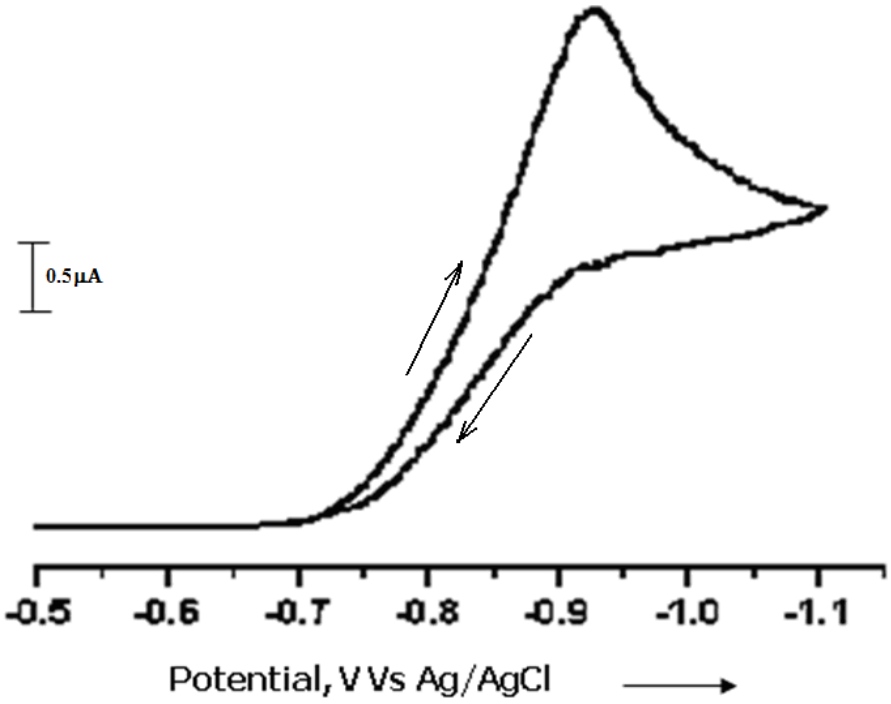


Fig. 1.7. Typical voltammogram for an irreversible process

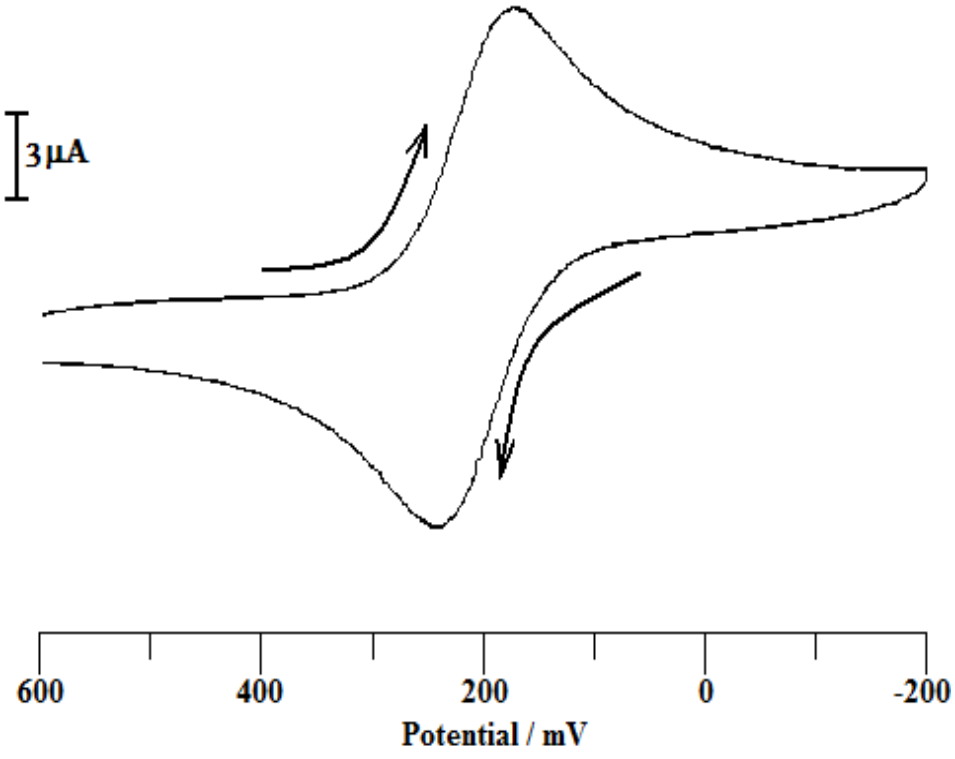


Fig. 1.8. Typical voltammogram for a quasi-reversible process

1.15. References

[1] R.L. McCreery and K.K. Kline, "Laboratory Techniques in Electroanalytical Chemistry", 2nd Edition (Eds. P.T. Kissinger and W.R Heineman) Dekkar, New York, 1995, Chapter 10.

[2] R.L. McCreery, "Electroanalytical Chemistry", Vol. 17 (Ed. A.J. Bard) Dekkar, New York, 1991, pp. 21-374.

[3] F. Gonon, M. Buda, R. Cespuglio, M. Jouvet and J.F. Pujol, *Nature*, 286 (1980) 902.

[4] R.D. O'Neill, *Analyst*, 119 (1994) 767.

[5] A. Salimi, H. Mam-Khezri and R. Hallaj, *Talanta*, 70 (2006) 823.

[6] J. Wang, "Analytical Electrochemistry", VCH Publishers Inc., New York (1994).

[7] J. Wang, D.B. Luo, P.A.M Farias and J.S. Mahmoud, *Anal. Chem*., 57 (1985) 158.

[8] J.G. Osteryoung, *Acc. Chem. Res*., 26 (1993) 77.

[9] J.G. Osteryoung and R.A. Osteryoung, *Anal. Chem*., 57 (1985) 101A.

[10] A.J. Bard, "Electroanalytical Methods", 3rd Edition (2000).

[11] Randles, *Trans. Far. Soc*., 44 (1948) 327.

[12] D.B. Hibbert, "Introduction to Electrochemistry", MacMillan, London (1993).

[13] A.E. Kaifer and M. Gomez-kaifer, "Supramolecular Electrochemistry", Willey, VCH, New York (1999).

[14] A.J. Bard and L.R. Faulkner, "Electrochemical Methods: Fundamentals and Applications", John Willey and Sons (1996).

[15] D.G. Davis, In: *Physical Chemistry: The Porphyrins*, Part A (Ed. D. Dolphin), Vol. III, Academic Press, New York (1978) Chapter 4.

[16] E.R. Brown and R.F. Large, In: *Physical Methods of Chemistry* (Eds. A. Weissberger and B. Rossiter), Vol. 1, Part II, A: Electrochemical Methods, Wiley Interscience, New York (1971).

[17] J.P. Renault, A. Bernard, A. Bietsh, B. Michel, H. R. Bosshard, E. B. Dalamarche, M. Kleiter, B. Hecht and U.P Wild, *J. Phys. Chem*. B., 107 (2003) 703.

[18] R.S. Nicholson, *Anal. Chem*., 37 (1965) 1351.

[19] R.S. Nicholson and I. Shain, *Anal. Chem*., 37 (1965) 190.

[20] R.S. Nicholson, *Anal. Chem*., 37 (1965) 1351.

[21] R.S. Nicholson, *Anal. Chem*., 37 (1965) 190.

[22] C.M.A. Brett and A.M.O. Brett, "Electrochemistry: Principle Methods and Application", Oxford University Press (1993).

[23] J. Bockris and S.U.M. Khan, "Surface Electrochemistry: A Molecular Level Approach", Plenum Press, New York and London (1993).

[24] R. Parson, *Surface Science*, 90 (1990) 813.

[25] R.S. Nicholson and I. Shain, *Anal. Chem*., 36 (1977) 706.

[26] D.H. Evans, *Acc. Chem. Res.,* 10 (1997) 313.

[27] W.R. Heineman and P.T. Kissinger, *Am. Labar*., 14 (1982) 29.

[28] D.H. Evans, K.M.O. Onnell, R.A. Peterson and M.J. Kelly, *J. Chem. Edu*., 60 (1983) 290.

[29] P.T. Kissinger and W.R. Heinman, *J. Chem. Edu*., 60 (1983) 702.

[30] A.W. Bott, *Curr. Seperat*., 16 (1997) 61.

[31] M. Noel and K.I. Vasu, "Cyclic Voltammetry and the Frontiers of Electrochemistry", 1st Edition, Oxford & IBH Publishing Co. Pvt. Ltd, New Delhi (1990).

[32] B.E. Conway, H. Angerstein-Kozlowska, W.B.A. Sharp and E. Criddle, *Anal. Chem.*, 45 (1973) 1331.

[33] R. Lines and V.D. Parker, *Acta. Chem. Scand*., B31 (1977) 369.

[34] E. Peled, A. Mitavski, A. Reger and E. Gileadi, *J. Electroanal. Chem.,* 75 (1977) 677.

[35] M. Elam, I. Eahatt, E. Peled and E. Gileadi, *J. Phys. Chem*., 88 (1984) 1609.

[36] D.T. Sawyer and J.E. Roberts, Jr. "Experimental Electrochemistry for Chemists", Wiley Interscience, New York (1974).

[37] J.K. Mann, *Electoanal. Chem*., 3 (1969) 57.

[38] H. Lund, In: *Organic Chemistry* (Eds. M.M. Baizer and H. Lund), Marcel Dekker, New York (1983).

[39] O. Hammerich and V.D. Parker, *Electrochim. Acta*., 18 (1973) 537.

[40] L. Meites, "Polarographic Techniques", 2nd Edition, Wiley Intersceince, New York (1958).

[41] A. Guindilis, P. Atanasov and E. Wilkins, *Electroanalysis*, 9 (1997) 661.

[42] C. Henry, *Anal. Chem*., 70 (1998) 594A.

[43] J. Marty, D. Garcia and R. Rouillon, *Trends Anal. Chem*., 14 (1995) 329.

[44] L. Gorton, *J. Chem. Soc. Faraday Trans*., 82 (1986) 1245.

[45] M. Lobo, A. Miranda and P. Tunon, *Electroanalysis*, 9 (1997) 191.

[46] Lei Zhang, Yugang Sun and Xiangqin Lin, *Analyst*, 126 (2001) 1760.

[47] L. Zhang, Y. Sun and X. Lin, *Analyst*, 126 (2001) 1760.

[48] H. Zhao, Y. Zhang and Z. Yuan, *Analyst*, 126 (2001) 358.

[49] L. Zheng, S. Wu, X. Lin, L. Nie and L. Rui, *Analyst*, 126 (2001) 736.

[50] B.E.K. Swamy and B.J. Venton, *Analyst*, 132 (2007) 876.

[51] X. Jiang and X. Lin, *Analyst*, 130 (2005) 391.

[52] M.L.A.V. Heien, P.E.M. Phillips, G.D. Stuber, A.T. Seipel and R.M. Wightman, *Analyst*, 128 (2003) 1413.

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

[54] L. Ozcan, M. Sahin and Y. Sahin, *Sensors*, 8 (2008) 5792.

[55] S.S.L. Castro, R.J. Mortimer, M.F. de Oliveira and N.R. Stradiotto, *Sensors*, 8 (2008) 1950.

[56] C.Y. Wang, Z.X. Wang, A.P. Zhu and X.Y. Hu, *Sensors*, 6 (2006) 1523.

[57] N. Chen, C.G. Trowbridge and J.B. Justice, Jr., *J. Neurochem*., 71 (1998) 653.

[58] H. Sun, C. Zang, K. Lian, *J. Pharm. Sci*., 4 (2009) 200.

[59] C. Li, *Coll. Surf. B Bioint*., 50 (2006) 147.

[60] R. Zhang, G.D. Jin, D. Chen and X.Y. Hu, *Sens. Act. B*., 138 (2009) 174.

[61] Y.X. Li and X. Lin, *Sens. Act. B*., 115 (2006) 134.

[62] Y. Zhang, G. Jin, Z. Yang and H. Zhao, *Microchem. Acta*., 147 (2004) 225.

[63] X.Y. Liu, C.Y. Li and S. Hu, *Microchem. Acta*., 154 (2006) 275.

[64] T.H. Kim and S.M. Park, *Electrochim. Acta*., 50 (2005) 1461.

[65] A.M Yu, H. Zhang and H.Y. Chin, *Analyst*, 122 (1997) 839.

[66] Y. Zhang, G. Jin, Y. Wang and Z. Yang, *Sensors*, 3 (2003) 443.

[67] L. Zhang and X. Lin, *Analyst*., 126 (2001) 367.

[68] T. Selvaraju and R. Ramaraj, *J. Appl. Electrochem*., 33 (2003) 759.

[69] X. Lin, G. Kang and L. Lu, *Bioelectrochem*., 70 (2007) 235.

[70] H.S. Mohran, *Amer. J. App. Sci*., 6 (2009) 964.

[71] B.J. Venton and R.M. Wightman, *Anal. Chem*., 75 (2003) 414A.

[72] J.W. Mo and B. Ogorevc, *Anal. Chem*., 73 (2001) 1196.

[73] Z. Nasri and E. Shams, *Electrochim. Acta*., 54 (2009) 7416.

[74] K. Wu, J. Fei and S. Hu, *Anal. Biochem*., 318 (2003) 100.

[75] Y. Sun, J. Fei, K. Wu and S. Hu, *Anal. Bioanal. Chem*., 375 (2003) 544.

[76] U. Chandra, B.E.K. Swamy, O. Gilbert, S. Redy and B.S. Sherigara, *Amer. J. Anal. Chem.,* 2 (2011) 262.

[77] E.C. Orozco, S.C. Avendano, M.T.R. Silva, M.R. Romo and M.P. Pardave, *Int. J. Electrochem. Sci*., 7 (2012) 6097.

[78] Z. Zhu, L. Qu, Y. Guo, Y. Zeng, W. Sun and X. Huang, *Sens. Actu*., 151 (2010) 146.

[79] J.B. Raoof, A. Kiani, R. Ojani and R. Valiollahi, *Anal. Bioanal. Electrochem*., 3 (2011) 59.

[80] S.C. Avendano, M.T.R. Silva, M.P. Pardave, L.H. Martinez, M.R. Romo and G.A. Angeles, *J. Appl. Electrochem*., 40 (2010) 463.

[81] A.J. Bard and L.R. Faulkner, "Electrochemical Methods Fundamentals and Applications", 2nd Edition, John Wiley & Sons Inc. (2001) Chapters 1,6,10 & 14.

[82] R.S. Nicholson and I. Shain, *Anal. Chem*., 36 (1964) 706.

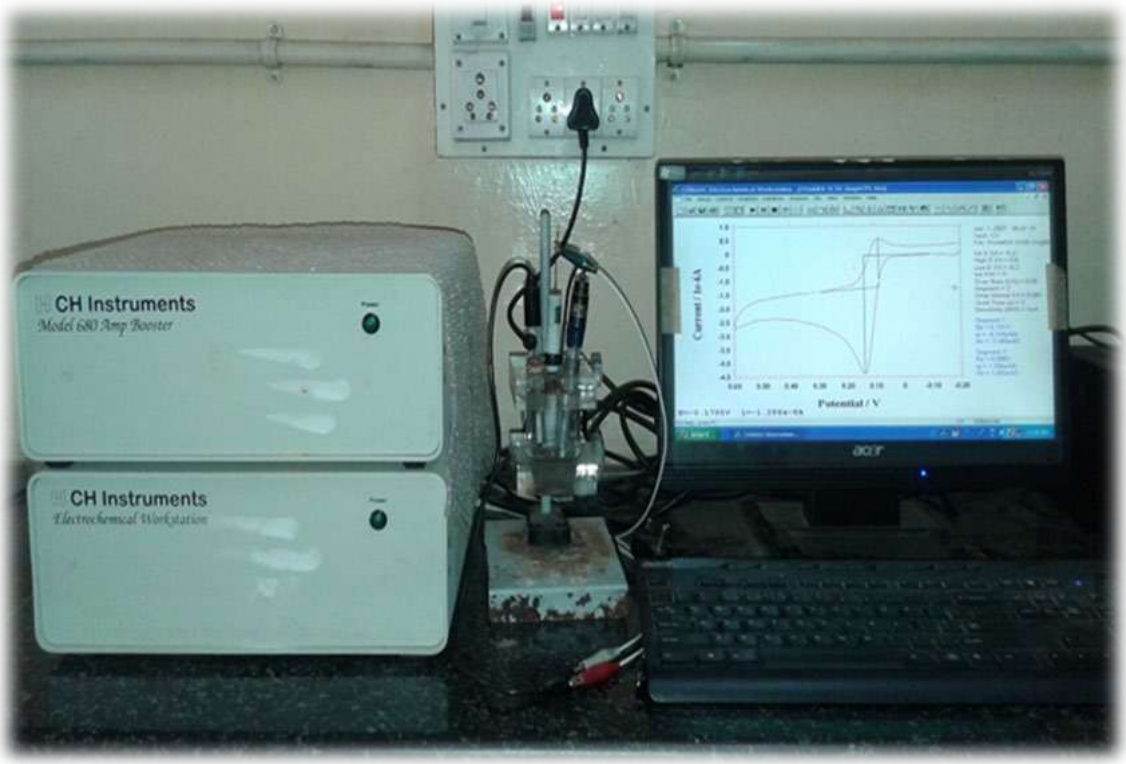
[83] J.E.B. Randles, *Trans. Farad. Soc*., 44 (1948) 334.

[84] E.R. Brown and R.F. Large, In: *Physical Methods of Chemistry*, Vol. 1, Part-IIA.

[85] "Electrochemical Methods" (Eds. A. Weissberger and B. Rossiter), Willey-Interscience, New York (1971).

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. The electrode system with special emphasis on carbon paste electrodes used in the course of this research are outlined. Preparation and characterisation of carbon paste electrodes and polymer film modified carbon paste electrodes surfaces, and procedures used in the present work are detailed. In addition, an overview of the theories and related equations are described.

2.2. Experimental Techniques

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

2.2.1. Cyclic Voltammetry

Cyclic voltammetry (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 to in the study of the electrochemical behaviour 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 synthesis the polymer film modified carbon paste electrode.

2.2.2. Differential Pulse Voltammetry

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

2.3. Instrumentation and Basic Equipments

2.3.1. Potentiostat

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 auxillary electrode (AE). The potentiostat performs three functions:

i. Controls the applied potential, which is potential difference between the WE and RE (the applied potential controls what half reactions occur at the WE);

ii. 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

iii. 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. Potentiostat Employed in the Present Work for CV and DPV Experiments

The electrochemical experiments were carried out using potentiostat provided with the Data Acquisition PC interface Card Model EA-201 Electro Analyzer fabricated by Chemi Link Systems, Trombay, Mumbai, India; compatible with an IBM PC and coupled to a printer (Fig. 2.2). This instrument is capable of performing more than six electro analytical techniques. The instrument incorporates a high speed, high accuracy and an electrolysis mode that consists of high-gain operational amplifier with circuits for controlled potential.

The WE current signal is handled a bit differently. This signal line is also presented as a voltage signal, but the voltage level is actually proportional to the current flowing at the WE. The potentiostat has an internal 'current converter' circuit that performs the necessary current-to-voltage conversion automatically. The current converter has a number of ranges and the operator is expected to choose the range most appropriate for the experiment being performed. Each range is associated with a particular proportionality constant, such as '100 mA/V' or '1 mA/V.'

2.3.2. Recording Device

Computers entered into electroanalytical instrumentation in 1967 [1] or even earlier. Computer applications in stationary electrode voltammetry [2, 3] and CV [5-6] were reported. Computers can be used to apply potential programme to the WE through the potentiostat. The initial potential, final potential, sweep rate, the nature of the pulse, current sensitivity etc, may be instructed to the computer in the digital form. Computers can be used very effectively in data acquisition. The applied potential values and the resulting current values may be converted 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. Apparatus (Electrolysis Cell)

In its simplest form, the electrolysis cell was a glassware capable of holding an appropriate volume of a test solution containing one or more electro active analytes. 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, 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.

2.3.4. 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.

2.4. 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.

2.4.1. 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).

2.4.1a. 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 effected 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 fibre, carbon films, or carbon composites.

2.4.1b. Carbon Paste Electrodes: Important Developments

Carbon paste electrodes (CPE) and their modifications underwent an attractive development in the field of biosensors. 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 of the so called solid state electrochemistry [14]. The era of chemically modified electrodes culminated at the beginning of 80's. Modification of carbon paste by impregnating the carbon particles with methanolic solutions of dimethyl glyoxime [15] represents another milestone in the history of CPE.

2.4.2. Carbon Paste as Electrode Material

2.4.2.1. Unmodified Carbon Paste

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

2.4.2.2. Modified Carbon Pastes

The base of modified carbon pastes is usually a mixture of powdered graphite and non-electrolytic binder [16, 18, 21]. Another constituent in the mixture is then a modifier

itself. Modifying agent is usually one substance; but, the pastes can also be modified with two or even more components, which is the case of carbon paste-based biosensors containing enzyme (or its carrier) together with appropriate mediator [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 main reason 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 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 pastes 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 modifiers recently used, one can find single compounds [26] sophisticated chemical agents [22, 29-28] 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.

2.4.3. Construction and Design of Carbon Paste Electrodes

The proper construction and design of CPE is based on short Teflon rod (shaped as a robust plug) with a well drilled in and a Pt-wire which provides electrical contact with the external circuit. Up till now the design similar to Adam's CPE is still the most frequently used construction. Still, the most popular bodies are various glass-, PVC tubes and Teflon rods whose end-hole can be easily re-filled with a new portion of carbon paste [59, 60]. Simple constructions equipped with a piston for extrusion of the paste [20, 30, 43] are also frequently employed. For common CPEs, the actual diameter of the end-hole forming the proper carbon paste surface is being chosen from 2 to 10 mm, which is convenient for a majority of electrochemical measurements [22]. Both above-mentioned construction variants of CPEs for batch measurements allows to utilise fully one of the most valuable property of carbon pastes easy and quick surface renewal or, in necessary cases, even removal and renewing of a larger portion of the paste. Practically immediate surface renewal can be achieved by wiping some paste off using a wet filter paper. If being performed carefully [20, 22] this procedure provides surface reproducibility nearly comparable to that attained by rather time-consuming circle-like polishing of the electrode surface upon a paper pad [45].

More interesting designs of CPEs are usually reported in association with carbon paste-based flow cells [61] electrochemical detectors [62] coulometric [63] amperometric [64, 65] and potentiometric [66] sensors, or some sensing devices for special measurements *in vivo* [67, 68]. For instance, electrochemical investigations on modulation of the electrode response can be performed with periodically renewed carbon paste by means of a special cell with doubled carbon paste filling [65]. Among others, such a design with intimate surface renewal is very effective in analysis of organic and biological materials where the surface of an electrode may readily be poisoned either with matrix constituents or by electrode reaction products [61].

2.4.4. Choice of Carbon (Graphite) Powder

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

Suitable carbonaceous materials should obey the following criteria:

i) Particle size in micrometers

ii) Uniform distribution of the particles

iii) High chemical purity and

iv) Low adsorption capabilities.

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

2.4.5. Pretreatment of Carbon Powder

In order to lower adsorption capabilities of graphite, Lindquist [69] 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.

2.5. Choice of Pasting Liquids or Binders

The pasting liquids should be non conducting non volatile, immiscible with aqueous solutions and should exhibit a high chemical and electro chemical inertness. Paraffin oil such as nujol and various silicon oils are most popularly used. Some organic esters like tri cresyl phosphate [70-72], dioctyl phthalate and dinitrophenyl octyl which are found to have ion pairing capabilities have been used as pasting liquids. Binders give rise to hydrophobic character of the carbon paste surface, which is in principle the main reason for different behaviour of CPEs compared to carbon solid electrodes. The presence of pasting liquid at the surface decreases the transfer rate (slower kinetics), causing the higher over potential compared to homogeneous electrodes. The increasing lipophility of the pasting liquid enhances the electrode overpotential (irreversibility). This is due to the marked hydrophobicity of the liquid which hinders the access of analyte towards the surface [73, 74]. The degree of surface hydrophobicity of the surface can be decreased by pretreatments.

2.6. 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 [75-77]. 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.

2.7. 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 [76] 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.

2.8. The Highest Potential Limits Attained at CPEs

For anodic polarizations, such a priority can be attributed to a value of +1.85 V vs. SCE specified for a CPE with impregnated graphite [78]. 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 vs. Ag/AgCl.

2.9. Preparation and Standardization of CPE Used in the Present Study

In the present study the ratio of carbon powder to nujol was optimized to 1g : 0.4 ml. For the preparation of silicone oil based CPE, the graphite powder and the

pasting liquid silicone oil was optimized to 1g: 0.62 ml. An increase in the volume of the pasting liquid greatly affected the reversibility of the electrode. This was reflected in the greater peak separations ΔEp in potassium ferrocyanide model system used for the study. The lower volumes of the binder affected the stability of the electrode. The paste was homogenized by careful grinding in an agate mortar with the help of a pestle. The well or the cavity in the electrode is filled with the prepared paste. Fresh electrode surfaces were obtained by polishing on a weighing paper until they showed a smooth and shiny appearance. After every measurement, the electrode surface was mechanically regenerated by removing some paste off (3 mm) and again polishing it on the weighing paper.

2.10. Construction of CPE

Carbon paste electrode 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).

2.11. Storage of CPE

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.

2.12. Model Systems for Basic Characterization of CPE in Voltammetry

2.12.1. Potassium Ferricyanide 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 [79, 80].

2.12.2. Calculation of Surface Area of the Electrode

The surface area of CPE was determined using potassium ferricyanide (1 mM) system in 1 M KCl. The effect of scan rate on cyclic voltammograms of 1 mM solution of ferricyanide has been studied at 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 Vs^{-1} as shown in Figure 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 $\Delta Ep = Epa-Epc/n \approx 0.059$ 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 ipa/ipc 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) [81]

$$ip = 2.65 \times 105 \times n3/2\times A \times C0^* \times D_0½ \times \nu ½ \qquad (1)$$

where, ip is the peak current in A, A is the area of the electrode in cm^2, n the number of electrons, C0* is the concentration in mol cm^{-3}, D_0 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.

2.13. 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.

2.14. Glassy Carbon Electrode used as Working Electrode

2.14.1. Electrode Pretreatment

Obtaining reproducible results and activating the electrode surface to obtain reproducible results are the main objectives of a great variety of pretreatment procedures reported. Reproducible results can be obtained if the electrode is maintained in the same state of cleanliness. Mechanical polishing is carried out with metallographic papers of increasing fineness. The electrode may also be polished using alumina or diamond powder of 0.05 μm sizes. This would remove all surface impurities. However, care must be taken to clean the electrode thoroughly afterwards to ensure that the polishing materials are completely removed. Otherwise, these polishing materials themselves may show some specific catalytic or inhibitive effects [82]. The most serious problem encountered in solid electrode methodology is the difficulty in understanding the true surface conditions and their possible effects on electrode processes. The surface oxidation of noble metal electrodes and the presence of adsorbed gas influence the electrode kinetics in a variety of ways. Thus, it becomes necessary to adopt some standard procedure that will produce identical surface conditions and hence enable one to interpret experimental observations in a useful way. It is possible to obtain reproducible results, provided the general pretreatment of the electrode is duplicated every time. Hence it is always essential to clean the electrode surface to rid it of the history effects before subjecting it to the appropriate procedures.

2.14.2. Pretreatment of GCE

Glassy carbon electrode has been 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 [83] 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 GCE surface (0.0337 cm^2) was polished to a mirror-like surface with 1 and 0.05 μm 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 potential cycling between -1.4 and 1.8 V at 0.1 Vs^{-1} for 11 multiple cycles in 0.1 M H_2SO_4 until a stable cyclic voltammogram for the cleaned GCE dried was obtained and the electropolymerisation of AZ was carried out with potentiostatic technique. The electro active species was added and the first potential sweep was registered.

2.15. Graphite Pencil Electrode Used as Working Electrode

The graphite pencil electrode (GPE) has been successfully acting as a biosensor in modern electroanalytical field. A porous composite 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 and ease of modification, renewal, low background current and miniaturization, the GPE has good application in elctroanalytical research [84-87]. GPE has a larger active electrode surface area and is therefore able to detect low concentrations and/or volume of the analyte [88]. From our research group reported the GPE is relatively new type of carbon electrode, it is less expensive, more convenient, and renewable compare to the commonly used CPE or GCE [89, 90]. This type of electrode has been successfully applied to design various biosensors [91-94]. Therefore the development of electrochemical sensor was a great challenging thing in electrochemistry research areas.

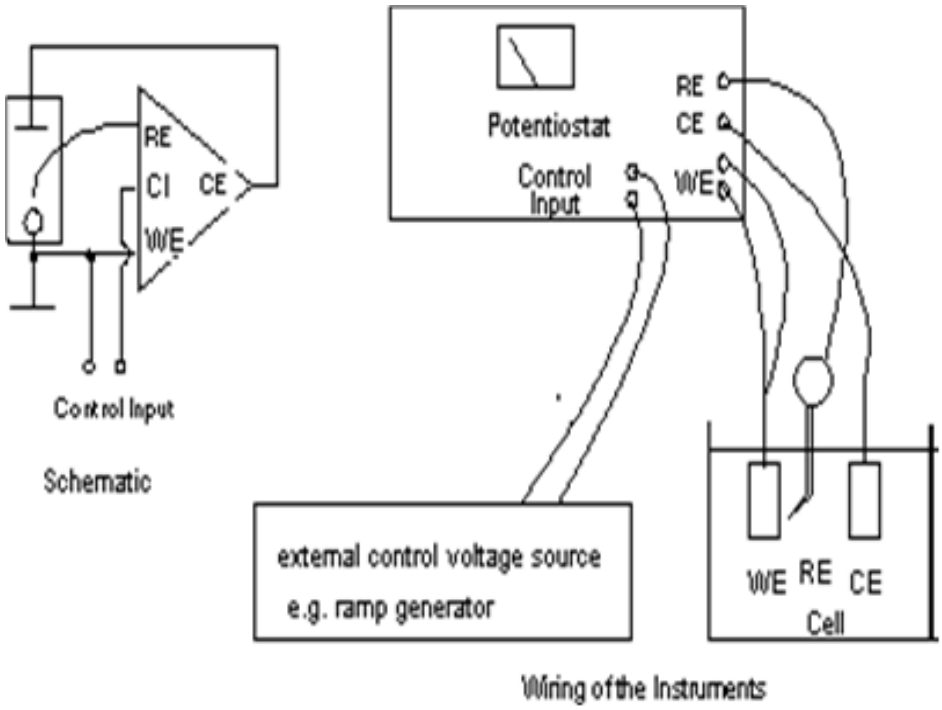


Fig. 2.1. Schematic representation of the experimental setting consisting of an external control voltage source, a potentiostat and the electrochemical cell

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

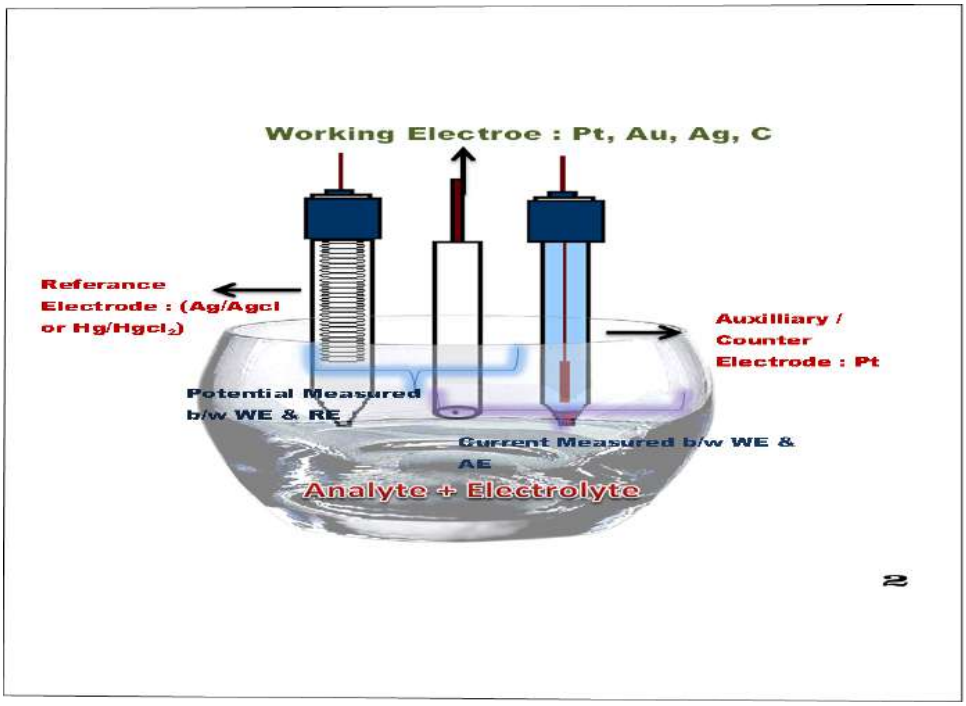


Fig. 2.3. Schematic representation of an assembled electrochemical cell containing an electrolyte solution, sparging device, and the three electrodes (WE, RE, and CE) for cyclic voltammetric experiments

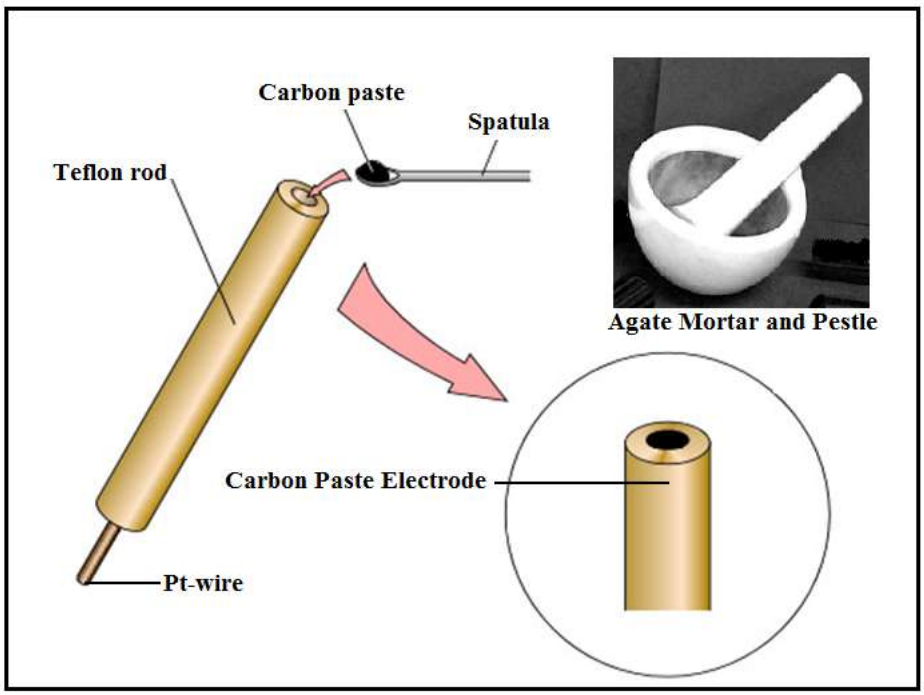


Fig. 2.4. Carbon Paste electrode, Preparation and Filling

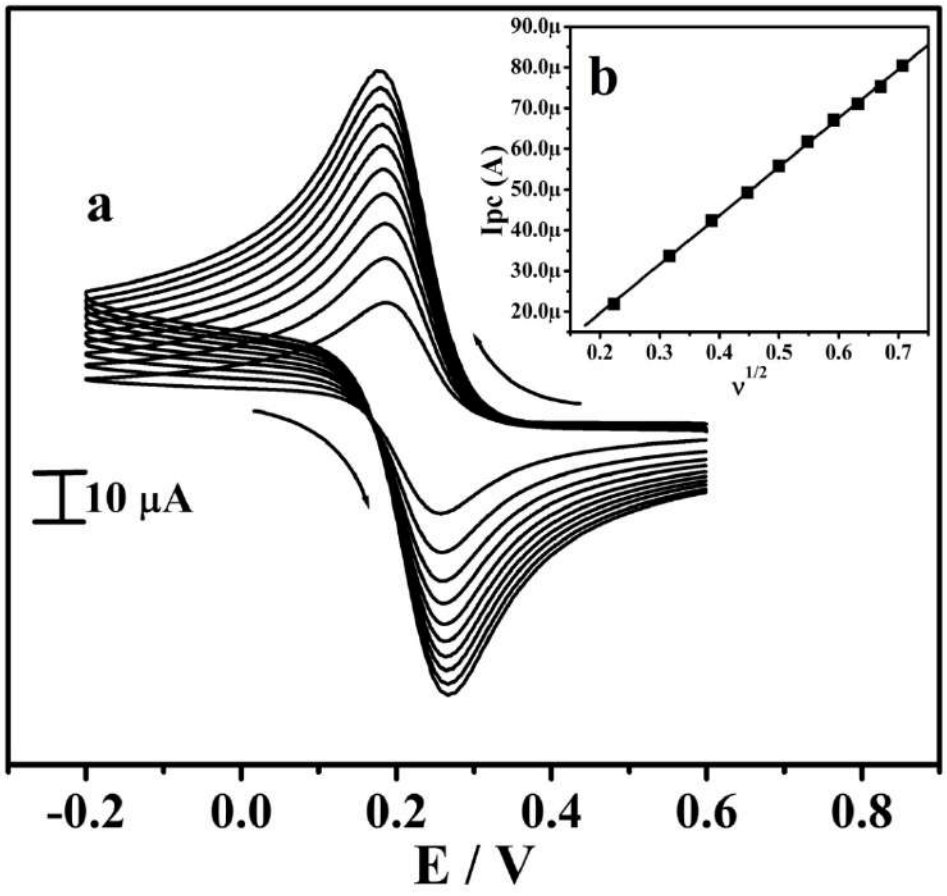


Fig. 2.5. (a) Cyclic voltammograms of 1 mM potassium ferricyanide at bare CPE in 1 M KCl at different scan rate (0.05 to 0.5 Vs^{-1}). (b) Graph of anodic peak current vs square root of scan rate

2.16. References

[1] G. Lauer, R. Abel and F.C. Anson, *Anal. Chem*., 48 (1976) 1616.

[2] S.P. Perone, J.E. Harrar, F.B. Stephens and R.E. Anderson, *Anal. Chem*., 40 (1968) 899.

[3] S.P. Perone, D.O. Jones and W.F. Gutknecht, *Anal. Chem*., 41 (1969) 1154.

[4] S.P. Perone, J.W. Frazer and A. Kray, *Anal. Chem*., 43 (1971) 1485.

[5] S.C. Creason, R.J. Loyd and D.E. Smith, *Anal. Chem*., 44 (1972) 1159.

[6] P.E. Whiston, H.W.V. Born and D.H. Evans, *Anal. Chem*., 45 (1973) 1298.

[7] B. Aalstad and V.D. Parker, *J. Electroanal. Chem*., 112 (1980) 163.

[8] R. Eliason and V.D. Parker, *J. Electroanal. Chem*., 170 (1984) 347.

[9] R.N. Adams, *Anal. Chem*., 30 (1958) 1576.

[10] R.N. Adams, *Electrochemistry at Solid Electrodes*, (Ed. M. Dekker), New York, (1969).

[11] J. Wang, *Electroanalysis*, 11 (1999) 283.

[12] T. Kuwana and W.G. French, *Anal. Chem*., 36 (1964) 241.

[13] L.S. Marcowx, K.G. Prater, B.G. Prater and R.N. Adams, *Anal. Chem*., 37 (1965) 1446.

[14] D. Bauer, M. Gaillochet, *Electrochim. Acta*, 19 (1974) 597.

[15] K. Kalcher, J.M. Kauffmann, J. Wang, I. Svancara, K. Vytras, C. Neuhold and Z. Yang, *Electroanalysis*, 7 (1995) 5.

[16] K. Kalcher, J.M. Kauffmann, J. Wang, I. Svancara, K. Vytras, C. Neuhold and Z. Yang, *Electroanal*., 7 (1995)5.

[17] K. Kalcher, X.H. Cai, G. Kolbl, I. Svancara, K. Vytras, Sb. Ved. Pr. and Vys. Sk, *Chemickotechnol., Pardubice*, 57 (1994) 5.

[18] K. Kalcher, K. Schachl, I. Svancara, K. Vytras and H. Alemu, *Sci. Pap. Univ. Pardubice, Ser., A* (1997).

[19] L. Gorton, *Electroanal*., 7 (1995) 23.

[20] I. Svancara and K. Schachl, *Chem. Listy*, 93 (1999) 490.

[21] K. Kalcher, *Electroanalysis*, 2 (1990) 419.

[22] Z. Q. Zhang, H. Liu, H. Zhang and Y.F. Li, *Anal. Chim. Acta*, 333 (1996) 119.

[23] I. Svancara, K. Kalcher, W. Diewald and K. Vytras, *Electroanalysis*, 8 (1996) 336.

[24] I. Svancara, J. Konvalina, K. Schachl, K. Kalcher and K. Vytras, *Electroanalysis*, 10 (1998) 435.

[25] S.S. Huang, Y.D. Cheng, B.F. Li and G.D. Liu, *Mikrochim. Acta*, 130 (1998) 97.

[26] R. Metelka, K. Vytras and A. Bobrowski, *J. Solid State Electrochem*., 4 (2000) 348.

[27] R. Martinez, M.T. Ramirez and I. Gonzalez, *Electroanalysis*, 10 (1998) 336.

[28] Q.J. Chi, W. Goepel, T. Ruzgas, L. Gorton and P. Heiduschka, *Electroanalysis*, 9 (1997) 357.

[29] D.F. Qui, Q. Zhao, K.Z. Liu and H. Yanjiu, *Electroanalysis*, 8 (1997) 41.

[30] Q.T. Cai and S.B. Khoo, *Electroanalysis*, 7 (1995) 379.

[31] Q.T. Cai and S.B. Khoo, *Analyst*, 120 (1995) 1047.

[32] M.R. Khan, *Analyst*, 123 (1998) 1351.

[33] S.B. Khoo, M.K. Soh, Q.T. Cai, M.R. Khan and S.X. Guo, *Electroanalysis*, 9 (1997) 45.

[34] M. Stadlober, K. Kalcher, G. Raber and C. Neuhold, *Talanta*, 43 (1996) 1915.

[35] M. Stadlober, K. Kalcher and G. Raber, *Sci. Pap. Univ. Pardubice, Ser. A.*, 3 (1997) 103.

[36] M. Stadlober, K. Kalcher and G. Raber, *Electroanalysis*, 9 (1997) 225.

[37] M. Stadlober, K. Kalcher and G. Raber, *Anal. Chim. Acta*, 350 (1997) 319.

[38] M.M.O. Viana, M.P.D. Silva, R. Agraz, J.R. Procopio, M.T. Sevilla and L. Hernandez, *Anal. Chim. Acta.*, 382 (1999) 179.

[39] K. Kalcher, I. Grabec, G. Raber, X.H Cai, G. Tavcar and B. Ogorevc, *J. Electroanal. Chem.*, 386 (1995) 149.

[40] P. Kula, Z. Navratilova and Fresenius, *J. Anal. Chem*., 354 (1996) 692.

[41] Kalcher and M. Stadlober, *Sci. Pap. Univ. Pardubice, Ser. A*, 3 (1997) 163.

[42] I.S. Grabec, M. Kolar, B. Ogorevc, B. Pihlar and Fresenius, *J. Anal. Chem*., 361 (1998) 358.

[43] A. Walcarius, *Anal. Chim. Acta*, 338 (1999) 79.

[44] A. Walcarius, P. Mariaaulle and L. Lamberts, *J. Electroanal. Chem*., 4653 (1999) 100.

[45] Z. Navratilova and P. Kula, *J. Solid State Electrochem*., 4 (2000) 342.

[46] I. S. Grabec, B. Ogorevc and Fresenius, *J. Anal. Chem*., 367 (2000) 701.

[47] Z. Navratilova and P. Kula, *Sci. Pap. Univ. Pardubice, Ser. A*, 3 (1997) 195.

[48] C.M. Wang, H.L. Zhang, Y. Sun and H.L. Li, *Anal. Chim. Acta*, 136 (1998) 133.

[49] C.M. Wang and H.L. Li, *Electroanal*., 10 (1998) 44.

[50] Li, H.L. and Li Fresenius, *J. Anal. Chem*., 363 (1999) 114.

[51] C.M. Wang, B. Zhu and H.L. Li, *Electroanalysis*, 11(1999)183.

[52] A. Walcarius, C. Despas, P. Trens, M.J. Hudso and J. Bessiere, *J. Electroanal. Chem.,* 453 (1998) 249.

[53] A. Walcarius, J. Devoy and J. Bessiere, *Environ. Sci. Technol*., 33 (1999) 4278.

[54] A. Walcarius, N. Luthi, L. Blin, B.L. Su and L. Lamberts, *Electrochim*. *Acta*, 44 (1999) 4601.

[55] H. Yao and G.J. Ramelow, *Talanta*, 45(1998) 1139.

[56] Z.U. Bae, Y.L. Kim, H.Y. Chang and F. Song, *Anal. Sci. Technol*., 8 (1995) 611.

[57] R.Z. Hu, W. Zhang, Fu, W. Zhang and Y.Y. Liu, *Anal. Commun*., 36 (1999) 147.

[58] K. Sugawara, T. Miyasita, S. Hoshi and K. Akatsuka, *Anal. Chim. Acta*, 353 (1997) 301.

[59] L. Hernandez, P. Hernandez, M.H. Blanco and M. Sanchez, *Analyst*, 113 (1988) 41.

[60] W. Diewald, K. Kalcher, C. Neuhold, I. Svancara and X.H. Cai, *Analyst*, 119 (1994) 299.

[61] J. Wang, *Anal. Chim. Acta*. 234 (1990) 41.

[62] A. Moeller, F. Scholz and Fresenius, *J. Anal. Chem*., 356 (1996)160.

[63] F. Alvarez, C. Hua, R. Munde and M.R. Smyth, *Analyst*, 117 (1992) 1125.

[64] Taliene, T. Ruzgas, V. Razumas and J. Kulys, *J. Electroanal. Chem*., 372 (1994) 85.

[65] J. Kulys, P. Klitgaard and H.E. Hansen, *Mat. Sci. Eng*. *C*, 4 (1996) 39.

[66] R.I. Stefan, J.F.Van Staden and H.Y. Aboul-Eneim, *Anal. Lett*., 31 (1998) 1787.

[67] J. Wang, L.D. Hutchins, S. Selim and L.B. Cummins, *Biochem. Bioenerg*., 12 (1984) 193.

[68] R.D. O'Neill, *Analyst*, 119 (1994) 767.

[69] J. Lindquist, *Anal. Chem*., 45 (1973) 1006.

[70] Svancara, K. Kalcher, W. Diewuld and K. Vytras, *Electroanalysis*, 8 (1996) 336.

[71] I. Svancara, M. Pravda, Hvizdalova K. Vytras and K. Kalcher, *Electroanalysis*, 6 (1994) 663.

[72] I. Svancara and K. Vytras, *Anal. Chim. Acta*, 273 (1993) 195.

[73] K. Kalcher, J.M. Kauffmann, J. Wang, I. Svancara, K. Vytras, C. Neuhold and Z. Yang, *Electroanalysis*, 7 (1995) 5.

[74] K. Kalcher, *Electroanalysis*, 2 (1990) 419.

[75] R.N. Adams, *Rev. Polarog. (Kyoto*), 11 (1963) 71.

[76] R.N. Adams, "Electrochemistry at Solid Electrodes", (Ed. Marcel Dekker), New York (1969) 26.

[77] K. Kalcher, J.M. Kauffmann, J. Wang, I.S. Vancara, K. Vytras, C. Neuhold and Z. Yang, *Electroanalysis*, 7 (1995) 5.

[78] S.B. Hocevar, I.S. Vancara, B. Ogorevc and K. Vytras, *Electrochim. Acta.*, 51 (2005) 706.

[79] Galus, R.N. Adams, *J. Phys. Chem*., 67 (1963) 866.

[80] P. Doderhgelm, *J. Electroanal. Chem*., 71 (1976) 109.

[81] H.P. Agarwal, *J. Electrochem. Soc*., 110 (1963) 237.

[82] G.P. Mamatha, B.S. Sherigara, K.M. Mahadevan and B.E.K. Swamy, *Bull. Electrochem*., 21 (2005) 9.

[83] J. Zak and T. Kuwana, *J. Electroanal. Chem*., 150 (1983) 645.

[84] M.R. Majidi, K.A. Zeynali and B. Hafezi, *Int. J. Electrochem. Sci*., 6 (2011) 162.

[85] N. Aladag, L. Trnkova, A. Kourilova, M. Ozsoz and F. Jelen, *Electroanal*., 22 (2010) 1675.

[86] W. Gao, J. Song and N. Wu, *J. Electroanal. Chem*., 576 (2005) 1.

[87] D. Demetriades, A. Economou and A. Voulgaropoulos, *Anal. Chim. Acta*., 519 (2004) 167.

[88] H. Karadeniz, B. Gulmez, F. Sahinci, A. Erdem, G.I. Kaya, N. Unver, B. Kivcak and M. Ozsoz, *J. Pharm. Biomed. Anal.,* 33 (2003) 295.

[89] U. Chandra, B.E.K. Swamy, O. Gilbert, M. P. Char, S. Reddy, S.S. Shankar and B.S. Sherigara, *Chin. Chem. Lett*., 21 (2010) 1490.

[90] U. Chandra, B.E.K. Swamy, O. Gilbert, S. Reddy, S.S. Shankar, M.T. Shreenivas and B.S. Sherigara, *Anal. Bioanal. Electrochem*., 3 (2011) 316.

[91] A. Levent, Y. Yardim and Z. Senturk, *Electrochim. Acta*., 55 (2009) 190.

[92] M. Vestergaard, K. Kerman and E. Tamiya, *Anal*. *Chim. Acta*., 538 (2005) 273.

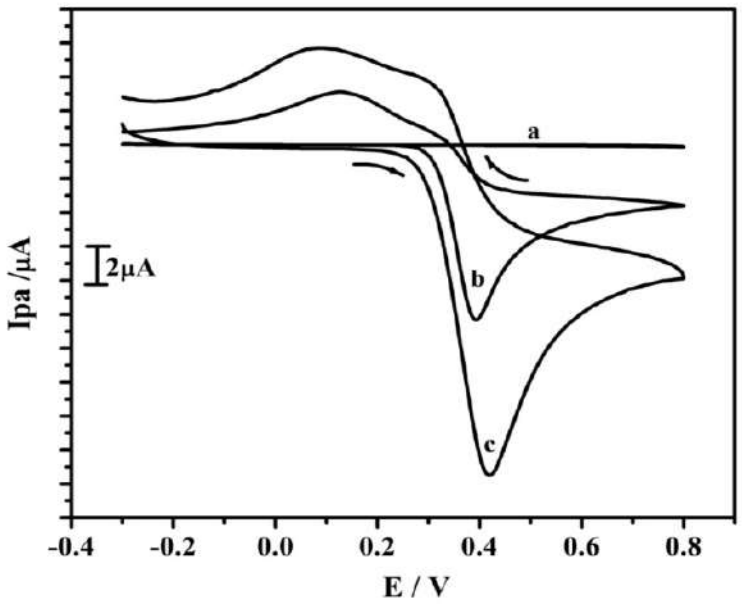
[93] A. Erdem, P. Papakonstantinou and H. Murphy, *Anal. Chem*., 78 (2006) 6656.

[94] M. Ozsoz, A. Erdem, K. Kerman, D. Ozkan, B. Tugrul, N. Topcuoglu, H. Ekren and M. Taylan, *Anal. Chem*., 75 (2003) 2181.

CHAPTER-3

Part-A

Electrosensitive Determination of Paracetamol Using a Poly (glycine) Film Coated Graphite Pencil Electrode: A Cyclic Voltammetric Study



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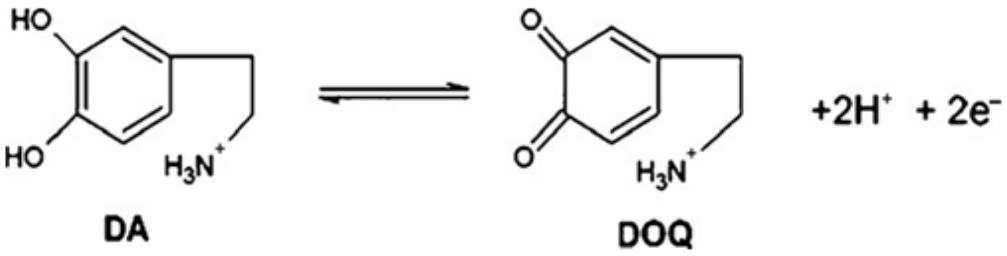
3.1. Introduction

The poly (glycine) film was deposited on the surface of graphite pencil electrode (GPE) by cyclic voltammetric technique. The modified film coated graphite pencil electrode exhibits excellent electrocatalytic activity towards the detection of paracetamol at pH 7.0. The scan rate effect was found to be diffusion controlled electrode process. The concentration effect of paracetamol was linear with current. This developed method can also be applied for some neurotransmitters.

3.1.1. Chemistry and Biosynthesis of Dopamine

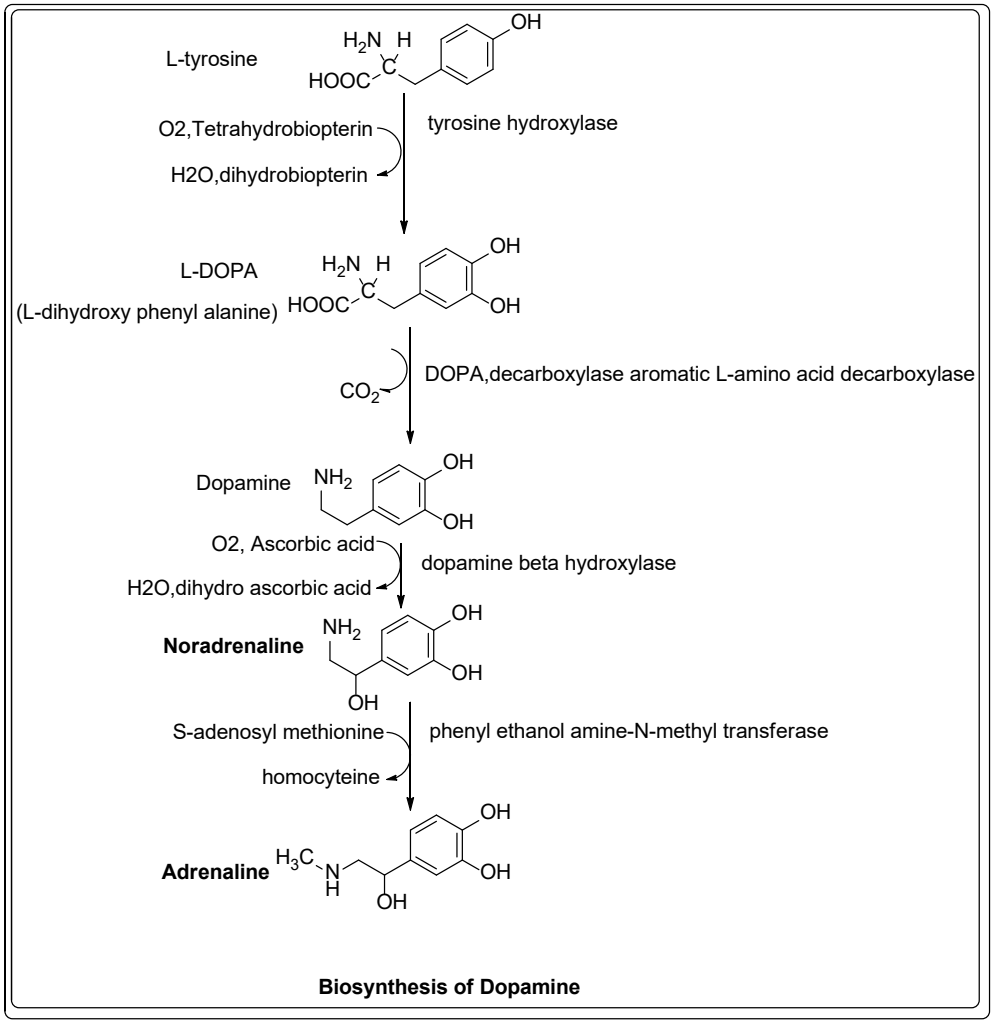
Neurotransmitters allow transmission of nerve impulses between neurons through synapse. On the behavior of neurons they are classified as excitatory or inhibitory, inhibitory neurotransmitters calm the brain and excitatory neurotransmitters stimulate the brain. Dopamine is a unique neurotransmitter as it possesses both excitatory and inhibitory classification. Dopamine vital function lies in regulating attention, cognition, movement, emotional response, sleep, mood, attention, memory, hormonal processes and ability to experience pleasure, pain cognition, memory [1, 2]. In physiological condition in brain tissue and body fluids, dopamine occurs as large organic cations [3-7]. Dopamine (3,4-dihydroxyphenethylamine) is one of the most important neurotransmitters in the brain, belongs to the catecholamine family and it performs diverse functions. Catecholamines have a ring-shaped chemical structure called a catechol with the molecular formula $C_6H_4(OH)_2$ and an amine functional group. In 1950s dopamine was discovered as neurotransmitter [8], in 1958 Arvid Carlsson and Nilsake Hillap discovered the function of dopamine as a neurotransmitter in the mammalian central nervous system. DA is generated by neurons in the various region of the mammalian brain such as the substantial nigra and the ventral segmental area, major dopamine containing area of the brain is the *Corpus straiatum* [9]. DA is also a neurohormone released by the hypothalamus. Its main function as a hormone is to inhibit the release of prolactin from the anterior lobe of the pituitary. Dopamine can be supplied as a medication that acts on the sympathetic nervous system, producing effects such as increased heart rate and blood

pressure. However, since dopamine cannot cross the blood-brain barrier, dopamine given as a drug does not directly affect the central nervous system. Low levels dopamine in the central nervous system is a marker of Parkinson's disease, solitary strategy for treating Parkinson's disease is the administration of a synthetic DA precursor L-DOPA (levodopa) to increase dopamine synthesis in the remaining neurons. The dopamine undergoes oxidation to form dopaquinone as shown in scheme 3.1.



Scheme 3.1. Electrochemical oxidation mechanism of dopamine

Dopamine is synthesized in the body from the compound L-dihydroxyphenylalanine (L-dopa) via the enzyme dopa decarboxylase as shown in Scheme 3.2. Dopamine is biosynthesized in the body (mainly by nervous tissues and medulla of the adrenal glands) first by the hydroxylation of the amino acid L-tyrosine to L-DOPA via the enzyme tyrosine 3-monooxygenase, also known as tyrosine hydroxylase, then by the decarboxylation of L-DOPA by aromatic L-amino acid decarboxylase. In some neurons, dopamine is further processed into norepinephrine by dopamine beta hydroxylase. Neuron is a nerve cell in the nervous system that is responsible for processing and transmitting messages by electrochemical signaling. In neurons dopamine is packaged after synthesis into vesicles, which are then released into the synapse in response to a presynaptic action potential dopamine, is inactivated by reuptake via the DA transporter, then enzymatic breakdown by catechol-O-Methyl transferase(COMT) and monoamine oxidase (MAOA and MAOB). DA that is not broken down by enzymes is repacked into vesicles for reuse. Dopaminergic neurons are present chiefly in the ventral segmental area (VTA) of the mid brain, the substantial nigra pars compact and the accurate nucleus of the hypothalamus.



Scheme 3.2. Biosynthesis of dopamine

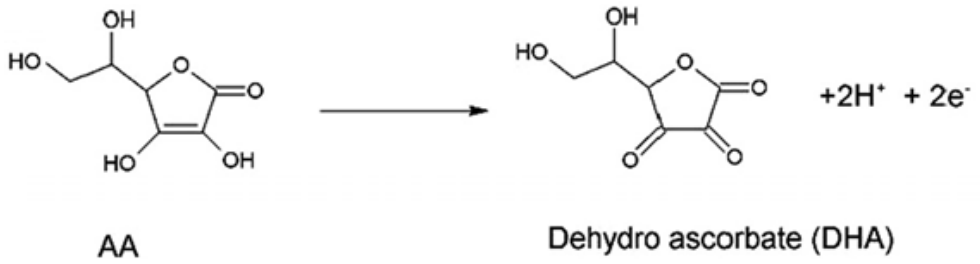
3.1.1.1. Biological Relevance of Dopamine

Dopamine is more associated with anticipatory desire and motivation. As opposed to actual consummator pleasure [10], Dopaminergic neurons of the midbrain are the main source of DA in the brain. Dopamine has been shown to be involved in the control of movements, the signaling of error in prediction of reward, motivation, and cognition. Cerebral DA depletion is the hallmark of several important diseases such as Parkinson's disease, schizophrenia, autism and attention deficit, hyper activity disorder as well as drug abuse [11]. The schizophrenia involves altered levels of dopamine activity, and most antipsychotic drugs used to treat this are dopamine antagonists which reduce

dopamine activity [12], similar dopamine antagonist drugs are also some of the most effective anti-nausea agents. Restless legs syndrome and attention deficit hyperactivity disorder (ADHD) are associated with decreased dopamine activity [13]. Dopamine is significantly concerned in so many physiological, behavioral and pathological functions, deficiency of dopamine implicated in several neurological disorders [14-17]. Humiliation of dopamine, motor symptoms, refuse of cognitive function and other problems lead to reduce the efficiency and function of brain and body. The complete depletion of dopamine in the central nervous system is strongly related with illness like Parkinson's disease. In Parkinson's disease the dopaminergic neurons in the brain slowly degenerate and eventually die. DA has many role in the brain, dopamine influence on the basal ganglia motor loop which intern influence the way the brain control movements. Neurotransmitter release is beginning by an electrical impulse called an action potential. each neuron has resting membrane when a suitable neurotransmitter binds to receptors on the cell bodies, ion channels open, permits an influx of Na^+ that changes the membrane potential and start an action potential or firing. It then spread down the axon to the terminal [18]. This firing causes voltage-gated Ca^{2+} channels to open in the terminals. The resultant Ca^{2+} influx triggers the vesicles to fuse with the cell membrane and release their contents, processes termed as an exocytosis, because some vesicles are docked adjacent to the membrane, exocytosis occurs on milliseconds time scale [19]. Neurotransmission involves the conversion of an electrical impulse to a chemical event and then to another electrical event, is extremely rapid. Action potentials and neurotransmitters represents the bricks with which the internal representation of the external world is build.

