KUVEMPU UNIVERSITY

Department of P.G. Studies and Research in Industrial Chemistry,
Jnana Sahyadri, Kuvempu University
Shankaragahtta – 577451

ELECTROCHEMICAL SENSOR FOR THE DETERMINATION OF SEROTONIN USING DIFFERENT MODIFIED ELECTRODES: A VOLTAMMETRIC STUDY

Thesis to be submitted to the Faculty of Science, Kuvempu University As a partial fulfillment for the requirements of degree of

DOCTOR OF PHILOSOPHY

In

INDUSTRIAL CHEMISTRY

Submitted by

RUKAYA BANU M.Sc

2023

Research Guide

Prof. B. E. Kumara Swamy

Department of P.G. Studies and Research in Industrial Chemistry
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2023

Dedicated To

My beloved Parents, Family Members, Teachers and Friends…

KUVEMPU UNIVERSITY

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Declaration

I hereby declare that the Ph.D., thesis entitled "**ELECTROCHEMICAL SENSOR FOR THE DETERMINATION OF SEROTONIN USING DIFFERENT MODIFIED ELECTRODES: A VOLTAMMETRIC STUDY"** embodies the results of my investigation and this has been composed by me under the Supervision of **Prof. B. E. Kumara Swamy,** Department of P.G. Studies and Research in Industrial Chemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shimoga and the same has not been previously formed the basis for the award of any degree, diploma, associateship, fellowship of any other University or Institution.

Date: 01/02/2023
Place: Shankaraghatta

Rukaya Banu
(RUKAYA BANU)

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Certificate

This is to certify that the work reported in this thesis entitled **"ELECTROCHEMICAL SENSOR FOR THE DETERMINATION OF SEROTONIN USING DIFFERENT MODIFIED ELECTRODES: A VOLTAMMETRIC STUDY"** submitted by **Ms. Rukaya Banu** to the Faculty of Science, Kuvempu University, for the award of **Doctor of Philosophy in Industrial Chemistry** is a record of the bonafide and original research work carried out by him under my guidance.

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

Date: 1/2/23
Place: Shankaraghatta

(Prof. B.E. KUMARA SWAMY)

Prof. B.E. Kumara Swamy
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Great thanks to my well-wishers…

Rukaya Banu…

LIST OF ABBREVIATIONS

AE	=	Auxiliary electrode
ASV	=	Anodic stripping voltammetry
AD	=	Adenine
CE	=	Counter electrode
CNS	=	Central nervous system
CPE	=	Carbon paste electrode
CV	=	Cyclic voltammetry
DA	=	Dopamine
DCE	=	Dropping carbon electrode
DME	=	Dropping mercury electrode
DMF	=	Dimethyl formamide
DMSO	=	Dimethyl sulphoxide
DNA	=	Deoxyribonucleic acid
D_0	=	Diffusion coefficient
DPV	=	Differential pulse voltammetry
E_f,	=	Final potential
E_i	=	Initial applied potential
Epa	=	Anodic peak potential
Epc	=	Cathodic peak potential
EP	=	Epinephrine
E_v,	=	Vertex potential
E_o	=	Formal potential
EDS	=	Energy dispersive spectroscopy
FA	=	Folic Acid
FMWNT	=	Functionalized Multiwall carbon nanotube
FSCV	=	Fast scan cyclic voltammetry
G	=	Gibb's free energy
GC	=	Glassy carbon
GI	=	Gastrointestinal
GU	=	Guanine
HOPG	=	Highly oriented pyrolytic graphite

5-HT	=	5-hydroxytryptamine (Serotonin)
Ipa	=	Anodic peak current
Ipc	=	Cathodic peak current
IPE	=	Ideally polarisable electrode
K_0	=	Heterogeneous rate constant
LOD	=	Limit of Detection
LOQ	=	Limit of Quantification
LSV	=	Linear sweep voltammetry
LT	=	L-Tryptophan
MCPE	=	Modified carbon paste electrode
mM	=	Millimolar
mV	=	Millivolt
mVs^{-1}	=	Millivolt per second
NPP	=	Pulse polarographic techniques
NPs	=	Nanoparticles
NTs	=	Neurotransmitters
PBS	=	Phosphate buffer solution
PGE	=	Pencil Graphite Elecrode
RE	=	Reference electrode
SE	=	Serotonin
SCE	=	Saturated calomel electrode
SEM	=	Scanning electron microscopy
SHE	=	Standard hydrogen electrode
SWV	=	Square wave voltammetry
WE	=	Working electrode
XRD	=	X-ray Diffraction
ν	=	Scan rate
$\upsilon^{1/2}$	=	Square root of scan rate

Summary of the Thesis

The main focus of the thesis is to formulate an electrochemical sensing approach for the detection of 5-HT. In present investigation, the different carbon based electrodes (pencil graphite electrode, carbon paste electrode and glassy carbon electrode) are modified by incorporating the various methods (Electropolymerization, mobilization/immobilization, electrochemical pretreatment and hand blending) to examine the electrocatalytic characteristics of serotonin in presence of some biologically important molecules like levodopa, epinephrine, L-tryptophan, adenine and guanine by the application of cyclic and differential voltammetric techniques. The several experimental conditions such as influence of pH of the supporting buffer, sweep rate, analyte concentration and interferences were optimized. The simultaneous analysis and real sample were carried out at different modified electrodes which show excellent performance toward serotonin detection.

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

Chapter-1

Introduction and Overview of Voltammetric Techniques and Cyclic Voltammetry

This chapter explains about voltammetric techniques and brief introduction to cyclic voltammetry and Differential pulse voltammetry. Electrode system, types of electrodes, solvents, electrode processes, electrochemical parameters and various types of voltammograms involved in this technique. A brief literature survey of cyclic voltammetric investigations of serotonin has been reviewed. The importance of serotonin is discussed. Also, objectives and scope of the present thesis were discussed in this chapter.

Chapter-2

Experimental

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

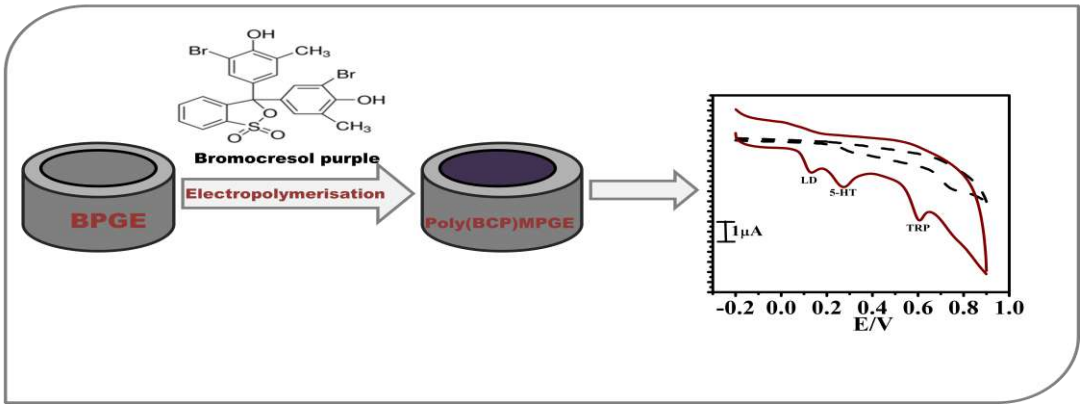
Chapter-3

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

Part-A

Poly (Bromocresol Purple) incorporated Pencil Graphite Electrode for Concurrent Determination of Serotonin and Levodopa in Presence of L-Tryptophan: A Voltammetric Study

In this chapter, the new electrochemical sensor was established based on the electropolymerisation of Bromocresol purple on pencil graphite electrode for the sensitive detection of serotonin (5-HT) and levodopa (LD) in assistance with L-Tryptophan(TRP). The morphological characterisation of developed working electrode was done by Scanning Electron Microscope (SEM). The modified sensor presents admirable electrocatalytic performance towards specific and selective examination of 5-HT, LD and TRP at 0.2 M Phosphate buffer solution of physiological pH having the speed rate of 50mV/s using cyclic voltammetric(CV) and differential pulse voltammetric(DPV) methods. The distinct experimental conditions like impact of supporting electrolyte, dissimilar concentration and varied sweep rates were optimised to accomplish a better peak current. The designed sensor effectually lowers the detection limits of 5-HT (0.49μM) and LD (2.3μM) and it is easy to fabricate, disposable, highly stable and applicable to practical analysis of bioactive molecules.



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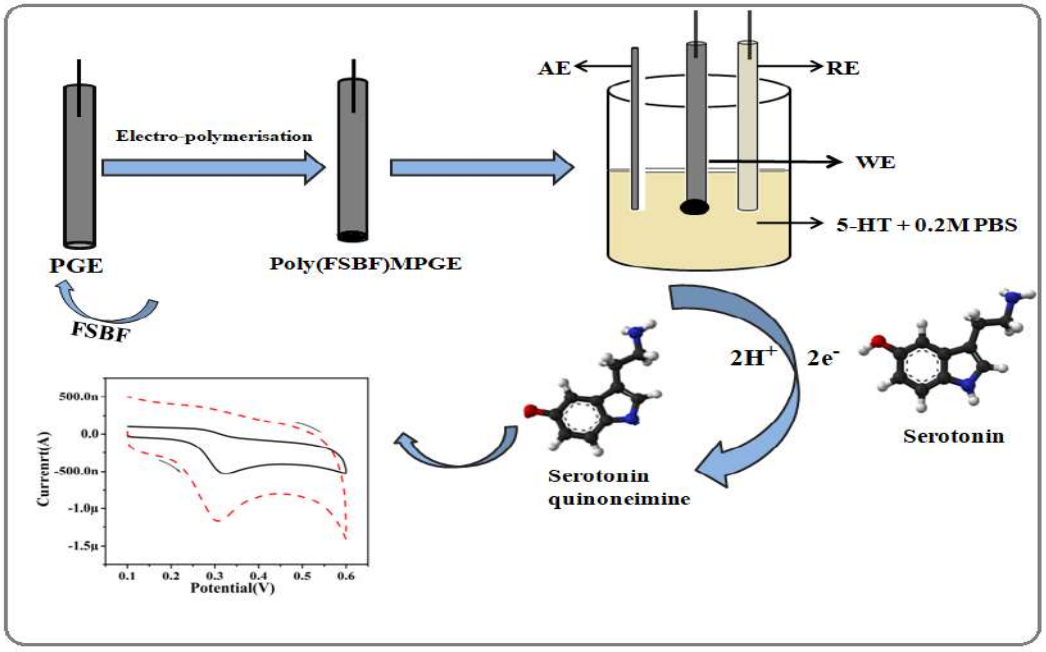
Part-B

Poly(Fast Sulphone Black F) Modified Pencil Graphite Electrode Sensor for Serotonin

This chapter involves, the development of sensitive and rapid biosensor for the investigation of serotonin has great significance because it is a key neurotransmitter and its unusual concentrations associated with serious mental disorders. In this study, an electrochemically modified serotonin-sensing electrode was fabricated by simple electropolymerisation of fast sulphone black F on pencil graphite electrode (PGE) using cyclic voltammetric technique. This modified pencil graphite electrode was applied for selective determination of serotonin(5-HT) and shows increased current responses of 5-HT in 0.2M PBS of pH 7.4. The various analytical parameters such as effect of scan rate, concentration of 5-HT and solution pH were investigated. The diffusion controlled electrode process was observed for 5-HT and detection limit was found to be 1.7μM. Interference study of 5-HT was analysed in presence of dopamine(DA) by cyclic voltammetry(CV) and differential pulse voltammetry(DPV).



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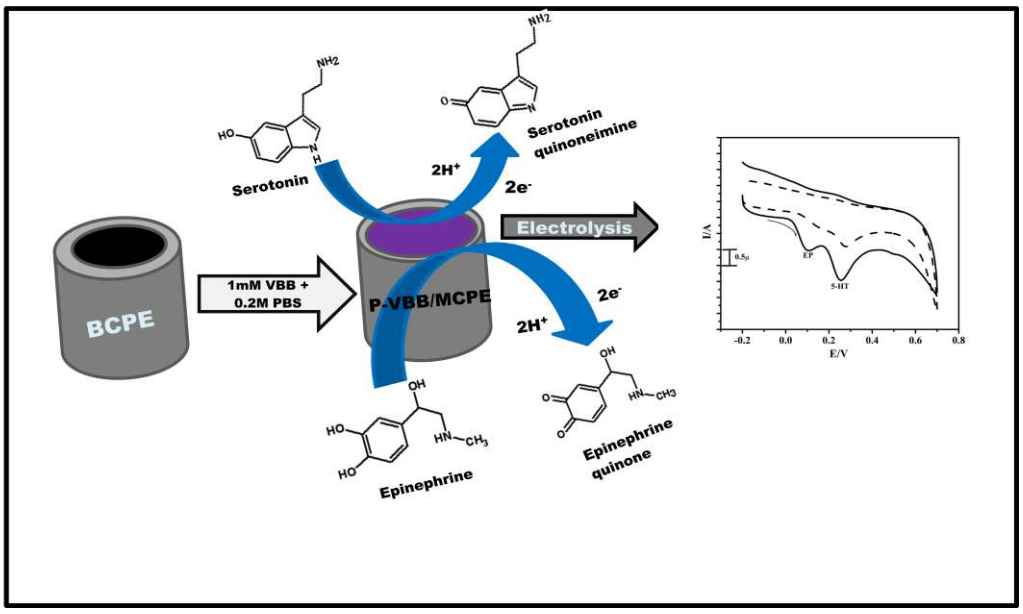
Chapter-4

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

Part-A

Simultaneous Resolution of Serotonin and Epinephrine at poly(Victoria blue B) Amplified Carbon Paste Electrode: A Voltammetric Study.

Herein, a polymerised film of Victoria blue B monomer was deposited on carbon paste electrode surface and characterised by using scanning electron microscope and electrochemical methods. The polymer film amplified electrode exemplified the elevated electrocatalytic oxidation of 5-HT and EP in presence 0.2 M PBS of physiological pH. The various experimental settings such as effect of supporting electrolyte, scan rate and varying the concentration of targeted analytes were scrutinized and the designed sensor depicts the lower detection limit for 5-HT (0.89μM) and EP (0.33μM). The proposed modified electrode also implemented for concurrent resolution and determination of serotonin (5-HT) and epinephrine (EP) by cyclic and differential pulse voltammetry.



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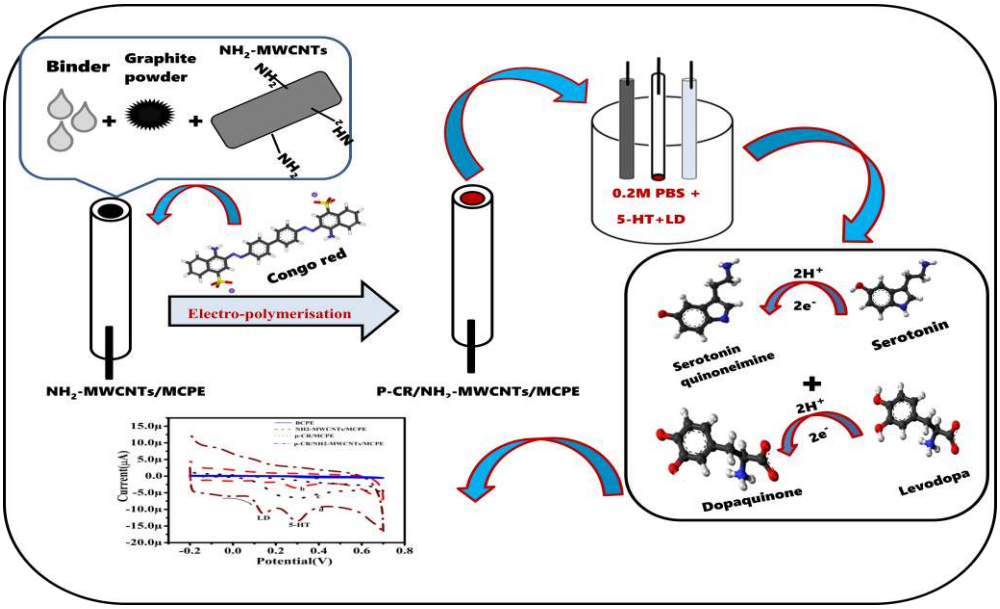
Part-B

Poly (Congo Red) Functionalised-MWCNT Composite Electrodes for the Simultaneous Voltammetric Detection of Serotonin and Levodopa in Human Serum

Serotonin (5-HT) and Levodopa (LD) are the imperative biomolecules that concurrently exist in body fluid and show undesirable effects on the functions of one another. LD depletes the levels of 5-HT in the brain in proportions to increase the dopamine level. The reduced 5-HT level can lead to severe neurodegenerative disorders. Hence, the simultaneous detection of 5-HT and LD has great importance in disease diagnosis. In the present study, an electrochemical sensing platform was formulated by amending carbon paste with functionalized multi-walled carbon nanotubes followed by electropolymerization of congo red (p-CR/NH_2-MWCNTs/MCPE). The established electrode shows excellent electrochemical properties for individual and concurrent detection of 5-HT and LD in a phosphate buffer solution of neutral pH at a sweep rate of 50 mV/s through cyclic voltammetric (CV) and differential pulse voltammetric (DPV) approaches. Various analytical variables like scan rate effect, concentration, and effect of pH were investigated. The modified sensor effectively reduces the detection limit values found to be 1.7 μM and 3.0 μM for 5-HT and LD respectively. Moreover, the proposed method offered favourable selectivity, stability, reproducibility and reliable recoveries of molecules in serum samples.



Communicated to Microchemical Journal (2022)

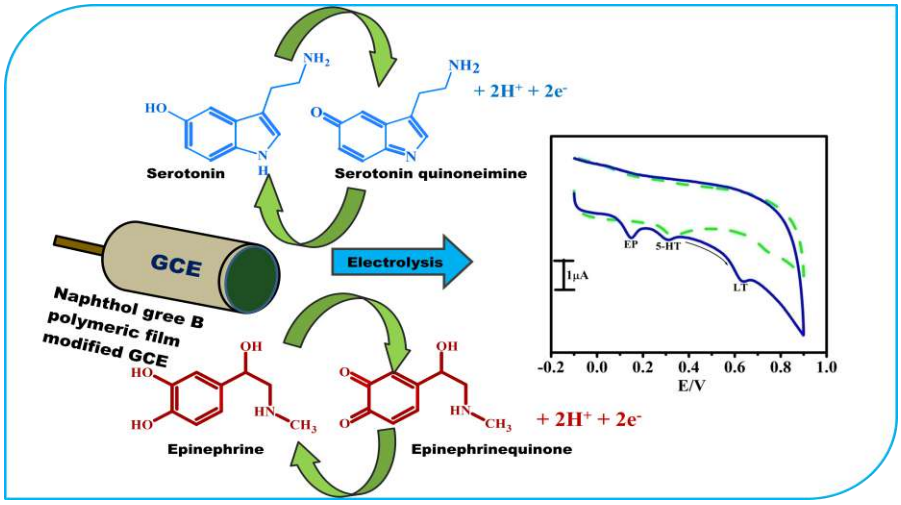
Chapter-5

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

Part-A

A Glassy Carbon Electrode Modulated with poly(Naphthol green B) for Simultaneous Electroanalysis of Serotonin and Epinephrine in Presence of L-Tryptophan.

Present study reports the simplified and efficient method for the quantification of Serotonin and Epinephrine by using a glassy carbon electrode customised with poly(Naphthol green B) film. The electrochemical measurements were performed through Cyclic and differential pulse voltammetry. The experimental outcomes authenticates that the developed sensor accelerates the electrocatalytic current response with reduced overvoltage for SE and EP. The operating system and certain experimental variables includes Sweep rate, differed pH values of supporting medium and the impact of varying concentration of SE and EP were adjusted. The sweep rate study reveals the diffusion controlled kinetics for SE and EP at P-NGB/MGCE. At the optimum reaction conditions, the fabricated electrode exhibits lower limit of detection of 2.3μM & 1.3μM for SE and EP respectively. The selectivity of the constructed electrode was achieved by concurrent determination of SE and EP in presence of L-Tryptophan. This provides the advantageous of the fabricated sensor as it shows good sensibility, convenient method for fabrication and excellent selectivity for specific and simultaneous study of SE and EP.



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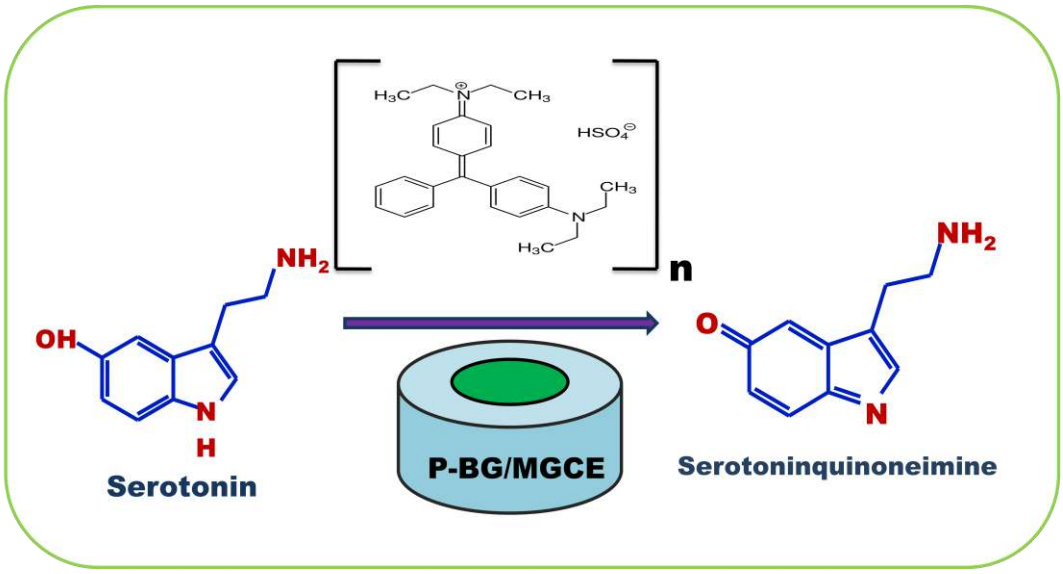
Part-B

A Selective Electrochemical Sensing of Serotonin and Epinephrine at Glassy Carbon Electrode Modulated with Brilliant Green: A Voltammetric Study

In the present investigation, a novel electrochemical sensing approach based on the modulation with electropolymerisation of Brilliant green on glassy carbon electrode was introduced to rapid and sensitive identification of SE and EP by Cyclic (CV) and differential (DPV) pulse voltammetric procedures. Under the adequate circumstances, the analytical variable like pH of the supporting solution was performed over the range of 6.2-8.0. Furthermore, the electro-kinetic parameter was surveyed and the electrode depicts the proportionality between the current intensities with the concentration of analytes with a low detection limit. The modulated sensor portrays the supreme electrocatalytic characteristics toward simultaneous quantification of SE and EP in a sample mixture.

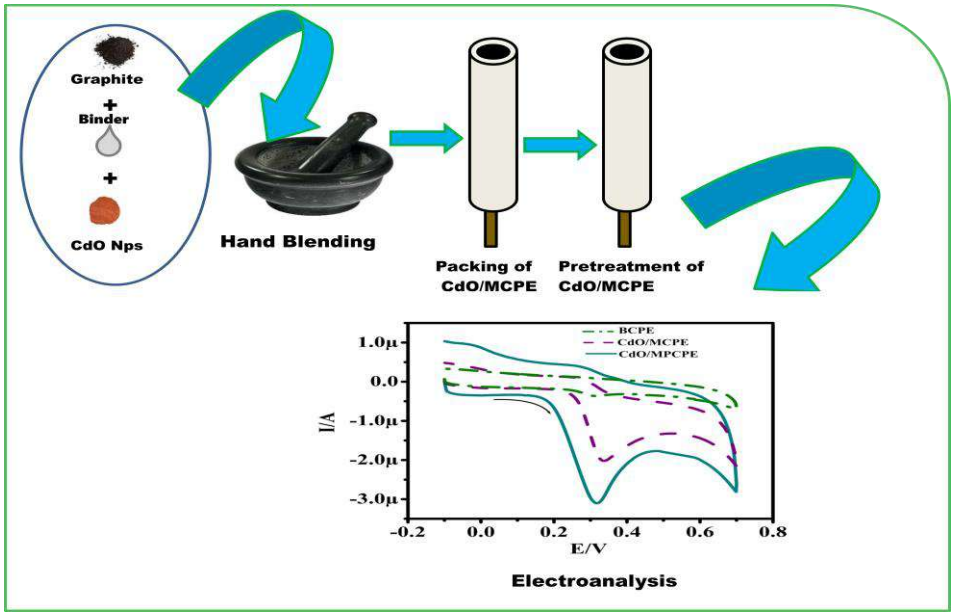


Communicated to Current Analytical Chemistry (2022)

Chapter-6

Electrochemical Sensor Facilitated by Synthesis of CdO Nanoparticles Amplified Pre-treated Carbon Paste Electrode for Quantification of Serotonin in the Presence of Epinephrine

Herein, the modest co-precipitation mode was implemented to fabricate the cadmium oxide nanoparticles (CdO). The size, elemental composition and morphological characteristics of the synthesized nanomaterials were confirmed by XRD, EDS and SEM measurements. The designed CdO nanoparticles were exploited for the amplification followed by pre-treatment of carbon paste electrode (CdO/MPCPE) and successfully utilised for the quantification of serotonin(SE) in the presence of epinephrine(EP) at biological pH. The tailored composite sensor proclaims the rapid electron transport behaviour which results in accretion in the oxidation peak signals for SE and EP. The several experimental conditions includes pH of supportive buffer, speed rate and concentration of analytes species were idealised. The CdO/MPCPE displays better sensing capability towards specific and simultaneous quantifications and lower detection limits were achieved. The customised electrode facilitates the high selectivity, excellent electrocatalytic stability and agreeable results for rapid diagnosis of identical bioactive entities.

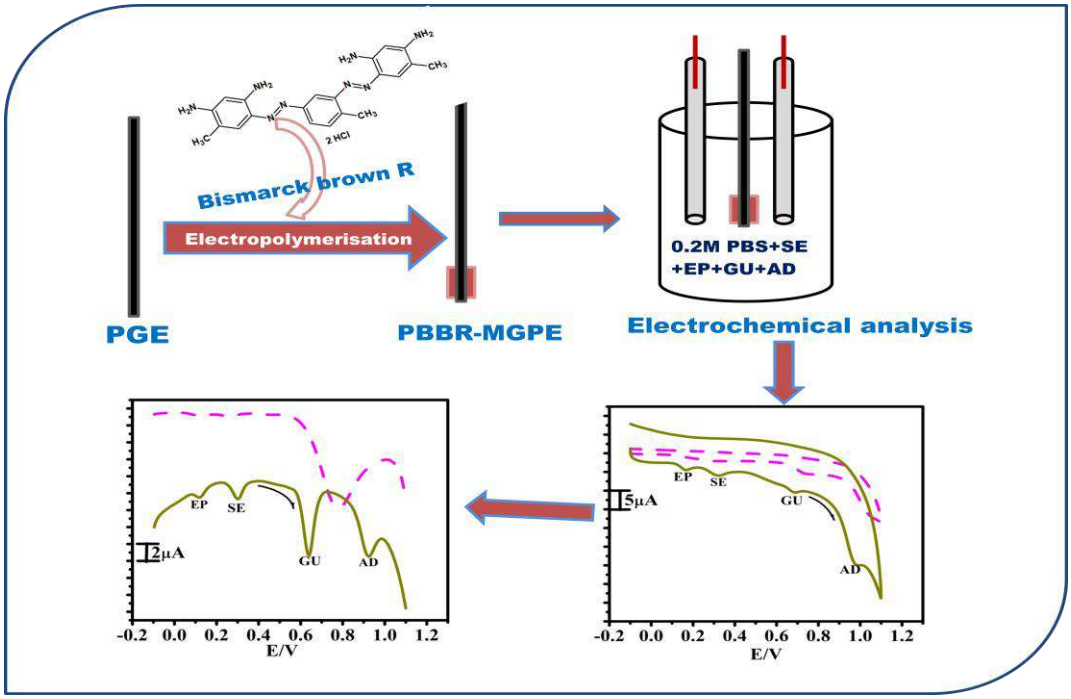


Communicated to Analytical and Bioanalytical Electrochemistry (2022)

Chapter-7

Voltammetric Analysis of Serotonin and Epinephrine in Presence of Guanine and Adenine at Bismarck brown R Amplified Graphite Pencil Electrode.

In present study, the novel sensing platform was electrochemically designed by incorporation of Bismarck brown R (BBR) as a enhancing substance onto the graphite pencil electrode(GPE) surface and successfully implemented for the electroanalysis of serotonin(SE) and epinephrine(EP) in assistant with adenine (AD) and guanine(GU) through cyclic and differential pulse voltammetric methods(CV and DPV). The morphology of electrode surface was characterised by scanning electron microscopy (SEM). The test solutions and operating conditions such as scan rate, varied supporting electrolyte pH and concentration of targeted analytes were optimised. The electrode kinetics of a tailored sensor was governed by both adsorption and diffusion phenomenon and gives a reduced detection limits for SE and EP respectively. The configured electrode proffers the potent and persistent specificity and sensibility followed by distinctly separated four anodic signals of SE, EP, AD and GU with appreciable increments in peak currents. Therefore, the offered sensor was cost effective, simple and applied in electrochemical monitoring and biosensing applications.



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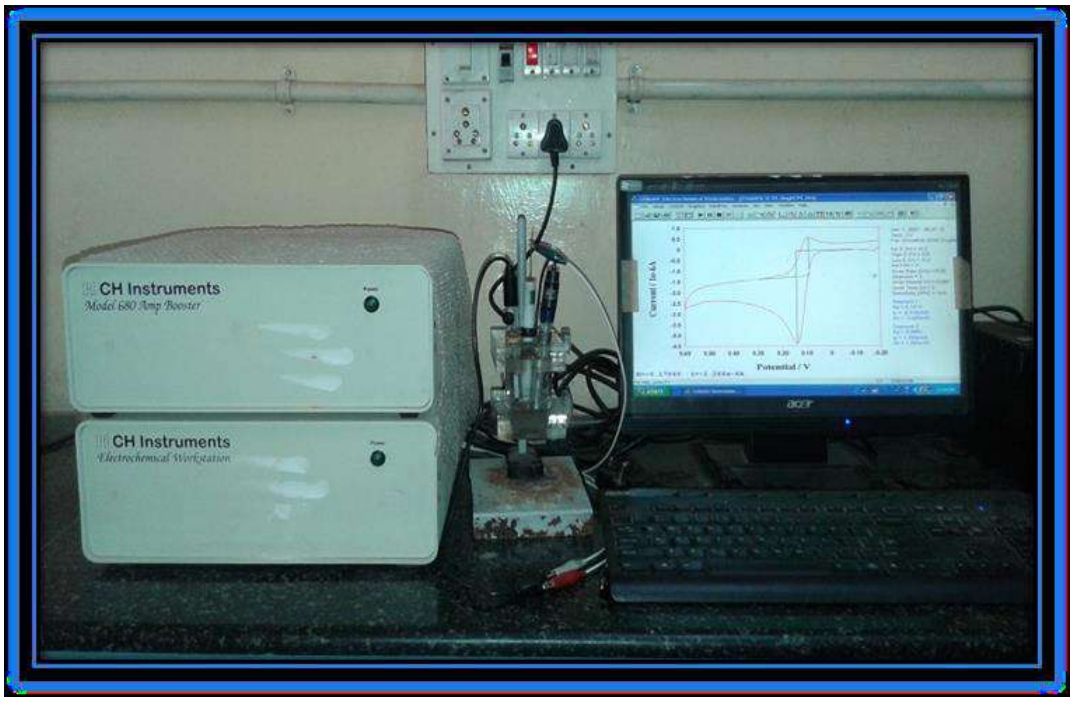
Chapter-I

INTRODUCTION



1.1. Introduction

Analytical chemistry is the science and imperative branch of advanced chemistry that concerned with isolation, chemical characterization and resolution of composition present in the natural and artificial material of interest [1]. Analytical chemistry incorporates the application of a series of techniques to extract and estimate the quantitative, qualitative and structural evidences of the matters [12]. In recent trends analytical chemistry makes significant contributions in nearly all aspects of science such as agricultural industry, clinical verdicts, biological investigations, forensic, metallurgical and pharmaceutical chemistry. Also employed to supervise and control the pollutants in environmental outflows. The series of instrumental approaches were utilised for analytical evaluations to obtain results with high accuracy and precision [3]. The most familiar analytical methods that served principle applicabilities are titrimetry, gravimetric, molecular and atomic spectroscopy, mass spectrometry, electrophoresis, thermal analysis, chromatography, radiochemical analysis and electrochemical investigation.

Electroanalytical methods involves the electroanalysis and it recognized as a new colonial approach of chemistry that consists a group of quantitative analytical techniques based on the electrochemical characteristics of the analyte solution which appears to be a part of an electrochemical cell [4]. It is applied for the measurement of electrical variables like potential (E), current (I), resistance (R) and charge (Q) and chemical parameters like analyte's concentration. The electrochemical techniques have vast impact as it provides superior selectivity, sensibility, easy method of amplification and fast response. Also serves as eco-friendly because of the less consumption of organic solvents and employed for splitting of ionic species in addition to their determination [5-7].

A sensor is a tool that detects and reacts to some type of inputs such as light, heat and moisture from the physical environment and the output is normally a signal that is converted to a human readable display at the sensor location or transmitted electronically [8-10]. The sensors were categorized into two kinds' biosensors and electrochemical sensors. A biosensor is an analytical device that combines a biological sensing component with physiochemical detector (transducer). The electrochemical

sensors are the devices that render the information about the composition of a system in real time by coupling a chemically selective layer or the recognition element to an electrochemical transducer. In simple way, the electrochemical sensor senses a chemical changes and transduced into an analytically beneficial electrical signal. The sequence of these two divergent techniques of classifications has provoke to a new kind of sensing device named as electrochemical biosensors, where the electrochemical procedures are applied for the production and working of a biosensor [11-14].

Electrochemical biosensors are recognised as a striking tool in scientific research for the exposure of the biologically significant substances, which acts as beneficial sources for the disease biomarkers [15, 16]. These sensors are portable, less expensive, provides accurate, rapid and real time analysis than other conventional methods and reproducible. Therefore there is a pressing need for the development of electrochemical sensing based biosensors for invasive clinical monitoring from treatment to prevention of diseases [17, 18].

1.2. Advantageous of Electroanalytical techniques

Electroanalytical methods are the utensils accustomed in advanced electroanalytical chemistry for the detection of the analyte of concern as an outcome of signals originated by redox reaction at electrolyte-electrode interface [19]. The potential benefits of Electroanalytical methods over other techniques are

- Easy to conduct, rapid, portable and highly proficient.
- Highly sensible and accurate results.
- Less instrumentation cost than spectroscopic equipments.
- Possibility of giving results in real time or close to real time particularly in flow systems for online monitoring.
- Determination of analyte in lower concentration without complications.
- Involvement of number of electrons in an electrode reaction can be quantified which is difficult with other methods.
- Environmental friendly approach.

1.3. Types of Electroanalytical techniques

In electroanalytical approach of analysis, one measures potential (voltage) and/or current signals [8]. The Electroanalytical methods are widely categorized and briefly discussed as follows:

- Potentiometry
- Coulometry
- Conductometry
- Voltammtery

1.3.1. Potentiometry

Potentiometry involves the measurement of solution potential created across the indicator electrode is measured in the absence of appreciably zero current. These techniques generally employ ion selective electrodes for rapid and simple determination of specific ionic moieties in solution and concentration of solute in solution [20].

1.3.2. Coulometry

Coulometry is an analytical technique in which the number of coulombs that passes through a solution during an electrochemical reaction or the quantity of electrical charges needed to transform the analyte quantitatively to a different oxidation state is measured. It usage either applied potential or current to completely convert oxidation state of an analyte at the working electrode [21].

1.3.3. Conductometry

It is based on measurement of electrolytic conductivity in ionized form to monitor a progress of chemical reaction. It is the quantification of the materials efficacy of the electric current. The value of conductance depends on the density ions and their mobility. This technique has great attention where the suitable indicator is not existing in volumetric experiments such as in acid-base titrations and also utilized to titrate very dilute solutions.

1.3.4. Voltammetry

Voltammetry is the widespread electrochemical technique in several areas of applied and pure science. Unlike polarography, voltammetry measures the flowing of

current at a stationary electrode as a function of the applied potential. The beginning of voltammetry was accompanied by the invention of polarography in 1922 by the Czech chemist Jaroslav Heyrovsky, he received the 1959 Nobel Prize in chemistry for this discovery. Voltammetry includes the monitor of current (I) passing at an electrode as a function of time-dependent potential (E) implied to the electrode. Because of this, the voltammetry can be recognized as a function of E, I and time (t). The graph of current (I) with respect to potential (E) is referred as voltammogram which gives information about the analyte both qualitatively and quantitatively in redox reaction.

1.4. Classification of voltammetric techniques

The disparate approaches practised in the voltammetry were differentiated from each other by the function of potential that is applied to the working electrode to stimulate the electrochemical reaction and by the elements used as the working electrode. There are various types of voltammetric techniques originated from distinct voltage waveforms (E-t profile) or excitation signals exploited to carry out the redox reactions at an electrode. Depending on this conviction voltammetric techniques are mainly classified into, as follows [22-26]:

- 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 Voltammetry (DPV)
- Fast Scan Cyclic Voltammetry (FSCV)
- Cyclic Voltammetry (CV)

Cyclic voltammetry (CV) is a multifaceted electrochemical technique because of its immensive applicability in electrochemistry field. CV is predominantly assisted to track the electroanalytical response of the analyte in the system. In earlier 1938 Randles first illustrated theoretically [27]. CV is often the first experiment conducted in the electroanalytical investigation. This process is established upon altering the applied potential at a working electrode in both forward and reverse

directions through inspecting the current at a constant sweep rate [24]. For example, the initial scan could be in the negative direction to the switching potential. At that point, the scan would be reversed and run in a positive direction. Based on the study, one full cycle, a partial cycle, or a series of cycles can be executed. Hence, it is completely favorable in the analytical field for scrutinizing the electroactive biomolecules.

1.5. Some of the terms frequently used in CV

1.5.1. Scan rate

A basic potential waveform that is used regularly in electrochemical inquiries is the linear waveform i.e., the potential is consistently adjusted as a linear function of time. The rate of change of potential with time is referred as scan rate.

1.5.2. Circuit

Voltammetric evaluations contain two circuits one of which is a polarizing circuit that applies the potential to the cell and another is a measuring circuit that extract the cell current. The working electrode managed potentiostatically. The potential is differed in some systematic manner and corresponding current against potential plot is termed as voltammogram.

1.5.3. Potential control

The potentiostat and a three electrode system is consumed to control the potential of the external circuit in which the working electrode (WE) potential is maintained related to reference electrode(RE), saturated calomel electrode (SCE) or Silver-Silver chloride(Ag/AgCl) electrode. The current flows between WE and counter electrode (or auxiliary electrode).

1.6. Principle and theory of cyclic voltammetry

The fundamental principle of voltammetry is it plays a key role in transfer of electrons that regulating the roadways to chemical reactions; the implications of the applied potential and the redox current response are expressed by various well-known laws. The concentration of redox species is restricted by applied potential at the

surface of electrode (C_O^o and C_R^o) and the rate of the reaction (k^o), as stated by the Nernst or Butler-Volmer equations accordingly. In the cases where diffusion acts as controlling part, the current resulting from the redox mechanism (termed as the faradic current) is related to the material flux at the electrode-solution interface and is reported by Ficks law.

For electrochemically reversible process (that is a reaction so fast that equilibrium is always reestablished as modifications are done), which is denoted by O + ne- $\Leftrightarrow$ R, the respective O and R concentration by the application of potential E troops at the electrode surface (i.e., C_O^o and C_R^o) to a ratio in accordance with the Nernst expression.

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

Where, T is the absolute temperature (k), R is the molar gas constant (8.3144 J mol^{-1} k^{-1}), n is the number of electrons transferred, F= Faraday constant (96,485 C/equivalence) and E_o is the standard reduction potential for the redox couple. If the applied potential of the electrode is altered, the ratio of C_R^o /C_O^o at the surface will also vary, so as to meet the expression (1.1). If the potential is made more negative the ratio becomes greater (i.e. O is reduced) and inversely, if the potential is made more positive the ratio becomes smaller (i.e. R is oxidized).

For few methods it is convenient to use the relationship that connects the parameters for potential, current and concentration, termed as Butler-Volmer formula.

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

where, θ= nF (E-E^o)/RT, k^o is the heterogeneous rate constant, α is known as the transfer coefficient, A is the area of the electrode. By using the equation one can get the values of the two analytically important variables, i and k^o, CV is essential approach for getting consequential facts about the electroanalytical procedures. CV primarily acquired to examine the electroactive systems especially bio-related components. Also utilized to explore the mechanistic investigations of systems in which the chemical reactions are coupled with electron transfer kinetics. CV is an eminent sweep method that includes the sweeping of electrode potential within steady potential limits E_1 and E_2 at a noted speed rate. To achieve the cyclic scan, on arriving the limit E_2 the sweep is reversed to E_1. The CV scan is a graph of potential with current and suggest the potential at which the redox reaction occur. The potential axis is also a time axis that is affiliated to the scan rate [27]. The excitation signal for CV is

a linear potential scan with a triangular waveform as shown in Fig. 1.1 [28]. This triangular potential excitation signal sweeps the potential of an electrode between two values, eventually named as the switching potential.

1.6.1. Characteristic parameters of cyclic voltammogram

The substantial specifications of cyclic voltammogram are the extent of cathodic peak current (Ipc), the anodic peak current (Ipa), cathodic peak potential (Epc) and the anodic peak potential (Epa) are displayed in Fig. 1.2. The outcomes withdrawn from these variables can be practiced to acquire the information on thermodynamics and kinetics of the redox probe [29]. If the potential is scanned towards negative side makes the electrode a stronger reductant, whereas, the potential scanned towards positive side makes it an excellent oxidant as portrayed in Fig. 1.3.

1.6.2. Faradaic current in CV

The faradaic phenomenon obeys Faraday's law and includes electron/charge transfer around the electrode-solution interface [30]. That is, the number of moles of reactant converted during the chemical process is directly proportional to the amount of charge (electricity) passed. So, the current that arrives from the redox process of the analyte is known as faradaic current. It may be operated to detect the kinetic, thermodynamic and transfer particulars of redox system [31].

1.6.3. Non-faradaic current in CV

It originates from the formation of double layer at the electrode and monolayer surface when the adsorption and desorption of ions, it is defined by time-dependent capacitive current in the electrochemical measurements. The interface between working electrode and electrolyte acts as a capacitor. Hence, the charging current is obligatory to convert the applied potential to the working electrode and is designated as non-faradaic current or residual current. It is because of the contaminates in the solvent, electrolyte or electrode and the dissolved oxygen [1, 2]. Since the potential in CV measurements is constantly varying, there is an approximately persistent charging current, which is the significant contributor to the background current. This charging current is proportional to the speed rate [30].