3.1.2. Chemistry and Biosynthesis of Ascorbic Acid

Ascorbic acid (AA) is a sugar of molecular weight 176.13. The molecule, which is partially ionized at physiological pH, contains two acid-ionized groups (pK_a 4.04 and 11.34). Though stable to air and light when dry, in aqueous solution it is powerful reducing agent, with redox potential of about 0.05 V at 30^{o}C and pH 7.4. It readily undergoes reversible oxidation to dehydro ascorbic acid (Scheme 3.3).



Scheme 3.3. Oxidation Mechanism of Ascorbic acid

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

3.1.2.1. Biological Relevance of Ascorbic Acid

This a water-soluble vitamin which is important in forming collagen, a protein that gives structure to bones, cartilage, muscle, and blood vessels. It also helps maintain capillaries, bones, and teeth and aids in the absorption of iron. AA, a reducing agent, is necessary to maintain the enzyme prolyl hydroxylase in an active form, most likely by keeping its iron atom in a reduced state. The precursor molecule to the protein collagen, procollagen, contains an unusual amino acid sequence in that every third amino acid is a glycine and contains a high frequency of two amino acids not found in any other proteins - hydroxyproline and hydroxylysine. These latter two amino acids are converted from proline and lysine, respectively, after the procollagen molecule has been synthesized. The hydroxylation of proline and lysine in procollagen is carried out by the enzyme prolyl hydroxylase using AA as a cofactor. The natural form of the vitamin is the L-isomer. AA plays an important role as a component of enzymes involved in the synthesis of collagen and carnitine; however, its most vital role is as a water-soluble vitamin in the human body [20, 21]. AA is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical. As a scavenger of reactive oxygen and nitrogen oxide species, AA has been shown to be effective against the superoxide radical

ion, hydrogen peroxide, the hydroxyl radical and singlet oxygen [22]. AA protects folic acid reductase, which converts folic acid to folinic acid, and may help release free folic acid from its conjugates in food. AA facilitates the absorption of iron. It helps maintain elasticity of the skin aids the absorption of iron and improves resistance to infection, treatment of scurvy and may prevent the occurrence and development of cancer.

3.1.3. Review of Electrochemistry of Paracetamol

Paracetamol, (N-acetyl-p-aminophenol) known as acetaminophen, is a pain killer that is popular throughout the world because it is remarkably safe to the stomach. Paracetamol (PC) was firstly introduced into medicine as an antipyretic/analgesic by Von Mering in 1893. Prior to this cinchona bark, which was also used to make the anti-malaria drug quinine, had been used to treat fevers. paracetamol is one of the most commonly used analgesics in pharmaceutical formulations, for the reduction of fever and also as a pain killer for the relief of mild to moderate pain associated with headache, backache, arthritis and postoperative pain in adults and children. It is the most used medicine after acetylsalicylic acid in many countries as an alternative to aspirin and phenacetin [23-27].

The graphite pencil electrode (GPE) 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, electrical conductivity, good mechanical rigidity, low cost, electrochemical reactivity, ease of modification, renewal, low background current and miniaturization, the GPE has good application in the analysis of neurotransmitter and in the detection of traces of metal ions [28-31]. GPE has a larger active electrode surface area and is therefore able to detect low concentrations and or volume of the analyte. This type of electrode has been successfully applied to design various biosensors [32-35].

Electropolymerisation is a good approach to immobilize polymers to prepare polymer modified electrodes (PME's) as adjusting the electrochemical parameters can coated film thickness permeation and charge transport characteristics. Polymer-modified electrodes have many advantages in the detection of analytes because of its selectivity

and homogeneity in electrochemical deposition, strong adherence to electrode surface and chemical stability of the film [36-38].

Surfactants, due to their favorable physicochemical properties are extensively used in many fields of technology and research, i.e. in pharmacy, in cosmetics, textile industry, agriculture, biotechnology. Normally surfactant is a linear molecule with a hydrophilic (attracted to water) head and a hydrophobic (repelled by water) end. Surfactants, a kind of amphiphilic molecules with a hydrophilic head on one side and a long hydrophobic tail on the other, have been widely applied in electrochemistry to improve the property of the electrode solution interface and also improve the detection limits of some bimolecular. The results showed that the electrochemical responses of these compounds were greatly enhanced in the presence of trace surfactants [39-42].

In the present work, it describes the electrochemical investigation of paracetamol on poly (glycine) modified graphite pencil electrodes. Many of the analytes have been detected by cyclic voltammetric technique by our research group [43-45]. The aim of the work reported here was to investigate the electrochemical properties of paracetamol on graphite pencil electrode and SDS/GPE as well as the electrochemical characterization of electrodes by cyclic voltammetric technique. It can be applied to routine investigations of pharmaceutical preparations in the form of tablets by using the cyclic voltammetric technique.

3.2. Experimental Part

3.2.1. Reagents and Chemicals

The pencil-lead rods were HB 0.5 mm in diameter and 6 cm length purchased from local bookstore. Sodium dodecyl sulphate, Sodium dihydrogen orthophosphate dihydrate, di-sodium hydrogen phosphate anhydrous was obtained from Merck. Glycine and Paracetamol obtained from Hi-media, 25×10^{-4} M Glycine and 25×10^{-4} M paracetamol and 0.2 M phosphate buffer solution (PBS) solutions all stock solutions were prepared with double distilled water. All experiments were performed at room temperature.

3.2.2. Apparatus

The electrochemical experiments were carried out using a CHI-660c (CH Instrument-660 electrochemical workstation). All the experiments were carried out in a conventional three electrochemical cell. The electrode system contained a working electrode was bare GPE and poly (glycine) modified GPE (0.5 mm in diameter), a platinum wire as counter electrode and saturated calomel electrode (SCE) as reference electrode. All potentials reported were versus the SCE.

3.2.3. Preparation of Poly (glycine) Modified GPE

The poly (glycine) modified GPE was prepared by 1mM glycine solution was placed in the electrochemical cell with 0.2 M PBS. The GPE was scanned by immersing 3 mm length in that solution. Electropolymerisation was achieved by the formation of film that grew between –100 mV to 1600 mV at a scan rate of 50 mVs^{-1} for 10 cycles by using cyclic voltammetry [46, 47].

3.3. Results and Discussion

3.3.1. Electrochemical Polymerization of Glycine on GPE

The Figure 3.1 shows the cyclic voltammograms for electro polymerization of glycine on the surface of GPE in the range from –100 to 1600 mV at the sweep rate of 50 mVs^{-1} at 10 multiple cycles. For 10 cycles the glycine was deposited on the surface of GPE by electropolymerization. After electropolymrization, the modified electrode was carefully rinsed with double distilled water and was used for electrochemical analysis.

3.3.2. Electrochemical Characterization of Poly (glycine) Modified GPE

Cyclic voltammetric technique was used for the estimation of electroactive surface of the modified electrode. Figure 3.2 shows the cyclic voltammograms of 1mM $K_3[Fe(CN)_6]$ at bare GPE (dashed line) and at poly (glycine) modified GPE (solid line) in 1M KCl at scan rate of 50 mVs^{-1}. Well defined oxidation and reduction peaks due to the Fe^{2+}/Fe^{3+} redox couple were observed. A comparison between the anodic and cathodic

peaks for ferricyanide shows that (ΔEp), is 60 mV for poly (glycine) modified GPE and 97 mV for bare GPE. As ΔEp is a function of the rate of electron transfer, the lower ΔEp, shows the higher electron transfer rate. The results obtained greatly improved the voltammetric response of potassium ferricyanide at poly (glycine) modified GPE reflected by the enlargement of peak current and the decline of peak potential. This indicates that the surface property of the modified electrode has been significantly changed. Based on the above observations the poly (glycine) modified GPE had favorable and stable electrochemical behavior. It might be used as a chemically modified electrode to explore electrochemical sensor applications.

3.3.3. Electrochemical Oxidation of Paracetamol at Poly (glycine) Modified GPE

Electrochemical study of paracetamol was studied by using cyclic voltammetric technique. Figure 3.3 shows the cyclic voltammograms of 2 mM paracetamol in PBS (pH 7) at scan rate of 50 mVs^{-1} at bare GPE (curve b), poly (glycine) modified GPE (curve c) and blank solution (curve a). Figure 3.3 shows that there is no characteristic peak of the modifier in the blank PBS solution and exhibited the redox nature of the paracetamol in the PBS solution. At bare GPE, for paracetamol shows significant increases of oxidation peak currents. At the poly (glycine) modified GPE for paracetamol shows increase in oxidation peak currents as compared to bare. This results shows that poly (glycine) modified GPE good catalytic effect and is irreversible electrode.

3.3.4. Effect of Scan Rate

Investigation of the effect of scan rate on the electrochemical oxidation of paracetamol at poly (glycine) modified GPE by using cyclic voltammetric technique. Figure 3.4a shows the scan rate was increased from 50 to 300 mVs^{-1} the anodic peak current was increased with increase in scan rate. The graph of Ipa vs. scan rate was plotted in Figure 3.4b the correlation co-efficient was found to be 0.99309 and Figure 3.4c shows the graph of Ipa vs. square root of scan rate was plotted. The resulted graph shows good linearity with the correlation coefficient was found to be 0.99965, suggested that the electrode process was controlled by diffusion [48].

3.3.5. Effect of Surfactant

The Figure 3.5 shows the graph of anodic peak current of paracetamol vs. concentration of SDS. The electrochemical response of paracetamol at SDS mobilized GPE at pH 7. The SDS/GPE was mobilized by adding 5 to 50 µL at 50 µL shows high current signal as the SDS concentration increases. This is due to subsequently electrostatic interaction between adsorbed substrate and hydrophobic character at SDS/GPE. This result shows that the method of modification shows maximum increases in the current signals [49, 50].

3.3.6. Electrochemical Response of Paracetamol at SDS/ GPE by Mobilization Method

The Figure 3.6 shows the cyclic voltammograms were recorded for a GPE containing 2mM paracetamol in Phosphate buffer solution of pH 7 at the scan rate of 50 mVs^{-1}. At bare GPE for paracetamol shows low redox peak currents (dashed line) and SDS/GPE (solid line) shows good increase in peak currents for paracetamol. This result shows that SDS (anionic surfactant) exhibited good electrosensing effect in the presence of paracetamol.

3.3.7. Effect of Concentration of Paracetamol

The electrocatalytic oxidation of paracetamol was carried out by varying the concentration at poly (glycine) modified GPE. Figure 3.7a shows that by increasing the concentration of paracetamol from 1 to 6 mM, the anodic peak current and cathodic peak current goes on increasing with negligible shifting anodic peak potential towards positive and cathodic peak potential towards negative side. The graph of anodic peak current vs. concentration of paracetamol was plotted and it shows increase in electrochemical peak currents (Fig. 3.7b). The obtained correlation coefficient was found to be 0.9986 and detection limit for paracetamol in the lower region was found to be 0.45 mM. The detection limit was calculated by using the formulas (1) [51-53], where S is the standard

deviation and M is the slope obtained from the three calibration plots. From the data, a lower limit of detection (LOD) can be achieved using the proposed method [54-57].

$$LOD=3S/M$$

3.3.8. Conclusion

The modified poly (glycine) GPE shows electrochemical sensor was used for the electrochemical determination of paracetamol. The effect of scan rate and concentration shows overall electrode process was diffusion controlled. The modified electrode shows good sensor application for electrochemical investigation of paracetamol with detection limit 0.45 mM. The surfactant SDS modified electrode shows significant increases for the determination of paracetamol. The proposed modified electrodes shows selectivity, sensitivity and stability towards paracetamol and the same method can also be applied for some bioactive molecule.

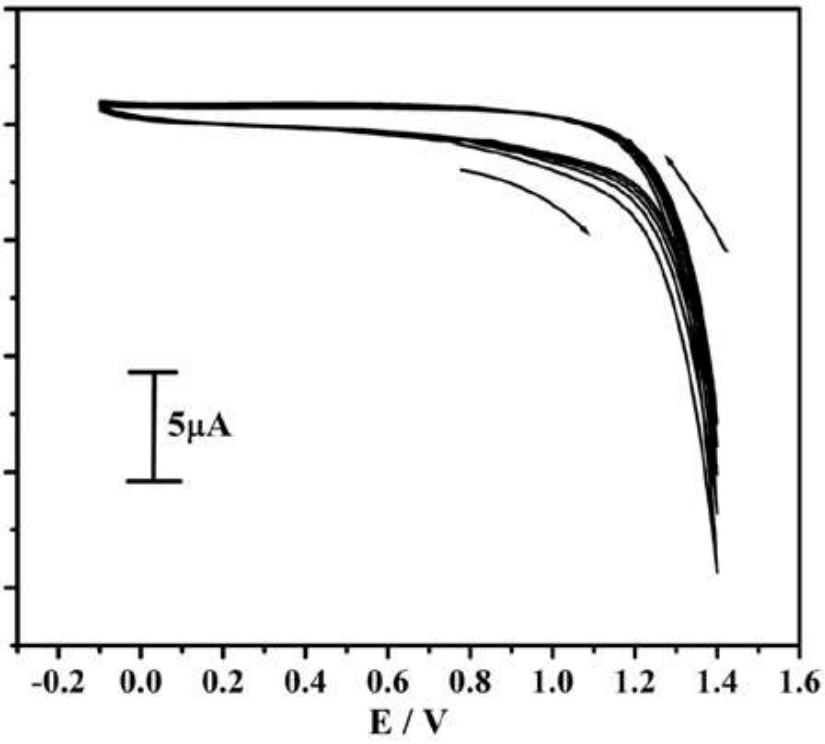


Fig. 3.1. Cyclic voltammogram for the electrochemical polymerization of glycine at the Graphite pencil electrode

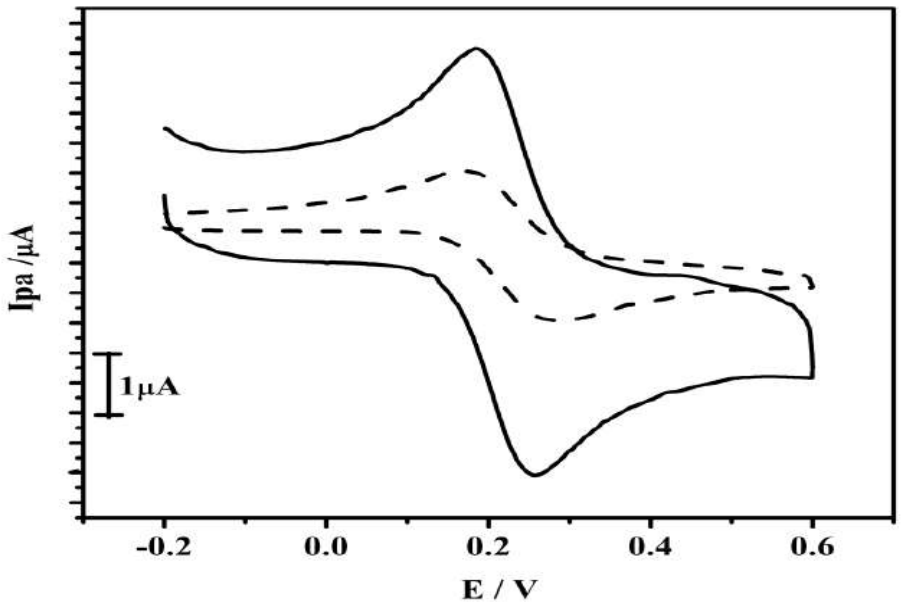


Fig. 3.2. Cyclic voltammograms for the electrochemical responses of $K_3[Fe(CN)_6]$ at bare (dotted line) and poly (glycine) modified GPE (solid line) in 1M KCl containing1 mM $K_3[Fe(CN)_6]$ at scan rate 50 mVs^{-1}

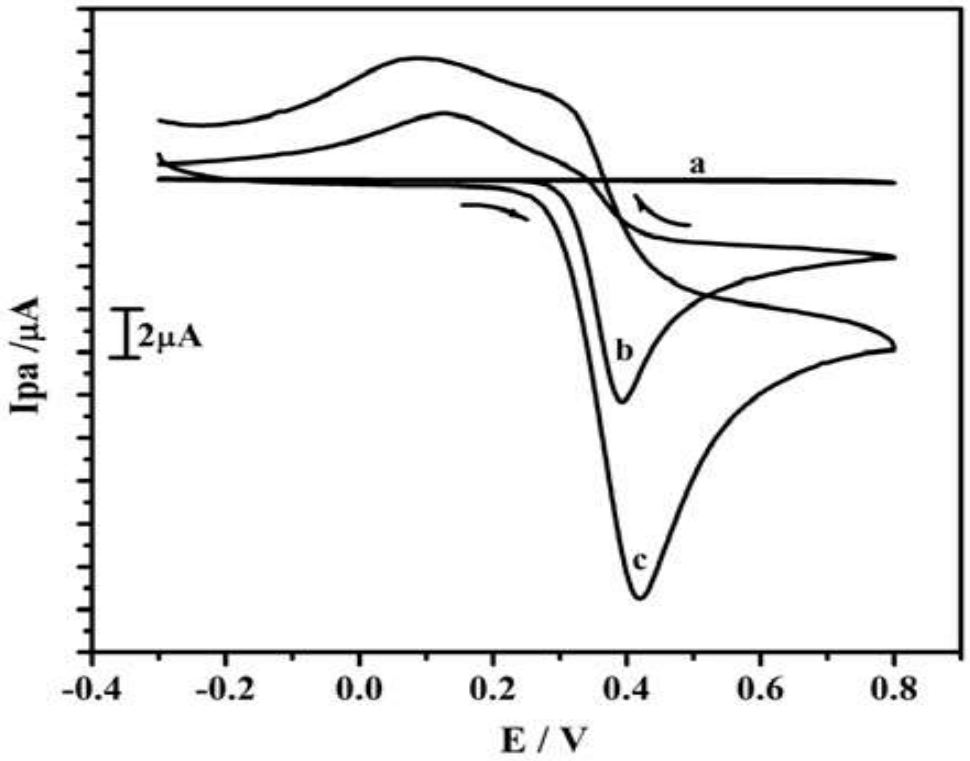


Fig. 3.3. Cyclic voltammograms of 2×10^{-4} M paracetamol obtained at the bare GPE (curve b), Poly (glycine) modified GPE (curve c) and in the absence of paracetamol at bare GPE (curve a) in 0.2 M PBS (pH.7.0) at scan rate 50 mVs^{-1}

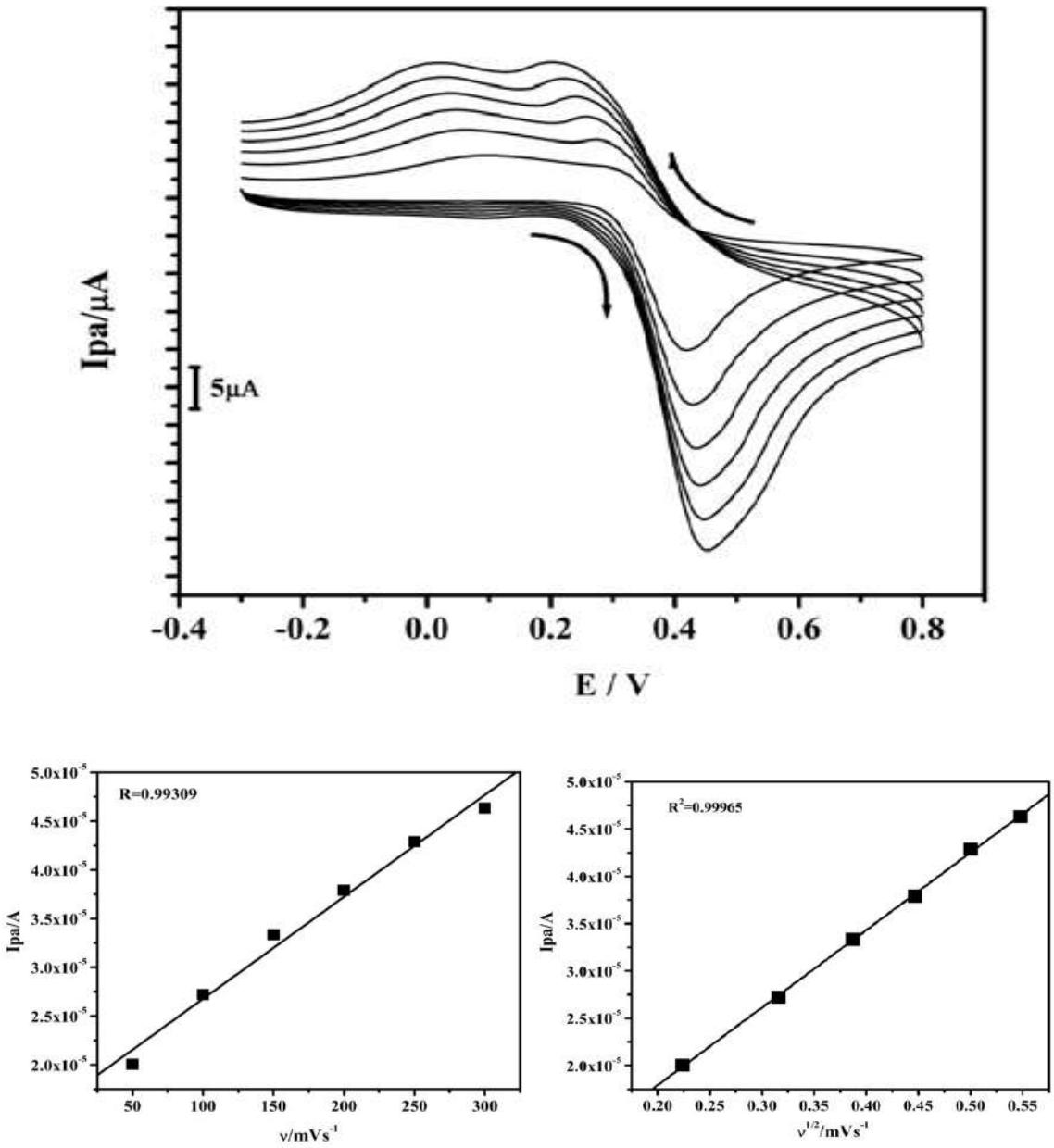


Fig. 3.4a. Cyclic voltammograms of 2×10^{-4} M paracetamol on the Poly (glycine) modified GPE at different scan rates (a–f: 50, 100, 150, 200, 250, 300 mVs^{-1}) in 0.2 M PBS (pH 7.0); b) shows the plot of the anodic peak current versus scan rate; c) shows the plot of the anodic peak current versus square root of scan rate

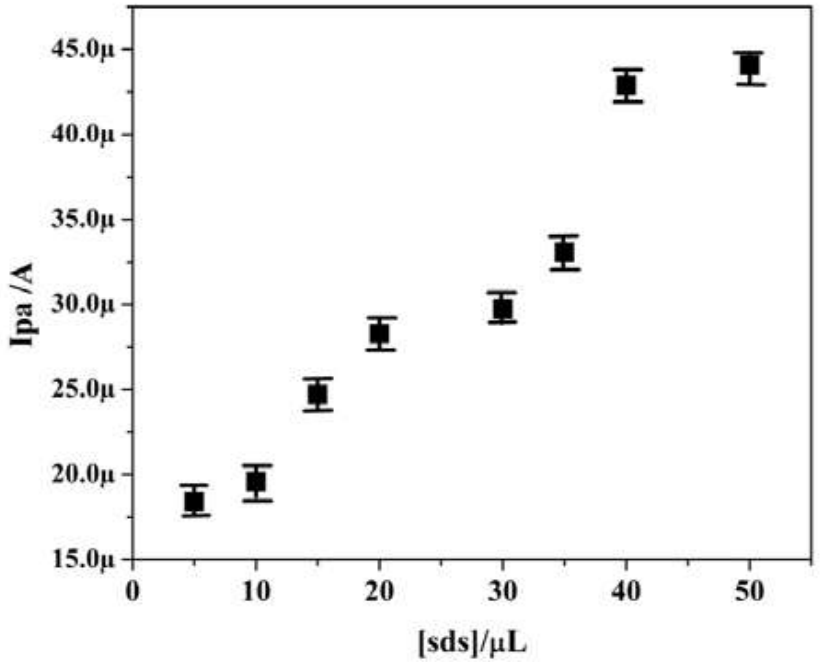


Fig. 3.5. Plot of anodic peak current versus concentration of SDS at scan rate 50 mVs^{-1}

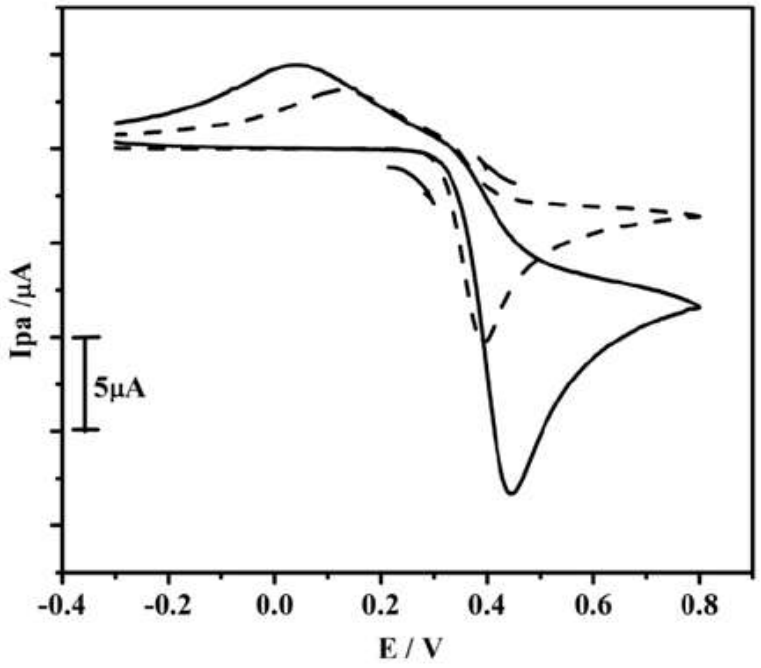


Fig. 3.6. Cyclic voltammograms of 2×10^{-4} M paracetamol at bare (dotted line) and SDS/GPE (solid line) scan rate 50 mVs^{-1} in 0.2 M PBS pH 7.0

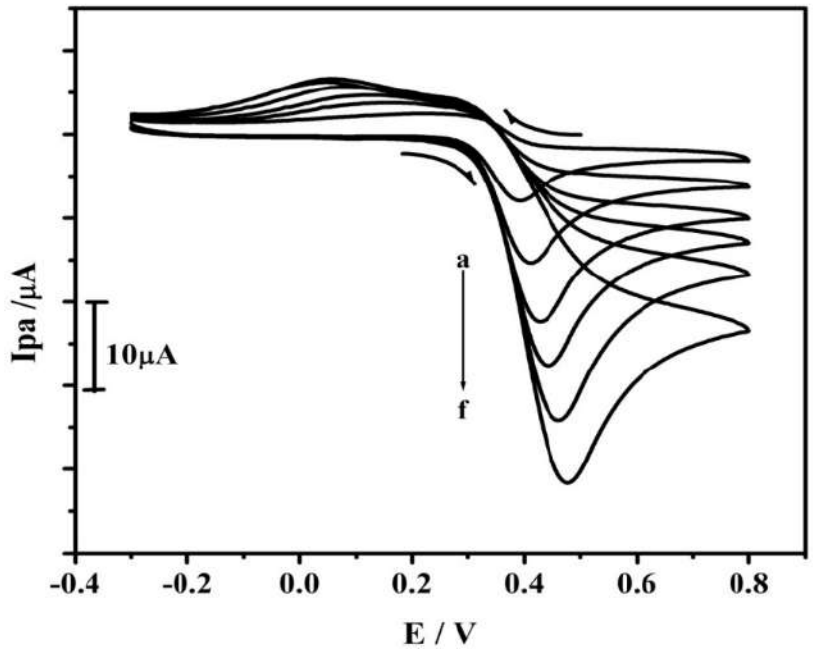


Fig. 3.7a. Cyclic Voltammograms of different concentration of paracetamol (a-f: 1 to 6 mM) at Poly (glycine) modified GPE in 0.2 M PBS. scan rate 50 mVs^{-1}

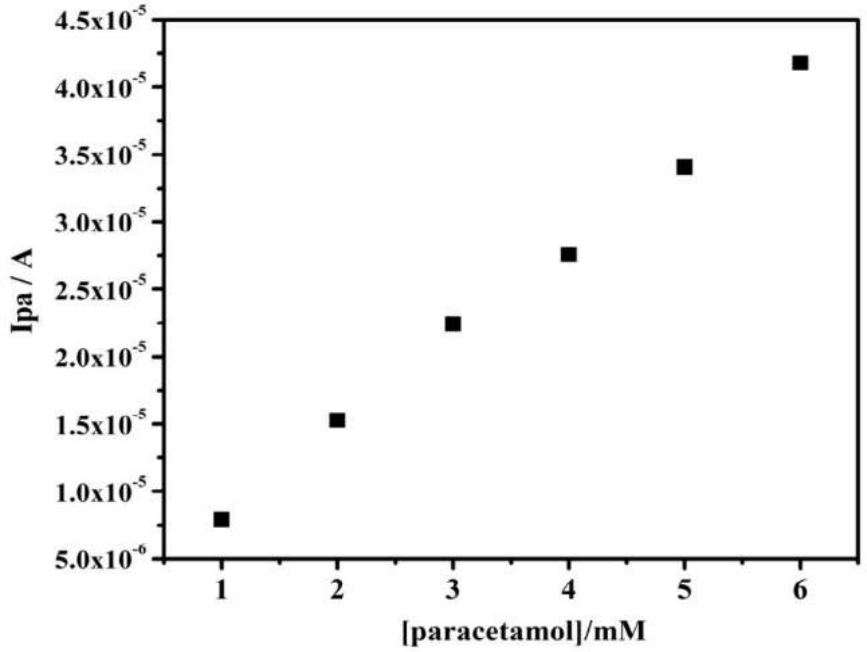


Fig. 3.7b. Plot of anodic peak current versus concentration of paracetamol

3.3.9. References

[1] R.M. Wightman, C. Amatorh, R.C. Engstrom, P.D. Hale, E.W. Kristensen, W.G. Kuhr and L.J. May, *Neuroscience*, 25 (1988) 513.

[2] S.S. Castro, R.J. Mortimer, M.F. De Oliveira and N.R. Stradiotto, *Sensors*, 8 (2008) 1950.

[3] K. Jackowska and P. Krysinski, *Anal. Bioanal. Electrochem*., 405 (2013) 3753.

[4] D.L. Robinson, A. Hermans, A.T. Seipel and R.M. Wightman, *Chem. Rev*., 108 (2008) 2554.

[5] R.L. McCrery, *Chem. Rev*., 108 (2008) 2646.

[6] C. Iuga, J. Alvarez-Idaboy and A. Vivier-Bunge, *J. Phy. Chem., B.,* 115 (2011) 12234.

[7] S.C. Avendaño, G.A. Angeles, M.T.R. Silva, G.R. Pina, M.R. Romo and M.P. Pardavé, *J. Electroanal. Chem*., 609 (2007) 17.

[8] G.B. Bunney, Dopamine, In: *Neurotransmitter Actions in the Vertebrate Nervous System*, (Ed. M. Barker), Rogawski, Plenum Press, New York (1985).

[9] B.N. Engl, *J. Med*., 321 (1989) 1725.

[10] S. Pecina, B. Cagniard, K. Berridge, J. Aldridge and X. Zhuang, *J. Neurosci*., 23 (2003) 9395.

[11] O. Arias-Carrion and E. Poppel, *Act Neurobiol. Exp*., 67 (2007) 481.

[12] J. Moncrieff, The Myth of the Chemical Cure : A Critique of Psychiatric Drug Treatment, Palgrave MacMillan, New York, (2008).

[13] N.D. Volkow, G.J Wang, S.H. Kollins, T.L. Wigal, J.H. Newcorn, F. Telang, J.S. Fowler, W. Zhu, J. Logan, Ma Y, K. Pradhan, C. Wong and J.M. Swanson, *JAMA*., 302 (2009) 1084.

[14] J. Girault and P. Greengard, *Arch. Neurol*., 61 (2004) 641.

[15] O. Hornykiewicz, *Pharmacol. Rev*., 18 (1966) 925.

[16] W. Schultz, *Trends Neurosci*., 30 (2007) 203.

[17] N. Hnasko and R. Ben-Jonathan, *Endocr. Rev*., 22 (2001) 724.

[18] A.A. Grace and B.S. Bunny, *Science*, 210 (1980) 654.

[19] N. Pothos, V. Davila and D. Sulzer, *J. Neurosci*., 18 (1998) 4106.

[20] H. Sies and W. Stahl, *Am. J. Clin. Nutr.,* 62 (1995) 1315.

[21] S.M. Levine, K.R. Dhariwal, R.W. Welch, Y. Wang and J.B. Park, *Am. J. Clin. Nutr*., 62 (1995) 1347.

[22] S.P. Weber, A. Bendich and Schalch, *Int. J. Vit. Nutr. Res*., 66 (1996) 19.

[23] R.N. Goyal and S.P. Singh, *Electrochim. Acta.,* 51 (2006) 3008.

[24] M.E. Bosch, A.J.R.S´anchez, F.S. Rojas and C.B. Ojeda, *J. Pharm. Biomed. Anal.,* 42 (2006) 291.

[25] L. Ozcan and Y. Sahin, *Sens. Actuators B,* 127 (2007) 362.

[26] J.M. Smith, W.O Robert, S. M. Hall, T.A. White and A.A. Gibertson, *Br. Med. J.,* 1 (1978) 331.

[27] B.B. Brodie and J. Axelrod, *J. Pharm. Exp. Ther*., 94 (1948) 29.

[28] W. Gao, J. Song and N. Wu, *J. Electroanal. Chem*., 576 (2005) 1.

[29] D. Demetriades, A. Economou and A. Voulgaropoulos, *Anal. Chim. Acta.,* 519 (2004) 167.

[30] A. Levent, Y. Yardim and Z. Senturk, *J. Electrochim. Acta.,* 55 (2009) 190.

[31] M. Vestergaard, K. Kerman and E. Tamiya, *Anal. Chim. Acta.,* 538 (2005) 273.

[32] A. Erdem, P. Papakonstantinou and H. Murphy, *Anal. Chem*., 789 (2006) 6656.

[33] M. Ozsoz, A. Erdem and K. Kerman, *Anal. Chem*., 75 (2003) 2181.

[34] J. Wang, A.N. Kawde and E. Sahlin, *Analyst,* 125 (2000) 5.

[35] U. Chandra, B.E.K. Swamy and O. Gilbert, *Chinese Chem. Lett*., 21 (2010) 1490.

[36] P.R. Roy, T. Okajima and T. Ohsaka, *J. Bioelectron. Chem*., 59 (2003) 11.

[37] J.G. Manjunatha, B.E.K. Swamy, G.P. Mamatha, S. Shankar, O. Gilbert, B.N. Chandrashekar and B.S. Sherigara, *Int. J. Electrochem. Sci*., 5 (2010) 1236.

[38] M.B. Deepa, G.P. Mamatha, B.S. Sherigara and Y. Arthobanaik, *Int. J. Res. Chem. Environ*., 2 (2012) 153.

[39] C.C. Vishwanath, B.E.K. Swamy, T.V. Sathisha and G.M. Madhu. *J. Anal. Bioanal. Electrochem.*. 5 (2013) 341.

[40] H. Jerabkova, K. Blanka and J. Nahlik, *Int. Biodeteriorat*., 44 (1999) 233.

[41] J. Wang, B.Z. Zeng, C. Fang and X.Y. Zhou, *J. Electroanal. Chem*., 484 (2000) 88.

[42] E. Niranjana, B.E.K. Swamy, R. Raghavendra Naik, B.S. Sherigara and H. Jayadevappa, *J. Electroanal. Chem*., 631 (2009) 1.

[43] B.N. Chandrasheka, B.E.K. Swamy, M. Pandurangachar, S.S. Shankar, O. Gilbert, J.G. Manjunatha and B.S. Sherigara, *Int. J. Electrochem. Sci*., 5 (2010) 578.

[44] B.E.K. Swamy, E.V.S. Subrahamanyam, B.S. Sherigara and G. Venkateswaran, *Bull. Electrochem*., 16 (2000) 533.

[45] B.N. Chandrashekar, B.E.K. Swamy, M. Pandurangachar, T.V. Sathisha, and B.S. Sherigara, *Anal. Bioanal. Electrochem.,* 3 (2011) 227.

[46] O. Gilbert, U. Chandra, B.E.K. Swamy, M. Panduranga Char, C. Nagaraj and B.S. Sherigara, *Int. J. Electrochem. Sci*., 3 (2008) 1186.

[47] O. Levent, S. Mutlu and S. Yucel, *Sensors,* 8 (2008) 5792.

[48] J. Wang and A.N. Kawde, *Anal. Chim. Acta.,* 431 (2001) 219.

[49] M. Fojta, L. Havran, S. Billova, P. Kostecka, M. Masarik and R. Kizek, *Electroanalysis*, 15 (2003) 431.

[50] M. Pandurangachar, B.E.K. Swamy, B.N. Chandrashekar and B.S. Sherigara, *Int. J. Electrochem. Sci.,* 4 (2009) 1319.

[51] E. Niranjana, R.R. Naik, B.E.K. Swamy, B.S. Sherigara and H. Jayadevappa, *Int. J. Electrochem. Sci*., 2 (2007) 923.

[52] W. Sun, M. Yang and K. Jiao, *Anal. Bioanal. Chem*., 389 (2007) 1283.

[53] U. Chandra, B.E.K. Swamy, O. Gilbert and B.S. Sherigara, *Electrochim. Acta.,* 55 (2010) 7166.

[54] M. Kumar, B. E. K. Swamy and M. Schell, *Chem. Sens*., 4 (2014) 1.

[55] R. N. Hegde, B.E.K. Swamy, N.P. Shetty and S.T. Nandibewoor, *J. Electroanal. Chem*., 635 (2009) 51.

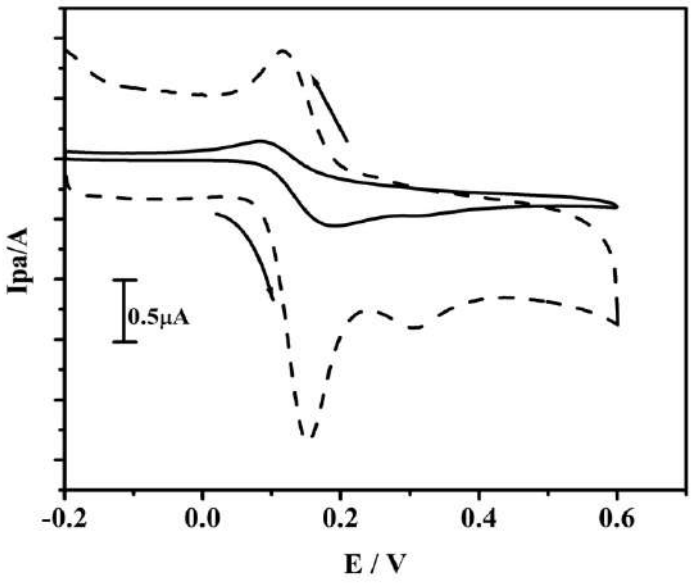
[56] Q. Wang, N. Li and W. Wnag, *Anal. Sci.*, 18 (2002) 635.

[57] N.B. Li, W. Ren and H.Q. Luo, *Electroanalysis,* 19 (2007) 1496.

CHAPTER-3

Part-B

Simultaneous Determination of Serotonin and Dopamine at Poly (Patton and Reeder's) Modified Graphite Pencil Electrode: A Cyclic Voltammetric Study



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3.4. Introduction

Electrochemical oxidation of serotonin has been studied at Graphite pencil electrode in 0.2M Phosphate buffer using cyclic voltammetric (CV) technique. The poly (Patton and Reeder's) film was synthesized on the surface of graphite pencil electrode in alkaline solution by cyclic voltammetric (CV) technique after pretreatment by H_2SO_4 media. The poly (PR) film coated on Graphite pencil electrode (GPE) exhibited excellent electrocatalytic activity towards the Detection of Serotonin [5-HT] at 7.0 pH. This polymer film coated electrode was very good at simultaneous study of Serotonin in presence of high concentrated DA.

3.4.1. Chemistry and Biological Relevance of Dopamine

The chemistry and biological relevance of dopamine has been explained details in chapter 3 Part A section 3.1.1 and 3.1.1.1.

3.4.2. Chemistry and Biological Relevance of Serotonin

Serotonin (5-HT) is a monoamine neurotransmitter of enormous biological importance widely distributed in the central nervous system, which primarily functions as chemical messenger between nerve cells. Biochemically derived from tryptophan, 5-HT is primarily found in the gastrointestinal (GI) tract, platelets, and in the central nervous system (CNS) of humans and animals. It is believed to play an important role in wide variety of activities such as inhibition of aggression, appetite, cardiovascular function, muscle contraction, endocrine regulation and pathological states (psychiatric disorders, depression, mental retardation etc.) [1-4]. Modulation of serotonin at synapses is thought to be a major action of several classes of pharmacological antidepressants.

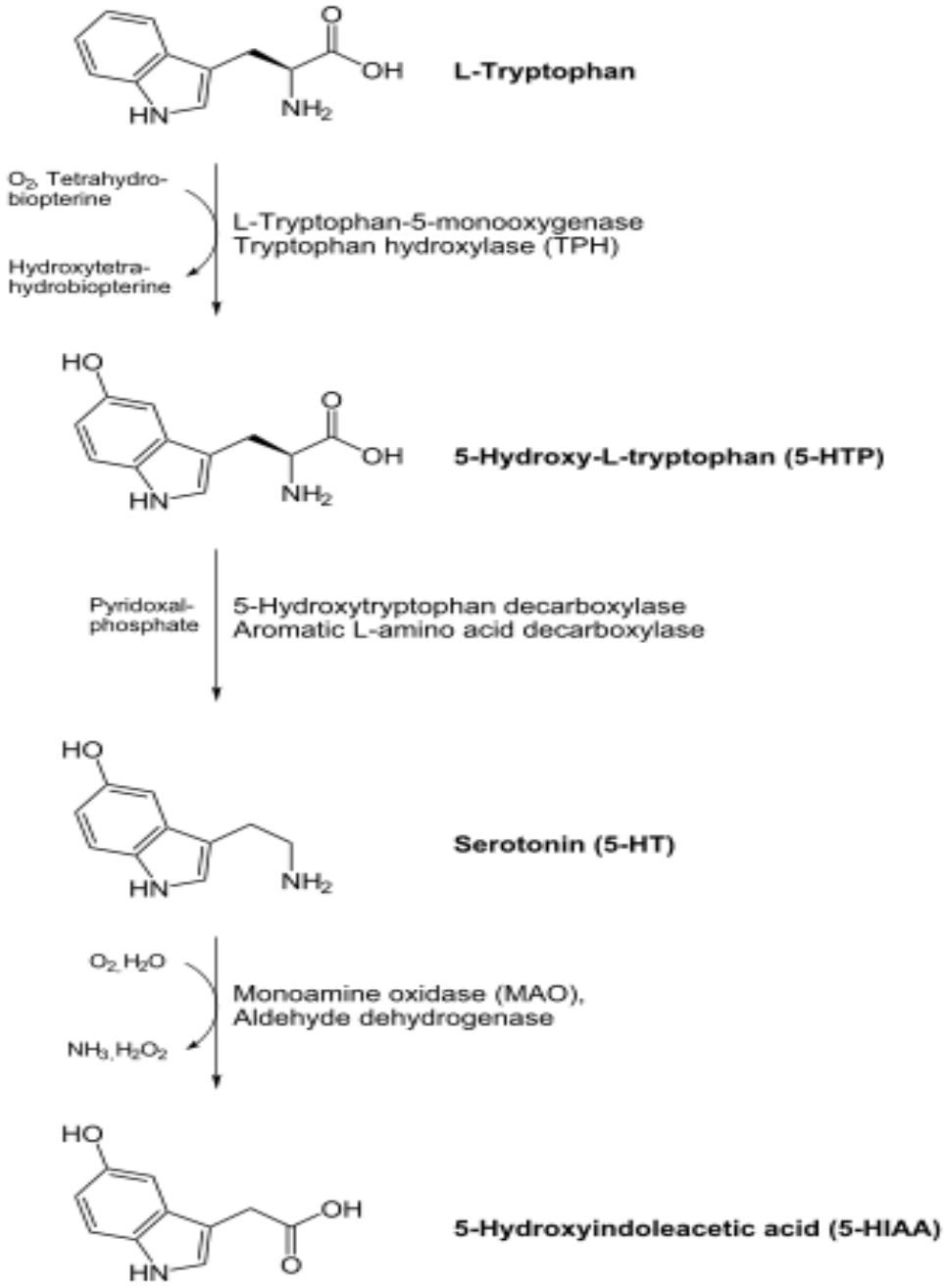
3.4.2.1. Biosynthesis of Serotonin

In animals including humans, 5-HT is synthesized from the amino acid L-tryptophan by a short metabolic pathway consisting of two enzymes: tryptophan

hydroxylase (TPH) and amino acid decarboxylase (DDC). The TPH-mediated reaction is the rate-limiting step in the pathway. TPH has been shown to exist in two forms: TPH1, found in several tissues, and TPH2, which is a brain-specific isoform. 5-HT taken orally does not pass into the serotonergic pathways of the CNS, because it does not cross the blood-brain barrier. However, tryptophan and its metabolite 5-hydroxytryptophan (5-HTP), from which serotonin is synthesized, can and do cross the blood-brain barrier. These agents are available as dietary supplements, and may be effective serotonergic agents. One product of 5-HT breakdown is 5-hydroxyindoleacetic acid (5 HIAA) (Scheme 3.4), which is excreted in the urine. 5-HT and 5 HIAA are sometimes produced in excess amounts by certain tumors or cancers, and levels of these substances may be measured in the urine to test for these tumors [5].

3.4.2.2. Dysfunction of Serotonin

In humans, defective signalling of 5-HT in the brain may be the root cause of sudden infant death syndrome (SIDS). If neurons that make 5-HT (serotonergic neurons) are abnormal in infants, there is a risk of SIDS [6]. Extremely high levels of 5-HT have toxic and potentially fatal effects known as serotonin syndrome [7]. Phenomenon of natural ageing has been found to exhibit profound effect on 5HIAA/5-HT ratio in human body [8]. Certain tumorous cells have been found to produce excess 5-HT (I) and 5-HIAA (II) and their altered urinary level may act as a marker of the starting stage of these tumors [9].



Scheme 3.4. Mechanism for the Biosynthesis of Serotonin

3.5. Review of Electrochemistry of Dopamine and Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is an important catecholamine neurotransmitter in biological systems. Although the central nervous system contains less

than 2% of the total serotonin in the body, serotonin plays a very important role in the range of brain functions. It is synthesized from the amino acid tryptophan. Serotonin regulates mood and sleep and is a major target for pharmaceutical treatments of depression. 5-HT plays a crucial role in an emotional system together with other neurotransmitters [10-13]. The physiological functions such as, sleep, thermoregulation, food intake, and sexual activity, as well as in psychopathological states such as depression, anxiety, alcoholism, and drug dependency were directly related to the concentration of serotonin. Serotonin was highly unstable and easily metabolized to its major metabolite, 5-hydroxyindoleaceticacid and other compounds [14-16].

Dopamine (DA) is one of the most important catecholamine neurotransmitters in mammalian central nervous system [17-20]. The loss of DA in human body may result in some serious diseases such as Parkinson's diseases [21]; 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 electrochemist to develop the detection method of DA no matter in route or in vivo analysis. However, in assay of DA, the electrochemical methods suffer from inferior selectivity because of the presence of Serotonin at have higher concentration than DA in physiological fluids and whose oxidation potentials always are close to that of DA. Therefore, it is a significant attempt to separate the oxidation peak potentials of DA, Serotonin of many electrochemical approaches have been used to implement the above goal [22-28]. Among these methods, using electroactive excellent selectivity and sensitivity.

In this paper, an electropolymerization film of Patton and Reeder's (PR) was prepared on to the surface of GPE by CV technique. The poly (PR) on to the surface of the GPE had high Concentration of negative charged function group $-SO_3^-$ and COO^- and the electron rich oxygen atoms on its surface. The poly (PR) film at the electrode consciously enhanced the redox peak current and could separately determine Serotonin. The graphite pencil electrode (GPE) has been successfully applied to analyses of certain compounds in recent years. The GPE is relatively new type of carbon electrode, it is less expensive, more convenient, and renewable compare to the commonly used CPE or GCE.

The reaction mechanism could be explained as fallows [Scheme 3.6] PR (A) was first deposited at surface of GPE and oxidized to form a benzoquinone diimine structure (B) and then the benzoquinone diimine structure (B) was reduced to PR (A) at the surface of GPE.

3.6. Experimental Section

3.6.1. Reagents

The pencil-lead rods were HB 0.5 mm in diameter and 6 cm length purchased from local bookstore. DA stock solution was prepared by dissolving in 0.1M perchloric acid, serotonin was prepared by double distilled water. All are Analytical grade and are used without further purification. Phosphate buffer solution (PBS) prepared by standard method. All experiments were performed at room temperature.

3.6.2. Apparatus

The electrochemical experiments were carried out using a CHI-660c (CH Instrument- 660 electrochemical workstation). All the experiments were carried out in a conventional three electrochemical cell. The electrode system contained a working electrode was bare GPE and poly (PR) modified GPE (0.5 mm in diameter), a platinum wire as counter electrode and the SCE.

3.6.3. Preparation of Pre-treated and Poly (PR) Modified GPE

The 1mM (PR) was placed in the electrochemical cell with 0.05M H_2SO_4. The GPE was pretreated by scanning in the solution from –400 to 1600mV at $100mVs^{-1}$ for 10 times. After this, the same GPE has enforced under sweeping from –400 to 1600mV at $100mVs^{-1}$ for multiple cycles (10 cycles) in the solution containing 1mM (PR) with 0.01M NaOH. The poly (PR) fabricated modified GPE after polymerization washed with water and data were recorded in pH 7.0 PBS.

3.7. Results and Discussion

3.7.1. Electrochemical Polymerization of Patton's and Reeder's on GPE

Figure 3.8 showed the cyclic voltammogram for electropolymerisation of Patton's and Reeder's (PR) on the surface of GPE in the range from –400 to 1600 mV at the sweep rate of 100 mVs^{-1} at 10 multiple cycles. Before the electropolymerisation process the GPE was pretreated by scanning in the solution containing in 1mM (PR) 0.05 M H_2SO_4 for 10 times in the same potential range. After this the electrode is made to undergo multiple cycles in 0.01M NaOH containing 1mM (PR). During the process of multiple cycles the voltammograms has gradually descended with increase of cyclic time. This indicates that the poly (PR) film was formed and deposited on the surface of GPE.

3.7.2. Electrocatalytic Oxidation of Serotonin at Poly (PR) Modified GPE

Cyclic voltammogram of serotonin in pH 7.0 PBS at a bare GPE and poly (PR) film modified GPE was recorded (Fig. 3.9). At bare GPE (solid line) of oxidation peak showed poor electrocatalytical activity with anodic peak potential of 334mV and not showed cathodic peak potential. Under the same condition poly (PR) modified GPE (dashed line) gave birth to significantly enhanced peak current and more reversible electron transfer process to serotonin with slight shift in redox peak potentials. A well defined redox wave of serotonin was observed with anodic and cathodic peak potential at 326 and 233 mV respectively. This suggests an efficient oxidation reaction toward serotonin at the poly (PR) modified GPE.

3.7.3. Effect of Scan Rate

The effect of scan rate on the anodic peak current of serotonin was studied at poly (PR) modified GPE by using CV technique (Fig. 3.10a). The scan rate was increased from 50 to 350 mVs^{-1}. The anodic peak current was increased with increase in scan rate. The graph of Ipa vs. square root of scan rate was plotted (Fig. 3.10b). The resulted graph showed excellent linearity with correlation co-efficient of (R^2=0.9929). This result showed that the electrode process was diffusion controlled.

3.7.4. Effect of Concentration of Serotonin

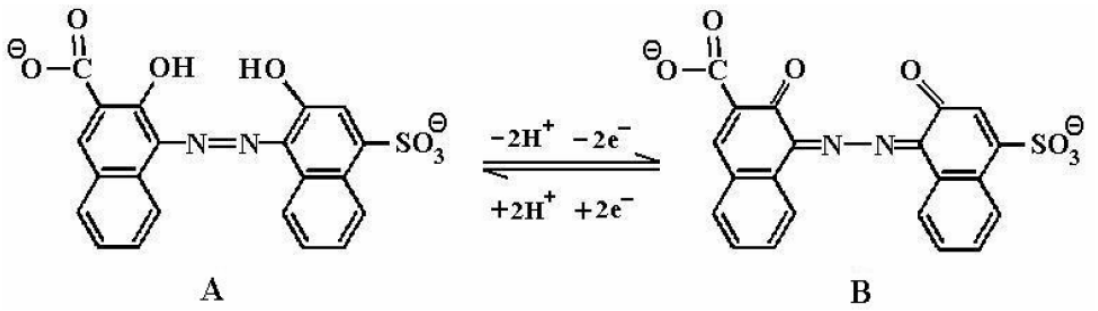
The electrocatalytic oxidation of serotonin was carried out by varying its concentration at poly (PR) MGPE (Fig. 3.11a). By increasing the concentration of serotonin, the electrochemical anodic peak and cathodic peak current goes on increasing with negligible shifting in redox peak potentials. Serotonin from 10 to 50 μM concentrations showed increase in redox peak currents. The graph of anodic peak current vs. concentration of serotonin was plotted (Fig. 3.11b).

3.7.5. Simultaneous Determination of Serotonin and DA

The main objective of our present work was the simultaneous determination of serotonin and DA in phosphate buffer solution. Figure 3.12 shows the cyclic voltammagram that are obtained for Serotonin and DA coexisting in phosphate buffer solution at the bare and modified Graphite pencil electrode. The bare GPE (solid line) showed only one broad anodic peak but not cathodic peak. The poly (PR) modified GPE has able to separate the oxidation peak (dashed line). The electrocatalytical anodic peak of serotonin was obtained at 303 mV and DA was found to be at 151 mV. The cathodic peaks for DA was found to be at 115 mV. The separation between serotonin – DA was found to be at 152 mV.

3.7.6. Conclusion

The prepared poly (PR) film modified GPE exhibits highly electrocatalytic activity to the oxidation of serotonin and DA. The modified electrode displaces higher selectivity in voltammetric measurements of serotonin and DA in the mixture solutions. The separations of the oxidation peak potentials for serotonin - DA are about 303 and 151 mV respectively by cyclic voltammetry with good sensitivity.