1.7. The Solvent

The selection of solvent is a principal aspect in cyclic voltammetry. Numerous considerations like characteristics of electroactive species, conductance and solubility of electrolyte are deliberated [31]. The solvents for electrochemical analysis are picked depending on the variety of physiochemical features such as follows,

- It should be in liquid fashion and pure.
- Ability to dissolve electroactive components in it.
- It should have excellent solvent power.
- A broad potential window to examine the desired redox processes.
- It should not interact with analyte or product.
- It must have mandated acid-base properties.
- It must have high dielectric constant.

The broadly accustomed solvent is water due to its affordable and polar feature and has capability to dissolve the ionic substances and form highly conducting solutions. Few non-aqueous solvents like N, N-dimethylformamide (DMF), propylene carbonate, acetonitrile, dimethylsulfoxide (DMSO), methylene chloride and methanol are also being utilized for the oxidation of organic molecules. Blended solvents may also be used for specific objectives.

1.8. The supporting electrolyte

The supporting electrolyte is one of the most required components in electroanalytical determinations. These are the chemical components that are not electroactive and which has an ionic strength and conductivity greater than those due to the electroactive moiety mixed to the electrolyte [32]. All ionic salts or ionizable compounds in a solvent are defined as supporting electrolytes. The supporting electrolyte should accomplish the following capabilities,

- It is highly dissolvable in the chosen solvent.
- These electrolytes ensures the solution with some conductivity by addition of an electrolyte.
- It is chemically and electrochemically inert in the circumstances of the experiment.

- ♦ They must be electro-inactive in the potential region of interest.
- ♦ The ions of the supporting electrolyte should not adsorbed on the surface, in that case they can catalyze or restrict other reactions.
- ♦ Generally they maintain the acidity of the ionic solution.
- ♦ The concentration of the supporting electrolyte should be very high, so that they can form a space charge near the surface and the space charge potential can not Influence the charge transfer kinetics.
- ♦ The supporting electrolyte should not form ion pairs with anion radicals produced during electrode reaction nor form complexes with the reactants or products Some ions may form complexes with the reactants and products.

Supporting electrolytes should be prepped out of extremely pure chemicals. Otherwise, contaminants present in them may interfere with the examination. H_2SO_4, $HClO_4$, and HCl are routinely used for analysis in acidic aqueous solutions and NaOH or KOH are occupied for alkaline media. In the neutral region, if buffering is essential, acetate, citrate, pyrophosphate and phosphate buffers are generally applied. If the redox process does not involve acid-base reactions, no buffer is required and any electrolyte may be utilized. Tetra-alkyl ammonium salts ($NR_4^+X^-$) are usual in organic media.

1.9. Electrodes in voltammetry

The emergence of advanced electrochemistry generated the necessity of modern electrodes and electrode set-ups. The most conventional arrangement used is the electrochemical cell with three different electrodes.

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

1.9.1. Reference electrodes

In a voltammetric experiment the working electrode potential was always maintained for some standard and that standard is the RE. In other words, RE can be defined as a constant potential device [33]. The principle requirements of RE is it should have stable and reproducible potential reached by employing a redox system

with constant (buffered or saturated) concentration each participant of the redox reaction when the experiments are conducted, In terms of a potential difference, RE provides constant potential value to which other potentials can be referred. It is impossible to evaluate the accurate value of the interfacial potential difference of a single electrode-solution interface. Potentials can only be recorded as the difference for a selected reference value. RE can be classified as aqueous, calomel, non-aqueous and own-constructing as hold as standard and plays a monumental role in managing and measuring the potential of working electrode in voltammetric measurements. Most frequently applied REs in voltammetric methods and their cell representation was shown below,

- Silver-silver chloride electrode (Ag/AgCl/KCl (saturated), E°=0.222V vs. NHE at 25°C).
- Mercury-mercury oxide electrode (Hg/HgO/H_2O, E°=0.098V vs. NHE at 25°C) etc.
- Saturated calomel electrode (SCE, Hg/Hg_2Cl_2/KCl (saturated), E°=0.244V vs. NHE at 25°C).

In present study, the SCE was used as a reference in the entire analysis. The fabrication of RE is as follows, an adequate piece of glassware that can store a little quantity of mercury in direct contact with solid calomel (Hg_2Cl_2) paste, while at the similar period retaining the paste in interaction with a saturated aqueous solution of potassium chloride. Electrical contact is built by dipping platinum wire into the liquid mercury and the potassium chloride solution controls ionic contact with the test solution in the electrochemical cell through a salt bridge or porous glass frit. Such electrodes can be "home-made" or purchased from a variety of manufacturers. The half cell representation for the SCE is as follows:

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

1.9.2. Auxiliary electrode

The auxiliary electrode often called as counter electrode is used in voltammetric analysis involving three electrode electrochemical cell and stipulates a means of applying input potential to the working electrode. The main purpose of AE is to complete the circuit and offers a substitute to allow the charge to flow. The AEs potential is adjusted so as to balance the reaction appearing at the working electrode.

This type of composition allows the potential of the working electrode to be evaluated against a known reference electrode without altering the constancy of that RE by streaming current trough it. The AE may be separated from the WE using glass frit to avoid any byproducts originated at the AE from contaminating the main test solution. The AE often has a much greater surface area than those of WE. Design choices are generally based on finding a material that is chemically inert in the specific test solution being explored. Normally a thin foil of platinum wire is utilized. However, sometimes carbon and gold have also been used.

1.9.3. Working electrode

In electrochemical system the WE is the electron conductor on which the reaction of interest takes place. The interpretations of the voltammetric processes are highly affected by the working electrode components. The most widespread solid inert WEs are Pb, Pt, Au, Ag, graphite, carbon paste, glassy carbon, vitreous carbon, indium tin oxide, diamond etc. WE can be of the shape of a small sphere, small disc, or short wire. It can also be a single crystal of metal or semiconductor, an evaporated thin film, or pressed to discs/pellets. The surface of the electrode should be smoothened to assure the shiny and crack free surface that may result in erratic responses. WE should follow some of these features:

- It should have a surface reproducibility and high purity.
- The standard working electrode is a very clean metal surface with a specific geometry that is in direct contact with an electrochemical test solution.
- It should be both chemically and electrochemically inert with long-lasting stability.
- The geometrical area of the WE should be as small as possible so that it has the maximum current density.
- It should have a large potential window for both anodic and cathodic applications.

1.10. Electrode process

The reaction progressing between the surface of electrode and component within the solution can move ahead with series of stages that drives the transmutation

of the dissolved oxidized species (O) to reduced species (R) in solution. The rate of electrode reaction is presided by the reaction rates such as

- Mass transfer
- Electron transfer of non-adsorbing species

The basic reaction comprises only mass transfer of reactant to the electrode, heterogeneous electron transfer concerning non adsorbed substance and the mass transfer of the product to the bulk solution. More complicated reaction progression involving a sequel of electron transfer, protonations, branching mechanisms, parallel paths or amplifications of the electrode surfaces are completely normal. When a steady state current is obtained, the rates of all reactions sequel are identical. The magnitude of this current is continually restricted by the instinctive slowness of one or more reactions called rate determining steps. The more uncomplicated reactions are then restrained from extreme rates by the slowness with which such steps adjusted of their products or originate their participants [34, 35].

1.10.1. Mass transfer process

In charge transport phenomenon which is appear at the electrode surface, the electroactive component gets diminished and the concentration slope was established. Mass transfer in electrochemistry depicts the net transfer of chemical substance from one location to another. Under such situation the reactant diffuse towards the electrode surface and the corresponding product of the electrode reaction diffuses away from the electrode surface. There are three kinds of mass transport namely, diffusion, migration and convection which influence and electrolysis reaction depicted in Fig. 1.4.

1.10.1a. Diffusion

Diffusion is particularly significant in Electroanalytical experiments. It is a spontaneous transmits of matter from a high concentration region to the lower concentration region and it happens whenever there is a chemical modification at a surface. When the analyte species undergo electrolysis, the diffusion arises because of the deduction of the concentration of electroactive ion at the WE surface and this deduction only due to transfer of charge occurring at the surface. Therefore, more analyte strikes towards the WE from the bulk of solution through diffusion. It is the most frequent mass transport in quiescent solution voltammetry.

1.10.1b. Convection

Convection as it employed to electrochemistry is forced movement of solution species by mechanical (stirring) or other means. It is observed due to the dissimilarities in density and temperature at various portions of the solution and then the natural convection cannot be replicated or reproduced. The natural convection due to its non-reproducible behavior makes electrode process more difficult. Hence, it is necessary to remove it. This can be done by conducting the electrolysis in a thermostat without stirring or vibrating.

1.10.1c. Migration

The movement of charged particles in response to a local electric field is called migration. It is the mechanism by which the charge passes through the electrolyte. The electrostatic forces cause the migration. Mass transfer is not an imperative in voltammetry. Migration is removed by the addition of an excess of supporting electrolyte so that ions of electrolytes migrate and not the analyte.

1.11. Electron transfer process

The transfer of electrons at the electrode and electrolyte interface is principal to an electrode reaction. An electroactive component relocated from the bulk of the solution by either diffusion or under forced convection enters in the electrical double layer, which is under the direct control of the electrode. There are three kinds of electron transfer phenomenon namely,

- Reversible process
- Irreversible process
- Quasi-reversible process

1.11.1. Reversible electron transfer

The reaction is considered to be electrochemically reversible if the electron transfer is rapid as compared to mass transport. In reversible systems, both oxidation and reduction peaks are witnessed as shown in Fig. 1.5. It obeys Nernst behavior and accepts that both oxidized and reduced substances are in equilibrium. The peak

current noticed in CV for electrochemically reversible systems is provided by Randles-Sevick's expression as follows.

$$Ip = (2.69 \times 10^5) (n)^{3/2} A D_0^{1/2} C_0 \nu^{1/2} \text{ ……..... (1.1)}$$

Where A is the area of the working electrode in cm^2, D_0 is the diffusion coefficient of the substance O in cm^2s^{-1}, n is the number of electron transferred in the electrode reaction, ν is the scan rate in Vs^{-1} and C_0 is the concentration of the species O in mol/cm^{-3}. The diagnostic check for cyclic voltammogram of reversible system at 25°C was as follows:

- $\Delta Ep = Epa - Epc = 59.1/n$, where n is the number of electrons change.
- $E_0 = [Epa + Epc] / 2$
- $Ep - E_{p/2} = 59.1/ n$
- $Ipa / Ipc = 1$
- $Ip \alpha \nu^{1/2}$
- Ep is independent of ν

1.11.2. The irreversible electron transfer process

The reaction is considered to be electrochemically irreversible if the electron transfer is sluggish as compared to the mass transport phenomenon. Irreversible systems are typified by the aspects of only either oxidation signal or reduction signal as presented in Fig. 1.6. But at times both the peaks are noted which is separated by a potential difference of more than 59.1/n mV. Irreversible systems do not obey Nernstian manners. The peak current perceived in CV for electrochemically irreversible systems is given by the following expression

$$Ip = (2.69 \times 10^5) n (\alpha n)^{1/2} A D_0^{1/2} C_0 \nu^{1/2}$$

$$(\alpha n) = 47.7/ Ep - E_{p/2}$$

Where, the term α is the charge transfer coefficient, the diagnostic check for an electrochemically irreversible system at 25°C was as follows:

- No reverse peak
- $Ip \alpha \nu^{1/2}$
- Ep shifts = 30/ αn mV, where α is the charge transfer coefficient.

- dE_P / dlog ν = -30/ αn
- [Ep – $E_{p/2}$] = 47.7/ αn mV

1.11.3. Quasi reversible electron transfer

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 system as portrayed in Fig 1.7. The current due to quasi-reversible process is controlled by both mass transport and charge transfer kinetics .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. The diagnostic check for an electrochemically quasi reversible system at 25°C was as follows:

- Ip increases with ν, but not proportional to it.
- Ipa/Ipc = 1, provided α = 0.5.
- ΔEp is greater than 59.1/n mV and it increases with increasing ν
- Ep shifts with increasing ν

1.12. Applications of cyclic voltammetry

CV is the most flexible and popular electroanalytical approach available for the mechanic study of redox reactions [36]. The technique offers a vast range of potential to be quickly scanned for oxidizable or reducible components. This beneficial trait along with its changeable time scale and appreciable sensibility makes this electroanalytical technique as a multifarious method. Once the redox couples are situated then can be categorized from the peak potential on the cyclic voltammogram and changes caused by differences of the scan rate. CV has capabilities to rapidly provide information about:

- Reaction mechanism, the rate constant, transfer co-efficients, diffusion co-efficients of redox process, the kinetics of electron-transfer reactions, and detection of chemical reactions coupled to electron transfer or adsorption processes.
- The number of electrons involved in each of the observed redox processes.

- Rapid location of redox potentials of the electroactive species present in new molecules.
- Interpretations of the organic compounds, neuroactive compounds in pharmaceutical formations.
- In-vivo analysis in the rat brain, bacteria and plants [37-41].
- Whether the redox behavior is affected by a change in the concentration of the electroactive species, solvent system, or the electrode surface.
- Reaction intermediates and their identification in the electrode reaction.
- Products formed in the electrochemical reactions.

1.13. Chemistry of Serotonin

Serotonin (5-HT), often known as "3-(2-aminoethyl)-1H-indol-5-ol, 5-Hydroxytryptamine" is an electroactive indolamine entity acts as a neurotransmitter and vasoconstrictor. 5-HT is a prominent mood modulating neurochemical extensively secreted by the essential amino acid L-tryptophan and principally found in peripheral and central nervous systems and body utilises it to relays the information between the nerve cells and regulates their intensity. The biological role of 5-HT is complex and diversified. 5-HT along with other monoamine neuromodulator plays a crucial role and fulfils the various physiological and biological actions of the body include mood-stabilizing, cognition, reward, learning, alcoholism, psychosis, eating disorder and thermoregulation [42-48]. 5-HT positively improves the sleeping patterns for more soothing and rejuvenates sleep. It also assists in blood clotting and bowel functions of the body. Any variations in the serotonin level leads to multiple psychiatric illnesses such as the deficit 5-HT levels attributed to severe mental sickness such as panic attacks, depression, insomnia, stress and unexplained irritability. The extreme 5-HT concentration is associated with the group of symptoms known as serotonin syndrome and toxicity [49-51].

1.14. A brief literature review of cyclic voltammetric investigation on serotonin sensor

A series of studies has been conducted by numerous researchers and their group towards the electrocatalytic evaluation of several analytes via cyclic voltammetric approaches. The modulation of working electrodes such as carbon paste

electrode, pencil graphite electrode and glassy carbon electrode by using variety of methods like electropolymerization, electrochemical grounding and pretreatment and utilized for the electroanalysis of serotonin in the presence of various neuromediators like dopamine, levodopa, epinephrine, L-trypophan, adenine and guanine. The specific properties of chemically modified working electrode can limiting the over-voltage, enhance the rate of reaction and increases the selectivity and sensibility of the detection. The literature survey discloses the following illustrious references

M. Lufti. Yola *et al* developed a sensitive electrode 5-HT detection based on graphene quantum dots/two-dimensional (2D) hexagonal boron nitride nanosheets with molecularly imprinted polymer (GQDs/2D-hBN). The modified electrode displayed the better stability and selectivity and the sensors offers the promising application for 5-HT [52].

A. L. Sanati *et al* report the modification of electrode using hybrid of graphene quantum dots and ionic liquid, later which was utilized for the sensitive determination of 5-HT. The electrochemical behavior of 5-HT at the modified electrode was investigated by DPV. The proposed method exhibited good stability and reproducibility [53].

O. E. Fayemi *et al* record the electrochemical response of serotonin on the modified electrode based on multiwalled-carbon-nanotube (MWCNT) doped metal oxide nanoparticles coating on glassy carbon electrode (GCE). The modified electrode exhibited excellent electrocatalytic activity towards the detection of serotonin at large peak current and lower oxidation potentials.[54].

I. Cesarino *et al* a sensitive and simple Pt/MWCNT/PPy/AgNPs electrode was applied to the detection of serotonin in plasmatic serum samples. It presents the good stability repeatability and reproducibility and can be an effective material to be used in biological sample [55].

M. Tertis *et al* construct a highly selective electrochemical detection of serotonin on polypyrrole and gold nanoparticles-based 3D architecture. The analytical characteristics obtained suggest that this sensor could be usefully incorporated in point-of-care devices for use in clinical analysis [56].

K. Reddaiah *et al* a new stable and sensitive electrochemical sensor was prepared by two-fold modification of glassy carbon electrode (GCE) with poly-alizarin red S (AzrS) and multiwalled carbon nanotubes (MWCNTs) and The developed electrochemical sensor exhibited a potent and persistent electron mediating behaviour towards the serotonin determination[57].

F. Wang *et al* designed a simple but highly sensitive and selective calixarene-based voltammetric sensor for serotonin. The developed electrode improves the electron transfer and the overall electrochemical behavior of 5-HT and exhibited a good linear relationship [58].

H. Yao *et al* developed a serotonin sensor that was simply prepared by amplifying the glassy carbon electrode with erichrome cyanine R. This work demonstrated a simple, efficient design for a chemical sensor Owing to its superior selectivity and anti-interference capability [59].

G. Ran *et al* customized a electrode using a Fe3O4–MWCNT–poly(BCG) nanocomposite film. The electrode presents a strong electrocatalytic effect in the oxidation of 5-HT and enhanced electron transfer between the target analytes and the electrode interface [60].

H. Filik *et al* reported on voltammetric determination of 5-HT in the presence of DA and AA using glassy carbon electrode amplified with safranin O by electropolymerization method. The sensor facilitates the simultaneous determination of 5-HT, DA and AA with good stability, sensitivity and selectivity [61].

1.15. Objectives of the thesis

The current thesis is targeted at explorating the electrochemical depiction of serotonin to fulfill the following objectives:

- Collection of modifying agents to develop the modified working electrode.
- To promote the functioning of bare electrode through its modification by adopting the multiple modes followed by its characterization and revealing its benefits for the electroanalysis.

- To identify the electrochemical sensor for the electroanalysis of serotonin of interest.
- Kinetic quantification of the modified electrode phenomenon.
- Concurrent and interference free resolution of analytes in a mixture by using CV and DPV methods.
- To establish a lower limit of detection.

1.16. Scope of the present thesis

The present thesis intended towards the fabrication of electrochemical sensing element for serotonin. The modulated electrodes have fascinated the researchers, because of its unique and versatile applicabilities in the area of electroanalytical chemistry. The prime prospect of the work is to interpret the electrochemical attitude of the serotonin at differently modified working electrodes. The electron transport behavior as well as kinetics occurs at the electrode surface, the impact of supporting electrolyte pH on redox process, linear dynamic range, detection limit and interference investigations were practiced. All these features were contrived by amplifying the working electrode with distinct modifiers and modification procedures like electropolymerization and pretreatment. The paramount reasons of modification were to upgrade the lower detection limit and the interference free detection of targeted analyte bearing with other possible molecules. Special attention was needed to ascertain our goal by simplifying the modification methods by employing organic molecules such as dyes, nanoparticles and surfactants. The modified electrode fulfills the requirements of an electrochemical sensor.

The aspect of the present thesis is to define the usability of different modified electrode as a sensing platform to detect the serotonin in existence with interferences like dopamine, epinephrine, adenine, guanine, L-tryptophan and levodopa. The stated customized electrodes displays the rapid electron transportation, elevated electrocatalytic reactivity, excellent constancy and reduced detection limit as compare to bare working electrodes. The modification approaches utilized in this offers a wide-spectrum of applicabilities such as ease of fabrication, affordable and simple instrumentation.

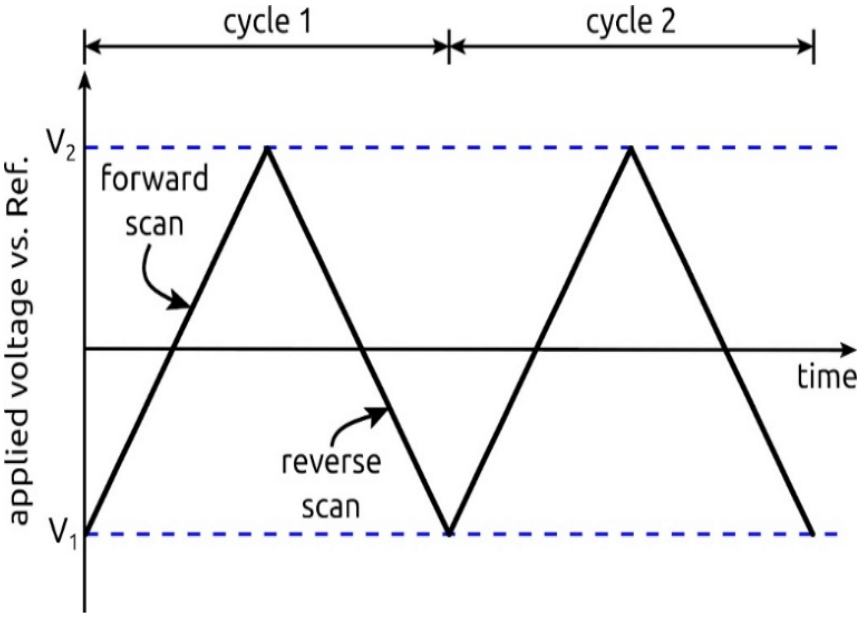


Fig.1.1. Potential excitation signal used for cyclic voltammetry by varying the applied potential as a function of applied potential.

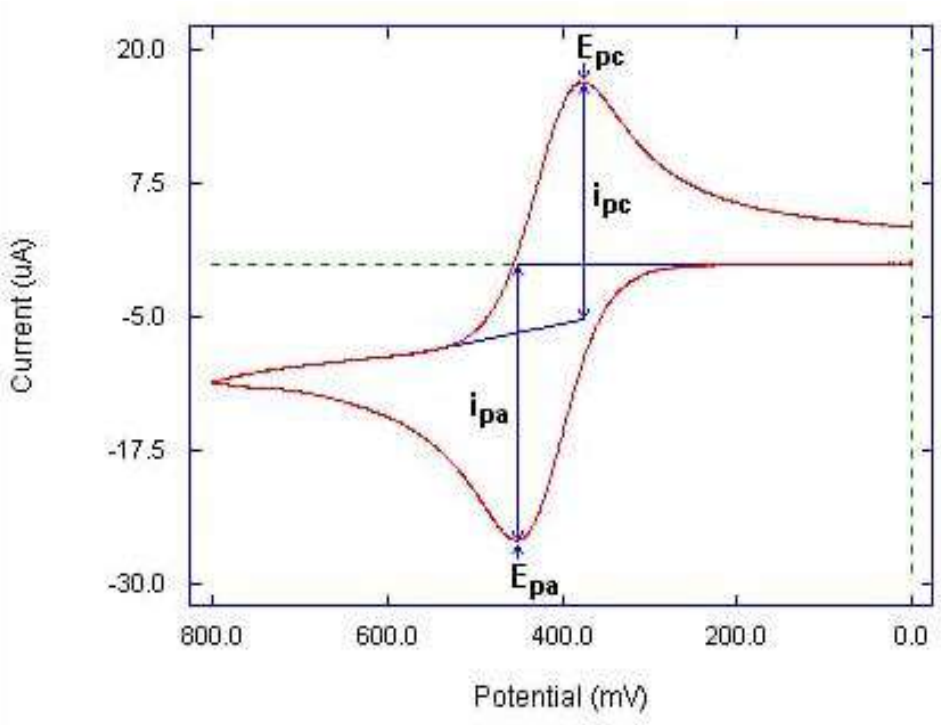


Fig. 1.2. Typical cyclic voltammetric response for reversible system.

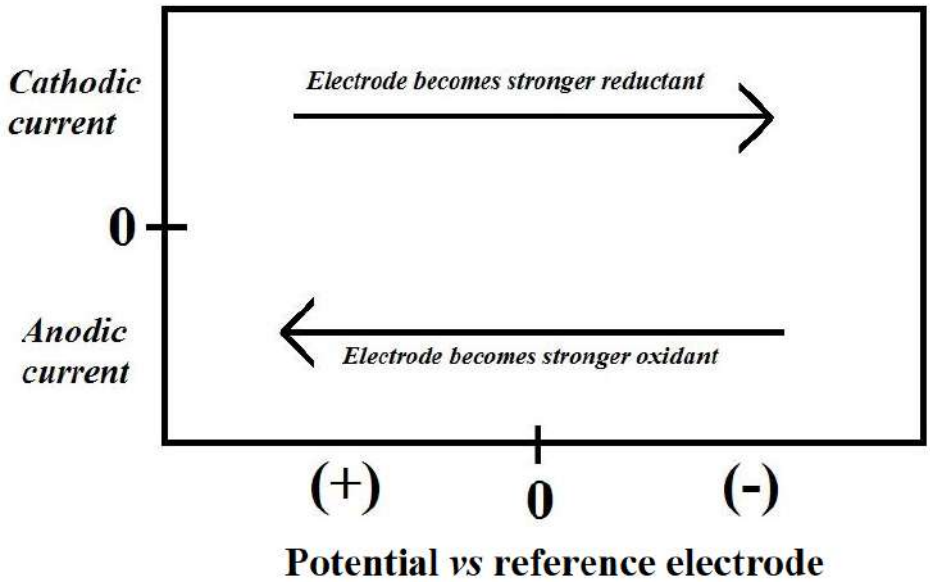


Fig. 1.3. Potential-current axes for cyclic voltammetry.

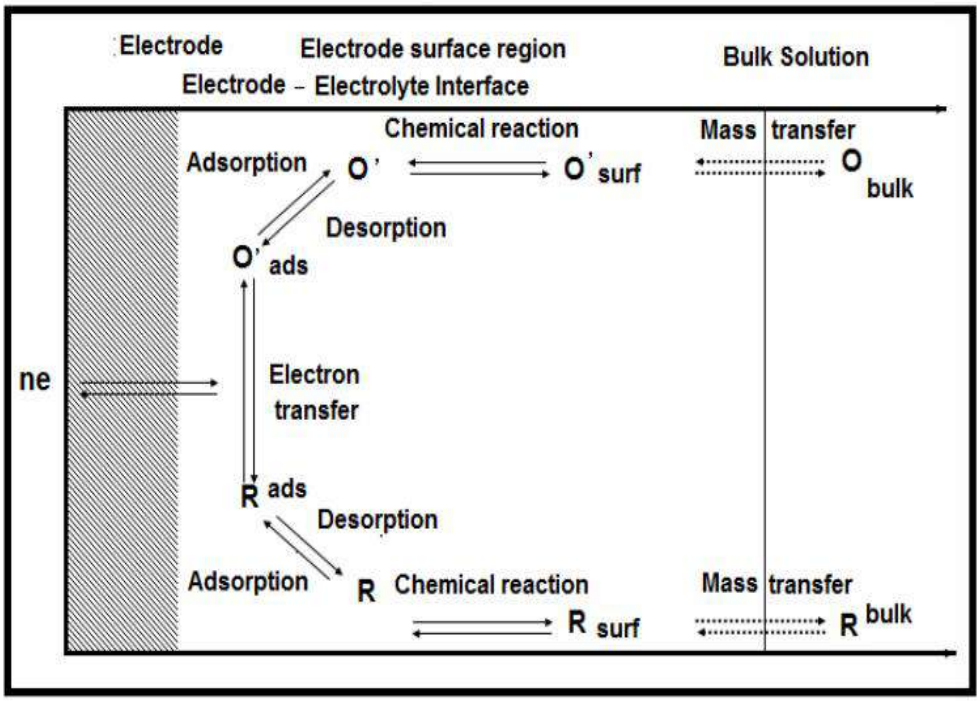


Fig. 1.4. Pathway of the general electrode reaction.

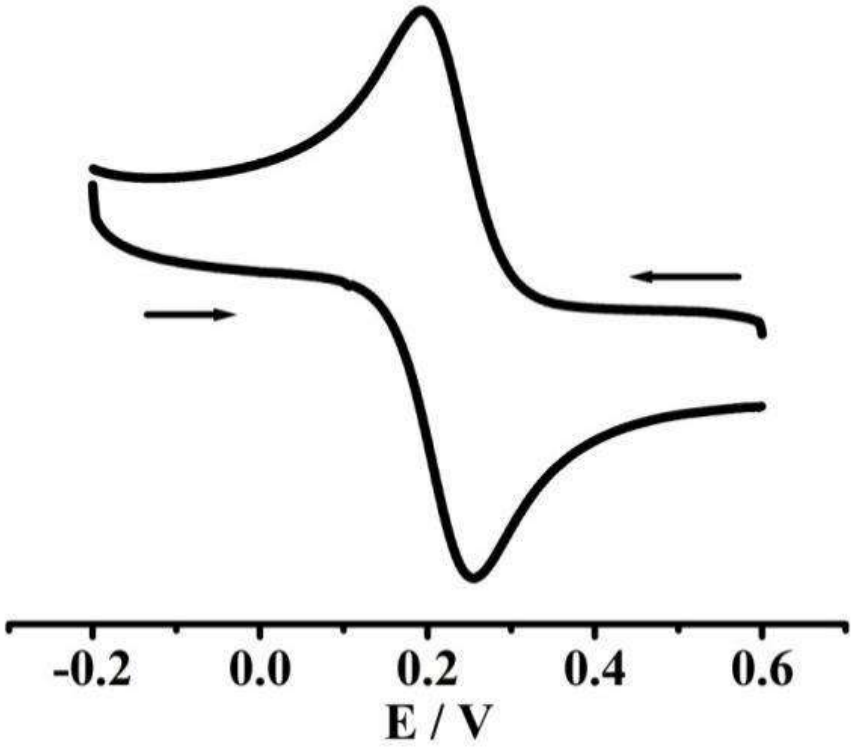


Fig. 1.5. Typical voltammogram for a reversible process.

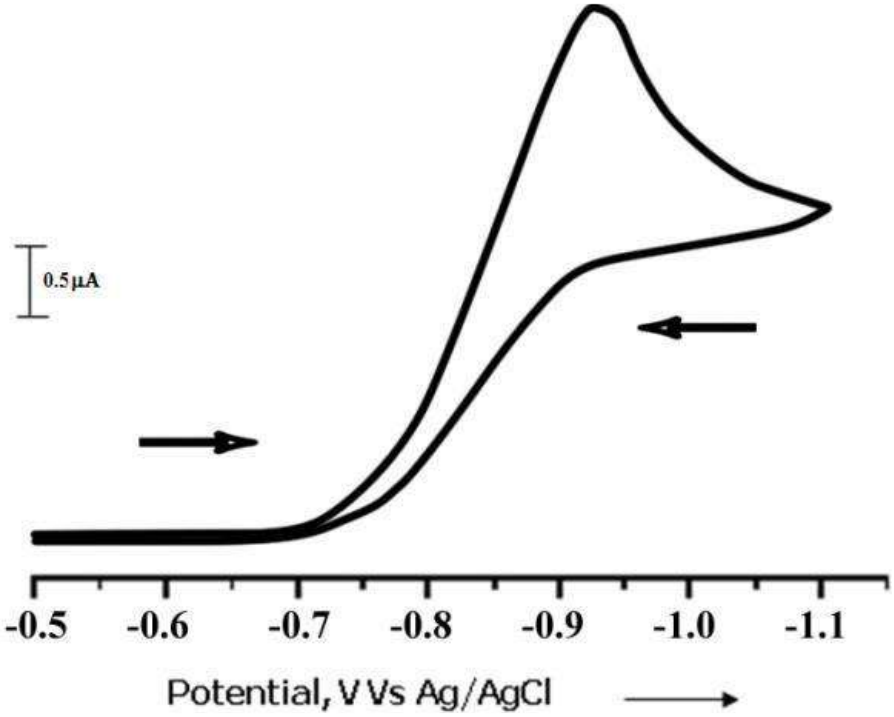


Fig. 1.6. Typical voltammogram for an irreversible process.

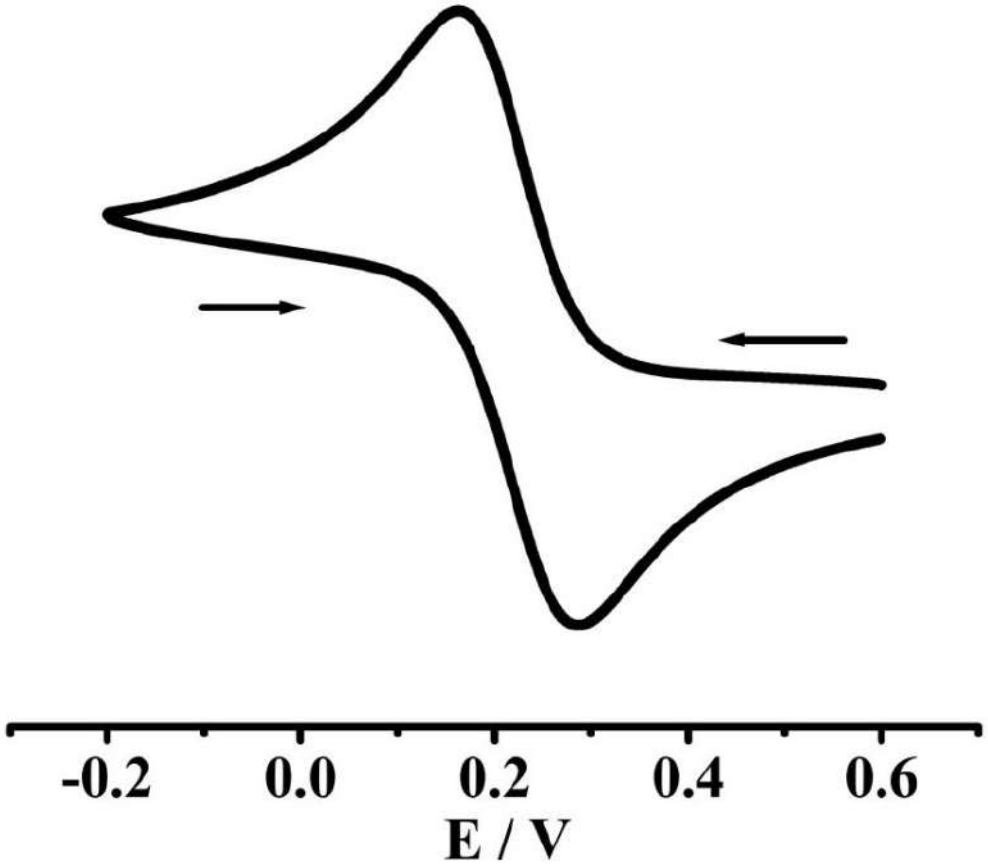


Fig. 1.7. Typical voltammogram for a quasi-reversible process.

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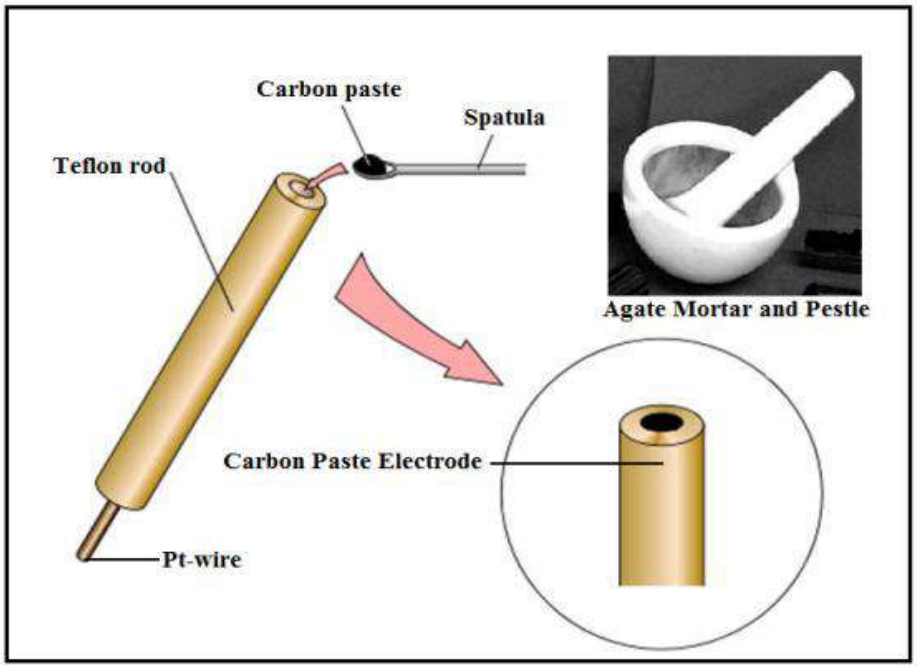
Chapter-II

EXPERIMENTAL



2.1. Introduction

The current chapter describes about the basic apparatus, instrumentation, experimental methods essential for the electrochemical measurements like an electrochemical cell, a recording device and a potentiostat. The electrode arrangements with specific attention on carbon paste electrode, pencil graphite electrode and glassy carbon electrode utilized in this research course is summarized. The preparation and characterization of nanomaterials, carbon paste electrode and the modification of the electrodes by several approaches such as electrochemical polymerization, blending and pre-treatment procedures were detailed.

2.2. Experimental Procedures

The prime electrochemical/analytical methods employed throughout this investigation were cyclic voltammetry and differential pulse voltammetry. Each technique is briefly defined below.

2.2.1. Cyclic Voltammetry

Cyclic voltammetry (CV) is a class of potentiodynamic electroanalytical device, popularly employed to examine the oxidation and reduction mechanisms of the electroactive species in solution that generates the electrochemical current response. Cyclic voltammetry is a method where the potential applied at the working electrode is linearly cycled between the two set potential values and variation in current is computed at the fixed rate defined as scan rate. The potential of the working electrode is ramped in the opposite orientation to return to the starting potential. This produces the cyclic impact and the cycles of potential ramps may be replicated as many times as required. It is a powerful approach for instantly get considerable information about the thermodynamics of the redox phenomenon for understanding the reaction intermediates, steadiness of the reaction products, energy levels of the analyte and the electron transfer kinetics. CV was especially exploited to study the electrocatalytic performances of bio-related molecules at unmodified and modified carbon based electrodes individually as well as being with the probable interfering compounds.

2.2.2. Differential Pulse Voltammetry

Differential pulse voltammetry is a pulse technique which is introduced in electrochemical investigations to enhance the speed and sensitivity of the voltammetric measurements. Differential pulse voltammetry is designed to minimize the background charging currents by decreasing the double layer capacitance to zero so that the recorded current is faradic. Related pulse type methods include normal pulse, differential and square wave voltammetry. In differential pulse voltammetry, each potential pulse is constant, of small amplitude and superimposed on a slowly varying base potential. The current is measured at two points of each pulse, at first just before the pulse is employed and the second at the end of the pulse. The difference between current measurements at these two points for every pulse is estimated and plotted as a function of base potential.

2.2.3. Scanning electron microscope (SEM)

Scanning electron microscope (SEM) is an electron type microscope designed for direct examination of the surface of solid materials. It generates images of the sample by scanning the surface with a focused beam of high energy electrons. The derived signals reveal the information about the specimen including texture (surface morphology), chemical composition, crystalline structure and orientation of materials of the sample. From the SEM observation one can characterize the heterogeneous nature of material on a nanometer (nm) to a micrometer (mm) length scale.

2.3. Instrumentation and basic equipments

In the modest Electroanalytical system for voltammetry, the basic device consisted potentiostat, computer, and the electrochemical cell show in Fig. 2.1. The potentiostat and computer are grouped into one set in certain cases and in other cases, the potentiostat can operate independently.

2.3.1. Potentiostat

The primary purpose of a potentiostat is to manage potential and measure current. The traditional three-electrode potentiostat is connected to the working, reference, and auxiliary (counter) electrodes dipped in the test solution placed in the

electrolytic cell. It controls the potential of the working electrode (WE), regarding the reference electrode (RE) while simultaneously measuring the current flowing between the WE and the auxiliary electrode (AE). The potentiostat accomplishes three functions:

I. Transforms the cell current to a voltage for recording devices.
II. Enables the current to pass between the WE and AE without passing current through the RE.
III. Controls the applied potential, which is the potential difference between the WE and RE.

A potentiostat must be efficient to impart the potential of the WE (for the RE) to the required level in a short enough time. The time occupied by the potentiostat for controlling the WE potential is referred as rise time. The potentiostat inner feedback circuits hinder all but a very limited current from streaming between the WE and RE Because, the fundamental characteristics of voltammetry is the control of electrode potential, a function generator is needed to provide the potential sweep or pulse sequence to be applied to the WE. Most modest potentiostat incorporated with a built-in sweep and/or pulse generator and those which are interfaced to a computer usually rely on the computer to generate the desired waveform. The inputs to the potentiostat are the connections to the electrodes in the cell. The outputs from the potentiostat are signal lines reflecting the current and potential of the WEs.

The electrochemical examinations were executed using potentiostat fitted out with the Data Acquisition PC interface Card Model CHI-660c (CH instrument-660 electrochemical workstation, USA). This device is able to practicing more than six Electroanalytical methods. The equipment comprises a high accuracy, speed and an electrolysis mode that contains high-grain operational amplifier with circuits for controlled potential. All the voltammograms were registered at an ambient temperature of 25±0.5◦C.

2.3.2. Recording device

Computers introduced to electroanalytical instrumentation in 1967 [1] or even earlier. Computer implementations in stationary electrode voltammetry [2, 3] and CV [2-6] were identified. Computers can be served to apply the potential program to the

WE through the potentiostat. Computers are very influential in data acquisition. The starting potential, final potential, speed rate, nature of the pulse, current sensitivity etc. may be commanded to the computer in the digital form. The applied potential values and the corresponding current values may be regenerated into digital information by an A/D converter and this upgrades the signal to noise ratio of the experimental cyclic voltammograms. Computers can replicate each experiment under similar conditions. Computers are utilized for data evaluation. It estimates 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 data thus collected such as peak current, peak potential and peak width at varied concentration may then be connected with theoretical predictions for confirming the type of processes and for computing the rate variables.

2.3.3. Electrochemical cell

The conventional electrochemical cell consisted three-electrode assembly was applied for electroanalysis in the present experiments. In its arrangement, the electrolytic cell is single compartment glassware capable of holding a proper volume of a test solution comprised with one or more electroactive components. The cell is then kept oxygen-free by passing nitrogen over the solution through a nitrogen inlet. The electrolytic cell associated with three electrodes that are immersed in this solution are electrically attached to the potentiostat. The SCE used as RE throughout the analysis which is separated from the solution by salt bridge to avoid contamination by leakage from the RE. The platinum wire was employed as AE in all measurements. The WEs (carbon paste, glassy carbon and pencil graphite electrodes) were directly placed into the solution (Fig. 2.2). Custom glassware designs include appropriate fittings for mounting electrodes, gas inlet and outlets. The current in any type of voltammetry is depends on temperature. Hence, the cell is thermostated for the desired temperature.

2.4. pH meter

The pH meter of Systronics Digital Model 335 was exploited to adjust and measure the pH of the solution which consist of a Special measuring probe of a combination of glass and saturated calomel electrode.

2.5. Electrodes

In this study, the three-electrode mode is utilised i.e. AE / WE / RE. The RE is a saturated calomel electrode (SCE) which is usually insulated from the solution by a salt bridge to inhibit contamination by leakage from the RE. The platinum foil as AE and WEs are carbon paste electrode or modified carbon paste electrode.

2.5.1. Reference Electrodes

In voltammetric experiments, the working electrode potential was always maintained with respect to some standard and that standard is the RE. The calomel electrode is most frequently used reference electrode for aqueous solutions along with that some other commercially available reference electrode in several of sizes and shapes.