Scheme 3.6

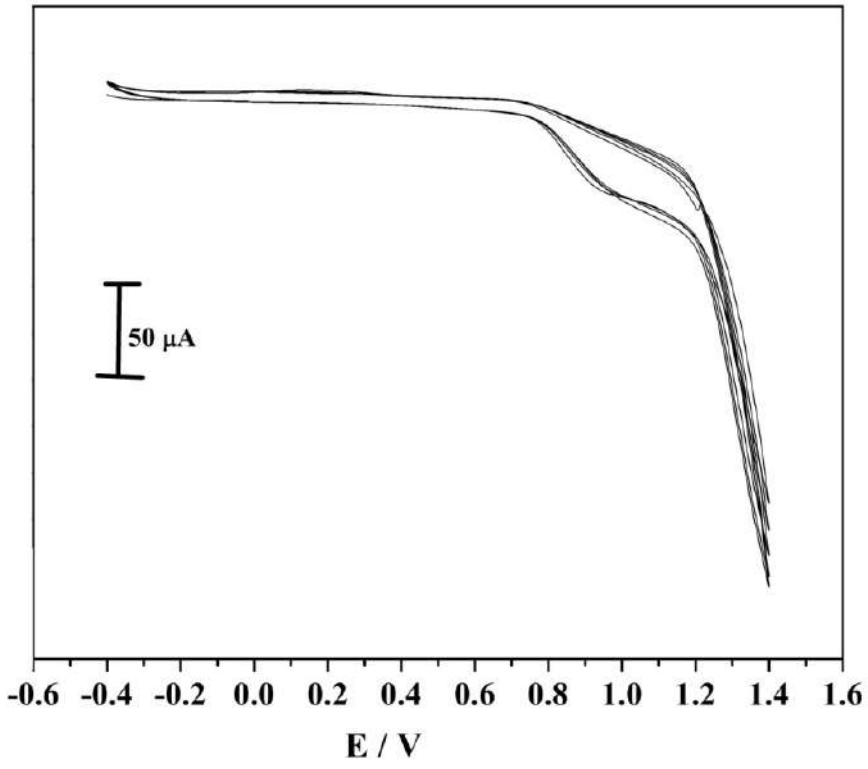


Fig. 3.8. Cyclic voltammogram of preparation of poly (PR) film coated GPE. 1×10^{-3} M PR in 0.01M NaOH at 10 cycles with sweep rate of $100mVs^{-1}$

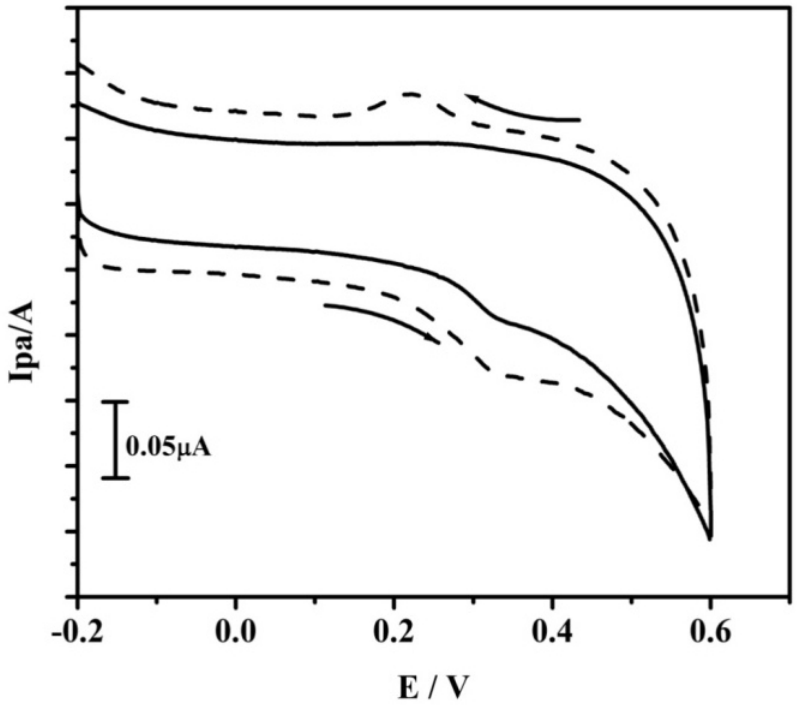


Fig. 3.9. Cyclic voltammogram of 1×10^{-4} M 5-HT in 0.2 M phosphate buffer solution of pH 7 at bare GPE (solid line) and poly (PR) film coated GPE (dashed line)

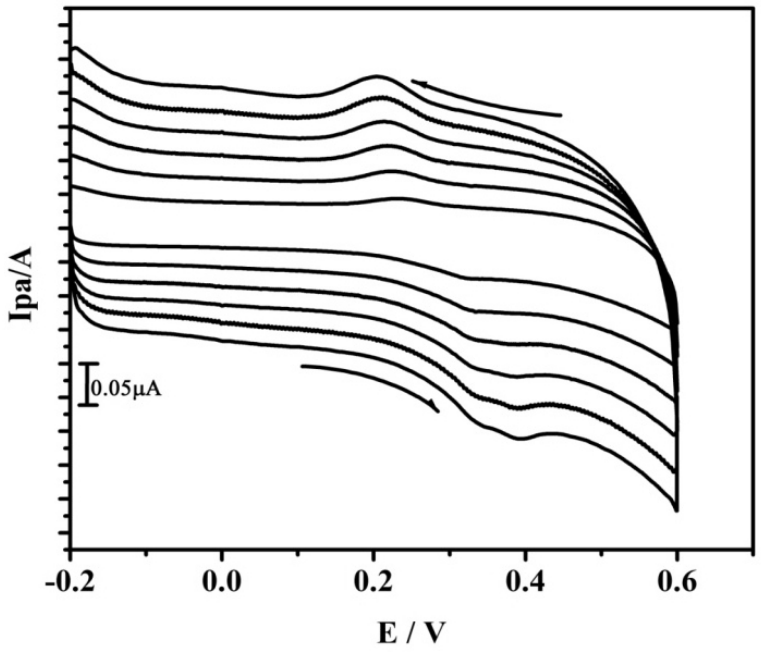


Fig. 3.10a. Variation of scan rat for Serotonin at poly (PR) film coated GPE (50 to 350 mVs^{-1})

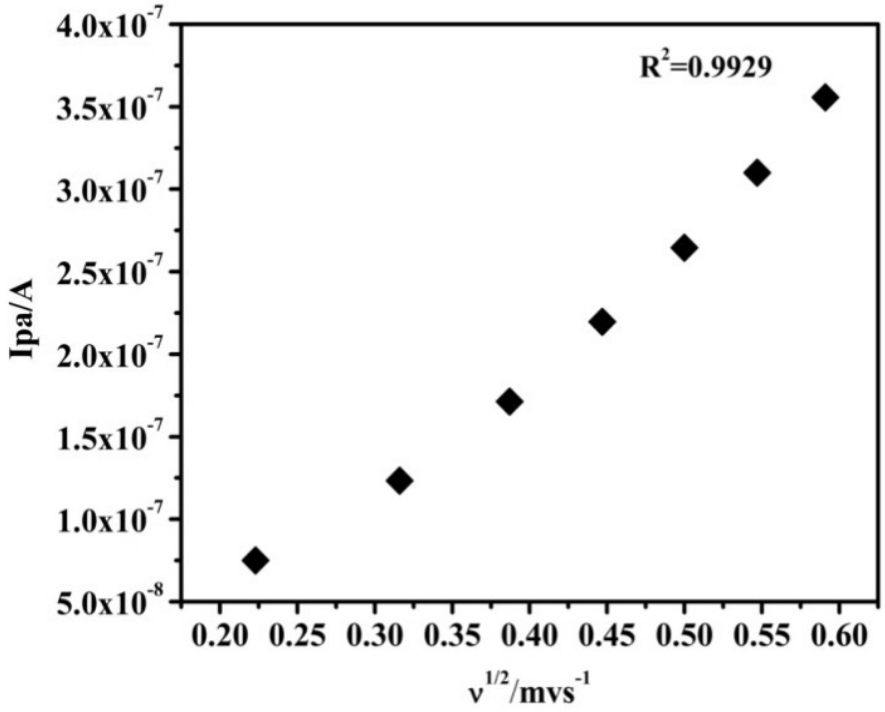


Fig. 3.10b. Graph of current vs. square root of scan rate

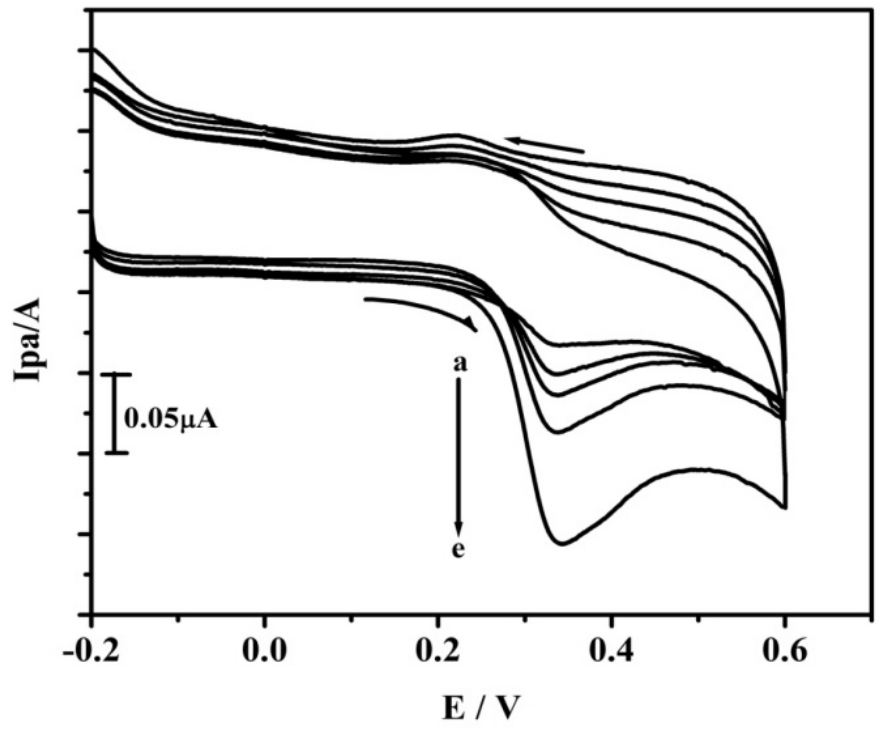


Fig. 3.11a. Cyclic voltammogram of Serotonin at different concentration (a-e: 10 to 50 μM)

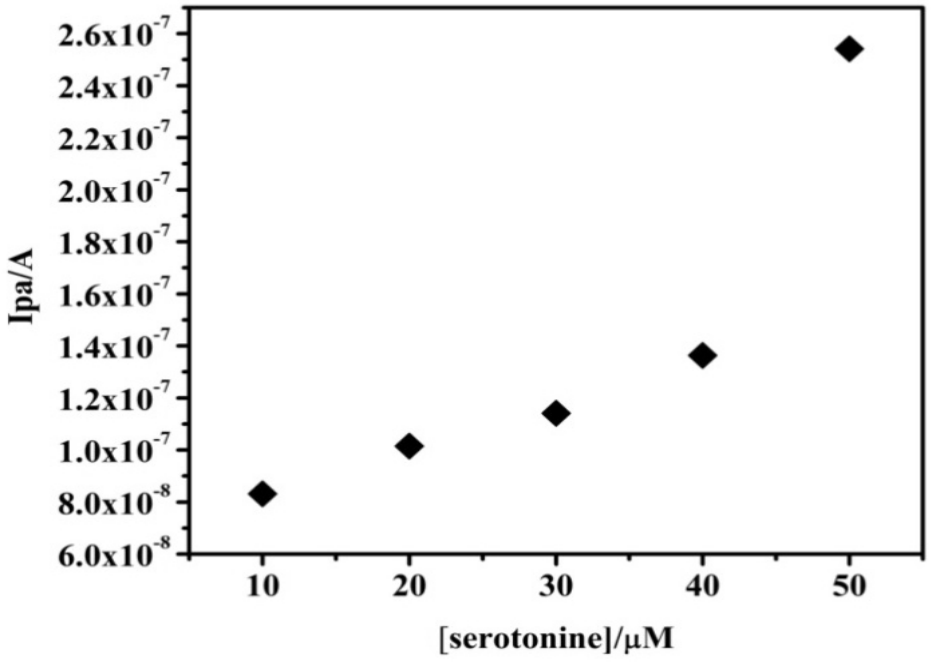


Fig. 3.11b. Graph of current vs. concentration of Serotonin

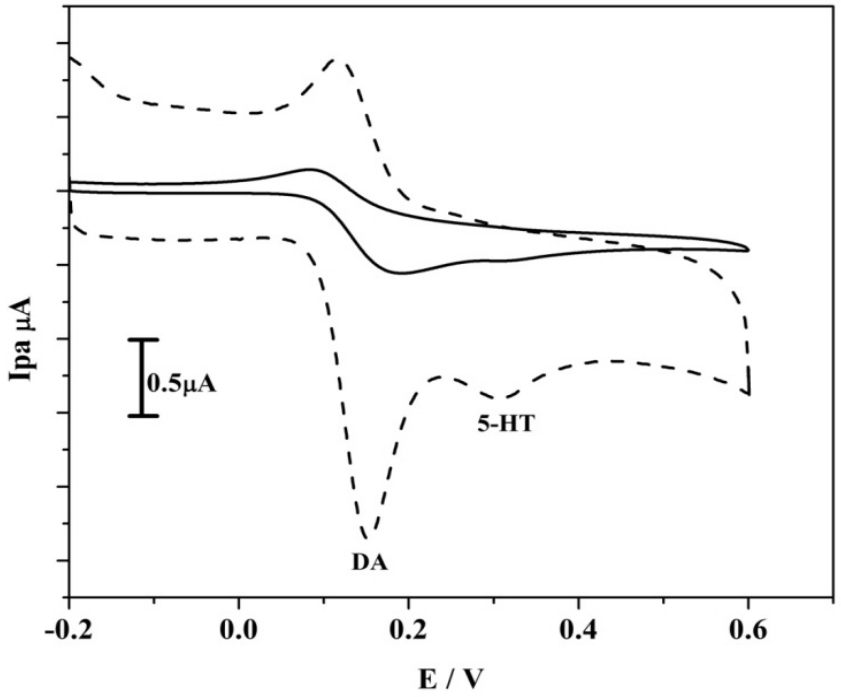


Fig. 3.12. Simultaneous determination of 1×10^{-4} M 5-HT and 1×10^{-3} DA at bare GPE (solid line) and at poly (PR) film coated GPE (dashed line)

3.7.7. References

[1] S.M. Stahl, *J. Affect Disord*., 51 (1998) 215.

[2] Y. Sun, J. Fei, J. Hou, Q. Zhang, Y. Liu and B. Hu, *Microchim. Acta*., 165 (2009) 373.

[3] M. Some and A. Helander, *Life. Sci*., 71 (2002) 2341.

[4] L.A. Houghton, W. Atkinson, R.P. Whitaker, P.J. Whorwell and M.J. Rimmer, *Gut*., 52 (2003) 663.

[5] D.J. Walther, J.U. Peter, S. Bashammakh, H. Hörtnagl, M. Voits, H. Fink and M. Bader, *Science*, 299 (2003)76.

[6] D. S. Paterson, F.L. Trachtenberg, E.G. Thompson, R.A. Belliveau, A.H. Beggs, R. Darnall, A.E. Chadwick, H.F. Krous and H.C. Kinney, *J. Am. Med. Assoc.,* 296 (2006) 2124.

[7] G.K. Isbister, S.J. Bowe, A. Dawson and I.M. Whyte, *J. Toxicol. Clin. Toxicol.,* 42 (2004) 277.

[8] S.M. Dursun, R.P. Whitaker, H. Andrews and M.A. Reveley, *Hum. Psychopharm*., 12 (1997) 365.

[9] S. Banik and T. Lahiri, *Biomed. Res*., 21 (2000) 255.

[10] P. Damier, E.C. Hirsch, Y. Agid and A.M. Graybiel, *Int. J. Electrochem. Sci*., 122 (1999) 1448.

[11] E. Dremencov, H. Gaspar, I.M. Rosentein, A. Mendelman, D.H. Overstreet, J. Zohr and G. Yadid, *Progress in Neuro Psychopharmacology & Biological Psychiatry,* 28 (2004) 147.

[12] K. Hayash, Y. Iwaski, R. Kurita, K. Sunagawa and O Niwa, *Electrochem. Commun.,* 5 (2003) 1042.

[13] Q. Li, Y. Wang and G. Luo, *Mater. Sci. Eng*., 11 (2000) 74.

[14] C. Nagaraja, B.E.K. Swamy, E. Niranjana and B.S. Sherigara, *Int. J. Electrochem. Sci.*, 4 (2009) 434.

[15] G. Ongera, C. Umesh, B.E.K. Swamy, C.M. Panduranga, C. Nagaraj and B.S. Sherigara,. *Int. J. Electrochem. Sci*., 3 (2008) 1195.

[16] K.W. Perry and R.W. Fuller, *Life Sciences*, 22 (1992)1690.

[17] K.J. Ressler and C.B. Nemeroff, *Depression Anxiety*, 12 (2000) 4.

[18] H. Razmi, M. Agazadeh and A.B. Habibi, *J. Electroanal. Chem*., 25 (2003) 552.

[19] N.R. Raghavendra, E. Niranjana, B.E.K. Swamy, B.S. Sherigara and H. Jayadevappa, *Int. J. Electrochem. Sci*., 3 (2008) 1581.

[20] M.H. Sorouraddin, J.L. ManZoori, E. Kargarzadch and A.M. Hajishahani, *J. Pharm. Biomed. Anal.,* 18 (1998) 81.

[21] T. Selvaraju and R. Ramaraj, *Electrochem. Commun*., 5 (2003) 672.

[22] Y. Shuai and H. Shengshui, *Electrochim. Acta*., 49 (2004) 4293.

[23] S.S. Sharath and B.E.K. Swamy, *Int. J. Electrochem. Sci*., 9 (2014) 1339.

[24] R.M. Wightman, L.J. May and A.C. Michael, *Anal. Chem.,* 60 (1988) 793.

[25] J. Wang, M. Li, Z. Shi, N. Li and Z. Gu, *Microchem. J.,* 73 (2002) 333.

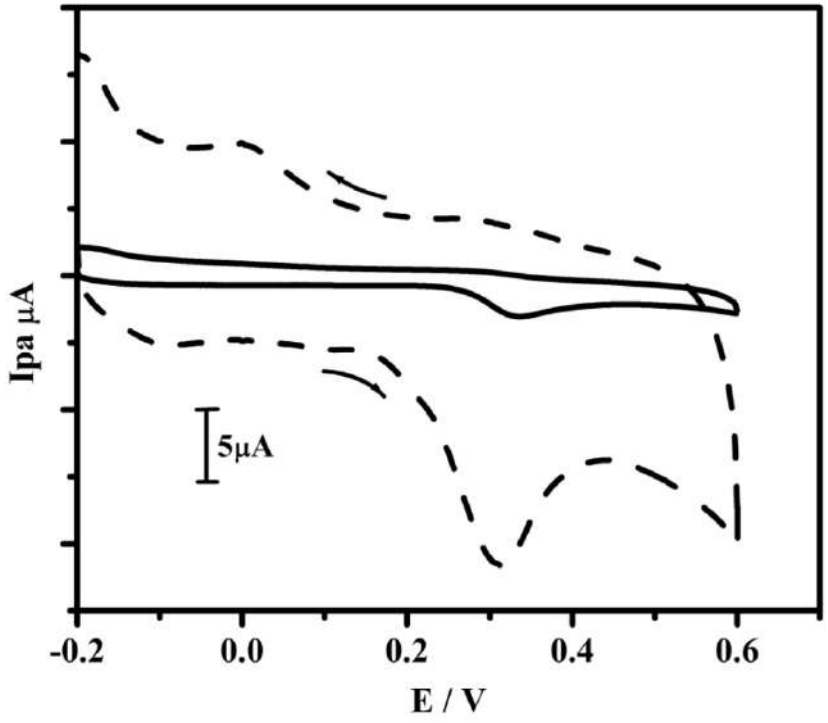
[26] K. Wu, J. Fei and S. Hu, *Anal. Biochem.*, 318 (2003) 106.

[27] K.H. Xue, F.F. Tao, W. Xu, S.Y Yin, W. Shen and W. Xue, *Chem. Phy. Lett.,* (2004) 401.

[28] W. Yan, L. Maoguo, J. Shoufeng, H. Qinan, W. Guangfeng and F. Bin, *Electrochim. Acta.,* 52 (2006) 772.

CHAPTER-4

Voltammetric Determination of Serotonin in Presence of Dopamine at Poly (Eriochrome Black-T) Film-Coated Graphite Pencil Electrode



Chinese Chemical Letters (Revised and submitted)

4.1. Introduction

A Graphite Pencil Electrode (GPE) was modified by electropolymerisation of Eriochrome Black-T (EBT) in alkaline solution by cyclic voltammetric technique (CV) and the electrochemical properties of the polymer film were studied. The poly (EBT) modified electrode was developed for the electrochemical determination of serotonin and it shows an excellent electrocatalytic activity towards the oxidation of serotonin in 0.2M Phosphate buffer solution (pH 7.0). The scan rate and the concentration effects at the modified electrode were found to be a diffusion-controlled electrode processes. The simultaneous study shows excellent result with good potential difference between serotonin and dopamine by using both cyclic voltammetric and differential pulse voltammetric (DPV) techniques.

4.2. Chemistry and Biological Relevance of Dopamine

The chemistry and biological relevance of dopamine has been explained details in chapter 3 Part A section 3.1.1 and 3.1.1.1.

4.3. Chemistry and Biological Relevance of Serotonin

The chemistry and biological relevance of serotonin has been explained details in chapter 3 Part B section 3.4.2, 3.4.2.1 and 3.4.2.2.

4.4. Review of Electrochemistry of Dopamine and Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is an important catecholamine neurotransmitter in biological systems. Although the central nervous system contains less than 2% of the total serotonin in the body and serotonin plays a very important role in the range of brain functions. It is synthesized from the amino acid tryptophan. Serotonin regulates mood and sleep and is a major target for pharmaceutical treatments of depression [1]. 5-HT plays a crucial role in an emotional system together with other neurotransmitters [2, 3, 4].

Dopamine (DA) is one of the most important neurotransmitters and is present in the mammalian central nervous system. It is a catecholamine in the form of large organic cations and belongs to the family of excitatory chemical neurotransmitters [5]. It plays a crucial role in the functioning of the central nervous, cardiovascular, renal, and hormonal systems as well as in Parkinson's disease [6-8]. Changes in DA concentration in biological samples are an important indication of possible body abnormalities or diseases. Therefore, determination of DA has become important and been given tremendous attention by neuroscientists and chemists in biomedical and bioanalytical research since its discovery during the 1950s. DA possesses high electrochemical activity and has been widely studied by electroanalytical techniques to significantly benefit biosciences [9, 10- 13].

The graphite pencil electrode (GPE) 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, high electrochemical reactivity, ease of modification, renewal, low background current, and miniaturization, the GPE has good application in analysis of neurotransmitter and in the detection of traces of metal ions [14-17]. GPE has a larger active electrode surface area and is therefore able to detect low concentrations [18]. Therefore this type of electrode has been successfully applied to design various biosensors [19-23].

Exploration of many kinds of chemically modified electrodes to detect DA selectively has occurred in past years. Several approaches based on polymer-modified electrode [24-33], carbon ionic liquid electrodes [34-36], nanomaterials modified electrodes [37-42] and self-assembled monolayers [42-46] have been tried to solving the problems. These films can carry negative charges and so they can selective detect the DA cation by electrostatic effect [47]. In recent years polymer modified electrodes have attracted great attention as polymeric film has good stability and reproducibility [48-49]. A number of researchers have employed polymeric film modified electrode to detect DA. Recently different methodologies have also been used for depositing polymeric films. Electropolymerisation is a good approach to immobilize polymers because adjusting the

electrochemical parameters can control film thickeners, permeation and charge transport characteristics. Recently poly (Eriochrome Black T) modified glassy carbon electrode [50-53] have attracted more attention because of their novel electrode material which exhibits several excellent electrochemical properties and high electrochemical stability. These properties enable the poly (EBT) GPE to render good reproducibility.

As part of our research work on the development of new electrochemical sensors for the determination of DA [53-54]. Present work reports the voltammetric behavior of serotonin at bare and poly (EBT) film modified graphite pencil electrode. The modified electrode showed an electrocatalytic activity for the oxidation of serotonin and DA. The results indicate that the modified electrode could be used to detect serotonin in the presence of DA.

EBT is a metallochromic indicator widely used in complexometric titration. It is an electroactive species with an azo group (-N=N-) in its molecular structure shown in (Scheme 4.1). This azo group is easily reduced on graphite pencil electrode by cyclic voltammetry. The EBT reduced in the two-step one-electron reduction of the azo group, which is similar with the literature [55]. The reduction mechanism of EBT was illustrated as below (scheme 2). The multiple cycle was applied, which results in greatly decrease in reductive peak current with the increase of scanning cycle. This was the characteristic of the strong adsorption behavior of EBT on graphite pencil electrode.

4.5. Experimental Part

4.5.1. Reagents

The pencil-lead rods were HB 0.5 mm in diameter and 6 cm length purchased from local bookstore. All other chemicals such as perchloric acid, potassium chloride, eriochrome black –T, sulphuric acid and sodium hydroxide were of certified analytical grade and obtained from Merck. DA stock solution was prepared by dissolving in 0.1M perchloric acid. Serotonin was prepared by double distilled water and the studies were carried out in phosphate buffer solution of pH-7.0. All reagents used were of analytical grade and used without further purification.

4.5.2. Apparatus

Electrochemical measurements were carried out with a model CHI-660c (CH Instrument-660 electrochemical workstation) at three electrode system was employed. The poly (EBT) modified graphite pencil electrode used as working electrode with a saturated calomel electrode as reference electrode (SCE) and the platinum electrode as auxiliary electrode for all experiment.

4.5.3. Preparation of Poly (EBT) Graphite Pencil Electrode

The poly (EBT) modified electrode was prepared by electrochemically pre-treating Graphite pencil electrode potential scan between –400 to 1400 mV in 0.05M sulphuric acid containing 1mM EBT at the scan rate of 100 mVs^{-1} for 10 times. Finally polymerization was carried out by immersing the pencil electrode in 0.01M NaOH solution containing 1mM EBT and was conditioned by cyclic potential sweeping from –400 to 1400 mV for 5 cycles at 100 mVs^{-1}. After polymerizations the poly (EBT) film could be formed uniformly on the surface of GPE. After that, the electrode was rinsed with double distilled water and kept in the 0.2M PBS at pH 7 and was used for determination of Serotonin in presence of DA [53].

4.6. Results and Discussion

4.6.1. Electropolymerisation of Eriochrome Black-T on Graphite Pencil Electrode

The poly (EBT) GPE was fabricated in 0.01 M sodium hydroxide solution containing 1mM of Eriochrome black-T. The film was grown on GPE by cyclic voltammetric scans between -400 to +1400 mV. The optimized scan number under the experimental conditions was determined as five for reaching the steady response. As shown in Figure 4.1, in the first cycle, with the potential scanning from –400 to 1400 mV. The peak descended gradually with the increase in cyclic time; such decrease indicates the poly (EBT) membrane forming and depositing on the surface of the GPE by electropolymerisation. After polymerization the poly (EBT) modified GPE was carefully rinsed with distilled water and was used for the determination of serotonin.

4.6.2. Electrochemical Response of Serotonin at poly (EBT) Modified GPE

The Figure 4.2 shows the Cyclic voltammogram was utilized to investigate the electrochemical behavior of Serotonin at the EBT polymer film GPE (dashed line), a bare GPE (solid line). It showed that only one oxidation peak at 0.33V at bare GPE, whereas an oxidation peak at 0.31V at the poly (EBT) GPE, in the potential range -100 to +600 mV. No reduction peak was observed in the reverse scan for bare, suggesting that the electrochemical reaction is a totally irreversible process. It was observed that the peak currents enhanced greatly at the polymer modified GPE, which provides more evidence for asserting that the polymer on the surface of the GPE possessed high electrocatalytic activity to the electrochemical response of serotonin.

4.6.3. Effect of Scan Rate

The effect of scan rates on the electrochemical response of serotonin at poly (EBT) modified GPE was studied between the range 50 to 350 mV/s and the cyclic voltammograms were shown in Figure 4.3. From Figure 4.3a it was found that the oxidation peak current increases linearly with the increase in scan rate with a correlation coefficient of 0.9971 which indicates diffusion GPE. However linearity was also obtained for the plot of square root of scan rate vs. the oxidation peak current with a correlation coefficient of 0.9898 (Fig. 4.3b). The observed shift in peak potential towards more positive values with increase in scan rate is a typical behavior of an irreversible electron transfer process [56].

4.6.4. Effect of Serotonin Concentration

The electrocatalytic oxidation of serotonin was carried out by varying its concentration at poly (EBT) modified GPE. The Figure 4.4a showed that by increasing concentration of serotonin, the electrochemical anodic peak current goes on increasing with shifting Epa towards positive direction. The plot of ipa vs. concentration of serotonin showed the linear relationship between the anodic peak current (ipa) and the serotonin concentration in the range of 10 to 50μM with a correlation co-efficient of 0.9864 in Figure 4.4b and limit of detection was found to be 0.32μM.

The detection limit was calculated by using the formula (1) [57-58] where S is the standard deviation and M is the slope obtained from the three calibration plots. From the data, a lower limit of detection (LOD) can be achieved using the proposed method [59-62].

$$LOD = 3S/M \text{ ---------- (1)}$$

4.6.5. Simultaneous Determination of Serotonin and Dopamine by Cyclic Voltammetry

DA is present along with serotonin in mammalian brain. The concentration of DA is much higher than that of serotonin. Since the oxidation potential of DA is nearly same as that of serotonin result is sometimes overlapped voltammetric response at bare GPE. However, the poly (EBT) modified GPE has ability to separate the oxidation peak potentials of serotonin and DA. Figure 4.5a showed the voltammogram for solution containing a mixture of both 10µM serotonin and 0.1mM DA in pH 7.0 phosphate buffer at sweep scan rate 100mVs^{-1}.The bare GPE (solid line) showed only one broad anodic peak. The poly (EBT) modified GPE has able to separate the oxidation peaks of serotonin and DA by showing two well separated anodic peaks and one cathodic peak (dashed line).The electrocatalytical anodic peak of serotonin was obtained at 0.33V and DA was found to be 0.166V. The cathodic peak for dopamine was found to be 0.116V. The separation between serotonin –DA was found to be 0.16V.

To study the effect of serotonin concentration in the presence of DA. The Figure 4.5b shows the cyclic voltammograms at poly (EBT) modified GPE for 0.1 mM DA and serotonin with different concentrations in 0.2M Phosphate buffer solution at the scan rate 100 mVs^{-1}. The anodic current of serotonin increases with increase in concentration while the anodic current of DA keeps constant due to its constant concentration in the experiments. Furthermore, it was observed that even in the presence of high concentration of serotonin it did not interfere with the determination of low concentration of serotonin. Therefore the poly (EBT) modified GPE electrode shows its good selectivity and sensitivity in the electrochemical detection of serotonin in the presence of DA.

4.6.6. Simultaneous Determination of Serotonin and Dopamine by DPV

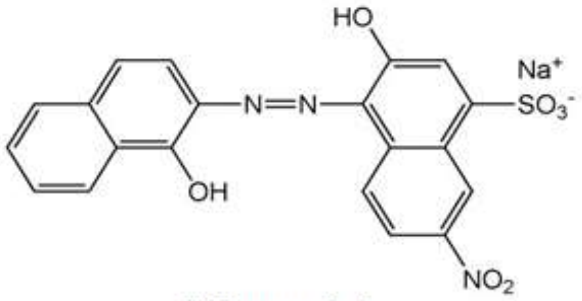
DPV was used for the determination of Serotonin and DA because it has more sensitivity and selectivity. The simultaneous study was carried out in the potential range from -200 to +600mV and DPV showed the simultaneous determination of serotonin and DA in the mixture was carried out at poly (EBT) modified GPE when concentration of one species changed whereas the other kept constant. Figure 4.6a shows the differential pulse voltammograms for a fixed concentration of DA and serotonin with concentrations varying from 10 to 50μM in 0.2 M phosphate buffer (pH 7.0) at the scan rate of 100 mVs^{-1}. Obviously anodic peak current increases with increasing concentration of serotonin, while the anodic peak current of DA remain constant. Furthermore, it was observed that in the presence of high concentration of DA the detection of lower concentrations of serotonin is still possible (Fig. 4.7a). The poly (EBT) modified GPE showed a good selectivity for electrochemical detection of serotonin in the presence of DA. The corresponding graphs of anodic peak current versus various concentrations of DA (0.1 to 0.5mM), serotonin (from 10 to 50μM) showed linear relationships with linear regressions for (serotonin) Ipa (μA) =0.4858C + 0.1634 and (DA) Ipa (μA) =2.0519C + 0.08829, the correlation coefficient for these linear graphs was 0.9795 and 0.9958 respectively for this poly (EBT) GPE which were shown in Figures 4.6b, 4.7b, respectively.

4.6.7. Conclusion

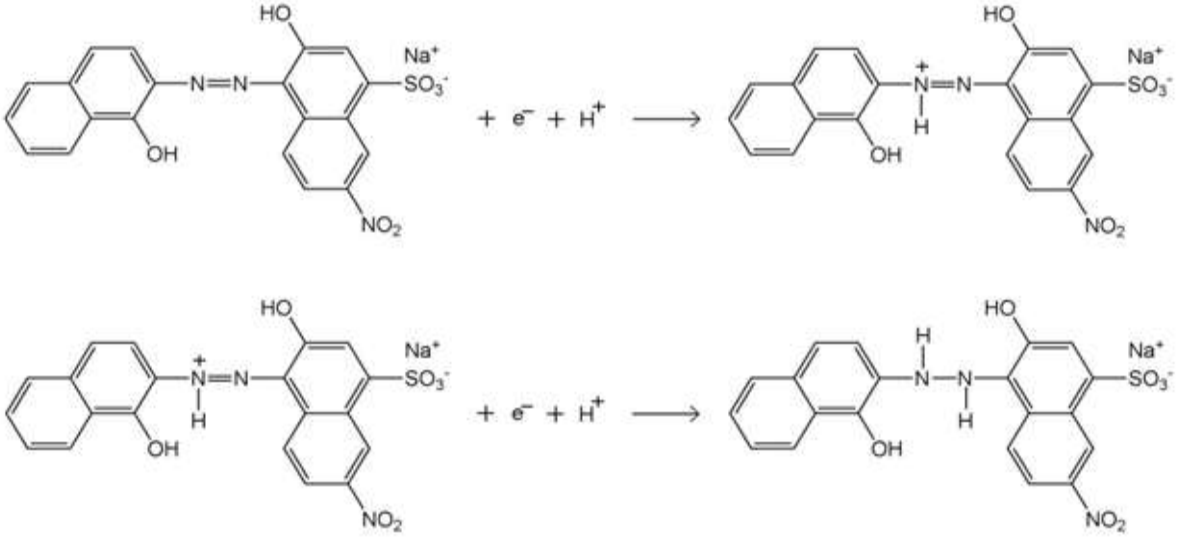
In this work, the electropolymerisation of EBT on the graphite pencil electrode produces a stable polymeric film. The modified electrode showed high electrocatalytic activities towards the oxidation of serotonin in the presence of DA with good selectivity and sensitivity. The poly (EBT) film coated GPE has very low detection limit 0.32 μM. Hence, poly (EBT) modified graphite pencil electrode could hold great application in the fields of electroanalytical chemistry and biosensors.

Table 4.1

Sl.No	Electrode	Detection limit (μM)	Techniques	Reference
1	MCPE/PR	0.53	DPV	[63]
2	PolyDAN-RB4/GCE	0.083±0.003	DPV	[64]
3	Bi_2O_3/GCE	0.2	DPV	[65]
4	f-MWCNTs/GCE	0.039	DPV	[66]
5	PEDOT/GCE	1.13	DPV	[67]
6	CF/GCE	0.036	CV	[68]
7	**Poly (EBT)/MGPE**	**0.32**	**CV**	**Present work**



Scheme 4.1



Scheme 4.2

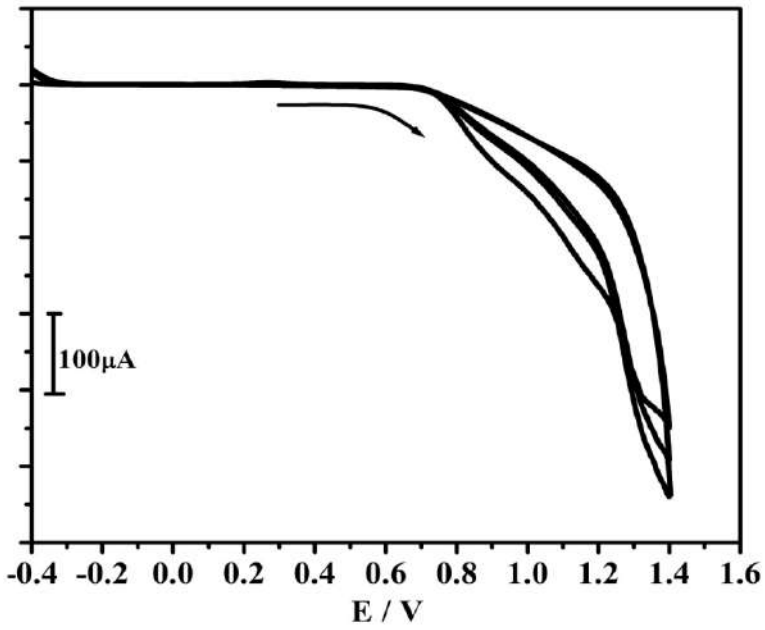


Fig. 4.1. Cyclic voltammogram for the electrochemical polymerisation of 1 mM Eriochrome black –T in 0.01 M NaOH solution on a GPE. Terminal potential 1400 mV; Initial potential – 400 mV. scan rate: 100 mVs^{-1}

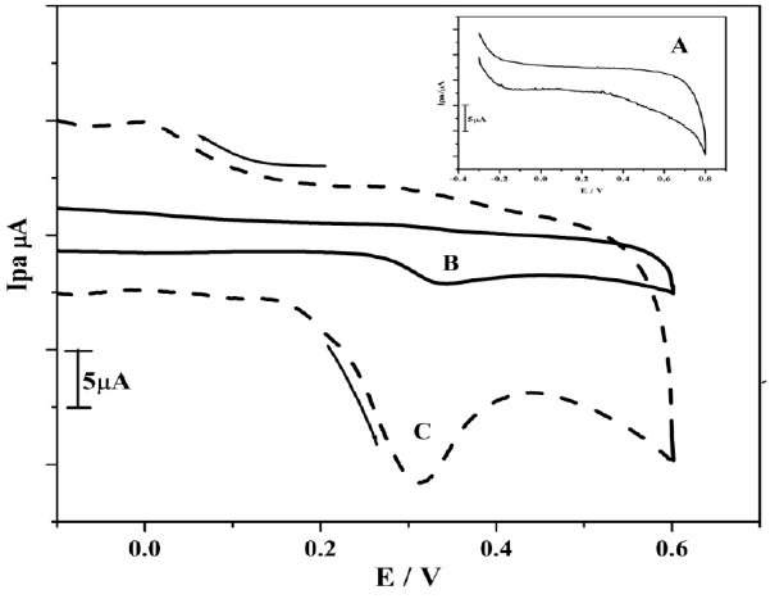


Fig. 4.2. Cyclic voltammogram of BGPE (curve B) and poly (EBT) MGPE (curve C) and absence of serotonin at bare GPE (insert curve A) in the presence of 10 μM serotonin and 0.2M Phosphate buffer, in pH 7.0 scan rate: 100 mVs^{-1}

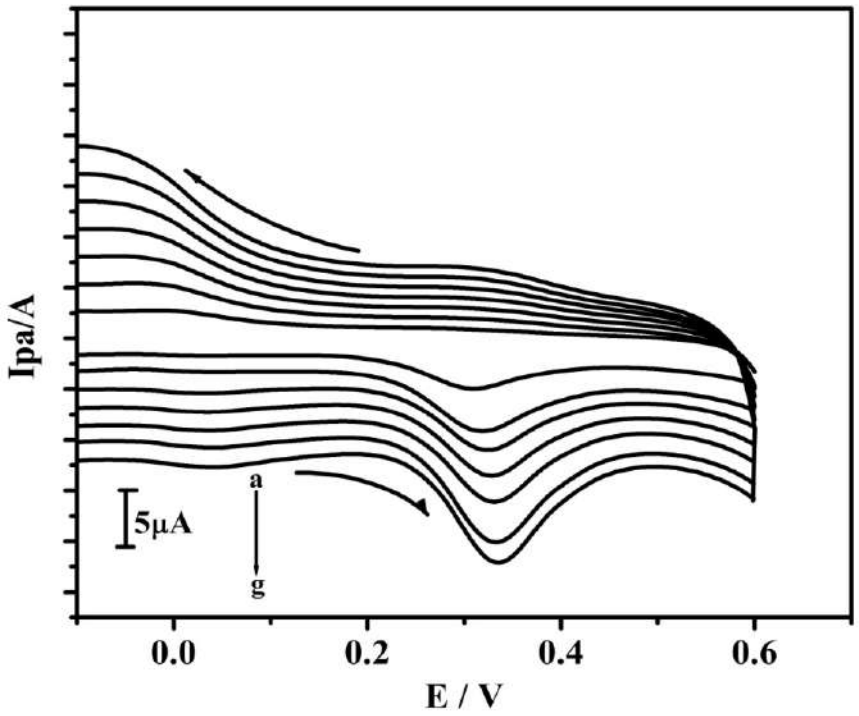


Fig. 4.3. Cyclic voltammograms of different scan rate in the presence of 10 μM serotonin and 0.2 M Phosphate buffer, in pH 7.0 scan rate (a-g: 50 to 350 mVs^{-1})

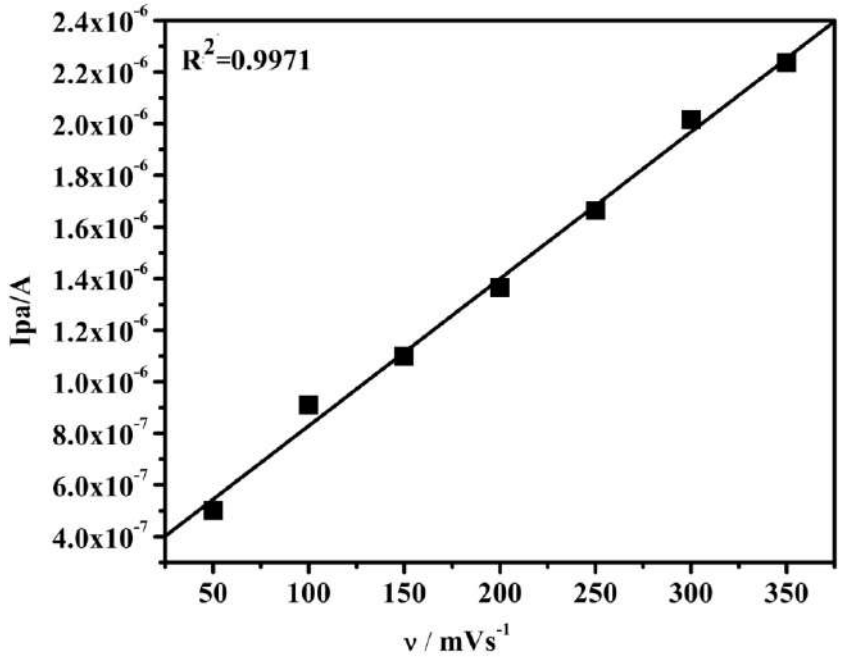


Fig. 4.3a. Effect of variation of scan rate on the anodic peak current of 10 μM serotonin and 0.2 M Phosphate buffer, in pH 7.0 scan rate: 100 mVs^{-1}

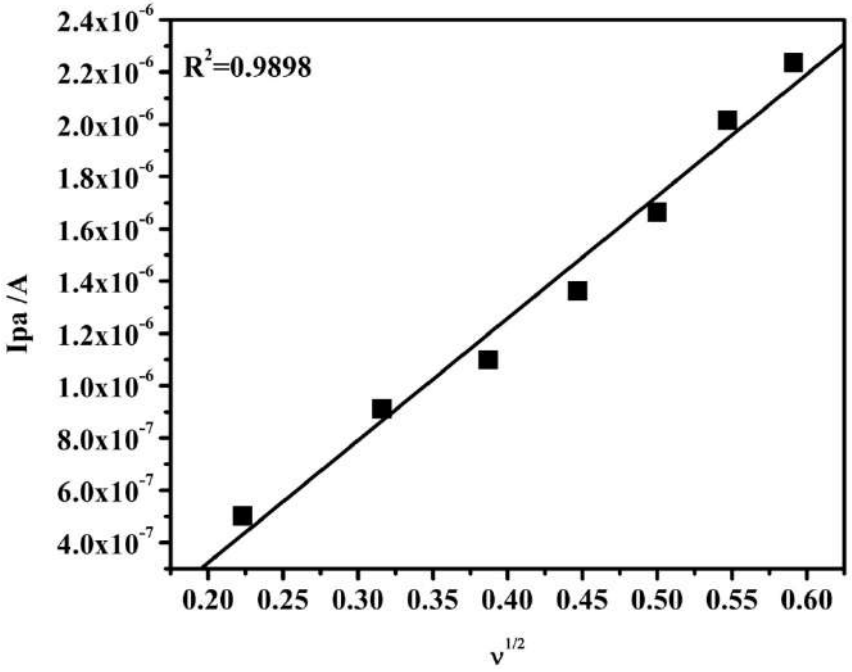


Fig. 4.3b. Effect of variation of square root of scan rate on the anodic peak current of 10 µM serotonin and 0.2M Phosphate buffer, in pH 7.0 scan rate: 100 mVs^{-1}

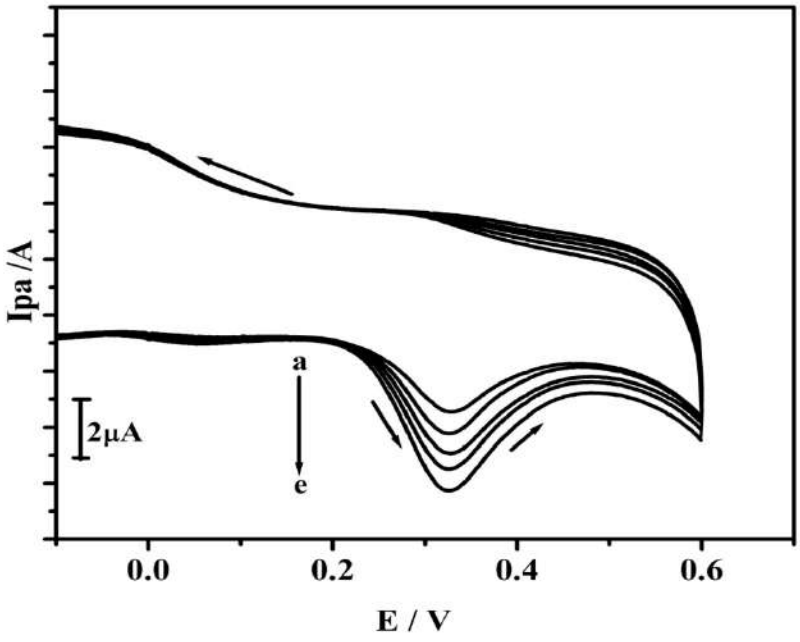


Fig. 4.4a. Cyclic voltammogram of variation of concentration of serotonin (a–e: 10, 20, 30, 40 and 50 µM) in 0.2M Phosphate buffer, in pH 7.0 scan rate: 100 mVs^{-1}

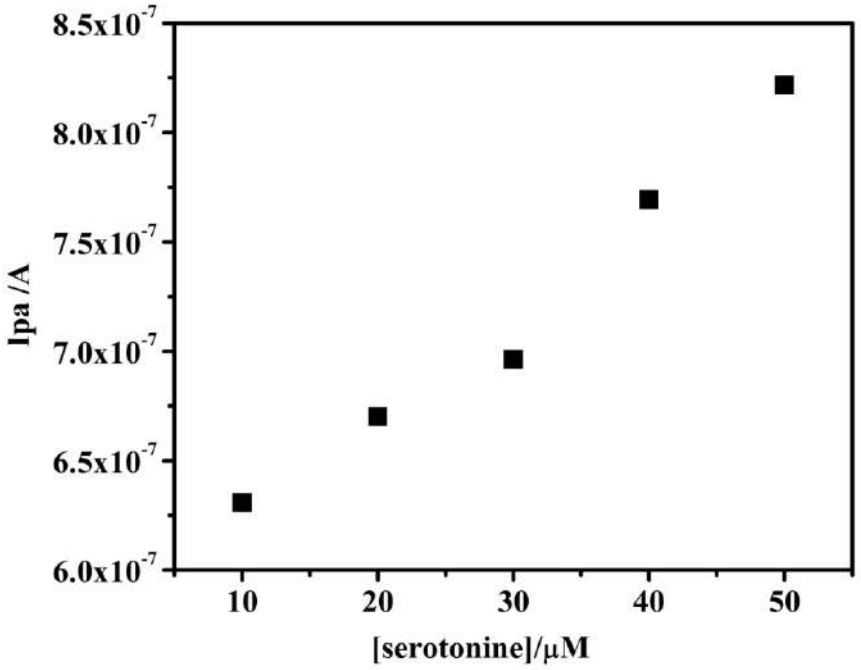


Fig. 4.4b. Effect of variation of concentration of serotonin on the anodic peak current in 0.2 M Phosphate buffer, in pH 7.0 scan rate: 100 mVs^{-1}

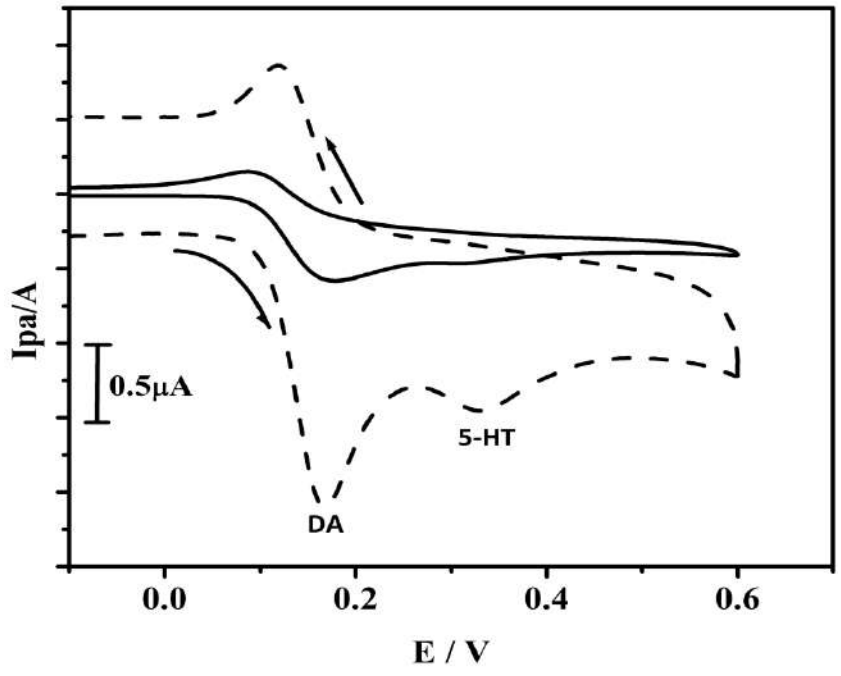


Fig. 4.5a. Cyclic voltammogram for simultaneous determination of serotonin and DA at bare GPE (solid line) and poly (EBT) MGPE (dotted line) and 0.2 M Phosphate buffer, in pH 7.0 scan rate: 100 mVs^{-1}

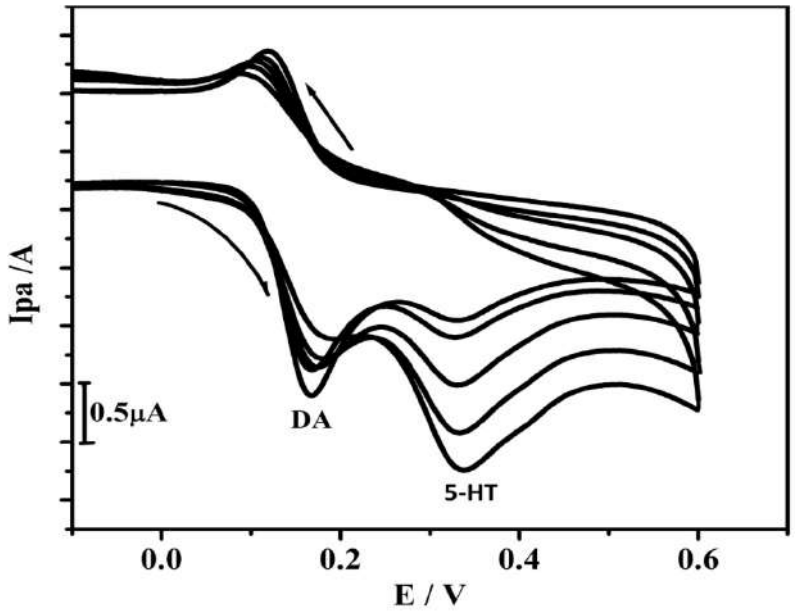


Fig. 4.5b. Cyclic voltammogram of different concentration of serotonin (10, 20, 30, 40 and 50 μM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.1 mM DA at poly (EBT) MGPE

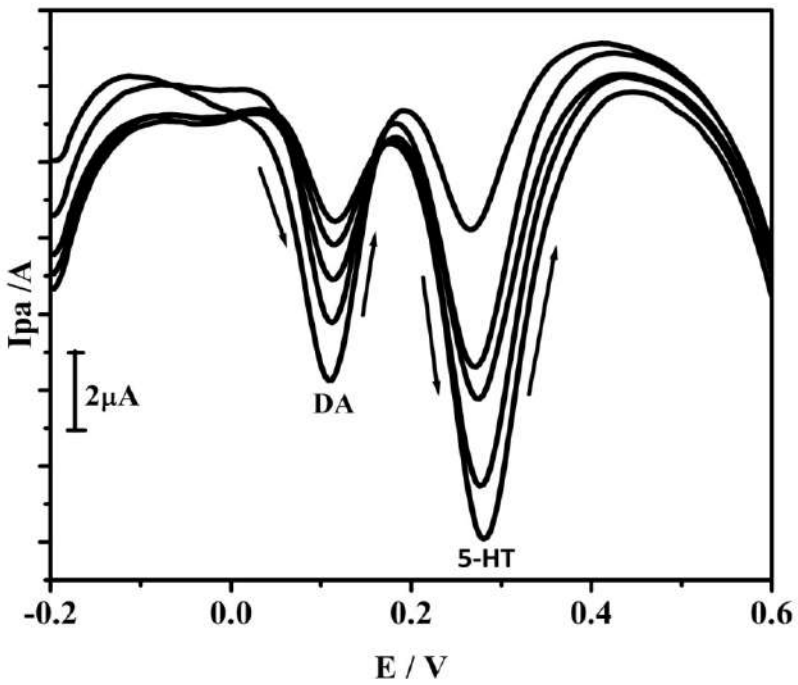


Fig. 4.6a. Differential pulse voltammogram of serotonin (10, 20, 30, 40 and 50 μM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.1 mM DA at poly (EBT) MGPE with scan rate: 100 mVs^{-1}

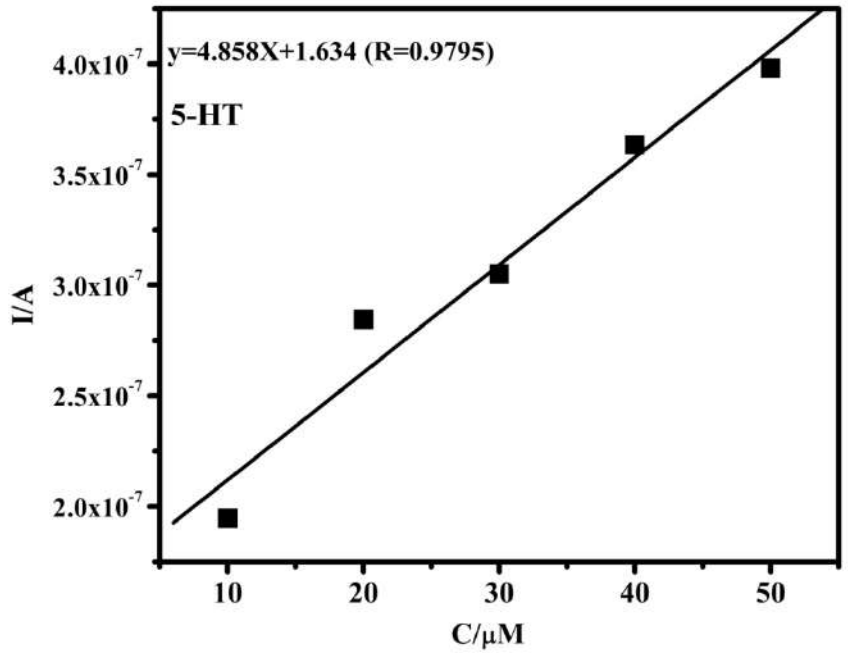


Fig. 4.6b. Plot of anodic peak current (Ipa) versus serotonin concentration

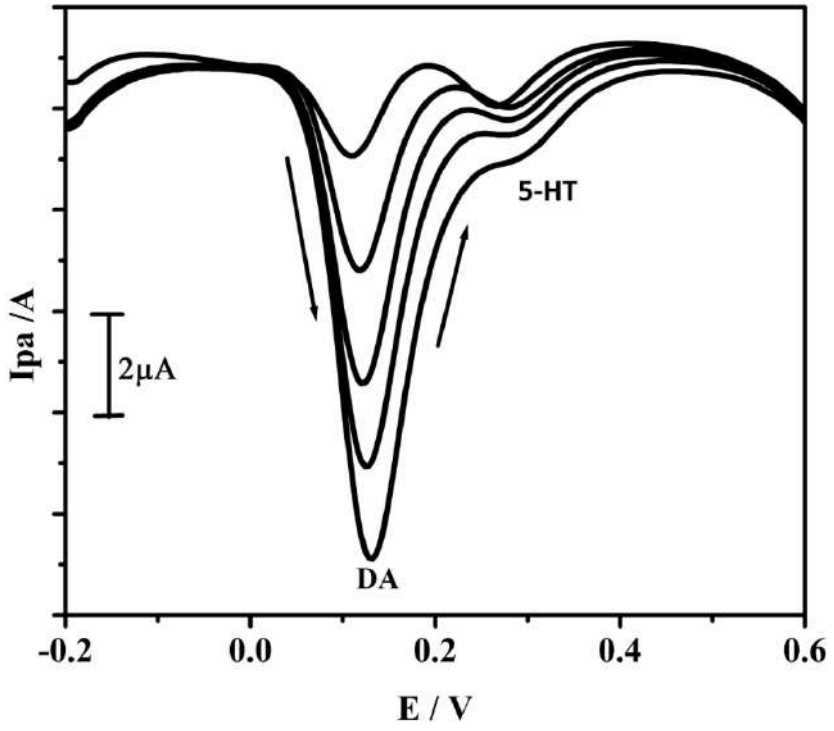


Fig. 4.7a. Differential pulse voltammogram of DA (0.1, 0.2, 0.3, 0.4 and 0.5 mM) in 0.2M phosphate buffer solution of pH 7.0 in the presence of 10 μM serotonin at poly (EBT) MGPE with scan rate: 100 mVs^{-1}

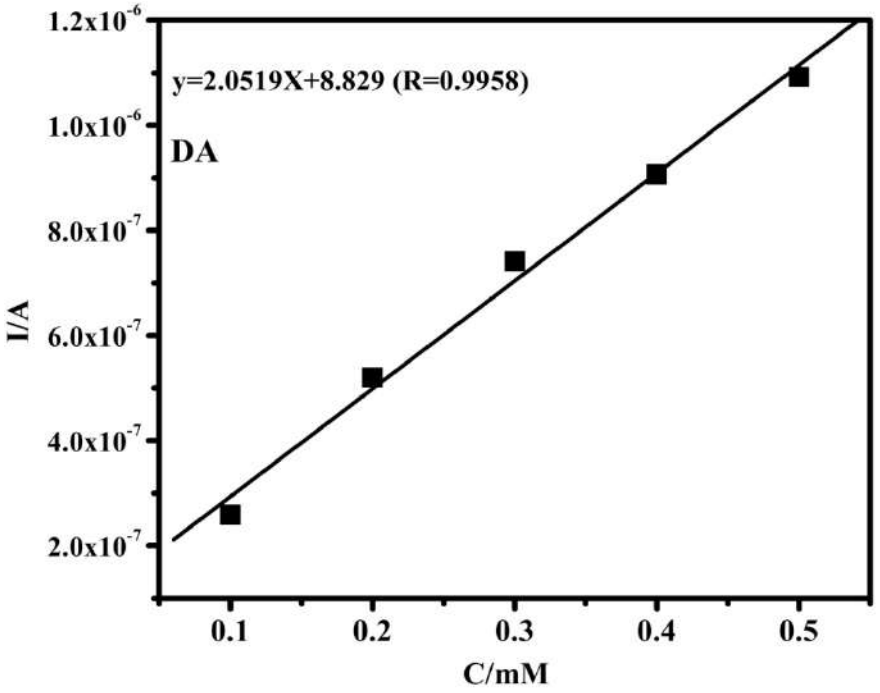


Fig. 4.7b. Plot of anodic peak current (Ipa) versus dopamine concentration

4.6.8. References

[1] K.J. Ressler and C.B. Nemeroff, *Depression and Anxiety,* 12 (2000) 19.

[2] E. Dremencov, I.G. Harman, M. Rosentein, A. Mendelman, D.H. Overstreet, J. Zohr and G. Yadid, *Prog. Neuro-Psychopharmacology and Biological Psychiatry,* 28 (2004) 147.

[3] K.W. Perry and R.W. Fuller, *Life Sciences,* 50 (1992) 1690.

[4] N. Chowdappa, B.E.K. Swamy, E. Niranjana and B.S. Sherigara, *Int. J. Electrochem. Sci*., 4 (2009) 434.

[5] A. Sawa and S.H. Snyder, *Schizophrenia: Diverse Approaches to a Complex Disease, Science,* 296 (2002) 5.

[6] R.M. Wightman, L.J. May and A.C. Michael, *Anal. Chem*., 60 (1988) 779.

[7] M. Velasco and A. Luchsinger, *Am. J. Ther.,* 5 (1998) 7.

[8] J.W. Mo and B. Ogorevc, *Anal. Chem*., 73 (2001) 1202.

[9] G.A. Junter, *Electrochemical Detection Techniques in the Applied Biosciences,* Halsted, New York (1988).

[10] Q. Wang, D. Dong and N.Q. Li, *Bio electrochem*., 54 (2001) 175.

[11] K.H. Xue, F.F. Tao and W. Xu, *J. Electroanal. Chem*., 578 (2005) 329.

[12] M. Chicharroa, A. Sancheza, A. Zapardiel, M.D. Rubianesc and G. Rivasc, *Anal. Chim. Acta.,* 523 (2004) 191.

[13] B.N. Chandrashekar, B.E.K. Swamy, M. Pandurangachar, S.S. Shankar, O. Gilbert, J.G. Manjunath and B.S. Sherigara, *Int. J. Electrochem. Sci*., 5 (2010) 592.

[14] W. Gao, J. Song and N. Wu, *J. Electroanal. Chem*., 576 (2005) 7.

[15] D. Demetriades, A. Economou and A. Voulgaropoulos, *Anal. Chim. Acta*., 519 (2004) 172.

[16] H. Karadeniz, B. Gulmez and F. Sahinci *et al*., 33 (2003) 302.

[17] A. Levent, Y. Yardim and Z. Senturk, *Electrochim. Acta*., 55 (2009) 195.

[18] A. Sathisha and B.E.K. Swamy, *Anal. Bioanal. Electrochem*., 7 (2015) 21.

[19] M. Vestergaard, K. Kerman and E. Tamiya, *Anal. Chim. Acta*., 538 (2005) 281.

[20] A. Erdem, P. Papakonstantinou and H. Murphy, *Anal. Chem*., 78 (2006) 6659.

[21] J. Wang, A. N. Kawde and E. Sahlin, *Analyst*, 125 (2000) 7.

[22] U. Chandra, B.E.K. Swamy and O. Gilbert *et al*., *Chinese Chemical Letters*, 21 (2010) 1492.

[23] U. Chandra, B.E.K. Swamy, Ongera Gilbert and B.S. Sherigara, *Int. J. Electrochem*., 20 (2011) 8.

[24] M. Aslanoglu, S. Abbasoglu, S. Karabulut and A. Kutluay, *Acta Chim. Slov.,* 54 (2007) 839.

[25] Z.G. Gao, D. Yap and Y. Zhang, *Anal. Sci.,* 14 (1998) 1063.

[26] Y.H. Zhang, G.Y. Jin, Y.L. Wang and Z.S. Yang, *Sensors,* 3 (2003) 450.

[27] Y.H. Zhang, G.Y. Jin, Z.S. Yang and H. Zhao, *Microchimica Acta.,* 147 (2004) 230.

[28] P.R. Roy, T. Okajima and T. Ohsaka, *Bioelectrochem*, 59 (2003) 19.

[29] P.F. Huang, L. Wang, J.Y. Bai, H.J. Wang, Y.Q. Zhao and S.D. *Fan, Micro Chimica Acta*., 157 (2007) 47.

[30] Y.L. Chen, J.H. Yuan, X.Z. Wang and C.X. Tian, *Anal. Sci.,* 20 (2004) 17263.

[31] W. Ren, H.Q. Luo and N.B. Li, *Biosens. Bioelectron*., 21 (2006) 1097.