2.5.2. Auxiliary Electrodes

The auxiliary (counter) electrode offers a substitute route for current flow so that only a very diminutive current flows through the reference electrode. Design choices are generally based on finding a material that is chemically inert in the specific test solution being explored. Thin platinum wire most often served as counter electrode. However, sometimes graphite and Au have also been used. If the electrochemical cell is composed from metal, then the cell itself might be acted as counter.

2.5.3. Working Electrodes

In electrochemical system the WE is the electron conductor on which the reaction of interest takes place. The interpretations of the voltammetric processes are highly affected by the working electrode components. Therefore, the choice of working electrodes depends on the redox behavior of the target analyte and the background current over the potential window required for the measurement. The main qualities of WE is it should permits the high signal-to-noise attributes. A further attention involves the potential window, electrical conductivity, surface reproducibility, mechanical characteristics, prize, availability and toxicity. A vast array of materials is implemented as working electrodes for electroanalytical

applications. The most popular solid inert WEs are Pb, Au, Ag, glassy carbon, graphite, carbon paste, vitreous carbon, diamond etc.

2.5.3a. Carbon Electrodes

Carbon based solid electrodes are more auspicious and has high prevalence in the recent circumstances for electroanalysis. They are extensively applicable mainly due to their vast potential window, small background current, affordable with ease of fabrication, chemical inertness and efficiency for diverse sensing and detection applications. Unlike, the rate of electron transport is slower at carbon surfaces as compared at metal surfaces. The activity of electron transfer is greatly influenced by the structure of carbon surface. The most common carbon-electrode materials are glassy carbon, carbon paste, carbon fiber, carbon films, and graphite pencil and carbon composites.

2.6. Carbon paste as electrode material: Major Developments

The carbon paste electrodes are a peculiar type of heterogeneous electrode. At the beginning electrochemistry has been connected to carbon paste electrode by Adams in 1958[10]. The carbon paste electrode and their modification underwent an appealing growth in the biosensing and Electroanalytical field. Initially, the CPE was helpful in examining the mechanisms of electrode reaction of distinct organic compounds [11]. The carbon paste electrode was first modified in 1964, in which an organic compound was dissolved with binder [11] and this MCPE was accommodate to inspect the electrode actions of the substance itself, was believed as a pioneering step in the field of CPE. In 1965, CPE was made by rubbing a modifier into the paste had represented its case with which a CPE could be modified [12]. The replenishment of non-electroactive pasting liquids by electrolyte solution [13] in 1974, a new branch of carbon paste electroactive electrodes were unfolded. Which at present belong to a special field called solid state electrochemistry [14]. The age of chemically modified electrodes concluded at the beginning of 80's. Modification of carbon paste by impregnating the carbon particles with methanolic solutions of dimethyl glyoxime [15] designates another benchmark in the history of CPE.

2.7. Carbon (Graphite) Powder

Finely powdered carbon (graphite) is a vital ingredient in carbon paste that assures the accurate functions of a sensor or an electrode in electrochemical computation. The features of graphite powder and pasting solution and its ratio greatly impact the practical aspects of CPE. Suitable carbonaceous components should fulfil the following guidelines:

- Uniform distribution of the particles.
- The size of the particle should be in the range of micrometers.
- High chemical purity.
- Low adsorption capabilities.

Naturally, the nature and characters of graphite used, as well as its overall amount in the carbon paste combination, are considers in all typical properties of the respective mixture. From the initial age of CPEs up until now, the most frequently chosen carbon powder is spectroscopic graphite with particles in the low micrometric scale (typically, 20-50 μM).

2.8. Pasting Liquids (Binders)

The pasting liquids are the crucial moiety of carbon paste co-determines its particularities. The optimal variables needed for pasting liquids should be as follows,

- It must shows high chemical and electrochemical inactiveness.
- It should have high viscosity and less volatility.
- Immiscibility with aqueous solution and non conducing

The well-known binding agents consumed are mineral oil like nujol and diverse silicone oils. Different organic esters such as tri cresyl phosphate [16-18], di-octyl phthalate, and di-nitrophenyloctyl are also applied as pasting liquids. Higher the ratio of binding agents, slower the surface kinetics and led to higher overvoltage than homogeneous electrodes. This is because of the hydrophobicity of the liquid that inhibits the accessibility of the analyte towards the surface[19-23]. However, the pretreatment can reduces the degree of surface hydrophobicity.

2.9. Bare (unmodified) carbon paste

The commixture of carbon powder and viscous liquid of non-electrolytic character with an adequate ratio is designated as bare (unmodified) carbon paste [24]. The exact electroactive element in carbon pastes is graphite powder with micrometric (20-50 μM) particles of superior purity and dispersion regularity. Such materials are now normally procurable in the market as spectroscopic graphite's. Non-electrolytic organic binders such as Nujol [25-27] and Silicone oil [28] are non-polar highly viscous pasting liquids that satisfy all the eminent standards; both are chemically inert, insulating, non-volatile, water-immiscible, and forming paste mixtures of fine texture. Liquid organophosphate binders have also been exploited. Though they have a fascinating attributes like high ion-pairing ability, they are less firm and rather a typical signal-to-noise characteristic requires special pre-treatments.

2.10. Major purpose for the modification carbon paste

The basis of modified carbon paste is normally a blend of powdered graphite and non-electrolytic binder and the constituent is modifier [29]. The motive to modify an electrode matrix is to get a new sensor with required, often pre-defined particularities. Chemically modified carbon paste carries conductive substrates modified with electroactive thin films, monolayers, or thick coatings. The modified carbon paste electrodes are developed for a specific application which is impractical with a bare carbon paste electrode. Modification of the carbon paste may elevated the electron transfer rate. These modifications involve mechanical crushing, self-assembled layers, covalently bonded electrolyzers and surfactant immobilization, etc. Surface modifications be of considerable interest and very small alterations in surface qualities ascertains the sensibiliity of measurement in electroanalytical applications.

Modified surfaces in general result in the following,

- Principally, to eradicate the fouling of the electrode surface.
- To transfer physicochemical properties of the modifier to the electrode.
- Improvement in the electrocatalytic activity, due to the use of materials with a higher surface area which in turn gives excellent current sensitivity.

- Selectivity and reproducibility towards analyte due to immobilized functional groups and dopants.
- Fast diffusion kinetics in the case of some material

2.11. Design and construction of carbon paste electrode

The suitable formation and pattern of CPE are founded on a short Teflon rod (structures as a strong plug) with a proper drilled in and a copper wire which connected with external circuit to supply electrical contact. The cavity of the Teflon rods can be easily re-filled with a new portion of carbon paste every time (Fig. 2.3) [23, 24]. Different type of glasses, PVC tubes, simple preparations equipped with a piston for extrusion of the paste are also generally used [25-27]. For standard CPEs, the substantial diameter of the cavity forming the suitable carbon paste surface is being selected from 2 to 10 mm, which is appropriate for a most of the electrochemical investigations [28].

More fascinating models of CPEs are usually described in association with carbon paste-based flow cells [29] Coulometry [30] electrochemical detectors [31] Amperometry [32, 33] and potetiometric [34] sensors. For instance, electrochemical interpretations on the alteration of the electrode response can be conducted with periodically renewed carbon paste utilizing a special cell with doubled carbon paste filling [35]. Among others, such a structure with intimate surface renewal is very potent in the evaluation of organic and bio-essential molecules where the surface of an electrode may easily be fouled either with matrix constituents or by electrode reaction products [36].

2.12. Uniqueness of CPE

The following reasons made CPEs special among all other WEs

- Effortless procedure for the fabrication.
- Low background current (below 1μA).
- Easily renewable surface.
- Higher compatibility to numerous kinds of modifiers.
- Cost effective and wide potential window.

- Possibilities of distinct modification practice with the accomplishment of low detection limit up to nanomolar.

2.13. Graphite pencil electrode employed as working electrode

Pencil graphite electrodes (PGE) is a carbon based novel type of electrode materials which has been adopted for electroanalytical quantification of various types of bio significant matrices [37-39]. Pencil electrodes increasingly earned traction with their superior electrical, mechanical, biocompatible and physical properties. Thanks to exceptional adaptability, portability and easy method of utilizing without any pre-concentration stages.

2.14. Glassy carbon electrode used as working electrode

Glassy carbon electrode (GCE) was very predominant due to its outstanding electrical and mechanical affluence, extended potential window, solvent resistance and relatively reproducible achievements. Glassy carbon was initially operated as sensing platform by Zittel and Miller [40] numerous workers continues to use the electrode. The qualities of glassy carbon have been stated by Yamada and Sato [19].

2.15. Electrochemical model systems for characterizations of CPE in voltammetry

2.15.a. Potassium ferrocyanide system

The properties of the electrode surface can be probed by its influence on the speed of electron transfer. This can be confirmed qualitatively by reviewing the peak potential difference in cyclic voltammogram of a substance whose electron transport kinetics are familiar to ne sensible to the state of the surface. A traditional potassium ferrocyanide ($[Fe(CN)6]^{4-}$/$[[Fe(CN)6]^{3-}$) redox probe was employed as standard to examine the overall surface assets of the CPE.

2.15.b. Estimation of surface area of the electrode

The availability of reactive surface area for electrode phenomenon was demonstrated using potassium ferrocyanide probe in supporting medium of 1M KCl. The speed rate impact on current signals of 1mM potassium ferrocyanide has been

tested at 0.05 V/s to 0.5 V/s. For a reversible redox couple, the number of electrons involved in the electrode reaction can be depicted by the separation between the peak potentials $\Delta Ep = Epa-Epc/n \approx 0.059$ V. The value obtained between 0.061 to 0.066 V which signifies to one electron. Also, the Ipa/Ipc ratio was got close to be one (0.9905) which is a typical action displayed by a reversible electrochemical charge transfer. The surface area of the electrode can be evaluated using ensuing equations [41, 42].

$$Ip = (2.69\times10^{5})\, n^{3/2}\, A\, D_0^{1/2}\, C_0\, \nu^{1/2}$$

Where, Ip is the peak current, A is area (cm^2) of working electrode, C_0 is the concentration (mol/cm^3) of the electro active substance, ν is sweep rate and D_0 is diffusion co-efficient (cm^2s^{-1}).

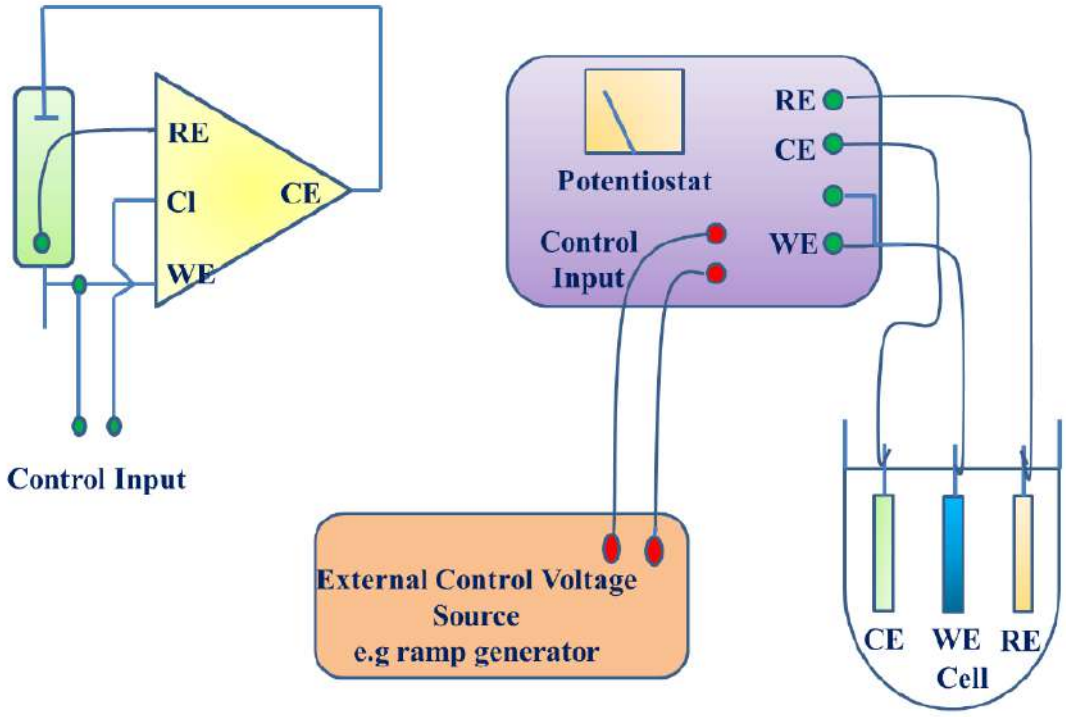


Fig. 2.1. Experimental set up consisting of an external control voltage source, a potentiostat and the electrochemical cell.

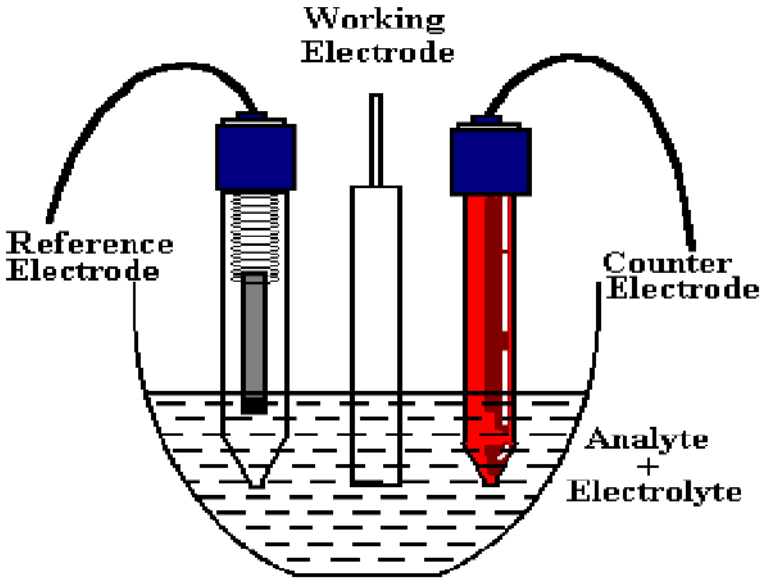


Fig. 2.2. Graphic representation of an assembled electrochemical cell includes an electrolyte solution and three electrodes (WE, RE and AE) for CV determination.

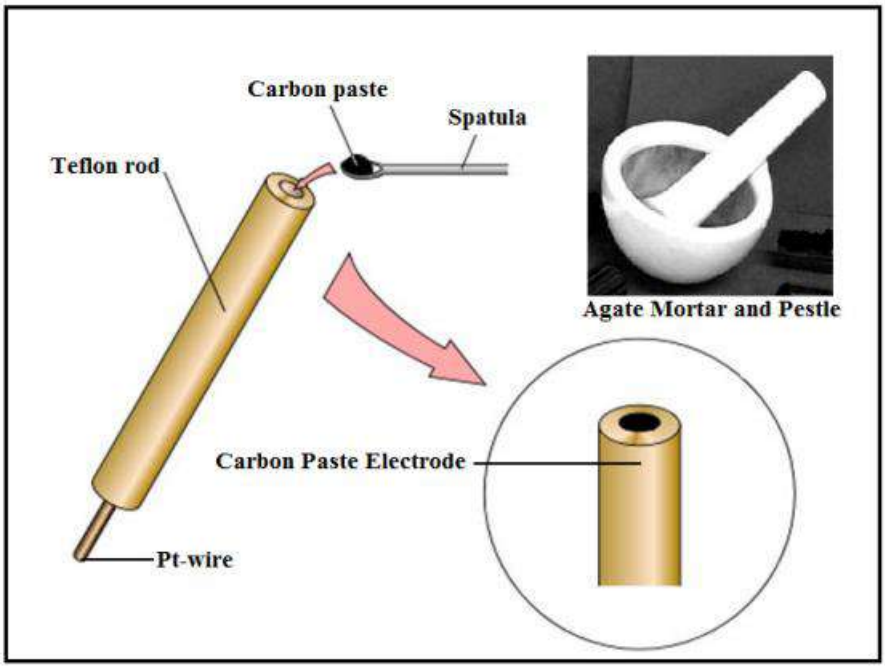


Fig. 2.3. carbon paste electrode preparation and packing.

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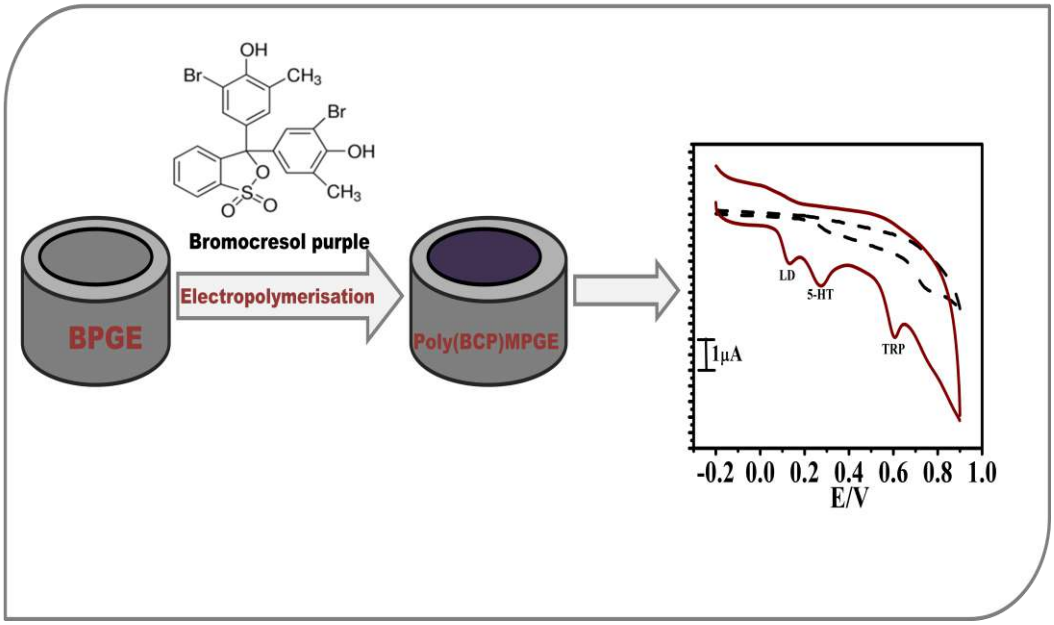
Chapter-III

PART-A

POLY (BROMOCRESOL PURPLE) INCORPORATED PENCIL GRAPHITE ELECTRODE FOR CONCURRENT DETERMINATION OF SEROTONIN AND LEVODOPA IN PRESENCE OF L-TRYPTOPHAN: A VOLTAMMETRIC STUDY



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3.1. Introduction

The exploitation of pencil graphite leads (PGE) as a sensing element and their great success profoundly influenced the field of biosensors. It opens new approaches of research for the detection of materials and bio-matters. Pencil electrodes increasingly earned traction with their superior electrical, mechanical, biocompatible and physical properties. Thanks to exceptional adaptability, portability and easy method of utilizing of pencil electrode in relatively complex samples make them one of the cornerstones of analytical electrochemistry [1-7]. A variety of analytical methods are known to be useful for the discrimination of biologically as well as environmentally beneficial entities such as high performance liquid chromatography, electrophoresis [8], photo kinetics [9] and spectrophotometry [10]. Even though the reported methods are completely validated, they possess a lower selectivity, highly expensive equipments and longer time of analysis. Although, the electroanalytical techniques have been considered as promising strategies to overcome all the above mentioned difficulties as it provides superior selectivity, sensibility, easy method of amplification and fast response. Also serves as eco-friendly because of the less consumptions of organic solvents [11-15].

Serotonin (5-Hydroxytryptamine, 5-HT) is an eminent biogenetic neurochemical that mainly identified in blood platelets, bowels, intrinsic and central nervous system of the brain. 5-HT is produced by essential serotonergic neurons and body ulitlises it to relays the information between the nerve cells and regulates their intensity. The biological role of 5-HT is complex and diversified [16-18]. 5-HT along with the other monoamine neuromodulators plays a leading role and contributes in mood modulating, reward, learning, cognition and numerous physiological functions like emesis and vascocontradiction [19-22]. Also serves as precursor for melatonin and thereby regulates the sleep-wake cycle and body clock [23].

L-dopa, also familiar as levodopa (LD) is a peculiar amino acid that produced and utilized as a portion of normal biology of humans, animals as well as in plants [24-26]. LD is a psychotropic drug and naturally derived dietary supplement found in specific type of herbs and foods. It is prepared by a way of biogenesis of nonessential amino acid L-tyrosine in brain and body of mammalian system. LD serves as

precursor for dopamine and used as medicine to cure the Parkinson's disorder by raising the dopamine levels in the brain. LD also employed in the medication of identical muscular conditions when they induced by drugs such as fluphenazine, chlorpromazine and others [27-28].

Tryptophan(2-amino-3-(1H-indole-3-yl)-propionic acid, TRP) is one of the crucial amino acid for mankind and herbivores. It is an indole molecule not naturally synthesized by the living organisms and also not sufficiently found in vegetables, it may be governed by the form of medicines and food supplements. It serves as a precursor for variety of neurochemicals such as serotonin, melatonin and niacin [29, 30]. It is a fundamental element of proteins and obligatory in human nutrition for fixing and sustaining of positive nitrogen balance. If the body fails to metabolize it correctly, then it is possible to cause schizophrenia and the hazardous waste product of TRP in the brain can leads to hallucinations and delusions [31, 32]. All 5-HT, LD and TRP concurrently exists in body fluids and their uneven concentration leads to severe heath issues it has been necessary to develop a accurate process for the determination of these molecules to secured a healthier and disease free living.

Bromocresol purple (Scheme 3.1) is a triphenylmethane dye and functioning as a pH indicator and also consumed to assess albumin in medical laboratories [33]. Electrochemical polymerisation is an influential method of depositing a polymer layer as it provides control over layer thickness, charge transfer and permeation [34]. The polymer film deposited electrode gets wide consideration in the construction of catecholamine sensors because they assures the foul-free electrode surfaces, greater stability, reproducibility, strong adhesion to the electrode and effective passivation [35, 36]. Herein, a uniform film of BCP was electro polymerised on BPGE surface which shows better electrochemical applications.

3.2. Material and Methods

3.2.1. Reagents and apparatus

The analytical purity chemicals were utilized throughout the analysis and employed as provided without further modification. 5-hydroxytryptamine (5-HT), L-dopa (LD) and L-tryptophan were acquired from Himedia, Mumbai, India. Graphite pencil leads (PGEs) were obtained from Camlin. The bromocresol purple (BCP) was

procured from Sigma, Bangalore, India. The chemicals used for the buffer preparation i.e. Disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4) were replevied from Sigma Aldrich, Bangalore, India. Doubly demineralised water was used to prepare all aqueous stock solutions and 0.5M HCl was employed to prepare L-dopa stock solution.

The computer controlled voltammetric equipment of model CHI-660C potentiostat (CH-instrument-660c electrochemical workstation) was opted for all the electrochemical evaluations and data extraction. The traditional three electrode single component glass cell was used which consist a saturated calomel(SCE) reference electrode, platinum foil counter electrode and bare and poly(bromocresol purple) modified pencil graphite working electrode. The surface characteristics of BPGE and Poly(BCP)MPGE were marked by SEM measurements using ZEISS Ultra-55. All the determinations were operated at room temperature.

3.3. Results and discussion

3.3.1 Fabrication of poly (BCP) embedded graphite pencil electrode

The poly(BCP)MPGE was primed via CV method followed by simple electropolymerisation process. Fig. 3.1A renders the CVs listed for electro-polymerisation of 1mM bromocresol pruple on PGE surface consisting of 0.1M NaOH as supporting electrolyte and cycling the potential between -0.2V to +1.8V having the sweep rate of 100 mV/s for 20 multiple cycles. During the successive scans, the peak current descended with raise in cyclic time affirms that the configuration of poly (BCP) layer on the bare surface of PGE. The thickness of Bromocresol purple layer directly affects the electrocatalytic action of modified sensor. It was known that the increased thickness slow down the electron transfer due to lacking of active sites on the electrode. However, the thickness of polymeric membrane can be optimised by fluctuating the number of cycles from 5-25 cycles. Inset Fig. 3.1B illustrates the graph of anodic peak current of 5-HT and LD with number of polymerisation cycles. The extreme current signal was ascertained at 20 scans. Hence it was chosen as optimal for further electroanalysis of 5-HT and LD and determination of all variables. After each electropolymerisation, the MPGE surface was sluiced with demineralised water to expel the unreacted molecules of modifier.

3.3.2 Characteristic property and surface area of working electrodes

The morphological characteristics of working electrodes were assayed by SEM analysis. Fig. 3.2A and 3.2B denotes the SEM images captured for BPGE and Poly(BCP)MPGE. From the images it can be clearly observed that the larger number of non-uniformly distributed flakes of bromocresol purple on the surface of BPGE and it indicates the formation of poly(BCP) film and successfully enhances the surface properties of the modified working electrode.

The ferrocyanide system was most frequently used for checking the adequacy of the developed working electrodes using CV technique. Fig. 3.3 depicts the voltammogram registered for 1mM freshly prepared potassium ferrocyanide ($K_4[Fe(CN)_6]$) sensor probe in existence with 1M KCl supporting electrolyte with 50 mV/s sweep rate at BPGE(dashed line) it displays the poor voltammetric response and wide difference in redox peak potential(ΔEp). The tremendous increment in the peak current and substantial reduction in ΔEp noted at poly(BCP)MPGE(solid line). This outcome implicit the change in surface functionalities and more rapid electron transfer kinetics of the modified sensor. The active surface area accessible for the reaction can be quantified using Randles-Sevick's Equation (1) [37].

$$Ip = (2.69\times10^5)\, n^{3/2}\, A\, D_0^{1/2}\, C_0\, \nu^{1/2} \quad \text{..... (3.1)}$$

The effective surface area values quantified to be 0.021 cm^2 for BPGE and 0.051 cm^2 for poly(BCP)MPGE inferred that the exceeded electrocatalytic features of fabricated electrode. An appropriate magnitude of embedded poly(BCP) film on electrode surface was accounted by using the expression(2) [38].

$$Ip = n^2F^2A\Gamma\upsilon/4RT \quad \text{……..(3.2)}$$

Where, Γ (M/cm^2) is the surface coverage concentration, n is the number of exchanged electrons. A and υ are scan rate and area of working electrode respectively. R, F and T are physical constants. The surface coverage concentration (Γ) on BPGE was found to be $0.0425\times10^{-11} M/Cm^2$.

3.3.3. Comparative voltammetric sensing of 5-HT and LD at unmodified and modified working electrodes

To track the enhanced electrocatalytic ability of developed sensors towards the oxidation of 5-HT and LD, the comparative study was conducted through the CV method. Fig. 3.4 and Fig. 3.5 interprets the CVs accounted for 10 μM 5-HT and 10 μM LD at BPGE (dashed curve) and poly(BCP)MPGE(solid curve) in assistance with 0.2M PBS supporting media of pH 7.4 having the sweep rate of 50 mV/s. The irreversible peak nature for both 5-HT and LD with wide anodic peak potential and an inadequate current sensitivity was noticed at BPGE. Despite that, the substantial enrichment in the anodic peak current of 5-HT and LD was noted at poly (BCP)/MPGE concerning that the rapidity in transfer of electron, an increase in the number of effective sites and excellent catalytic characteristic of sensor after modification. The adopted oxidation mechanism for 5-HT and LD was provided in Scheme.3.2 and scheme 3.3.

3.3.4. Effect of supporting electrolyte

It is requisite to optimise the pH of the supporting medium to obtain the excellent sensitivity because the anodic peaks current as well as peak potential of electro active molecules are greatly affected by pH of the solution. The impact of pH on voltammetric response of 10 μM 5-HT and 10μM LD were probed by employing 0.2M PBS of dissimilar pH ranges over 5.8-7.8 and the potential sweep rate was 50mV/s as illustrated in Fig. 3.6A and Fig. 3.7A. The graph testifies that the oxidation peak potential of 5-HT and LD were emigrated linearly to less positive potential values with raise in pH of buffer. This result proclaimed that the direct participation of electrons and protons in the electrochemical oxidation of 5-HT and LD at the surface of poly (BCP) MPGE. The potential diagram was plotted between the oxidation peak potential of 5-HT and LD with change in pH values. The appeared linear relation can be expressed as $Ep(V) = 0.7063 - 0.0568\ pH (R^2 = 0.99405)$ and $Ep(V) = 0.6530 - 0.06869\ pH\ (R^2 = 0.9975)$ provides the slope values of 56mV/pH and 68mV/pH for 5-HT and LD respectively. These slope values followed the theoretical Nernstian value for two electrons and two protons transfer reaction [39, 40]. Fig. 3.6B and Fig. 3.7B displays the graph of Ipa versus different pH proofed

that the Oxidation current of 5-HT and LD progressively raises as pH value increases upto pH 7.4 and above that pH, the peak current decreased. Although the sharper peak with excellent sensitivity for10μM 5-HT and 10μM LD (Fig. 3.6C and Fig. 3.7C) was accomplished at pH 7.4. So, the pH 7.4 was preferred as standard for further analysis.

3.3.5. Implication of Sweep rate on 5-HT and LD at poly(BCP)MPGE

The consequences of the potential sweep rate on peak currents of analyte species were confer the essential facts about the kinetics and mechanism of electrode process. Fig. 3.8A offers the CVs of 10μM 5-HT at distinct speed rate along with 0.2 M PBS of physiological pH at poly(BCP)MPGE. The amplitude of anodic peak current intensified with raise in scan rate in the range of 50-500mV/s with minute shift of peak potential to more positive side. The linearity relationship was obtained between the log of differed scan rates (log ν) versus log of oxidation peak current of 5-HT (log Ipa) and Ipa of 5-HT versus $\nu^{1/2}$ as presented in Fig. 3.8B and Fig. 3.8C. The ensued plot provides the slope value of 0.5970 (log Ipa (μA) = 0.5970 logν(mV/s) + 0.4792) imparts the ideal reaction requirement for diffusion controlled electrode phenomenon [41] and the regression coefficient (R^2) was found to be 0.98786. Fig. 3.9A reflects the CVs entered for 10μM LD at contrasting speed rates of 50-500mV/s. The augmented peak currents were registered with the raise in scan rate with a slight switching of anodic peak potential to positive direction. Fig. 3.9B and Fig. 3.9C presents the plot of log Ipa of LD versus logν and plot of Ipa of LD versus $\nu^{1/2}$ respectively. The resultant slope value was 0.6062(R^2 = 0.9895) shows closeness with the theoretical value of 0.5. Which confirms the diffusion controlled electrode process of LD at poly(BCP)MPGE[42].

3.3.6. Construction of calibration plot and limit of detection:

To analyze the linear dynamic range and sensing ability of formulated sensor, the impact of varied concentration of 5-HT and LD were valuated under optimal circumstances. Fig. 3.10A depicts the CVs of dissimilar concentrations of 5-HT by employing 0.2M PBS of pH 7.4 at poly(BCP)MPGE speckled from 10μM -70μM at the sweep rate of 50 mV/s. The remarkable enhancement in 5-HT peak current with raise in concentration was observed along with the shift in peak potential to more

negative side. The linearity was ascertained by plotting oxidation peak current of 5-HT versus concentration as shown in Fig. 3.10B. Likewise, the oxidation behaviour of LD at poly(BCP)MPGE was studied in 0.2 M PBS of pH 7.4 at scan rate of 50mV/s by changing thee LD concentration from 10μM-70μM presented in Fig. 3.11A. The esteemed upgrade in Ipa of LD with raise in concentration was perceived with displacement of peak potential to positive value from 136mVto 151mV. Fig. 3.11B interprets the plot of Ipa versus LD concentration. A calibration curve for both 5-HT and LD shows better linearity with regression coefficient (R^2) of 0.9945 and 0.9926 respectively. This consequence suggested that the proportionality between peak current and concentration of analyte species. The LOD and LOQ for 5-HT and LD were figured to be 0.49μM, 1.65μM and 2.3μM, 7.6μM respectively trough sequential expression [43].

$$LOD = 3\sigma/M \quad (3.3)$$

$$LOQ = 10\ \sigma/M \quad (3.4)$$

Where, σ denotes standard deviation and M is slope from calibration curve. The Reduced LOD values are compared with other modified electrodes in Table.3.1 and Table3.2.

3.3.7. Selective investigation of 5-HT, LD and TRP at Poly (BCP)MPGE

Since 5-HT, LD and TRP were coincide in biological solutions, the concurrent detection of these molecules in the combined solution was often tricky at unaltered electrode by means of closeness in peak potentials. To certify the effectiveness of the formulated sensor, the electrocatalytic behaviour of 5-HT, LD and TRP were examined by CV and DPV methods. Fig. 3.12 and inset Fig. 3.12 portrays the CVs and DPVs entered for uniform mixture of 10μM 5-HT, 10μM LD and 10μM TRP in presence of 0.2M PBS of pH 7.4 at BPGE (dashed curve) and Poly(BCP)MPGE (solid curve) about the sweep rate of 50mV/s. At BPGE the imperceptible oxidation signals of 5-HT, LD and TRP were observed and thereby it is impossible to identify the peak potentials from the coincided and wide voltammetric signals. However, at the same circumstances, poly(BCP)MPGE was able to resolve the voltammetric signals into three precisely defined peaks and the separate anodic potential for 5-HT, LD and TRP

were spotted at 277, 131 and 605 respectively. DPV provides the distinguished oxidation potentials at 232, 77 and 56 for 5-HT, LD and TRP respectively and the corresponding peak to peak separation of 5-HT to LD and 5-HT to TRP were found to be 145 and 330. These results concluded that our modified electrode has potency for specific and simultaneous detection of these molecules in their uniform mixture.

3.3.8. Interference study

The anti- interference stability of developed sensor was paramount and it was practiced by highly efficient DPV technique. The individual determination of 5-HT, LD and TRP in their single solution was conducted by varying the concentration of any one of the analyte at poly(BCP)MPGE bearing with 0.2M PBS of pH7.4 and the scan rate was 50mV/s. Fig. 3.13A interprets the DPV curves recorded for the trinary combination of 5-HT, LD and TRP in which the 5-HT concentration was adjusted and holding the constant concentration of LD (10μM) and TRP (10μM). The peak current intensity of 5-HT enlarged in the range of 10μM -50μM. Likewise, the concentration of the LD speckled in the sequence of 10μM-50μM and other two analytes was reserved as illustrated in Fig. 3.13B. The identical procedure was repeated for TRP (from 10μM -50μM) by maintaining the steady amount of 5-HTand LD (Fig. 3.13C). The inset figures illustrates the obtained linearity relation by plotting the Ipa versus varied concentration of 5-HT, LD and TRP. This outcome signifies the commendable selectivity of poly(BCP)MPGE approaching the sensing of 5-HT, LD and TRP.

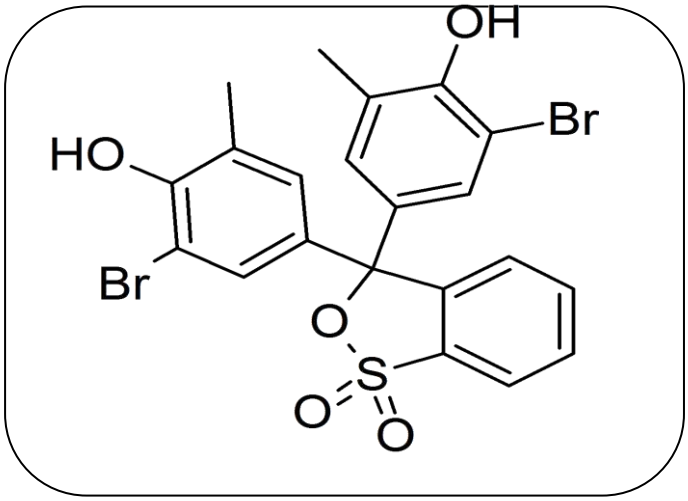
3.3.9. Stability and practical application of fabricated electrode

The stability of poly (BCP) MPGE was verified by measuring the current signals of a mixed solution with fixed concentration in physiological pH. The electrode was repeatedly scanned for 30 cycles having the sweep rate of 50mV/s as presented in Fig. 3.14. The unchanged peak potentials with small reduction peak currents were observed and the percentage degradation was appraised by the expression, % degradation = Ip_n/Ip_1 [44] where, $Ip_1$and Ip_n denotes the first and n^{th} oxidation peak current respectively. The stability recollected for poly(BCP)MPGE was found at 90.5 %, 85.38% and 86.56% for5-HT, LD and TRP respectively, which ensures the constancy of modified sensor. The practical utility of designed poly(BCP)MPGE was examined in the serum sample by standard addition process.

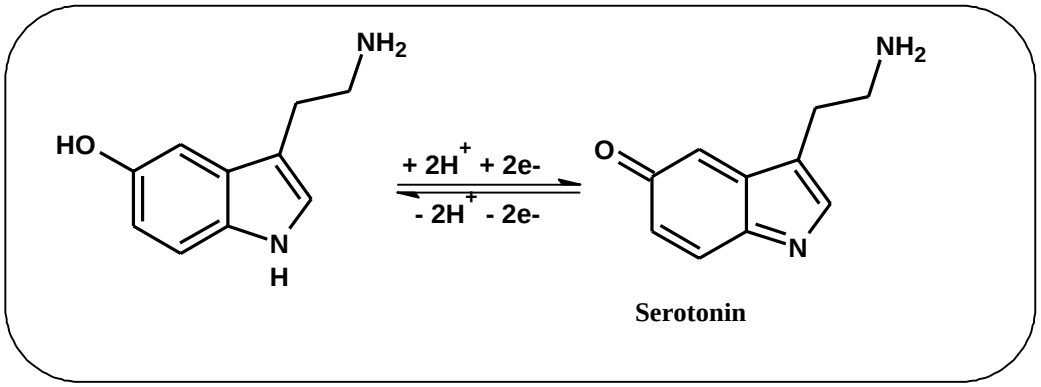
The admirable recoveries were obtained as revealed in table 3.3. This outcome concerned the profitability of the proposed sensor in biological field.

3.4. Conclusion

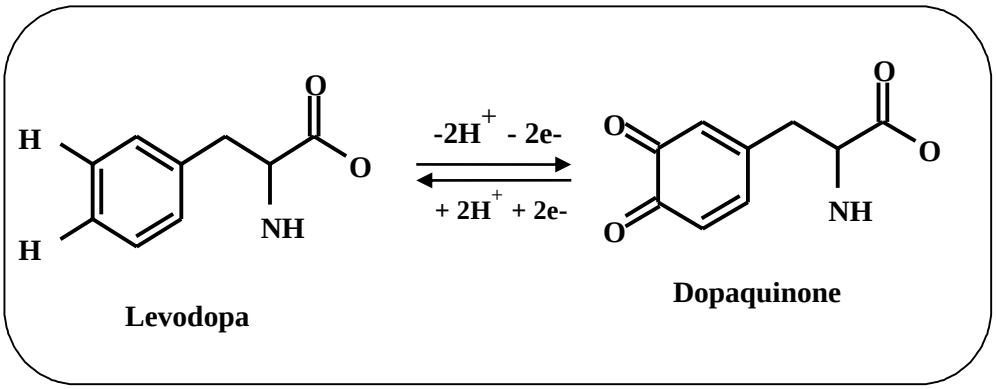
This study reports the formulation of bromocresol purple based facile electrochemical device made by simple electropolymerisation on pencil graphite electrode. The proposed sensor depicts the justifiable analytical efficacy, stability, selectivity and sensitivity with respect to the specific and concurrent determination of 5-HT and LD in presence of L-TRP at physiological pH. The poly(BCP)MPGE effectually solved the overlapped current signals of target analytes into well distinguished peaks by CV and DPV methods. The overall electrochemical investigation shows that the diffusion controlled kinetic phenomenon of the modified electrode and successfully reduces the detection limit. The practical utility of customised sensor was examined in real sample provides agreeable recoveries. All these outcomes recommended that the designed electrode is favourable for bio-sensing applications.



Scheme 3.1. Structure of Bromocresol purple



Scheme.3.2. Electrochemical oxidation mechanism of Serotonin.



Scheme 3.2. Electrochemical oxidation mechanism of Levodopa.

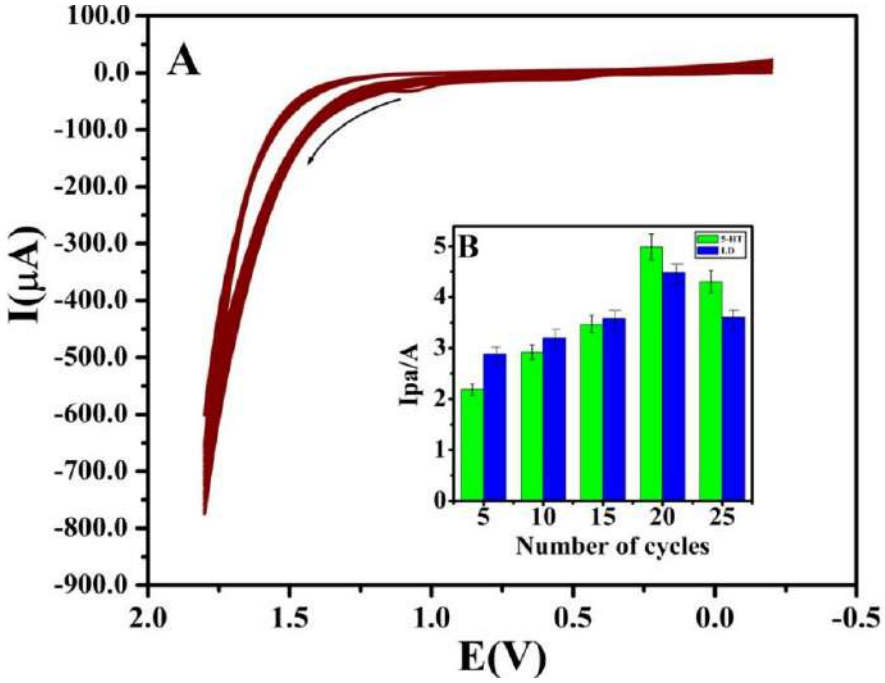


Fig. 3.1A. CVs for Electropolymerisation of 1mM BCP on surface of PGE in presence of 0.1M NaOH at 20 multiple cycles with sweep rate of 100 mVs^{-1}. **B.** Plot of anodic peak current (Ipa) of 5-HT and LD versus number of polymerization cycles.

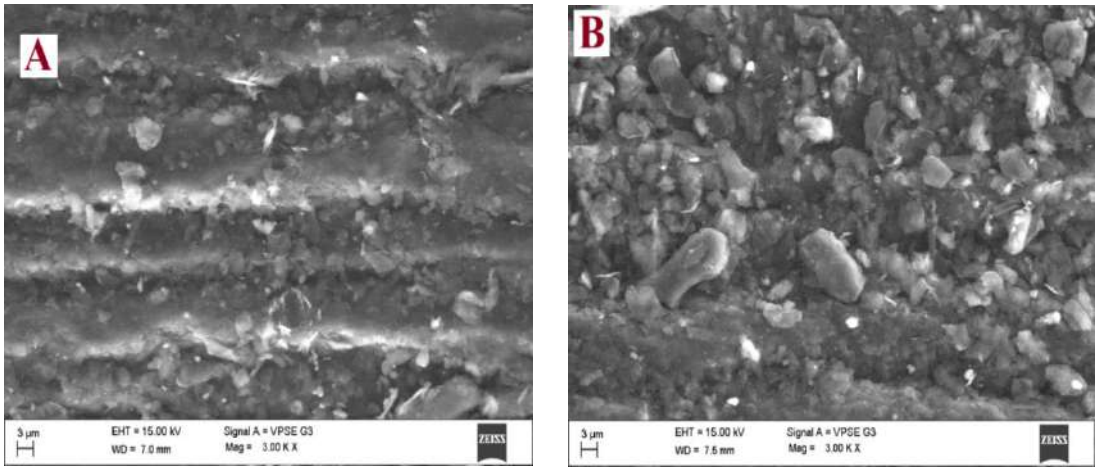


Fig. 3.2. SEM images for BPGE**(A)** and Poly(BCP)MPGE **(B)**.