[32] T.F. Kang, G.L. Shen and R.Q. Wu, *Anal. Chim. Acta*., 356 (1997) 251.

[33] L.Z. Zheng, E.G.X.Q. Lin, L. Nie and L. Rui, *Analyst,* 126 (2001) 738.

[34] W. Sun, M. Yang and K. Jiao, *Anal. Bioanal. Chem*., 389 (2007) 1291.

[35] A. Safavi, N. Maleki, O. Moradlou and F. Tajabedie, *Anal. Biochem*., 359 (2006) 229.

[36] Y.F. Zhao, Y.Q. Gao, D.P. Zhan, H. Liu, Q. Zhao, Y. Kou, Y.H. Shao, M.X. Li, Q.K. Zhuang and Z.W. Zhu, *Talanta*, 66 (2005) 57.

[37] Z. Wang, J. Liu, Q. Liang, Y. Wang and G. Luo, *Analyst,* 127 (2002) 658.

[38] P. Zhang, F.H. Wu, G.C. Zhao and X.W. Wei, *Bioelectrochem.*, 67 (2005) 114.

[39] S.B. Hocevar, J. Wang, R.P. Deo, M.M. Usameh and B. Ogorevc, *Electroanal.*, 17 (2005) 422.

[40] Z.H. Wang, Q.L. Liang, Y.M. Wang and G.A. Luo, *J. Electroanal. Chem*., 540 (2003) 134.

[41] K.H. Xue, F.F. Tao, W. Xu, S.Y. Yin and J.M. Liu*, J. Electroanal. Chem*., 578 (2005) 329.

[42] Q. Wang, N. Jiang and N.Q. Li, *Microchem. Journal,* 68 (2001) 85.

[43] H.M. Zhang, N.Q. Li and Z.W. Zhu, *Microchem. Journal,* 64 (2000) 284.

[44] T. Liu, M.X. Li and Q.Y. Li, *Talanta*, 63 (2004) 1059.

[45] Q. Wang, D. Dong and N.Q. Li, *Bioelectrochem*., 54 (2001) 175.

[46] L. Zhang, J.B. Jia, X.Q. Zou and S.J. Dong, *Electroanal*., 16 (2004) 1413.

[47] Z.G. Hua, L.M. Fang and L.M. Li, *CEJC,* 5(4) (2007) 1114.

[48] C.Y. Wang, Z.X. Wang, A.P. Zhu and X.Y.Hu, *Sensors,* 6 (2006) 1536.

[49] C.M.A. Brett, G. Inzelt and V. Kertesz, *Anal. Chim. Acta*., 385 (1999) 123.

[50] H. Zhao, Y.Z. Zhang and Z.B. Yuan, *Electroanalysis,* 14 (2002) 1035.

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

[52] H. Yao, Y.Y. Sun, X.H. Lin, Y.H. Tang and L.Y. Huand, *Electrochim. Acta*., 52 (2007) 6165.

[53] X.H. Lin, W. Li, H. Yao, Y.Y. Sun, L.Y. Huang and Y.J. Zheng, *Coll. Czech. Chem. Comm.,* 72 (2007) 1177.

[54] O. Gilbert, B.E.K. Swamy, U. Chandra and B.S. Sherigar, *Int. J. Electrochem. Sci*., 4 (2009) 591.

[55] W. Sun, K. Jiao, J.Y. Han and G.Y. Xu, *J. Chinese Chem. Soc*., 53 (2006) 1141.

[56] B.E.K. Swamy and B.J. Ventron, *Analyst,* 132 (2007) 884.

[57] E. Niranjana, R. Raghavendra Naik, B.E.K. Swamy, Y.D. Bodke, B.S. Sherigara, H. Jayadevappa and B.V. Badami, *Int. J. Electrochem. Sci*., 3 (2008) 992.

[58] W. Sun, M. Yang and K. Jiao, *Anal. Bioanal. Chem*., 389 (2007) 1291.

[59] U. Chandra, B.E.K. Swamy, O. Gilbert and B.S. Sherigara, *Electrochim. Acta*., 55 (2010) 7174.

[60] R.N. Hegde, B.E.K. Swamy, N.P. Shetty and S.T. Nandibewoor, *J. Electroanal. Chem.,* 51(2009) 635.

[61] Q. Wang, N. Li and W. Wnag, *Anal. Sci*., 18 (2002) 635.

[62] N.B. Li, W. Ren and H.Q. Luo, *Electroanal.,* 19 (2007) 15.

[63] T. Thomas, R.J. Mascarenhas, F. Cotta, K.S. Guha, B.S.K. Swamy, P. Martis and Z. Mekhalif, *Colloids Surf. B*, 101 (2013) 91.

[64] P. Chandra, N.X. Son, H.B. Noh, R.N. Goyal and Y.B. Shim, *Biosens. Bioelectron*., 39 (2013) 144.

[65] M. Zidan, T.W. Tee, A.H. Abdullah, Z. Zainal and G.J. Kheng, *Int. J. Electrochem. Sci*., 6 (2011) 288.

[66] Y. Umasankar, B. Unnikrishnan, S.M. Chen and T.W. Ting, *Int. J. Electrochem. Sci*., 7 (2012) 498.

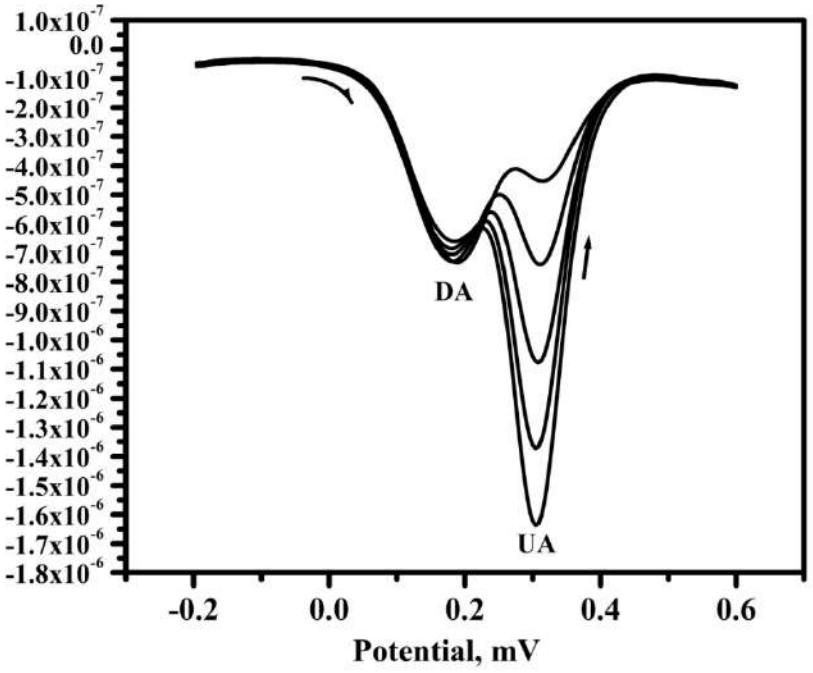
[67] S. Mehretie, S. Admassie, M. Tessema and T. Solomon, *Anal. Bioanal. Electrochem*., 3 (2011) 50.

[68] V. Anitha Kumary, J. Divya, T.E. Mary Nancy and K. Sreevalsan, *Int. J. Electrochem. Sci.,* 8 (2013) 6619.

CHAPTER-5

Part-A

Voltammetric Determination of Dopamine in Presence of Uric Acid at Glimepiride Modified Carbon Paste Electrode



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5.1. Introduction

The carbon paste electrode was modified with glimepiride (GM) and it was used for simultaneous determination of dopamine (DA), uric acid (UA) in 0.2 M phosphate buffer of pH 7.0. Based on its strong electrocatalytic action towards the oxidation of dopamine and uric acid. The modified electrode shows is increased in anodic peak currents. The glimepiride modified carbon paste electrode used for the detection of dopamine was stable, reproducible and low detection limit for DA. The effect of scan rate, pH, surfactant and concentration was studied. The effect of interferences was studied by differential pulse voltammetric technique. The modified electrode was used for the analysis of DA and UA in real samples with satisfactory results.

5.1.1. Chemistry and Biological Relevance of Dopamine

The chemistry and biological relevance of dopamine has been explained details in chapter 3 Part A section 3.1.1 and 3.1.1.1.

5.1.2. Chemistry and Biological Relevance of Uric Acid

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

As a bicyclic, heterocyclic purine derivative, UA does not protonate in the same manner as do carboxylic acids. X-Ray diffraction studies on the hydrogen urate ion in crystals of ammomium hydrogen urate, formed in vivo as gouty deposits, revealed that the keto-oxygen in the 2 position of a tautomer of the purine structure existed as a hydroxyl group and that the two flanking nitrogen atoms at the 1 and 3 positions shared the ionic charge in the six membered pi-resonance-stabilized ring [2]. Thus, whereas most organic acids are deprotonated by the ionization of a polar hydrogen-to-oxygen bond, usually accompanied by some form of resonance stabilization (resulting in a carboxylate

ion), this acid is deprotonated at a nitrogen atom and uses a tautomeric keto/hydroxy group as an electron-withdrawing group to increase the pK_1 value. The five membered ring also possesses a keto group (in the 8 position), flanked by two secondary amino groups (in the 7 and 9 positions), and deprotonation of one of these at high pH could explain the pK_2 and behavior as a diprotic acid. Similar tautomeric rearrangement and pi-resonance stabilization would then give the ion some degree of stability.

Generally the solubility of UA, its Alkali and Alkali Earth Metal salts in water are rather low and all exhibit greater solubility in hot water than cold allowing for easy recrystallization. The solubility of the acid and its salts in ethanol is very low or negligible. In ethanol water mixtures the solubility are somewhere between the end values for pure ethanol and pure water.

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

5.1.3. Review of Electrochemistry of Dopamine and Uric Acid

Dopamine (DA) and uric acid (UA) are some of the biological compounds which are electrochemically active in voltammetric techniques. The detection of these compounds is important not only for diagnostic studies but also for pathological research. Electrochemical techniques for the determination of these compounds in body fluid samples have attracted great interest, since these techniques are fast in detection, low in cost and with the merits of low detection limit and high accuracy.

Dopamine (DA) is one of the naturally occurring catecholamines. It is an important compound for message transfer in the mammalian central nervous system. Changes in its concentration may lead to serious diseases such as Parkinson's [3]. Quantitative determination of DA in human physiological fluids is of considerable significance in both biochemical and clinical diagnoses. Methods for the detection of DA include chemiluminescence [4], fluorimetry [5], ultraviolet-visible spectrometry [6], and capillary electrophoresis (CE-luminescence) [7-8]. Because of its electrochemical activity, DA can also be determined with electrochemical methods because it is an electrochemically active compound [9-10].

Uric acid (UA) and other oxypurines are main final products of purine metabolism in the human body. Disorder of purine biosynthesis and/or purine catabolism, such as gout, hyperuricemia and Lesch. Nyhan syndrome are generally considered due to the abnormal concentrations of UA dissolved in human urine and/or blood [11-12].

Surfactants, due to their favorable physicochemical properties are extensively used in many fields of technology and research, i.e. in pharmacy, in cosmetics, textile industry, agriculture, biotechnology. Normally surfactant is a linear molecule with a hydrophilic (attracted to water) head and a hydrophobic (repelled by water) end. Surfactants, a kind of amphiphilic molecules with a hydrophilic head on one side and a long hydrophobic tail on the other, have been widely applied in electrochemistry to improve the property of the electrode solution interface and also improve the detection limits of some biomolecules. The results showed that the electrochemical responses of these compounds were greatly enhanced in the presence of trace amounts of surfactants [13-17].

Alpha-olefin sulfonate surfactants (AOS) are produced by the direct reaction of olefin with strong sulfonating agents, such as sulfur trioxide. This leads to the formation of surface active anionic mixtures containing both alkene sulfonates and hydroxyalkane sulfonates. These surfactants may be used in place of linear alkyl benzene sulfonates in many formulas with resulting improvements in biodegradability, mildness to skin, foaming and detergency. In addition AOS surfactants are stable over a much broader pH

range than alkyl sulfates, alkyl ether sulfates and ester- type surfactants. They also exhibit excellent foaming and detergency in hard water. The INCI name of the primary AOS commerce is sodium C14-16 olefin sulfonate.

Carbon Paste Electrode (CPE) is one of the convenient conductive matrices to prepare the chemically modified electrodes (CMEs) by the simple mixing of graphite/binder paste and modifier [18-19]. These kinds of electrodes are inexpensive and possess many advantages such as low background current, wide range of used potential, ease of fabrication, and rapid renewal [20]. Carbon paste electrodes modified with different modifiers, have been reported for voltammetric determination of DA in the presence UA. Although the simultaneous determination DA and UA at chemically modified electrodes was reported [21-29] still there is a scope for the preparation of new electrode in electrochemical sensor field.

The structure could be explained as follows (Scheme 5.1) Glimepiride, 1-[[*p*-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulfonyl]-3-(*trans*-4-methylcyclohexyl) urea, is a new oral sulfonylurea hypoglycemic agent. It contains a sulfonylurea nucleus and a cyclohexyl ring. Glimepiride is a white to yellowish white, crystalline, odorless to practically odorless powder, which is practically insoluble in water [30]. Glimepiride is used in the management of non-insulin dependent (type II) diabetes mellitus and is completely absorbed from the GI tract after oral administration [31] and [32].

The aim of our work is to fabricate a type of stable electrode by coating a redox film which can show an excellent electrocatalytic property. The modification was done by glimepiride of carbon paste electrode and is used for the analysis of DA in physiological pH.

5.2. Experimental Section

5.2.1. Chemicals

Dopamine [DA], Uric acid [UA], Glimepiride [GM], Sodium alpha-olefin sulfonate were obtained from Himedia. Dopamine was dissolved using 0.1M Perchloric

acid ($HClO_4$). All other Chemicals were of analytical grade quality and were used without further purification. The water used was a double distilled in all the measurements. Phosphate Buffer 0.2 M was prepared by 0.2 M disodium hydrogen phosphate and 0.2 M sodium dihydrogen phosphate.

5.2.2. Apparatus and Procedure

The electrochemical experiments were carried out using a model CHI-660c (CH Instrument-660 electrochemical workstation). All experiments were carried out in a conventional three-electrode system. The electrode system contained a working carbon paste electrode, homemade cavity (3 mm diameter), a platinum wire as counter electrode and saturated calomel electrode as reference electrode. Bare carbon paste electrode was prepared by grinding 70% graphite powder and 30% silicon oil in an agate mortar by hand mixing for about 30 min to get homogenous paste. The paste was packed into the cavity CPE and smoothened on weighing paper.

5.2.3. Preparation of Glimepiride Modified Carbon Paste Electrode

Glimepiride Modified Carbon Paste Electrode (GMMCPE) was prepared by grinding the different weights of glimepiride (2, 4, 6, and 8 mg) with 70% graphite powder of 50 mm particle size and 30% silicon oil in an agate mortar by hand mixing for about 30 minute to get homogeneous glimepiride modified carbon paste. The paste was packed into the cavity of homemade CPE of 3mm in diameter and smoothened on weighing paper.

5.3. Results and Discussion

5.3.1. Electrochemical Response of Dopamine with Glimepiride Modified Carbon Paste Electrode (GMMCPE)

The effect of glimepiride concentration in the carbon paste electrode was investigated for 0.1mM DA in 0.2 M phosphate buffer solution at pH 7.0 by CV method. The carbon paste electrode with 4 mg of glimepiride showed high anodic peak current as

compared to 2, 6 and 8 mg of Glimepiride. The electrochemical response of 2, 4, 6 and 8 mg of GMMCPE is shown in Figure 5.1.

5.3.2. Electrocatalytic Response of DA at Glimepiride Modified Carbon Paste Electrode

Dopamine is being an easily oxidisable catecholamine, its voltammogram was recorded in the potential range of -200 to 600 mV in the 0.2 M phosphate buffer at pH-7.0 at 100 mVs^{-1}. Figure 5.2 shows the pair of redox peaks for 1 $\times 10^{-4}$ M DA at bare CPE (dashed line) with Epa 198 mV and Epc 130 mV. The peak to peak separation Ep was found to be 68 mV and ratio of redox peaks current ipa/ipc was found to be 1.83 μA. However for the GMMCPE a pair of redox peaks is obtained with good enhancement in both anodic and cathodic peak current (solid line). The Epa was located at 225 mV and the corresponding cathodic peak potential was located at 114 mV. Peak to peak separation was calculated as 111 mV and the value of ipa/ipc was about 1.85 μA. Hence, the voltammogram obtained for GMMCPE was also with good improvement in enhancement of oxidation and reduction peak current showed electron transfer kinetics.

5.3.3. Effect of Scan Rate

Figure 5.3 shows the cyclic voltammograms recorded for DA at different scan rates at GMMCPE The scan rate has a great influence on the peak current of DA on GMMCPE. The difference between the anodic peak potential and the cathodic peak potential was increasing with the increased in the scan rate The graph of current Ipa versus scan rate and square root of scan rate were plotted. The graph obtained were nearly straight line as shown in Figure 5.3a and 5.3b. In the range from 50 to 300 mV/s the anodic peak currents were proportional to the scan rate and also the to the square root of scan rate with correlation coefficient 0.99737 and 0.98137 for Ipa versus scan rate and Ipa versus square root scan rate respectively. This indicates that, the electrode transfer reaction is adsorption controlled.

5.3.4. Effect of Concentration of DA

The electrocatalytic oxidation of DA was carried out by varying its concentration at GMMCPE was shown in Figure 5.4. By increasing the concentration of DA from 0.1 to 0.6 mM, the Ipa and Ipc was found to be increasing with shifting of Epa towards positive potential and Epc towards slightly negative potential. The concentration curve of DA shows increase in electrochemical peak current shown in Figure 5.4a which indicates that Ipa was proportional to concentration of DA. The detection limit and quantification limit of DA of GMMCPE was calculated from the graph and was found to be 20μM and 67μM respectively.

The detection limit and quantification limit was calculated by using the formulas (1) and (2) where S is the standard deviation and M is the slope obtained from the three calibration plots. From the data, a lower limit of detection (LOD) and lower limit of quantification (LOQ) can be achieved using the proposed method [33-34].

$$LOD = 3S/M \quad \text{---------- (1)}$$

$$LOQ = 10S/M \quad \text{---------- (2)}$$

5.3.5. Effect of pH

Figure 5.5 shows the effect of pH on the electrocatalytic oxidation of dopamine on the GMMCPE. The Epc and Epa shifts to a more negative oxidation side indicating that an equal number of protons take part in the reaction. The dependence of Epa versus pH shown in Figure 5.5a was also investigated. From the plot it was found that the anodic peak potential decreases with the increase in pH, from 6.4 to 7.4 indicates that the electro-oxidation process becomes easier at higher pH.

5.3.6. Electrochemical Response of Dopamine with Glimepiride Modified Carbon Paste Electrode in Presence of Surfactant

The electrochemical responses of dopamine at carbon paste electrode was shown in Figure 5.6 with 0.2 M phosphate buffer as a supporting electrolyte at pH 7.0 at a scan

rate of 100 mVs^{-1} owing to the complex properties and the roughness of the electrode surface, the cyclic voltammogram of dopamine with modified carbon paste electrode the absence of Sodium alpha-olefin sulfonate surfactant is low signal (solid line) and showed high anodic peak current as compared with the bare carbon paste electrode (dotted line). However, the voltammetric response is apparently improved in the presence of 50 μM of sodium alpha-olefin sulfonate reflected by the good enlargement of anodic peak current (ipa) (dashed line). The probable mechanism is the Sodium alpha-olefin sulfonate surfactant molecule diffuses into the carbon paste electrode along with the dopamine results increase in the signal.

5.3.7. Influence of Concentration of Sodium Alpha-Olefin Sulfonate Surfactant on Voltammetric Response for Dopamine on Glimepiride Modified Carbon Paste Electrode

To study the effect of addition of surfactants the experiments were carried out using surfactant Sodium alpha-olefin sulfonate initially, the cyclic voltammogram were recorded for GMMCPE a solution containing dopamine in Phosphate buffer solution at pH 7.0. Keeping the concentration of dopamine constant, the concentration of the surfactant was increased from 10 to 50 μM by mobilization method. Figure 5.7 shows the effect of surfactant concentration by mobilized method both the ipa and ipc increases rapidly with the increases of surfactant concentration.

5.3.8. Electrocatalytic Oxidation of UA on Glimepiride Modified Carbon Paste Electrode

The cyclic voltammogram obtained for the oxidation of UA at the bare and at the GMMCPE are shown in Figure 5.8. The dashed line curve corresponds to bare carbon paste electrode (BCPE) and solid line corresponds to GMMCPE in 0.2 M phosphate buffer solution with 0.1 mM UA at GMMCPE, which confirmed that electrochemical reaction of UA was an irreversible process. At BCPE oxidation peak was observed for 0.1 mM UA with the potential of about 348 mV. After modification of the electrode the anodic peak current increased and the peak shifted slightly towards positive side

(390 mV). Increase in the current signal showed that GMMCPE film at electrode catalyzed the electrochemical oxidation of UA.

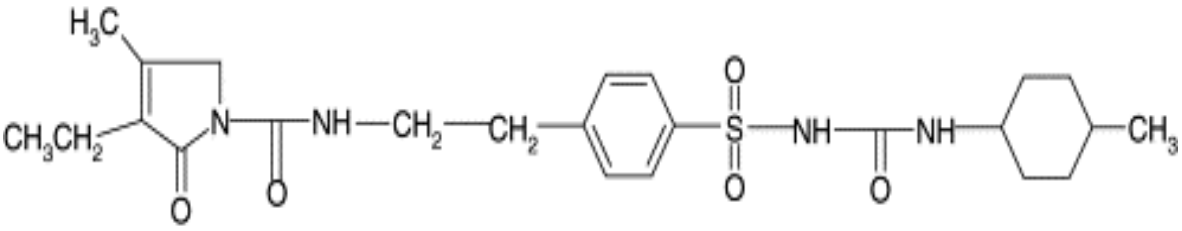
5.3.9. Simultaneous Determination of DA and UA

UA were present along with DA in mammalian brain and the concentration of these were much higher than that of DA. Since the oxidation potential of UA was nearly same as that of DA result in an overlapped voltammetric response at BCPE. The Figure 5.9 showed the cyclic voltammetric response of DA in the presence of UA in phosphate buffer solution of pH 7.0 at both BCPE and GMMCPE. The voltammogram obtained for mixture of sample at BCPE was less sensible (dotted line). However GMMCPE faced this challenge and achieved the separation (solid line). The resulted voltammogram at GMMCPE had two well defined peaks for DA and UA at different potentials. The oxidation peak potentials of DA and UA were at 205 and 385 mV respectively. The peak to peak separation of DA–UA was 180 mV. This results were large sufficient to identify DA in the presence of UA at GMMCPE.

The simultaneous determination of DA and UA in the mixture was carried out at GMMCPE when concentration of one species changed where as the other remained kept constant. From Figure 5.10 it can be seen that the peak current of DA was proportional to its concentration which was increased from 20 to 100 μM when keeping the concentration of UA 0.1 mM. There were no change in the peak current and peak potential occurred for UA. The Figure 5.11 self explains the concentration effect of UA from 0.1mM to 0.5mM respectively. These results show that the DA and UA were exist independently in their mixtures of samples. The corresponding graphs of anodic peak current versus various concentrations of DA (20-100 μM), UA (0.1-0.5 mM) showed linear relationships with linear regressions for A (DA) Ipa (μA) =0.005153 C(μM/L) +8.9606, B (UA) Ipa (μA) =2.9895 C(mM/L) +1.5787, the correlation coefficient for these linear graphs was 0.9986 and 0.9980 respectively for this GMMCPE which were shown in Figures 5.10a and 5.11a**,** respectively.

5.3.10. Conclusion

In this work, the modified glimepiride carbon paste electrode acts as a good sensor exhibited strong promoting effect and stability towards the electrochemical oxidation of dopamine at pH 7 in phosphate buffer solution (PBS). The scan rate effect was found to be adsorption controlled electrode process. Using cyclic voltammetric technique was well investigated the concentration effect, pH effect and surfactant effect and detection limit of 2×10^{-6} of dopamine is achieved. The proposed technique provides a suitable method for simultaneous detection of dopamine and uric acid in biological samples.



Scheme 5.1. Structure of Glimepiride

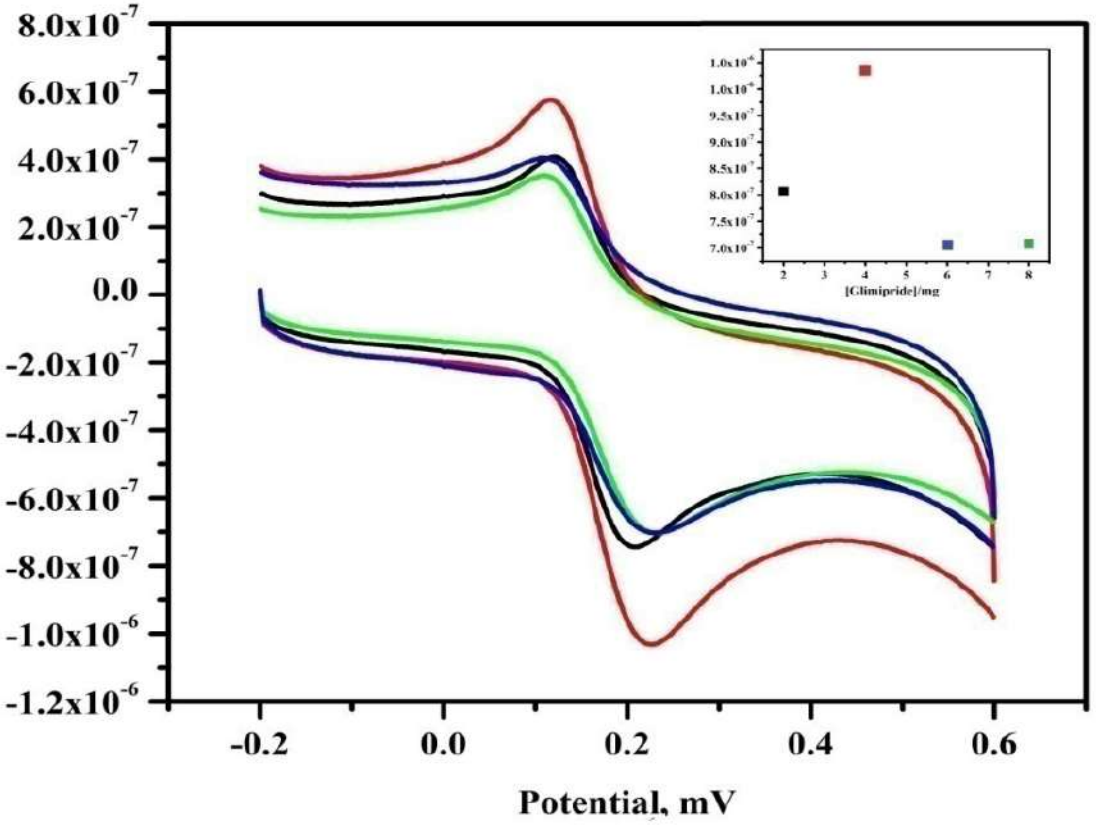


Fig. 5.1. Cyclic voltammogram of variation of concentration of glimepiride in 0.1 mM DA pH 7.0, Phosphate buffer solution, Plot of anodic peak current of DA verses concentration of glimepiride (insert curve)

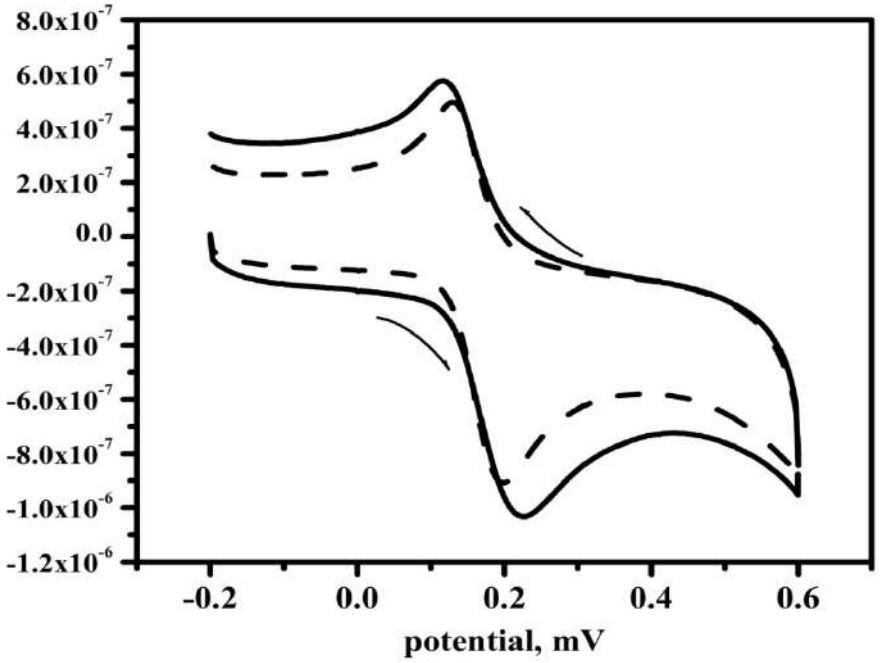


Fig. 5.2. Cyclic voltammograms obtained for the electrochemical response of DA at glimepiride MCPE (solid line) and bare carbon paste electrode (dashed line) in 0.2 M phosphate buffer solution pH 7.0 containing 0.1mM DA scan rate 100 mV/s

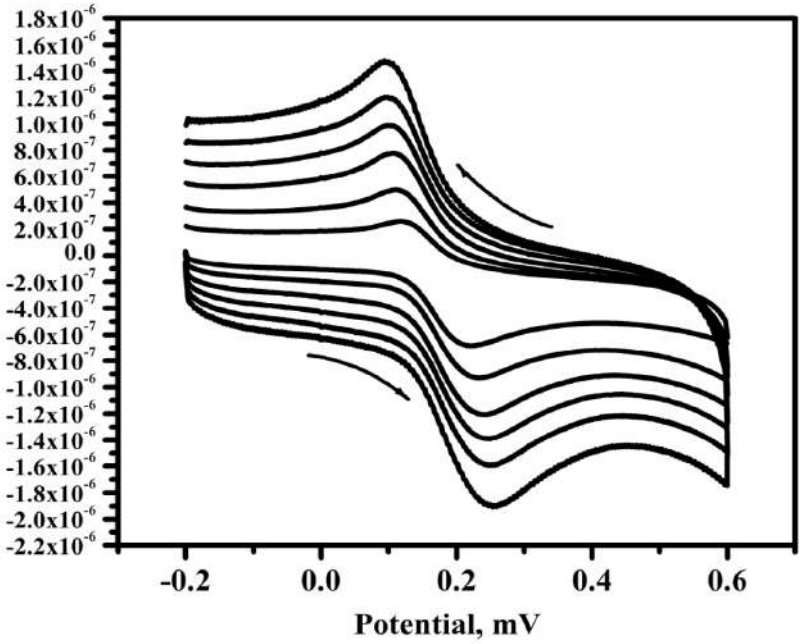


Fig. 5.3. Cyclic voltammogram of different scan rate in the presence of 0.1 mM dopamine and 0.2 M phosphate buffer solution at pH 7.0, scan rate 50 to 300 mV/s

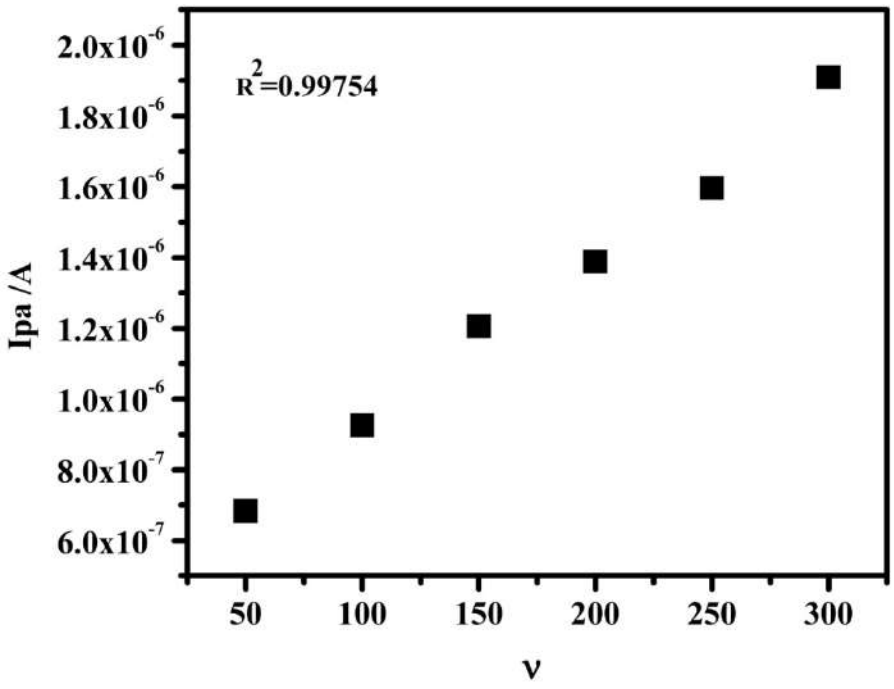


Fig. 5.3a. Plot of anodic peak current verses scan rate

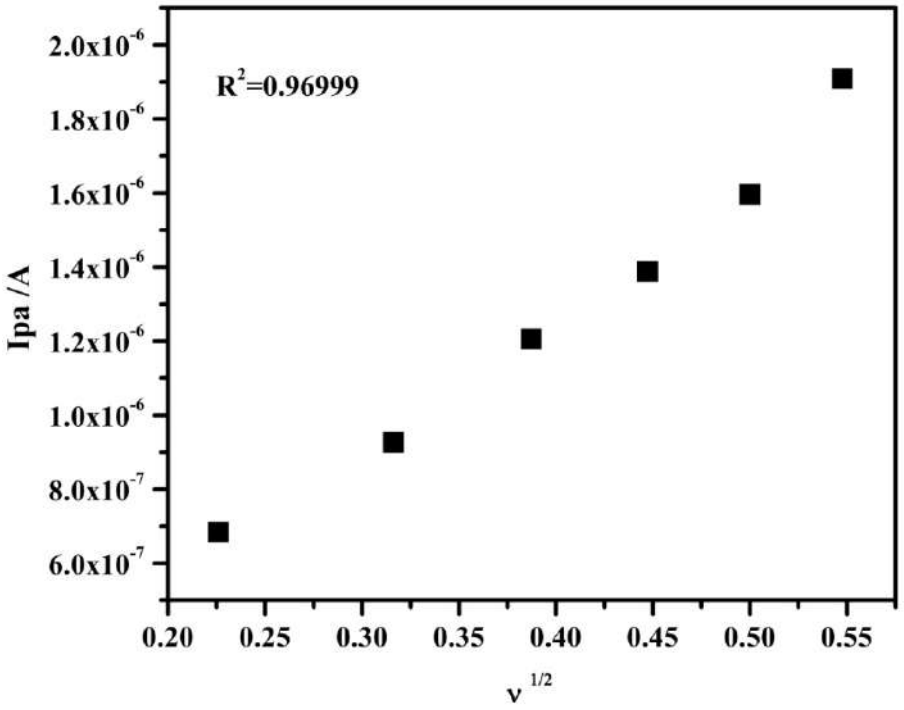


Fig. 5.3b. Plot of anodic peak current verses square root of scan rate

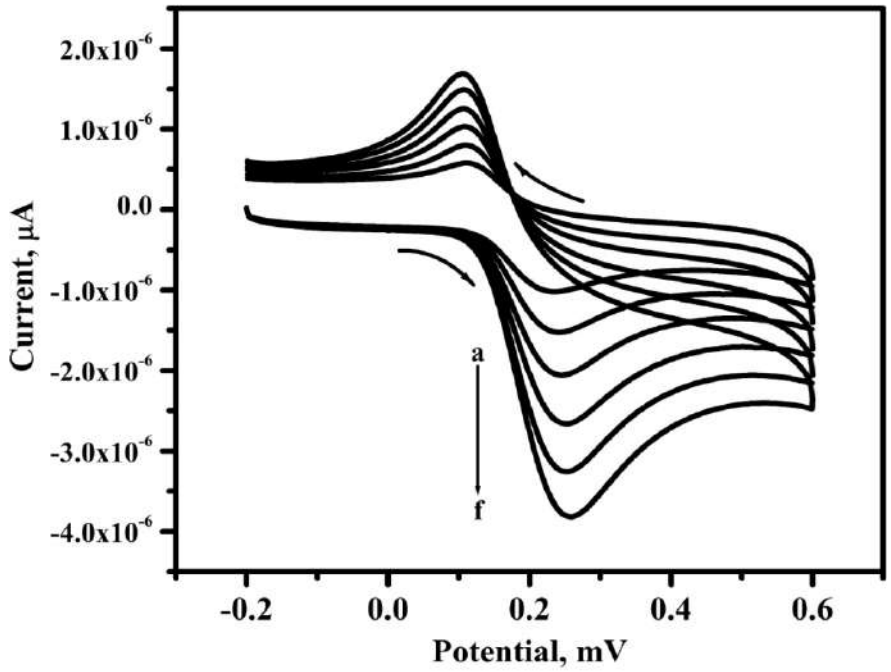


Fig. 5.4. Cyclic voltammogram of variation of concentration of dopamine from 0.1 to 0.6 mM in presence of phosphate buffer solution at pH 7.0

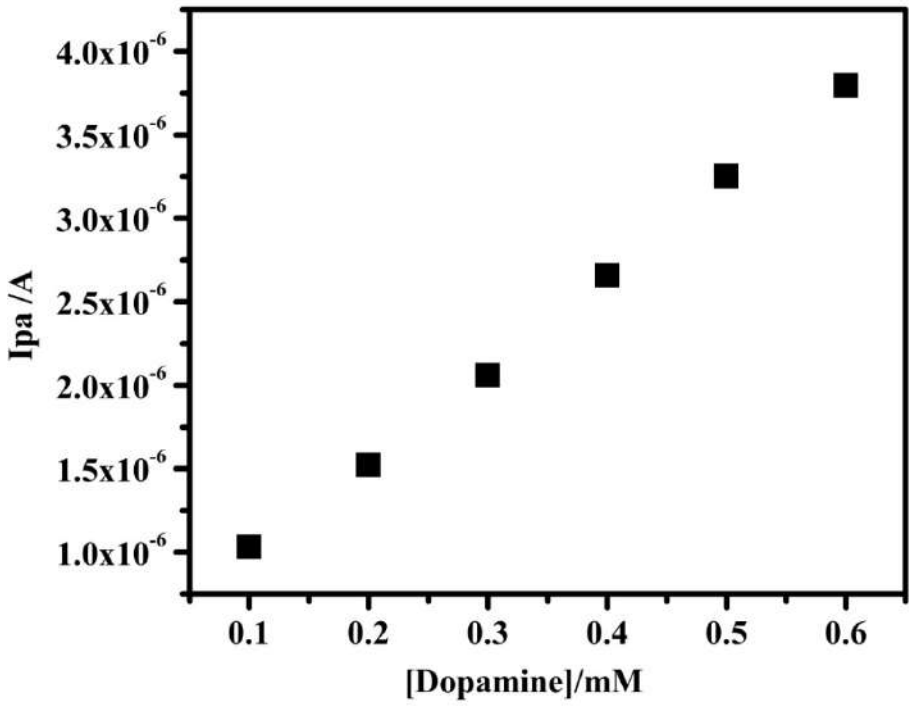


Fig. 5.4a. Plot of anodic peak current verses the concentration of DA

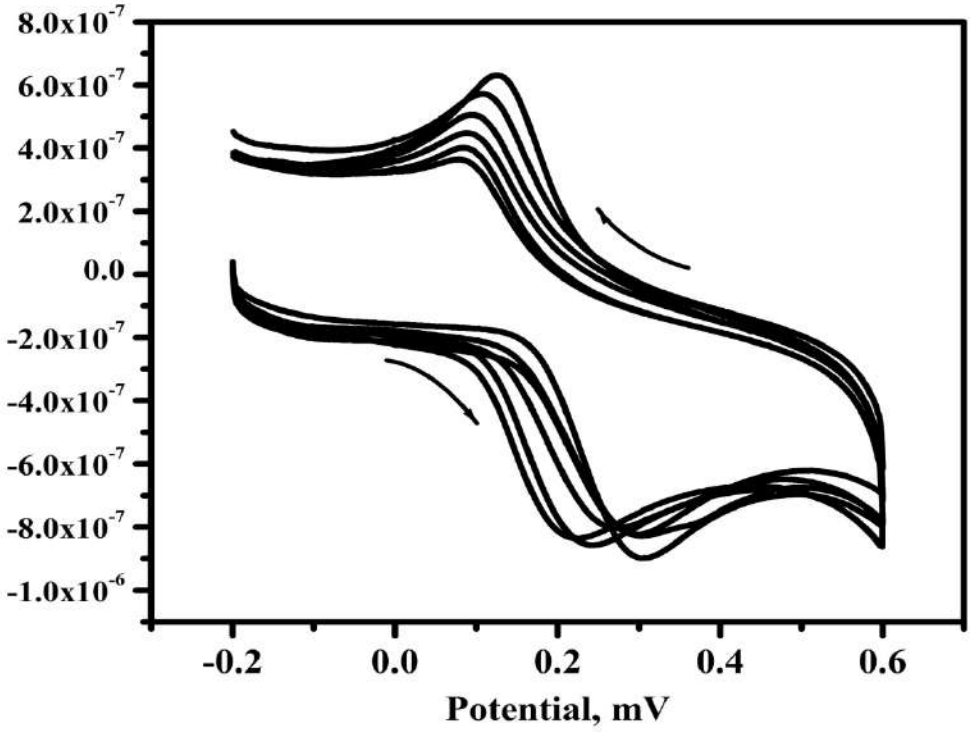


Fig. 5.5. Cyclic voltammograms of 0.1 mM DA for different pH (from 6.4 to 7.4 pH) at GMMCPE

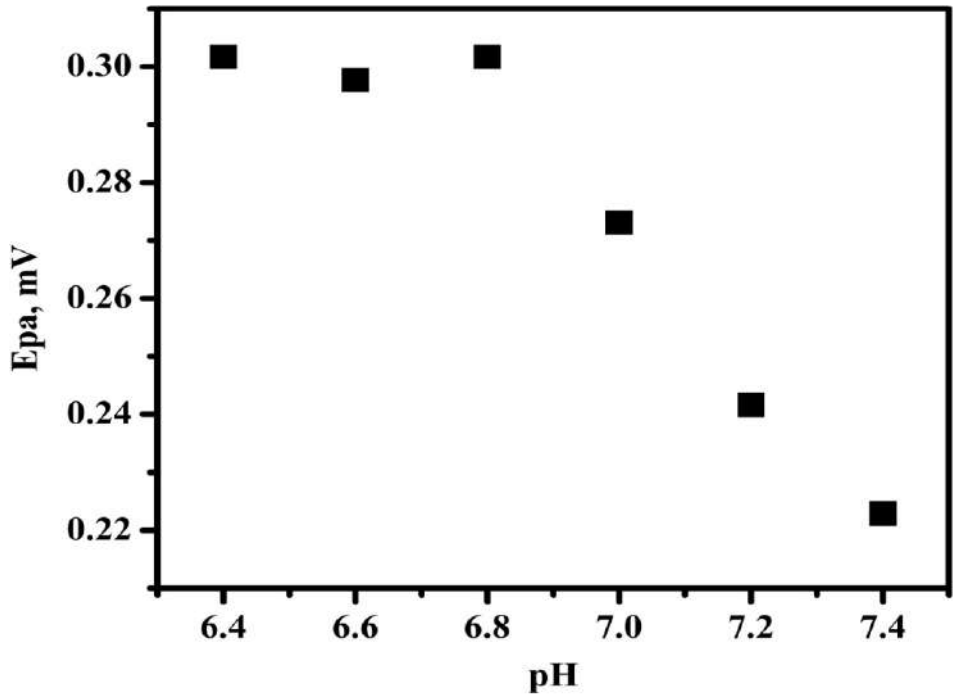


Fig. 5.5a. Plot of anodic peak potential verses pH

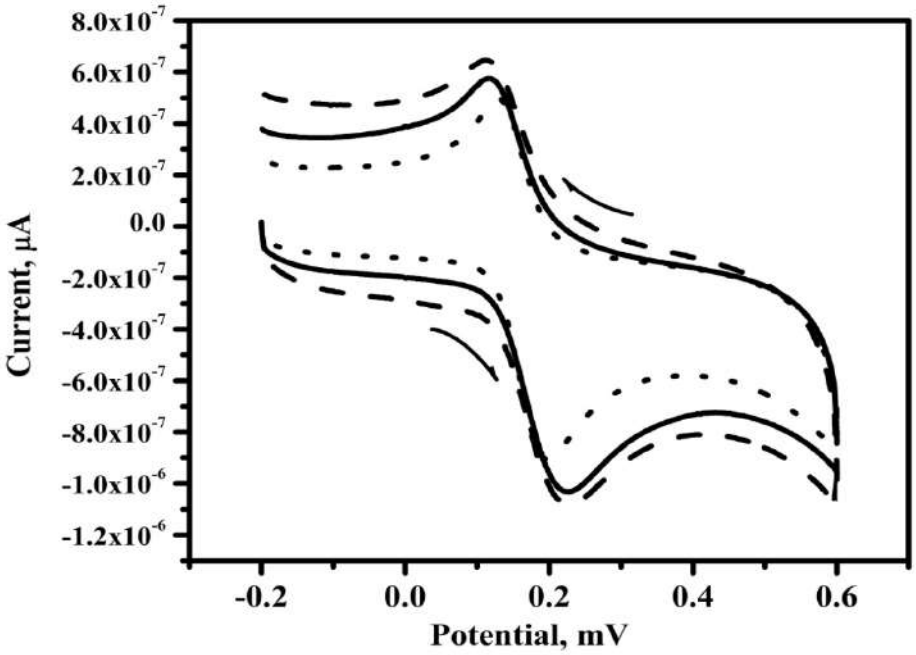


Fig. 5.6. Cyclic voltammogram of 0.1mM DA at BCPE (dotted line), GMMCPE (solid line), 50 µM Sodium alpha-olefin sulfonate on the modified CPE (dashed line) in 0.2 M phosphate buffer solution pH 7.0, scan rate 100 mV/s

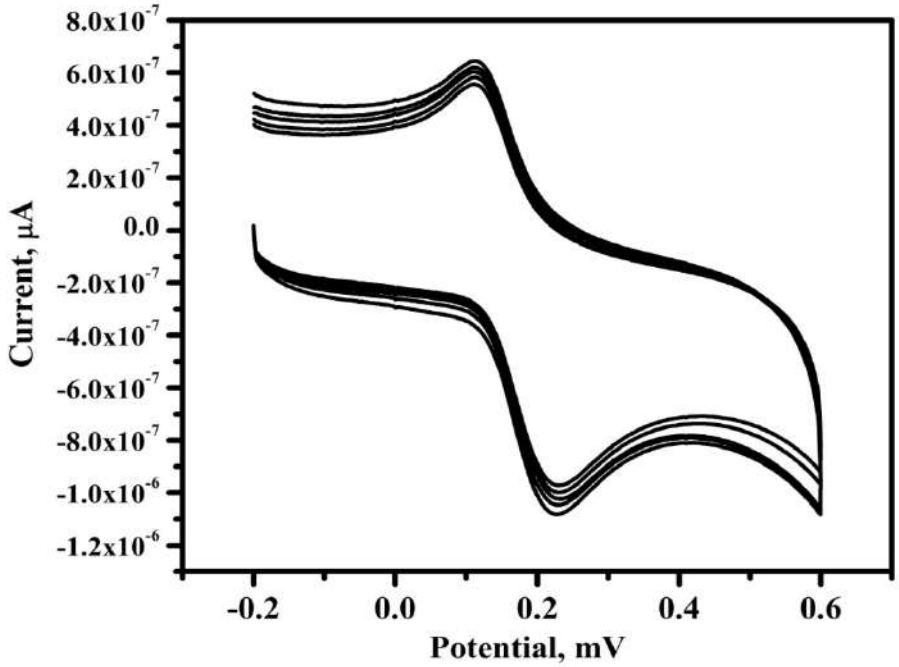


Fig. 5.7. Cyclic voltammogram of variation of concentration of surfactant from 10 to 50 µM for 0.1 mM Dopamine at GMMCPE in 0.2M PBS pH 7.0, scan rate 100 mV/s

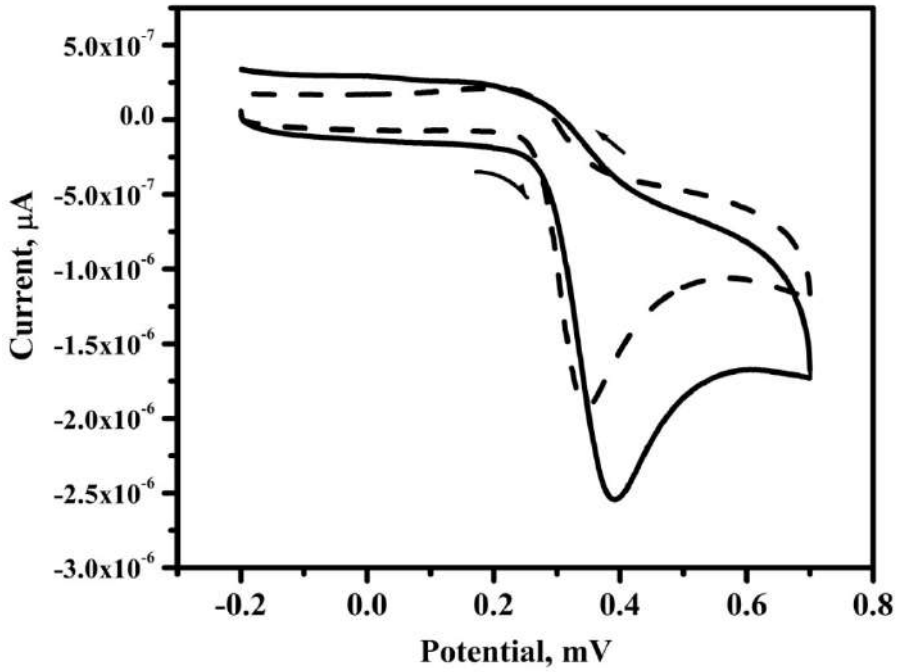


Fig. 5.8. Cyclic voltammogram of for 0.1 mM UA at BCPE (dashed line) and at GMMCPE (solid line) in 0.2 M PBS pH 7.0, scan rate 100 mV/s

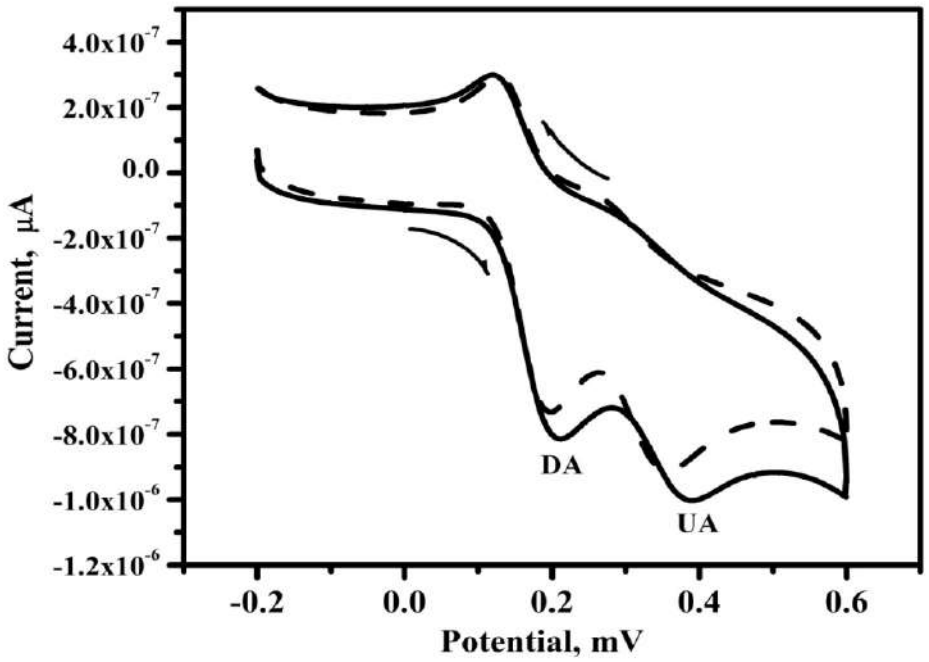


Fig. 5.9.Cyclic voltammogram for simultaneous determination of DA and UA at bare CPE (dotted line) and GMMCPE (solid line) in 0.2 M Phosphate buffer solution pH 7.0, scan rate 100 mV/s

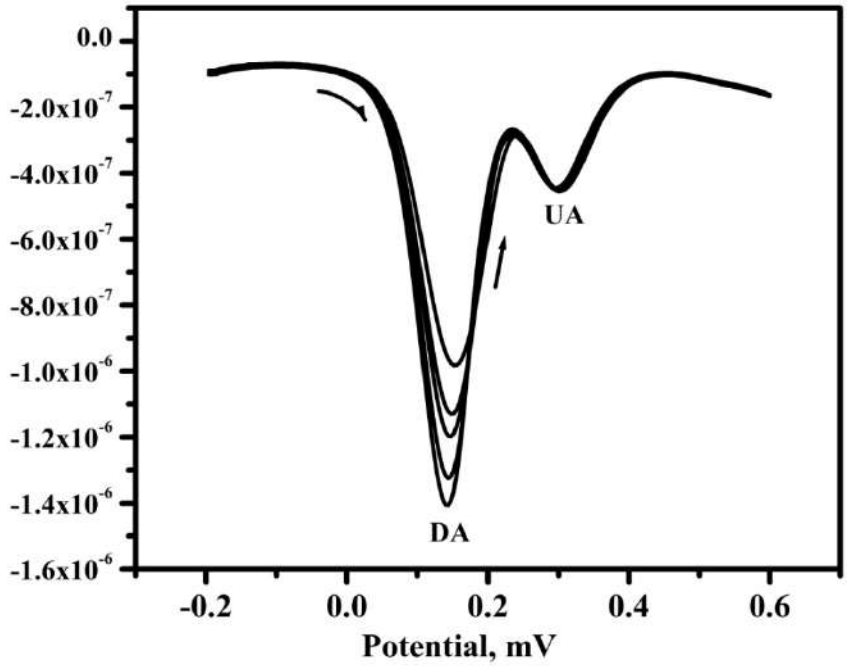


Fig. 5.10. Differential pulse voltammograms of DA (20, 40, 60 and 100 µM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.1 mM UA at GMMCPE with the scan rate of 100 mV/s

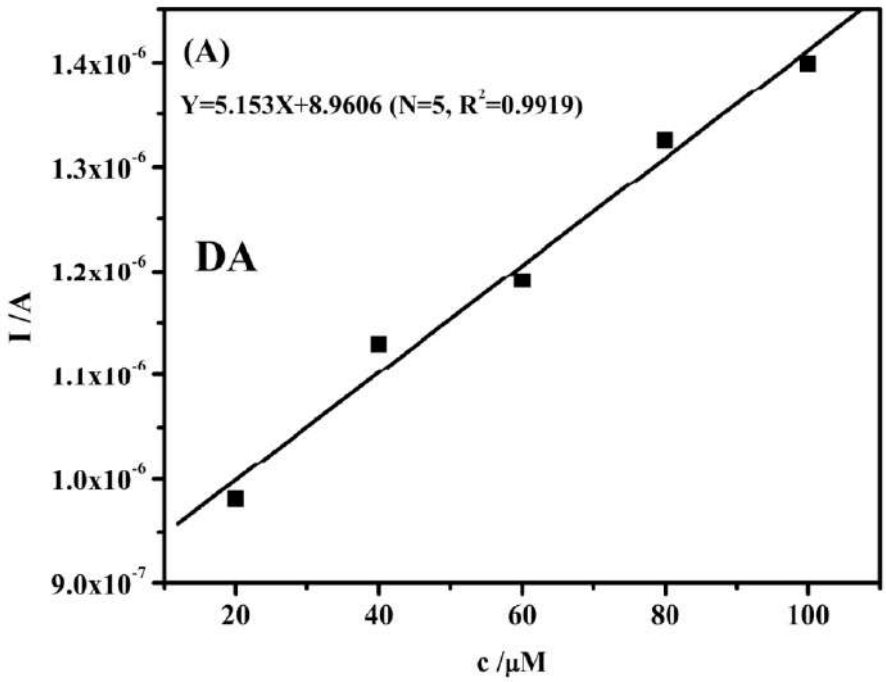


Fig. 5.10a. Plot of anodic peak current (Ipa) versus DA concentration

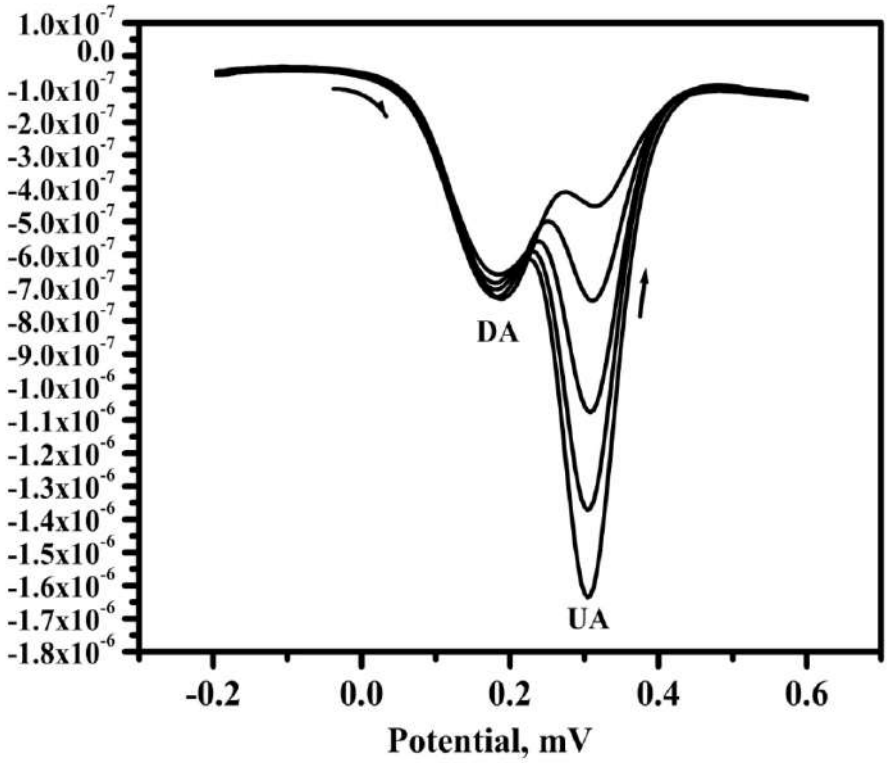


Fig. 5.11. Differential pulse voltammograms of UA (0.1, 0.2, 0.3, 0.4 and 0.5 mM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 20 µM UA at GMMCPE with the scan rate of 100 mV/s

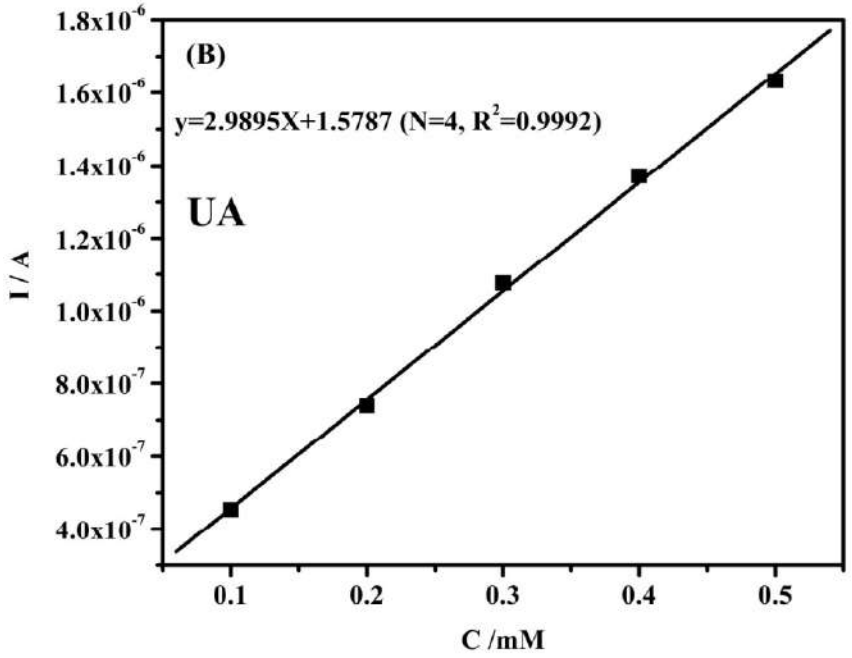


Fig. 5.11a. Plot of anodic peak current (Ipa) versus DA concentration

5.3.11. References

[1] K. Tausche, S. Unger and K. Richter, *Der. Internist*., 47 (2006) 509.