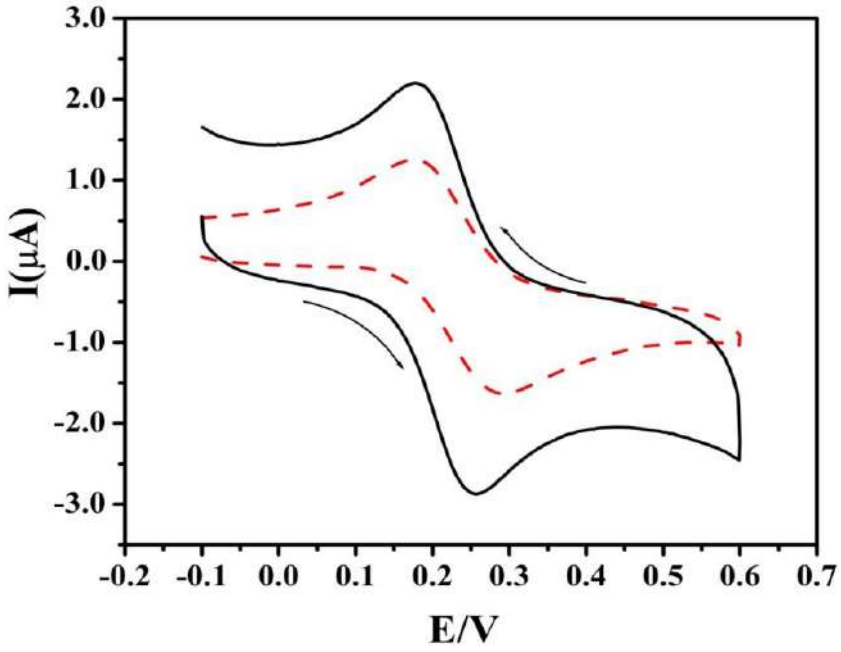


Fig. 3.3. CVs recorded for 1mM $K_4[Fe(CN)_6]$ in 1M KCl at BCPE(dashed line) and Poly(BCP)MPGE (solid line) at sweep rate of 50 mV/s.

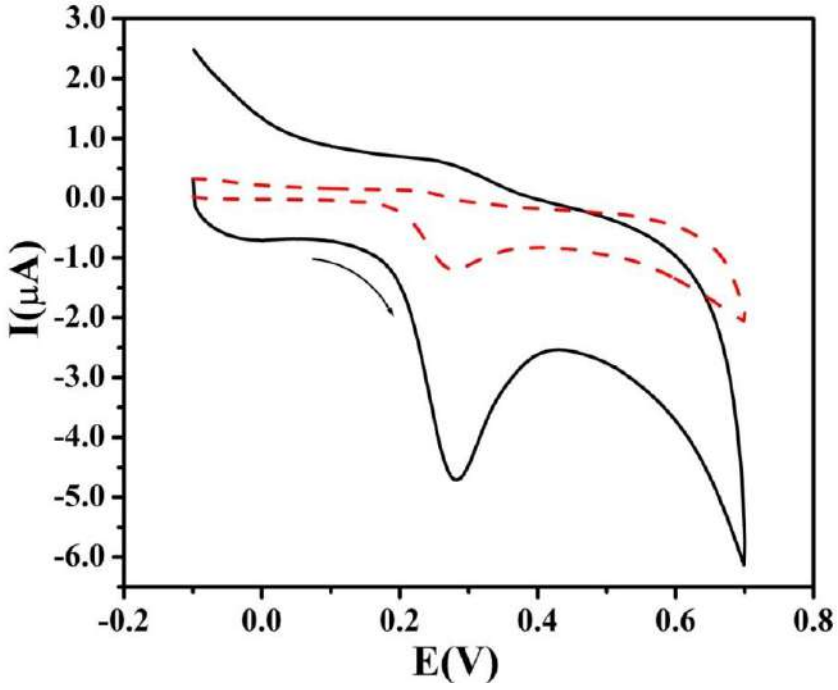


Fig. 3.4. CVs of 10 μM 5-HT in 0.2 M PBS of pH 7.4 at BCPE (dashed line) and Poly(BCP)MPGE (solid line) at sweep rate of 50 mV/s.

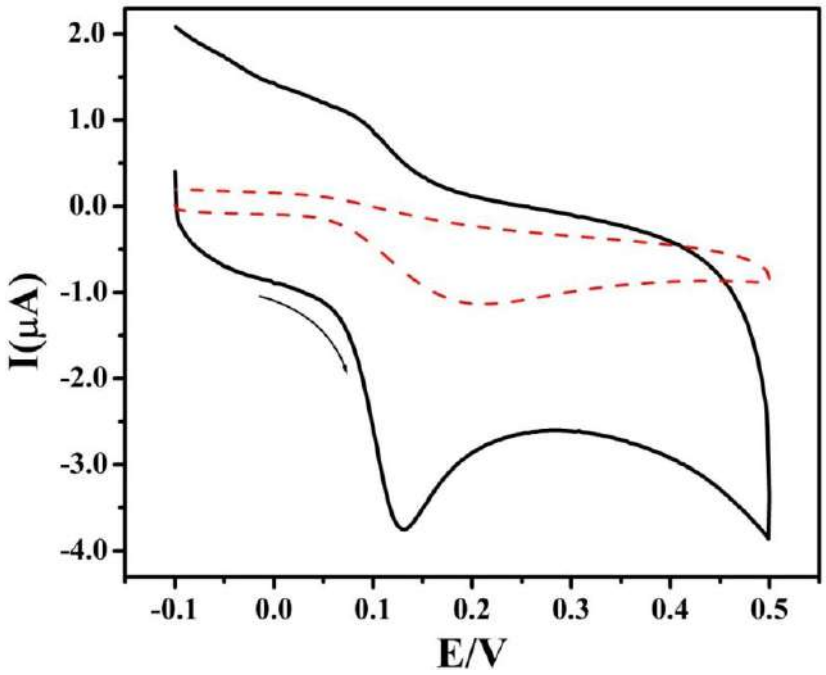


Fig. 3.5. CVs of 10 μM LD in 0.2 M PBS of pH 7.4 at BCPE (dashed line) and Poly(BCP)MPGE (solid line) at sweep rate of 50 mV/s.

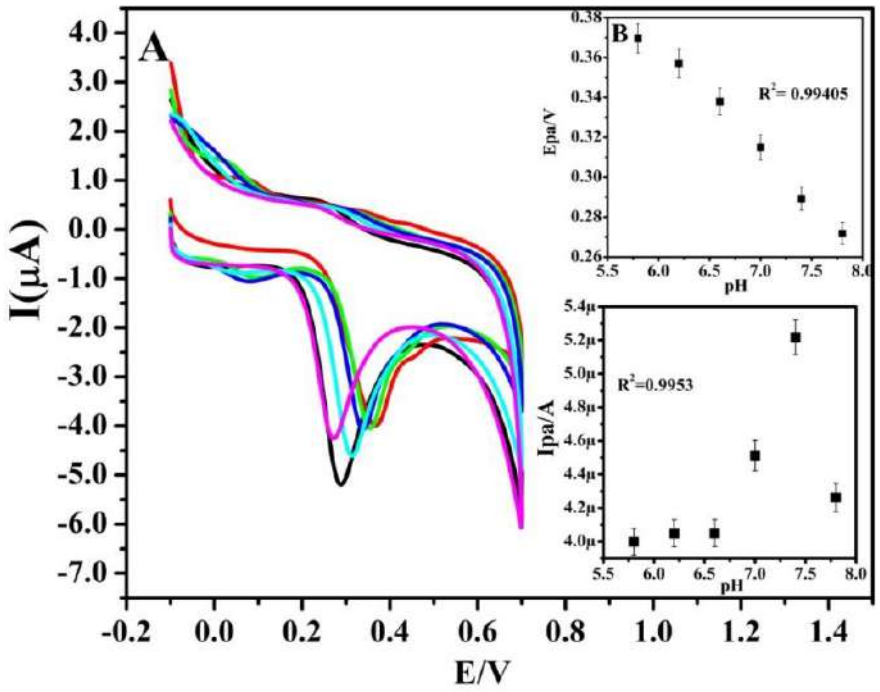


Fig. 3.6 A. CVs of 10 μM 5-HT at Poly(BCP)MPGE with dissimilar pH (5.8-7.8) with the sweep rate of 50 mV/s. **B.** Inset graph of Epa versus different pH values of 5-HT. **C)** Inset plot of Ipa of 5-HT versus varied pH.

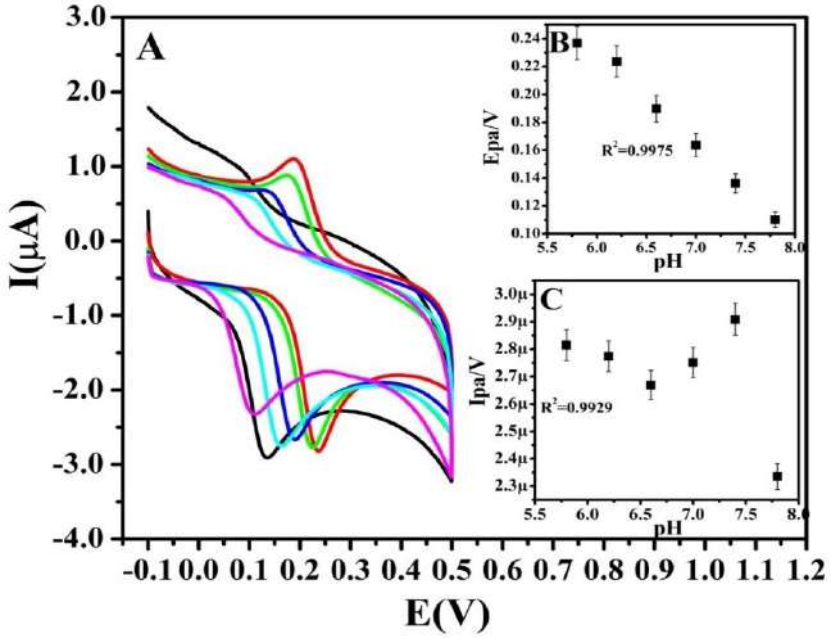


Fig. 3.7 A. CVs of 10 μM LD at Poly(BCP)MPGE with varied pH (5.8-7.8) with the sweep rate of 50 mV/s. **B.** Inset graph of Epa versus different pH values of LD. **C)** Inset plot of Ipa of LD versus varied pH.

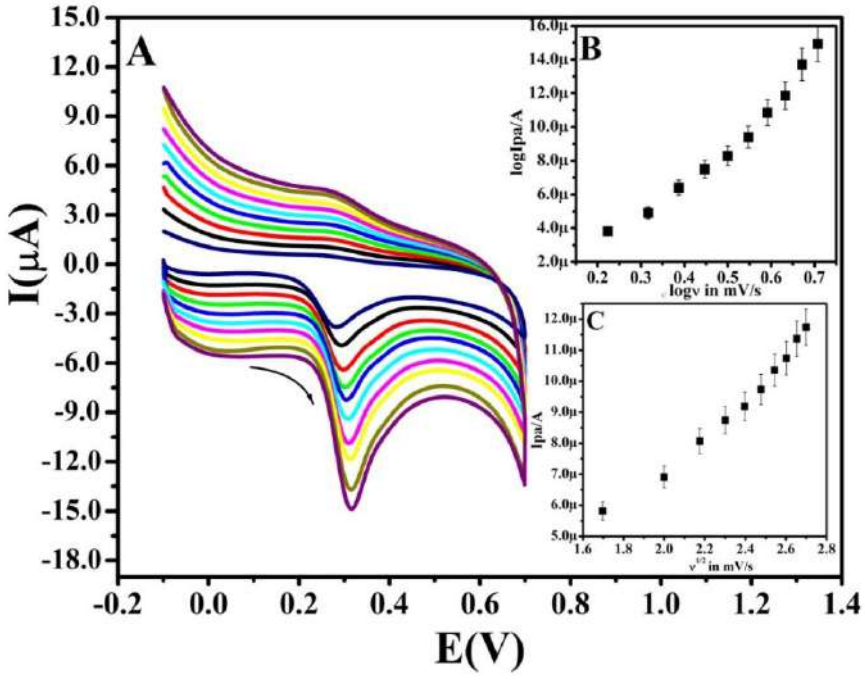


Fig. 3.8 A.CVs of 10 μM 5-HT at poly(BCP)MPGE with different sweep rates(50-500 mV/s) in 0.2 M PBS of pH7.4. **B.** Inset graph of log Ipa of 5-HT versus logν.**C)** Inset plot of Ipa of 5-HT versus $\nu^{1/2}$.

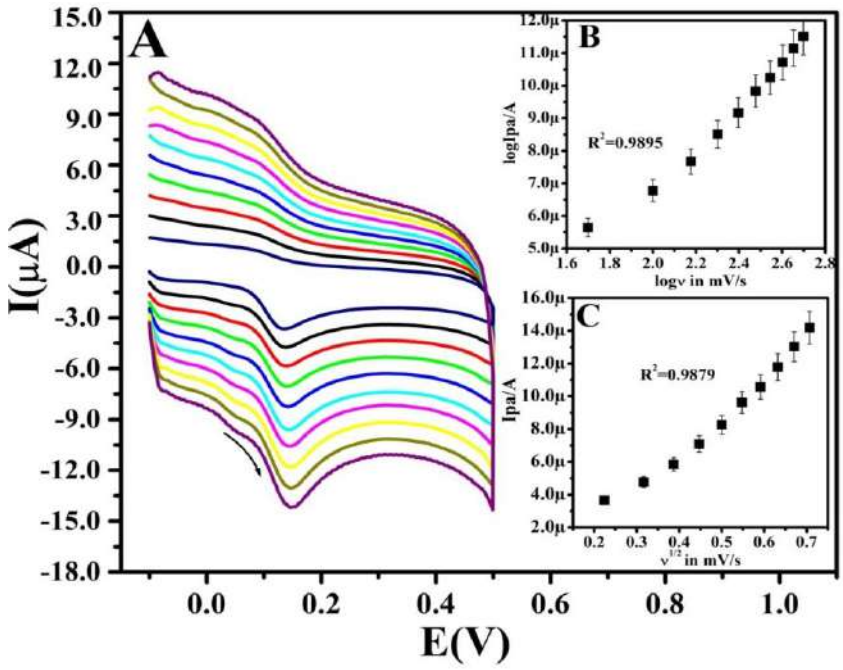


Fig. 3.9 A. CVs of 10 μM LD at Poly(BCP)MPGE with different sweep rates(50-500 mV/s) in 0.2 M PBS of pH 7.4. **B.** Inset graph of logIpa of LD versus logν. **C.** Inset plot of Ipa of LD versus $\nu^{1/2}$.

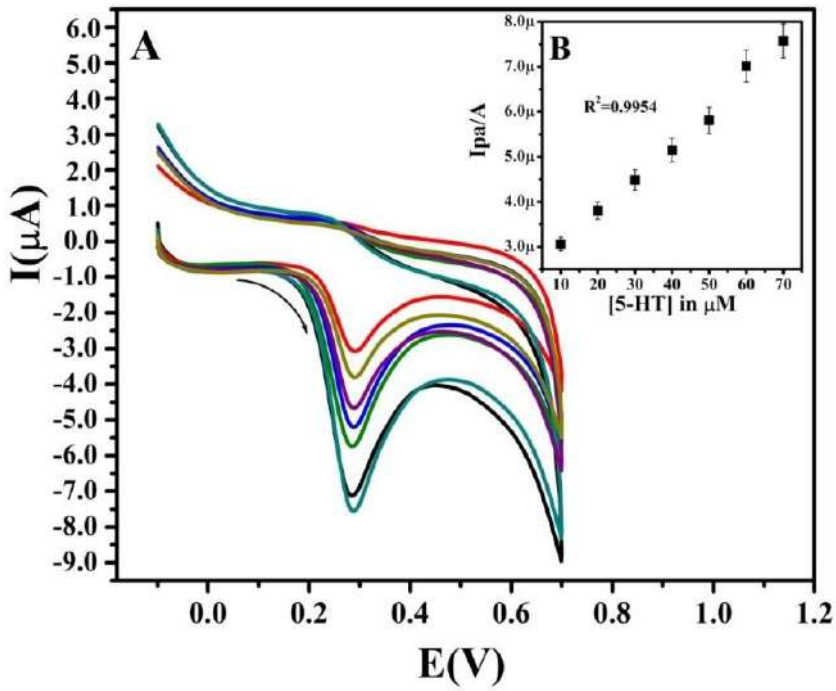


Fig. 3.10 A CVs of distinct concentration of 5-HT (10μM-70μM) in 0.2 M PBS of pH 7.4 with sweep rate of 50 mV/s at poly(BCP)MPGE. **B.** Inset graph of Ipa versus concentration of 5-HT in μM.

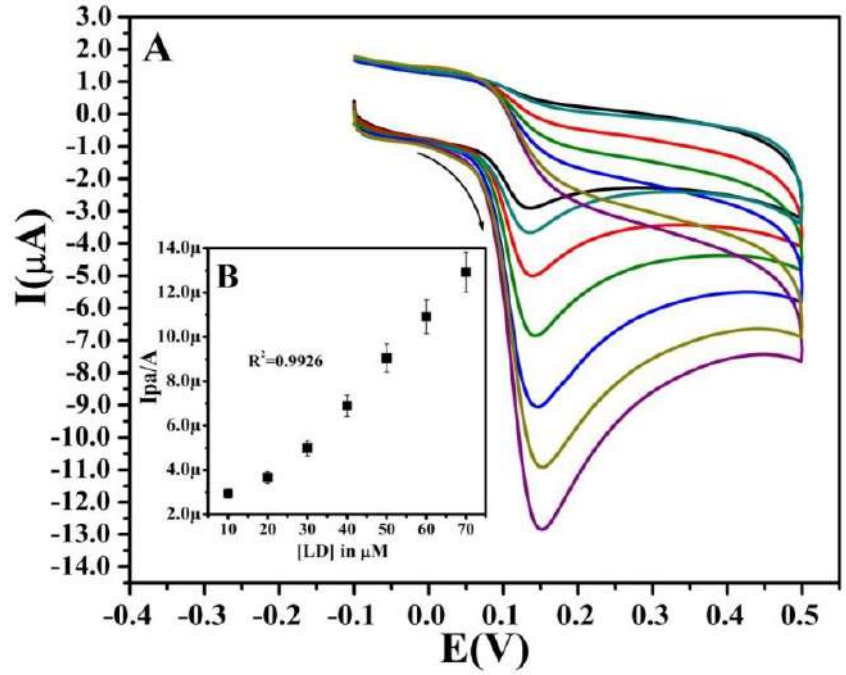


Fig. 3.11 A. CVs of distinct concentration of LD (a-g; 10 μM -70 μM) in 0.2 M PBS of pH 7.4 with sweep rate of 50 mV/s at poly(BCP)MPGE. **B.** Inset graph of Ipa versus concentration of LD in μM.

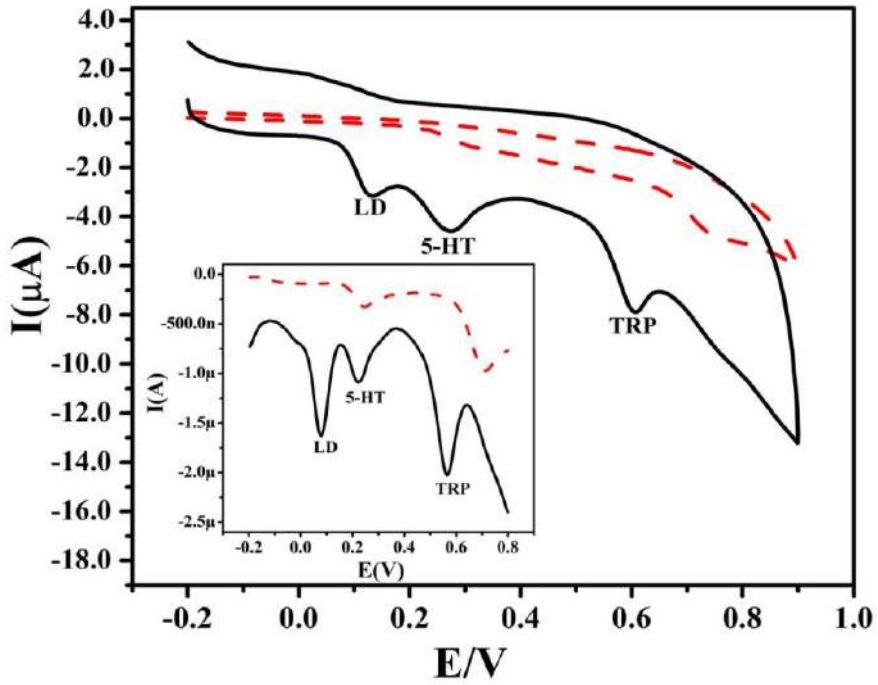


Fig. 3.12. CVs and DPVs obtained for concurrent determination of 10 μM 5-HT and 10 μM LD and 10 μM TRP at BCPE(dashed curve) and Poly(BCP)MPGE(solid curve) in 0.2 M PBS of pH 7.4 at the sweep rate of 50 mV/s.

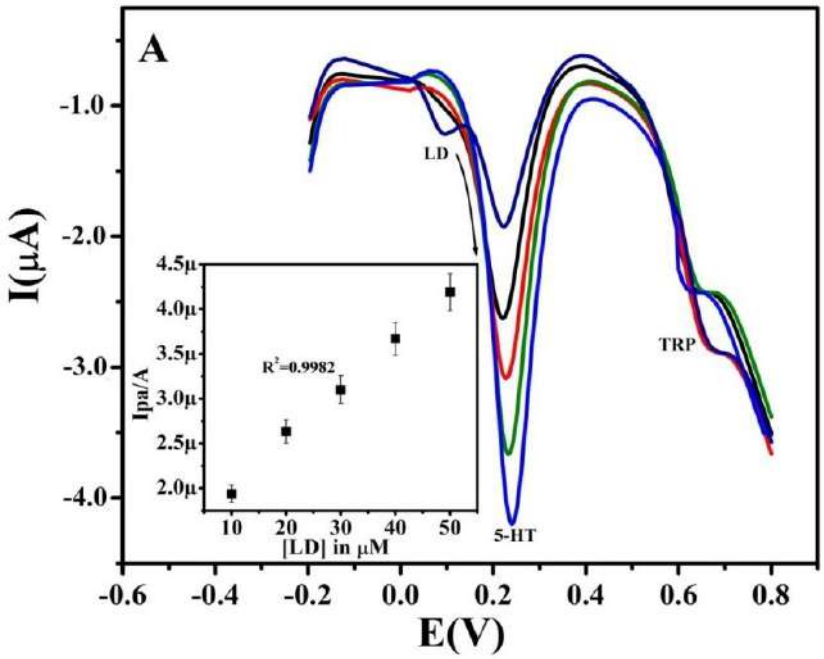


Fig. 3.13. DPVs obtained for 5-HT at varied concentration (10 μM -50 μM) in presence of constant LD (10 μM) and TRP(10 μM) in 0.2 M PBS of pH 7.4 at Poly(BCP)MPGE at sweep rate of 50 mV/s.

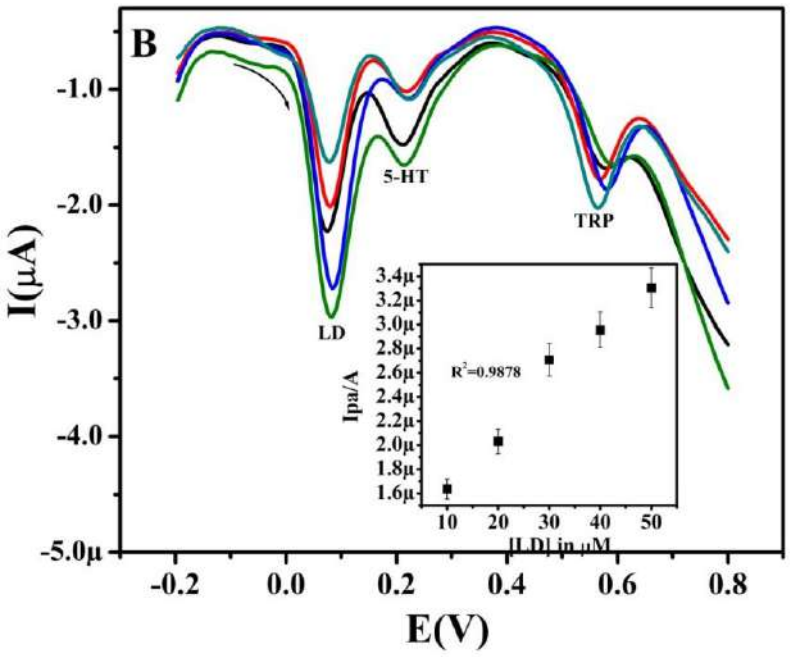


Fig. 3.14. DPVs obtained for LD at varied concentration(10 μM -50 μM) in presence of constant 5-HT(10 μM) and TRP(10 μM) in 0.2 M PBS of pH 7.4 at Poly(BCP)MPGE at sweep rate of 50 mV/s.

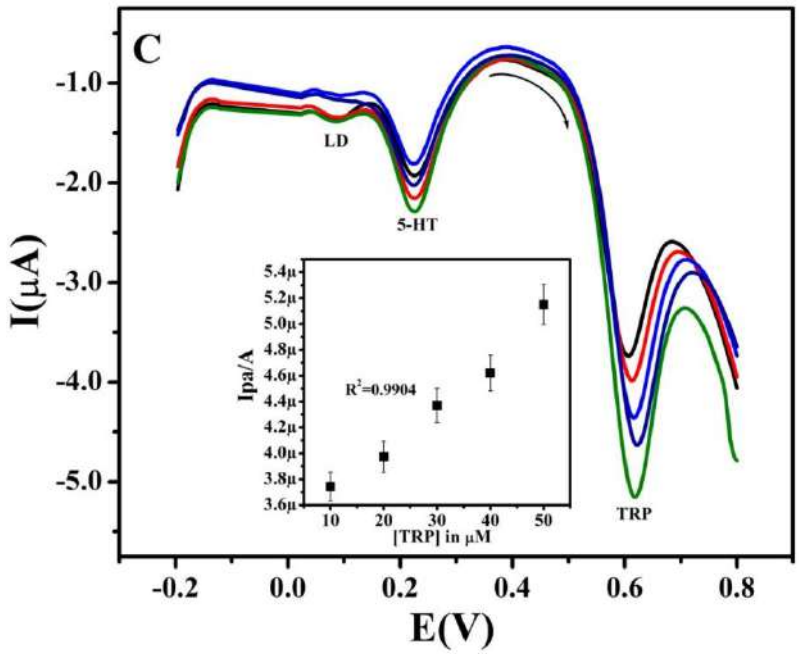


Fig. 3.15. DPVs obtained for TRP at varied concentration (10 μM -50 μM) in presence of constant 5-HT (10 μM) and LD (10 μM) in 0.2 M PBS of pH 7.4 at Poly(BCP)MPGE at sweep rate of 50 mV/s.

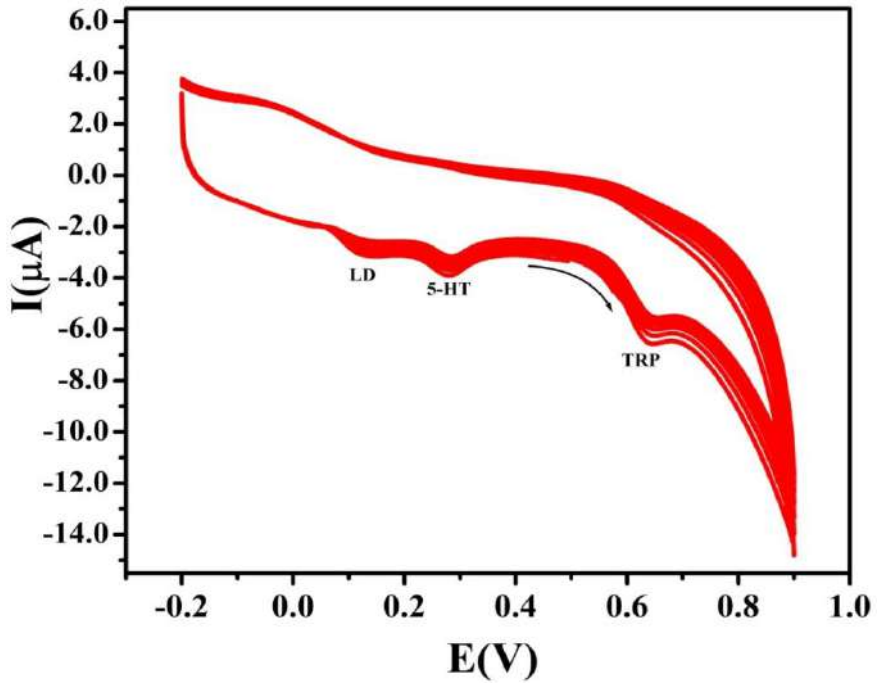


Fig. 3.16. CVs for stability analysis for a mixture of 10 μM 5–HT,10 μM LD and 10 μM TRP in 0.2 M PBS of pH 7.0 at Poly(BCP)MPGE with the sweep rate of 50 mV/s for 30 cycles.

Table 3.1. Comparative analytical efficacy of the different modified electrodes for 5-HT detection.

Working Electrode	Electrochemical Techniques	Linear range(μM)	LOD (μM)	Reference
GPE	CV	10-90	4.0	[45]
AuNPs@PPy/GSPE	SWV	0.1-15	33.2	[46]
Poly(FSBF)MPGE	CV	10-50	1.7	[47]
t-ZrO_2/MCPE	DPV	10-50	0.58	[48]
Poly(BCP)MPGE	CV	10-70	0.49	**This work**

Table 3.2. Comparative analytical efficacy of the different modified electrodes for LD detection.

Working electrode	Electrochemical Techniques	Linear range(mM)	LOD (μM)	Reference
Gold screen printed electrode	CV	99-1200	68	[49]
SWCNT/PPR/GCE	DPV	10-50	2.4	[50]
ZnO-GF	DPV	5-50	5.0	[51]
PbO2-MCPE	DPV	260-1200	25	[52]
Poly(BCP)MPGE	CV	10-70	2.3	**This work**

Table 3.3. Detection of 5-HT and LD in real sample (n=2)

Sample	5-HT added(μM)	Found (μM)	Recovery (%)
5-HT	20 30	19.81 29.30	99.05 97.66
LD	20 30	19.9 30.32	99.5 101.06

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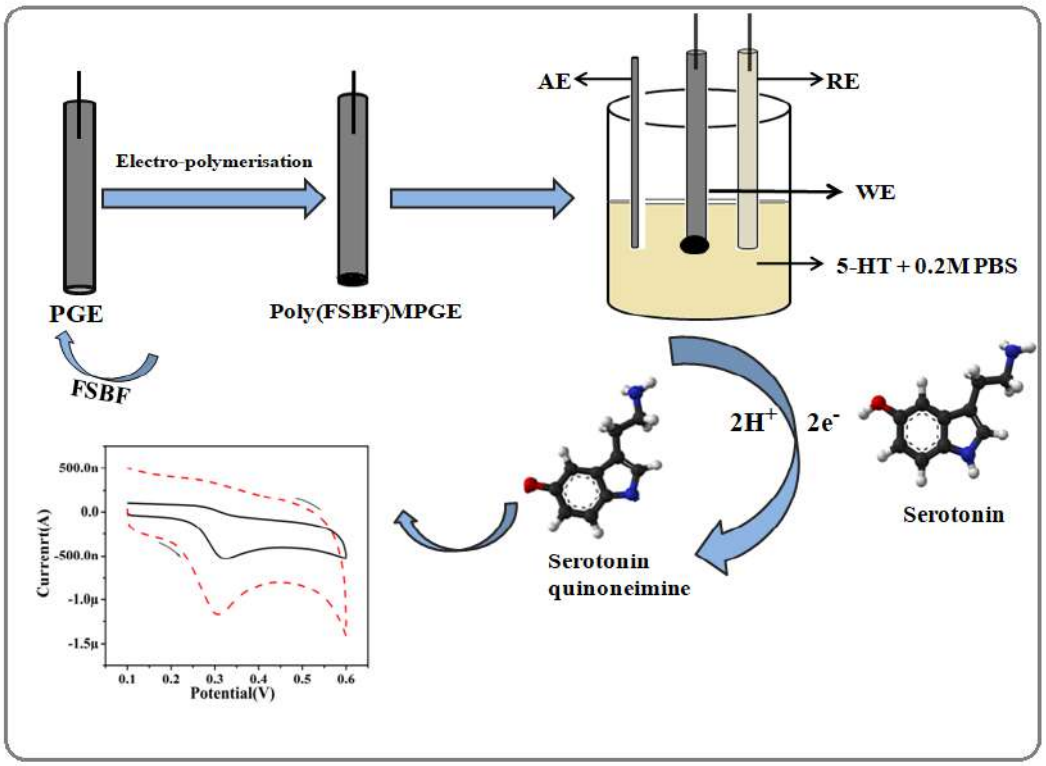
Chapter-III

PART-B

POLY (FAST SULPHONE BLACK F) MODIFIED PENCIL GRAPHITE ELECTRODE SENSOR FOR SEROTONIN



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3.6. Introduction

Pencil leads often referred as pencil graphite electrodes(PGEs) gained more prominence in recent days as a working electrode material for various electrochemical applications. PGEs act as a crucial substitution for other carbon electrodes due to their affordable price and thin dimensions. Also, shows great potential in designing a disposable biosensing electrode materials [1-2]. A fine particle of graphite is used to produce a pencil leads, which is composed of graphite powder mixing with clay or mica and a high polymeric binders are sometimes added [3]. PGEs are simple and easy to use because of their good adsorption properties, high conductivity, good mechanical strength and easy methods of modifications. The renewal of electrode surfaces is simple and faster in case of PGEs among various solid electrodes involving common polishing and cleaning techniques and has larger surface area. Therefore, it is able to detect the analyte in its lower concentration [4-6].

Serotonin and Dopamine are important neurotransmitters belong to catecholamine family and serves as chemical carriers for transporting information between the nerve cells [7]. Both serotonin and dopamine are responsible for various phenomenons occur in the living organisms. Serotonin, which is chemically known as 5-hydroxytryptamine (5-HT) derived from α–amino acid tryptophan and widely distributed inside and outside of brain tissues. 5-HT together with other neurotransmitter plays a significant role in regulating and controlling the several biological and physiological functions like sleep disturbances, memory, wound healing, appetite, thermoregulation, behaviour and drug dependency [8-11]. Many life functions are depends on the concentration of 5-HT level in blood. The extent of 5-HT levels in blood is 101-283 ng/mL. Any unbalance in the concentration of 5-HT leads to numerous health issues such as low concentration level causes anxiety, chronic pain, blood clotting. While extremely high concentration leads to potentially fatal effects like serotonin syndrome, carcinoid syndrome, liver regeneration and autism [12-14].

3, 4-dihydroxyphenylethylamine commonly known as dopamine(DA) is an inhibitory neurotransmitter. It exhibits significant contribution in the proper functioning of hormonal, renal and cardiovascular system [15, 33]. The normal level

of DA in blood plasma is in the range of 0.04-450 nM [16]. Lower concentration of DA than the normal level causes severe neuronal afflictions namely Parkinson's disease, Huntington's chorea, schizophrenia, drug addiction and HIV infection whereas an excess level can leads to hallucinations, mania and Euophia [17, 18].

Analysis of 5-HT is more complicated as a result of interference of other neurotransmitters present in the biological fluids. In this regard, several conventional analytical methods were employed for the detection of 5-HT such as, capillary electrophoresis, luminescence [19] and HPLC provides a certain results. However, these methods are time-taking, complex, costly equipments and required sample management. The use of electrochemical methods are more advantageous than other techniques because of its experimental simplicity, good selectivity, usage of small quantity of sample, rapid monitoring and inexpensiveness [20-23]. The polymeric film coated electrodes have excellent stability, greater number of reactive sites, uniformity and strong adherence nature to the surface of the electrode [24-26]. Due to this properties, the electropolymer film modified electrodes attracted more in the field of sensor. The main purpose of our study was to frame a stable and sensible working electrode via electropolymerising fast sulphone black F (Scheme 3.5), a metallochromic dye on the surface of bare pencil graphite electrode to achieve the 5-HT detection in the presence of DA in biological pH.

3.7. EXPERIMENTAL PROCEDURES

3.7.1. Chemicals and reagents

The pencil-leads (HB 0.7mm) were purchased from bookstore. Serotonin(5-HT), dopamine(DA) and fast sulphone black F (FSBF) purchased from Himedia (Bangalore, India). Stock solution of 25×10^{-4}M Serotonin, 25×10^{-3}M fast sulphone black F and 0.1M NaOH were prepared by dissolving in doubly distilled water and 25×10^{-4}M dopamine stock solution was prepared in 0.1M perchloric acid. Phosphate buffer solution (PBS) of different pH was obtained by mixing a appropriate proportions of standard 0.2M disodium hydrogen phosphate (Na_2HPO_4) and 0.2M sodium dihydrogen phosphate (NaH_2PO_4). All the materials were of analytical grade quality and used without any additional treatment.

3.7.2. Instruments and Procedure:

The electrochemical investigations executed in analytical instrument of Model CHI-660c potentiostat (CH Instrument-660 electrochemical workstation). The standard three-electrode one component glass cell used to perform all experiments. The cell containing a bare pencil graphite electrode (BPGE) and poly(fast sulphone black F) modified pencil graphite electrode (poly(FSBF)MPGE) as working electrode. Additionally, platinum wire auxiliary electrode and a saturated calomel electrode (SCE) as a reference electrode. All the redox potentials of electroanalysis of 5-HT were referenced to SCE at an optimum temperature.

3.8. Results and discussion

3.8.1. Electropolymerization of FSBF on BPGE

The electrochemical polymerization process of fast sulphone black F onto the PGE surface was achieved in the potential window of 0.0 V and +1.4 V at the sweep rate of $50mVs^{-1}$ through cyclic voltammetry for 15 multiple cycles. During the process of multiple scanning, the voltammogram gradually decreased as the cyclic time increases. This result shows that the film of poly(FSBF) was coated on the surface of BPGE [27-28]. The extent of thickness of the polymer film formed on the BPGE will also affect the electrocatalytic response of the electrode and thickness of the polymeric film can be managed by changing the number of cycles on the BPGE (from 5 to 25 multiple cycles). Fifteen cycles shows the maximum anodic peak current as shown in the Fig.3.17A. Therefore, 15 cycles were considered as most favorable for the electro-polymerization of FSBF on BPGE. The electrocatalytic response of 5-HT enhanced at initially upto 15 cycles. After that, the peak currents of 5-HT was decreased as shown in Fig.3.17B. Because of increase in thickness of the polymeric film that would prohibit the electron transfer process. The corresponding electrocatalytic performance towards oxidation of 5-HT in PBS of pH 7.4 was investigated. An appropriate amount of incorporated FSBF polymeric film on BPGE surface was estimated by using the formulae (1) [29].

$$Ip = n^2F^2A\Gamma\upsilon/4RT \quad \text{.....(3.5)}$$

Where, Ip is the peak current(A), Γ (M/cm^2) is the surface coverage concentration, n is the number of exchanged electrons A and υ are the area and scan rate of the working electrode respectively, R, T and F are physical constants with their normal meanings. The surface coverage concentration (Γ) of FSBF on BPGE was estimated to be $0.0274\times10^{-10} M/cm^2$.

3.8.2. Characterisation of fabricated poly(FSBF)MPGE

The electrochemical aspects of poly(FSBF)MPGE was examined by using 1mM potassium ferrocyanide ($[K_4Fe(CN)_6]$) in presence of supporting electrolyte(1M KCl) with the sweep rate of $50mVs^{-1}$ by CV techniques. Fig.3.18 represents the cyclic voltammograms for 1mM potassium ferrocyanide recorded at BPGE (dashed line) shows lower redox peak current signals and large difference between the redox peak potentials (ΔEp). While at poly(FSBF)MPGE the notable enhancement in the redox peak current was observed. This results indicates that the modified electrode shows better surface properties and electrocatalytic properties for potassium ferrocyanide system. The total electroactive surface area of modified pencil electrode can be computed by Randles– Sevick's equation (2) [30, 31].

$$I_p = (2.69\times10^5)n^{3/2}AD^{1/2}C_0\nu^{1/2} \quad \text{.....(3.6)}$$

Where, Ip is the peak current(A), A is the active surface area of electrode(cm^2), n is number of exchanged electrons, C^0 is the concentration of electroactive species ($mol\ cm^{-3}$) in solution, D is the diffusion co-efficient (cm^2s^{-1}) and ν is the scan rate (Vs^{-1}). The electro active surface area calculated for BPGE and poly(FSBF)MPGE found to be $0.0561cm^2$ and $0.0523\ cm^2$ respectively.

3.8.3. Electrocatalytic behaviour of 5-HT at bare and poly (FSBF) modified PGE

Fig. 3.19 exhibits the cyclic voltammograms recorded for the electrocalatytic response of 10µM 5-HT at BPGE (solid line) and poly(FSBF)MPGE (dashed line) in 0.2 M PBS of pH 7.4 with the scan rate of 50 mVs^{-1}. It is observed that voltammogram obtained at BPGE shows poor voltammetric signal and low current signal. However, in the identical condition, the poly(FSBF)MPGE exhibited well defined oxidation peak with a higher magnitude of oxidation current than bare PGE. Which is ascribed

to the larger surface area of the poly(FSBF) film. This results demonstrated that the poly (FSBF) modified electrode possessed a strong electrocatalytic action for the oxidation of 5-HT. Cathodic peak could not be found at the potential swept of +0.1V to +0.6 V, denoting that the electrochemical oxidation of 5-HT at MPGE was an irreversible process. The possible electro oxidation of 5-HT at poly(FSBF)MPGE was represented in scheme 3.6.

3.8.4. Influence of sweep rate on peak current of 5-HT

The effect of potential sweep rate on the electrocatalytic current response of 5-HT was illustrated for different scan rates. Fig. 3.20A presents the voltammograms reported for 10µM 5-HT in 0.2M PBS at pH 7.4 with different sweep rates from 50 to 500 mVs^{-1} at poly(FSBF)MPGE. The oxidation peak current (Ipa) of 5-HT increases linearly with the increase in potential scan. To study the type of electrode process, log of anodic peak current (Ipa) of 5-HT was plotted with log of scan rates (ν) for poly(FSBF)MPGE as shown in Fig. 3.20B. The plotted graph shows excellent linearity between anodic peak currents and scan rates. The corresponding linear regression equation is Ipa(µA)= 0.7251(log υ) + 4.6037 with R^2 0.9798. This result suggested that diffusion controlled electrode process occurs at poly(FSBF) modified pencil graphite electrode [32].

3.8.5. Effect of solution pH on 5-HT at poly (FSBF) MPGE

The influence of varying the pH of the supporting electrolyte towards the electrocatalytic performance of 5-HT was examined. Cyclic voltammograms of 10µM 5-HT in 0.2 M PBS at poly(FSBF)MPGE at different solution pH values from 5.8-7.8 are shown in Fig. 3.21A. The peak potentials of 5-HT were moved towards less positive direction with increasing the pH value of the electrolyte. The oxidation peak potential of 5-HT moved from 400mV to 290mV with respect to pH from 5.8 to 7.8. The pH 7.4 was selected for all subsequent electroanalysis of 5-HT because pH 7.4 is a biological pH. A linear relationship was established by plotting oxidation peak potential(Epa) with pH of the solution as illustrated in Fig. 3.21B. From the plot, the slope of 53mV/pH was found. The obtained value near to the theoretical Nernstian value of 59mV/pH [33]. This result corresponds to the electrochemical oxidation of 5-HT was two-electron and two-proton transfer reaction process.

3.8.6. Concentration influence of 5-HT at poly (FSBF) MPGE

The poly(FSBF)MPGE was employed towards the electrocatalytic oxidation of 5-HT by varying it's concentration in between 10-50µM in 0.2M PBS of pH 7.4 at sweep rate of 50 mVs^{-1} using cyclic voltammetric(CV) method as shown in Fig. 3.22A and Fig. 3.22B. From the graph it confirms that the oxidation peak current(Ipa) eventually increases with increase in 5-HT concentration. The calibration plot of Ipa versus cocentration of 5-HT gives good linearity which was given by equation Ipa (µA) = 0.1435(C_0µM/L) +1.9078 and R^2 was 0.9867. The detection limit (LOD) and quantification limit (LOQ) for 5-HT at poly (FSBF)MPGE were calculated through the ensuing formulas [34,35].

$$LOD = 3\ S/M \quad \text{.....(3.7)}$$

$$LOQ = 10\ S/M \quad \text{.....(3.8)}$$

Where 'S' is standard deviation of oxidation peak currents and 'M' is the slope of the calibration plot. The LOD of 5-HT was found to be 1.7µM and LOQ was found to be 5.8µM for 5-HT. The comparison of LOD of 5-HT at poly (FSBF) MPGE with other reported modified electrodes is provided in Table 3.4.

3.8.7. Simultaneous analysis of 5-HT and DA at poly(FSBF)MPGE

The prime objective of the present investigation is to apply the fast sulphone black F modified electrode for the determination of 5-HT in presence of DA. Both 5-HT and DA are co-exist in biological fluid. The simultaneous detection of these molecules in a binary mixture was difficult at most solid electrodes because of similar oxidation potentials of the molecules. The cyclic voltammetric response of 10µM 5-HT and 10µM DA in 0.2 M phosphate buffer solution of pH 7.4 at the BPGE (dashed line) and the poly (FSBF) MPGE (Solid line) with the sweep rate of 50mVs^{-1} is displayed in Fig. 3.23. Voltammetric signals of 5-HT and DA shows less sensitive and broad anodic peaks at BPGE, so the peak potentials for 5-HT and DA are not well separated at unmodified electrode. On the other hand, in case of Poly (FSBF) MPGE the problem of overlapped peak was resolved into two well-identified peaks of 5-HT and DA with different peak potentials located at 325mV and 142mV respectively. The corresponding peak potential difference of 5-HT to that of DA was 183mV. This

output result was large enough to determine 5-HT and DA individually and simultaneously.

3.8.8. Interference study

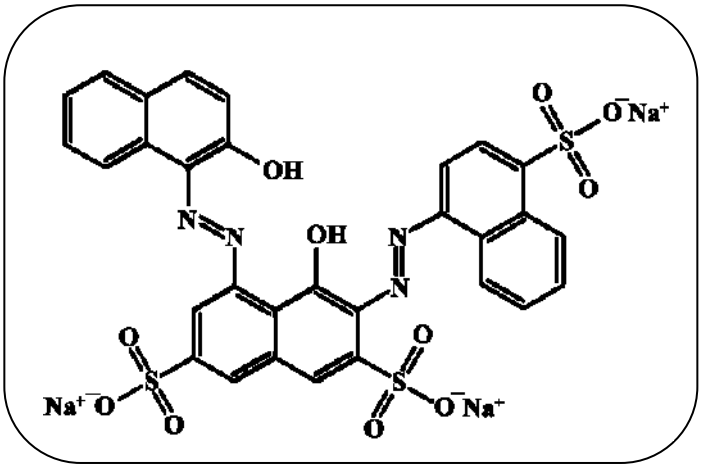
Differential pulse voltammetry(DPV) was selected to examine the anti-interference ability of the poly(FSBF)MPGE due to better resolution and high current sensitivity of the technique. The Fig. 3.24 shows the voltammogram recorded by DPV for homogeneous mixture of 10μM 5-HT and 10μM DA in 0.2M PBS of pH 7.4 with the sweep rate of $50mVs^{-1}$ gives well separated voltammograms corresponding to their oxidation at poly(FSBF)MPGE. The oxidation potentials of 5-HT and DA positioned at 243mV and 95mV respectively. The difference between the peak potential of 5-HT to the peak potential of DA was 148mV. This suggested that the fabricated modified electrode exhibits good tendency for the determination of 5-HT in presence of DA.

3.8.9. Analysis of 5-HT in serum sample

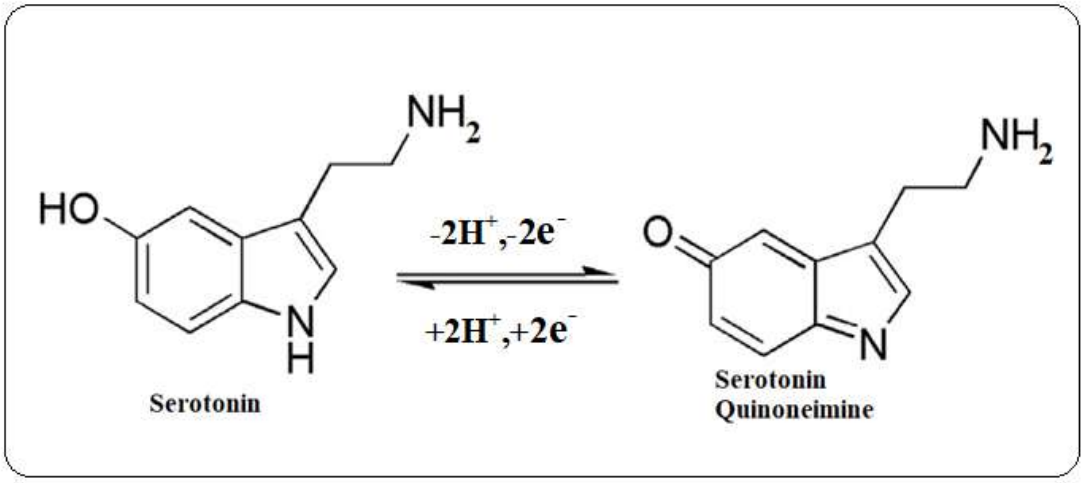
To validate the practical implementation of prepared electrode, the poly (FSBF) MPGE was utilized for the sensing of 5-HT in human serum sample. The human sample obtained from hospital was diluted with 0.2M PBS of pH 7.4 and spiked with 5-HT. The analysis results were given in table 3.5. Implied that the sensing activity of modified electrode was reliable for 5-HT detection in real sample.

3.9. Conclusion

In this work, Poly (FSBF) MPGE was developed by electropolymerisation of FSBF on the surface of PGE and successfully employed for the study of 5-HT and DA. The modified electrode showed better electrocatalytic properties with higher sensitivity and selectivity. The poly (FSBF) MPGE resolves the problem of broad oxidation peaks of 5-HT and DA and gives well separated oxidation peaks for the binary mixture of 5-HT and DA. This result indicates the anti-interference ability of the modified electrode. The offered electrochemical sensor was simple, cost effective and can be used in the detection of other bioactive molecules.



Scheme 3.3. Structure of Fast Sulphone Black F



Scheme.3.4. Electro-oxidation of 5-HT

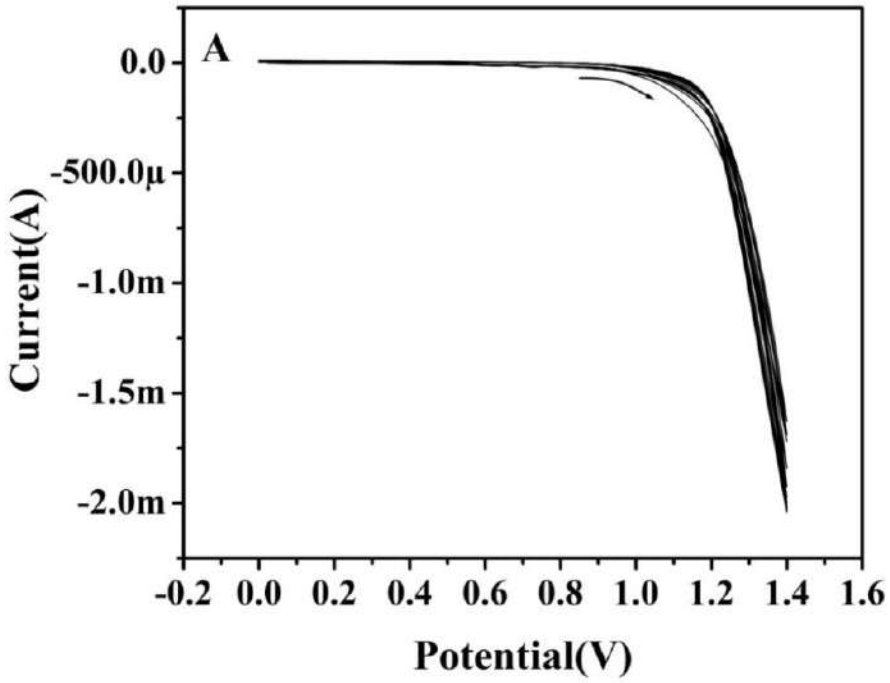


Fig. 3.17A. Electropolymerisation of 1mM FSBF on surface of BPGE recorded in presence of 0.1M NaOH at 15 multiple cycles with sweep rate of 50 mVs^{-1}.

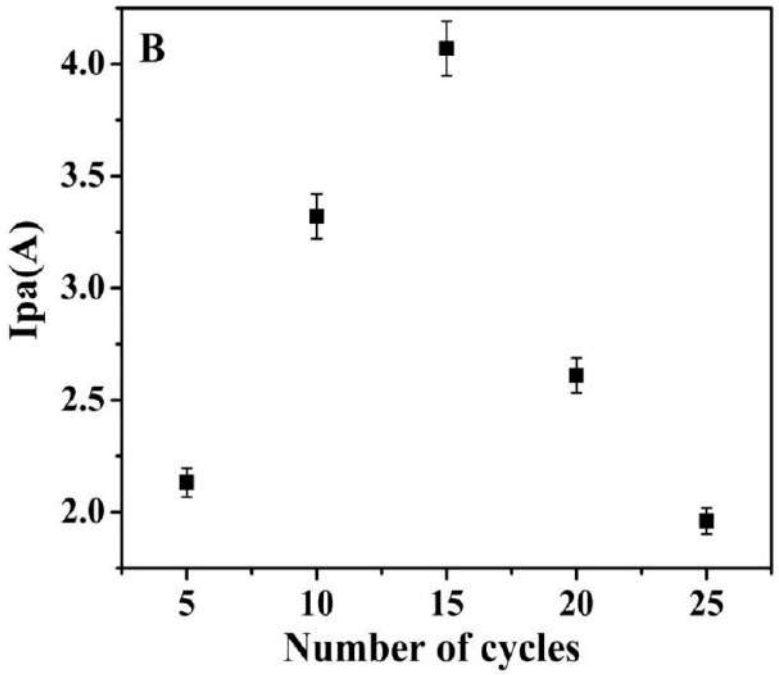


Fig. 3.17 B. Plot of anodic peak current (Ipa) versus number of polymerization cycles.

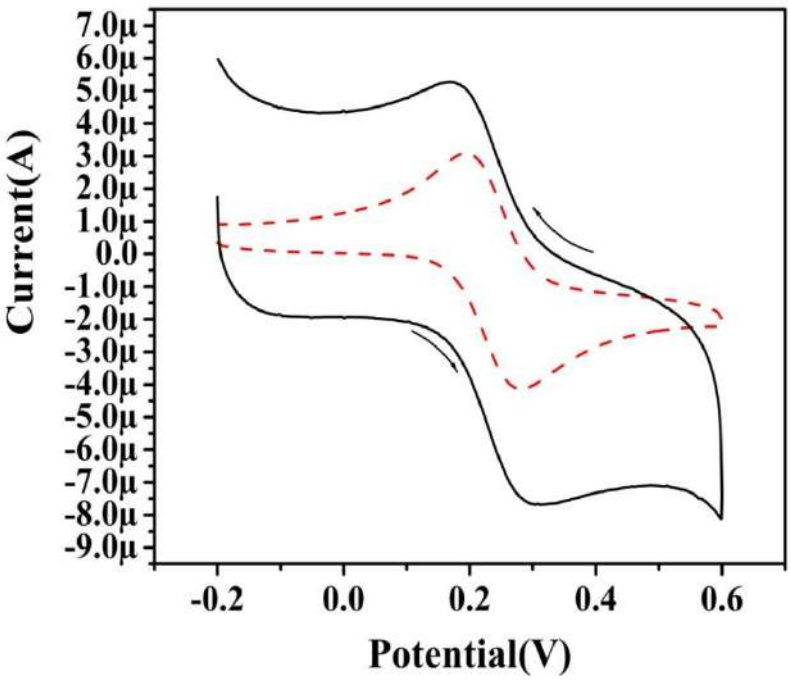


Fig. 3.18. Cyclic voltammograms of BPGE(dashed line) and poly(FSBF)MPGE (solid line) for 1mM $[K_4Fe(CN)_6]$ in presence of 1M KCl at sweep rate of 50mVs^{-1}.

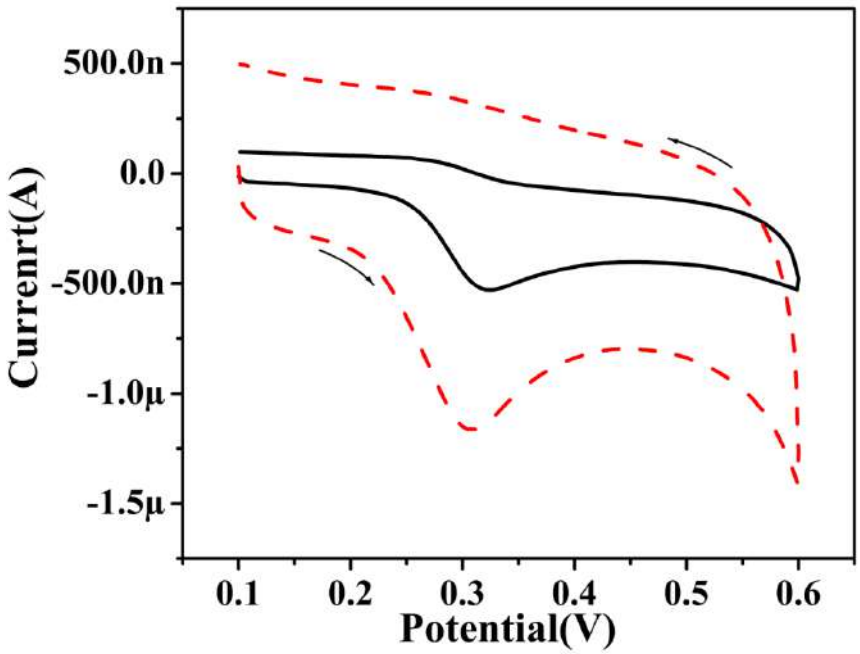


Fig. 3.19 Cyclic voltammogram of 10µM 5-HT in 0.2 M phosphate buffer solution of pH 7.4 at BPGE (solid line) and poly(FSBF) MPGE (dashed line) with scan rate of 50mVs^{-1}.

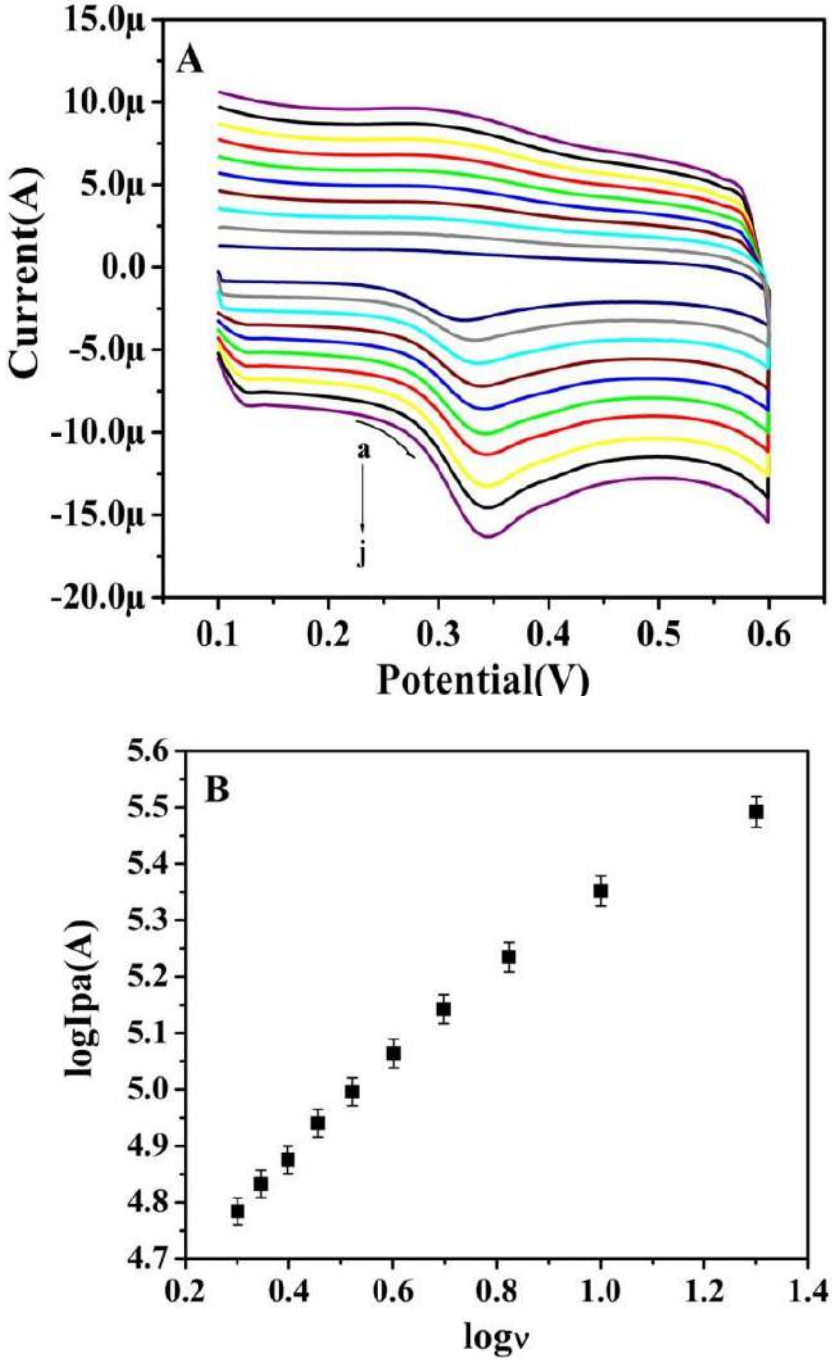


Fig. 3.20A. Cyclic voltammograms for 10µM 5-HT at poly (FSBF)MPGE with different scan rates (a-j:50-500 mVs^{-1}) using 0.2 M phosphate buffer solution at pH 7.4. **B.** The plot of log Ipa versus log υ.

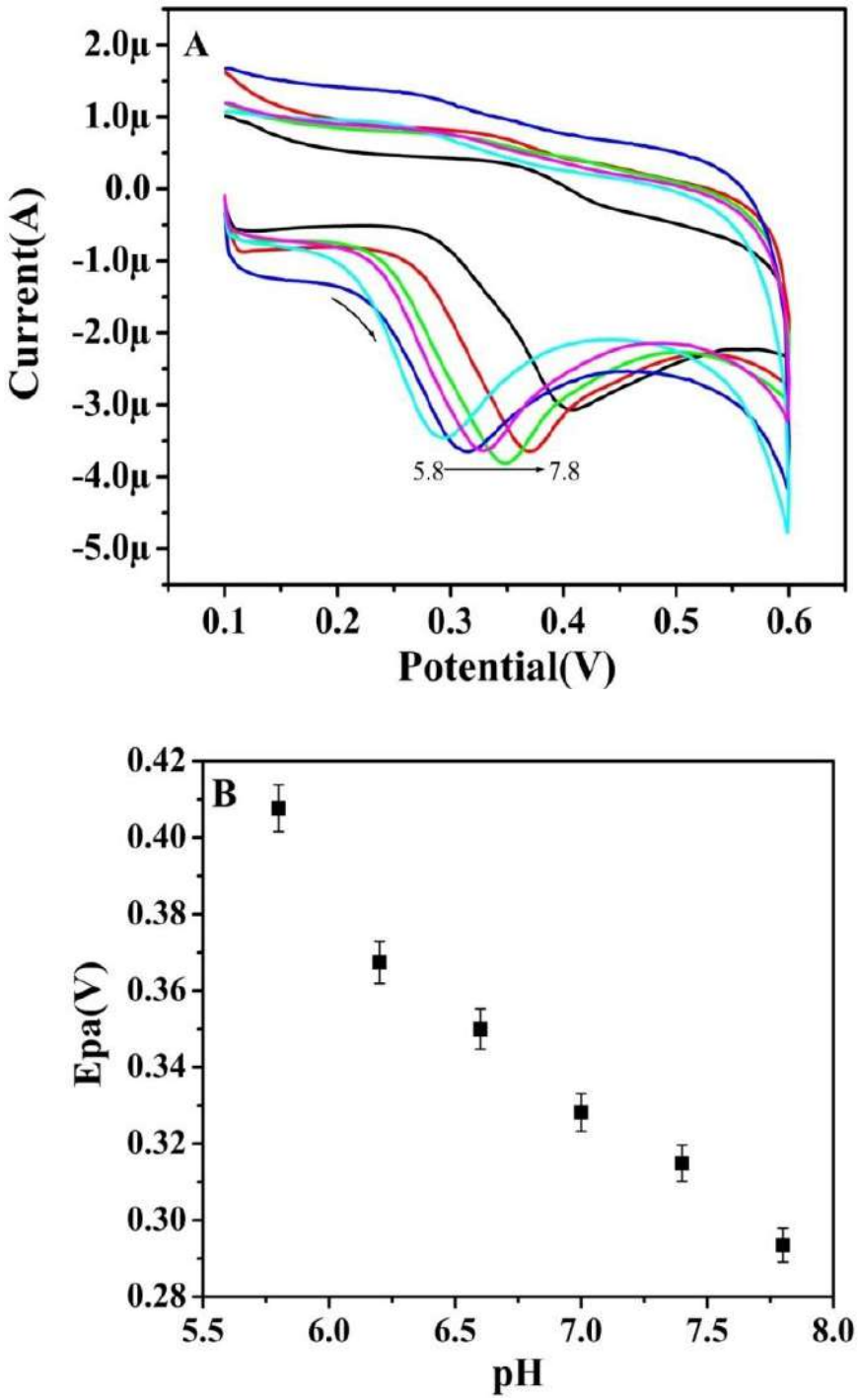


Fig. 3.21 A. Cyclic voltammograms of 10µM 5-HT at different pH (5.8 to 7.8) in 0.2 M PBS of pH 7.4 with sweep rate of 50mVs^{-1} at poly (FSBF) MPGE.
B. The plot of oxidation peak potential versus different solution pH.

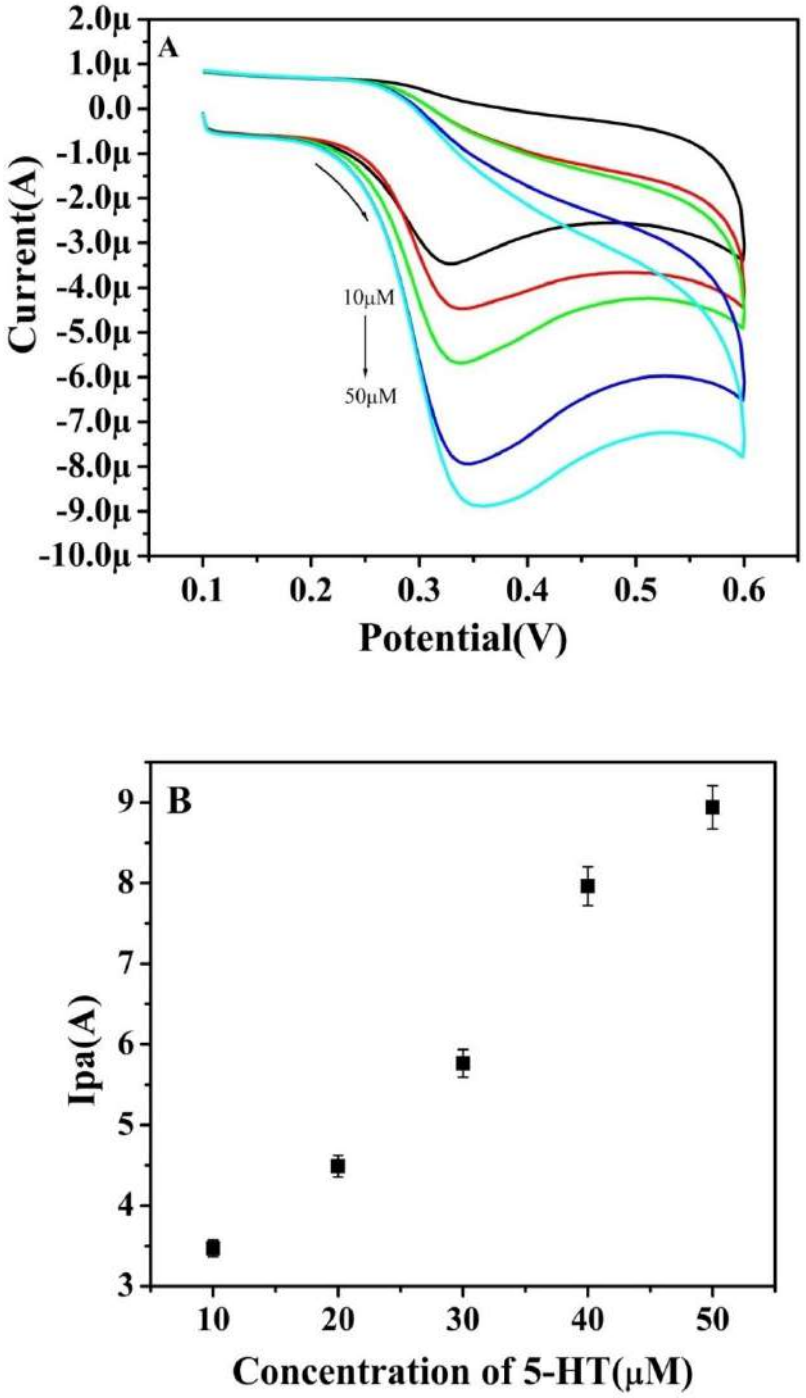


Fig. 3.22A. Cyclic voltammograms of variation in 5-HT concentration (10-50 μM) at poly (FSBF) MPGE in 0.2M PBS of pH 7.4 at scan rate of 50 mVs^{-1}. **B.** Graph of the oxidation peak current (Ipa) versus the concentration of 5-HT (μM).

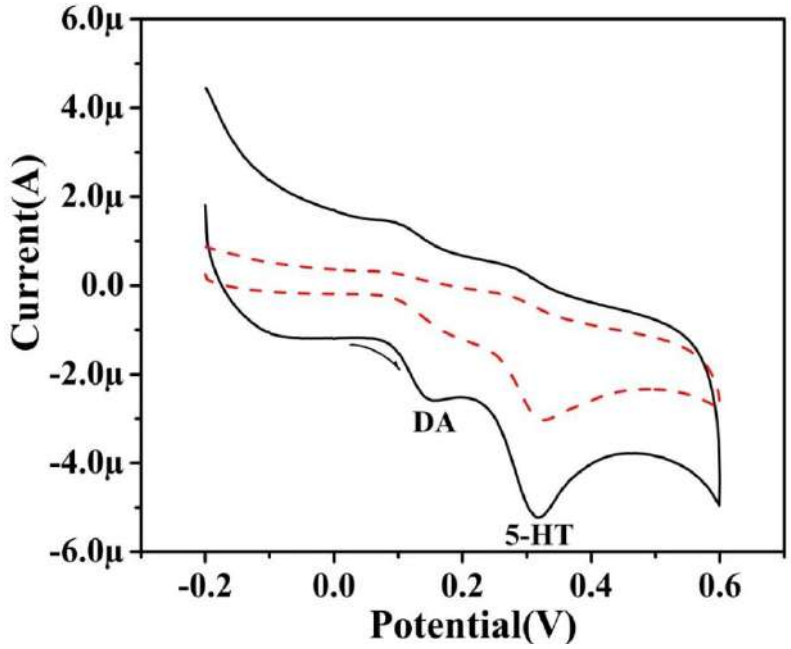


Fig. 3.23. Voltammograms for a mixture of 10µM 5-HT and 10µM DA at BPGE (dashed line) and poly (FSBF)MPGE (solid line) in 0.2 M PBS of pH.7.4 at sweep rate of 50 mVs^{-1}.

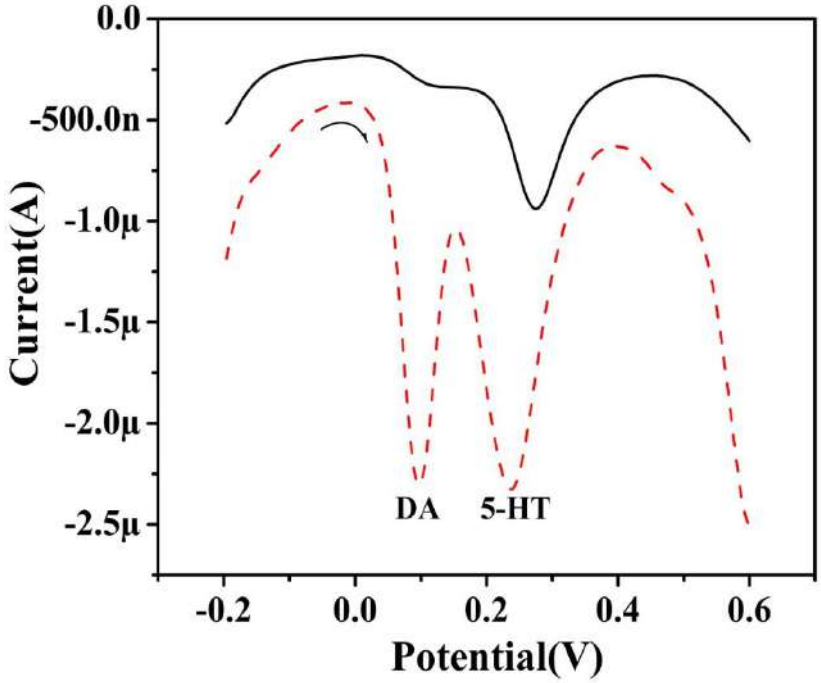


Fig. 3.24. Differential pulse voltammograms recorded for 10µM 5-HT and 10µM DA in 0.2 M PBS of pH.7.4 with scan rate of 50 mVs^{-1} at BPGE (solid line) and poly (FSBF)MPGE (dashed line).

Table.3.4. Comparison of LOD of 5-HT with different modified electrodes:

SI. No	**Working Electrode**	**Techniques**	**Linear range(μM)**	**LOD (μM)**	**Reference**
01	3D – ITO	DPV	50-1000	7.5	[34]
02	PGE	CV	10-90	4.0	[35]
03	IL-DC-CNT/GCE	DPV	5.0-900	4.0	[28]
04	Poly(FSBF)MPGE	CV	10-50	**1.7**	**This study**

Table.3.5. Detection of 5-HT in real sample (n=3)

Sample	**5-HT added (μM)**	**Found (μM)**	**Recovery (%)**
01	10	9.32	93.2
02	20	18.91	94.55
03	30	28.30	94.33

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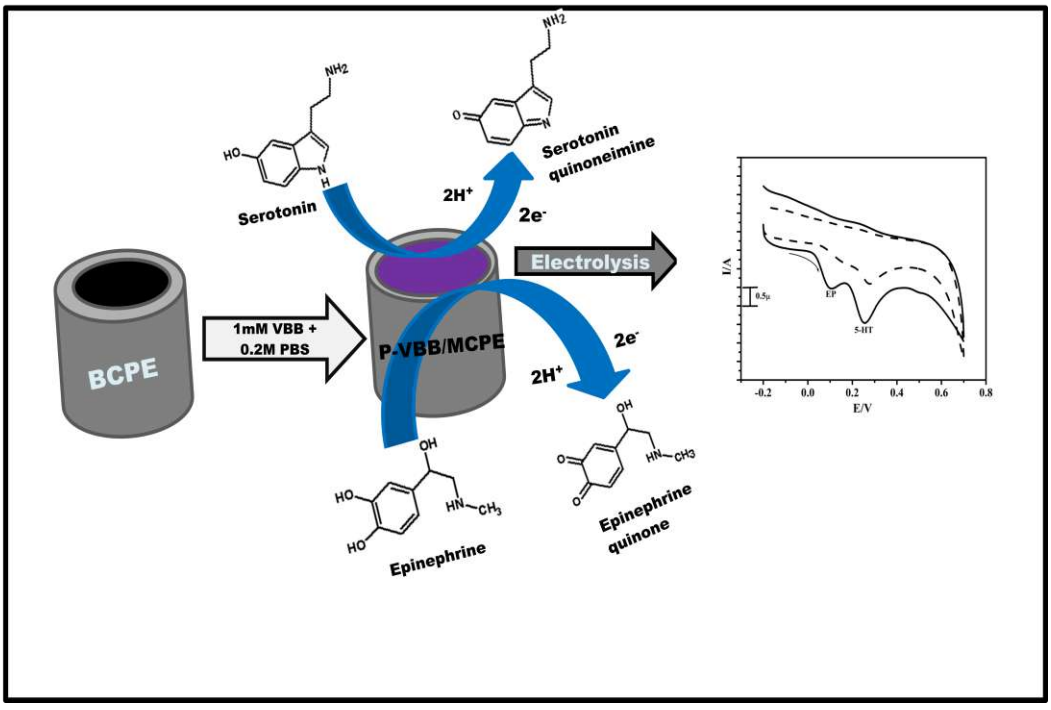
Chapter-IV

PART-A

SIMULTANEOUS RESOLUTION OF SEROTONIN AND EPINEPHRINE AT POLY (VICTORIA BLUE B) AMPLIFIED CARBON PASTE ELECTRODE: A VOLTAMMETRIC STUDY



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4.1. Introduction

In recent decades, the formulation of carbon based voltammetric sensors has great deal of interest in electrochemical studies as a sensitive and powerful method to examine the characteristics of the biologically important oxidizable compounds[1,2].

Serotonin(5-HT) and Epinephrine(EP) both are neuromodulators and serves as signaling substances that carries balances and promotes the messages between the neurons[3]. 5-HT is a prominent mood modulating neurochemical extensively secreted by the essential amino acid L-tryptophan and principally found in peripheral and central nervous systems and fulfils the various physiological and biological actions of the body [4-7]. However the highest amount of 5-HT (~90%) is located in enterochromaffin cells of gastrointestinal tract and involved in multiple gastrointestinal ailments such as irritable bowel syndrome, food hypersensitivity and secretion [8, 9]. The insufficiency in 5-HT levels results in distinct neuro problems like Down's syndrome, depression and suicidal inclinations [10-12]. The intensified level of 5-HT leads to toxicity and Huntington's syndrome [13,14]. Also, DNA damage was influenced by 5-HT in presence of copper ions [15].

EP, also referred as adrenaline is a larger organic cation exists in biological fluids and neural tissues [16, 17]. EP is an adrenergic hormone and presumably acts as an inhibitory neuromodulator which is released by the adrenal glands and by the neurons in medulla oblongata of mammalian system [18]. It trained the body for action at emergency circumstances by promoting the glucose and oxygen flow to the brain and muscles. EP also serves as a medicant and employed to cure the numerous conditions including superfacial bleeding, cardiac arrest and anaphylaxis. Orthostatic hypotension and parkinsonism are the results of deficit EP concentration. Whereas, the extreme EP levels leads to stress and inadequacy of thyroid hormone [19-24]. Both 5-HT as well as EP plays influential duties in maintaining the human health. In this regard, it is requisite to formulate a sensible method for quantitative determination of these molecules for studies of physiological activities and diagnostics in clinical medicine [25].

At present, Victoria blue B (Scheme 4.1) was employed to chemically modifying carbon paste electrode and used for the simultaneous resolution of 5-HT and EP. Victoria blue B is a stain commonly known as basic blue B utilised in quantitative detection of phospholipids and as a nuclear stain [26].

4.2. Experiments

4.2.1. Reagents and Chemicals

Serotonin (5-HT), KCl, $K_4[Fe(CN)_6]$, Na_2HPO_4 and NaH_2PO_4 were procured from Himedia laboratories. Epinephrine(EP) was obtained by sigma Aldrich and its stock solution(25×10^{-4}) was prepared by dissolving in 0.1M perchloric acid solution. Victoria blue B(VBB) was replieved from nice chemicals. Highly viscous silicone oil and graphite powder were from Merck and Fluka. High purity chemicals were utilised and all the aqueous solutions were prepared from doubly demineralised water. The supporting electrolyte employed in all the investigations was 0.2 M phosphate buffer solution of required pH.

4.2.2. Investigation methods

All the voltammetric quantifications were functioned by using CHI-660c electro analyser (CH instrument-660 electrochemical workstation). The three electrode arrangement inhered with bare and poly (Victoria blue B) modified carbon paste working electrodes. Saturated calomel reference electrode and platinum wire auxiliary electrode. All evaluations were conducted at room temperature.

4.3. Results and discussion

4.3.1. Preparation of bare and p-VBB/MCPE

The BCPE was formulated by grinding the commixture of graphite powder and paraffin oil in relevant ratio of 70:30(w/w) using mortar and pestle to homogenised a mixture. The small proportion of homogeneous paste was stuffed into the cave of homemade Teflon tube and polished over smooth paper to attain crack free shiny surface. The electrical contact was accomplished at the end of the PVC tube through copper wire [32].

The electropolymerisation of Victoria blue B was done on the BCPE surface by placing 1mM Victoria blue B monomer in an electrochemical cell comprises of phosphate buffer (pH 7) supporting media having the speed rate of 100 mVs^{-1} using simple and convenient CV method (Fig. 4.1A). The potential was continually cycled from -0.4 to 1.2 V for 15 sequential scans. The dropping of peak current was observed with increasing the cyclic time endows the clue about the deposition of a polymeric layer on BCPE surface. The thickness of polymeric film was asserted by adjusting the number of multiple sweeps (from 5 to 25) and resultant current response of 5-HT (0.1×10^{-4}) and EP (0.1×10^{-4}) were reported as shown in inset Fig. 4.1B. The supreme catalytic properties were noticed for the electrode modified by 15 polymerisation sweeps. Thus 15 cycles were elected as optimum for electropolymerisation and cleaned with deionised water and utilised for detection of all electrochemical parameters.

4.3.2 Surface characterisation and area of the electrodes

Scanning electron microscopic (SEM) images were opted to characterise the morphology of the bare and modified working electrode surface as showed in Fig. 4.2A and 4.2B respectively. From the Fig. it was perceived that after electropolymerisation the electrode surface is relatively smoother and well distributed with poly (VBB) layer than bare surface and posses a higher responsive surface area after modification.

Fig. 4.3C displays the CV response tested for 1mM $K_4[Fe(CN)_6]$ as a probe at BCPE(solid line) and p-VBB/MCPE(dashed line) with 100 mVs^{-1} speed rates in 1M KCl supporting medium. The lower redox response was noticed at BCPE. Whereas, the current response was intensively upgraded at p-VBB/MCPE with minimisation of peak potential (ΔEp) as a function of higher rate of electron trasnfer. The total available surface area for electron transfer was evaluated by Randles-Sevick's formula (1) [33]. The greater electroactive surface area value found for p-VBB/MCPE(0.0399 cm^2) than BCPE(0.0298 cm^2). The variance between the surface area values of bare and modified sensors confirms that substantial change in morphological characteristics of the sensor. The magnitude of surface coverage concentration of

poly(VBB) membrane on BCPE was valued to be 0.024×10^{-11}M/ cm^2 by using equation (2)[34] .

$$Ip = (2.69\times10^5)\, n^{3/2}\, A\, D_0^{1/2}\, C_0\, \nu^{1/2} \quad \text{..... (4.1)}$$

$$Ip = n^2F^2A\Gamma\upsilon/4RT \quad \text{.....(4.2)}$$

Where, Γ (M/cm^2) is the surface coverage concentration, n is transferred electrons, A is area(cm^2) of developed electrode, C_0 is the concentration(mol/cm^3) of the electro active species, ν is sweep rate and D_0 is diffusion coefficient(cm^2s^{-1}).

4.3.3. Impact of supporting electrolyte and pH

The pH of the supporting media has huge impact on the electrode reaction of the analyte molecules. Fig. 4.4A depicts the CVs reviewed for 0.1×10^{-4} M 5-HT over 5.8 to 7.8 pH ranges in 0.2M PBS having the speed rate of $50mVs^{-1}$. As seen from the Fig., the 5-HT peak potentials relocated to negative direction with ascended pH values of the buffer solution. The relationship established between Epa of 5-HT with distinct pH values as portrayed in Fig. 4.4B and follows the regression expression, Ep(V) = 0.7289-0.055pH (R^2=0.9974) and the obtained slope value of 55mV/pH which is closer to Nernstian value. This instructed that the electro-oxidation of 5-HT includes the transport of equal number of protons and electrons [35]. However, the maximum peak sensitivity was achieved at pH 7.4. Hence it was selected for further analysis of 5-HT.

4.3.4. Voltammetric response of 5-HT at p-VBB/MCPE

The comparison investigation was carried out by employing CV method to attest the enhancement effect of designed electrode. Fig. 4.5 represents the CVs listed for 0.1×10^{-4} M 5-HT at BCPE(scattered line) and p-VBB/MCPE(solid line) in 0.2M PBS of pH7.4 with the speed rate of $50mVs^{-1}$. The irreversible oxidation of 5-HT was witnessed at both the electrodes (scheme 2). The less sensitive and higher magnitude of background current of 5-HT was obtained at BCPE. Despite that, a well-established anodic wave of 5-HT with improved peak current was noticed at p-VBB/MCPE. The increased sensitivity and declined over potential proofed the supreme electrocatalytic mediation effect of p-VBB/MCPE towards 5-HT detection.