[2] Y.C. Luo, J.S. Do and C.C. Liu, *Biosens. Bioelectron*., 22 (2006) 482.

[3] R.A. Vargas, A. Garciá and M.A. Vargas, *Electrochim. Acta*., 43 (1988) 1271.

[4] H. Zhao, Y.Z. Zhang and Z.B. Yuan, *Analyst*, 126 (2001) 360.

[5] J. Li and J. Lu, *Chinese J. Anal. Chem*., 25 (1997) 317.

[6] H. Nohta, T. Yukizawa, Y. Ohkura, M. Yoshimura, J. Ishida and M. Yamaguchi, *Anal. Chim. Acta*., 344 (1997) 240.

[7] Y. Wu, R. Fan and J. Di, *Chinese J. Anal. Chem*., 24 (1996) 873.

[8] R. Zhu and W.T. Kok, *Anal. Chem*., 69 (1997) 4016.

[9] L.F. Xiao, J. Chen and C.S. Cha, *J. Electroanal. Chem*., 495 (2000) 27.

[10] U. Chandra, B.E.K. Swamy, O. Gilbert, M. Pandurangachar and B.S. Sherigara, *Int. J. Electrochem. Sci*., 4 (2009) 1488.

[11] Liu, H.S. Honma and Zhou, *Biosens. Bioelectron*., 21 (2005) 816.

[12] R.N. Goyal, V.K. Gupta, A. Sangal and N. Bachheti, *Electroanalysis*, 17 (2005) 2223.

[13] C.C. Vishwanath, B.E.K. Swamy, T.V. Sathisha and G.M. Madhu, *J. Anal. Bioanal. Electrochem*., 5 (2013) 351.

[14] H. Jerabkova, K. Blanka and J. Nahlik, *Int. Biodeteriorat*, 44 (1999) 241.

[15] J. Wang, B.Z. Zeng, C. Fang and X.Y. Zhou, *J. Electroanal. Chem*., 484 (2000) 92.

[16] E. Niranjana, B.E.K. Swamy, R. Raghavendra Naik, B.S. Sherigara and H. Jayadevappa, *J. Electroanal. Chem*., 631 (2009) 9.

[17] A. Sathisha and B.E.K. Swamy, *Anal. Bioanal. Electrochem*., 7 (2015) 21.

[18] S.S. Shankar, B.E.K. Swamy, U. Chandra and B.S. Sherigara, *Anal. Bioanal. Electrochem*., 3 (2011) 477.

[19] J. Zima, I. Svancara, J. Barek and K. Vytras, *Crit. Rev. Anal. Chem*., 39 (2009) 227.

[20] N. Adams, *Anal. Chem*., 48 (1976) 1138.

[21] R.R. Naik, B.E.K. Swamy, U. Chandra, E. Niranjana, B.S. Sherigara and H. Jayadevappa, *Int. J. Electrochem. Sci*., 4 (2009) 862.

[22] S. Hou, N. Zheng, H. Feng, X. Li and Z. Yuan, *Anal. Biochem*., 381 (2008) 184.

[23] A. Liu, I. Honma and H. Zho, *Biosens. Bioelectron*., 23 (2007) 80.

[24] H. Zhao, Y. Zhang and Z. Yua, *Analyst*, 126 (2001) 360.

[25] Y. Zhang, Y. Cai and S. Su, *Anal. Biochem*., 350 (2006) 291.

[26] G.R. Xu, M.L. Xu, J.M. Zhang, S. Kim and Z.U. Bae, *Bioelectrochem*., 72 (2008) 93.

[27] X. Lin, Q. Zhuang, J. Chen, S. Zhang and Y. Zheng, *Sens. Actuat. B*., 125 (2007) 245.

[28] S.A. Kumar, C.F. Tang and S.M. Chen, *Talanta*, 74 (2008) 866.

[29] L. Chen and G. Lu, *Sens. Actuat. B*, 121 (2007) 429.

[30] Physicians' Desk Reference (PDR) Medical Economics Inc., Montvale, NJ, USA, 55 (2001) 678.

[31] The Martindale Pharmaceutical Press, London, UK, 32 (1999) 320.

[32] Isam Ismail Salem, Jafer Idrees, I. Jaafar and Al Tamimi, *J. Chromatograph. B.,* 799 (2004) 109.

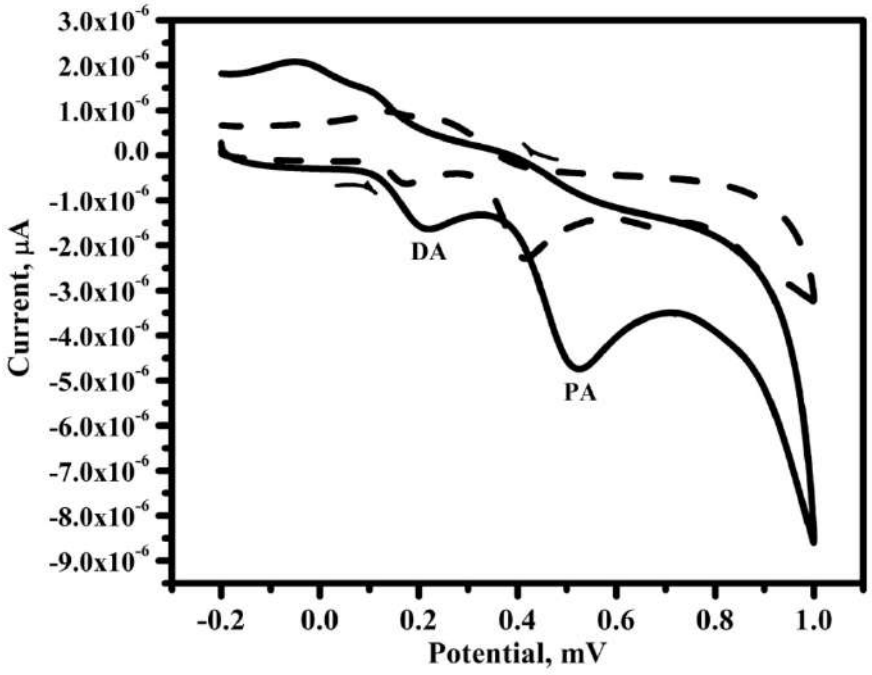
[33] R.N. Hegde, B.E.K. Swamy, N.P. Shetty and S.T. Nandibewoor, *J. Electroanal. Chem*., 635 (2009) 51.

[34] Q. Wang, N. Li and W. Wnag, *Anal. Sci*., 18 (2002) 635.

CHAPTER-5

Part-B

Simultaneous Electrochemical Determination of Paracetamol, Dopamine and Diclofenac at Diacerein Modified Carbon Paste Electrode: A Voltammetric Study



Analytical and Bioanalytical Electrochemisty (Revised and submitted)

5.4. Introduction

Diacerein was used for the modification of carbon paste electrode (CPE) to determine the electrochemical behavior of paracetamol (PA) in 0.2M phosphate buffer solution (PBS) at pH 7. The effect of concentration, scan rate, pH and surfactant was studied for electrochemical studies of paracetamol. The Diacerein modified carbon paste electrode showed an excellent electrocatalytic activity for the selective determination of PA in the presence of DA and DF by using CV and differential pulse voltammetric techniques (DPV) respectively. The catalytic peak current obtained was linearly related to PA concentrations in the ranges of to 0.1 to 0.6 mM with correlation co-efficient of 0.9981 which reveals the adsorption controlled process. The detection limit of paracetamol was found to be 3.8×10^{-6} M. The present technique provides a novel method for the simultaneous determination of PA, DA and DF in their mixture sample.

5.5. Chemistry and Biological Relevance of Dopamine

The chemistry and biological relevance of dopamine has been explained details in chapter 3 Part A section 3.1.1 and 3.1.1.1.

5.6. Chemistry and Biological Relevance of Paracetamol

The chemistry and biological relevance of paracetamol has been explained details in chapter 3 Part A section 3.1.3.

5.7. Chemistry and Biological Relevance of Diclofenac

Diclofenac (sold under a number of trade names) [1] is a nonsteroidal anti-inflammatory drug (NSAID) taken or applied to reduce inflammation and as an analgesic reducing pain in certain conditions. It is supplied as or contained in medications under a variety of trade names.

The name "diclofenac" derives from its chemical name: 2-(2,6-dichloranilino) phenylacetic acid. Diclofenac was first synthesized by Alfred Sallmann and Rudolf Pfister and introduced as Voltaren by Ciba-Geigy (now Novartis) in 1973 [2].

The primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is thought to be inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis. Inhibition of COX also decreases prostaglandins in the epithelium of the stomach, making it more sensitive to corrosion by gastric acid. This is also the main side effect of diclofenac. Diclofenac has a low to moderate preference to block the COX 2-isoenzyme and is said to have, therefore, a somewhat lower incidence of gastrointestinal complaints than noted with indomethacin and aspirin. Diclofenac is often used to treat chronic pain associated with cancer, in particular if inflammation is also present for treatment of chronic pain. Diclofenac can be combined with opioids if needed such as a fixed combination of diclofenac and codeine.

5.8. Review of Electrochemistry of Paracetamol, Dopamine and Diclofenac

In recent years, efforts have been exerted in the development of voltammetric methods for the determination of PA and DA in biological samples. Paracetamol, (N-acetyl-p-aminophenol) known as acetaminophen, is a pain killer that is popular throughout the world because it is remarkably safe to the stomach. Paracetamol (PC) was firstly introduced into medicine as an antipyretic/analgesic by Von Mering in 1893. Prior to this cinchona bark, which was also used to make the anti- malaria drug quinine, had been used to treat fevers. paracetamol is one of the most commonly used analgesics in pharmaceutical formulations, for the reduction of fever and also as a pain killer for the relief of mild to moderate pain associated with headache, backache, arthritis and postoperative pain in adults and children. It is the most used medicine after acetylsalicylic acid in many countries as an alternative to aspirin and phenacetin [3-8].

Dopamine is one of the excitory neurotransmitters that play an important role in physiological events. It is involved in the functioning of renal, cardiovascular, hormonal and nervous systems. Dopamine is involved in neurological diseases such as Parkinson's [9], Alzheimer's disease [10] and schizophrenia [11-12]. As a result of these discoveries, catecholamines, and drugs are now widely used in the treatment of bronchial asthma, hypertension, Parkinson's disease, myocardial infarction and cardiac surgery. Consequently various approaches have been made to develop selective and sensitive

methods for the determination of DA concentrations. Dopamine is an electrochemically active compound that can be directly oxidized at an appropriate potential and a suitable electrode material.

Diclofenac is a synthetic nonsteroidal anti-inflammatory drug (NSAID), has been proven as a safe and efficacious drug in the treatment of a variety of inflammatory and rheumatoid disorders [13]. Diclofenac is well absorbed after oral administration with extensive hepatic metabolism. This compound exhibits a terminal half life of 1–2 h, volume of distribution of 0.17 l/kg, 99% protein binding and enters the synovial fluid [14]. The determination of small amounts of diclofenac in pharmaceutical preparations is very important for medical and pharmaceutical needs where it is used for the treatment of various diseases. Therefore it is vital to develop a simple, fast, selective and cost-effective method of determining the trace amounts of diclofenac in different pharmaceutical formulations.

In this study, the surfactant (sodium alpha olefin sulphonate) is used as a modifier for the electrochemical determination of Paracetamol. 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 structures, surfactant has been extensively used in the fields of electrochemistry and electroanalytical chemistry [15-17] for various purposes. To improve the detection limits of some biomolecules Hu's group [18-20] has introduced surfactants to electroanalytical chemistry.

Diacerein, a purified compound with anthraquinonic structure [scheme.5.2], has been shown to inhibit, *in vitro and in vivo,* the production and activity of IL-1 and the secretion of metalloproteases without affecting the synthesis of prostaglandins [21-22]. Diacerein is a drug with interleukin-1 (IL-1)–inhibitory activity developed for the treatment of osteoarthritis (OA).In animals, oral administration of diacerein resulted in anti-inflammatory activity as manifested by an inhibition of edema induced by the injection of carrageen an into the footpad. Diacerein inhibited adjuvant arthritis induced in rats by the injection of Mycobacterium tuberculosis. It also exhibited analgesic effects and antipyretic activities in animal models [23-24].

The aim of the work is to establish a simple and sensitive electrochemical method for the determination of Paracetamol in the presence of Diacerein and surfactant. Diacerein MCPE shows excellent electrocatalytic activity for the oxidation of Paracetamol, it accelerates the electron transfer rate and lowers the over potential. Some electrochemical parameters of Paracetamol electrochemical oxidation were measured by different electrochemical methods.

5.9. Experimental

5.9.1. Reagents and Materials

Diacerein, Dopamine, Paracetamol, Diclofenac and Sodium alpha-olefin sulfonate were obtained from Himedia Chemicals. Perchloric acid, sodium dihydrogen orthophosphate dihydrate, and di-sodium hydrogen phosphate anhydrous were obtained from Merck. 25×10^{-4} M DA, 25×10^{-4}M PA and 25×10^{-4}M DF stock solutions were prepared by dissolving in 0.1M perchloric acid solution and double distilled water respectively. All other reagent solutions were prepared in double distilled water. All chemicals are of analytical grade quality and were used without further purification.

5.9.2. Apparatus and Procedure

Electrochemical measurements were carried out with a CHI Model 660c Electrochemical Work station connected to a personal computer for control and data storage. All electrochemical experiments were performed in a standard three-electrode cell. The bare carbon paste electrode or the modified electrode used as a working electrode. The counter electrode was platinum wire and the reference electrode was a saturated calomel electrode (SCE). All potentials reported are with respect to the SCE.

5.9.3. Preparation of Bare and Modified Carbon Paste Electrode

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

in the end of the tube. Similarly the modified carbon paste electrode was prepared by grinding 8mg concentration of diacerein along with graphite powder.

5.10. Results and Discussion

5.10.1. Electrocatalytic Oxidation of Paracetamol at Diacerein Modified Carbon Paste Electrode

The cyclic voltammograms of 1×10^{-4}M PA in 0.2 M PBS showed slightly shifted towards positive side with the increase in the oxidation current of diacerein modified carbon paste electrode as compared to the bare carbon paste electrode as shown in Figure 5.12. The oxidation peak current enhancement of paracetamol at the Diacerien MCPE electrode was caused by the electrocatalytic effect.

5.10.2. Effect of Scan Rate

The effect of scan rate on 1×10^{-4} M PA was studied at Diacerien MCPE by using pH 7 of PBS as a supporting electrolyte with CV technique. Figure 5.13a gives the information about the effect of scan rate on the peak currents and peak potentials. The peak current gradually increases with the increase in scan rates. In Figure 5.13b and 5.13c gives the plot between scan rate(ν) and square root of scan rate ($\nu^{1/2}$) versus peak currents (Ipc), was found to be linear with a correlative coefficient (R^2)=0.9866 and (R^2) = 0.9984.This indicating adsorption controlled process at the electrode surface [25].

5.10.3. Effect of pH

The effect of pH on the modified electrode with the oxidation peak potential was investigated by cyclic voltammetry of the solution containing 1×10^{-4} M PA in Figure 5.14a. The Epa versus pH graph clearly indicates that the catalytic peak shifts to a more negative potential with increase of pH. From the Figure 5.14b, it could also be seen that the current reached the maximum at pH 7.0.

5.10.4. Effect of Concentration of Paracetamol

The effect of concentration of 1×10^{-4} M PA was studied at Diacerein MCPE in 0.2M PBS of pH 7 (Fig. 5.15a). From the figure it is clear that with increase in the

concentration of PA the peak current increases. The plot of Ipa versus concentration of PA shows a linear relation with correlation coefficient of 0.9981 in Figure 5.15b. The catalytic peak current has a linear relationship with PA concentration over the range of 0.1 mM to 0.6mM. The detection limit for PA was found to be 3.8×10^{-6} M and quantification limit was 1.28×10^{-7}M. The detection limit and quantification limit was calculated by using the formulas (1) and (2) [26–28] and the corresponding results were tabulated in Table 1, where S is the standard deviation and M is the slope obtained from the three calibration plots. The comparison of this electrode with other modified electrode for the determination of PA is listed in Table 5.1.

$$LOD = 3S/M \quad \ldots\ldots (1)$$

$$LOQ = 10S/M \quad \ldots\ldots (2)$$

5.10.5. Electrochemical Response of Paracetamol at Carbon Paste Electrode on to the Surface with Sodium Alpha Olefin Sulphonate

The electrochemical response of paracetamol at carbon paste electrode was shown in Figure 5.16 with 0.2M PBS of pH 7.0.Owing to the roughness of the electrode surface, the cyclic voltammogram of paracetamol in bare carbon paste electrode (solid line) and Diacerein modified carbon paste electrode the absence of SAOS is low signal (dashed line). However, the voltammetric response is apparently improved in the presence of 50µL of SAOS, reflected by the enlargement of anodic peak current (ipa) (dotted line). The probable mechanism is the SAOS surfactant molecule diffuses into the carbon paste electrode along with the paracetamol results increase in the signal [29].

5.10.6. Simultaneous Determination of PA and DA

Diacerein modified carbon paste electrode was introduced for analysis of PA in the containing DA. The sample mixture concentrations were 1×10^{-4} M PA and 1×10^{-4} M DA in PBS of pH 7. As shown in Figure 5.17, the voltammogram obtained for the mixture of sample at BCPE (dashed line) was less sensible the oxidation peaks as while two separated well-defined oxidation peaks can be found at Diacerein MCPE (solid

line).The anodic peak potentials located around at 520 and 220mV for PA and DA respectively. The difference of the Epa for PA-DA was 300 mV by CV.

5.10.7. Influence of PA, DA and DF on Each Other

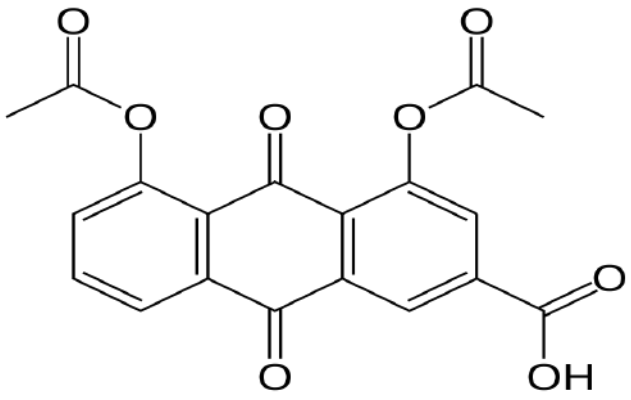
Figure 5.18a shows the DPVs obtained by increasing the concentrations of DA (from 0.2mM -0.5mM) in the presence of 0.2mM PA and 0.2 mM DF. An increase in the peak current of DA was observed with the increasing DA concentration and the voltammetric peak of PA and DF was almost unaltered during the oxidation of DA. Similarly in Figures 5.19a and 5.20a self explains the concentration effect of PA from 0.2 to 0.5 mM and DF from 0.2 to 0.5 mM respectively. These results shows that the DA, PA and DF were exist independently in their mixtures of samples. The corresponding graphs of anodic peak current versus various concentrations of DA (0.2-0.5 mM), PA (0.2-0.5 mM), DF (0.2-0.5 mM) showed linear relationships with linear regressions for A (DA) Ipa (μA) =2.227 Cm M L^{-1} + 2.10, B (PA) Ipa (μA) =7.6 Cm M L^{-1} + 2.089, C (DF) Ipa (μA) =4.36 Cm M L^{-1} + 2.154, the correlation coefficient for these linear graphs was 0.9990, 0.9913 and 0.9009 respectively for this Diacerein MCPE and the detection limit for PA was found to be 2.4×10^{-6}M.Which were shown in Figures 5.18b, 5.19b and 5.20b respectively.

5.10.8 Conclusion

In this work, the modified diacerein carbon paste electrode was shows electrochemical sensor for electrochemical determination of PA. The prepared modified electrode has detection limit of 3.8 $X10^{-6}$M and it shows excellent sensitivity, selectivity and anti-fouling properties. The surfactant (sodium alpha olefin sulphonate) is used as a modifier showed have made better sensor for the detection of PA. Moreover, valid response to paracetamol and the good potential separations obtained for DA, PA and DF are prospective pointers to the application of this sensor to other biological molecules as well.

Table 5.1. Comparison of the performances of paracetamol electrochemical sensors

Modified electrodes	**pH used**	**Detection limit (M)**	**References**
Graphene/GCE	9.3	3.2×10^{-8}	[30]
PAY/nano-TiO_2/GCE	7.0	2.0×10^{-6}	[31]
MWCNT-BPPGE	7.5	1.0×10^{-8}	[32]
C60/GCE	7.2	0.5×10^{-4}	[33]
PANI-MWCNTs/GCE	5.5	2.5×10^{-7}	[34]
C–Ni/GCE	3.0	6.0×10^{-7}	[35]
Nafion/TiO_2–graphene/GCE	7.0	2.1×10^{-7}	[36]
Diacerein/MCPE	**7.0**	**3.8×10^{-6}**	**[This work]**



Scheme 5.2. Structure of Diacerein

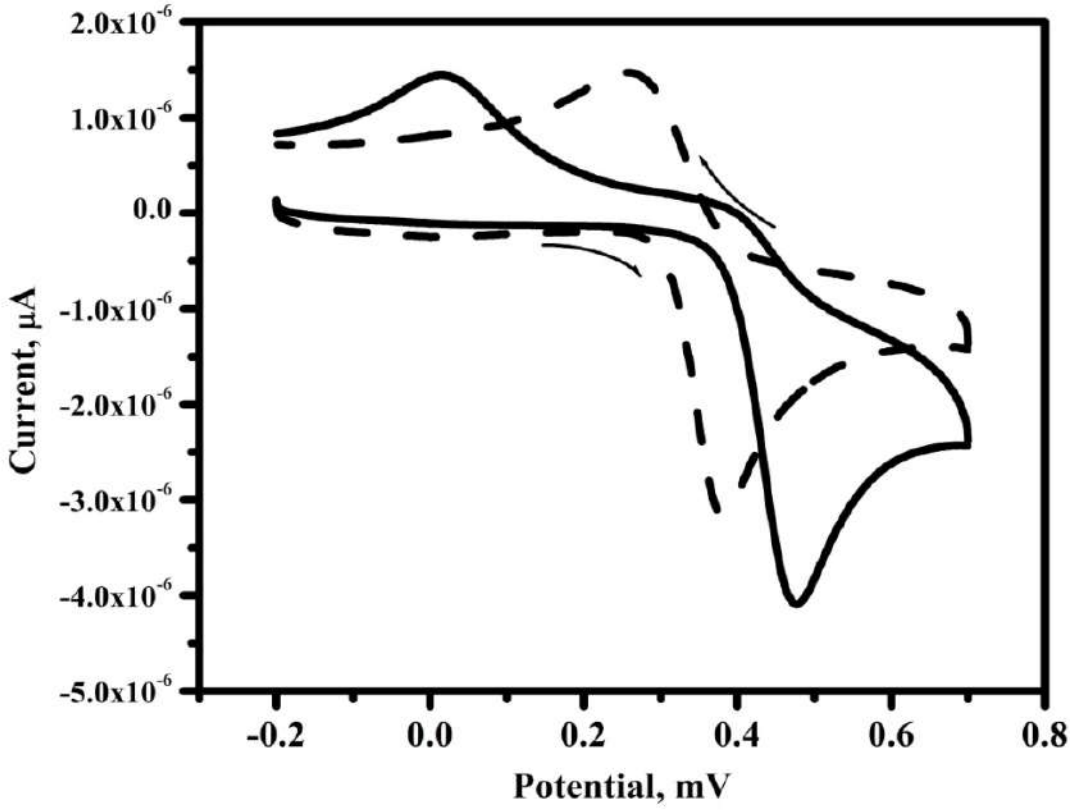


Fig. 5.12. Cyclic voltammograms obtained for the electrochemical response of PA at Diacerein MCPE (solid line) and bare carbon paste electrode (dashed line) in 0.2 M phosphate buffer solution pH 7.0 containing 0.1 mM PA scan rate 100 mV/s

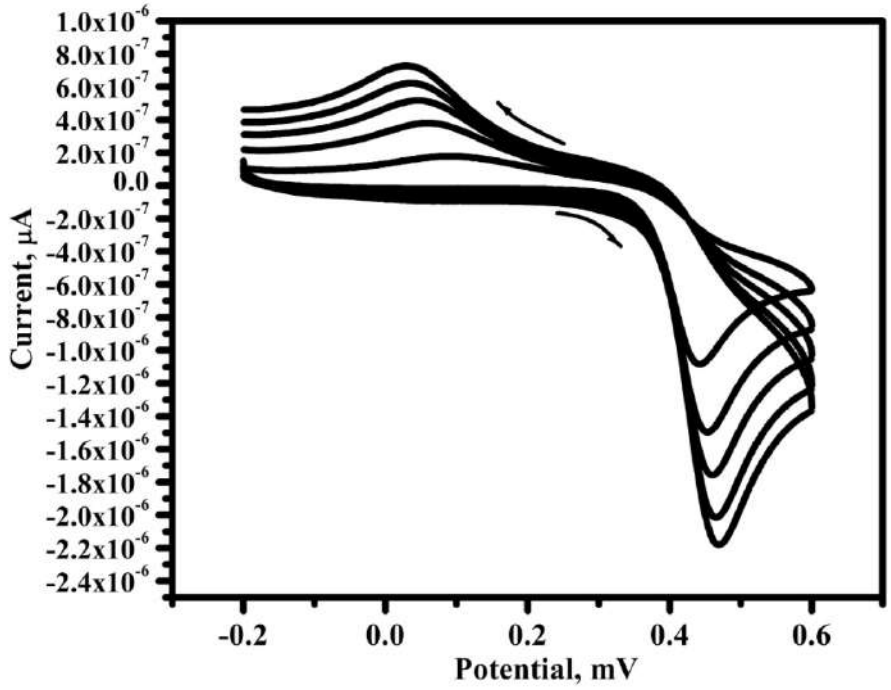


Fig. 5.13a. Cyclic voltammogram of different scan rate in the presence of 0.1 mM PA at Diacerein MCPE in 0.2 M phosphate buffer solution at pH 7.0, scan rate 10 to 50 mV/s

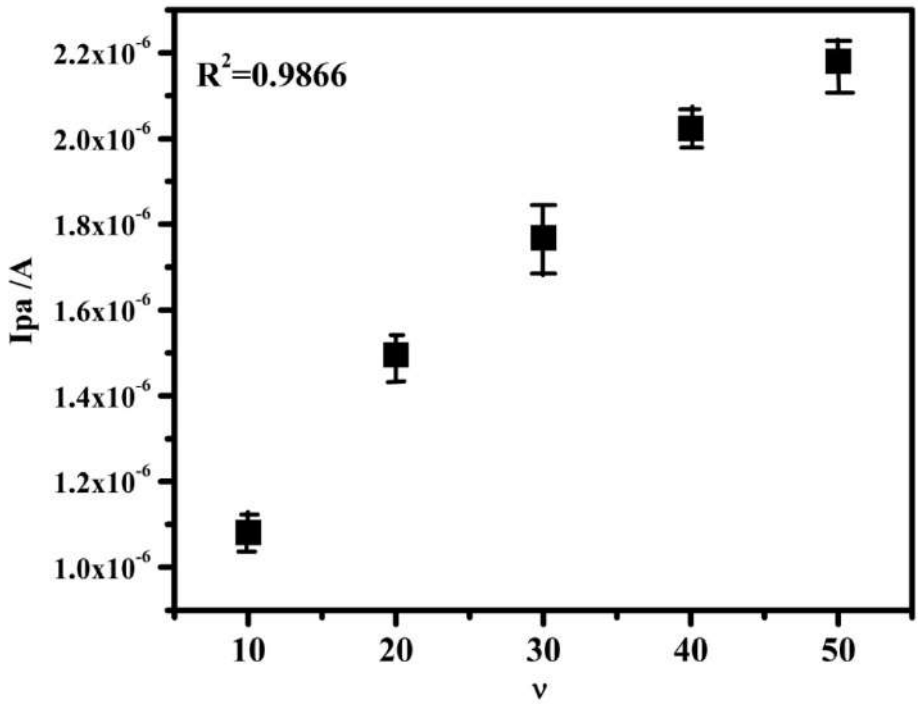


Fig. 5.13b. Plot of anodic peak current versus scan rate

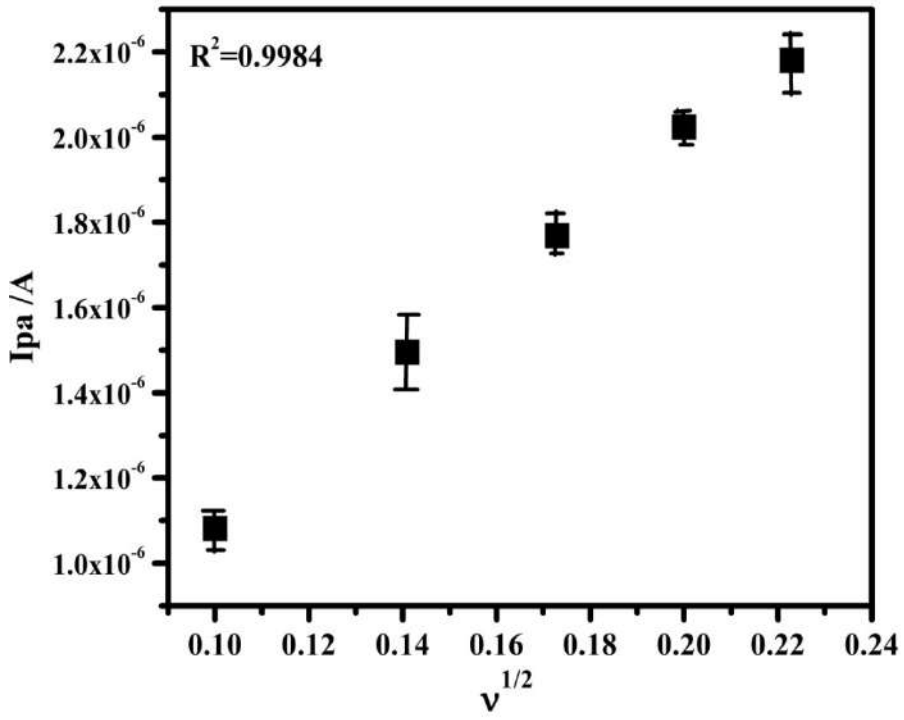


Fig. 5.13c. Plot of anodic peak current versus square root of scan rate

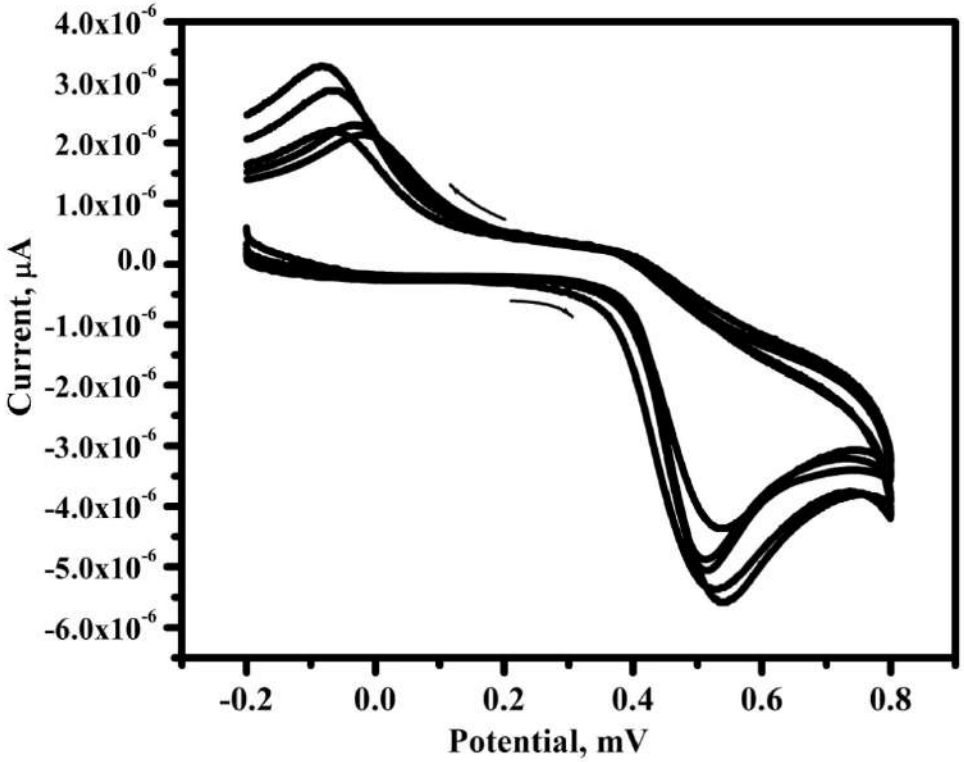


Fig. 5.14a. Cyclic voltammograms of 0.1 mM PA for different pH (from 6.6 to 7.4 pH) at Diacerein MCPE

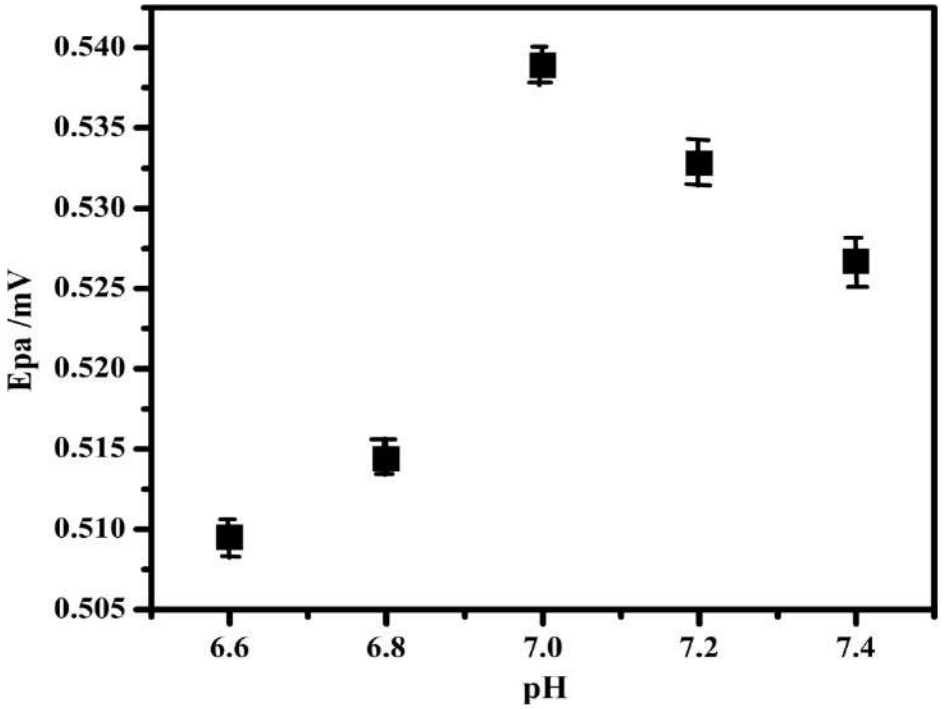


Fig. 5.14b. Plot of anodic peak potential versus pH

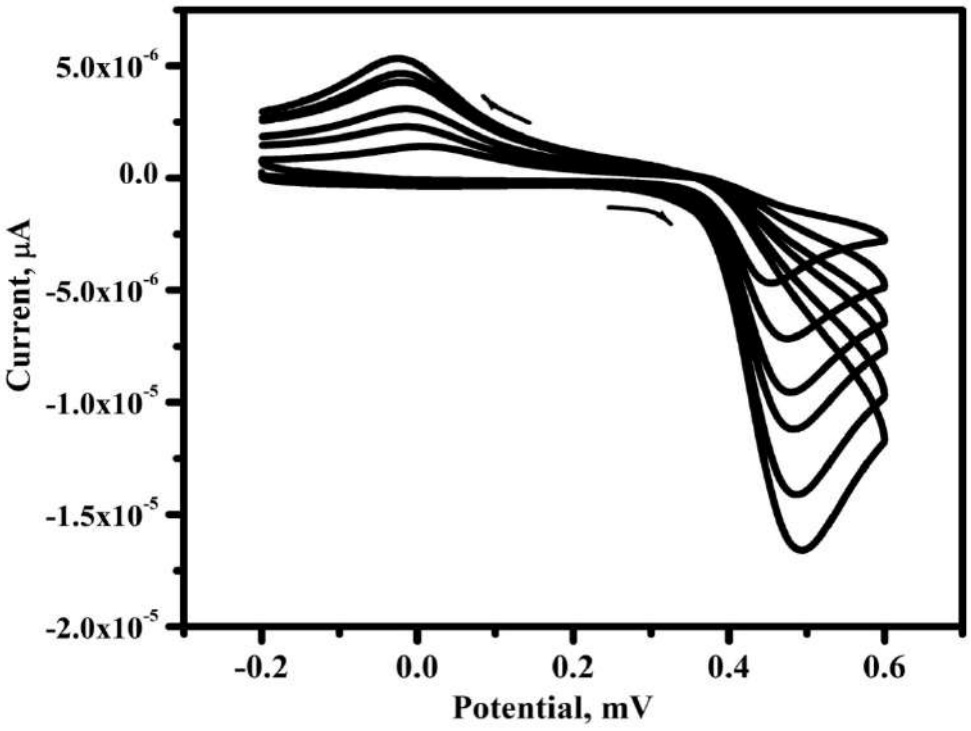


Fig. 5.15a. Cyclic voltammogram of variation of concentration of paracetamol from 0.1 to 0.6 mM in presence of phosphate buffer solution at pH 7.0

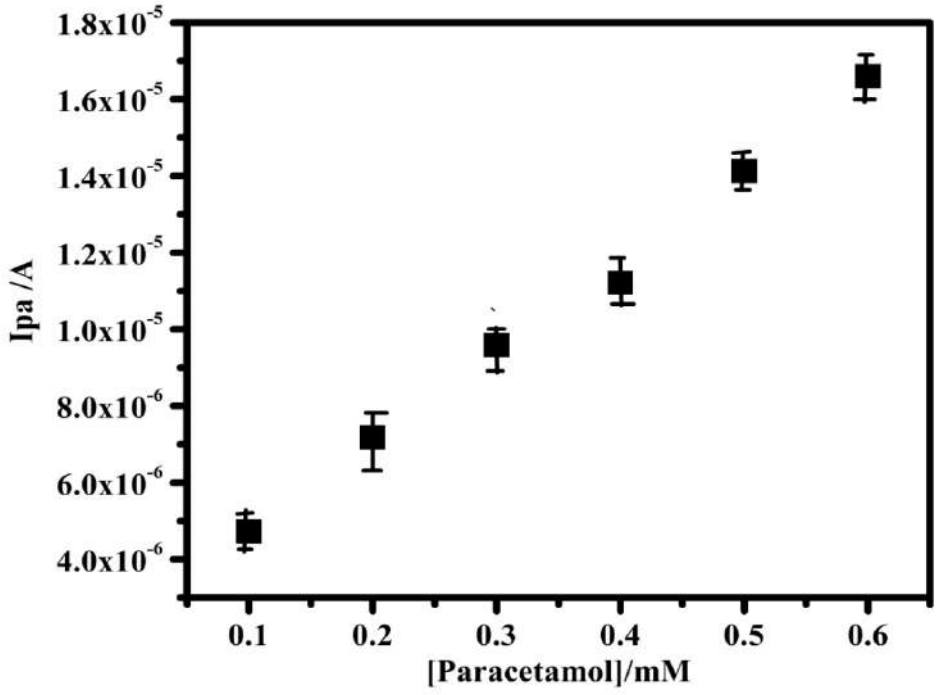


Fig. 5.15b. Plot of anodic peak current versus the concentration of PA

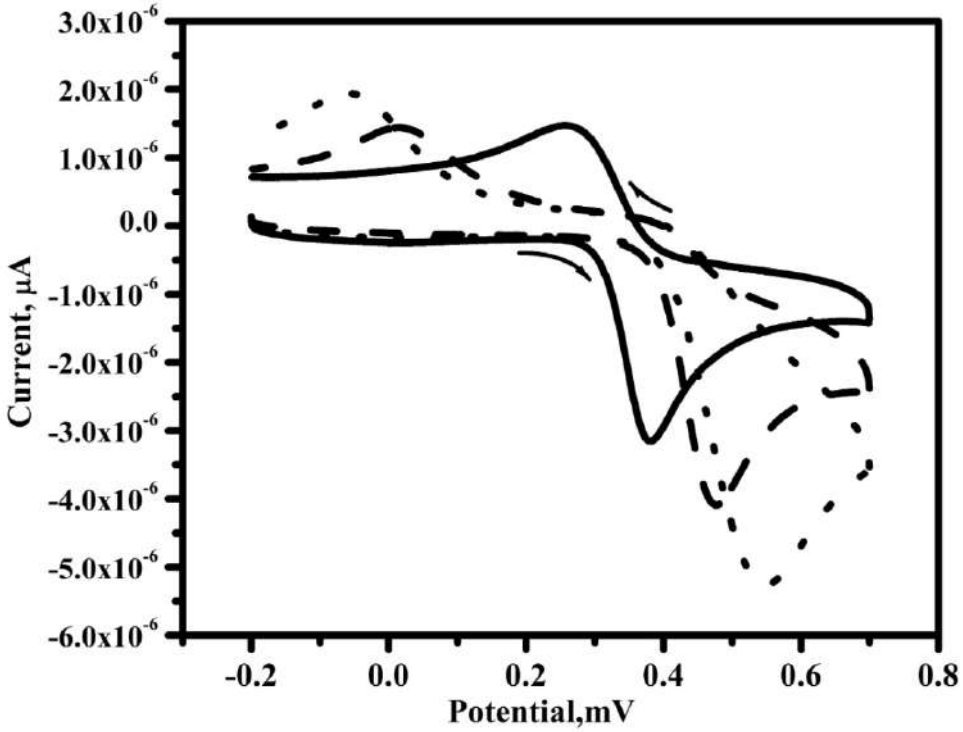


Fig. 5.16. Cyclic voltammogram of 0.1mM PA at BCPE (solid line), Diacerein MCPE (dashed line) and 50µM SAOS on the modified CPE (dotted line) in 0.2 M phosphate buffer solution pH 7.0. scan rate 100 mV/s

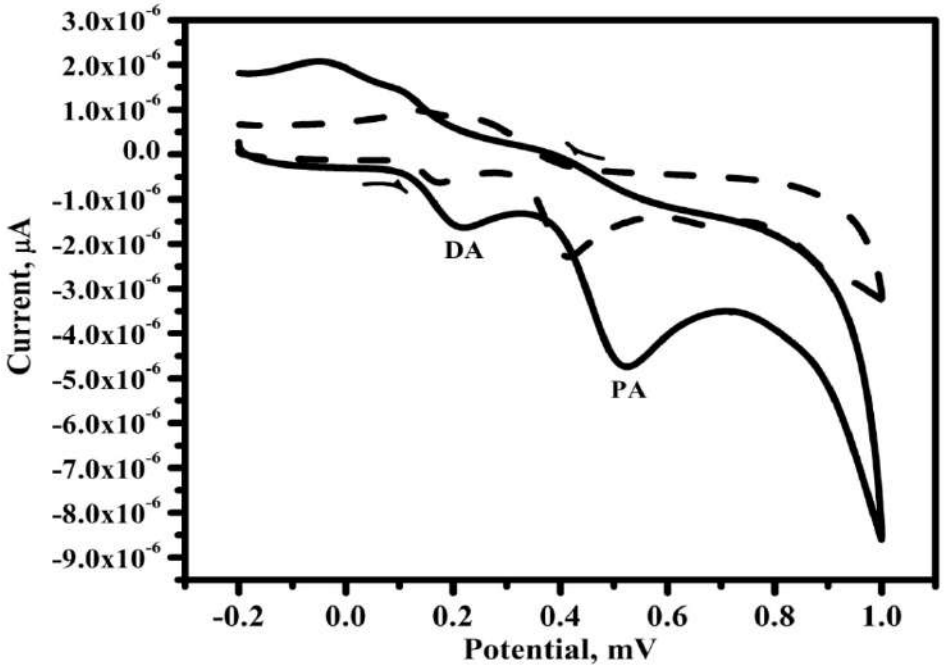


Fig. 5.17. Cyclic voltammograms recorded containing mixture of PA and DA at bare CPE (dashed line) and Diacerein MCPE (solid line) in 0.2 M PBS pH 7.0 at scan rate 100 mV/s

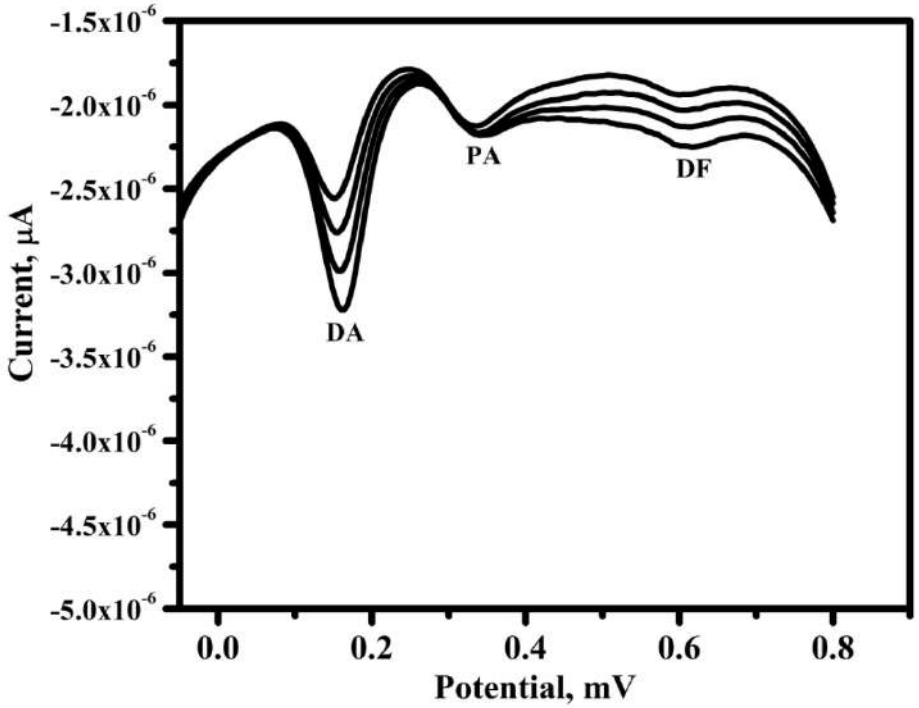


Fig. 5.18a. Differential pulse voltammograms of DA (0.2, 0.3, 0.4 and 0.5 mM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.2 mM PA and 0.2 mM DF at Diacerein MCPE with the scan rate of 100 mV/s

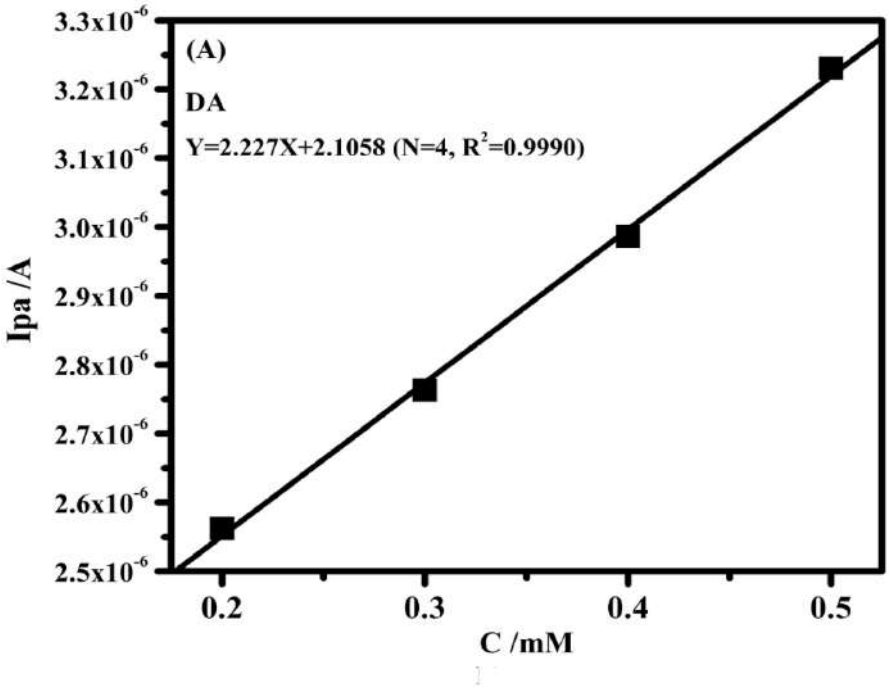


Fig. 5.18b. Plot of anodic peak current (Ipa) versus DA concentration

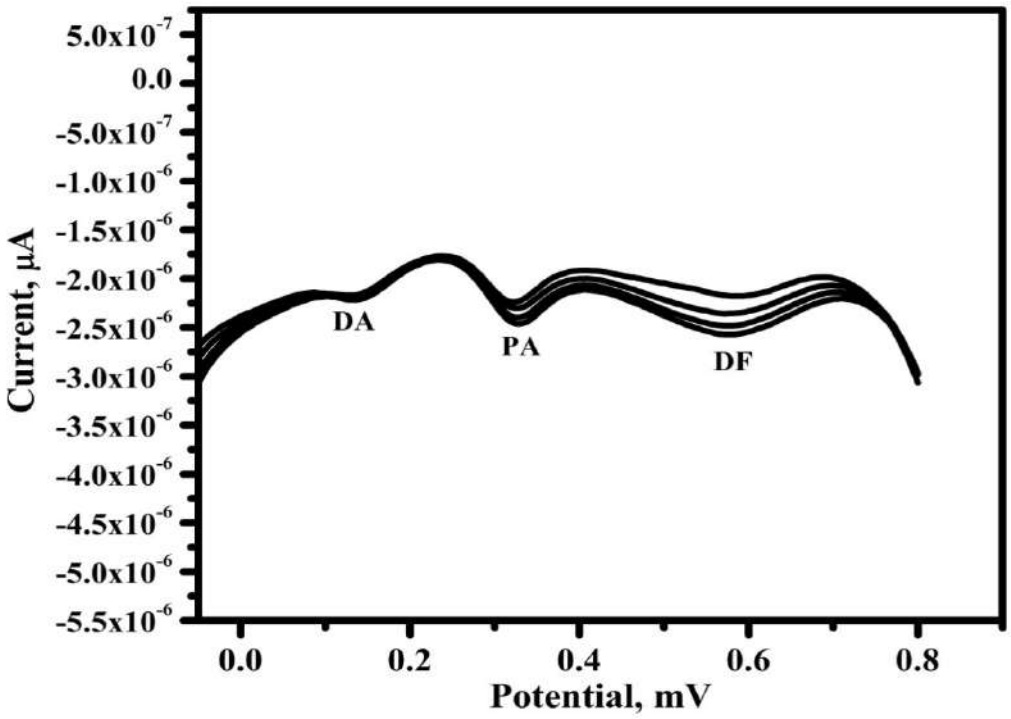


Fig. 5.19a. Differential pulse voltammograms of PA (0.2, 0.3, 0.4 and 0.5 mM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.2 mM DA and 0.2 mM DF at Diacerein MCPE with the scan rate of 100 mV/s

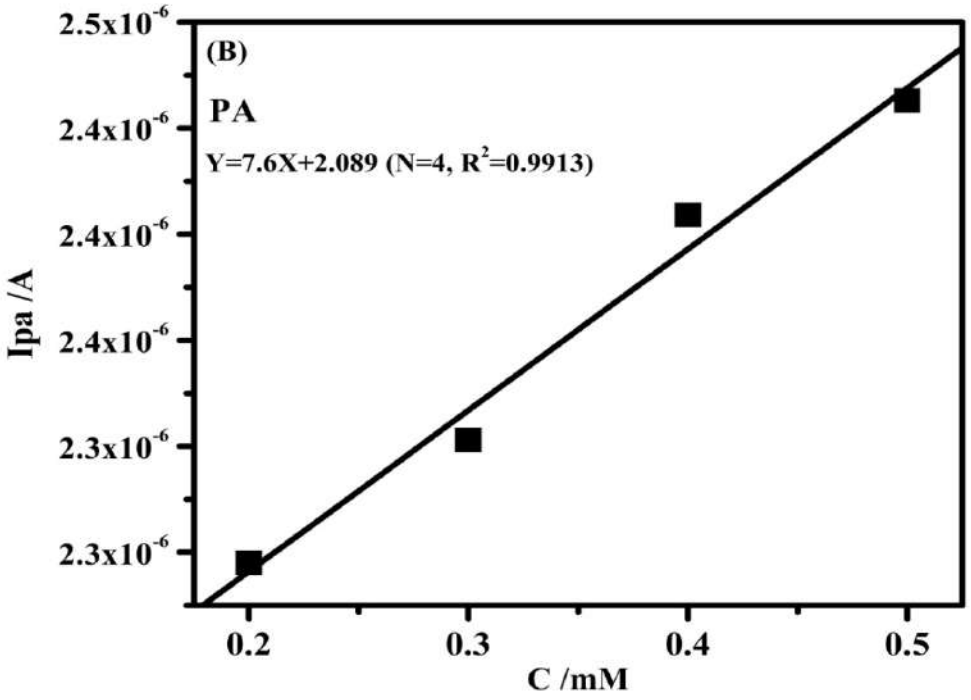


Fig. 5.19b. Plot of anodic peak current (Ipa) versus PA concentration

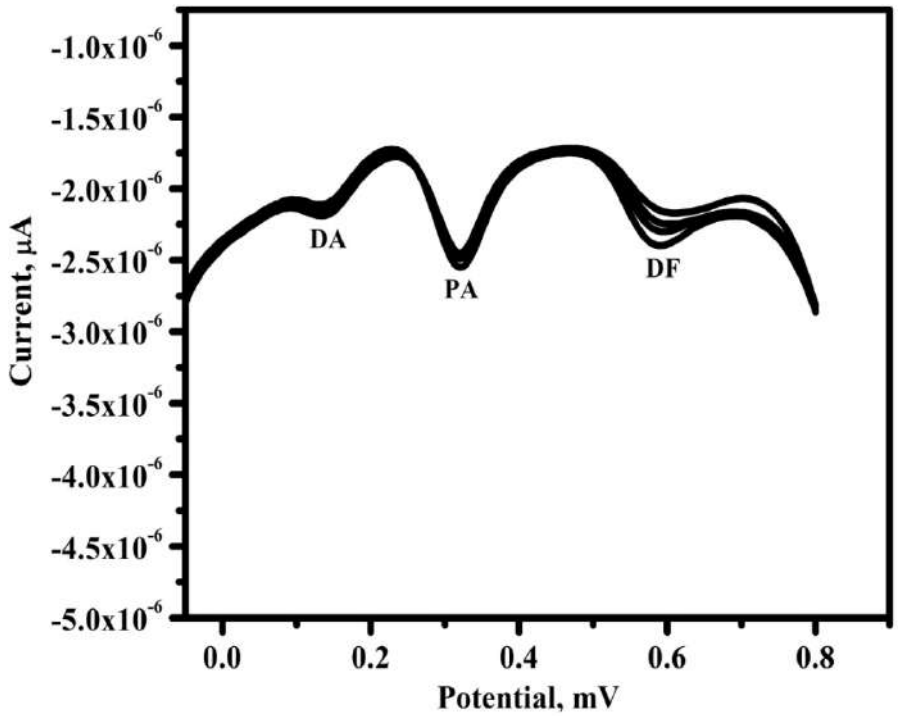


Fig. 5.20a. Differential pulse voltammograms of DF (0.2, 0.3, 0.4 and 0.5 mM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.2 mM DA and 0.2 mM PA at Diacerein MCPE with the scan rate of 100 mV/s

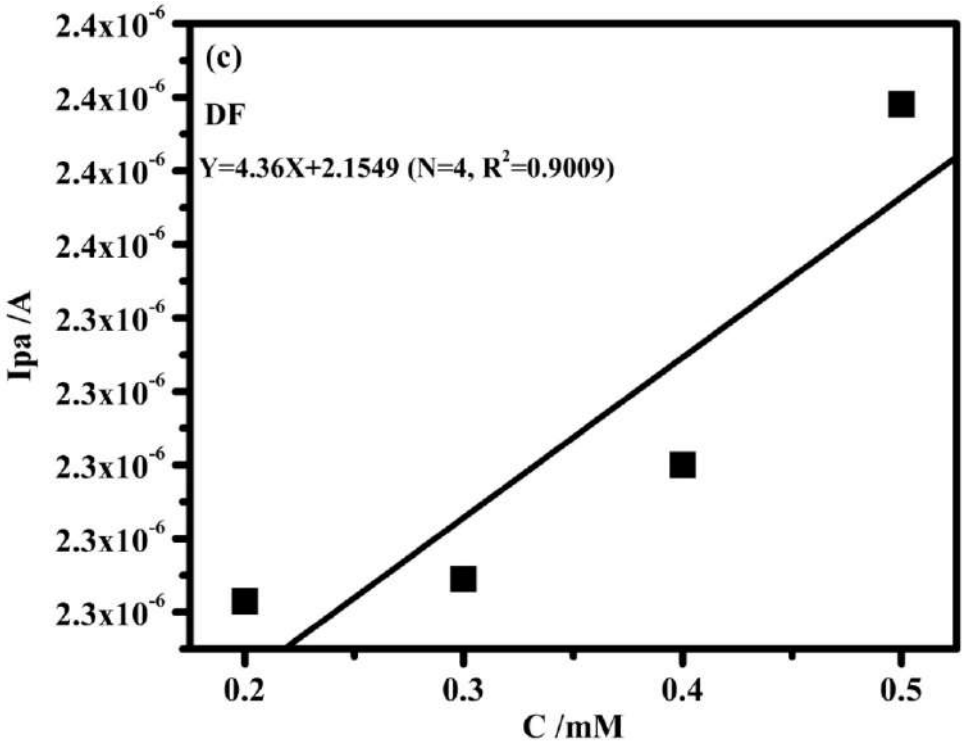


Fig. 5.20b. Plot of anodic peak current (Ipa) versus DF concentration

5.10.9. References

[1] R.N. Goyal and S.P. Singh, *Electrochim. Acta.,* 51 (2006) 3008.

[2] M.E. Bosch, A.J.R. S´anchez, F.S. Rojas and C.B. Ojeda, *J. Pharm. Biomed. Anal*., 42 (2006) 291.

[3] L. Ozcan, and Y. Sahin, *Sens. Actuators B,* 127 (2007) 362.

[4] J.M. Smith, W.O Robert, S.M. Hall, T.A. White and A.A. Gibertson, *Br. Med. J.,* 1 (1978) 331.

[5] B.B. Brodie and J. Axelrod, *J. Pharm. Exp. Ther*., 94 (1948) 29.

[6] A. Sathisha and B.E.K. Swamy, *J. Anal. Bioanal. Electrochem.,* 7 (2015) 12.

[7] S. Marceglia, G. Foffani, A. Bianchi, G. Baselli, F. Tamma, M. Egidi and A. Priori, *J. Physiol.,* 571 (2006) 579.

[8] N. Kemppainen, P. Marjamaki, M. Roytta and J.O. Rinne, *J. Neural Transm.*, 108 (2001) 827.

[9] L. Schwieler, G. Engberg and S. Erhardt, *Synapse,* 52 (2004) 114.

[10] B.N. Chandrashekara, B.E.K. Swamy, M. Pandurangachar, T.V. Sathisha and B.S. Sherigara, *J. Colloids and Surfaces B: Biointerfaces,* 88 (2011) 413.

[11] W. Thongchai, B. Liawruangrath, C. Thongpoon and T. Machan, *J. Sci*., 33 (2006) 123.

[12] J. Emami, N. Ghassami and R. Talari, *DARU,* 15 (2007) 132.

[13] C. Hu and S. Hu, *J. Electrochim. Acta.,* 49 (2004) 405.

[14] J.F. Rusling, *J. Acc. Chem. Res.,* 24 (1991) 75.

[15] M. Kumar, B.E.K. Swamy, T.V. Sathisha and Manjanna, *J. Anal Methods,* 5 (2013) 735.

[16] S. Hu and Y. Yan, *J. Anal. Chim. Acta.,* 248 (1991) 108.

[17] H. Yi, K. Wu, S. Hu and D. Cui, *J.* 55 (2001) 1205.

[18] S. Zhang, K. Wu and S. Hu, *J*. 58 (2002) 747.

[19] J.M. Pelletier, F. Mineau, F.C. Jolicoeur, J.M. Cloutier and J.P. Pelletier, 25 (1998) 753.

[20] J.P. Pelletier, F. Mineau, J.C. Fernandes, N. Duval, and J. Martel-Pelletier, *J. Rheumatol.,* 25 (1998) 2417.

[21] C.M. Spencer and M.I. Wilde, *Diacerein. Drugs,* 53 (1997) 98.