4.3.5. Sweep rate implications on 5-HT at p-VBB/MCPE

The catalytic dependency of 5-HT peak current on the potential sweep rate was examined in presence of supporting solution of pH 7.4 containing 0.1×10^{-4} M of analyte. The voltammetric outcome suggested that the anodic peak current of 5-HT progressively raised with the sweep rate in the range of 50-500mVs^{-1}(Fig. 4.6A). The peak potentials switched slightly towards more positive values with increasing sweep rate. The graph of log Ipa of 5-HT with log ν presents the linearity as displayed in inset Fig. 4.6B with regression equation, log Ipa(μA) = 0.5389 logν(mV/s)+0.6544 (R^2=0.9833) which implies that the electrode phenomenon was controlled by diffusion[36]. The inset Fig. 4.6C depicts the plot of Ipa of 5-HT against $\nu^{1/2}$ with regression coefficient of R^2= 0.9848 indicates the proportionality between anodic peak current and square root of sweep rate.

4.3.6. Consequences of 5-HT concentration

The redox functioning of 5-HT at dissimilar concentration was probed to examine the responsiveness of the developed electrode. Fig. 4.7A exhibits the voltammogram logged for speckled concentration of 5-HT from 0.1×10^{-4} M to 0.7×10^{-4} M at p-VBB/MCPE by applying 0.2M PBS of physiological pH along with the scan rate of 50mVs^{-1}. The progressive increment of oxidation peak current with enhancing 5-HT concentration was spotted with a negligible transfer of Epa to positive values. The plot of Ipa of 5-HT with differed 5-HT concentration was graphed in inset Fig. 4.7B, it shows a excellent linearity with regression coefficient (R^2) of 0.9957. The configured sensor offers comparatively lower detection as well as quantification limits for 5-HT than other modified sensors(Table 4.1) and LOD measured to be 0.89μM and corresponding LOQ was 2.96μM by equating in equation(3) and (4)[37].

$$LOD = 3\sigma/M \quad \text{..... (4.3)}$$

$$LOQ = 10\ \sigma/M \quad \text{..... (4.4)}$$

Where, σ denotes standard deviation and M is slope from calibration plots.

4.3.7. Sensing of EP at BCPE and p-VBB/MCPE

The catalytic behaviour of EP was explored at bare and modified working electrodes through CV technique. Fig.4.8 demonstrate the CVs accounted for 0.1×10^{-4} M EP consisted 0.2 M supporting medium of pH 7.4 at BCPE (scattered curve) and p-VBB/MCPE(solid curve) and the sweep rate was $50mVs^{-1}$. The smaller oxidation peak was ascertained at BCPE. However, the extensive boost up of analytical signal with depletion in oxidation potential was seen with the incorporation of p-VBB/MCPE film onto the BCPE surface which validates the favourable electrode response of p-VBB/MCPE sensor towards EP. The electro oxidation of EP was provided in scheme 4.3.

4.3.8. Influence of scan rate variation and detection limit of EP at p-VBB/MCPE

Fig. 4.9A illustrates the voltammogram listed for 0.1×10^{-4} M EP at distinct scan rate at p-VBB/MCPE employing 0.2 M PBS of pH 7.4. The Fig. clearly evidenced that the magnitude of oxidation signals of 5-HT intensified with increasing scan rate in the range of 50-$500mVs^{-1}$ denotes the accelerated electron transfer on polymeric film of VBB. Fig. 4.9B Fig. 4.9C reveals the linear graphs obtained for the log Ipa of EP with log ν and peak current(Ipa) against square root of scan rate($\nu^{1/2}$) respectively. The plots exhibit the linear correlation with a slope of 0.51 and a good regression factor (R^2) of 0.9838 and 0.9798 respectively. It was confirmed that the diffusion controlled mechanism occurred at the modified sensor [38].

The impact of altering the EP concentration on its peak signals was analysed at P-VBB/MCPE in assistant with 0.2 M PBS of pH 7.4 at $50mVs^{-1}$ sweep rate as presented in Fig. 4.10A. The oxidation peak signals sharply amplified with addition of EP speckled from 0.1×10^{-4} M to 0.7×10^{-4} M with a minute change in peak potentials to positive side. The correlation between Ipa and dissimilar concentration of EP was plotted as illustrated in inset Fig. 4.10B, it gives a straight line and a regression factor (R^2) of 0.9772. Moreover, using the expression (3) and (4) the LOD and LOQ values quantified to be 0.33μM and 1.17 μM respectively (Table 4.2).

4.3.9. Concurrent resolution of 5-HT and EP at p-VBB/MCPE

The p-VBB/MCPE was implemented to investigate the sensing ability and specificity of the developed sensor towards simultaneous resolution of targeted analytes in their homogeneous mixture. Fig. 4.11 verified the CVs documented for the concurrent analysis of equimolar solution of 0.1×10^{-4} M 5-HT and 0.1×10^{-4} M EP at BCPE(dashed line) and p-VBB/MCPE(solid line) in occurrence of 0.2M PBS of physiological pH and $50mVs^{-1}$ sweep rate. The conventional BCPE failed to differentiate the peak signals because of their closer peak potentials. Whereas, the simultaneous separation of 5-HT and EP achieved at embedded p-VBB/MCPE, it gives individualised peak signals of 5-HT and EP with differed peak potentials situated at 0.25V and 0.10V respectively and the corresponding peak to peak potential difference was 0.15V. This examination proved the catalytic sensitivity of the tailored sensor in simultaneous experiment.

4.4.0. Selective study of 5-HT and EP at p-VBB/MCPE

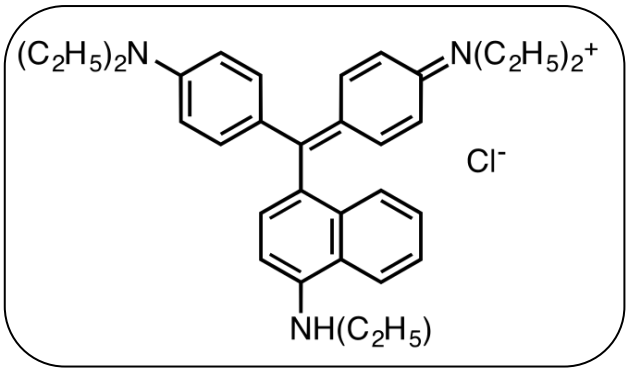
Highly sensible DPV technique was adopted to check the anti-interference stability of the customised sensor in a mixture of the sample by adjusting the concentration of each analyte and retaining the constant concentration of remaining species. Fig. 4.12A reveals the DPVs reported for varying the 5-HT concentration from 0.1×10^{-4} M to 0.8×10^{-4} M in presence of 0.2M PBS of pH7.4 having the speed rate of $50mVs^{-1}$ and holding the stable EP concentration(0.1×10^{-4}M). Furthermore, the EP concentration was deviated in the range of 0.1×10^{-4} M to 0.8×10^{-4} M with steady concentration of 5-HT (Fig. 4.13A). It can be seen from the Fig., the current intensity of 5-HT and EP positively uplifted with raise in their concentration without modifying the peaks of another molecule. The inset Fig. 4.12B and 4.13B represents the graph of Ipa with concentration of 5-HT and EP provides linearity with R^2 0f 0.9981 and 0.9879 respectively. By observing the above results, it can be concluded that the formulated sensor possessed acceptable specificity towards concurrent determination of 5-HT and EP.

4.4.1. Analytical applications and stability study of p-VBB/MCPE

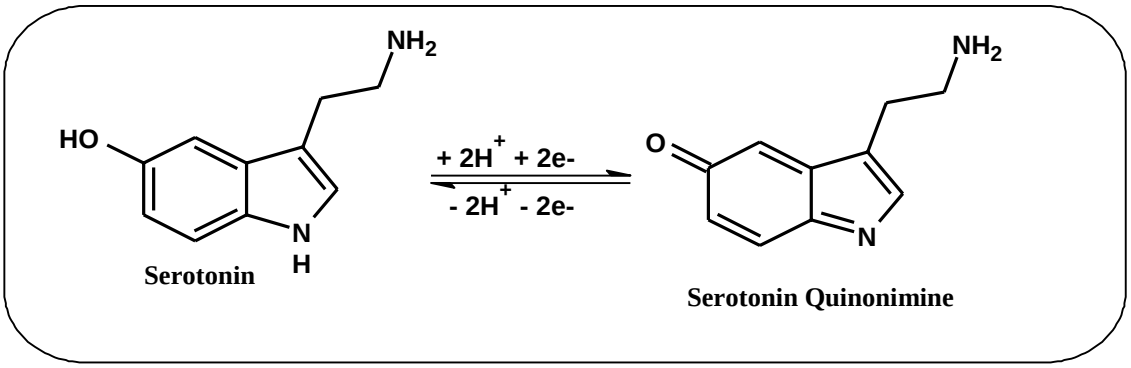
The tailored p-VBB/MCPE sensor was practiced for the exploration of 5-HT in serum sample by standard addition process. The obtained results show commendable recoveries as proved in Table 4.3. This outcome suggested the practical efficacy of the proposed method. The effectiveness of the fabricated electrode was assessed by stability study in mixture of 5-HT and EP (0.1×10^{-4} M) in presence of 0.2M PBS of pH 7.4 at the sweep rate of $50mVs^{-1}$ for 20 tedious measurements as showed in Fig. 4.14A. The peak current remains steady with constant peak potential for 5-HT and EP. As well, the developed sensor was conserved for a week at room temperature. Even after that the prepared sensor retained approximately 92.1% of its previous peak current response (Fig. 4.14B). This outcome discloses the steadiness and long service life of the proposed sensor.

4.5. Conclusion

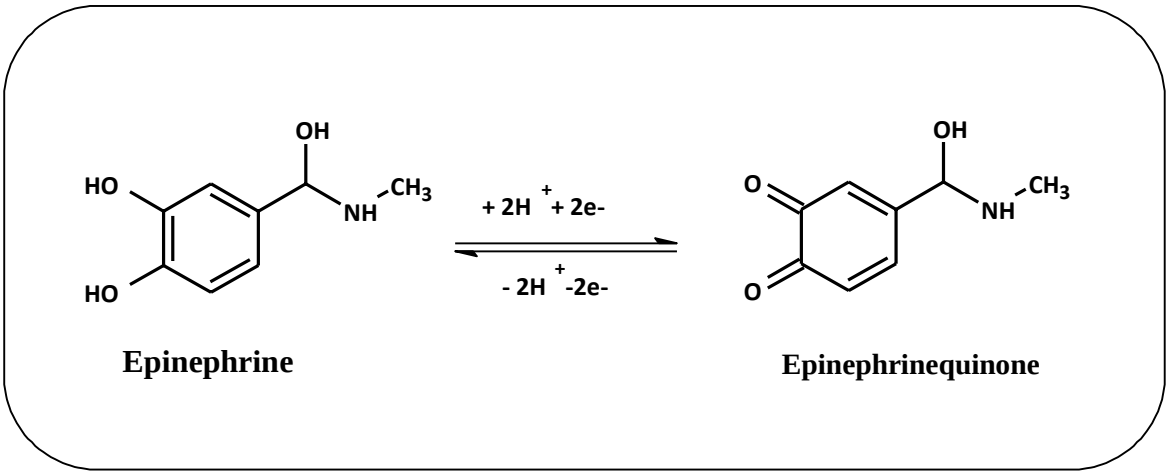
This study presents the establishment of an efficient sensor based on electropolymerisation of Victoria blue B monomer on BCPE surface by CV technique. The prepared electrode provides acceptable sensibility, specificity and robustness towards individual and concurrent determination of 5-HT and EP at physiological pH using CV and DPV technique. The overall electrode phenomenon reveals that the diffusion controlled kinetic at modified sensor and lower detection limit was achieved. The designed electrode also favourably detect5- HT in real sample. All these findings present the viability of the proposed method in sensing application of biologically active species.



Scheme. 4.1. Structure of Victoria blue B



Scheme. 4.2. Electrochemical oxidation of 5-HT



Scheme. 4.3. Electrochemical oxidation of EP.

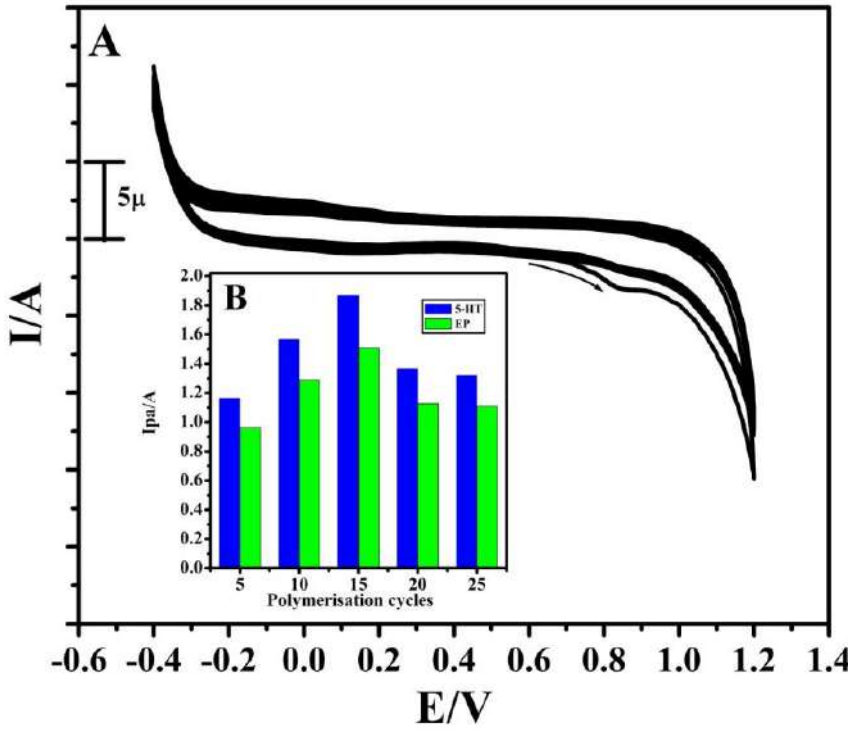


Fig. 4.1A. CVs of formation of p-VBB(1mM VBB) film deposited BCPE in presence of 0.2M PBS(pH 7.4) at sweep rate of 100 mVs^{-1}. **B.** Plot of Ipa of 5-HT and EP versus number of polymerization sweeps.

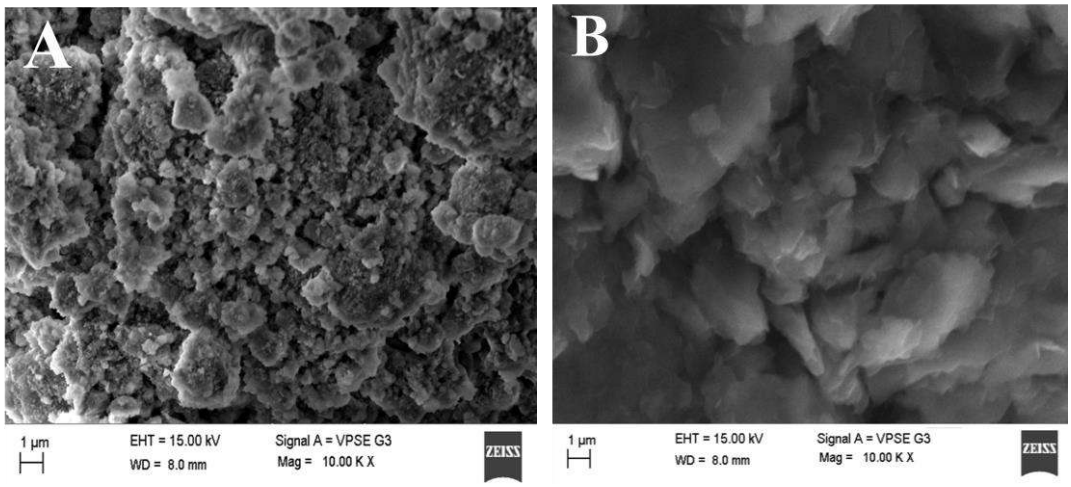


Fig. 4.2.A and 2B. The SEM images captured for BCPE and p-VBB/MCPE

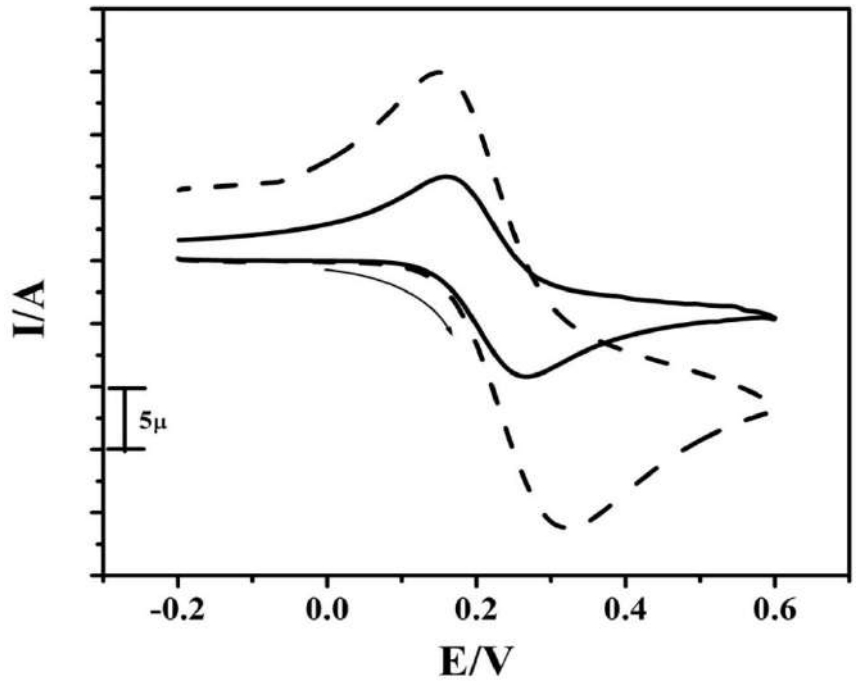


Fig. 4.3. CVs recorded for 1mM $K_4[Fe(CN)_6]$ in 1M KCl at BCPE(solid line) and p-VBB/MCPE(dashed line) with the scan rate of 50 mVs^{-1}.

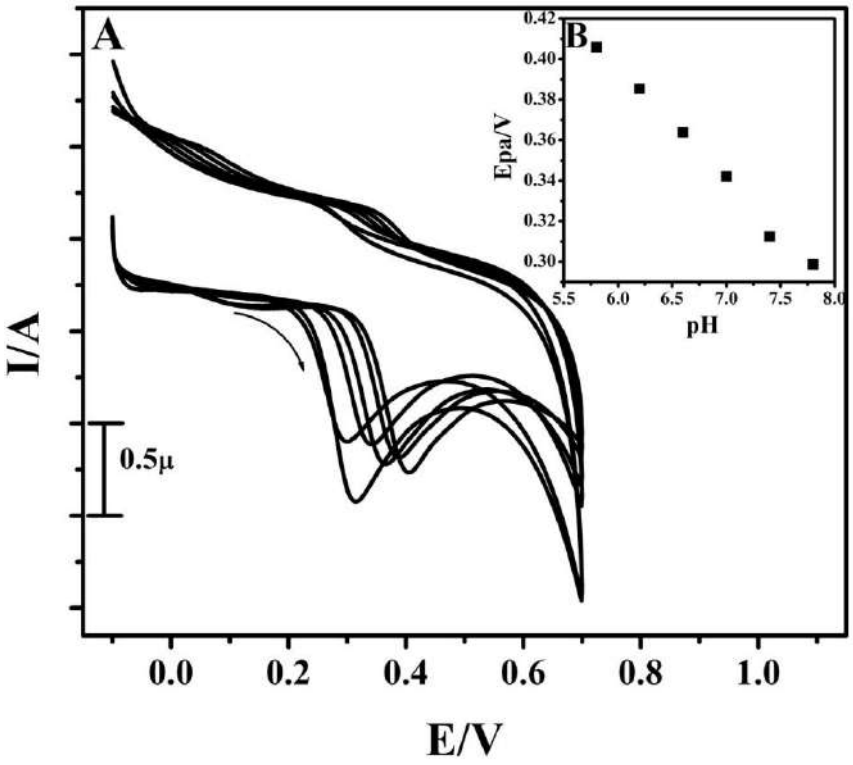


Fig. 4.4A CVs for influence of buffer pH on the oxidation peak for 0.1×10^{-4} M 5-HT at distinct pH (5.8-7.8) at p-VBB/MCPE. **B.** Plot of Epa of 5-HT versus varied pH.

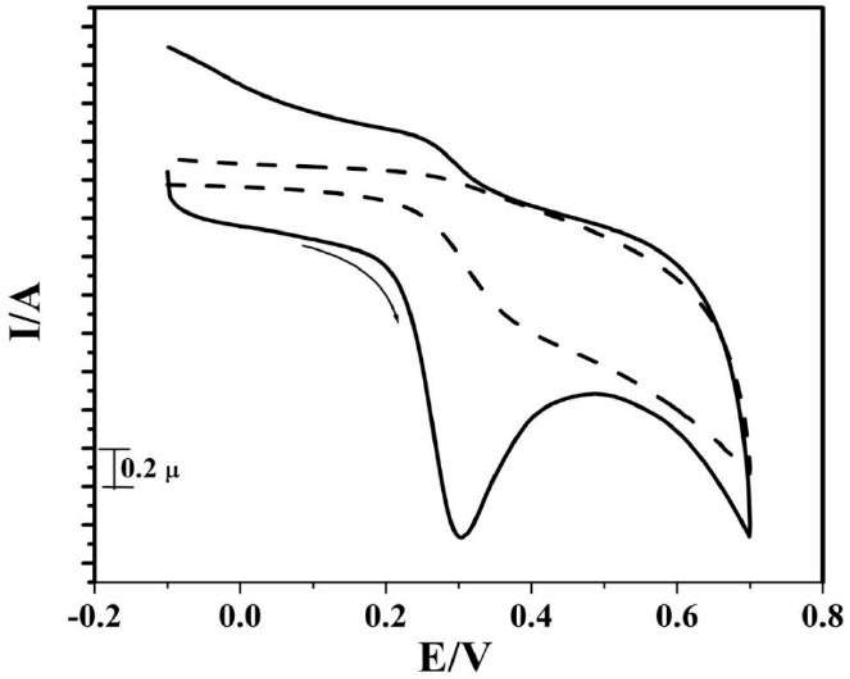


Fig. 4.5. CVs of 0.1×10^{-4} M 5-HT in 0.2M PBS of pH 7.4 at BCPE(scattered line) and p-VBB/MCPE(solid line) using 50 mVs^{-1}.

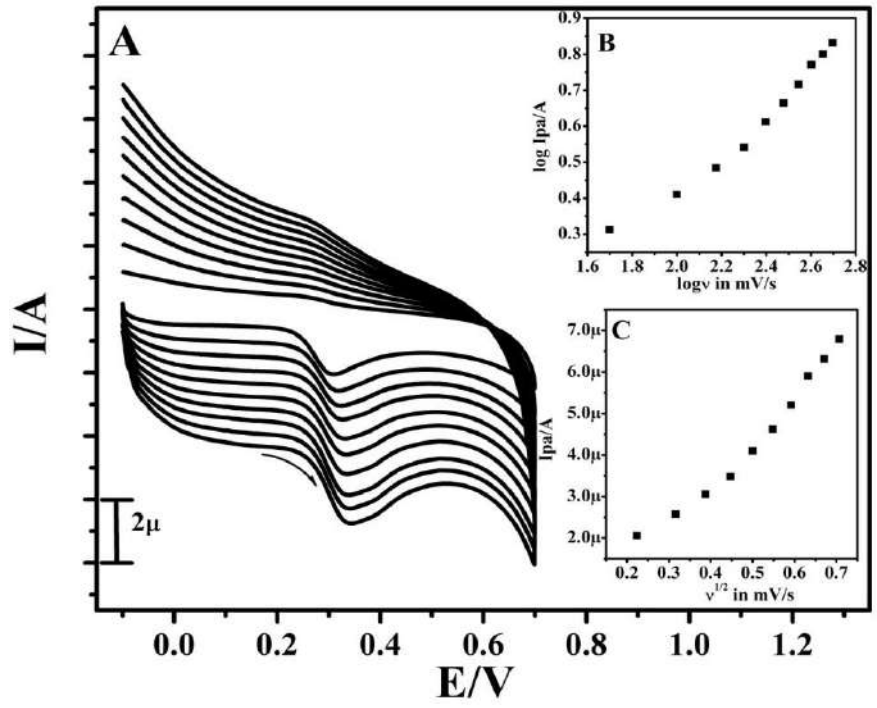


Fig. 4.6A CVs of p-VBB/MCPE in 0.2M PBS of pH7.4 in 0.1×10^{-4} M 5-HT at differed sweep rates (50-500 mVs^{-1}). **B.** Plot of log Ipa of 5-HT with log ν. **C.** Graph of Ipa of 5-HT versus square root of scan rate**(**$\nu^{1/2}$).

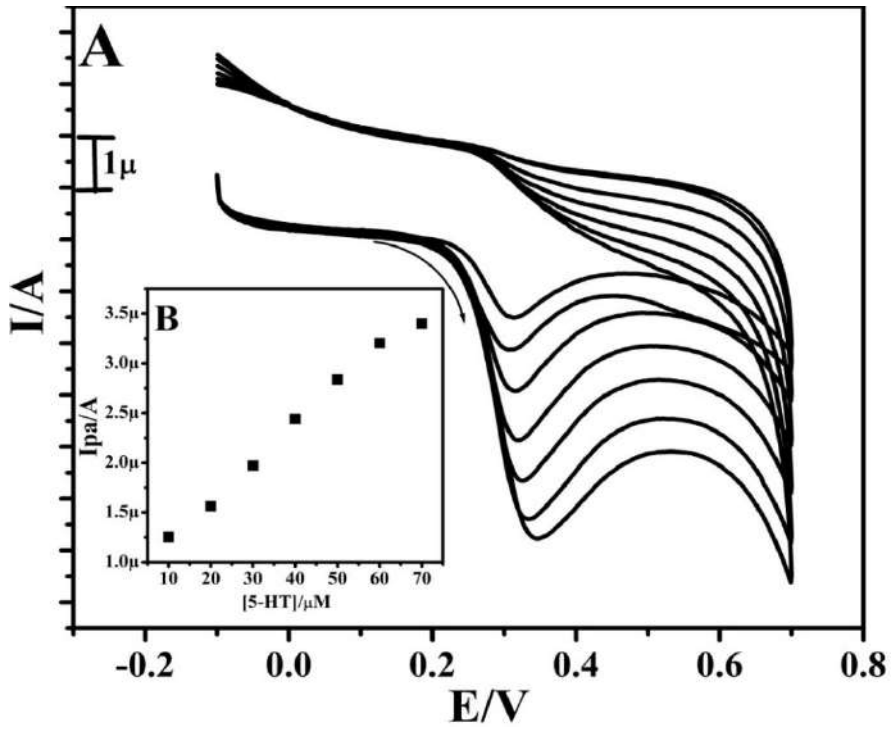


Fig. 4.7A CVs of 5-HT in 0.2M PBS of pH 7.4 at sweep rate of 50mVs^{-1} for dissimilar concentration form 0.1×10^{-4} M - 0.7×10^{-4} M. **B.** Plot of Ipa versus concentration of 5-HT at p-VBB/MCPE.

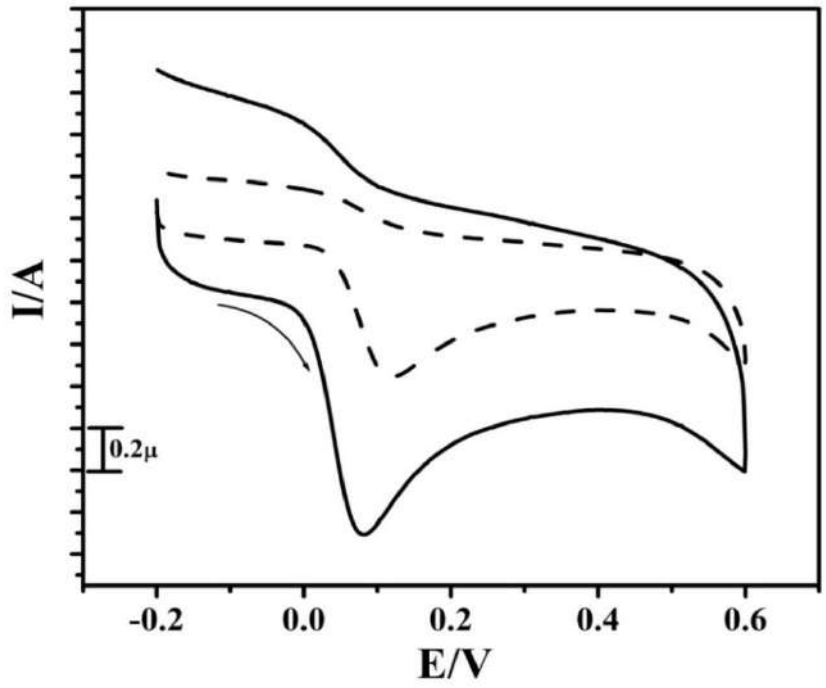


Fig. 4.8. CVs 0.1×10^{-4} M EP in 0.2M PBS of pH 7.4 at BCPE(scattered curve) and p-VBB/MCPE(solid curve) using 50 mVs^{-1}.

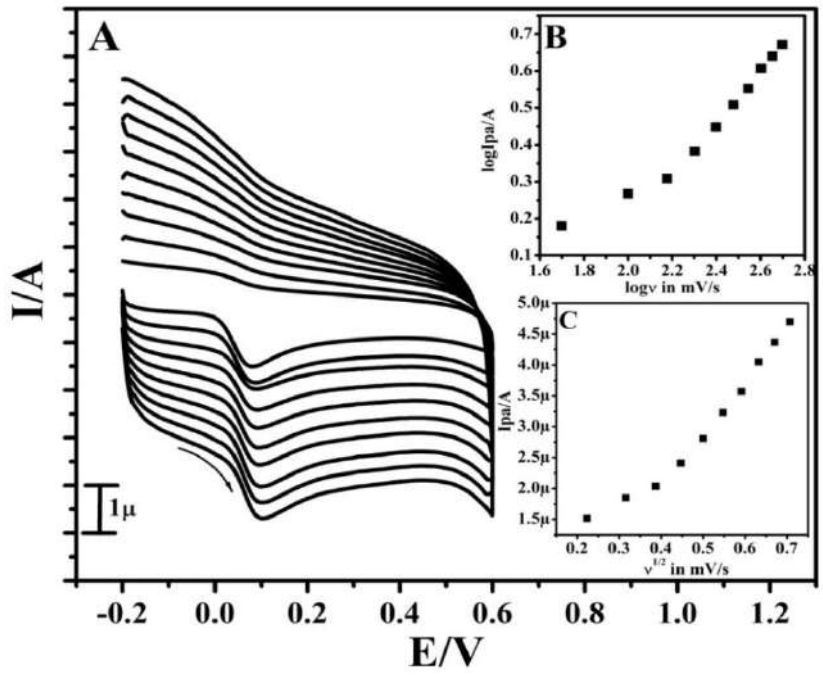


Fig. 4.9A. CVs of 0.1×10^{-4} M EP at p-VBB/MCPE in 0.2M PBS of pH7.4 at differed sweep rates (50-500 mVs^{-1}). **B.** Plot of log Ipa of EP with log ν. **C.** Graph of Ipa of EP versus square root of scan rate **(**$\nu^{1/2}$).

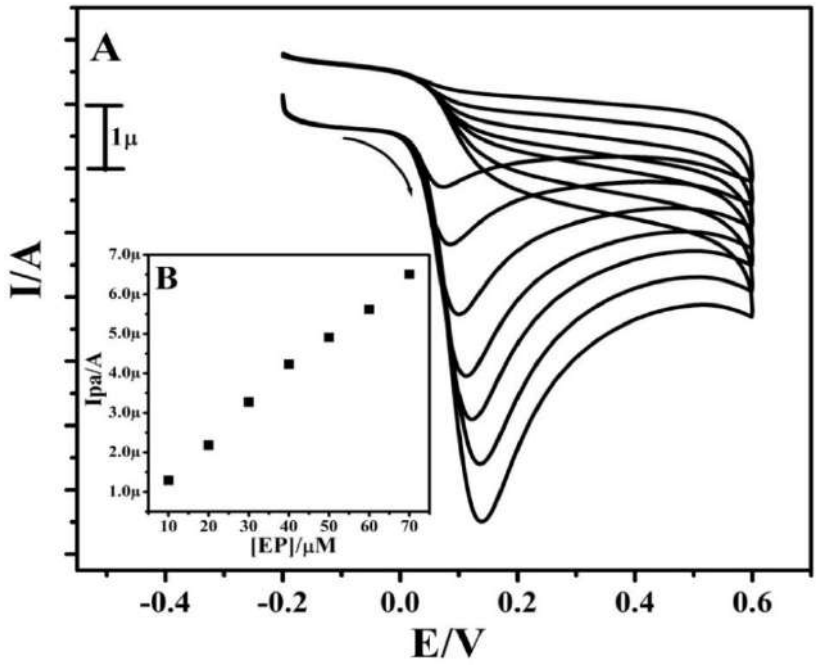


Fig. 4.10A. CVs of EP in 0.2M PBS of pH 7.4 at sweep rate of $50mVs^{-1}$ for dissimilar concentration form 0.1×10^{-4} M - 0.7×10^{-4} M. **B.** Plot of Ipa versus concentration of Ep at p-VBB/MCPE.

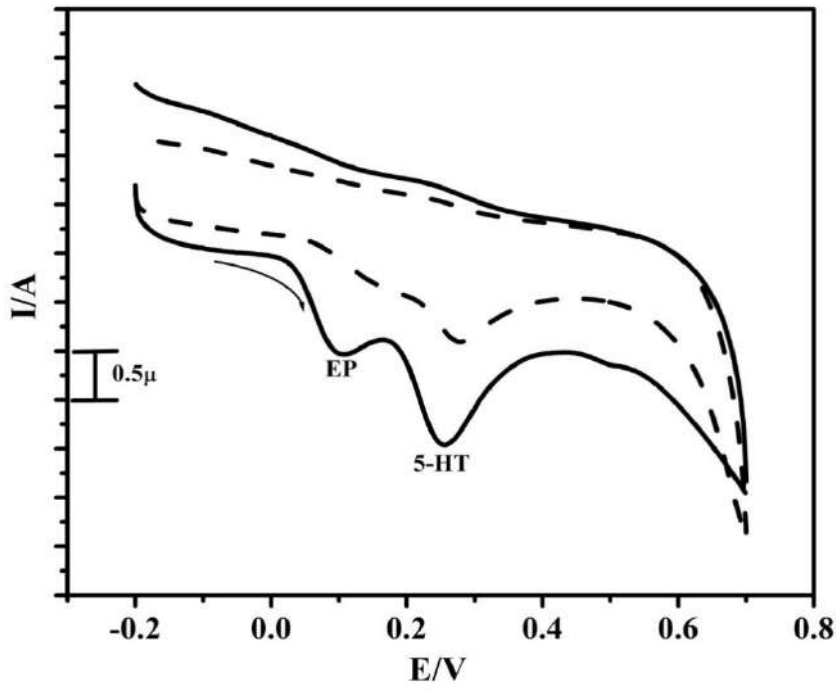


Fig. 4.11. CVs recorded for concurrent resolution of 0.1×10^{-4} M 5-HT and 0.1×10^{-4} M EP with the scan rate of 50 mVs^{-1} using 0.2M PBS of ph 7.4 at BCPE(dashed line) and p-VBB/MCPE(solid line).

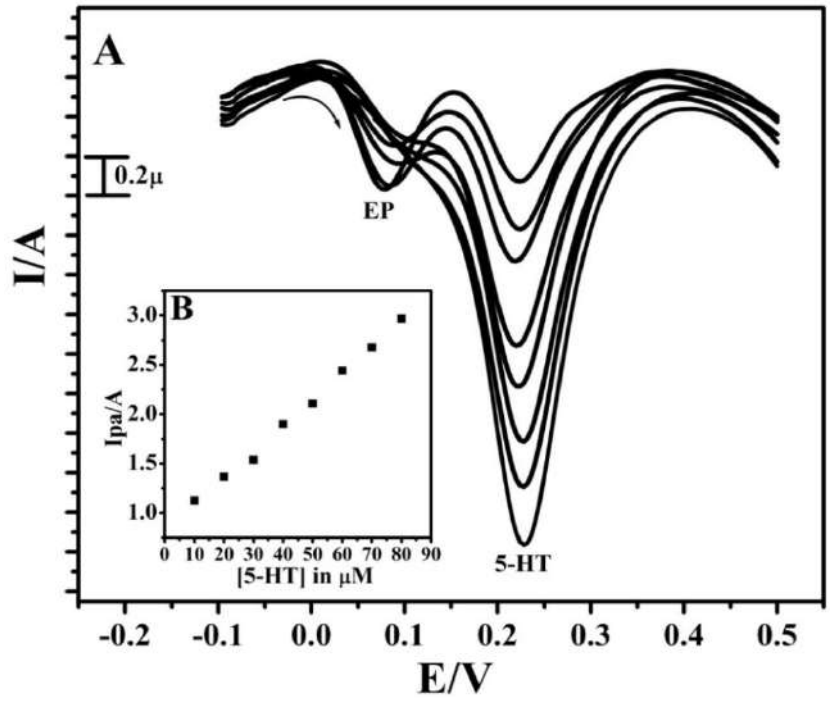


Fig. 4.12A. DPVs for variation of 5-HT concentration from 0.1×10^{-4} M - 0.8×10^{-4} M in PBS of pH 7.4 with 0.1×10^{-4} M EP at p-VBB/MCPE and 50mVs^{-1} sweep rate. **B.** Graph of Ipa v/s different 5-HT concentration.

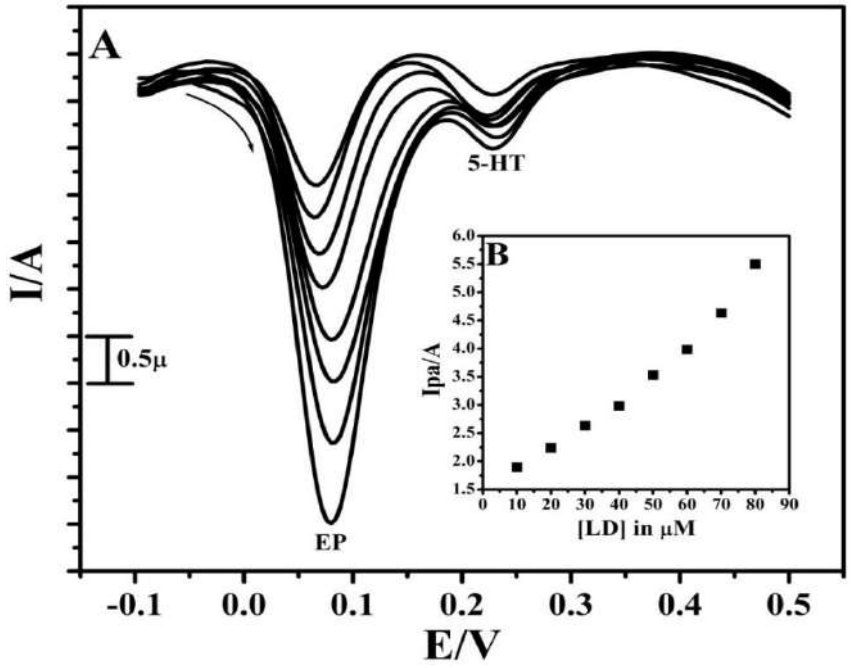


Fig. 4.13A. DPVs for altering EP concentration from 0.1×10^{-4} M - 0.8×10^{-4} M in PBS of pH 7.4 with 0.1×10^{-4} M 5-HT at p-VBB/MCPE and 50mVs^{-1} sweep rate. **B.** Graph of Ipa v/s different EP concentration.

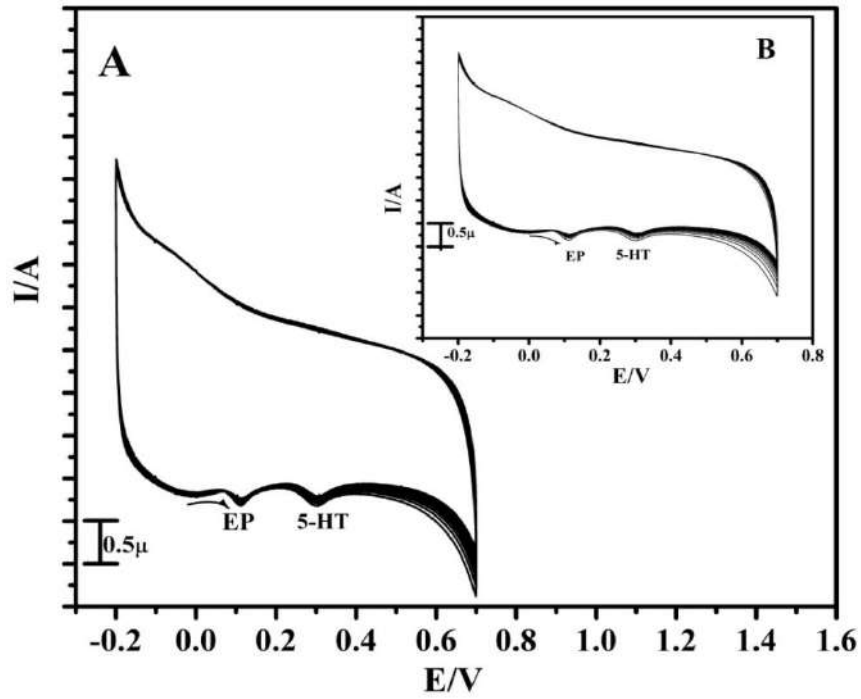


Fig. 4.14A.CVs recorded for the mixture of 5-HT and EP (0.1×10^{-4}) in presence of 0.2M PBS of pH 7.4 with the sweep rate of 50mVs^{-1} at p-VBB/MCPE for 20 cycles. **B.** CVs noted for mixture of 5-HT and EP (0.1×10^{-4}) after a 7 days at p-VBB/MCPE for 20 scans.

Table.4.1. Comparative analytical behavior of 5-HT at p-VBB/MCPE with other modified electrodes.

SI No	Electrode	Detection limit(μM)	Method	Reference
01	AuNPs@PPy/GSPE	33.2	SWV	[39]
02	CDP-Choline/MCPE	5.81	CV	[40]
03	3D-ITO	7.5	DPV	[41]
04	IL-DC-CNT/GE	2.0	DPV	[42]
05	P-VBB/MCPE	0.89	CV	**This work**

Table.4.2. Comparative analytical behavior of EP at p-VBB/MCPE with other modified electrodes.

SI No	Electrode	Detection limit(μM)	Method	Reference
01	TX-100/CPE	1.0	CV	[43]
02	AlpM-CPE	0.46	CV	[44]
03	Au 4MpyAuNPs	4.5	CV	[45]
04	PSAF-MCPE	0.61	CV	[46]
05	MgO-MWCNTs-MCPE	0.83	CV	[47]
06	P-VBB/MCPE	0.89	CV	**This work**

Table.4.3. Applicability of the p-VBB/MCPE for the detection of 5-HT in spiked serum sample.