[22] J. Pierrepelletier, M. Yaron, Boulosharaoui, P. Cohen, M.A. Nahir, D. Choquette, I. Wigler, I.A. Rosner and Andre´d Beaulieu, *J. Arthritis Rheumatism.,* 43 (2000) 2339.

[23] P. Raghu, B.E.K. Swamy, T.M. Reddy, B.N. Chandrashekar and K. Reddaiah, *J. Bioelectro. Chem.,* 83 (2012) 19.

[24] S.C. Avendaño, G.A. Ángeles, M.T.R. Silva, M.R. Romo, A. Cuán and M.P. Pardavé, *J. Electrochem. Soc.,* 156 (2009) 375.

[25] U. Chandra, B.E.K. Swamy, O. Gilbert and B.S. Sherigara, *J. Electrochim. Acta.,* 55 (2010) 7166.

[26] N.R. Hegde, B.E.K. Swamy, N.P. Shetti and S.T. Nandibewoor, *J. Electroanal. Chem.,* 635 (2009) 51.

[27] E. Niranjana, R.R. Naik, B.E.K. Swamy, B.S. Sherigara and H. Jayadevappa, *Int. J. Electrochem. Sci.*, 2 (2007) 923.

[28] X. Kang, J. Wang, H. Wu, J. Liu, I.A. Aksay and Y. Lin, *Talanta,* 81 (2010) 754.

[29] S.A. Kumar, C.F. Tang and S.M. Chen, *Talanta,* 76 (2008) 997.

[30] R.T. Kachoosangi, G.G. Wildgoose and R.G. Compton, *Anal. Chim. Acta*., 618 (2008) 54.

[31] R.N. Goyal and S.P. Singh, *Electrochim. Acta.,* 51 (2006) 3008.

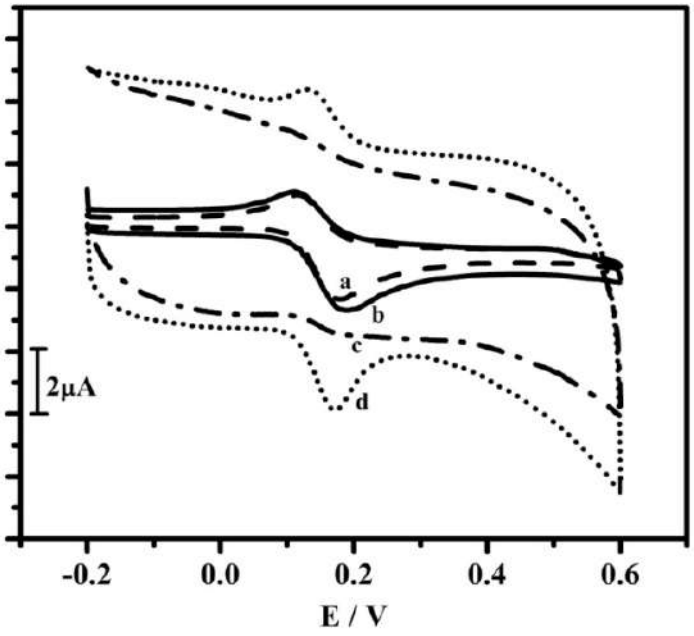
[32] M. Li and L. Jing, *Electrochim. Acta.,* 52 (2007) 3250.

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

[34] Y. Fan, J.H. Liu, H.T. Lu and Q. Zhang, *Colloids and Surfaces B: Biointerfaces,* 85 (2011) 289.

CHAPTER-6

Simultaneous Determination of Dopamine, Serotonin and Folic acid at Torasemide Modified Carbon Paste Electrode: A Cyclic Voltammetric Study



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6.1. Introduction

A carbon paste electrode modified by torasemide was used for determination of dopamine (DA). The modified electrode exhibited strong promoting effect and stability towards the electrochemical oxidation of dopamine at pH 7.0 in phosphate buffer solution (PBS). The parameters which influence the electrode response like paste composition; effects of scan rate, concentration, pH, surfactants and interferences have been studied. The linear range of DA 0.9990 and the detection limit for DA was found to be 2.4×10^{-6} M. Anionic surfactant Sodium Dodecyl Sulphate (SDS) showed very good electrocatalytic effect on the modified carbon paste electrode. The preparation of the modified electrode was easy and renewed by simple polishing gives very good reproducibility, high stability in its voltammetric response and low detection limit for DA.

6.2. Chemistry and Biological Relevance of Dopamine

The chemistry and biological relevance of dopamine has been explained details in chapter 3 Part A section 3.1.1 and 3.1.1.1.

6.3. Chemistry and Biological Relevance of Serotonin

The chemistry and biological relevance of serotonin has been explained details in chapter 3 Part B section 3.4.2, 3.4.2.1 and 3.4.2.2.

6.4. Chemistry and Biological Relevance of Folic Acid

Name of folic acid comes from the Latin word folium, which means leaf. Folic acid was first isolated from spinach, then from the liver. This vitamin is very common in biological systems. In 1935 it was noted the lack of some nutrient in monkeys. It is now known that this disease occurred due to a lack of folic acid. To factor that caused this disease was given name vitamin M. Later, similar symptoms were observed in chickens and men, and the name of folic acid was given by Mitchell, Snell and Williams, who were isolated it from spinach.

Folic acid is poorly soluble in cold water and ethanol, and very soluble in hot water. Folic acid is easily broken down by the sun's light, food processing (particularly boiling), heat and action of estrogen.

Folic acid (vitamin B9) is transformed into a tetra hydro folic acid (THFK, FH4) in the tissues, which can be called coenzyme. This vitamin is involved in the biosynthesis of nitrogen bases, nucleic acids (and thus in the synthesis of DNA), creatine, methionine and serine amino acid. The studies, which were conducted after the 1970s have shown that pre-cancerous cells can be protected if folic acid is consumed in large quantities. Folic acid has a role (along with vitamin B12) in the protection of various forms of cancer, especially lung cancer. Vitamin B9 is needed for the formation of red blood cells and plays an important role in the prevention of birth defects. The body folic acid is deposited in the liver and is excreted by urine. folic acid is found in significant quantities in higher plants and microorganisms, plant leaves, especially the dark green leaves (it was first found in the leaves of spinach), integral wheat, sesame, aronia, black rye flour, brewer's yeast, liver, egg yolk, beans, oranges, carrots, apricots, pumpkin, avocado and various other vegetables and fruits. Of the animal products the richest are liver and heart muscles.

Some of the bacteria can synthesise folic acid (vitamin B9) from p-amino benzoic acid. This discovery provided the ability to understand the mechanism of sulfanyl amides, which are used as medicines. Sulfanyl-amide inhibits the growth of pathogenic micro-organisms that have the need for p-amino benzoic acid. In patients with folic acid deficiency in taking of 300 to 500 micrograms of foliate per day causes positive hematologic response. Scientists from the U.S. National Cancer Institute in 1986. Was found that the smokers with abnormal bronchial cells, the level of folic acid in the blood decreases, leading to an increased risk of cancer. Subsequent addition of folic acid and vitamin B12 may reduce the number of disordered bronchial cells and thus reduce the possibility of this disease. Although this study reduced the care for smokers, it should not be their excuse to continue smoking. Folic acid (vitamin B9) plays an important role in the treatment of mental disorders. For children in prepuberty age adding this vitamin may increase the intelligence quotient. In older children, it is not observed. Folic acid has a

role in the treatment of atherosclerosis (narrowing of the arteries). Kurt Oster showed that giving patients up to 80 milligrams per day of folic acid may prevent heart attacks.

6.5. Review of Electrochemistry of Dopamine, Serotonin and Folic Acid

Voltammetric detection provides a highly sensitive approach to the electroanalysis of a wide range of analytes [1-3]. However, this approach can sometimes be restricted by limitations of selectivity due to the interference from the other redox active molecules which may undergo electrolysis at similar potentials to the target species in the medium [4–6]. The most important strategy to overcome such problems is to modify the surface of the electrode to produce a chemically modified electrode that aims to alter the electrode kinetics of both target species and the interfering species. The potential under which the target species undergo oxidation becomes shifted from that required to electrolyse the interfering species [7-12].

Dopamine is one of the well-known prevalent biogenic amine acting as a neurotransmitter present in mammals plays a significant role in physiological actions, occupying in functioning of nervous, and renal, hormonal and cardiovascular systems. Anomalous level of DA in the body fluids causes diseases such as Alzheimer's, Parkinson's and Schizophrenia [13-15] as a consequence of these innovations, catecholamine drugs are extensively used in the treatment of those diseases. DA is electrochemically active that oxidized to form dopamine-o-quinone (DOQ). There are different techniques have been employed for the detection of DA including fluorimetry, UV-visible spectroscopy, chemiluminesence, capillary electrophoresis and electrochemical methods. As a result, electrochemical techniques for the detection of neurotransmitter have received considerable interest because of its fast speed; low cost, high accuracy and low detection limit [16-19].

There is considerable interest in developing electrochemical techniques for measurement of neurotransmitters such as dopamine (DA) and serotonin (5-hydroxytryptamine (5-HT)). Dopamine (DA), 3,4-dihydroxyphenylethylamine, is an important neurotransmitter of the catecholamine group that exists in the mammalian central nervous system and is characterized by presenting very strong electrochemical

activity [20]. In recent years, there has been considerable interest in developing new methods to measure this neurotransmitter in biological samples such as brain tissues [21, 22]. It is therefore very important to take into account both selectivity and sensitivity in the development of new voltammetric sensors for the determination of DA. Several works in the literature describes the development of new methodologies that employ chemically modified electrodes for the determination of DA in the presence of AA [23-29]. The intensive use of chemically modified electrodes has gained much attention now a days because of their interesting features for analytical use, such as good sensitivity, high selectivity, reproducibility, better stability and anti-fouling behavior [30-31]. 5-HT is widely distributed in the brain, and together with other neurotransmitters, makes an important contribution to brain function. For example, 5-HT has been implicated in control and regulation of various physiological functions such as sleep, thermoregulation, food intake, and sexual activity, as well as in psychopathological states such as depression, anxiety, alcoholism, and drug dependency [32].

Folic acid (FA) is a water-soluble vitamin B that helps to build up healthy cells. The deficiency of FA is a common cause of anemia and is thought to increase the shells of heart attack and stroke. Many studies suggest that diminished foliate status is associated with enhanced carcinogenesis, as FA, along with vitamin B12, participates in nucleotide synthesis, cell division, and gene expression [33]. Per conceptual supplementation of FA has been demonstrated to significantly reduce the incidence and reoccurrence of neural tube defects, such as spina bifida of women [34]. A survey of the literature reveals that there are various methods available for the determination of FA, which include liquid chromatography [35], high performance liquid chromatography [36], flow-injection Chemiluminometry [37], Isotope Dilution-Liquid Chromatography/ Tandem Mass Spectrometry [38] and spectrophotometric method [39]. As FA is an electroactive component, some electrochemical methods have been reported for its determination [40]. Compared with other techniques, the electrochemical method is more desirable because of its convenience and low cost.

Surfactant belongs to a class of molecules with surface active properties. This behaviour is due to their amphiphilic structure, which contains both a polar or hydrophilic

head and non-polar or hydrophobic tail [41]. Surfactants are normally classified according to the head group type [42] Ionic (anionic and cationic), non-ionic and amphoteric (Zwitter ions). Adsorption of ionic surfactants onto the surface generates charge. Cationic surfactants will lead to a positively charged surface and anionic surfactants will give a negative charged surface. A non-ionic surfactant molecule has no charge in aqueous media but normally consists of a highly polar region such as polyoxyethyelene groups. Amphoteric surfactants develop a negative or positive charge depending on the pH of the solution. At low concentration, surfactant molecules are unassociated monomers. As the concentration of surfactant is increased, the attractive and repulsive forces between the molecules cause self-aggregation to occur resulting in the formation of monolayer or micelles. The concentration at which these micelles form is called the Critical Micelles Concentration (CMC). The characteristics of micelles can be controlled by small changes in the chemical structure of the surfactant molecules or by varying the conditions of the disperse phase. Changes in the pH, ionic strength and temperature are all known to influence the size and shape of surfactant micelles. Related works have been done by our research group [43-47].

Torasemide (TOR), 1-isopropyl-3-(4-m-toluidinepyridine-3-sulphonyl) urea, (Scheme 6.1) is the leader of sulphonylurea class of high ceiling loop diuretics, used mainly in the treatment of hypertension and edema associated with congestive heart failure. One adverse effect of loop diuretics is the induction of kaliuresis resulting from increased potassium excretion rates. TOR is a potent natri-uretic and potassium-sparing more than the most often used loop diuretics ‘furosemide’; consequently it is the most favorable one to be used [48-50].

Now a days, the use of diuretics is not limited to therapeutic aims, owing to their features that make them attractive in the world of sport and fitness, for some different purposes, including fast weight reduction through water loss (a) masking the presence of other drugs through ‘faster excretion, urine dilution and urine pH variation (b) and emphasizing muscles where it is essential for body building (c). Due to these reasons, diuretics have been banned by the International Olympic Committee (IOC) since 1988

and are currently included in the list of substances prohibited in- and out-of-competition [51]. Because of its high potency, low therapeutic doses are required, where in once daily dose; TOR is effective in the treatment of hypertension without either a significant hypokalemia, elevation of blood sugar or lipid disorders if compared with those of thiazides and indapamide. TOR is well absorbed, yields bioavailability of about 80-90% and highly bound to plasma proteins 99%. It undergoes extensive hepatic metabolism 'including hydroxylation at various positions, oxidation and reduction' and only 20-25% of the parent drug is excreted unchanged in urine [52-55].

The aim of the work is to establish a simple and sensitive electrochemical method for the determination of dopamine in the presence of Torasemide and surfactant; the oxidation peak current of dopamine remarkably increases at the CPE suggesting significant improvement of determining sensitivity and which might be able to explain the enhancement effects of surfactant in electroanalytical chemistry for Torasemide. There has been increased interest in the development of modified electrodes, especially for the use as biosensors and more recent development involves the direct electron transfer from the modified electrodes to the analytes The advantages of include the high sensitivity, extreme simplicity, rapid response and low cost. In future these electrocatalytic modified electrodes which act as sensors can be used in the medicine and biotechnology field and also the development of sensors that holds great promise for green chemistry applications [56-57].

6.6. Experimental Part

6.6.1. Apparatus and Reagents

The electrochemical experiments were carried out using a CHI-660c (CH Instrument-660 electrochemical workstation) connected to a personal computer for control and data storage. All electrochemical experiments were performed in a standard three-electrode cell. The bare or modified CPE was used as a working electrode, platinum electrode as counter electrode and saturated calomel electrode (SCE) as reference electrode. All potentials reported were versus the SCE.

Torasemide, Dopamine, Uric acid and Folic acid were obtained from Himedia and Serotonin, sodium dodecyl sulphate, cetyl trimethyl ammonium bromide and perchloric acid used from Sigma-Aldrich chemicals. The DA sample was prepared in 0.1 M perchloric acid. The water used was a double distilled and Phosphate Buffer [0.2 M] was prepared by 0.2 M disodium hydrogen phosphate and 0.2 M sodium dihydrogen phosphate. Freshly prepared solutions were used in each experiment. All chemicals were of analytical grade quality and were used without further purification.

6.6.2. Preparation of Bare and Modified Carbon Paste Electrode

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

6.7. Results and Discussion

6.7.1. Influence of Torasemide Concentration

Torasemide (TOR) used as a modifier in the preparation of Torasemide Modified Carbon Paste Electrode (TORMCPE). The characterization of TORMCPE was investigated by using cyclic voltammetric technique. Torasemide Modified Carbon Paste Electrode (TORMCPE) was prepared of different ratios by adding different amount of Torasemide. The effect of the modifier on the development of sensors for DA has been studied [17-22]. In our study Torasemide was varied from 2 to 10 mg for investigation of 0.1 mM DA in 0.2 M phosphate buffer solution pH 7.0. This is shown more distinctively in Figure 6.1, the plot of anodic peak current verses concentration of Torasemide. As the figure illustrates DA oxidation peak is enhanced at 8 mg of the modifier and then decreases when the amount of modifier was increased further. This may be due to a decrease in oxidation sites in the paste and consequent reduction of the actual electrode area. Therefore the role of modifier is to enhance the peak current and also decreases the over potential for the oxidation and reduction of DA.

6.7.2. Electrocatalytic Oxidation of Dopamine at Torasemide Modified Carbon Paste Electrode

Figure 6.2a and 6.2b shows the cyclic voltammograms obtained for the electrochemical response of DA at Torasemide Modified Carbon Paste Electrode (TORMCPE) solid line and bare carbon paste electrode (BCPE) dashed line in 0.2 M phosphate buffer solution pH 7.0. At BCPE, the oxidation and reduction peak potentials of DA occurs at 113.7 and 178 mV respectively. Under the identical conditions, TORMCPE produces significantly increased peak current and more reversible electron process of DA with the oxidation and reduction peak potentials at 227.8 and 98 mV respectively. The remarkable enhancement of peak currents provides clear evidence of electrocatalytic effect of TORMCPE.

6.7.3. Effect of Scan Rate

According to Randles-Sevick's equation increase in the scan rate increases the peak current. The TORMCPE showed increase in the peak current with increase in scan rate (50 mV/s to 350 mV/s) in 0.1mM dopamine and 0.2M phosphate buffer solution at pH 7.0. Cyclic voltammogram for dopamine at TORMCPE is shown in Figure 6.3a. The graph of current Ipa versus scan rate and square root of scan rate were plotted. The graph obtained were nearly straight line as shown in Figure 6.3b and 6.3c**.** In the range from 50 to 350 mV/s the anodic peak currents were proportional to the scan rate and also the to the square root of scan rate with correlation coefficient 0.9950 and 0.9895 .This indicates that, the electrode transfer reaction is adsorption controlled.

6.7.4. Effect of Concentration of DA

The electrocatalytic oxidation of DA was carried out by varying its concentration at TORMCPE in Figure 6.4a. By increasing the concentration of DA from 0.1 to 0.6 mM, the Ipa and Ipc was found to be increasing with shifting of Epa towards positive potential and Epc towards slightly negative potential. The concentration curve of DA shows increase in electrochemical peak current in Figure 6.4b which indicates that Ipa was proportional to concentration of DA. The plot of Ipa vs. concentration of DA shows a

linear relation with correlation coefficient of 0.9990 and the detection limit for DA was found to be 2.4×10^{-6} M (Table 6.1). The detection limit was calculated by using the formula (1) [58, 59], where S is the standard deviation and M is the slope obtained from the three calibration plots. From the data, a lower limit of detection (LOD) can be achieved using the proposed method [60-62].

$$LOD=3S/M \quad \text{---------- (1)}$$

6.7.5. Effect of pH

To optimize the electrochemical response of modified carbon paste electrode for the oxidation and reduction of DA, the effect of pH on the electrode response was studied. The voltammogram of dopamine for pH is shown in the Figure 6.5a. As the pH increases from 5.8 to 7.4, the anodic peak current shifted towards the negative side and the well redox peak is arrived at the neutral pH=7.0. The dependence of Epa vs pH (Figure 6.5b) was also investigated. From the plot it was found that the anodic peak potential decreases with the increase in pH, with slope of 71 mV / pH which indicates that equal number of protons take part in the reaction.

6.7.6. Effect of Surfactant

To study the effect of addition of surfactants the experiments were carried out using anionic surfactant sodium dodecyl sulphate (SDS) and cationic surfactant Cetyl Trimethyl Ammonium Bromide (CTAB). Initially, cyclic voltammogram were recorded for TORMCPE a solution containing Dopamine (0.1 mM) in phosphate buffer solution pH 7.0. Keeping the concentration of Dopamine constant, the concentration of the surfactant was increased from 1 to 14 μM by mobilization method**.** Figure 6.6a shows the effects of surfactant concentration in mobilization when the surfactant concentration is lower than 4 μM the critical micelle concentration (CMC) of surfactant at room temperature, both Ipa and Ipc increases rapidly with the increase of surfactant concentration. From these results SDS shows good enhancement in the peak current compared to CTAB. Figure 6.6b shows comparison of two surfactants SDS and CTAB on mobilization method at TORMCPE in the presence of 0.1 mM Dopamine in

Phosphate Buffer Solution pH 7.0. The more anodic peak current enhancement in SDS compared to CTAB. In this work, the SDS at TORMPCE and Dopamine was explored by cyclic voltammetry, which might be able to explain the enhancement effect of surfactant in electroanalytical chemistry.

6.7.7. Interference Study

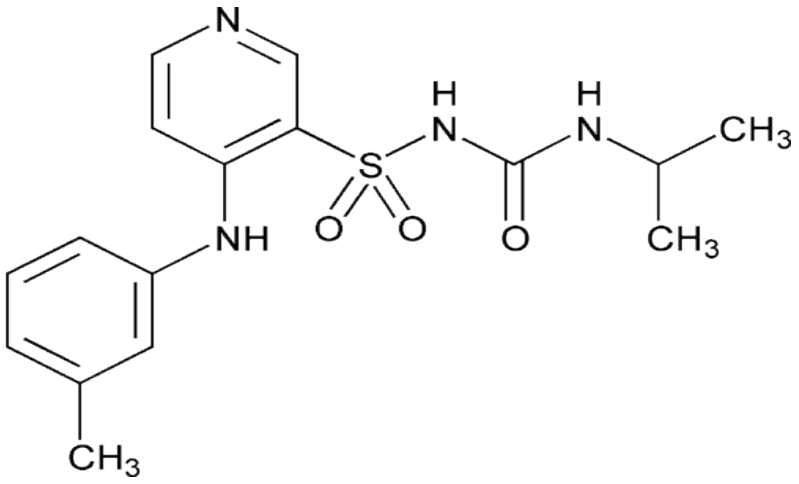
The simultaneous determination of DA, 5-HT and FA in the mixture was carried out at TORMCPE when concentration of one species changed, whereas the others kept constant. From the Figure 6.7a it can be seen that the peak current of DA was proportional to its concentration, which was increased from 0.1 to 0.4 mM when keeping the concentration of 5-HT 10 μM and FA 10 μM. There were no change in the peak current and peak potential occurred for 5-HT and FA. Similarly in Figs.6.8a and 6.9a self explains the concentration effect of 5-HT from 10 to 40 μM and FA from 10 to 25 μM respectively. These results shows that the DA, 5-HT and FA were exist independently in their mixtures of samples. The corresponding graphs of anodic peak current versus various concentrations of DA(0.1-0.4 mM), 5-HT(10-40 μM), FA(10-25 μM) showed linear relationships with linear regressions for A (DA) Ipa (μA)=3.928 Cm M L-1+8.2, B (5-HT) Ipa (μA)=4.6809 Cm M L-1+9.27, C (FA) Ipa (μA)=7.972 Cm M L-1+1.88, the correlation coefficient for these linear graphs was 0.9986, 0.9985 and 0.9980 respectively for this TORMCPE and the detection limit for DA was found to be 0.025×10^{-6} M. which were shown in Figs.6.7b, 6.8b, 6.9b respectively.

6.7.8. Conclusion

In this work, chemically modified Torasemide carbon paste electrode acts as a good sensor, exhibited strong promoting effect and stability towards the electrochemical oxidation of dopamine in presence of 5-HT and FA. The scan rate effect was found to be adsorption controlled electrode process. The concentration effect, pH effect and surfactant effect was well investigated by using cyclic voltammetric technique. Anionic surfactant SDS showed very good electrocatalytic effect on the Torasemide modified carbon paste electrode. The Torasemide modified electrode acts as a good sensor for DA and it can be further applied for the investigation of other neurotransmitter.

Table 6.1

Sl. No	Electrode	Detection limit (µmol/L)	Techniques	Reference
1	Triazole self-assembled monolayer modified gold electrode	0.5	DPV	[63]
2	Fc-MCPE	9.4	CV	[64]
3	Bi_2O_3/GCE	0.2	CV	[65]
4	Poly[L-methionine] modified electrode	0.42	CV	[66]
5	IL CPE	0.7	CV	[67]
6	CF/GCE	0.036	CV	[68]
7	**Torasemide Modified Carbon Paste Electrode**	**0.24**	**CV**	**This work**



Scheme 6.1

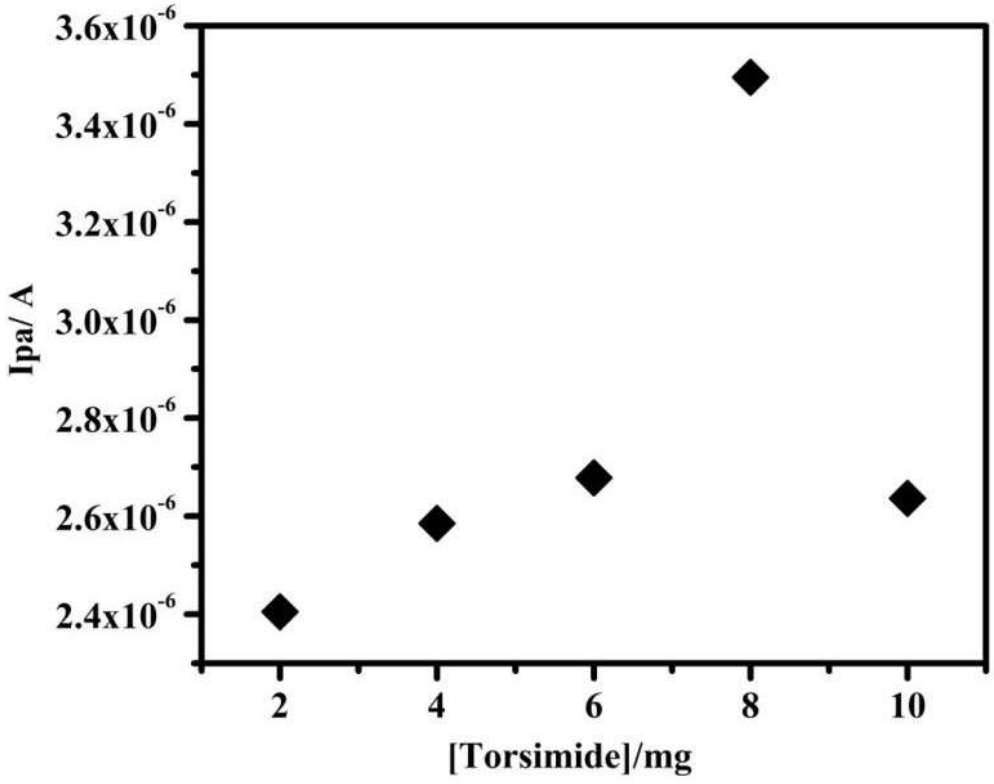


Fig. 6.1. Plot of anodic peak current of DA vs. concentration of Torasemide in 0.1 mM DA pH 7.0 phosphate buffer solution

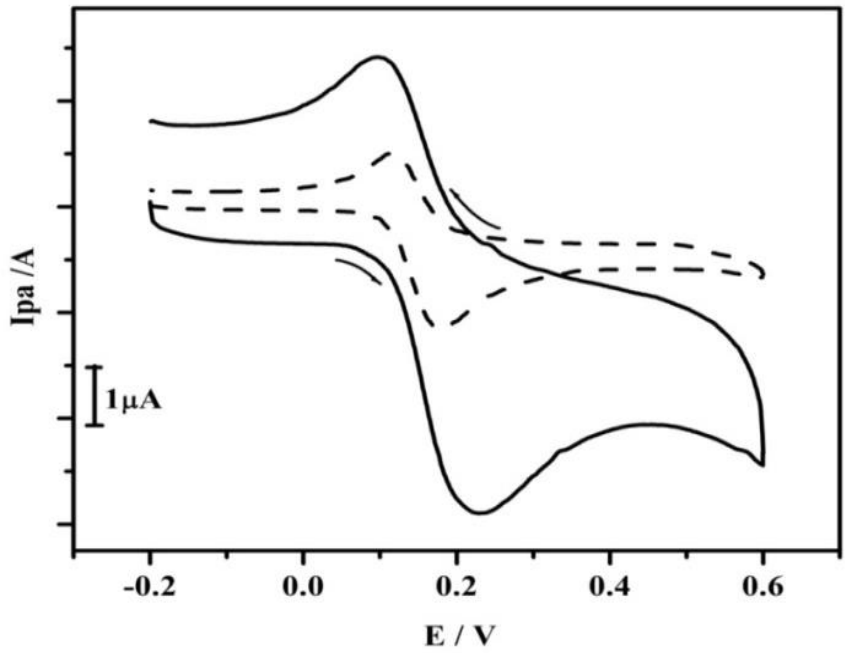


Fig. 6.2a. Cyclic voltammograms obtained for the electrochemical response of DA at torasemide MCPE (solid line) and bare carbon paste electrode (dashed line) in 0.2 M phosphate buffer solution pH 7.0 containing 0.1 mM DA scan rate 100 mV/s

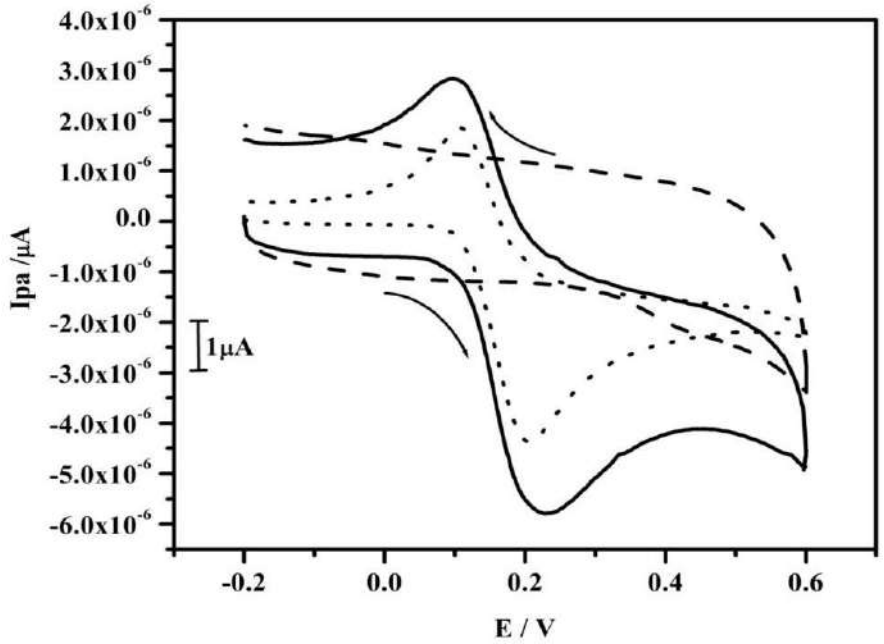


Fig. 6.2b. Cyclic voltammograms obtained for the electrochemical response of DA at torasemide MCPE (solid line), torasemide MCPE without DA (dashed line) and bare carbon paste electrode (dotted line) in 0.2 M phosphate buffer solution pH 7.0 containing 0.1 mM DA scan rate 100 mV/s

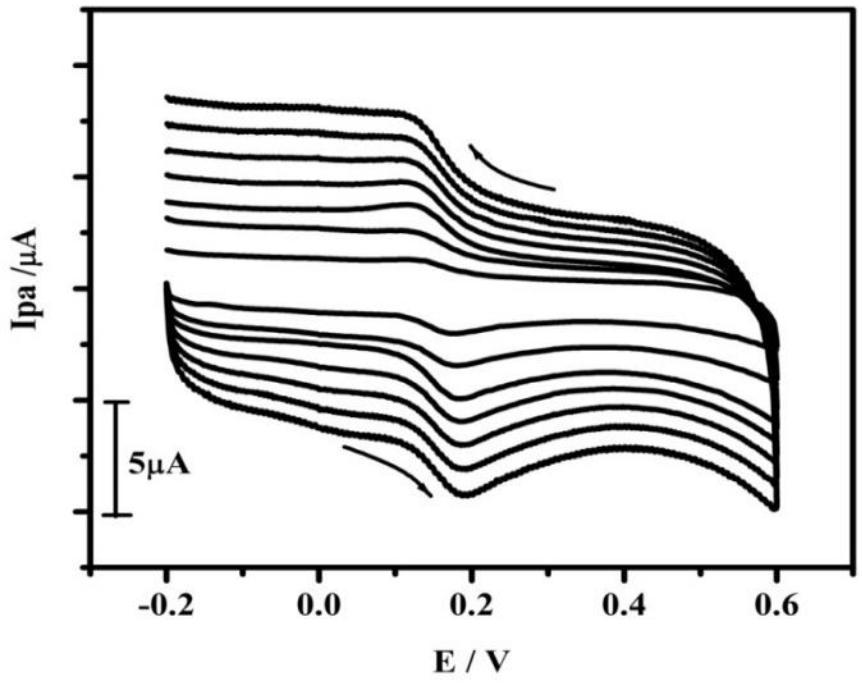


Fig. 6.3a. Cyclic voltammogram of different scan rate in the presence of 0.1 mM dopamine and 0.2 M phosphate buffer solution at pH 7.0, scan rate 50 to 350 mV/s

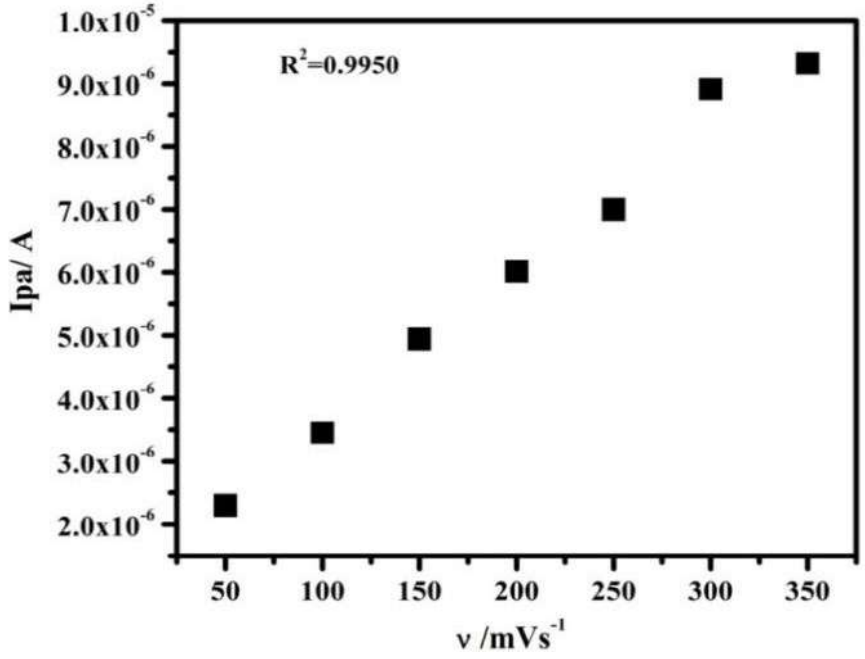


Fig. 6. 3b. Plot of anodic peak current verses scan rate

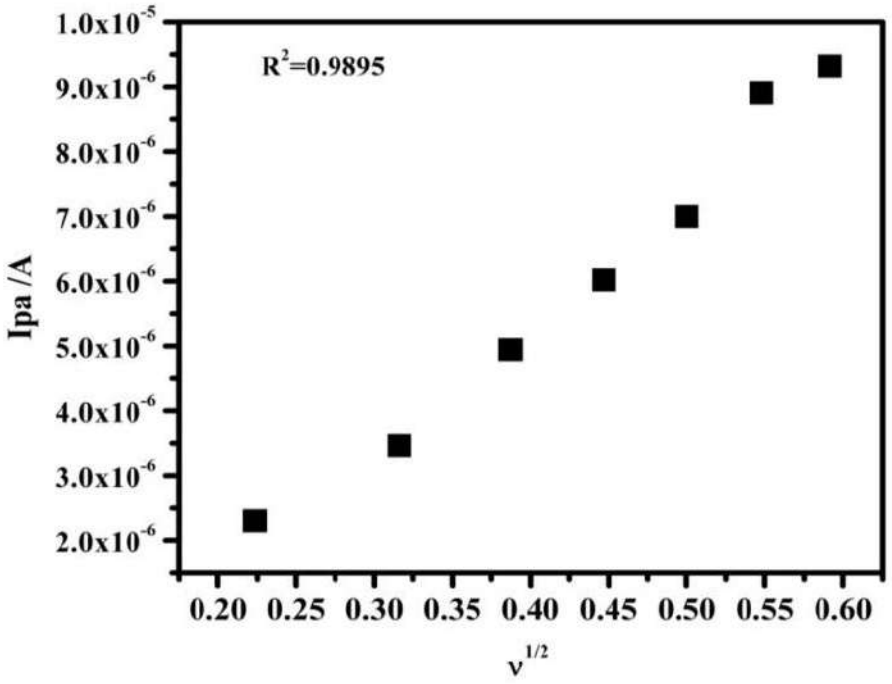


Fig. 6.3c. Plot of anodic peak current verses square root of scan rate

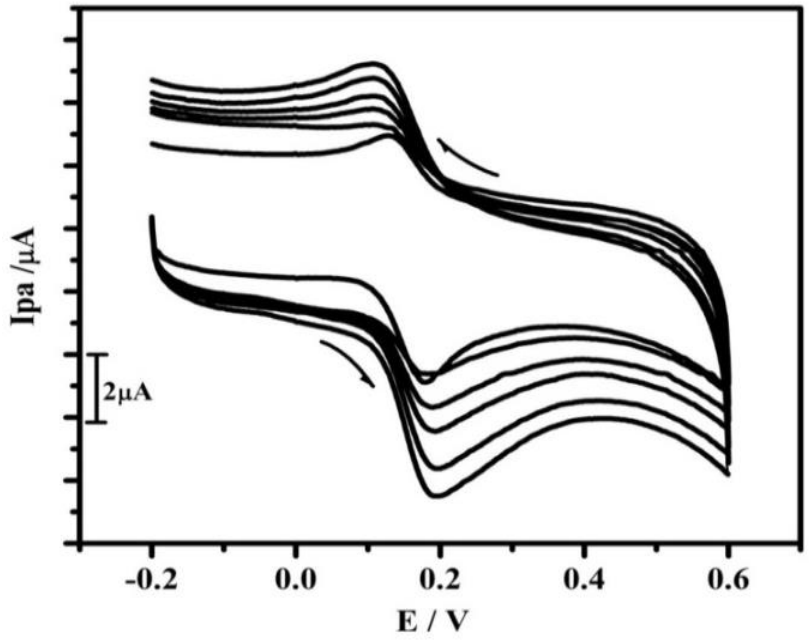


Fig. 6.4a. Cyclic voltammogram of variation of concentration of dopamine from 0.1 to 0.6 mM in presence of phosphate buffer solution at pH 7.0

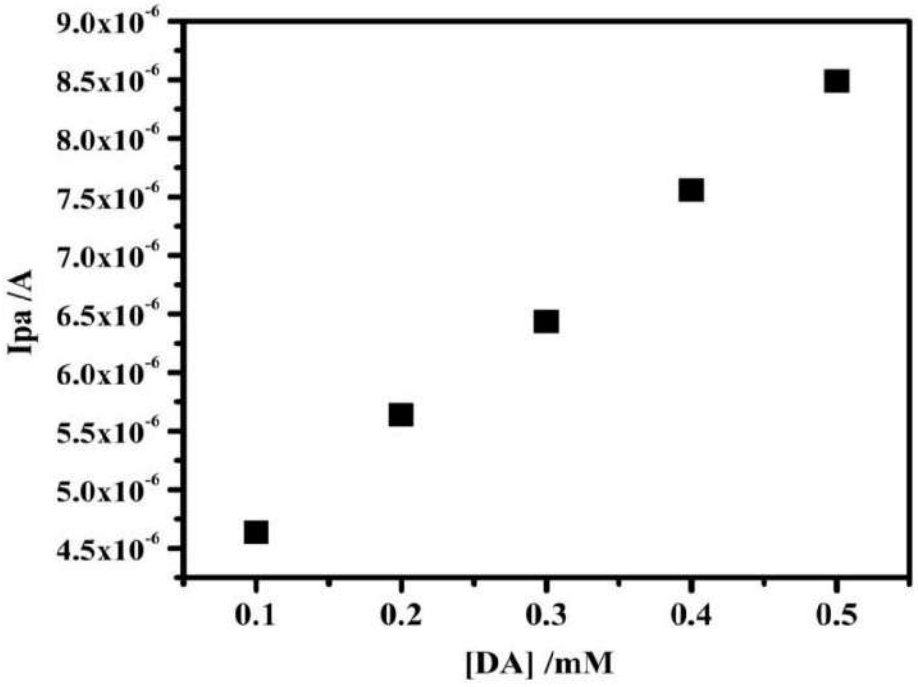


Fig. 6.4b. Plot of anodic peak current verses the concentration of DA

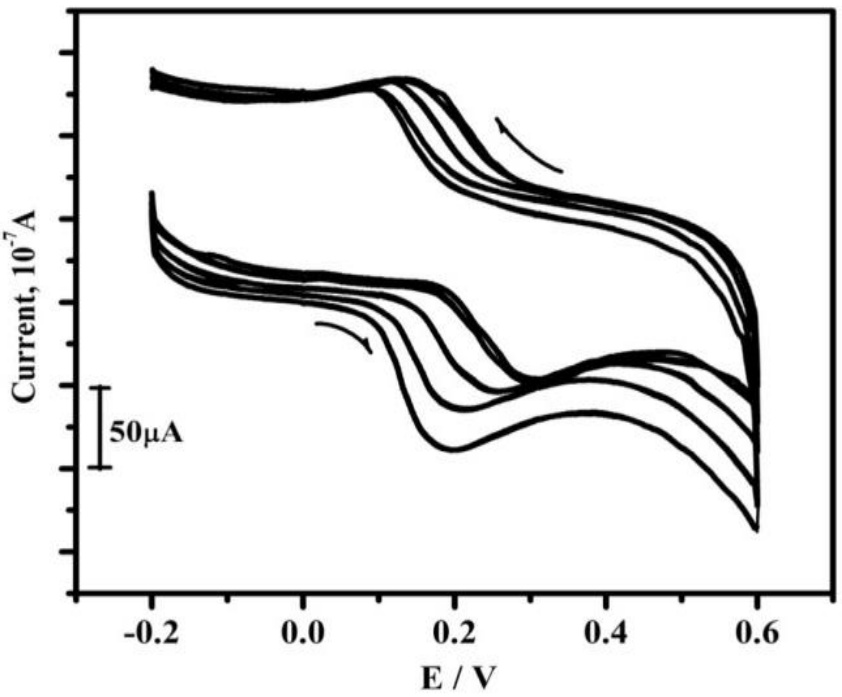


Fig. 6.5a. Cyclic voltammograms of 0.1 mM DA for different pH (from 5.8 to 7.4 pH) at TORMCPE

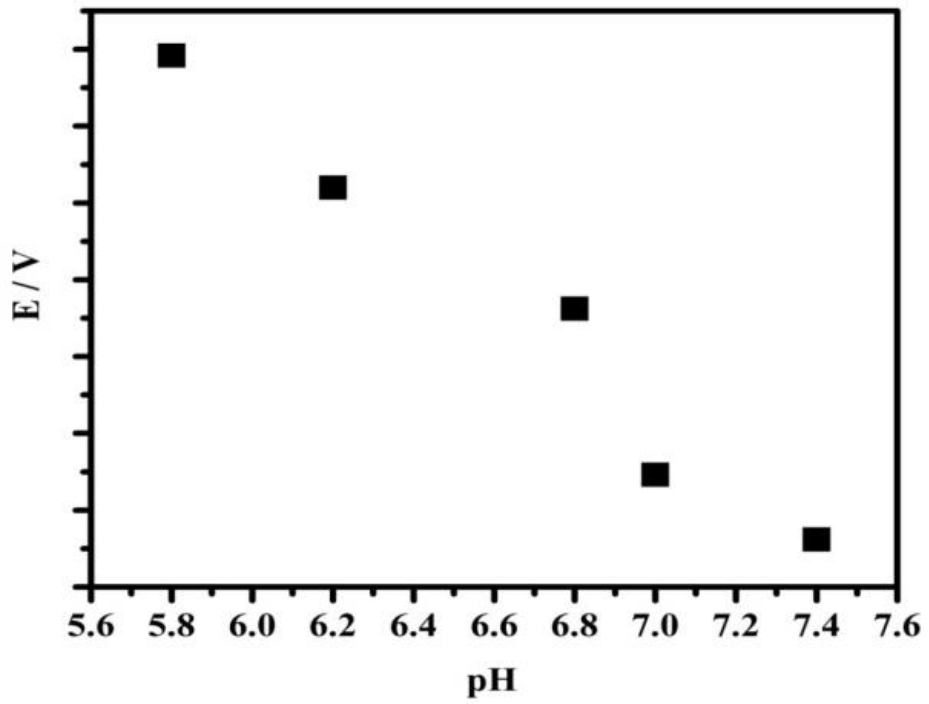


Fig. 6.5b. Plot of anodic peak potential verses pH

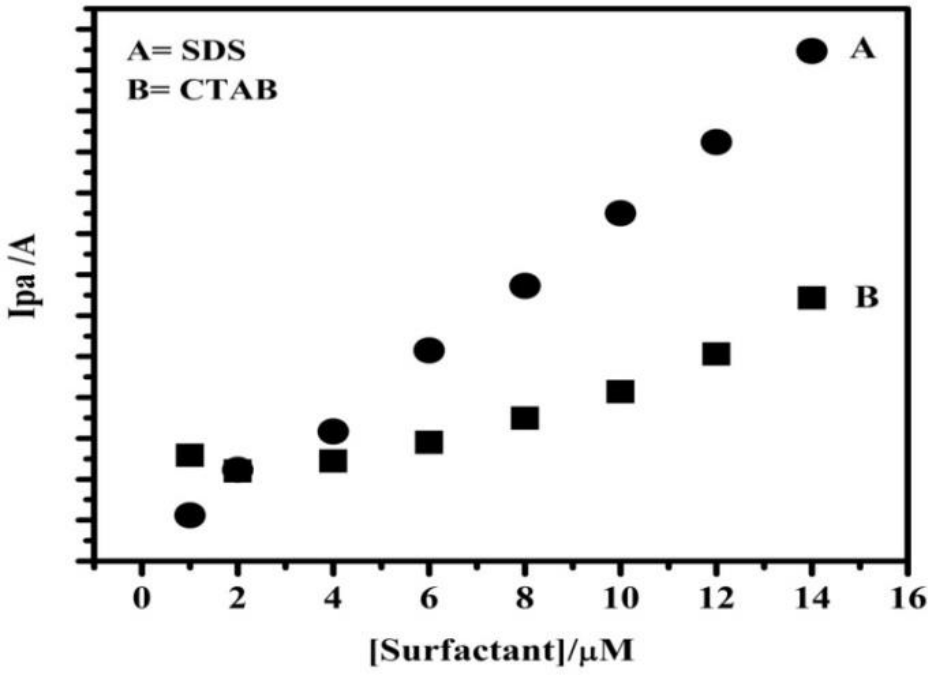


Fig. 6.6a. Effect of variation of concentration of surfactants mobilization for 0.1 mM Dopamine at TORMCPE in 0.2 M PBS pH 7.0, scan rate 100 mV/s

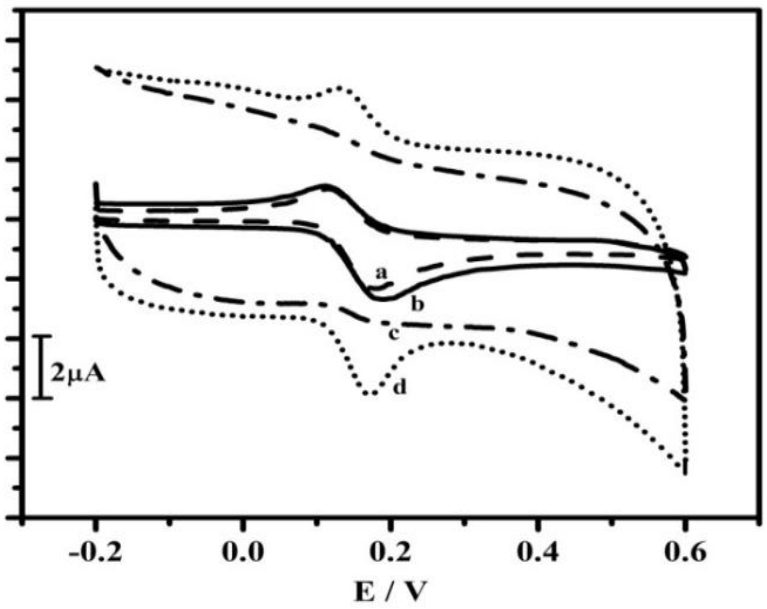


Fig. 6.6b. Cyclic voltammogram of 0.1 mM DA at BCPE (dashed line), TORMCPE (solid line), 14 μM CTAB (solid dashed line) and 14 μM SDS on the modified CPE (dotted line) in 0.2 M phosphate buffer solution pH 7.0, scan rate 100 mV/s

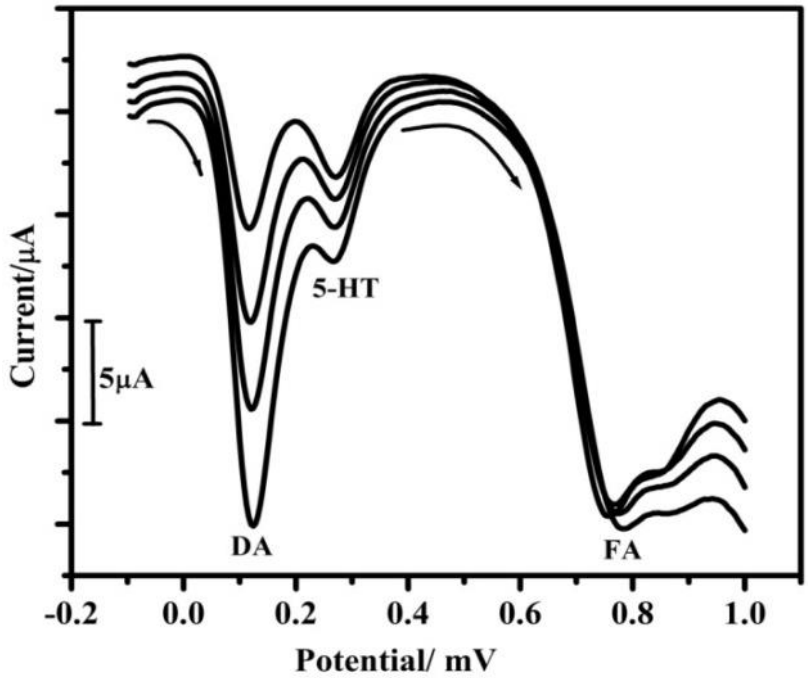


Fig. 6.7a. Differential pulse voltammograms of DA (0.1, 0.2, 0.3 and 0.4 mM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 10 μM 5-HT and 10 μM FA at TORMCPE with the scan rate of 100 mV/s

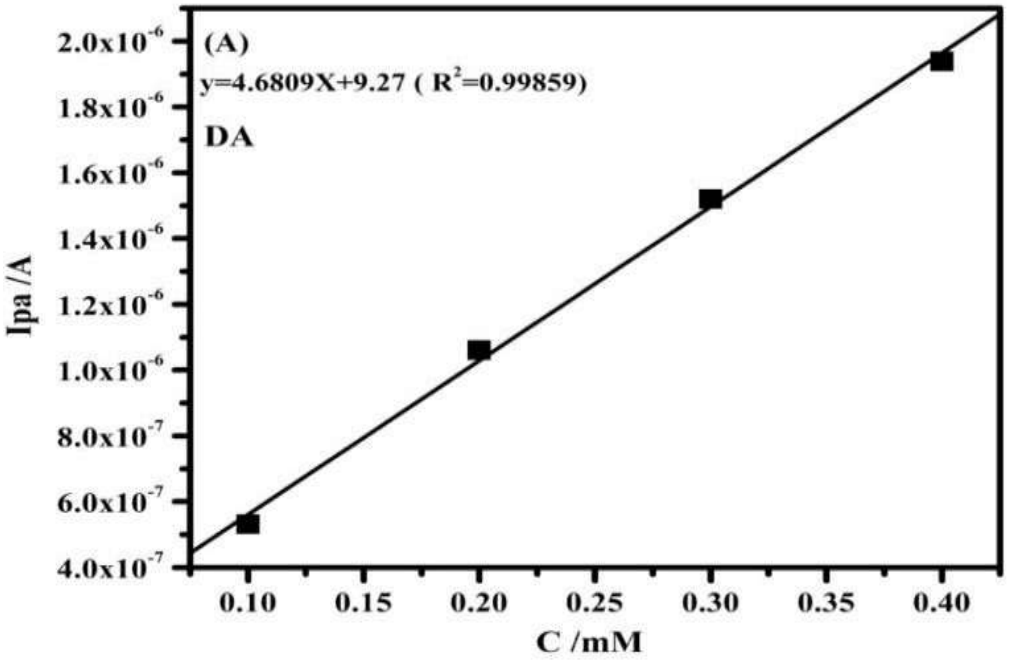


Fig. 6.7b. Plot of anodic peak current (Ipa) versus DA concentration

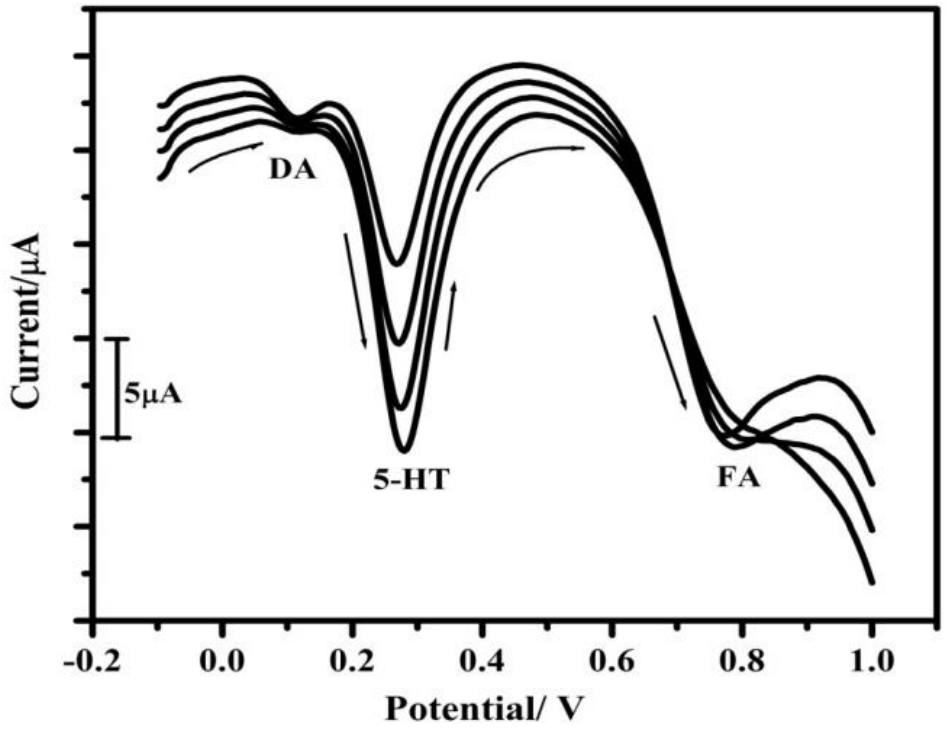


Fig. 6.8a. Differential pulse voltammograms of 5-HT (10, 20, 30 and 40 μM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.1 mM DA and 10 μM FA at TORMCPE with the scan rate of 100 mV/s

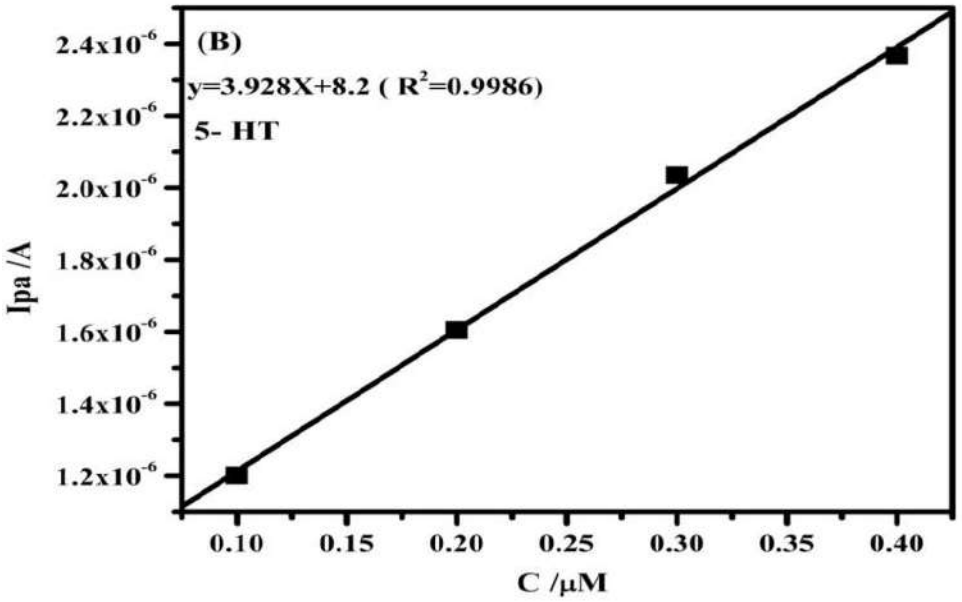


Fig. 6.8b. Plot of anodic peak current (Ipa) versus 5-HT concentration

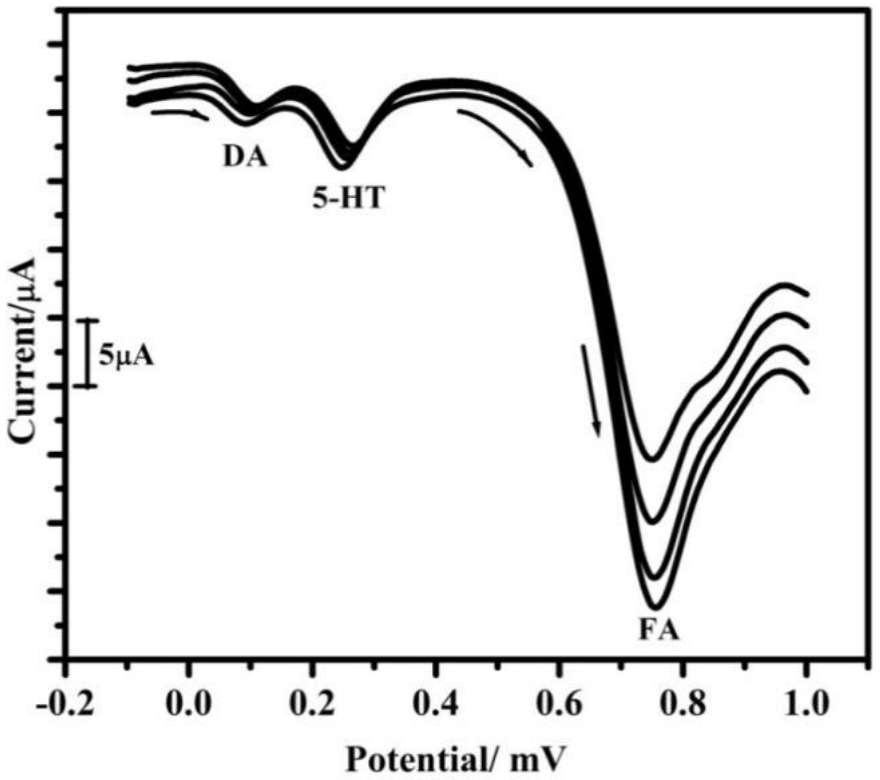


Fig. 6.9a. Differential pulse voltammograms of FA (10, 15, 20 and 25 μM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.1 mM DA and 10 μM 5-HT at TORMCPE with the scan rate of 100 mV/s

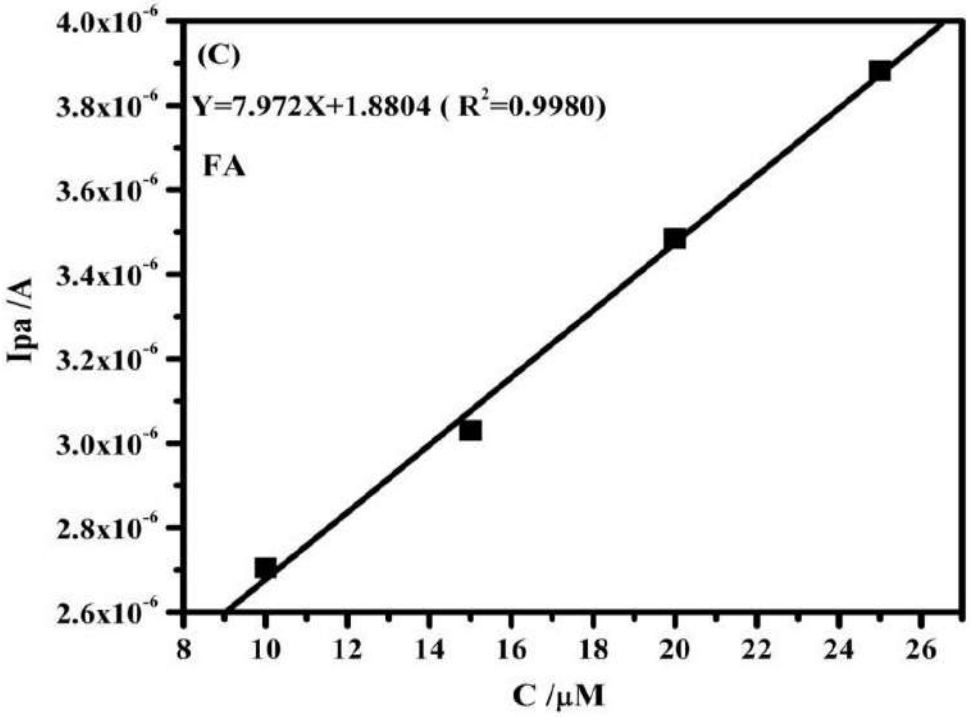


Fig. 6.9b. Plot of anodic peak current (Ipa) versus FA concentration

6.7.9. References

[1] R.T. Kachoosangi and R.G. Compton, *Anal. Bioanal. Chem*., 387 (2007) 2793.

[2] G.G. Wildgoose, C.E. Banks, H.C. Leventis and R.G. Compton, *Microchim. Acta.,* 152 (2006) 214.

[3] A.A. Ensafi, M. Taei and T. Khayamian, *J. Electroanal. Chem.,* 633 (2009) 220.

[4] I. Streeter, G.G. Wildgoose, L. Shao and R.G. Compton, *Sens. Actuators B,* 133 (2008) 466.

[5] R.T. Kachoosangi, G.G. Wildgoose and R.G. Compton, *Analyst,* 133 (2008) 895.

[6] R.T. Kachoosangi, G.G. Wildgoose and R.G. Compton, *Anal. Chim. Acta.,* 618 (2008) 60.

[7] A. Walcarius, *Electroanalysis,* 20 (2008) 738.

[8] S.E.W. Jones and R.G. Compton, *Curr. Anal. Chem.,* 4 (2008) 182.

[9] S. Griese, D.K. Kampouris, R.O. Kadara and C.E. Banks, *Electroanalysis*, 20 (2008) 1512.

[10] J.J. Gooding, *Electroanalysis,* 20 (2008) 582.

[11] E. Chow and J.J. Gooding, *Electroanalysis,* 18 (2006) 1448.

[12] A. Kutluay and M. Aslanoglu, *Sens. Actuators B*, 185 (2013) 404.

[13] S. Marceglia, G. Foffani, A. Bianchi, G. Baselli, F. Tamma, M. Egidi and A. Priori, *J. Physiol*., 571 (2006) 591.

[14] N. Kemppainen, P. Marjamaki, M. Roytta and J.O. Rinne, *J. Neural. Trans*., 108 (2001) 836.

[15] L. Schwieler, G. Engberg and S. Erhardt, *Synapse,* 52 (2004) 122.

[16] R. Zhu and W.T. Kok, *Anal. Chem*., 69 (1997) 4016.

[17] M. Kumar, B.E.K. Swamy and M. Schell, *Chem. Sens*., 4 (2014) 9.

[18] U. Chandra, B.E.K. Swamy, O. Gilbert, M. Pandurangachar and B.S. Sherigara, *Int. J. Electrochem. Sci.,* 4 (2009) 1488.

[19] A. Sathisha and B.E.K. Swamy, *Anal. Bioanal. Electrochem*., 7 (2015) 21.

[20] Y. Sun, Y. Baoxian, W. Zhang and X. Zhou, *Anal. Chim. Acta,* 363 (1998) 75.

[21] J.A. Stamford, P. Palij, C. Davidson and S.J. Trout, *Bioelectrochem. Bioenerg.,* 38 (1995) 289.

[22] C. Davidson, E.H. Ellinwood, S.B. Douglas and T.H. Lee, *J. Neurol. Methods,* 101 (2000) 75.

[23] L. Zhang, J. Jia, X. Zou and S. Dong, *Electroanal.,* 16 (2004) 1413.

[24] H. Zhao, Y. Zhang and Z. Yuan, *Electroanal.,* 14 (2002) 1031.

[25] C.R. Raj, K. Tokuda and T. Ohsaka, *Bioelectrochem.,* 53 (2001) 183.

[26] H. Zhao, Y. Zhang and Z. Yuan, *Anal. Chim. Acta,* 441 (2001) 117.

[27] M.M. Da´Vila, M.P. Elizalde, J. Mattusch and R. Wennrich, *Electrochem. Acta.,* 46 (2001) 3189.

[28] T. Liu, M. Li and Q. Li, *Talanta*, 63 (2004) 1053.

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

[30] T.K. Kawagoe and R.M. Wightman, *Talanta,* 41(1994) 865.

[31] J.G. Manjunatha, B.E.K. Swamy, G.P. Mamatha, O. Gilbert, M.T. Shreenivas, and B.S. Sherigara, *Int. J. Electrochem. Sci*., 4 (2009) 1718.

[32] K. Wu, J. Fei and S. Hu, *Anal. Biochem*., 318 (2003) 106.

[33] D. Hoegger, P. Morier, C. Vollet, D. Heini, F. Reymond and J.S. Rossier, *Anal. Bioanal. Chem*., 387 (2007) 275.

[34] E. Gujska and A. Kuncewicz, *Eur. Food. Res. Technol*., 221 (2005) 213.

[35] I.J. Holcomb and S.A. Fusari, *Anal. Chem*., 53 (1981) 609.

[36] J.F. Gregory, B.P.F. Day and K.A. Ristow, *J. Food. Sci.,* 47 (1982) 1571.

[37] A.A. Alwarthan, *Anal. Sci*., 10 (1994) 922.

[38] M. Jung, B. Kim, D.W. Boo and H.Y. Korean, *Chem. Soc*., 28 (2007) 750.

[39] M.M. Ardakani, H. Beitollahi, M.A.S. Mohseni, H. Naeimi and N. Taghavinia, *Applied Catalysis A: General,* 378 (2010) 201.

[40] O. Gilbert, U. Chandra, B.E.K. Swamy, M.P. Char, C.Nagaraj and B.S. Sherigara, *Int. J. Electrochem. Sci.,* 3 (2008) 1195.

[41] D.H. Everett, "Basic Principles of Colloids Science", The Royal Society of Chemistry, Cambridge, UK (1988).

[42] T. Cosgrove (Ed.), "Colloid Science: Principles Methods and Applications", Blackwell, Oxford, UK (2005).

[43] R. Naik, E. Niranjana, B.E.K. Swamy, B.S. Sherigara and H. Jayadevappa, *Int. J. Electrochem. Sci.,* 3 (2008) 1574.

[44] Pandurangachar, E. Niranjana, B.E.K. Swamy, B.S. Sherigara and K.V. Pai, *Int. J. Electrochem. Sci*., 3 (2008) 588.

[45] E. Niranjana, R.R. Naik, B.E.K. Swamy, B.S. Sherigara and H. Jayadevappa, *Int. J. Electrochem. Sci.,* 2 (2007) 923.

[46] U. Chandra, O. Gilbert, B.E.K. Swamy, D.Y. Bodke and B.S. Sherigara, *Int. J. Electrochem. Sci.,* 3 (2008) 1044.

[47] Rekha, B.E.K. Swamy, R. Deepa, V. Krishna, O. Gilbert, U. Chandra and B.S. Sherigara, *Int. J. Electrochem. Sci.,* 4 (2009) 845.

[48] A.C. Moffat, M.D. Osselton and B. Widdop, *Clarke's Analysis of Drugs and Poisons,* 1652 (2004).

[49] B. Masereel, M.S.J.M. Krzesinski, B. Pirotte, G. Rorive and J. Delarge, *J. Pharm. Pharmacol*., 45 (1993) 720.

[50] C.L. Dunn, A. Fitton and R.N. Brogden, *Drugs*, 49 (1995) 121.

[51] L. Politi, L. Morini and A. Polettini, *Clin. Chim. Acta*., 386 (2007) 46.

[52] F.C. Luft and J. Cardiovasc, *Pharmacol*., 22 (1993) 32.

[53] M.B. Barroso, H.D. Meiring, A. deJong, R.M. Alonso and R.M. Jiménez, *J. Chromatogr.*, 690 (1997) 105.

[54] S. Engelhardt, I. Meineke and J. Moller, *J. Chromatogr*., 831 (2006) 31.

[55] A.M. El-Kosasy, O.A. El-Aziz, N. Ebrahim and L.A. Fattah, *Anal. Bioanal. Electrochem*., 4 (2012) 371.

[56] B.N. Chandrashekar, B.E.K. Swamy, N.B. Ashoka and M. Pandurangachar, *J. Mol. Liquids,* 165 (2012) 168.

[57] B.N. Chandrashekar, B.E.K. Swamy, M. Pandurangachar, T.V. Sathisha and B.S. Sherigara, *Colloid. Surf. B*, 88 (2011) 413.

[58] W. Sun, M. Yang and K. Jiao, *Anal. Bioanal. Chem.,* 389 (2007) 1283.

[59] U. Chandra, B.E.K. Swamy, O. Gilbert and B.S. Sherigara, *Electrochim. Acta.,* 55 (2010) 7166.

[60] R.N. Hegde, B.E.K. Swamy, N.P. Shetty and S.T. Nandibewoor, *J. Electroanal. Chem*., 635 (2009) 51.

[61] Q. Wang, N. Li and W. Wnag, *Anal. Sci*., 18 (2002) 635.

[62] N.B. Li, W. Ren and H.Q. Luo, *Electroanalysis,* 19 (2007) 1496.

[63] S. Yixin and S.F. Wang, *Microchim. Acta.,* 154 (2006) 121.

[64] M.A. Kamyabi and F. Aghajanloo, *Croat Chem. Acta.,* 82 (2009) 606.

[65] M. Zidan, T.W. Tee, A.H. Abdullah, Z. Zainal and G.J. Kheng, *Int. J. Electrochem. Sci*., 6 (2011) 288.

[66] W. Ma and D.M. Sun, *Russian J. Electroanal. Chem.,* 12 (2007) 1389.

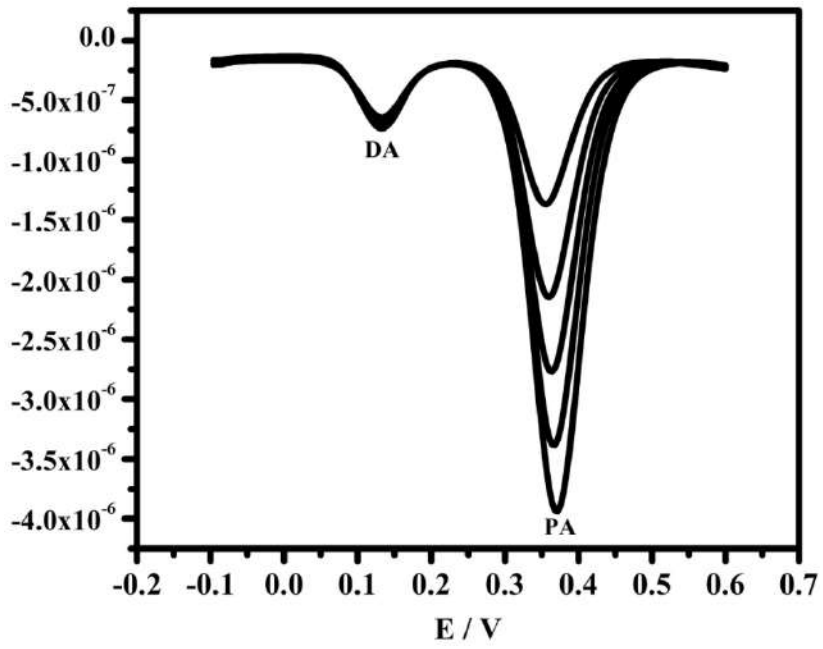
[67] W. Sun, M. Yang and K. Jiao, *Anal. Bioanal. Chem*., 389 (2007) 1291.

[68] V.A. Kumary, J. Divya, T.E.M. Nancy and K. Sreevalsan, *Int. J. Electrochem. Sci*., 8 (2013) 6619.

CHAPTER-7

Part-A

Electrochemical Selective Determination of Dopamine in Presence of Paracetamol in Mobilization Pregabalin Glassy Carbon Electrode: A Voltammetric Study



Analytical and Bioanalytical Electrochemistry (Revised and submitted)

7.1. Introduction

Electrochemical sensitive and selective determination of dopamine using a pregabalin mobilization glassy carbon electrode was developed by voltammetric technique. pregabalin mobilization glassy carbon electrode showed excellent electrocatalytic activity towards the oxidation of dopamine in phosphate buffer solution (pH 7) by cyclic voltammetric technique (CV) and differential pulse voltammetry (DPV). The effect of concentration, scan rate, and pH was studied for dopamine (DA). The detection limit (LOD) and quantification limit (LOQ) were calculated. The interference studies showed that the modified electrode exhibited excellent selectivity in the presence of dopamine and paracetamol (PA). The proposed method was applied for the detection of dopamine in biological samples.

7.1.1. Chemistry and Biological Relevance of Dopamine

The chemistry and biological relevance of dopamine has been explained details in chapter 3 Part A section 3.1.1 and 3.1.1.1.

7.1.2. Chemistry and Biological Relevance of Paracetamol

The chemistry and biological relevance of paracetamol has been explained details in chapter 3 Part A section 3.1.3.

7.1.3. Review of Electrochemistry of Dopamine and Paracetamol

The application of chemically modified electrodes in electroanalysis offers several advantages. They can lower the over potential, increase the reaction rate and sensitivity, and improve the selectivity. Dopamine (DA) is one of the most significant catecholamine belongs to the family of excitatory chemical neurotransmitter [1, 2]. It plays very important role in the function of central nervous, renal, hormonal and cardio vascular system [3, 4]. Because extreme abnormalities of DA level are symptoms of several diseases such as schizophrenia, Parkinson's disease [5], the determination of such compounds in a real biological samples and identify changes in neurotransmission that

correlate the behavioral state of animal are an obvious target in neurochemical studies. Generally the determination of DA is performed with high performances liquid chromatography (HPLC) [6], Ion Chromatography [7] and spectrophotometry. DA can be determined by electrochemical methods because it is an electrochemical active compound. The development of voltammetric sensor for the detection of neurotransmitter in the extracellular fluid of the central nervous system received much interest in the past few decades. The electrochemical methods have more advantages over other methods because the electrodes can be made conveniently to sense the neurotransmitter in the living organism [8-10].

Paracetamol, N-acetyl-p-aminophenol or acetaminophen, is one of the most commonly used drugs in the world. It is the preferred alternative analgesic and antipyretic to aspirin, to patients who cannot tolerate aspirin [11, 12]. Paracetamol is an acylated aromatic amide that was first introduced in medicine by Von Mering in 1893, has been in use as an analgesic for home medication for over 50 years, and is accepted as an effective drug for the relief of pain and fever in adults and children [13]. Standard usage of paracetamol has no any detrimental effect on the human body but over usage of the drug could lead to some serious side effects, such as liver disorders, skin rashes and inflammation of the pancreases [14]. Besides, p-aminophenol, the primary hydrolytic degradation product of paracetamol, can be present in pharmaceutical preparations as a synthetic intermediate or as a degradation product of paracetamol that can cause serious nephrotoxocity and tetragenic effects [15, 16].

Nevertheless, paracetamol is being increasingly used for therapeutic purposes. The development of a simple, precise and accurate method for the determination of paracetamol is therefore very important. The various methods reported for the determination of paracetamol in body fluids and pharmaceutical preparations include spectroscopy [17, 18], liquid chromatography [19, 20], capillary electrophoresis [21-23] and enzyme based assay methods [24]. However, these methods usually involve the hydrolysis of paracetamol sample to 4- aminophenol, which then required the formation of a colored complex using an appropriate reagent, which takes a long time to perform.

Pregabalin (PGB) is a novel antiepileptic drug. PGB is chemically (S)-3-(aminomethyl)-5-methylhexanoic acid [25] and it is approved in the US and Europe for adjunctive therapy of partial seizures in adults, and also has been approved for the treatment of pain from diabetic neuropathy or post-herpetic neuralgia in adults. Recently, it has been approved for treatment of anxiety disorders in Europe. Pregabalin is structurally related to the antiepileptic drug gabapentin and the site of action of both drugs is similar, the alpha2-delta (alpha2-delta) protein, an auxiliary subunit of voltage-gated calcium channels [26, 27]. The structure of the PGB is presented in (Scheme 7.1). According to the literature survey carried out, PGB is not official in any pharmacopoeia [28].

In the present work study, the electrochemical behaviors of dopamine were investigated in detail at pregabalin mobilization glassy carbon electrode [PGBMGCE]. The pregabalin mobilization glassy carbon electrode shows excellent electrocatalytic activity for the oxidation of dopamine, it accelerates the electron transfer rate and lowers the over potential. Some electrochemical parameters of dopamine electrochemical oxidation were measured by different electrochemical methods. The pregabalin mobilization glassy carbon electrode can be used for the determination of dopamine and simultaneous studies of dopamine and paracetamol.

7.2. Experimental Part

7.2.1. Chemicals

Dopamine [DA], Paracetamol [PA] and Pregabalin [PGB] were obtained from Himedia. Dopamine was dissolved using 0.1 M Perchloric acid ($HClO_4$). Paracetamol and pregabalin are dissolved using double distilled water. All other chemicals were of analytical grade quality and were used without further purification. The water used was a double distilled in all the measurements. Phosphate Buffer 0.2 M was prepared by 0.2 M disodium hydrogen phosphate and 0.2 M sodium dihydrogen phosphate.

7.2.2. Apparatus and Procedure

The electrochemical experiments were carried out using a model CHI-660c (CH Instrument-660 electrochemical workstation). All experiments were carried out in a conventional three-electrode system, consisting of mobilization glassy carbon working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode.

7.2.3. Preparation of Pregabalin Mobilization Glassy Carbon Electrode

The pregabalin mobilization glassy carbon electrode was prepared by 1 mM pregabalin solution was placed in the electrochemical cell with 0.2 M PBS in presence of 0.1 mM Dopamine. The glassy carbon electrode was scanned by immersing 3 mm length in that solution at a scan rate of 100 mVs^{-1} by using cyclic voltammetry.

7.3. Results and Discussion

7.3.1. Response of DA at the Bare GCE and PGBMGCE

The electrochemical response of dopamine at BGCE and PGBMGCE in the 0.2 M phosphate buffer at pH-7.0 at the scan rate of 100 mVs^{-1}. A pair of redox peaks observed for BGCE and with the mobilization GCE. The PGMGCE shows electrochemical property with lower the peak potential difference (ΔEp). As ΔEp is a function of electron transfer rate, lower the ΔEp higher will be the electron transfer rate. The result shows a dramatic change in the voltammetric response at fabricated electrode.

7.3.2. Electrocatalytic Response of DA at Pregabalin Mobilization Glassy Carbon Electrode

Figure 7.1 shows the cyclic voltammograms of DA in the 0.2M PBS at pH 7.0 of 100 mVs^{-1}. At a bare GC electrode, a pair of redox peak was observed with the oxidation peak potential at 0.17 V and the reduction peak potential at 0.13 V. Under the same conditions, the pregabalin mobilization GC electrode significantly enhanced peak current and a more reversible electron transfer process to DA. A well-defined redox wave of DA

was observed with the anodic and cathodic peak potentials at 0.18 and 0.13 V, respectively. The separation of peak potentials at the pregabalin mobilization GC electrode, ΔEp = (Epa −Epc) was 50 mV. This is the clear evidence that our mobilization electrode has better electrocatalytic activity by exposing large surface area for electrochemical oxidation of DA (Scheme 7.2).

7.3.3. Effect of Scan Rate

The effect of variation of applied scan rate was examined by CV technique at pregabalin mobilization glassy carbon electrode as shown in Figure 7.2a. The experimental results obtained at pregabalin mobilization glassy carbon electrode showed increase in the redox peak currents with increase in the applied scan rate and they are proportional to each other according to Randles–Sevcik equation. The observation shows that there is a shifting of anodic peak potential (Epa) to more positive side and cathodic peak potential (Epc) to the less negative side. In order to confirm the electrode process, the graph of peak current (Ip) versus scan rate (t) was plotted as shown in Figure 7.2b with the correlation coefficient (r^2) 0.9982. The Ip versus square root scan rate ($\nu^{1/2}$) was plotted as shown in Figure 7.2c with the correlation coefficient (r^2) 0.9962. This suggests the electrode process was an adsorption-controlled process.

7.3.4. Effect of Concentration of DA

The effect of varying concentration of DA was studied at pregabalin mobilization GC electrode in 0.2 M PBS pH 7.0 at a scan rate of 100 mVs^{-1} as shown in the Figure 7.3a. It is clearly observed that, anodic peak current of DA was increased as a result of increased concentration of DA in the range 0.2 to 0.6 mM. The plot of Ipa versus concentration of DA shows a linear relation with correlation coefficient of 0.99487 as shown in the Figure 7.3b. The limit of detection and limit of quantification was calculated by using equation (1) and (2).

$$LOD=3S/M \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$LOQ=10S/M \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where, S is the standard deviation and M is the slope of obtained from the calibration plot. The limit of detection and limit of quantification was found to be 5.5×10^{-7}M and 18.5×10^{-7}M. The proposed method exhibited relatively lower detection limit as compared with the early reports as shown in Table 7.1 [31-38].

7.3.5. Effect of pH

The effect of pH on the formal potential was investigated by cyclic voltammetry in the solution containing 0.1 mM DA. Cyclic voltammograms obtained at pregabalin mobilization GC electrode in 0.2 M phosphate buffer solution of different pH values at scan rate of 50 mVs^{-1}. As the pH gradually increases from 6.4 to 7.2 the anodic peak potential shifts to more negative potential as shown in Figure 7.4a. The peak potential of DA oxidation shifted negatively at a slope of −65 mV/pH unit, indicating equal number of electrons and protons involved in the oxidation process, which is close to the theoretical value of 59 mV/pH unit for one electron and one proton [29, 30].

7.3.6. Electrochemical Oxidation of DA and PA at the Pregabalin Mobilization GCE

Figure 7.5 shows cyclic voltammograms of mixture of 0.1mM DA and 0.1 mM PA. The dashed line in cyclic voltammogram represents bare glassy carbon electrode and solid line represents the pregabalin mobilization GCE. The cyclic voltammograms shows active sensitive enhancement in selective separation of DA and PA in a mixture and two well separated oxidation peaks were found at pregabalin mobilization GCE. The electrochemical oxidation peak of DA and PA was found at 190 mV and 410 mV at simultaneously. The difference of oxidation peak potentials for DA and PA is 220 mV. It is clear that pregabalin mobilization GCE possessed an excellent electrocatalytic activity for the selective oxidation of dopamine and paracetamol.

7.3.7. Effect of pH on the Oxidation of DA and PA in a Binary Mixture of CV and DPV

The effect of pH of solutions on the electrochemical response of the pregabalin mobilization GCE towards the single determination of DA and PA was studied and

variations of peak current with respect to the change in pH of the electrolyte in the pH range from 6.4 to 7.2 are shown in Figure 7.6a and 7.6b. The anodic peak current of DA increases slightly with an increase in the solution pH until it reaches 7.2; that of DA increases with increasing pH until it reaches about 7.0, and then it decreases when the pH increases further. For PA, a bigger anodic peak current was obtained at about pH 7.0. In addition, all the anodic peak potentials for the oxidation of DA and PA shifted towards negative direction with an increase in pH, showing that protons have taken part in their electrode reaction processes. Therefore, the optimum solution pH selected was pH 7.0 as shown in Figure 7.7.

7.3.8. Simultaneous Determination of DA and PA

The electro-oxidation processes of DA and PA in the mixture have also been investigated when the concentration of one species changed, whereas those of other species are kept constant. The results are shown in Figure 7.8a gives the DPV recordings at various DA concentrations at the pregabalin mobilization GCE in the presence of PA. It can be seen that, the peak current of DA increased with an increase in DA concentration when the concentrations of PA was kept constant. Similarly as shown in Figure 7.9a keeping the concentration of DA constant, the oxidation peak current of PA was increased with an increase in PA concentration. From the experimental results it can be obtained that the electrochemical response peaks for DA and PA oxidation at the pregabalin mobilization GCE were clearly separated from each other when they co-exist in pH-7.0. It is therefore possible to simultaneously studies DA and PA in samples at a pregabalin mobilization GCE.

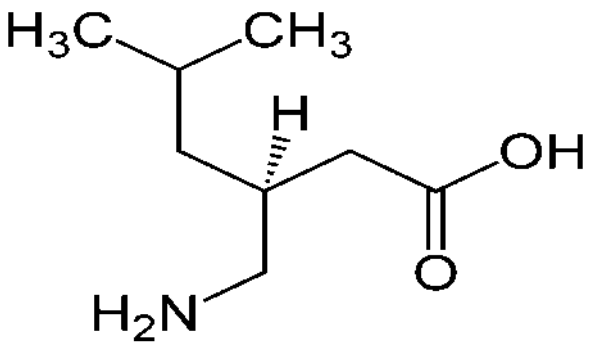
The corresponding graphs of anodic peak current versus various concentrations of DA (0.2-0.6 mM) and PA (0.2-0.6 mM) showed linear relationships with linear regressions for A (DA) Ipa (μA) =1.924 C(μM/L) +8.7684, B (PA) Ipa (μA) =6.344 C(mM/L) +1.832, the correlation coefficient for these linear graphs was 0.9911 and 0.9979 respectively for this pregabalin mobilization GCE which were shown in Figures 7.8b, 7.9b, respectively.

7.3.9. Conclusion

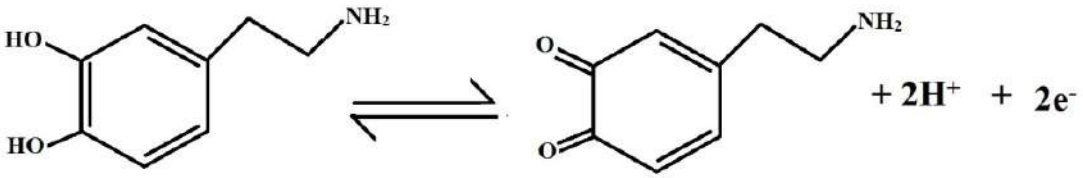
The mobilization of pregabalin glassy carbon electrode shows an excellent electrocatalytic activity and remarkable enhancement of peak current of dopamine as compared with electrochemical performances obtained at bare glassy carbon electrode. The mobilization electrode has superior functioning characteristics like sensitivity, selectivity and low detection limit. Consequently, a good approach towards the development of PGBMGCE has been presented for the determination of dopamine in the presence of excess of paracetamol. The developed method has a potential for other important biomolecules in developing sensor application.

Table 7.1

Electrode	pH	Linear dynamic range for DA (μM)	Detection limit for DA	Technique used	Reference
CPE/SnHCF/CTAB	7.0	200–25,000	–	CV	[31]
Ag/CNT-CPE	2.0	0.8–64	3.7×10^{-7} M	DPV	[32]
TBQ-MCPE	7.0	10–100	0.0074 μA/μM (Sensitivity)	DPV	[33]
SDS/CPE	7.4	0.5–800	5×10^{-8} M	DPV	[34]
CPE/SILICA GEL	5.0	0.2–1 & 2–150	4.8×10^{-8} M	DPASV	[35]
CNT-TNCPE	4.0	0.1–80	8×10^{-8} M	DPV	[36]
CoNSal/TOAB	5.0	1–100	5×10^{-7} M	DPV	[37]
MCPE-GO	7.0	0.7–70	1.5×10^{-8} M	DPV	[38]
PGBM/GCE	**7.0**	**0.1- 600**	**5.5×10^{-7}M**	**CV**	**Present Work**



Scheme 7.1



Dopamine **Dopa-O-Quinone**

Scheme 7.2

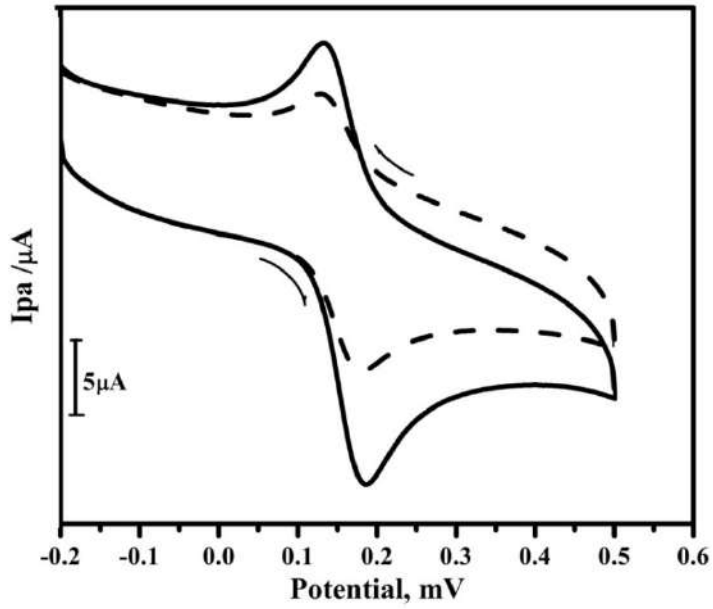


Fig. 7.1. Cyclic voltammograms obtained for the electrochemical response of DA at pregabalin MGCE (solid line) and bare GCE (dashed line) in 0.2 M phosphate buffer solution pH 7.0 containing 0.1mM DA at scan rate 100 mV/s

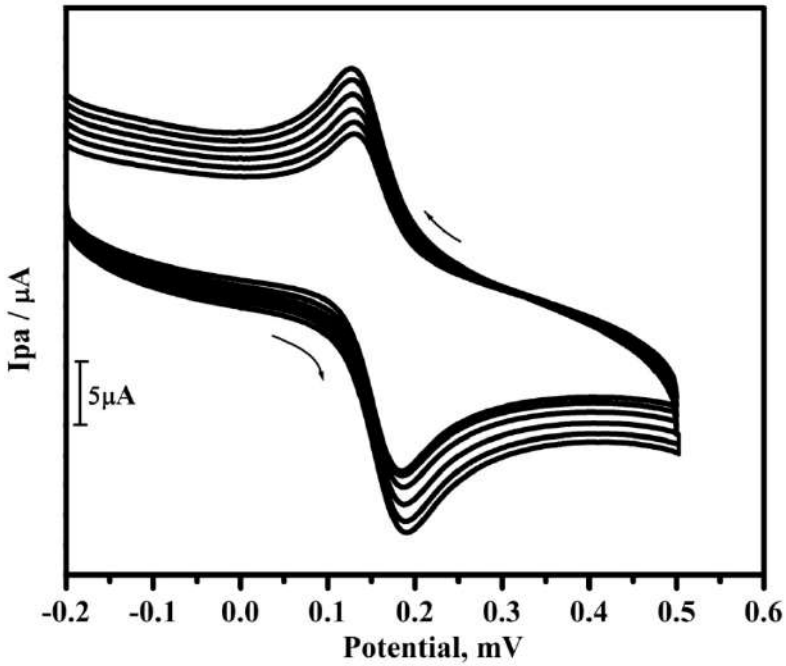


Fig. 7.2a. Cyclic voltammogram of different scan rate in the presence of 0.1 mM dopamine and 0.2 M phosphate buffer solution at pH 7.0, scan rate 50 to 100 mV/s

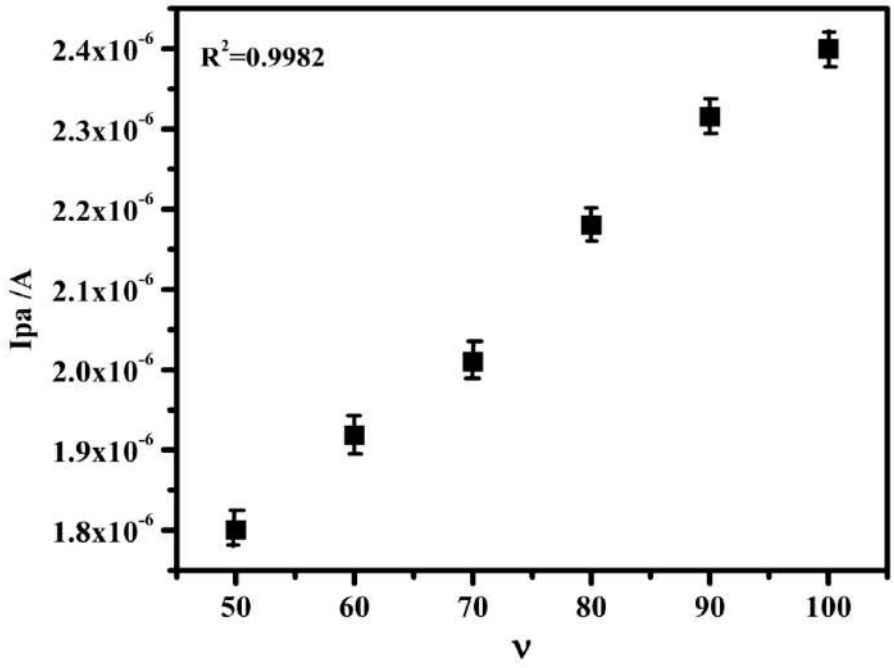


Fig. 7.2b. Plot of anodic peak current versus scan rate

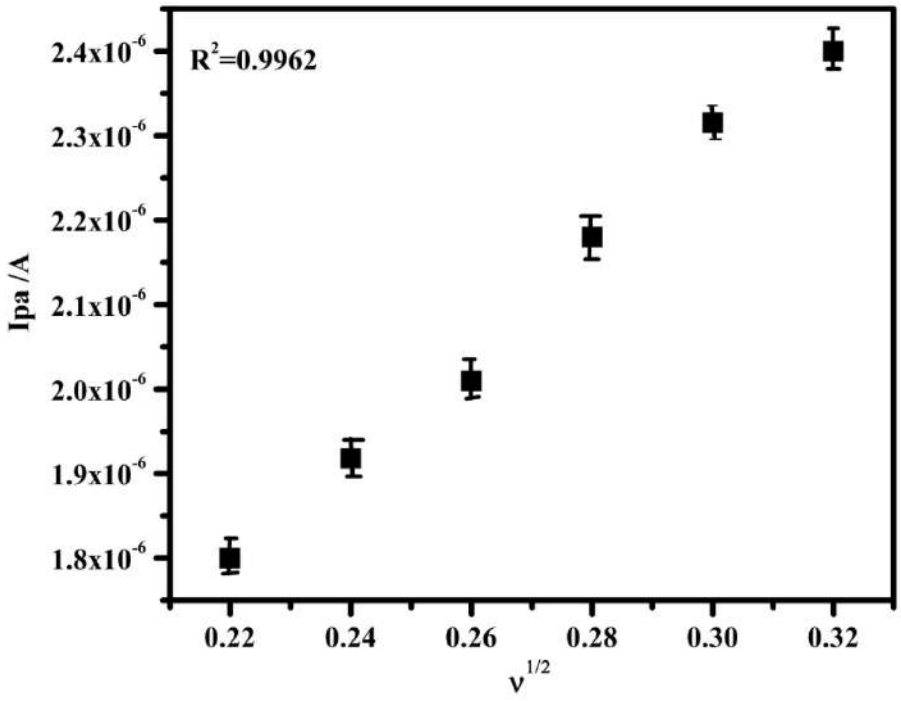


Fig. 7.2c. Plot of anodic peak current versus square root of scan rate

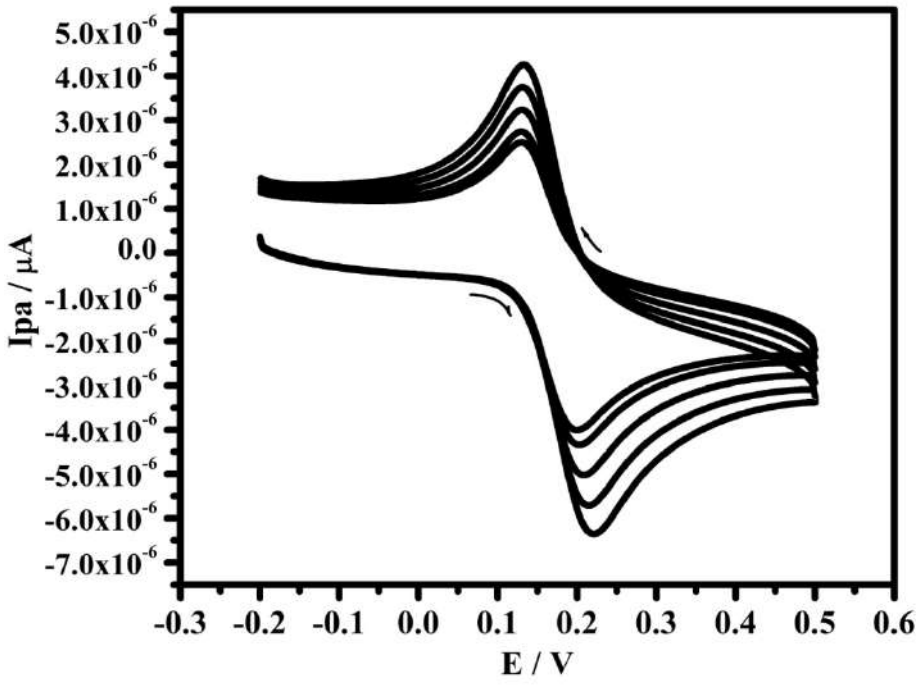


Fig. 7.3a. Cyclic voltammogram of variation of concentration of dopamine from 0.2 to 0.6 mM in presence of phosphate buffer solution at pH 7.0

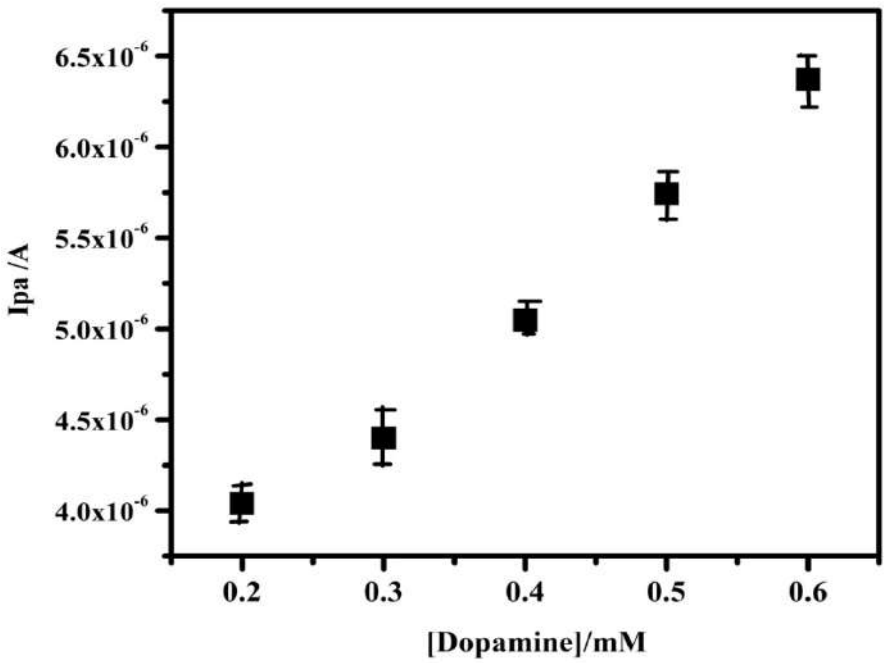


Fig. 7.3b. Plot of anodic peak current versus the concentration of DA

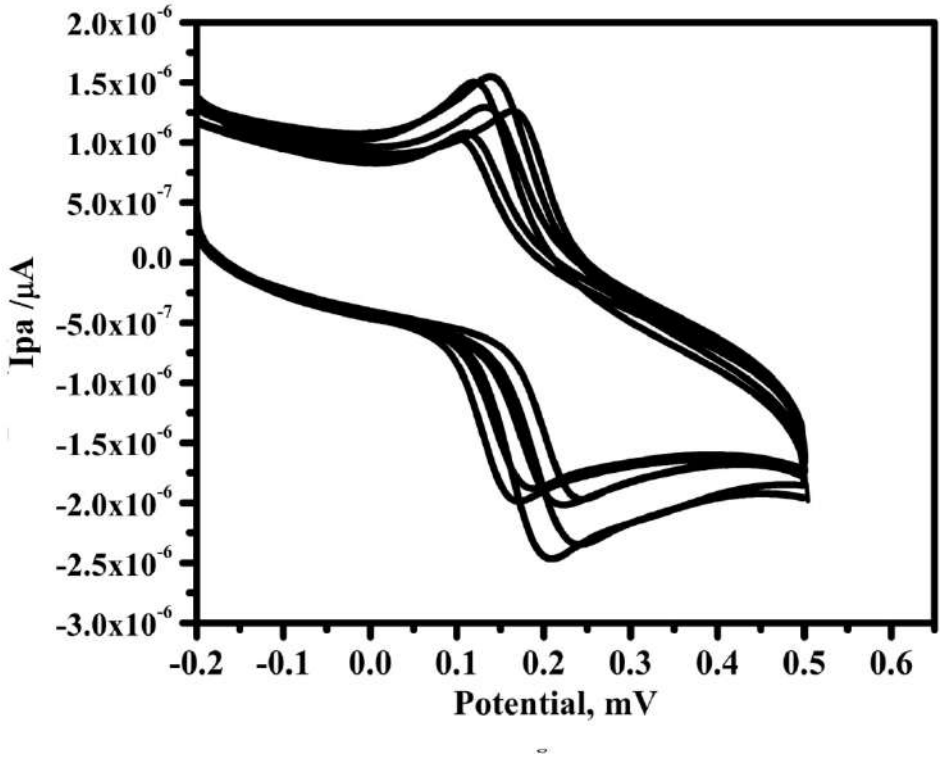


Fig. 7.4a. Cyclic voltammograms of 0.1 mM DA for different pH (from 6.4 to 7.2 pH) at PGBMGCE

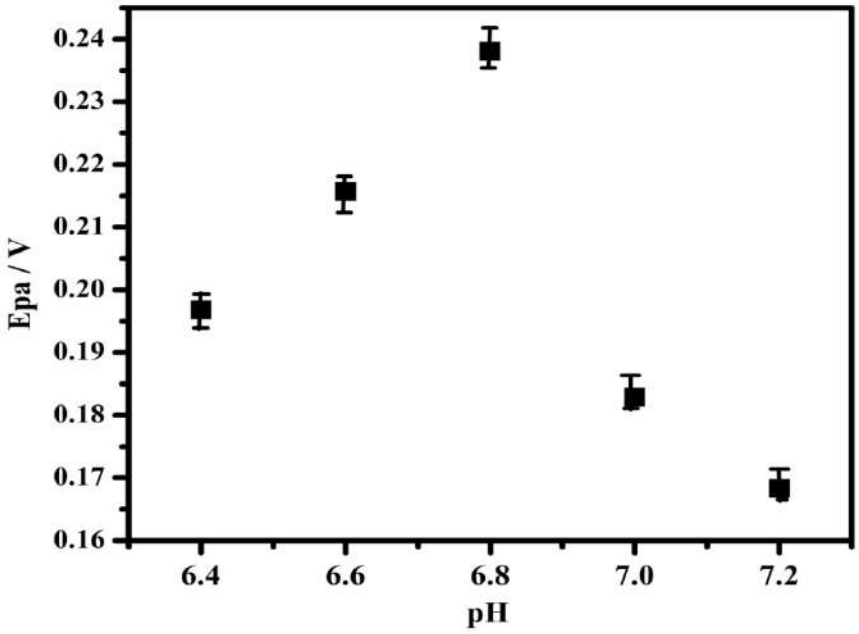


Fig. 7.4b. Plot of anodic peak potential versus pH

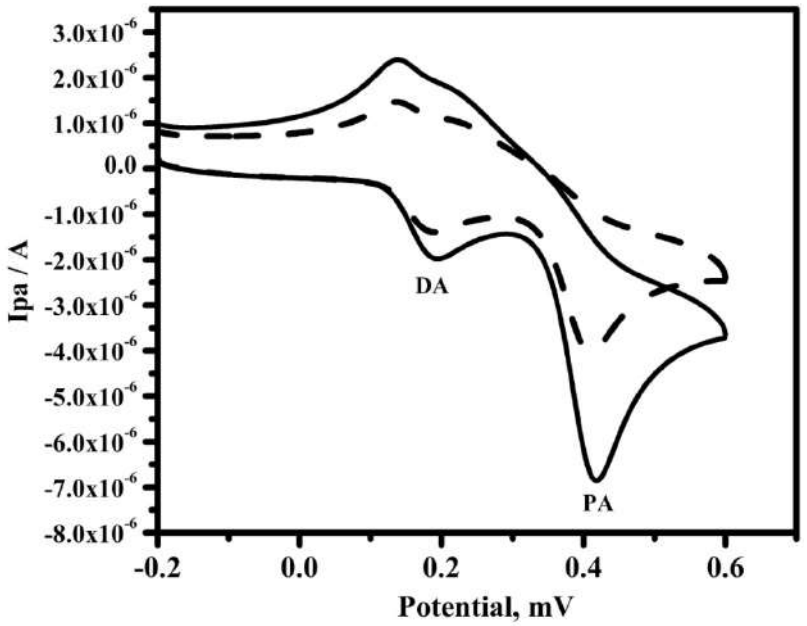


Fig. 7.5. Cyclic voltammogram for simultaneous determination of DA and PA at bare GCE (dotted line) and PGBMCPE (solid line) in 0.2 M Phosphate buffer solution pH 7.0, scan rate 100 mV/s

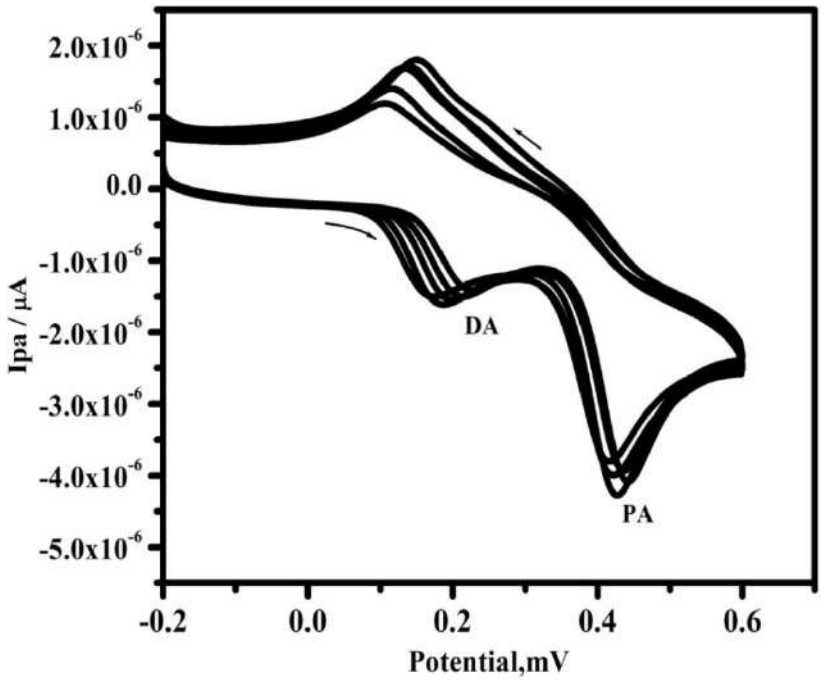


Fig. 7.6a. Cyclic voltammogram for different pH (from 6.4 to 7.2) in presence of binary mixture DA and PA at PGBMGCE in 0.2M PBS pH 7.0, scan rate 100 mV/s

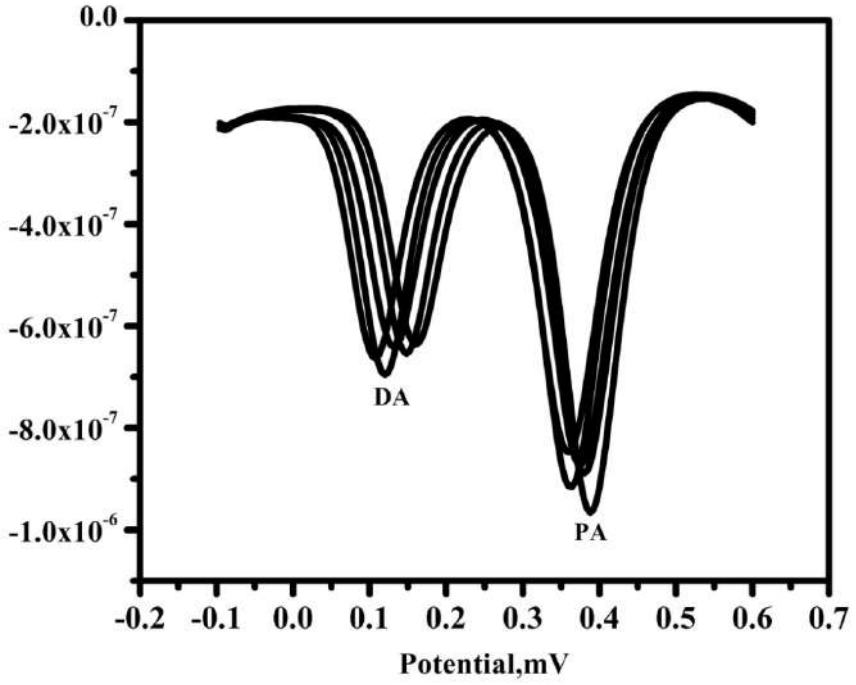


Fig. 7.6b. Differential pulse voltammogram for different pH (from 6.4 to 7.2) in presence of binary mixture DA and PA at PGBMGCE in 0.2 M PBS pH 7.0, scan rate 100 mV/s

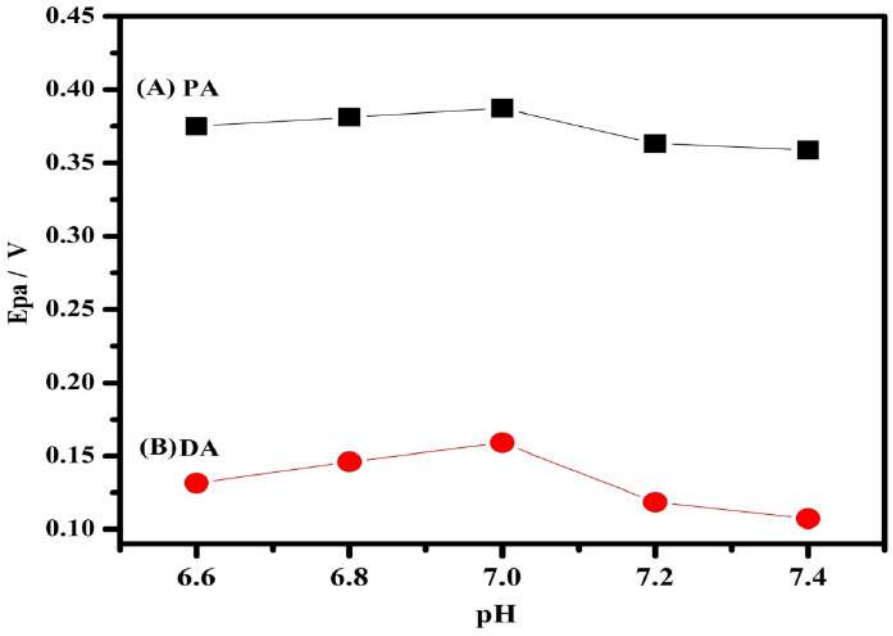


Fig. 7.7. Effect of pH on the peak potential for the oxidation of DA and PA. Concentrations: DA 0.1 mM, PA 0.1 mM in presence of phosphate buffer solution at pH 7.0

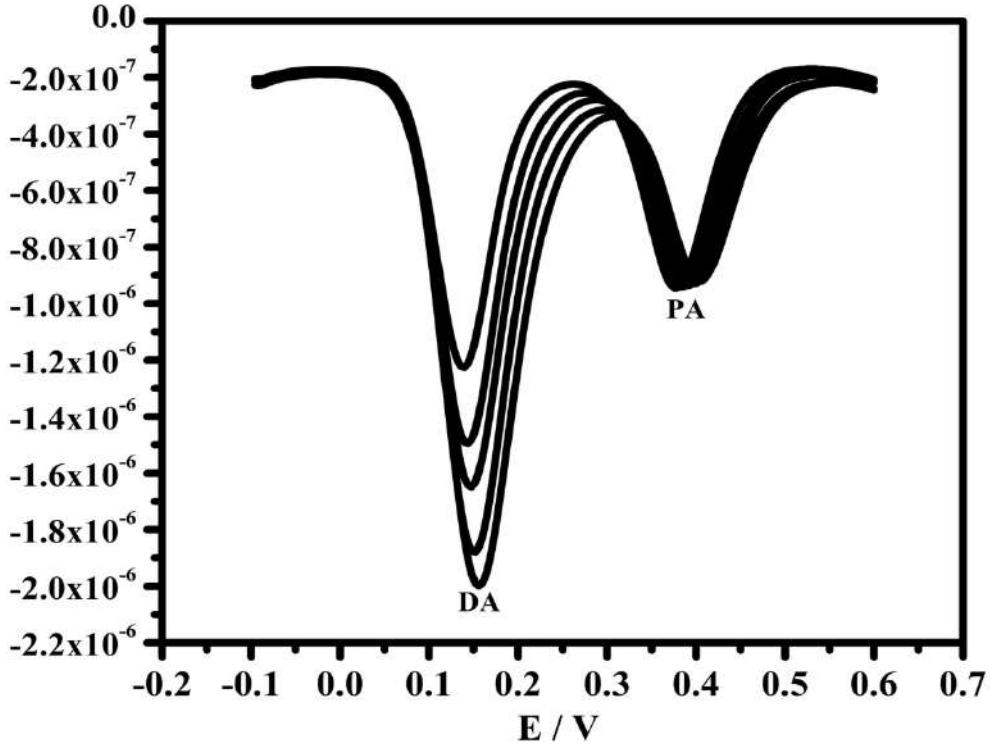


Fig. 7.8a. Differential pulse voltammograms of DA (0.2, 0.3, 0.4, 0.5 and 0.6 mM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.1 mM PA at PGBMGCE with the scan rate of 100 mV/s

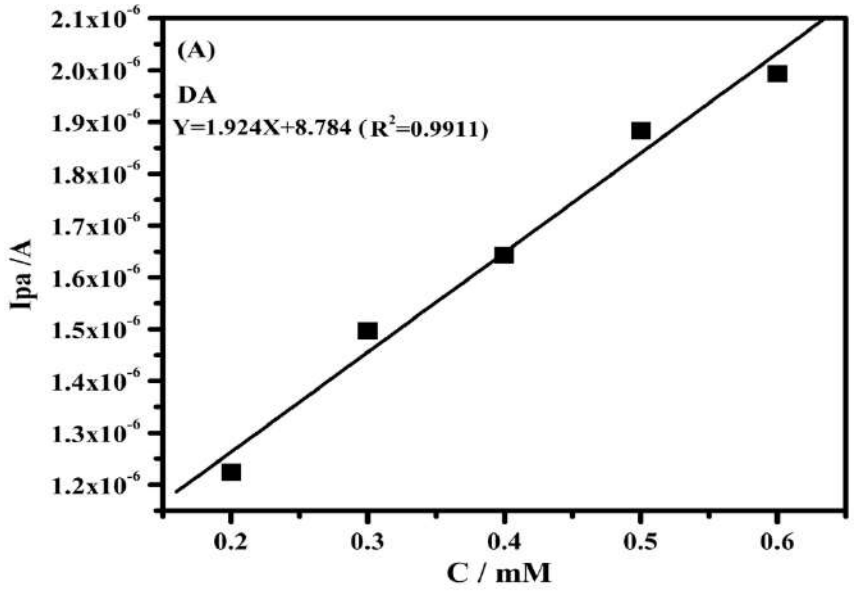


Fig. 7.8b. Plot of anodic peak current (Ipa) versus DA concentration

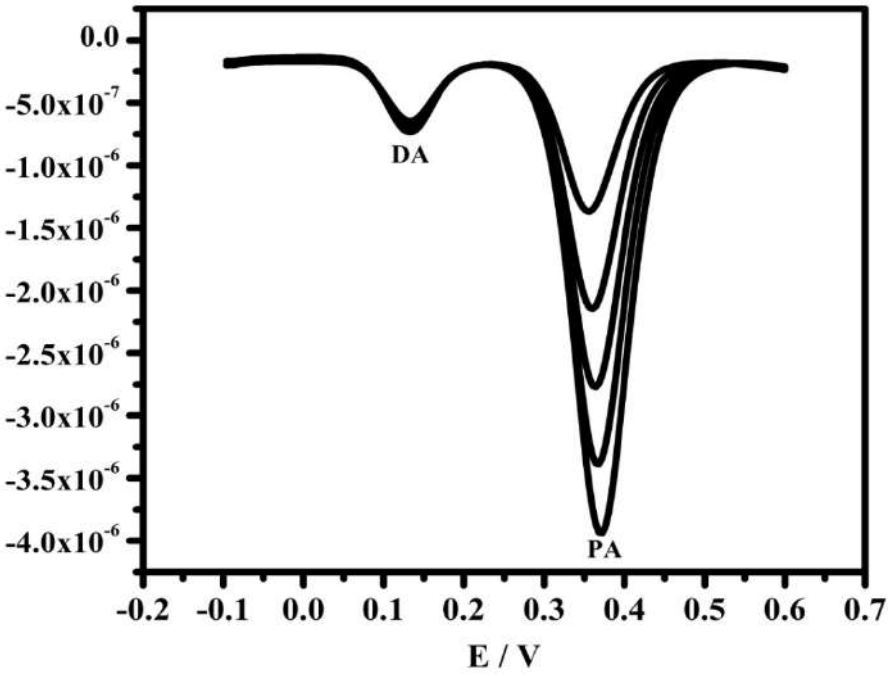


Fig. 7.9a. Differential pulse voltammograms of PA (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mM) in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 0.1 mM DA at PGBMGCE with the scan rate of 100 mV/s

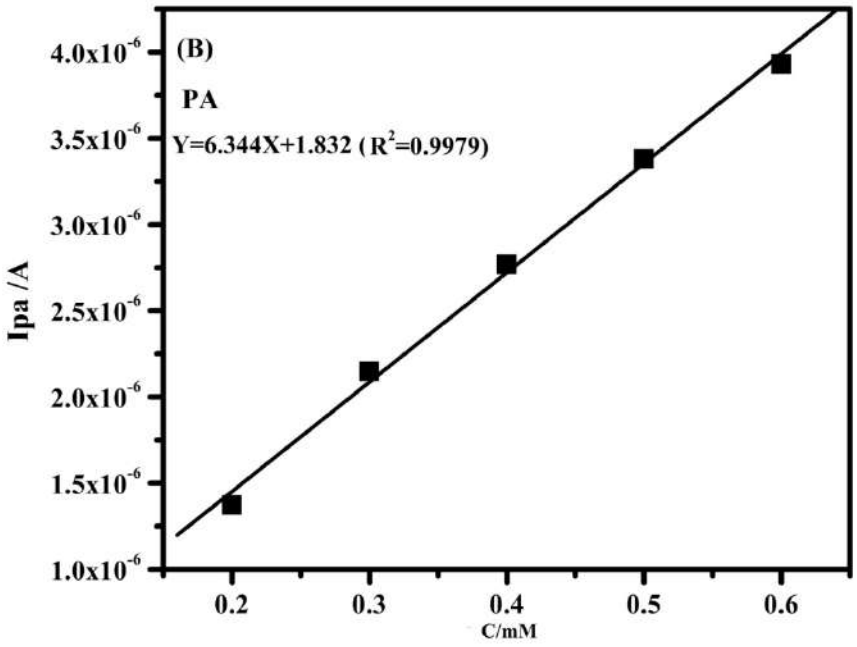


Fig. 7.9b. Plot of anodic peak current (Ipa) versus PA concentration

7.3.10. References

[1] R.M. Whiteman, L.J. May and A.C. Michael, *Anal. Chem.,* 60 (1988) 769.

[2] T.E. Smith and T.M. Devlin, "Textbook of Biochemistry with Clinical Corelations", Wiley Liss, NY (1992).

[3] J.R. Cooper, F.E. Bloom and R.H. Roth, "The Biochemical Basis of Neuropharmacology", Oxford University Press, Oxford, UK (1982).

[4] P. Damier, E.C. Hirsch, Y. Agid and A.M. Graybiel, 122 (1999) 1437.

[5] J.W. Mo and B. Ogorevc, *Anal. Chem.,* 73 (2001) 1196.

[6] S. Sarre, Y. Michotte, P. Herregodts, D. Deleu, N.D. Klippel and G. Ebinger. *J. Chromatogr*, 575 (2000) 207.

[7] L. Guan, Ouyang, Q.L. Li, B.H. Liu and W.R.G. Baeyens, *Talanta,* 50 (2000) 1197.

[8] F.B. Salem, *Talanta,* 34 (1987) 810.

[9] M. Pandurangachar, B.E.K. Swamy, B.N. Chandrashekar and B.S. Sherigara, *Int. J. Electrochem. Sci*., 4 (2009) 1319.

[10] A. Sathisha and B.E.K. Swamy, *Anal. Bioanal. Electrochem*., 8 (2016) 589.