Sample	5-HT added (μM)	Found (μM)	Recovery (%)
1	20	19.61	98.05
2	30	29.36	97.86
3	40	39.41	98.52

4.5. Reference:

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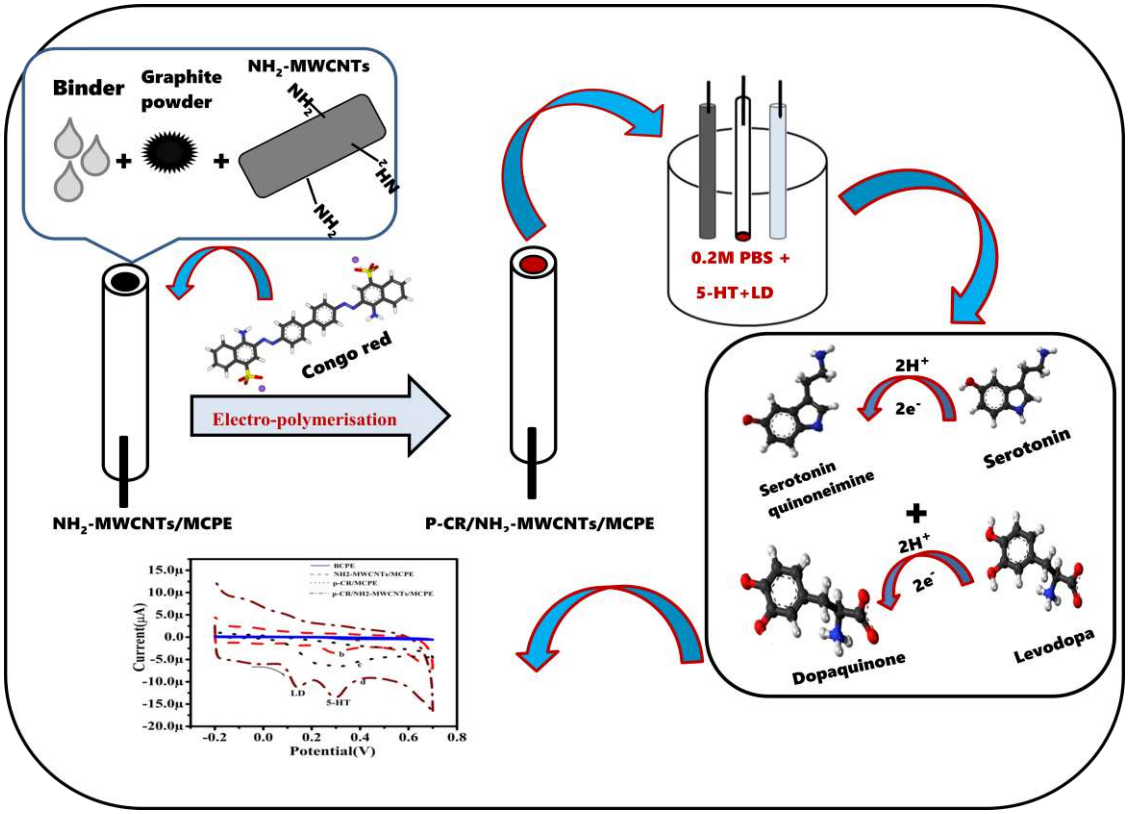
Chapter-IV

PART-B

POLY (CONGO RED) FUNCTIONALISED-MWCNT COMPOSITE ELECTRODES FOR THE SIMULTANEOUS VOLTAMMETRIC DETECTION OF SEROTONIN AND LEVODOPA IN HUMAN SERUM



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4.6. Introduction

Now-a-days, the emerging trend in the field of analytical electrochemistry is the designing of novel electrochemical biosensors combined with simplicity, excellent sensitivity, and developed by inexpensive and easily available materials [1-3]. These portable sensors can detect the analyte molecules in their lower concentration levels and can be disposable. This set of beneficial traits has impressively increased the implimentations of sensors to clinical, industrial, environmental, and food analysis and is economically desirable because of their affordable instrumentation and accuracy [4-7].

Serotonin (5-HT, 5-hydroxytryptamine) is an important neuromodulator and fulfilled as a putative biogenic neurotransmitter that occurs in the central and intrinsic nerve system of the brain. It is synthesized by serotonergic neurons and successfully acts as a chemical agent that transmits signals between nerve cells [8-10]. 5-HT is also found in blood platelets and enterochromaffin cells within the alimentary canal [11]. 5-HT makes a principal contribution in numerous psychopathological, biological and physical processes includes mood-stabilizing, cognition, reward, learning, alcoholism, psychosis, eating disorder, sleep, and thermoregulation [12, 13]. Change in 5-HT concentration than its normal range can leads to severe disorders for instance lower concentration of 5-HT has been associated with migraine, bipolar disorder, anxiety, and depression [14, 15]. The increased 5-HT levels lead to toxicity, carcinoid syndrome, obsessive-compulsive disorder, and autism [16, 17].

Levodopa (L-3, 4-dihydroxyphenylalanine, L-Dopa) is an unfamiliar amino acid that performs decisive responsibilities in the biological functions of humans, plants as well as in some animals [18]. LD is a precursor for various neurotransmitters like dopamine, adrenaline, and nor-adrenaline. Parkinson's disease is a neurological disorder produced due to a deficiency of dopamine in the brain. LD increases and acts as a prodrug of dopamine and is the main medication used to treat the symptoms of Parkinson's disorder [19, 20]. This catecholamine drug is also used to treat dopamine-responsive dystonia and reduces the symptoms of brady kinesia, resting tremor, flexed posture, and rigidity [21]. The elevated and long-term usage of LD causes adverse health effects on humans, for example, paranoia, dyskinesia, and gastritis [22]. Both

5-HT and LD are co-existed in thehuman system. The increase in dopamine level by LD can cause damage in serotonin neurons and lowers the 5-HT concentration. LD also interferes in 5-HT metabolism and inhibits its synthesis in humans [23, 24]. Therefore, the formulation of a sensitive and authentic analytical systems for the detection of 5-HT in presence of LD in clinical formulations is essential for the identification and treatment of disorders.

Multiwalled carbon nanotubes (MWCNTs) incorporated into carbon electrodes employed as extensive voltammetric sensors gained enormous attention in recent days because of their unique features[25]. These are the carbon molecules that possess a larger surface area, greater surface chemical functionality, high size stability. MWCNTs reduce the fouling of electrode surface and having good mechanical and electrical characteristics [26, 27].

Congo red (CR) has a complex chemical structure and belongs to the family of azo dyes derived from benzidine and formerly used in the dyeing of cotton. It is also employed in histology to stain tissues and serves as an acid-base indicator. It provides an excellent electrode response toward bioactive molecules when used as modifying material [28, 29]. In this present study, the CR dye was used to electropolymerized the amino-functionalized MWCNTs modified carbon paste electrode surface. The fabricated electrode was utilized for the selective and coincidental investigation of Serotonin and levodopa at neutral pH. The modified composite sensor exhibits good sensing ability towards 5-HT and LD carried out by CV and DPV techniques.

4.7. Experimental procedures

4.7.1. Materials and equipment

All the chemical agents used for the study were of high purity and served as supplied without any amendment. Fine graphite powder of appropriate particle size (50 μm) and a binding agent (paraffin oil) were procured from Loba Chemie and Himedia. 5-HT, LD, NaOH, and HCl were obtained from Himedia. CR and the reagents used to prepare a buffer solution i.e., Sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were supplied by Merck,

and Amino-functionalized multiwalled carbon nanotubes (NH_2-MWCNTs) were fetched from Sigma Aldrich. The stock solutions of 5-HT (25 μM) and LD (25 μM) were prepared by using demineralized water and HCl (0.5 M) respectively. 0.2 M Phosphate buffer of the desired pH was prepared by a mixture of the suitable ratio of 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4. Doubly distilled water was utilized to prepare all aqueous solutions.

The CH instrument of model 660c (CH instrument-660c electrochemical workstation) was used in the entire measurements and data extraction. The instrument is associated with three-electrode one component cell assembly includes platinum wire auxiliary electrode, saturated calomel (SCE) reference electrode, and working electrodes. Series of platforms were examined as working electrodes such as bare carbon paste electrode (BCPE), NH_2-MWCNTs modified carbon paste electrode(NH_2-MWCNTs/MCPE), electro-polymerized Congo red on carbon paste electrode (p-CR/MCPE), and p-CR on the surface of NH_2-MWCNTs/MCPE (p-CR/NH_2-MWCNTs/MCPE). All the measurements were exercised at ambient temperature.

4.7.2. Fabrication of working electrodes

The BCPE was designed by plodding the adequate ratio of graphite powder and a binding agent (70:30) in an agate mortar for about half an hour to achieve a uniformly wetted carbon paste. A minute quantity of derived paste was filled tightly into the cave of the Teflon tube and flattened over a piece of butter paper [30] to get the uniform surface. Copper wire was exploited to secure the electrical contact. The identical grinding and packing process was followed for the fabrication of NH_2-MWCNTs/MCPE. During this, enough amount of corresponding material (NH_2-MWCNTs) was combined with the unmodified carbon paste.

The p-CR/MCPE was fabricated by electro-polymerization of 1 mM Congo red on the surface of BCPE involving 0.1M NaOH by cyclic voltammetric techniques for 15 cycles at a potential extent of 0.0V to +1.5V at a sweep rate of 100 mV/s. Likewise, in contrast to BCPE, NH_2-MWCNTs/MCPE platform was used to electro-polymerize Congo red for the fabrication of p-CR/ NH_2-MWCNTs/MCPE. Thereafter the electrode surface was carefully cleansed with double deionized water to wipe out

the unpolymerised Congo red, and the electrode was employed for the 5-HT detection in presence of levodopa.

4.8. Results and discussion

4.8.1. Effect of modifiers

The amount and characteristics of modifying species directly affect the electrochemical action of the BCPE. Here, the NH_2-MWCNTs/MCPE was constructed by the addition of different weights of NH_2-MWCNTs to the bare carbon paste and applied for the investigation of electro-oxidation of 5-HT and LD. By raising the quantity of NH_2-MWCNTs, the oxidation peak currents increase upto a specific ratio (4mg) as shown in Fig. 4.15A. Beyond this ratio, an increase in NH_2-MWCNTs weight steadily reduces the peak currents. Thereby, 4mg was selected for modification. To enhance the detection sensitivity, The NH_2-MWCNTs/MCPE surface was electropolymerized by 1mM aqueous congo red in presence of supporting electrolyte 0.1M NaOH. The electropolymerized film was grown within the potential window of 0.0V to +1.5V at the sweep rate of 100 mV/s with assistance with cyclic voltammetry for 15 cycles (Fig. 4.15B). The film thickness can be restricted by deviating the multiple cycles (from 5cycles to 25 cycles). Fig. 4.15C represents the plot of the peak current of 5-HT and LD versus the number of polymerization cycles. The higher current sensitivity was assured at 15 scans. Therefore 15 cycles were chosen for all subsequent electroanalysis.

4.8.2. Characterization of BCPE and modified carbon paste electrodes

To evaluate the electrochemical performance, the bare and modified electrodes were examined using standard potassium ferrocyanide ($K_4[Fe(CN)_6]$) as a probe in presence of 1 M KCl supporting buffer by CV technique. Fig. 4.16 illustrates the CVs reported for 1mM $K_4[Fe(CN)_6]$ at BCPE(dotted line), NH_2-MWCNTs/MCPE(dashed line), p-CR/MCPE(dashed-dotted line), and p-CR/NH_2-MWCNTs/MCPE-MWCNTs/MCPE(solid line) at a sweep rate of 50 mV/s. The decreased redox peak response and wide separation of peak potentials (ΔEp) were ascertained at BCPE, NH_2-MWCNT/MCPE, and p-CR/MCPE owing to the delayed electron transfer rate. At the similar situation, the massive improvement in peak current values and

considerable diminution in ΔEp was noticed at p-CR/NH_2-MWCNTs/MCPE which ascribed the faster electron transfer kinetics due to modification. This illuminates the high affinity of CR for carbon nanotubes and the resulting sensor can bind the electroactive molecule (noncovalent pi-pi interactions) [31, 32]. The effective surface area for the separate electrodes was evaluated through Randle-Sevick'sequation (1) [33].

$$Ip = (2.69\times10^5)\, n^{3/2}\, A\, D_0^{1/2}\, C_0 \nu^{1/2} \ldots\ldots\ldots\ldots\ldots (4.5)$$

Where, Ip is the peak current in A, A is the active surface area (cm^2), D_0 is diffusion co-efficient (cm^2s^{-1}), C_0 denotes concentration of electroactive entity ($molcm^{-3}$), n is the number of transferred electrons. The values of surface area calculated for different electrodes were found to be 0.0254 $cm^2$for BCPE, 0.0298 $cm^2$for NH_2-MWCNTs/MCPE, 0.0366 cm^2 for p-CR/MCPE and 0.0511 cm^2 for p-CR/NH_2-MWCNTs/MCPE.

An adequate quantity of incorporated p-CR film on NH_2-MWCNTs/MCPE surface was estimated by practicing the formulae (2) [34].

$$Ip=n^2F^2A\Gamma\upsilon/4RT \ldots\ldots\ldots\ldots (4.6)$$

Where Γ (M/cm^2) indicates the surface coverage concentration, n is the number of electrons exchanged. The surface coverage concentration (Γ) of p-CR on NH_2-MWCNTs/MCPE was to be 0.0422×10^{-11} M/cm^2.

4.8.3. Comparative sensing behaviour of working electrodes towards 5-HT and LD detection

The electro-catalytic functioning of 5-HT and LD at bare and different modified electrodes was inspected through the CV technique. Fig. 4.17A and Fig. 4.17B depicts the CVs recorded for 10 μM 5-HT and 20 μM LD at BCPE (curve a), NH_2-MWCNTs/MCPE (curve b), p-CR/MCPE (curve c), and p-CR/NH_2-MWCNTs/MCPE (curve d) in occurrence of 0.2 M PBS of pH 7.0 at a sweep rate of 50 mV/s. Both 5-HT and LD show irreversible peak behaviour and a broad oxidation peak potential and weak oxidation peak currents were observed at BCPE and NH_2-MWCNTs/MCPE. A slight increment in electrochemical response was obtained at p-CR/MCPE. However, the p-CR/NH_2-MWCNTs composite electrode exhibits striking

improvement towards oxidation peak current of 5-HT and LD, illustrates that the boost up of the electron transfer rate after electro-polymerization. The greater increment in the current response of analytes implied that the composite electrode has a greater active surface area, excellent biocompatibility and good catalytic properties towards 5-HT and LD detection.

4.8.4. Impact of solution pH

The oxidation potential and peak current of electro-active species are relatively dependent on the pH of the supporting electrolyte. To get better sensitivity and good peak resolution, The influence of solution pH on current response of 10 μM 5-HT and 20 μM LD were investigated in presence of 0.2 M PBS at disparate pH values varied over the range of 6.2-7.8 along with sweep rate of 50 mV/s as shown in Fig. 4.18A and Fig. 4.19A. From the Figure, it is evidenced that the anodic peak potential for both 10 μM 5-HT and 20 μM LD were shifted linearly towards a more negative potential side with higher pH of the solution. This signifies the involvement of electrons and protons in the electro-oxidation of 5-HT and LD at p-CR/NH2-MWCNTs/MCPE. Fig. 4.18B and Fig. 4.19B depicts the plots of pH value of supporting electrolyte versus anodic peak potentials of 5-HT and LD. The graphs show good linearity with the slope value for 5-HT was 53 mV/pH (R^2=0.98927) and for LD the value was 63 mV/pH (R^2=0.99328). These values obey the theoretical Nernst equation for the uniform protons and electrons transfer in the reaction [35]. Meanwhile, as the pH of the solution increases, the oxidation current of 5-HT and LD gradually increases upto pH 7.0. When the pH value is above 7.0, the peak current density diminished. However, the maximum peak current for10 μM 5-HT and 20 μM LD was noticed at pH 7.0. Hence, this pH was selected as ideal for further investigations.

4.8.5. Influence of speed rate on 5-HT and LD at p-CR/NH2-MWCNTs/MCPE

The influences of potential sweep rate on anodic peak currents of 5-HT and LD at p-CR/NH_2-MWCNTs/MCPE were probed by the CV technique. The impact of changing speed rate for 10μM 5-HT in 0.2 M PBS of pH 7.0 is illustrated in Fig. 4.20A. By the raise of scan rate, the peak current of 5-HT raised from 50-500 mV/s and the peak potential relocated to slight positive side. Inset Fig. 4.20B and Fig. 4.20C

exhibits the plots of the relationship between the log of oxidation current (log Ipa) of 5-HT versus the log of varied scan rates (log ν) and graph of Ipa of 5-HT against square root of scan rate ($\nu^{1/2}$). The entrained graphs present good linearity i.e. log Ipa(μA) = 0.8053 log ν(mV/s) + 0.9634 associated with regression co-efficient r^2=0.9968. This proves that the nature of the electrode process for 5-HT at p-CR/NH_2-MWCNTs/MCPE was adsorption controlled [36]. Fig. 4.21A displays the CVs documented for LD at distinct scan rates (50-500mV/s). From the Figure, peak currents of LD gradually enhance with an increase in potential sweep rates. Inset Fig. 4.21B and Fig. 4.21C expresses the graph of log Ipa of LD versus logν and plot of Ipa of LD in contrary to $\nu^{1/2}$ respectively. The determined slope value from the graph was 0.6490(i.e logIpa (μA) = 0.6490 logν (mV/s) + 0.6908), which closer to the theoretical value for diffusion-controlled electrode process [37].

4.8.6. Concentration variation and limit of detection

To examine the detection sensibility of the modified electrode, voltammetric responses of 5-HT and LD at different concentrations were assayed under optimum conditions. The reported CV plot for 5-HT concentration from 10 μM– 50 μM in appearance with 0.2 M PBS of pH 7.0 at the sweep rate of 50mV/s at p-CR/NH_2-MWCNTs/MCPE was displayed in Fig. 4.22A. The appreciable improvement in the anodic peak currents (Ipa) of 5-HT with an intensifying the concentration was observed with a shift in anodic peak potential to the positive direction from 298 mV to 302 mV. Fig. 4.22B depicts the calibration plot between Ipa of 5-HT and varied in the concentration of 5-HT. The outcome implies that the peak current is proportional to the 5-HT concentration with excellent linearity and the value of r^2 was found to be 0.9973. Fig. 4.23A illustrates the CV curve for changing the concentration of LD in a linear dynamic range of 20μM−110 μM at p-CR/NH_2-MWCNTs/MCPE in 0.2 M PBS of pH 7.0 having the sweep rate of 50 mV/s. The Ipa of LD significantly elevated with increasing concentration of LD and the oxidation peak potential moved towards the positive side from 131 mV to 164 mV. A linear relationship was ascertained by plotting the Ipa of LD versus the concentration of LD as showed in Fig. 4.23B with an obtained regression coefficient of 0.9987. The LOD and LOQ values were computed via ensuing equations (2) & (3) [38].

$$LOD = 3S/M \quad (4.7)$$

$$LOQ = 10S/M \quad (4.8)$$

Where M is the slope of the calibration plot and S is the standard deviation of measurements. The LOD values for 5-HT and LD were found to be 1.7 μM and 3.0 μM respectively and the corresponding LOQs were 5.9 μM for 5-HT and 10.1 μM for LD. The LOD's of 5-HT and LD at p-CR/NH_2-MWCNTs/MCPE were compared with previously reported modified electrodes and the results were summarised in Table.4.4 and Table.4.5.

4.8.7. Simultaneous resolution of 5-HT and LD at p-CR/NH_2-MWCNTs/MCPE

The principal objective of the present study was to develop a capable modified electrode for sensible and selective detection of a binary mixture of 5-HT and LD. Individual detection of these species in a mixture has suffered from the overlapping of voltammetric signals because of their comparable oxidation potentials. To establish the efficiency of p-CR/NH_2-MWCNTs/MCPE, the electrochemical action of a mixture of 10μM 5-HT and 20 μM LD were analyzed by cyclic voltammetric and differential pulse voltammetric methods. Fig. 4.24A and inset Fig. 4.24B demonstrates the CVs and DPVs obtained for a mixture of 10 μM 5-HT and 20 μM LD in 0.2M PBS of pH 7 at the sweep rate of 50 mV/sat BCPE (curve a), NH_2-MWCNTs/MCPE (curve b), p-CR/MCPE (curve c) and p-CR/NH_2-MWCNTs/MCPE (curve d). The oxidation potentials of 10 μM 5-HT and 20 μM LD were less sensitive and incapable to separate at BCPE, NH_2-MWCNTs/MCPE, and p-CR/MCPE. Despite that, the feasibility of the modified electrochemical sensor for the simultaneous resolution of 5-HT and LD was accomplished. Because it gives two well-established sharp peaks of 5-HT and LD with enhanced sensitivity and reduces the over potential. The separate peak potentials were noticed at 298 mV and 142 mV for CVs of 5-HT and LD respectively and the potential difference between the two peaks was found to be 156 mV. The oxidation potentials of DPVs of 10 μM 5-HT and 20 μM LD were positioned at 246 mV and 94 mV respectively and the corresponding peak-to-peak separation were found to be 152 mV. This outcome was sufficient to recognize the 10μM 5-HT and 20 μM LD at p-CR/NH_2-MWCNTs/MCPE individually and simultaneously.

4.8.8. Interference investigation

A highly sensitive differential pulse voltammetric method was occupied towards the interference investigation of a mixture of a sample containing 10 μM 5-HT and 20 μM LD at p-CR/NH_2-MWCNTs/MCPE in the bearing with 0.2 M PBS of pH 7 at the sweep rate of 50 mV/s by varying one analyte concentration and keeping the fixed concentration of another. Fig. 4.25A provides the DPVs profile for raising 5-HT concentration from 10 μM-100 μM at a constant level of 20 μM LD. Furthermore, the 5-HT concentration kept constant (10 μM) and the levels of LD linearly varied in the range of 20 μM-110 μM as presented in Fig. 4.26A. The peak currents (Ipa) of 5-HT and LD regularly elevated with an increase in concentration without any significant influence from the other molecule in the mixture. The good linearity was varified by plotting the graph of concentration versus Ipa of 5-HT and Ipa of LD as shown in inset Fig. 4.25B and Fig. 4.26B respectively. This result suggested that the offered sensor is acceptable for the independent investigation of molecules in their binary mixture without interference.

4.8.9. Stability effect and Practical application of p-CR/NH_2-MWCNTs/MCPE at 5-HT and LD

The stability of the fabricated electrochemical sensor was authenticated through cyclic voltammetric technique by recording series of repetitive curves for 10 μM 5–HT and 20 μM LD in presence of 0.2 M PBS of neutral pH along with sweep rate of 50 mV/s as shown in Fig. 4.27. These findings of 25 successive measurements showed that the oxidation peak potentials were constant with a minute diminution in their peak currents. Also, the reproducibility of the composite sensor was examined by stored the developed electrode for 5 days in a dry state at room temperature. It was noticed that the peak responses of 90 % and 94.1 % for 5-HT and LD were retained and peak potentials were remains the same. From the detection outcome, it is proved that the p-CR/NH_2-MWCNTs/MCPE is stable, and the repeated measurements were possible for the selective investigation of 5-HT and LD.

To authenticate the practicality of the developed sensor, the detection of 5-HT and LD in serum samples was carried out at p-CR/NH_2-MWCNTs/MCPE. The standard addition practice was applied for the analysis and the real sample was diluted

with 0.2 M PBS (pH 7.0) and spiked separately by adding a known concentration of 5-HT and LD from the stock solutions. The percentage recoveries were calculated and obtained results were summarized in Table.4.6. These outcome results showed the feasibility of the proposed electrode for the investigation of 5-HT and LD in biological fluid.

4.9. Conclusion

Herein, for the fabrication of composite sensor, functionalized MWCNTs were first used to modify the carbon paste followed by electro-polymerization of Congo red dye to yield an electrode adequate for the detection of 5-HT and LD at neutral pH. The customized sensor shows the higher active surface area and elevated current responses of 5-HT and LD compared to other modified electrodes. The overall study reveals that the adsorption and diffusion-controlled mass transfer takes place at the modified electrode and exhibited satisfactory lower detection limits for 5-HT and LD. The p-CR/NH_2-MWCNTs/MCPE depicts the acceptable electro-catalytic performance and good selectivity for the simultaneous analysis of 5-HT in presence of LD. The established sensor gave good stability, reproducibility and admissible recovery in the real sample. The overall result implied that the designed electrode was simple and accurate for the examination of 5-HT and LD in blood samples.

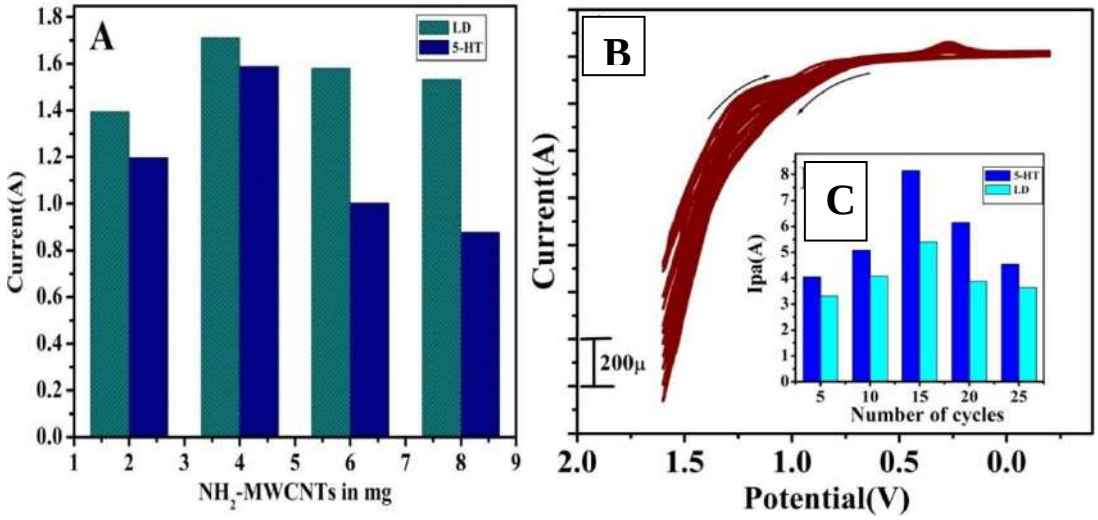

Fig. 4.15 A. A plot of different quantities of NH_2-MWCNTs in mg versus peak currents of 5-HT and LD. **B.** CVs for Electropolymerisation of 1mM CR on the surface of NH_2-MWCNTs/MCPE in presence of 0.1 M NaOH at 15 multiple cycles with a sweep rate of 100 mVs^{-1}. **C.** Plot of anodic peak current (Ipa) of 5-HT and LD versus several polymerization cycles.

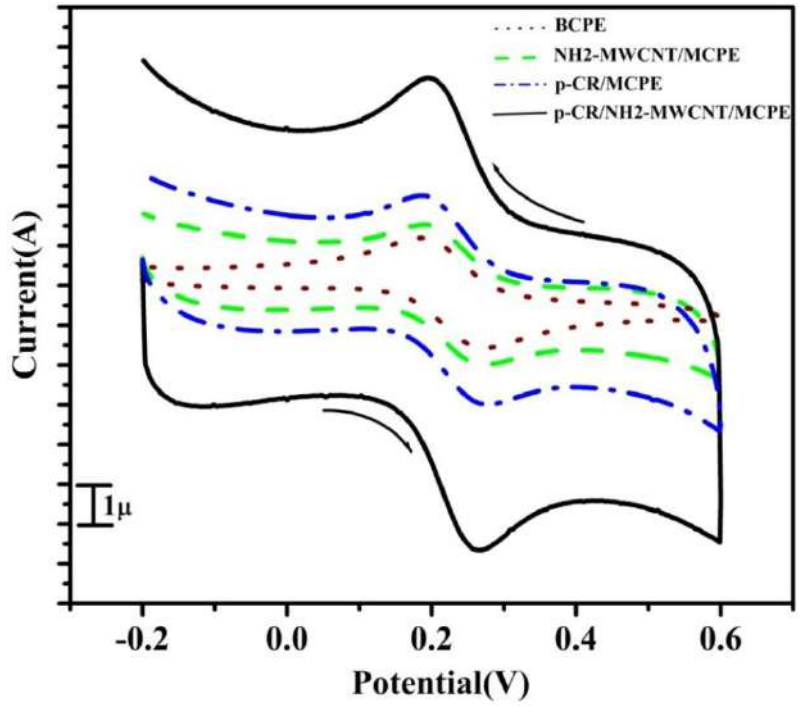

Fig. 4.16. CVs recorded for 1 mM $K_4[Fe(CN)_6]$ in 1M KCl at BCPE(dotted line), NH_2-MWCNTs/MCPE (dashed line), p-CR/MCPE(dashed dotted line) and p-CR/NH2- MWCNTs/MCPE(solid line) at sweep rate of 50mV/s.

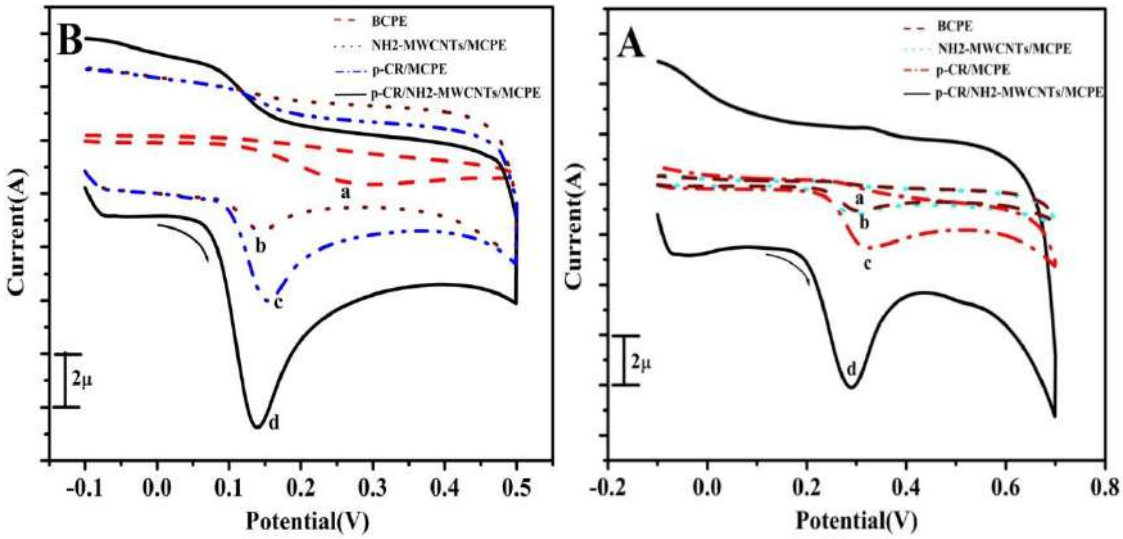

Fig. 4.17A. and B. CVs of 10μM 5-HT and 20μM LD in 0.2 M PBS of pH 7.0 at **a)**BCPE, **b)**NH_2-MWCNTs/MCPE, **c)** p-CR/MCPE, and **d)** p-CR/NH_2-MWCNTs/MCPE at the sweep rate of 50mV/s.

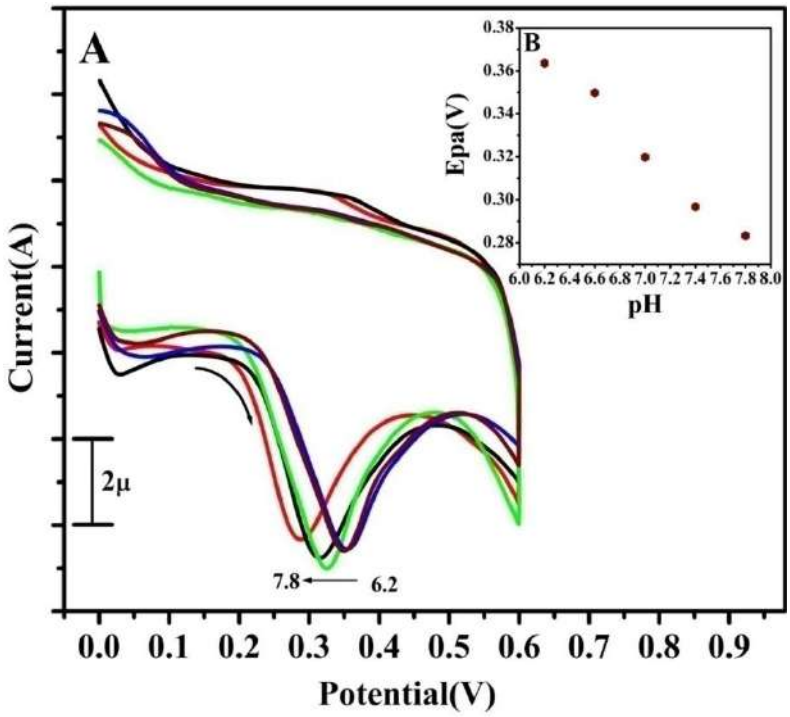

Fig. 4.18. CVs of 10μM 5-HT at p-CR/ NH_2-MWCNTs/MCPE with varied pH (6.2-7.8) with the sweep rate of 50 mV/s. **B.** inset graph of Epa versus different pH of 5-HT.

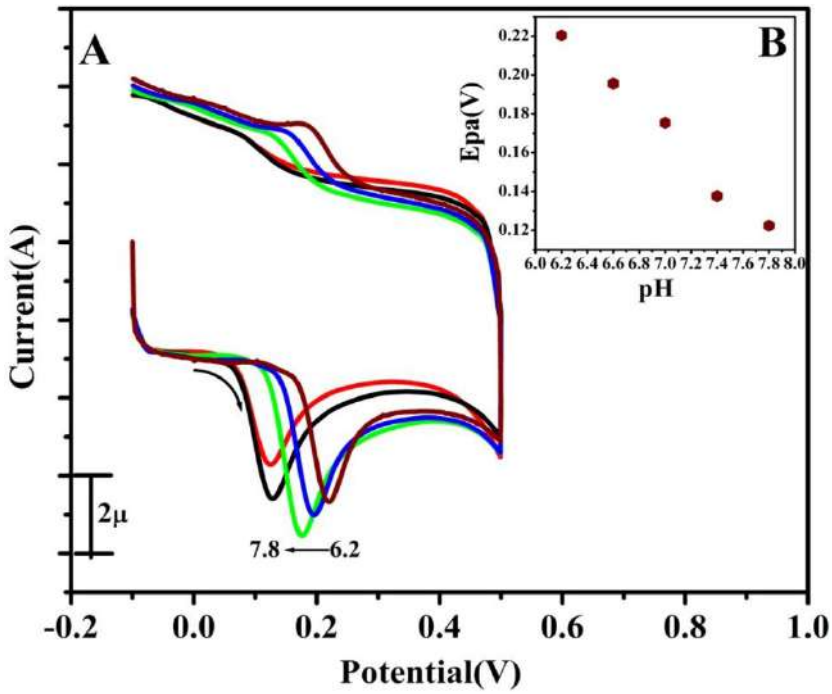

Fig. 4.19A. Graph of CVs of 20 μM LD at p-CR/ NH_2-MWCNTs/MCPE with varied pH(6.2-7.8)With the sweep rate of 50 mV/s. **B.** Inset graph of Epa versus different pH of LD.

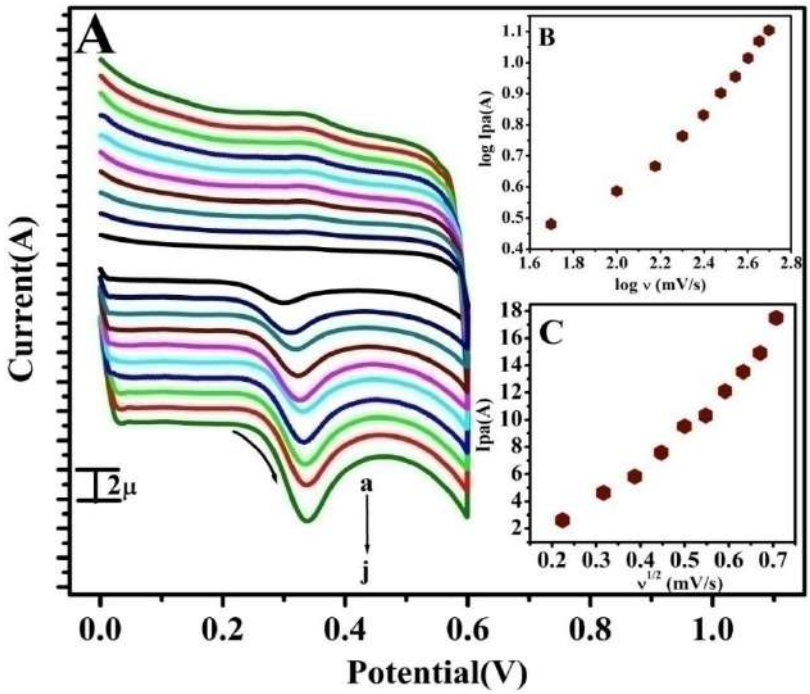

Fig. 4.20A. CVs of 10 μM 5-HT at p-CR/NH2-MWCNTs/MCPE with different sweep rate(a-j;50-500 mV/s) in 0.2 M PBS of pH7.0. **B.** Inset graph of log Ipa of 5-HT versus logν. **C.** Inset plot of Ipa of 5-HT versus $\nu^{1/2}$.

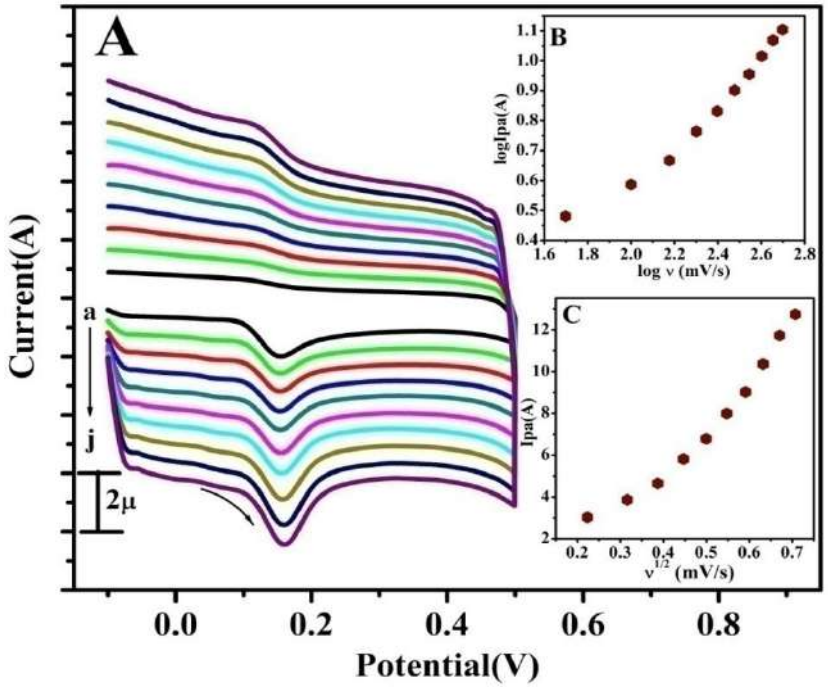

Fig. 4.21 A. CVs of 20 μM LD at p-CR/NH2-MWCNTs/MCPE with different sweep rate (a-j;50-500 mV/s) in 0.2 M PBS of pH 7.0. **B.** Inset graph of log Ipa of LD versus logν. **C.** Inset plot of Ipa of LD versus $\nu^{1/2}$.

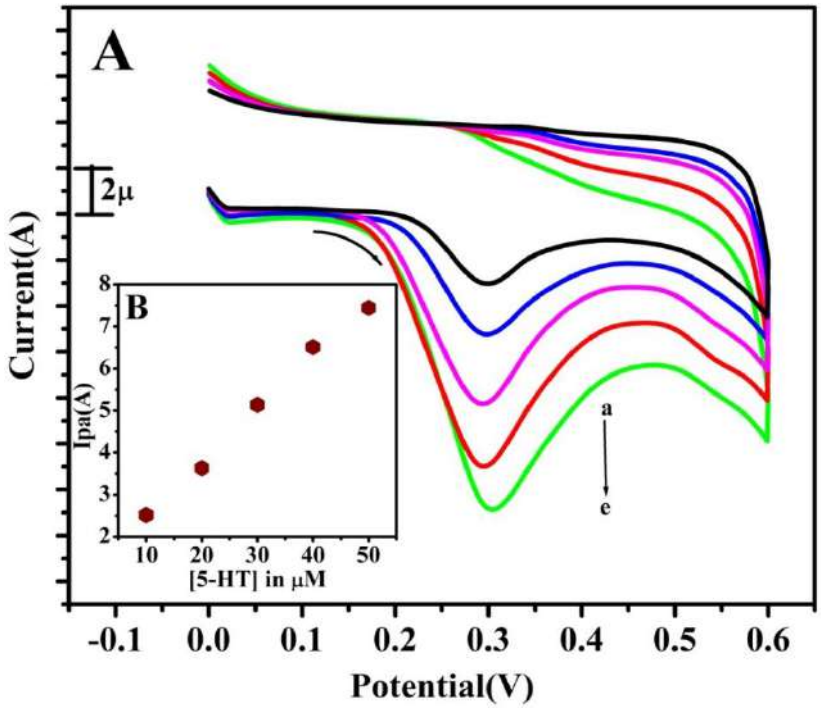

Fig. 4.22 A. CVs varied concentration of 5-HT (a-e; 10 μM-50 μM) in 0.2 M PBS of Ph 7.0 with sweep rate of 50 mV/s at p-CR/ NH_2-MWCNTs/MCPE. **B.** Inset graph of Ipa versus concentration of 5-HT in μM.

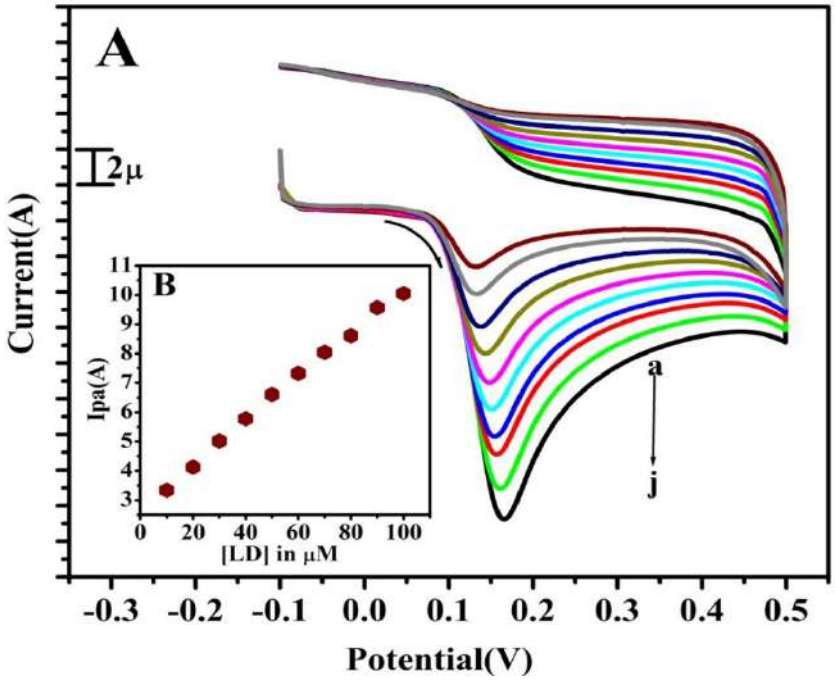

Fig. 4.23 A. CVs varied concentration of LD (a-e; 20μM -110 μM) in 0.2 MPBS of pH7.0 with sweep rate of 50 mV/s at p-CR/NH_2-MWCNTs/MCPE. **B.** Inset graph of Ipa versus concentration of LD in μM.