[11] M.E. Bosch, A.J.R. Sanches, F.S. Rojas and C.B. Ojeda, *J. Pharm. Biomed. Anal*., 42 (2006) 291.

[12] R.M. De-Carvalho, R.S. Freire, S. Rath and L.T. Kubota, *J. Pharm. Biomed. Anal*., 34 (2004) 871.

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

[14] S.J.R. Prabakar and S.S. Narayanan, *Talanta,* 72 (2007) 1818.

[15] J. Forshed, F.O. Andersson and S.P. Jacobsson, *J. Pharm. Biomed. Anal.,* 29 (2002) 495.

[16] A. Yesilada, H. Erdogan and M. Ertan, *Anal. Lett*., 24 (1991) 129.

[17] F.A. Mohamed, M.A.A. Allah and S.M. Shammat, *Talanta,* 44 (1997) 61.

[18] M.S. Bloomfield, *Talanta,* 58 (2002) 1301.

[19] A. Marin, E. Garcia, A. Garcia and C. Barbas, *J. Pharm. Biomed. Anal*., 29 (2002) 701.

[20] L.A. Shervington and N.A. Sakhnini, *J. Pharm. Biomed. Anal*., 24 (2000) 43.

[21] F. Bohnenstengel, H.K. Kroemer and B. Sperker, *J. Chromotogr. B. Biomed. Sci. Appl*., 721 (1999) 295.

[22] S. Heitmer and G. Blaschke, *J. Chromotogr. B. Biomed. Sci. Appl.,* 721 (1999) 93.

[23] L. Suntornsuk, O. Pipitharome and P. Wilairat, *J. Pharm. Biomed. Anal.*, 33 (2003) 441.

[24] E. Valero, P. Carrion, R. Varon and F.G. Carmona, *Anal. Biochem*., 318 (2003) 187.

[25] D.M. Tassone, E. Boyce, J. Guyer and D. Nuzum, *Pregabalin: Clin, Ter*., 29 (2007) 48.

[26] C.P. Taylor, T. Angelotti and E. Fauman, *Epilepsy Res*., 73 (2007) 137.

[27] K. Hamandi and J.W. Sander, *Pregabalin Seizure,* 15 (2006) 78.

[28] S. Budawari, 13th Edition, Merck and Co. Inc., NJ (2003).

[29] O. Gilbert, B.E.K. Swamy, U. Chandra and B.S. Sherigara, *J. Electroanal. Chem.,* 636 (2009) 80.

[30] W. Suna, Y. Wang, Y. Lub, A. Hub, F. Shia and Z. Sun, *Sensors Actuators* B, 188 (2013) 564.

[31] R. Hosseinzadeh, R.E. Sabzi and K. Ghasemlu, *Colloids Surf*., B68 (2009) 217.

[32] J. Tashkhourian, M.R.H. Nezhad, J. Khodavesi and S. Javadi, *J. Electroanal. Chem.*, 633 (2009) 91.

[33] H.R. Zare, N. Nsirizadeh and M.M. Ardakani, *J. Electroanal. Chem*., 577 (2005) 33.

[34] J. Zheng and X. Zhou, *Bioelectrochem.*, 70 (2007) 415.

[35] Z. Nasri and E. Shams, *Electrochim. Acta*, 54 (2009) 7421.

[36] S. Shahrokhian and H.R.Z. Mehrjardi, *Electrochim. Acta*, 52 (2007) 6317.

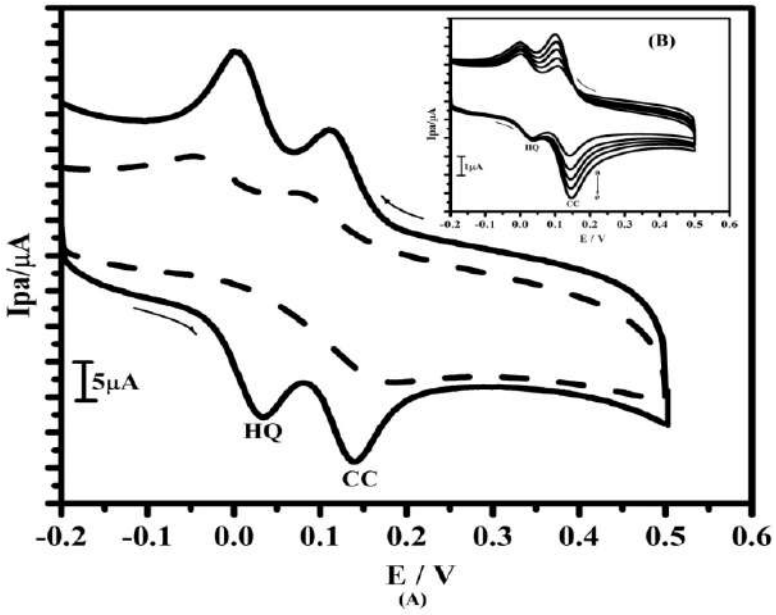
[37] S. Shahrokhian and H.R.Z. Mehrjardi, *Sens. Actuators*, B121 (2007) 537.

[38] T. Thomas, R.J. Mascarenhas, C. Nethravathi, M. Rajamathi and B.E.K. Swamy, *J. Electroanal. Chem*., 659 (2011) 119.

CHAPTER-7

Part-B

Simultaneous Determination of Catechol and Hydroquinone at Poly (Sunset yellow) Modified Glassy Carbon Electrode: A Voltammetric Study



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7.4. Introduction

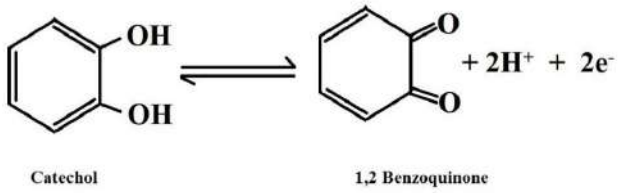
An electrochemical sensor for the sensitive determination of catechol has been developed by poly (sunset yellow) modified glassy carbon electrode. The electrochemical oxidation of catechol was studied by using cyclic voltammetric and differential pulse voltammetric techniques. The experimental results show that the poly (sunset yellow) modified glassy carbon electrode shows high electrochemical process towards the oxidation of catechol and hydroquinone. The lower detection limits were found to be 1.45×10^{-6} M and 2.6×10^{-7} M respectively. The peak to peak separation of CC and HQ was more than enough to identify individually by CV technique. This work provides a simple and easy approach for the simultaneous analysis of CC and HQ.

7.5. Chemistry and Biological Relevance of Catechol

Catechol is also known as pyrocatechol or 1,2-dihydroxybenzene it is a simple organic compound with the molecular formula $C_6H_4(OH)_2$. It is the ortho isomer of the three isomeric benzenediols [1]. This colorless compound occurs naturally in trace amounts. It was first discovered in 1839 by Edgar Hugo Emil Reinsch by destructive distillation of the plant extract catechin. Trace amounts of catechol naturally occurs in vegetable and fruits, along with the enzyme polyphenol oxidase (also known as catecholase, or catechol oxidase). Upon mixing the enzyme with the substrate and exposure to oxygen (as when a potato or apple is cut and left out) the colorless catechol oxidizes to reddish-brown melanoid pigments, derivatives of benzoquinone. The enzyme is inactivated by adding an acid, such as lemon juice. Benzoquinone is considered as an antimicrobial agent, which slows the spoilage of wounded fruits and other plant parts. It is one of the main natural phenols in argan oil and also found in Agaricus bisporus [2]. The oxidation mechanism of catechol was shown in Scheme 7.3.

Catechol moieties are widely spread in the nature. Arthropod cuticle consists of chitin linked by a catechol moiety to protein. The cuticle may be strengthened by cross-linking (tanning and sclerotization), in particular, in insects, and of course by biomineralization [3]. Catechol's such as DHSA (3,4-Dihydroxy-9, 10-secoandrosta-

1,3,5(10)-triene-9,17-dione) are produced through the metabolism of cholesterol by bacteria such as Mycobacterium tuberculosis [4]. Urushiols are naturally existing organic compounds that have the catechol skeleton structure and diphenol functionality but with alkyl groups substituted onto the aromatic ring. Urushiols are the skin-irritating poisons found in plants. Catecholamines such as, dopamine, norepinephrine and epinephrine are significant hormones/neurotransmitters that are phenyl amines in which the phenyl group has a catechol skeleton structure. About 20 million kg of catechol are now synthetically produced annually as a commodity organic chemical, mainly as a precursor to pesticides, flavors and fragrances.



Scheme 7.3. Oxidation mechanism of catechol

7.6. Chemistry and Biological Relevance of Hydroquinone

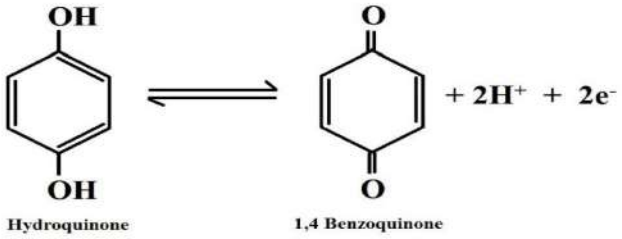
Hydroquinone is an aromatic organic compound also known as benzene-1, 4-diol. It is one of the derivative of benzene with the molecular formula $C_6H_4(OH)_2$. The two hydroxyl groups are bonded to a benzene ring in a para position. It is a white granular solid readily soluble in water. The name hydroquinone was coined by Friedrich Wohler in 1843 [5].

The reversible electrochemical oxidation of hydroquinone under mild condition gives 1, 4-benzoquinone as shown in the Scheme 7.4. Some of the naturally occurring hydroquinone derivatives exhibit this sort of reactivity, one example being coenzyme Q (ubiquinone). Industrially this reaction is exploited both with hydroquinone itself but more often with its derivatives where one OH has been replaced by an amine.

Hydroquinone has a variety of uses principally associated with its action as a reducing agent. There are various other uses associated with its reducing power. As a

polymerization inhibitor, hydroquinone prevents polymerisation of acrylic acid, methyl methacrylate, cyanoacrylate and other monomers that are susceptible to radical initiated polymerisation. This application exploits the antioxidant properties of hydroquinone.

In human medicine, hydroquinone is used as a topical application in skin whitening to reduce the color of skin. It does not have the same predisposition to cause dermatitis as metal does. This is a prescription only ingredient in some countries, including the member states of the European Union. In 2006, the United States food and drug administrative revoked its previous approval of hydroquinone and proposed a ban on all over the counter preparations. The FDA stated that hydroquinone cannot be ruled out as a potential carcinogen. This conclusion was reached based on the extent of absorption in humans and the incidence of neoplasms in rats in several studies where adult rats were found to have increased rates of tumors, including thyroid follicular cell hyperplasia's, anisokaryosis (variation in nuclei sizes), mononuclear cell leukaemia, hepatocellular adenomas and renal tubule cell adenomas. The campaign for safe cosmetics has also highlighted concerns.



Scheme 7.4. Oxidation mechanism of hydroquinone

Numerous studies have revealed that hydroquinone can cause exogenous ochronosis, a disfiguring disease in which blue-black pigments are deposited onto the skin, if taken orally; however, skin preparations containing the ingredient are administered topically. The FDA has classified hydroquinone currently as a safe product, as currently used [6].

While using hydroquinone as a lightening agent can be effective with proper use, it can also cause skin sensitivity. Using a daily sunscreen with a high PPD (persistent

pigment darkening) rating reduces the risk of further damage. Hydroquinone is sometimes combined with alpha hydroxy acids that exfoliate the skin to quicken the lightening process. In the United States, topical treatments usually contain up to 2% in hydroquinone. Otherwise, higher concentrations (up to 4%) should be prescribed and used with caution. Hydroquinone's are one of the two primary reagents in the defensive glands of bombardier beetles, along with hydrogen peroxide (and perhaps other compounds, depending on the species), which collect in a reservoir. The reservoir opens through a muscle-controlled valve onto a thick-walled reaction chamber. This chamber is lined with cells that secrete catalases and peroxidases. When the contents of the reservoir are forced into the reaction chamber, the catalases and peroxidases rapidly break down the hydrogen peroxide and catalyze the oxidation of the hydroquinone into p-quinone. These reactions release free oxygen and generate enough heat to bring the mixture to the boiling point and vaporize about a fifth of it, producing a hot spray from the beetle's abdomen [7].

Farnesyl hydroquinone derivatives are the principal irritants exuded by the poodle-dog bush, which can cause severe contact dermatitis in humans. Hydroquinone is thought to be the active toxin in Agaricus hondensis mushrooms [8]. Hydroquinone has been shown to be one of the chemical constituents of the natural product propolis [9]. It is also one of the chemical compounds found in castoreum. This compound is gathered from the beaver's castor sacs.

7.7. Review of Electrochemistry of Catechol and Hydroquinone

Hydroquinone (HQ, 1,4-dihydroxybenzene) and catechol (CC, 1,2-dihydroxybenzene) are the two positional isomers of a dihydroxybenzene [10, 11]. They widely exist in industrial effluents, such as the waste from oil refineries, coal tar, cosmetics, plastic, leather, paint, steel and pharmaceutical industries [12–14]. Even in a very low concentration itself these isomers are toxic to animals and human beings and they are difficult to degrade. Because of these factors they are one of the main sources for the environment pollution [15, 16]. The determination of these phenolic compounds is of

great importance in environmental control [17]. Furthermore, because of the similar structure and properties both HQ and CC coexist in the ecosystem. Therefore, it is very important to develop simple and rapid analytical methods for the qualitative and the quantitative estimation of these dihydroxybenzene isomers [18]. So far various methods have been reported for their determination, high performance liquid chromatography (HPLC) [19, 20], spectrophotometry [21], electrochemiluminescence [22], pH based flow injection analysis [23] and synchronous fluorescence [24]. All these methods are generally little bit complicated and tedious.

Simultaneous determination of hydroquinone and catechol levels is of great necessary because of their coexistence in environmental samples as environmental pollutants with high toxicity [25]. The established methods for the determination of catechol and hydroquinone are commonly performed after previous separation [26]. Disadvantages of the separation are operating complexity, time waste and reagent consuming. Thus, it is favorably necessary to develop a new method with possibility of the simultaneous determination without previous separations of these compounds. Both hydroquinone and catechol have a basic quinone structures that may be electrochemically oxidized at a platinum or carbon electrodes [27]. A simultaneous investigation of electrochemical behavior of hydroquinone and catechol was suggested at a poly (sunset yellow) modified electrodes with 105 mV of potential difference between the oxidation peak of hydroquinone and the oxidation peak of catechol [28]. Carvalho *et al*. [29] reported an electrochemical method for simultaneous determination of phenol isomers in binary mixtures by differential pulse voltammetry using carbon fiber electrode and neural network with pruning as a multivariate calibration tool. However, the calibration process of this method was tedious. To our best knowledge, the simultaneous determination of hydroquinone and catechol at sunset yellow modified electrode has not been reported.

Food dyes are the most interesting group of food additives. Frequently color of a product determinates its attractiveness for consumer [30]. Materials of natural origin have been used to provide color in foods, drugs and cosmetics for the thousands of years. Ash from fires, mineral compounds and plants were probably among the first materials used

for cosmetic purposes. Later, it was discovered that certain materials, mostly plant derived, could be used to enhance the appearance of foods and thus turmeric, paprika and saffron were used for more than just their flavoring properties [31]. However natural dyes are unstable and easily undergo degradation during the food processing. In the nineteenth century, synthetic organic dyes were developed to provide a more economical and extensive array of colorants. Synthetic dyes show several advantages compared with natural dyes such as high stability to light, oxygen and pH, color uniformity, low microbiological contamination and relatively lower production costs. However, many of them may exhibit adverse health effects (allergy, respiratory problems, thyroid tumours, chromosomal damage, urticaria, hyperactivity, abdominal pain, etc.) [32].

Red (E-129), sunset yellow (E-110), and tartrazine (E-102) are three highly used synthetic dyes which are added to many food products [33]. The color additive sunset yellow (SY) (Scheme 7.5) is principally the disodium salt of 1-4-sulphophenyl azo -2 naphthol-6 sulphonic acid. So SY belongs to “azo” family. Azo compounds are formed from arene diazoniumions reacting with highly reactive aromatic compounds, in what is called a diazo coupling reaction. Azo compounds are generally deeply colored because the azo linkage brings the two aromatic rings into conjugation. In addition to possessing extended conjugation, many azo dyes are also ring substituted with sulfonic acid substituents, which significantly increase polarity and water solubility and decrease absorption *in vivo*. Sunset Yellow, subject of this work, is a synthetic dye available as yellow powder that can be present in common foods (drinks, yoghurts, ice cream, sweets, etc.). The presence and content of this dye must be controlled because it is not totally innocuous.

The present study mainly reports the electropolymerization of sunset yellow on the bare glassy carbon electrode and its use for the simultaneous determination of catechol and hydroquinone. This modified glassy carbon electrode shows very good enhancement when compared to bare carbon paste electrode. This work reports about sensitivity, selectivity, stability and reproducibility of CC and HQ at poly (sunset yellow) film coated glassy carbon electrode.

7.8. Experimental Section

7.8.1. Reagents

Hydroquinone (HQ), Catechol (CC) and sunset yellow (SY)-were purchased from Himedia. The stock solution 25 × 10^{-4} M HQ and 25 × 10^{-4} M CC was prepared in double distilled water. Phosphate buffer solution (PBS) of same ionic strength was prepared (0.2 M) by mixing appropriate ratio of $NaH_2PO_4 \cdot H_2O$ and Na_2HPO_4. All the chemicals mentioned were of analytical grade and used as received without any further purification.

7.8.2. Apparatus

The electrochemical experiments were carried out using a model CHI-660c (CH Instrument-660 electrochemical workstation). A traditional three electrode system was employed in an electrochemical cell with a saturated calomel electrode (SCE) as a reference, a platinum counter electrode, and a bare or poly (sunset yellow) modified glassy carbon electrode (MGCE) as working electrode. The corresponding oxidation potential of analytes was recorded versus SCE.

7.9. Results and Discussion

7.9.1. Electrochemical Polymerization of Sunset Yellow on GCE

Cyclic voltammetry is a simple and convenient method to immobilize an organic dye on the surface of GCE. The poly (sunset yellow)- modified glassy carbon electrode (MGCE) was prepared by placing 0.2 mM sunset yellow with 0.1 M NaOH in an electrochemical cell. The potential window was maintained from −0.5 to 1.5 V with scan rate 100 mV/s for 20 multiple cycles. During the process of multiple cycles, the voltammogram has gradually descended with increase of cyclic times as shown in Figure 7.10. This indicates that the sunset yellow film was formed and deposited on the surface of BGCE [34–39]. The structure of sunset yellow was shown in Scheme 7.5.

7.9.2. Effect of Multiple Cycles in the Preparation of Poly (Sunset Yellow) MGCE

From the experimental results, the amount of coated polymer film affects the electrocatalyic property of that electrode. The coating was controlled by applying multiple cycles. The GCE was modified by applying different multiple cycles (from 5 to 25 multiple cycles) and the corresponding electrocatalytic activity towards oxidation of 0.2 mM catechol (CC) in phosphate buffer solution (PBS) of pH 7.4 were examined. From Figure 7.11, it can be noticed that the maximum current enhancement towards detection of CC occurred at 20 cycles. Therefore, twenty cycles were chosen as optimum for the electropolymerization of sunset yellow. The mechanism for the electrochemical processes of sunset yellow was shown in Scheme 7.6.

7.9.3. Electrochemical Response of CC at Poly (Sunset Yellow) MGCE

Figure 7.12 shows the cyclic voltammograms (CV) of catechol at the scan rate of 100 mV/s in phosphate buffer solution (PBS) of pH 7.4 at bare GC electrode (dashed line) and at poly (sunset yellow) MGCE (solid line), blank solution (dotted line) respectively. At bare GC electrode, the anodic peak potential and the cathodic peak potential of catechol were 205 mV and 65 mV, respectively. And ΔEp of catechol at bare GC electrode was 140 mV. At poly (sunset yellow) MGCE, the oxidation peak current of catechol greatly increased and the anodic peak and the cathodic peak appeared at 134 mV and 107 mV, respectively. ΔEp of catechol at poly (sunset yellow) MGCE was 27 mV. This result indicates that the oxidation peak current of catechol at the poly (sunset yellow) MGCE is significantly enhanced and the over potential of catechol at the sunset yellow is much lowered. The mechanism of oxidation of both CC and HQ was shown in Scheme 7.7.

7.9.4. Effect of Scan Rate on the Peak Current of CC

The effect of scan rate on 0.2 mM solution of catechol was studied at poly (sunset yellow) MGCE by using pH-7.4 of PBS as a supporting electrolyte with CV technique. Figure 7.13a gives the information about the effect of scan rate on the peak currents and

peak potentials. The effect of scan rate on peak currents of catechol at different scan rates. The peak current gradually increases with the increase in scan rates. In order to confirm the electrode process, the graph of peak current (Ipa) versus scan rate (υ) was plotted and the obtained graph is a straight line with good linearity in the range from 50 to 100 mV/s as shown in Figure 7.13b with the correlation coefficient (r^2) =0.9997. The Ipa versus square root scan rate ($\upsilon^{1/2}$) was plotted as shown in Figure 7.13c with the correlation coefficient (r^2) = 0.9997. This result suggests that the electrode process is adsorption-controlled [40-41].

7.9.5. Effect of CC Concentration

The electrocatalytic oxidation of CC was carried out by varying its concentration at poly (sunset yellow) MGCE. Figure 7.14a shows by increasing the concentration of CC from 0.1 to 0.6 mM, the Ipa and Ipc goes on increasing with shifting Epa towards less positive and Epc towards least negative side. The inset graph of Ipa versus concentration of CC was plotted and it shows almost straight line with good linearity as shown in Figure 7.14b. The linear regression equation Ipa (10^{-4}A) = 0.7048 (C_0 10^{-4}M/L) + 1.736, (r^2 = 0.9989). The detection limit in the lower concentration range for CC was 1.45×10^{-6} M for the poly (sunset yellow) MGCE and limit of quantification was 4.8X 10^{-6}M was calculated [42-44]. The proposed electrode exhibited a relatively lower detection limit than those reported as shown in Table 7.2.

7.9.6. Electrocatalytic Oxidation of HQ at Poly (Sunset Yellow) MGCE

The electrochemical response of hydroquinone (HQ) was studied at poly (sunset yellow) modified glassy carbon electrode. The Figure 7.15 shows the cyclic voltammograms of 0.2 mM HQ at BGCE (dotted line) and poly (sunset yellow) MGCE (solid line) in 0.2 M PBS at pH 7.4 with the scan rate of 100 mVs^{-1}. Comparatively poly (sunset yellow) MGCE shows great enhancement in the anodic peak current (Ipa) than BGCE. Thus remarkable improvement in electrochemical sensitivity towards HQ at poly (sunset yellow) MCPE gives an evidence for the catalytic effect of proposed electrode.

7.9.7. Effect of Scan Rate on the Peak Current of HQ

The effect of variation of applied scan rate for 0.2 mM hydroquinone in 0.2 M PBS of pH 7.4 was examined by CV technique at poly (sunset yellow) MGCE as shown in Figure 7.16a. The experimental results obtained at poly (sunset yellow) MGCE showed increase in the redox peak currents with increase in the applied scan rate and they are proportional to each other according to Randles–Sevcik equation. The observation shows that there is a shifting of anodic peak potential (Epa) to more positive side and cathodic peak potential (Epc) to the less negative side. The plot of anodic peak current (Ipa) versus scan rate (ν) shows good linearity with correlation coefficient value R^2=0.9990 as shown in Figure 7.16b. Along with the plot of anodic peak current (Ipa) versus square root of scan rate ($ν^{1/2}$) was also studied and correlation coefficient value was found to be R^2=0.9990 as shown in Figure 7.16c. This suggests the electrode process was controlled by adsorption.

7.9.8. Effect of HQ Concentration

The electrocatalytic oxidation of HQ was carried out by varying its concentration poly (sunset yellow) MGCE as shown in Figure 7.17a By increasing the concentration of HQ from 0.1mM to 0.6mM, the Ipa and Ipc was found to be increasing with shifting of Epa towards positive potential and Epc towards slightly negative potential. The inset graph of Ipa versus concentration of HQ was plotted and it shows almost straight line with good linearity as shown in Figure 7.17b. The linear regression equation Ipa (10^{-4}A) = 0.7296 (C_0 10^{-4}M/L) + 1.356, (r^2 = 0.9988). The limit of detection (LOD) was calculated by using the formula (1) [45-47] and it was found to be 2.6×10^{-7}M and the limit of quantification (LOQ) was calculated by using the formula (2) and it was found to be 8.0×10^{-7}M

LOD=3 S/M …………………………………… (1)

LOQ=10 S/M ……………………………………(2)

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

7.9.9. Simultaneous Determination of CC and HQ

Figure 7.18a shows the cyclic voltammogram of catechol in the presence of hydroquinone at bare GC electrode and at poly (sunset yellow) MGCE at a scan rate of 100 mV/s in 0.2 M phosphate buffer solution (pH 7.4), respectively. It can be seen that the oxidation peak of hydroquinone and the oxidation peak of catechol merged into a large peak of 173 mV at bare GC electrode. Hydroquinone and catechol at poly (sunset yellow) MGCE yielded two well-defined oxidation peaks at poly (sunset yellow) MGCE, whose potentials were 138 mV and 33 mV, respectively. Meanwhile, the oxidation peak currents also remarkably increased at poly (sunset yellow) MGCE. The peak to peak separation was 105 mV and this result was sufficient for the simultaneous measurement of CC and HQ in a mixture.

The cyclic voltammograms obtained for the mixture of samples in an electrochemical cell containing CC and HQ at poly (sunset yellow) MGCE. The concentration of one species was changed and the other are kept constant. The results in Figure 7.18b show that the peak current of CC was proportional to its concentration, which was increased from 0.1 to 0.5 mM respectively. There are no changes in the peak current and peak potential for HQ. These result show that the peaks remain well separated and CC and HQ can be simultaneously detected in mixtures at poly (sunset yellow) MGCE.

7.9.10. Interference Investigation

The interference investigation was performed in the mixture of samples containing both CC and HQ at the poly (sunset yellow) MGCE when concentration of one species changed where as the other remained kept constant. From Figure 7.19a it can be seen that the peak current of CC was proportional to its concentration which was increased from 0.1 to 0.5 mM when keeping the concentration of HQ 0.1 mM. There were no change in the peak current and peak potential occurred for HQ. The Figure 7.20a self explains the concentration effect of HQ from 0.1 to 0.5 mM respectively. These results show that the CC and HQ were exist independently in their mixtures of samples.

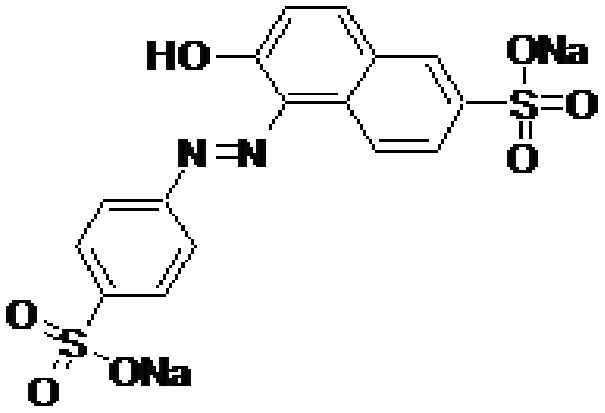
The corresponding graphs of anodic peak current versus various concentrations of CC (0.1-0.5 mM), HQ (0.1-0.5 mM) showed linear relationships with linear regressions for (A) (CC) Ipa (μA) =4.9563C(mM/L) +5.8338, (B) (HQ) Ipa (μA) =3.4932C(mM/L) +5.1232, the correlation coefficient for these linear graphs was 0.9960 and 0.9983 respectively for this poly (sunset yellow) MGCE which were shown in Figures 7.19b,7.20b, respectively.

7.9.11. Conclusion

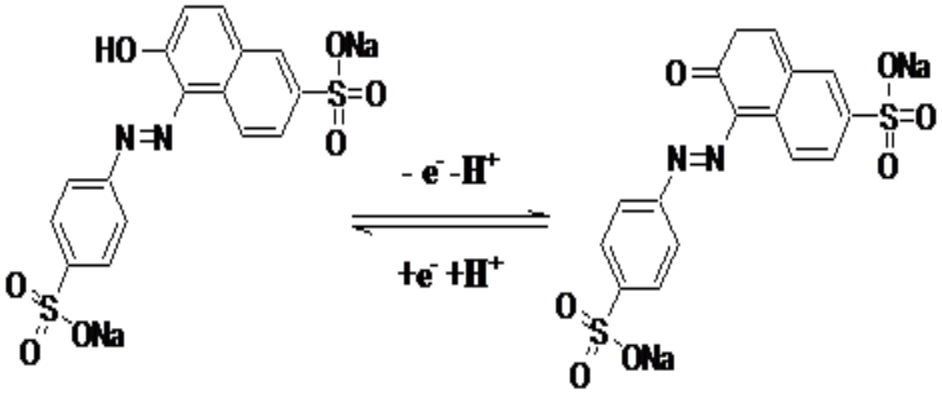
In the present study, a novel method of constructing poly sunset yellow modified GCE for the determination of both CC and HQ was developed. The modified electrode shows the significant increment in the oxidation of CC and HQ individually and simultaneously. The peak to peak separation was 105 mV by both CV and DPV techniques. Together with low cost and ease preparation, this film modified electrode seems to be of good utility for further sensor development. Finally, the applicability of this modified electrode was used for the determination of both CC and HQ in pharmaceutical and also in clinical samples by the standard addition method.

Table 7.2

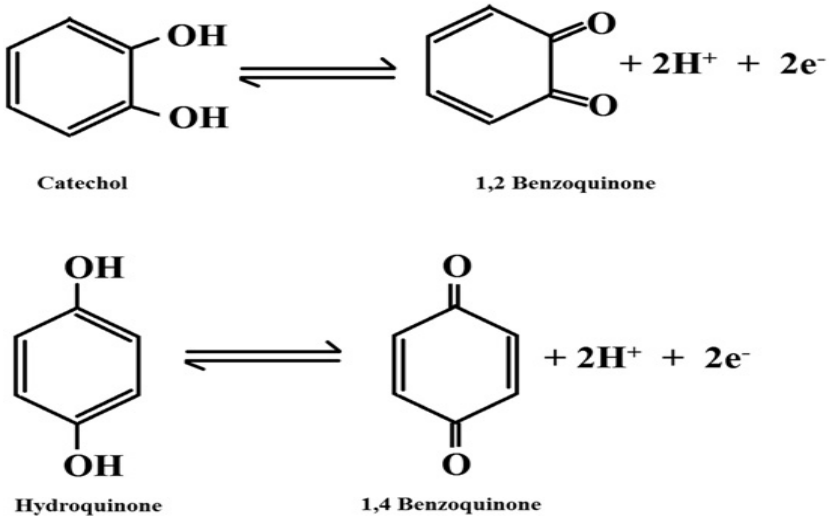
Working electrode	Limit of detection (μM)		Method	Reference
	HQ	CC		
SPC electrode	0.05	0.05	SWV	[48]
Boron-doped graphene	0.3	0.2	DPV	[49]
LRG/GCE	0.5	0.8	DPV	[50]
Poly(phenylalanine)	1	0.7	DPV	[51]
MWNT/GCE	0.75	0.20	DPV	[52]
PANI/MnO2-GCE	0.12	0.15	DPV	[53]
(LDHf/GCE)	9	1.2	DPV	[54]
Poly (sunset yellow) /GCE	**0.26**	**1.45**	**CV**	**[This work]**



Scheme 7.5



Scheme 7.6



Scheme 7.7

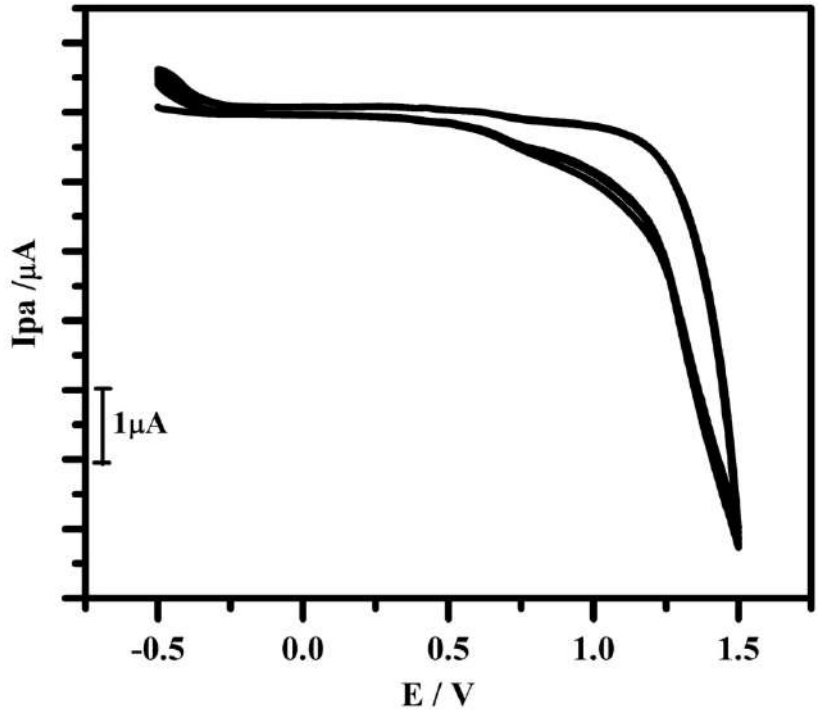


Fig. 7.10. Cyclic voltammograms of the preparation of poly (sunset yellow) MGCE. 0.2 mM aqueous solution in 0.1 M NaOH at 20 cycles with scan rate of 100 mVs^{-1}

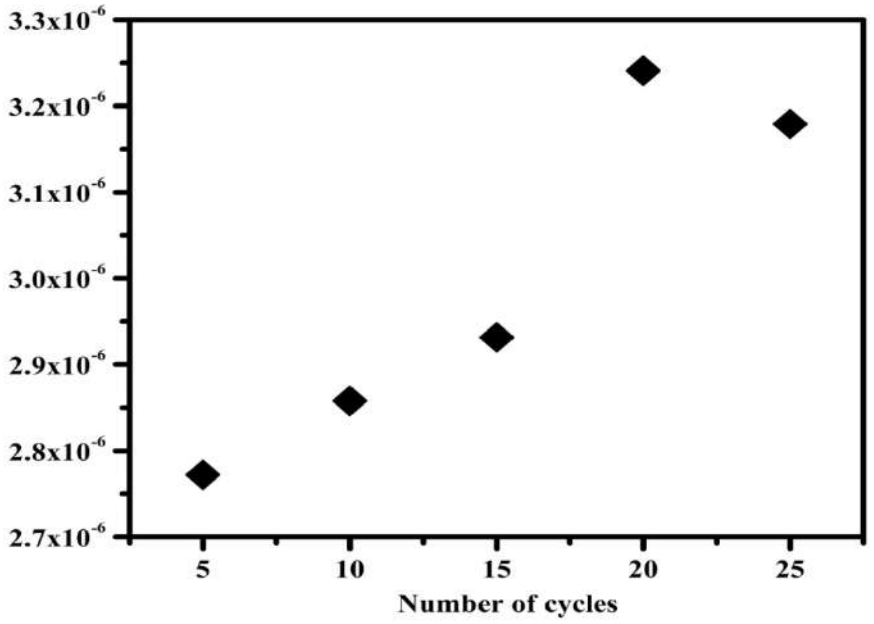


Fig. 7.11. Graph of anodic peak current of oxidation of 0.2 mM CC in 0.2 M PBS of pH 7.4 versus number of polymerization cycles

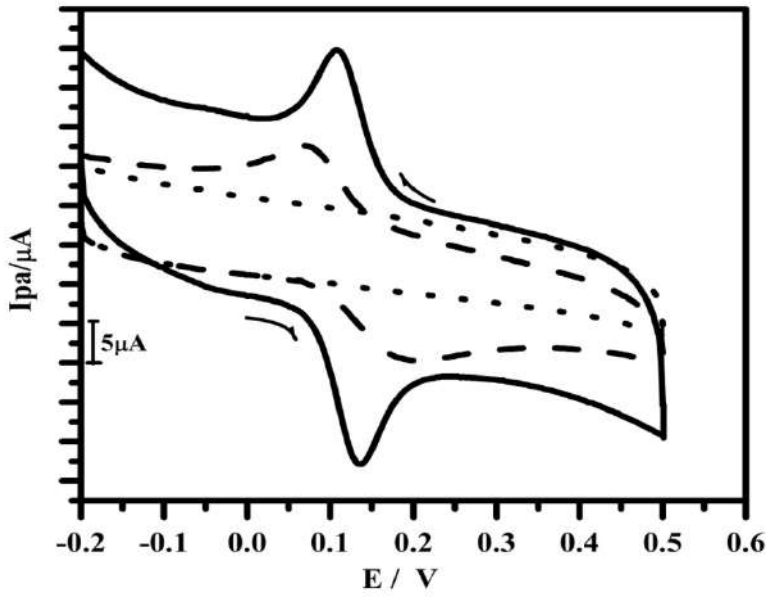


Fig. 7.12. Cyclic voltammograms of 0.2 mM CC in 0.2M PBS solution of pH 7.4 at BGCE (dashed line), poly (sunset yellow) MGCE (solid line) and in the absence of CC at bare GCE (dotted line) at scan rate of 100 mVs^{-1}

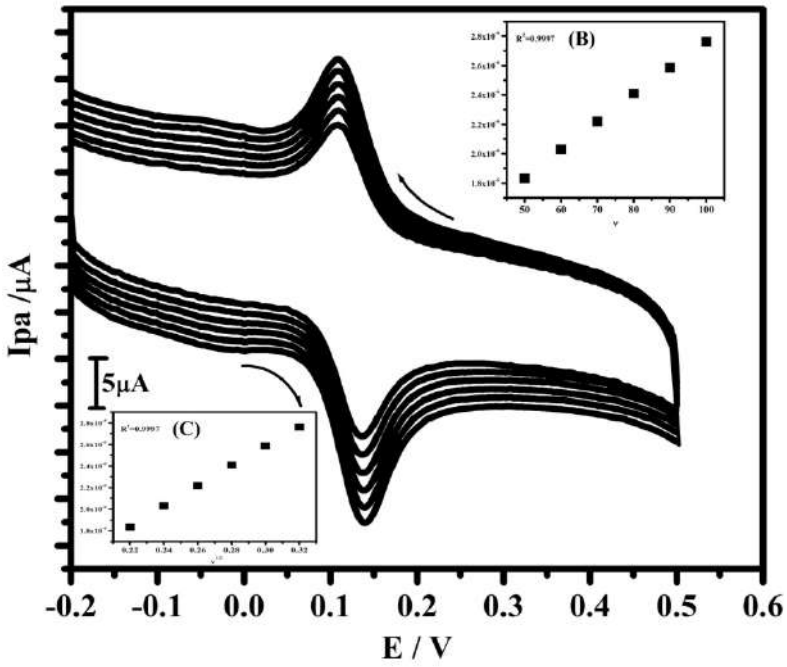


Fig. 7.13a. Cyclic voltammograms of 0.2 mM CC in 0.2 M PBS solution of pH 7.4 at poly (sunset yellow) MGCE at different scan rates (50 to 100 mVs^{-1}). (b) Graph of peak current versus scan rate. (c) Graph of peak current versus square root of scan rate

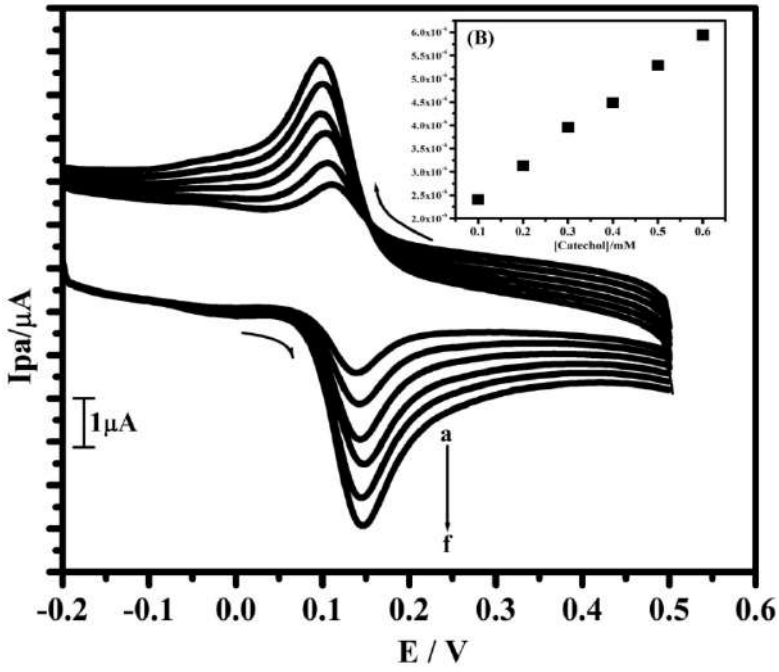


Fig. 7.14a. Cyclic voltammograms of CC in 0.2 M PBS solution of pH 7.4 at poly (sunset yellow) MGCE at scan rate of 100 mVs^{-1} with different concentrations (a–f: 0.1-0.6 mM). (b) Graph of anodic peak current versus concentration.

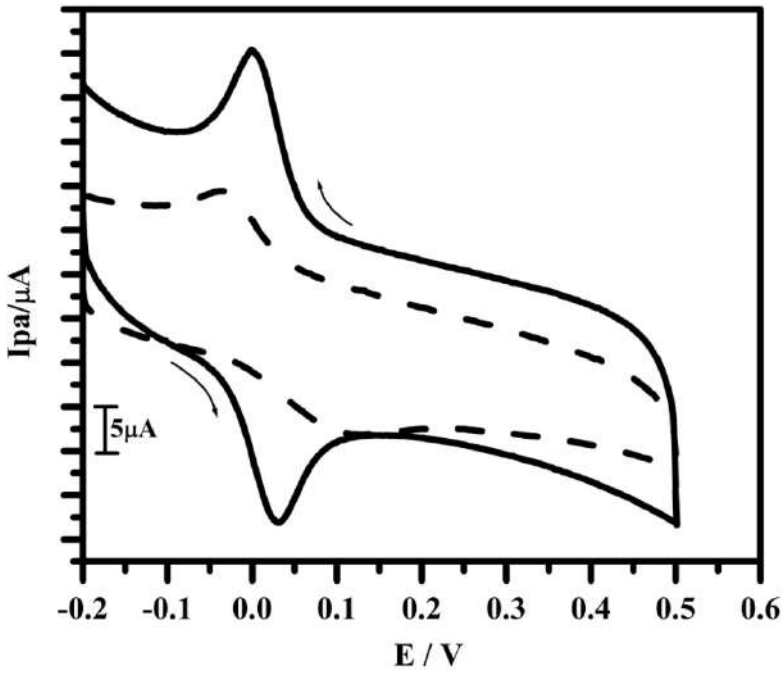


Fig. 7.15. Cyclic voltammograms of 0.2 mM HQ in 0.2 M PBS solution of pH 7.4 at BGCE (dashed line) and poly (sunset yellow) MGCE (solid line) at scan rate of 100 mVs^{-1}

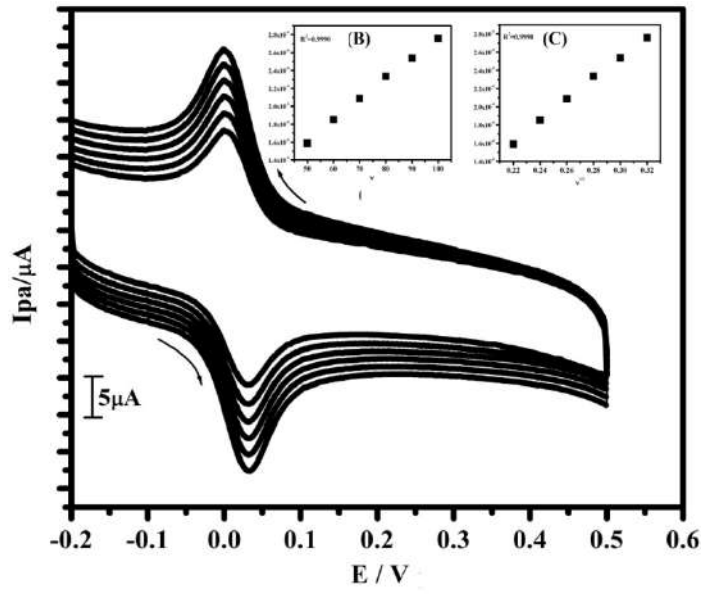


Fig. 7.16a. Cyclic voltammograms of 0.2 mM HQ in 0.2 M PBS solution of pH 7.4 at poly (sunset yellow) MGCE at different scan rates (50 to 100 mVs^{-1}). (b) Graph of peak current versus scan rate. (c) Graph of peak current versus square root of scan rate

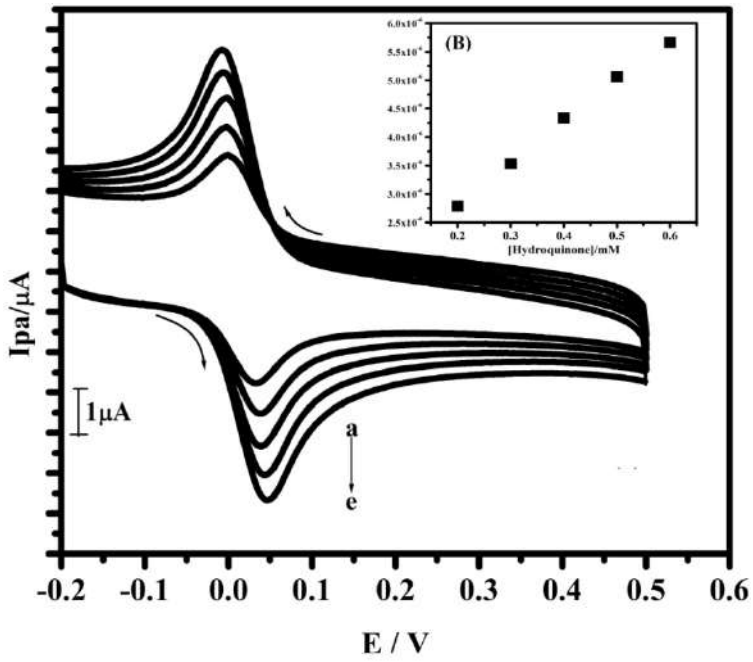


Fig. 7.17a. Cyclic voltammograms of HQ in 0.2 M PBS solution of pH 7.4 at poly (sunset yellow) MGCE at scan rate of 100 mVs^{-1} with different concentrations (a–e: 0.1-0.5 mM). (b) Graph of anodic peak current versus concentration.

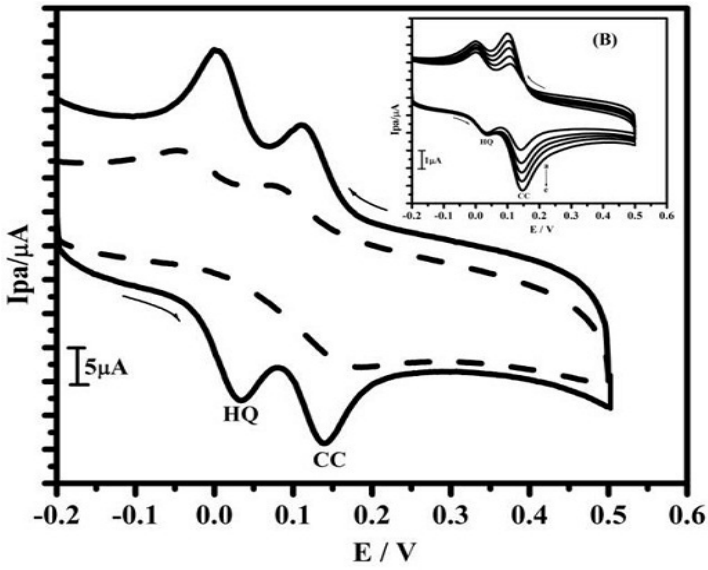


Fig. 7.18a. Cyclic voltammograms for simultaneous determination of 0.1mM CC, 0.1mM HQ at BGCE (dashed line) and poly (sunset yellow) MGCE (solid line) at scan rate of 100 mVs^{-1}. (b) Cyclic voltammograms of CC (a–e: 0.1, 0.2, 0.3, 0.4 and 0.5 mM) in 0.2 M PBS of pH 7.4 in the presence of 0.1 mM HQ at poly (sunset yellow) MGCE with the scan rate of 100 mVs^{-1}

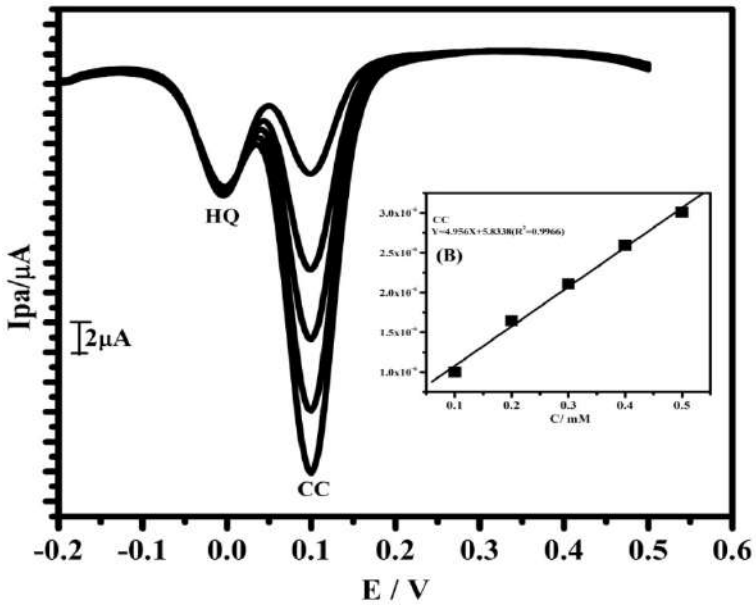


Fig. 7.19a. Differential pulse voltammograms of CC (0.1, 0.2, 0.3, 0.4 and 0.5 mM) in 0.2 M PBS of pH 7.4 in the presence of 0.1 mM HQ at poly (sunset yellow) MGCE with the scan rate of 100 mVs^{-1}. (b) The plot shows anodic peak current (Ipa) versus CC concentration

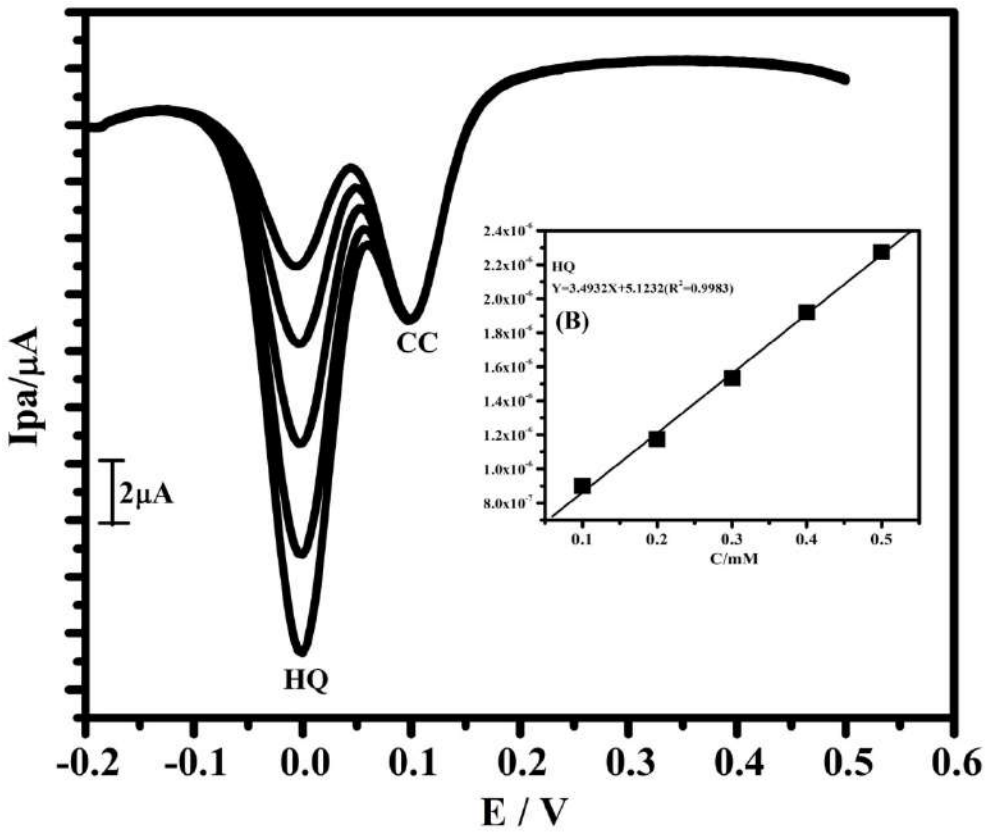


Fig. 7.20a. Differential pulse voltammograms of HQ (0.1, 0.2, 0.3, 0.4 and 0.5 mM) in 0.2 M PBS of pH 7.4 in the presence of 0.1 mM CC at poly (sunset yellow) MGCE with the scan rate of 100 mVs^{-1}. (b)The plot shows anodic peak current (Ipa) versus HQ concentration

7.9.12. References

[1] Z. Charrouf and D. Guillaume, *Am. J. Food Tech.,* 2 (2007) 679.

[2] A. Delsignore, F. Romeo and M. Giaccio, *Mycol. Res*., 101 (1997) 552.

[3] D.E.G. Briggs, *Phil. Trans. R Soc*. *B*, 354 (1999) 7.

[4] K.C. Yam, I.D. Angelo, R. Kalscheuer, H. Zhu, J.X. Wang, V. Snieckus. L.H. Ly, P.J. Converse, W.R. Jacobs, N. Strynadka and L.D. Eltis, *PLoS Pathog*, 5 (2009) e1000344.

[5] F. Wohler, *Annalen der Chemie and Pharmacie*, 51 (1844) 145.

[6] Olumide, Y.M. Akinkugbe, A.O. Altraide, D. Mohammed, T. Ahamefule, N. Ayanlowo, S. Onyekonwu and C. Essen, *J. Dermatol*, 47 (2008) 344.

[7] T.W.G. Solomon and C.B. Fryhle, "Organic Chemistry", 10th Edition, Wiley Publishing (2010).

[8] E. Joval, P. Kroeger and N. Twores, *Planta Med*., 62 (1996) 185.

[9] G.A. Burdock, *Food. Chem. Toxicol*., 36 (1998) 347.

[10] D. Song, J. Xia, F. Zhang, S. Bi, W. Xiang, Z. Wang, L. Xia, Y. Xia, Y. Li and L. Xia, *Sensors Actuators,* B206 (2015) 118.

[11] L. Wang, P. Huang, J. Bai, H. Wang, L. Zhang and Y. Zhao, *Int. J. Electrochem. Sci*., 2 (2007) 132.

[12] J. Yu, W. Du, F. Zhao and B. Zeng, *Electrochim. Acta,* 54 (2009) 988.

[13] C. Terashima, T.N. Rao, B.V. Sarada and D.A. Tryk, *Anal. Chem*., 74 (2002) 902.

[14] W. Xiao and D. Xiao, *Talanta,* 72 (2007) 1292.

[15] X. Li, G. Xu, X. Jiang and J. Tao, *J. Electrochem. Soc*., 161 (2014) H468.

[16] P.S. da Silva, B.C. Gasparini, H.A. Magosso and A. Spinelli, *J. Braz. Chem. Soc.,* 24 (2013) 699.

[17] R.M. Carvalho, C. Mello and L.T. Kubota, *Anal. Chim. Acta,* 420 (2000) 121.

[18] Y.P. Ding, W.L. Liu, Q.S. Wu and X.G. Wang, *J. Electroanal. Chem*., 575 (2005) 280.

[19] G.K.J. Chao and J.C. Suatoni, *J. Chromatogr. Sci.,* 20 (1982) 440.

[20] L.H. Wang and Y.P. Kuo, *Chromatographia,* 49 (1999) 211.

[21] P. Nagaraja, R.A. Vasantha and K.R. Sunitha, *J. Pharm. Biomed. Anal*., 25 (2001) 424.

[22] Y.G. Sun, H. Cui, Y.H. Li and X.Q. Lin, *Talanta,* 53 (2000) 666.

[23] J.A.G. Mesa and R. Mateos, *J. Agric. Food Chem*., 55 (2007) 3868.

[24] M.F. Pistonesi, M.S.D. Nezio, M.E. Centurión, M.E. Palomeque and A.G. Lista, *Talanta,* 69 (2006) 1268.

[25] H. Cui, C. He and G. J. Zhao, *J. Chromatogr*., A855 (1999) 179.

[26] R. Rella, D. Ferrara, G. Barison, L. Doretti and S. Lora, *Biotechnol. Appl. Biochem*., 24 (1996) 83.

[27] R.L. McCreey, In: *Electroanalytical Chemistry: A Series of Advances* (Ed. A.J. Bard), Marcel Dekker, New York, 27 (1991) 295.

[28] H. Zhao, Y.Z. Zhang and Z.B. Yuan, *Chin. J. Anal. Lab*., 20 (2001) 70.

[29] R.M. deCarvalho, C. Mello and L.T. Kubota, *Anal. Chim. Acta,* 420 (2000) 121.

[30] M. Kucharska and J. Grabkab, *Talanta,* 80 (2010) 1051.

[31] M.M. Hashem, A.H. Atta, M.S Arbid, S.A. Nada and G.F. Asaad, *Food Chem Toxicol.,* 48 (2010) 1586.

[32] N.E. Llamas, M. Garrido, M.S. Di Nezio and B.S. Fernández, *Anal. Chim. Acta,* 655 (2009) 424.

[33] Y.S. Al-Degs, *Food Chem.,* 117 (2009) 490.

[34] Y. Hong, S. Yuanyuan, L. Xinhua, T. Yuhai, L. Ailin, L. Guangwen, L. Wei and Z. Shaobo, *Anal. Sci*., 23 (2007) 682.

[35] L. Xinhue, L. Wei, Y. Hong, S. Yuanyuan, H. Liying, Z. Yanjie and C. Czechoslov. *Chem. Commun*., 72 (2007) 1188.

[36] P.S. Ganesh and B.E.K. Swamy, *J. Anal. Bioanal. Tech.,* 6 (2014) 7.

[37] A. Sathisha and B.E.K. Swamy, *J. Anal. Bioanal. Electrochem*., 7 (2015) 21.

[38] M. Pacios, M.D. Valle, J. Bartroli and M.J. Esplandiu, *J. Electroanal. Chem.,* 619 (2008) 124.

[39] D.K. Gosser Jr., *VCH, Weinheim*, (1993).

[40] J.B. Raoof, R. Ojani and Z. Mohammadpour, *Int. J. Electrochem. Sci*., 5 (2010) 188.

[41] P. Raghu, B.E.K. Swamy, T.M. Reddy, B.N. Chandrashekar and K. Reddaiah, *Bioelectrochem*., 83 (2012) 24.

[42] U. Chandra, B.E.K. Swamy, O. Gilbert and B.S. Sherigara, *Electrochimica. Acta,* 55 (2010) 7174.

[43] W. Suna, Y. Wang, Y. Lub, A. Hub, F. Shia and Z. Sun, *Sensors Actuators,* B 188 (2013) 570.

[44] S.C. Avendaño, G.A. Ángeles, M.T.R. Silva, M.R. Romo, A. Cuán and M.P. Pardavé, *J. Electrochem. Soc.,* 156 (2009) 375.

[45] U. Chandra, B.E.K. Swamy, O. Gilbert and B.S. Sherigara, *J. Electrochim. Acta,* 55 (2010) 7166.

[46] N.R. Hegde, B.E.K. Swamy, N.P. Shetti and S.T. Nandibewoor, *J. Electroanal. Chem*., 635 (2009) 51.

[47] S.M. Wang, W.Y. Su and S.H. Cheng, *Int. J. Electrochem. Sci*., 5 (2010) 1664.

[48] Y. Zhang, R. Sun, B. Luo and L. Wang, *Electrochim. Acta,* 156 (2015) 234.

[49] T. Lai, W. Cai, W. Dai and J. Ye, *Electrochim. Acta,* 138 (2014) 55.

[50] A.A. Shaikh, S.K. Saha, P.K. Bakshi, A. Hussain and A.J.S. Ahammad, *J. Electrochem. Soc.*, 160 (2013) B42.

[51] L. Wang, P. Huang, J. Bai, H. Wang, L. Zhang and Y. Zhao, *Int. J. Electrochem. Sci.*, 1 (2006) 413.

[52] H. Qi and C. Zhang, *Electroanalysis,* 17 (2005) 838.

[53] M.U.A. Prathapa, B. Satpatib and R. Srivastava, *Sensors Actuators,* B186 (2013) 77.

[54] M. Li, F. Ni, Y. Wang, S. Xu, D. Zhang, S. Chen and L.Wang, *Electroanalysis*, 21 (2009) 152.