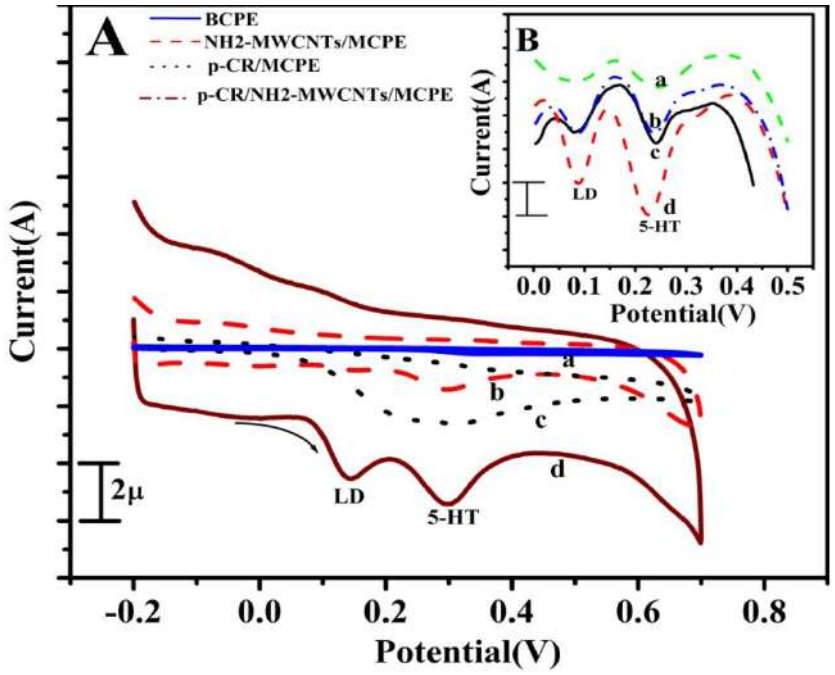

Fig. 4.24 A & B. CVs and DPVs obtained for simultaneous determination of 10 μM 5-HT and 10 μM LD at **a)**BCPE, **b)**NH_2-MWCNTs/MCPE, **c)**p-CR/MCPE, and d)p-CR/NH_2-MWCNTs/MCPE in 0.2 M PBS of pH 7.0 at the sweep rate of 50 mV/s.

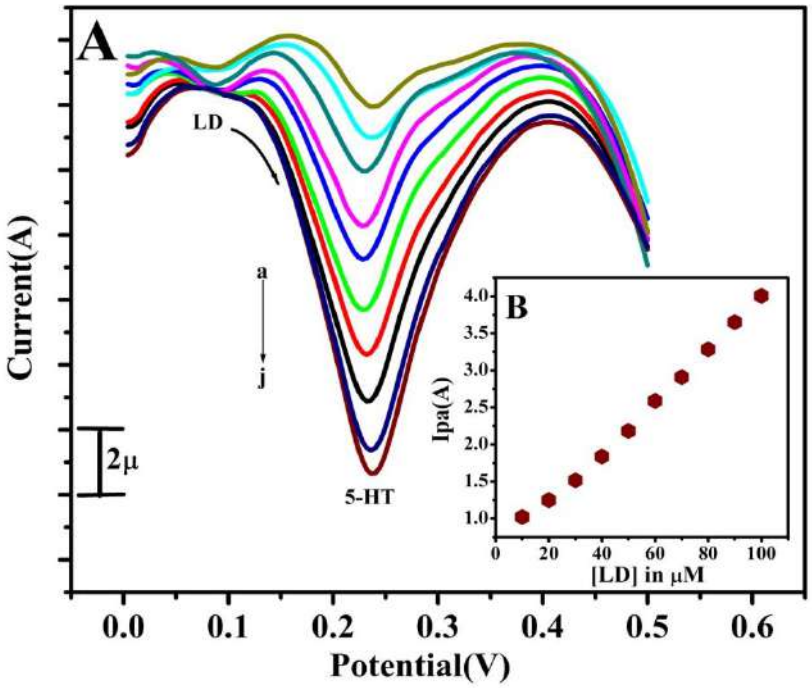

Fig. 4.25 A. DPVs were obtained for 5-HT at a varied concentration(a-j; 10 μM -100 μM) in presence of constant LD(10 μM) in 0.2 M PBS of pH 7.0 atp-CR/NH_2-MWCNTs/MCPE at a sweep rate of 50 mV/s. **B.** Inset graph of Ipa versus different concentrations of 5-HT.

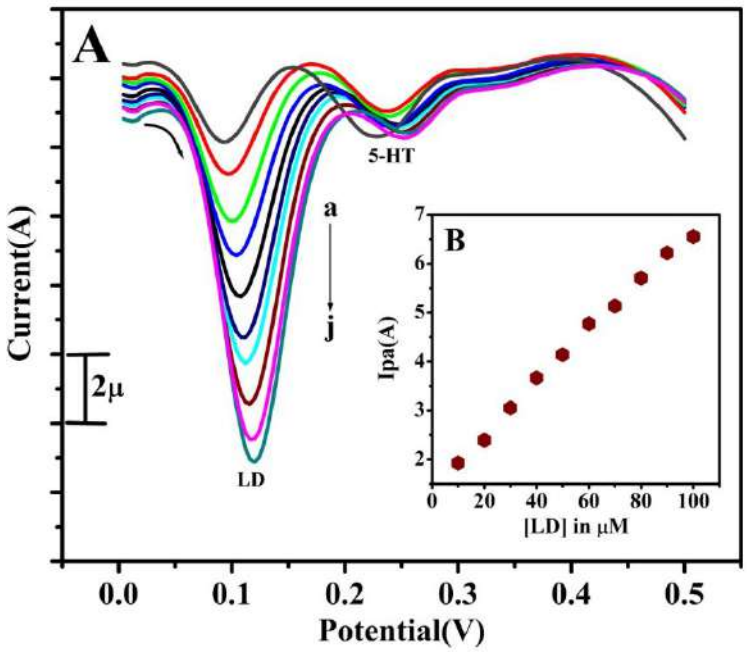

Fig. 4.26 A DPVs obtained for LD at varied concentrations (a-j;20 μM-110 μM) in presence of constant 5-HT(10μM) in 0.2M PBS of pH 7.0 at p-CR/NH_2MWCNTs/MCPE at a sweep rate of 50 mV/s. **B.** Inset graph of Ipa versus different concentrations of LD.

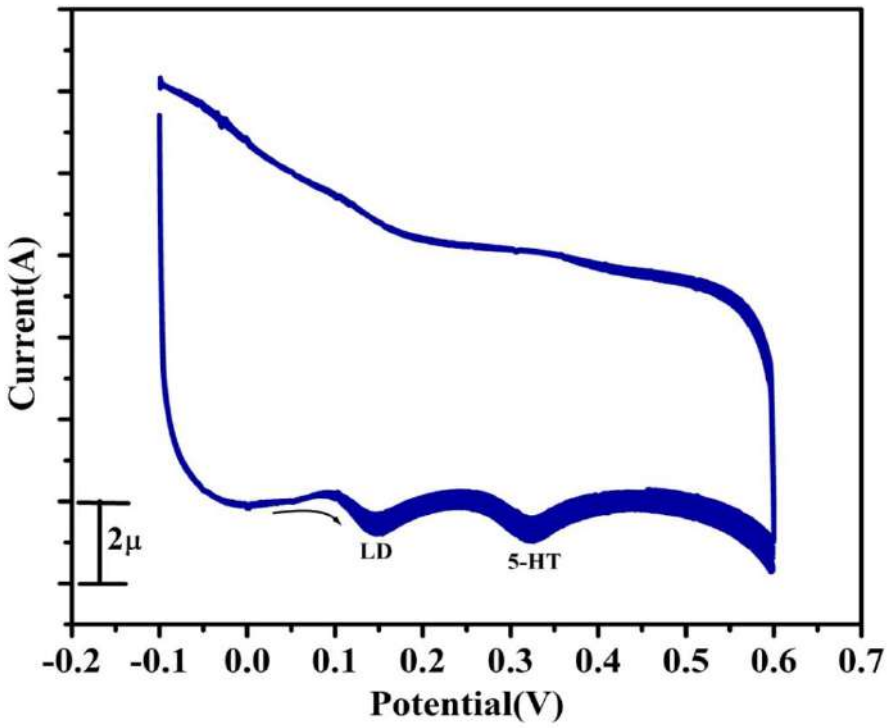

Fig. 4.27. CVs for stability analysis for a mixture of 10 μM 5–HT and 10 μM LD in 0.2 M PBS of pH 7.0 at p-CR/NH_2-MWCNTs/MCPE at the sweep rate of 50 mV/s for 25 cycles.

Table. 4.4. Comparison of analytical efficiency of the different modified electrodes for 5-HT detection.

Working Electrode	**Electrochemical Techniques**	**Linear range(μM)**	**LOD (μM)**	**Reference**
F-MWCNTs/BR9	DPV	1-10	9.0	[9]
3D – ITO	DPV	50-1000	7.5	[10]
CDP-Choline/MCPE	CV	10-30	5.81	[11]
AuNPs@PPy/GSPE	SWV	0.1-15	33.2	[12]
IL-DC-CNT/GE	DPV	10-50	2.0	[39]
p-CR/NH_2-MWCNTs/MCPE	CV	10-50	1.7	**This work**

Table. 4.5. Comparison of analytical efficiency of the different modified electrodes for LD detection.

Working Electrode	**Electrochemical Techniques**	**Linear range(μM)**	**LOD (μM)**	**Reference**
Gold screen printed electrode	CV	90-1200	68	[23]
ZnO-GF	DPV	5-50	5.0	[24]
PbO2-MCPE	DPV	260-1200	25	[25]
p-CR/NH_2-MWCNTs/MCPE	CV	10-100	3.04	**This work**

Table. 4.6. Detection of 5-HT and LD in real sample (n=3)

Sample	**5-HT added (μM)**	**Found (μM)**	**Recovery (%)**
5-HT	10	9.62	96.2
	20	19.81	99.05
	30	29.30	97.66
LD	10	9.91	99.1
	20	19.9	99.5
	30	30.32	97.06

4.10. References

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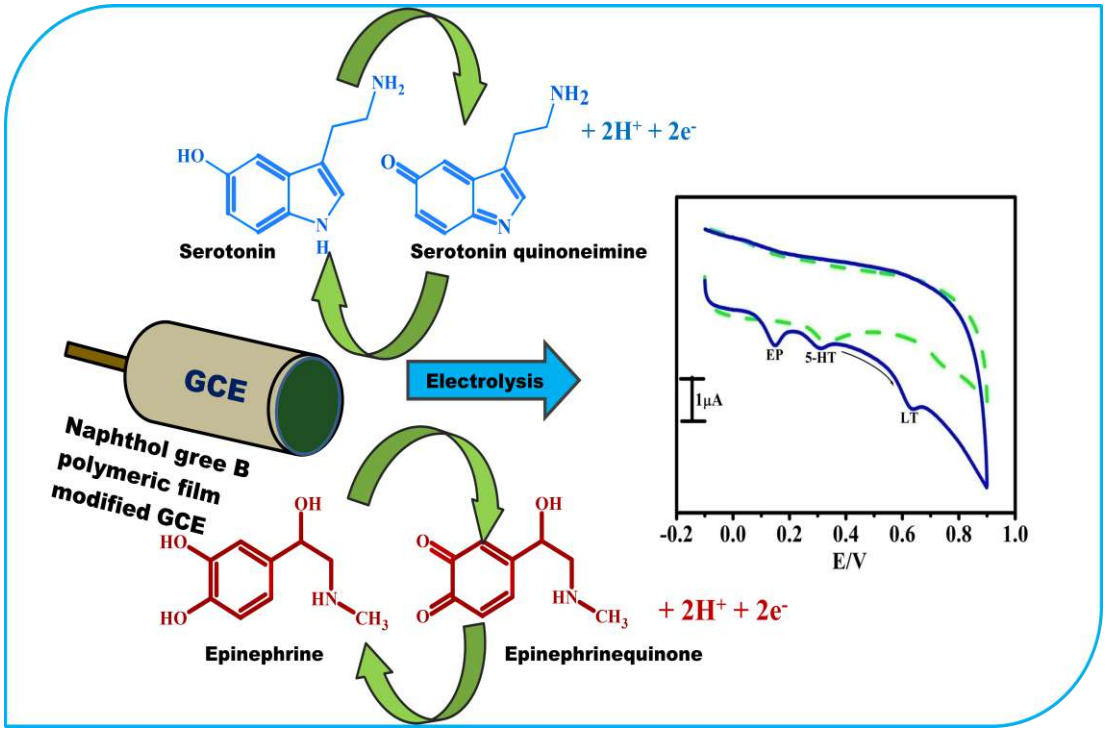
Chapter-V

PART-A

A GLASSY CARBON ELECTRODE MODULATED WITH POLY (NAPHTHOL GREEN B) FOR SIMULTANEOUS ELECTROANALYSIS OF SEROTONIN AND EPINEPHRINE IN PRESENCE OF L- TRYPTOPHAN.



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5.1. Introduction

5-HT is an electroactive indolamine entity acts as a neurotransmitter and vasoconstrictor [1, 2]. It supports in signal transport to regulate various bodily functions as well as mood enhancing chemical in the brain that influences the mood, feelings of well-being and helps to manage hunger. 5-HT positively improves the sleeping patterns for more soothing and rejuvenates sleep [3, 4]. It also assists in blood clotting and bowel functions of the body. The deficit 5-HT levels attributed to severe mental sickness such as panic attacks, depression, insomnia, stress and unexplained irritability. The extreme 5-HT concentration is associated with the group of symptoms known as serotonin syndrome and toxicity [5, 6].

EP is a fight or flight hormone also an important neurotransmitter of catecholamine class present in the mammalian nervous system [7, 8]. EP is a most powerful vasopressor drug and regulates the heart beat, bronchodilation, blood sugar and lipolysis. EP often employed as an emergency medication for the treatment of various conditions such as accidents, cardiac arrest, hypertension and bronchial asthma etc [9, 10]. Higher levels of EP are associated with hypoglycaemia, myocardial infarction and stress, while the deficiency in the EP level leads to orthostatic hypotension and Parkinson's disorder [11].

LT is a vital amino acid plays a crucial biochemical and clinical importance. It is needed for the normal growth and positive nitrogen balance in human and herbivores [12, 13]. Human body consumes it in the form of dietary supplements and medicines. L-TRP involved in the formation and maintenance of several bio-essential elements like proteins, muscles, enzymes and neurotransmitters. The level of LT in the body affects the brain serotonin level. Thus, the inappropriate metabolite of LT causes abnormalities in the level of serotonin and melatonin and thereby leads to depression, hallucination, Alzheimer's disorder and delusions [14, 15]. The concurrent determination of these biomolecules is an important task due to their concurrence and closer oxidation potentials which results in an overlapping of their signals. Hence, the selective and sensitive method of their determination is highly needful to track and diagnose the diseases. The several methods such as HPLC [16] and spectrophotometric [17] methods were employed for the detection of 5-HT, EP and

LT. Most of these techniques include laborious, delayed procedures, modification of analytes, lower selectiveness, costly instrumentation and required long duration for analysis [18-25].

Electrochemical polymerization of dyes is a most influential method of depositing a conducting polymer layer as it provides chemical stability, control over layer thickness by simply adjusting the numbers of potential sweep cycles, charge transfer and permeation [26]. Naphthol green B is sodium salt of naphthol green Y and shows better redox properties. It is a coordination complex of iron and used in histology for collagen staining and in industries it is employed for staining of nylon, paper and soap [29]. The present work describes a development of poly(naphthol green B) modified glassy carbon electrode by electropolymerization method. It possess an excellent electrochemical characteristics and employed for the individual and simultaneous detection of 5-HT, EP in L-TRP.

5.2. Experimental procedure

5.2.1. Chemicals

All the analytical grade reagents were employed in every part of the analysis. 5-HT, EP sodium hydroxide and potassium chloride were collected from Himedia laboratories (Mumbai, India) with highest available purity. Naphthol green B was purchased from Sigma Ltd (Bangalore, India). 25.0×10^{-4} M solutions of 5-HT and EP were prepared by using demineralised water and 0.1 M perchloric acid solution respectively. The supporting electrolyte of phosphate buffer solution was prepared by using a mixture of 0.2 M of Na_2HPO_4 and NaH_2PO_4. Doubly demineralised water was utilised to make all aqueous solutions.

5.2.2. Devices

A CHI-660c voltammetric analyser (CH-instrument-660c electrochemical workstation) was used to evaluate electrocatalytic characteristics by CV and DPV techniques. The electrolytic cell includes three electrode configured system with platinum foil, saturated calomel, bare and naphthol green B modified glassy carbon

electrodes (BGCE and p-NGB/MGCE) as a counter, reference and working electrodes respectively. All the evaluations were performed at room temperature.

5.2.3. Working Electrode preparation and modification

Prior to electrochemical modification, the BGCE was firstly polished with slurry containing aluminium oxide particles of 0.05μm size for 5 min and cleansed with deionised water to remove remained slurry particles from the electrode surface. After that, the p-NGB/MGCE was fabricated by electropolymerization through CV technique by dipping polished BGCE in electrochemical cell containing 1.0×10^{-3} M aqueous naphthol green B monomer in 0.1 M NaOH as supporting medium at the sweep rate of $100mVs^{-1}$ for 10 successive scans as shown in Fig. 5.1A. During the electro-deposition, the peak current slowly decreased with increased scanning time gives the confirmation about the formation of p-NGB membrane on BGCE. The membrane thickness can be decided by varying the number of sweep segments and the resultant peak current of 5-HT was recorded (Fig. 5.2B). For 5-HT, the 10 cyclic sweeps shows marvellous catalytic sensitivity. Hence, 10 cycles were opted for further modification process. After each modification, the electrode surface was sufficiently rinsed with doubly deionised water to expel the untreated monomer molecule.

5.3. Results and discussion

5.3.1. Electrochemical characterization of BGCE and p-NGB/MGCE

The Voltammetric response of $K_4[Fe(CN)_6]$ at different electrodes were tracked with the aid of cyclic voltammetry. Fig. 5.3 depicts the CV response accounted for 1.0×10^{-3} M $K_4[Fe(CN)_6]$ including 1.0 M KCl as supporting solution with the speed rate of $50mVs^{-1}$ at BGCE(Scattered line) and p-NGB/MGCE(solid line). It can be seen from the Fig., at BGCE, A well defined redox peak of $K_4[Fe(CN)_6]$ occurred with the lower peak currents and the redox peak separation(ΔEp) value found to be 136 mV. Whereas, the augmentation in the peak current after modification with the ΔEp of 64mV. This is the clear indication for the increase in the electron transfer rate and electro active sites after NGB modification. The surface area accessible for electroactive species in solution was assessed by Randles-Sevick's equation (1) [30]. The evaluated surface area values were found to

be 0.0241cm^2 for BGCE and 0.0351cm^2 for p-NGB/MGCE. These findings certified that p-NGB modified sensor has maximal electroactive surface area and superior catalytic properties.

$$Ip = (2.69\times10^5)\, n^{3/2}\, A\, D_0^{1/2}\, C0\, \nu^{1/2} \quad \text{..... (5.1)}$$

Where, A is area (cm^2) of working electrode, C_0 is the concentration (mol/cm^3) of the electro active substance, ν is sweep rate and D_0 is diffusion co-efficient(cm^{2}s^{-1}).

5.3.2. Influence of solution pH on 5-HT and EP peak current

The optimization of pH of supportive media would be crucial to achieve acceptable sensitiveness and to study the detailed redox behaviour of the targeted analytes. The pH dependent electrochemical profile of 5-HT and EP was encountered through CV technique. Fig. 5.4A and Fig. 5.5A exhibits the voltammetric signals of 0.1×10^{-4} 5-HT and 0.1×10^{-4} EP recorded at the surface of p-NGB/MGCE in 0.2 M PBS under variant pH value ranging over 5.8-7.8 with the speed rate of 50 mVs^{-1}. The displayed signals showed that the negative shift in the oxidation potentials of 5-HT and EP with increase in pH values. This could be attributed to the impact of protons in electrode reaction. Linearity was attained for plots between distinct pH with oxidation potentials of 5-HT and EP (Fig. 5.4B and Fig. 5.5B) with a regression expression of Epa(V)= 0.0496-6.677(pH), (r^2 = 0.97767) for SE and Epa(V)= 0.073-8.319 (pH), (r^2 = 0.9927) for EP. The retrieved slope value evidenced that the equal electron and proton contribution in the electrochemical oxidation of 5-HT and EP [31]. The best electrochemical outputs were observed at neutral pH (Fig. 5.4C and 5.5C). As the pH of the buffer rises from pH 7.0 the peak current started to decrease. The reduction in peak current at highly basic pH was may be due to less availability of protons, authenticates the oxidation process was pH dependent. Therefore the pH 7.0 was chosen for further electroanalysis.

5.3.3. Voltammetric profile of 5-HT and EP at different working electrodes

The electrochemical interpretations of targeted analytes were scrutinized at Bare and customised sensors by facilitating CV technique. Fig. 5.6 and Fig. 5.7 represents the CVs profile recorded for the 0.1×10^{-4}M 5-HT and 0.1×10^{-4}M EP in occurrence with the 0.2M PBS of neutral pH at BGCE(scattered curve) and p-

NGB/MGCE(solid curve) having the speed rate of 50mVs^{-1}. In the case of BGCE, small and almost negligible oxidation peak signals were perceived for 5-HT and EP. With the incorporation of NGB polymeric film onto BGCE surface, there is a considerable elevation in the analytical signals of 5-HT and EP with minimisation of over potential. This increment in peak current accredits the increased catalytic effectiveness and reactive sites of p-NGB/MGCE toward electro-oxidation of 5-HT and EP.

5.3.4. Study of scan rate variation on 5-HT and EP at p-NGB/MGCE

The catalytic dependency of peak current and peak potential of 5-HT and EP with scan rate were explored by varying the scan rate to examine the kinetic parameters with the help of CV method. Fig. 5.8A and Fig. 5.9A interprets the CVs of 0.1×10^{-4}M 5-HT and 0.1×10^{-4} EP in 0.2 M PBS of pH 7.0 at p-NGB/MGCE with varied speed rates differed from 50-500mVs^{-1}. According to the findings, the oxidation signals of 5-HT and EP were progressively raised with the increments in speed rates and the oxidation potentials were slightly approaches to positive side. The linear dependency was evaluated by plotting oxidation currents (Ipa) of 5-HT and EP with square root of scan rate ($\nu^{1/2}$) (Fig. 5.8B and Fig. 5.9B) exhibits fine linearity with the correlation factor (R^2) of 0.9995 and 0.9987 for 5-HT and EP respectively. Also the log of Ipa of 5-HT and EP showed linearity with log ν (Fig. 5.8C and Fig. 5.9C) with the regression expressions, log Ipa(μA) = 0.7105 logν(mV/s)+0.8990 (R^2=0.9960) for 5-HT and log Ipa(μA) = 0.7075 logν(mV/s)+0.7376 (R^2=0.9983) for EP. This is the clear evidence that the electrochemical oxidation at p-NGB/MGCE was under the control of diffusion process [32, 33].

5.3.5. Calibration of p-NGB/MGCE

The voltammetric performance of 5-HT and EP on altering their concentration was examined to validate the analytical efficacy and to track the detection and quantification limits at developed sensor. Fig. 5.10A and Fig. 5.11A portraits the CVs of contrasting concentrations of 5-HT and EP accounted in assistance with 0.2 M PBS of pH 7.0 at p-NGB/MGCE having the sweep rate of 50mVs^{-1}. The oxidation current steadily hikes with hiking the 5-HT and EP concentration in the dynamic range of 0.1×10^{-4} M to 0.8×10^{-4} M and 0.1×10^{-4} M to 1×10^{-4} M respectively with minutely

switching the oxidation potentials to positive direction. The linearity was noticed between the plot of distinct 5-HT and EP concentration with increased current intensity as illustrated in inset Fig. 5.10B and Fig. 5.11B with the regression equations Ipa (μA) = 0.0155(μM) + 1.96 (R^2=0.99859) and Ipa (μA) = 0.0636 (μM) + 2.10 (R^2=0.9976) respectively. The linearity was deviated at higher concentrations of targeted analytes as a result of the adsorption of reactive oxidation products on the electrode surface. The LOD and LOQ values were measured by applying the equations (3) & (4) [34]. The estimated values of LOD and LOQ were of 1.83μM & 6.13 μM for 5-HT and 0.44μM & 4.49 μM for EP respectively.

$$LOD = 3\sigma/M \quad \text{..... (5.2)}$$

$$LOQ = 10\sigma/M \quad \text{.....(5.3)}$$

Where, σ denotes standard deviation and M is slope from calibration plots. The efficacy of the modified sensor compared with other reported electrode for 5-HT and EP in Table.5.1 and Table.5.2.

5.3.6. Concurrent resolution of 5-HT, EP and LT at p-NGB/MGCE

The simultaneous discrimination of the targeted analytes in a sample mixture is a key factor to decide the sensibility and selectiveness of the fabricated sensor. The proficiency of the developed electrode to stimulate the voltammetric resolution of 5-HT, EP and LT was studied using CV and DPV methods. Fig. 5.12A signifies the CVs logged for a ternary solution containing equimolar concentration (0.1×10^{-4} M) of 5-HT, EP and LT in occurrence with 0.2 M PBS of neutral pH with the speed rate of $50mVs^{-1}$. As can be seen from the Fig., the less sensitive and coincided oxidation potentials were noted at BGCE (Scattered line). Meanwhile, the distinct and well resolved oxidation signals of 5-HT, EP and LT were observed at p-NGB/MGCE (Hard line) and the three separate oxidation potentials noticed at 307 mV, 148mV and 603mV for 5-HT, EP and LT respectively. Identically, the DPVs verified for homogeneous mixture of 5-HT, EP and LT in 0.2 M PBS of pH 7.0 with the speed rate of $50mVs^{-1}$ at p-NGB/MGCE as showed in inset Fig. 5.12B. The individual peak potentials observed at 270mV, 113mV and 615mV for 5-HT, EP and LT respectively. Hence, it attested that the electrocatalytic functioning of p-NGB/MGCE is superior to the unmodified electrode.

5.3.7. Selective Study and stability of p-NGB/MGCE

The selective quantification of 5-HT, EP and LT in their single solution was examined by using highly efficient DPV system. The anti interference capability of the p-NGB/MGCE was investigated by altering the any one species concentration. Fig. 5.13A portrays the DPV curve verified for uneven concentration of 5-HT in presence of 0.2 M PBS of pH 7.0 at p-NGB/MGCE over the range of 0.1×10^{-4} M to 1×10^{-4} M having the speed rate of $50mVs^{-1}$ by holding the constant EP and LT concentration (0.1×10^{-4}). Next the concentration of EP is altering in the range of 0.1×10^{-4} M to 1×10^{-4} M at the retained concentration of 5-HT and LT (Fig. 5.13B). Comparably, the LT concentration (0.1×10^{-4} M to 0.6×10^{-4} M) was deviated and the concentration of 5-HT and EP (0.1×10^{-4}) kept constant (Fig. 5.13C). As observed from the figures, the peak current rapidly raises with increasing the respective analytes concentration. The relation between the anodic peak current with differed concentration was displayed in inset figures. The constancy of the formulated electrode is an essential characteristic of the sensor. To prove the steadiness of the p-NGB/MGCE, the sensor was scanned continuously for 20 consecutive cycles for the mixed solution containing targeted analytes in 0.2 M PBS of neutral pH along with the sweep rate of $50mVs^{-1}$. As interpreted in Fig. 5.14, the anodic potential was retained with slight diminishing in peak signals. This experimental outcome suggested that the modified sensor provides higher stability towards electroanalysis.

5.3.8. Practical application

In order to conclude the relevancy of the proposed method, the designed p-NGB/MGCE sensor was practiced for the exploration of 5-HT and EP in serum sample by standard addition process. The observed results show commendable recoveries as endowed in Table 5.3. This outcome suggested the practical efficacy of the customized.

5.4. Conclusion

In current study, a newly modulated electrode system has been stacked and implemented for the voltammetric detection of 5-HT and EP in occurrence with LT. According to the findings, the p-NGB/MGCE presents agreeable electrocatalytic

functionality, sensibility, selectiveness and enhances the electrode kinetics. Using cyclic and differential voltammetric methods, the distinct parameters were electrochemically investigated. The diffusion controlled electrode procedure occurs for 5-HT and EP at drafted electrode. The proposed sensor offers the reduced limit of detection values. The p-NGB/MGCE could greatly improve the specific and concurrent recognition of 5-HT and EP in presence of LT. The p-NGB/MGCE successfully employed to analyse 5-HT and EP in real samples with an acceptable recoveries and the conferred electrode was employed for further electrocatalytic investigations of some notable electroactive biomolecules.

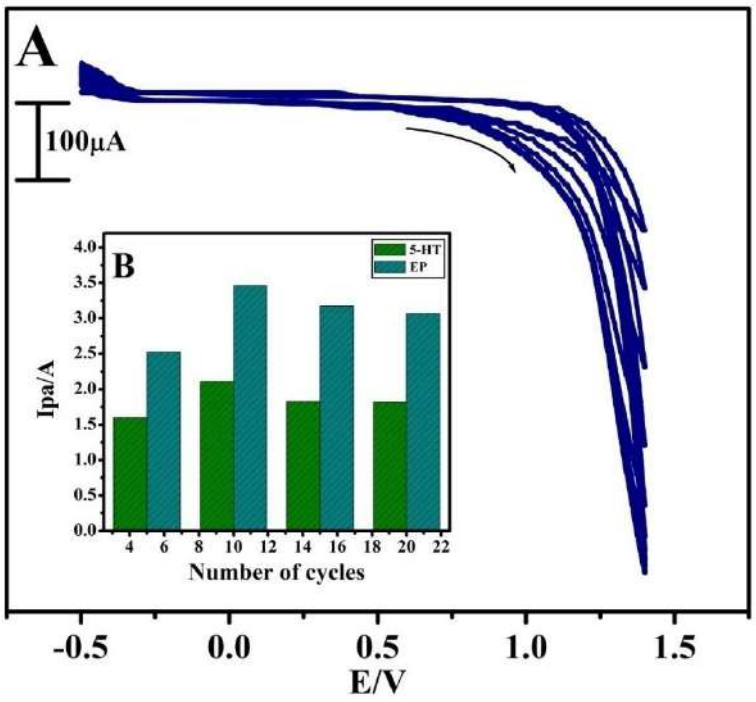

Fig. 5.1A. CVs gotten for continual cycling of 1mM naphthol green B occurrence with 0.1M NaOH for 10cycles with the sweep rate of 50mVs^{-1}. **B.** Plot of oxidation signals of 5-HT and EP v/s distinct polymerisation segments.

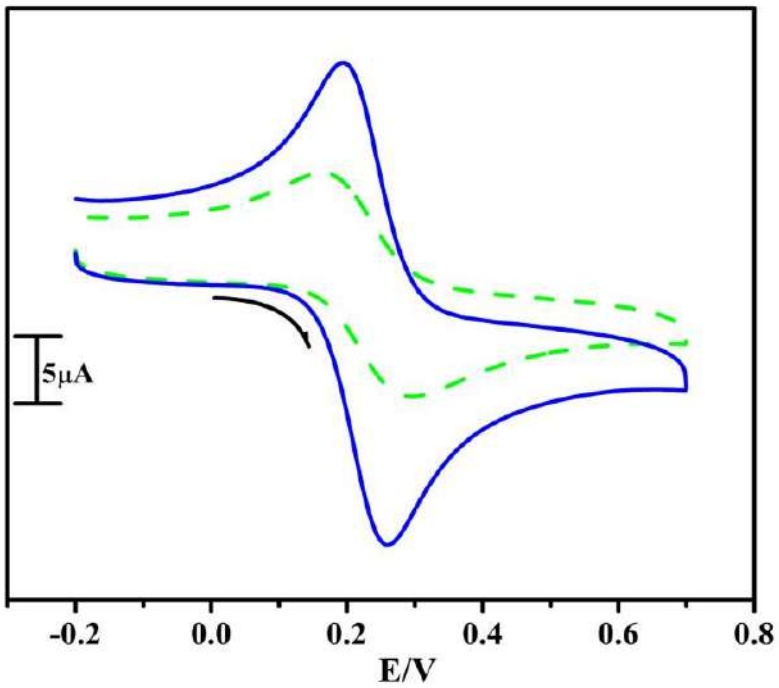

Fig. 5.2. CVs of BGCE(Scattered line) and p-NGB/MGCE(solid line) in 1mM $K_4[Fe(CN)_6]$ including 1M KCl as supporting solution with the speed rate of 50mVs^{-1}.

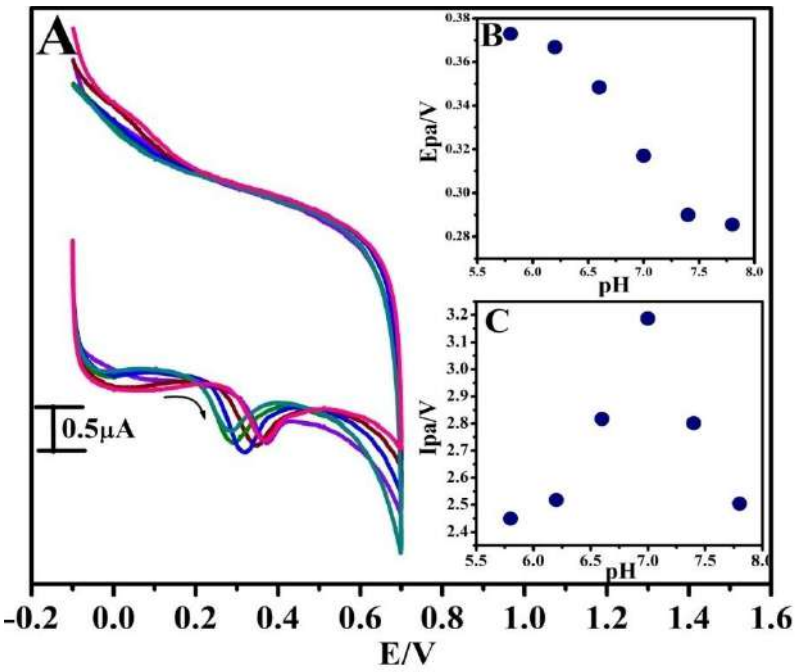

Fig. 5.3A. Voltammetric signals of 0.1×10^{-4} M 5-HT recorded at p-NGB/MGCE in 0.2 M PBS under variant pH value ranging over 5.8-7.8 with the speed rate of 50m Vs^{-1}. **B.** Plot of Epa of 5-HT v/s differed pH. **C.** Plot of Ipa of 5-HT v/s differed pH.

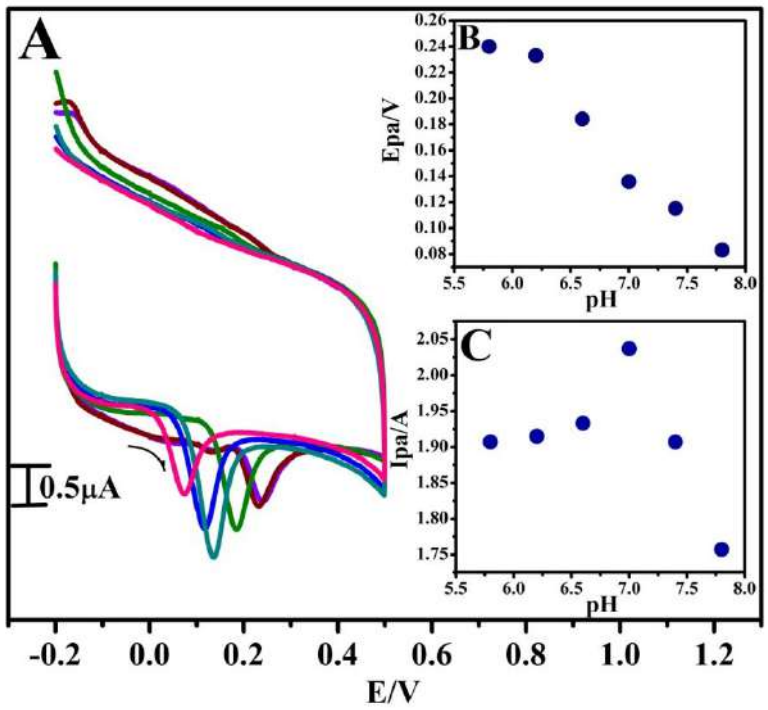

Fig. 5.4A. voltammetric signals of 0.1×10^{-4} M EP recorded at p-NGB/MGCE in 0.2 M PBS of separate pH values (5.8-7.8) with the speed rate of 50mVs^{-1}.
B. Plot of Epa of EP v/s differed pH values. **C.** Plot of Ipa of EP v/s differed pH.

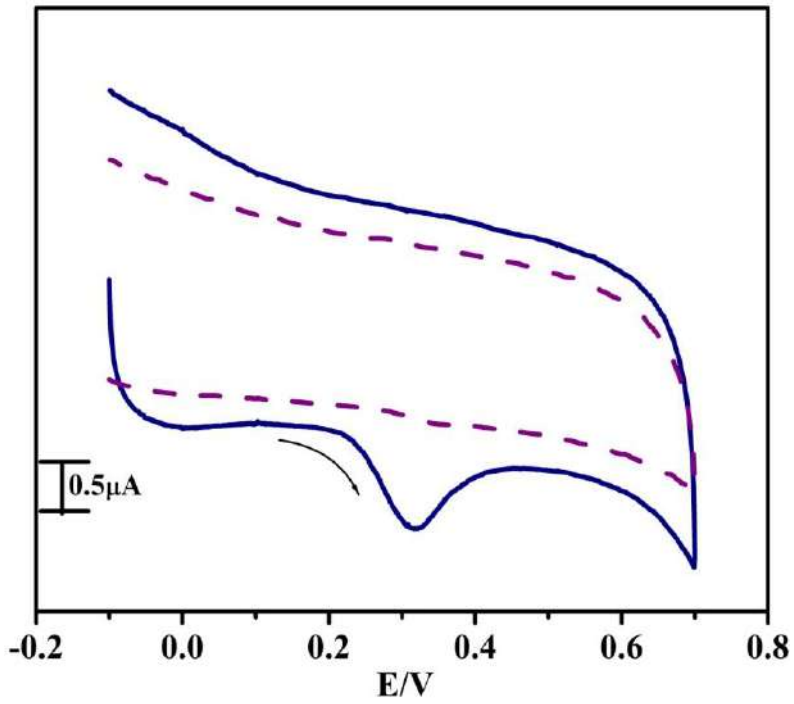

Fig. 5.5. CVs profile for the 0.1×10^{-4} M 5-HT in occurrence with the 0.2 M PBS of pH 7.0 at BGCE(scattered curve) and p-NGB/MGCE(solid curve) having the speed rate of $50mVs^{-1}$.

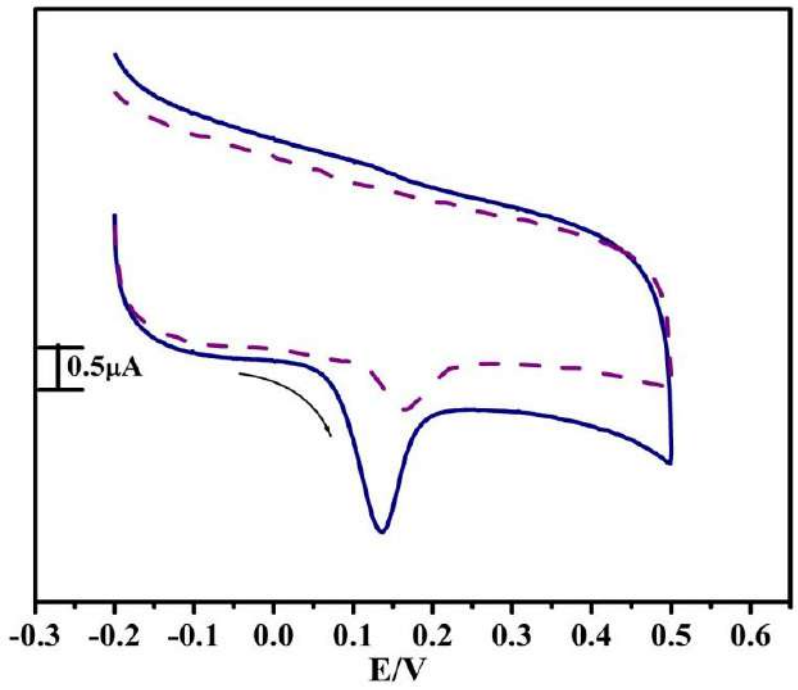

Fig. 5.6. CVs profile for 0.1×10^{-4}M EP in 0.2M PBS of pH 7.0 at BGCE(scattered curve) and p-NGB/MGCE(solid curve) having the speed rate of $50mVs^{-1}$.

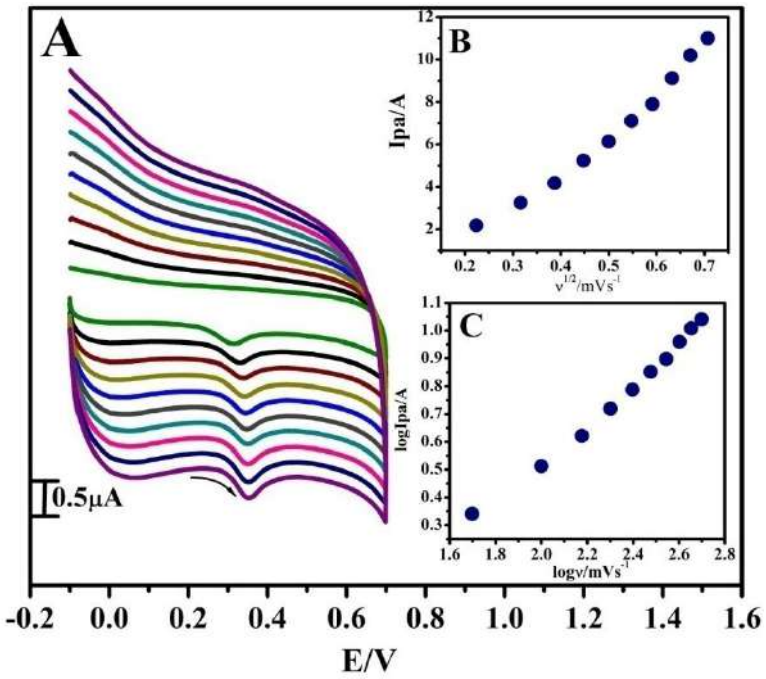

Fig. 5.7A. CVs of 0.1×10^{-4}M 5-HT in assistance with 0.2 M PBS of pH 7.0 for distinctive sweep rates (50-500mV s^{-1}) at p-NGB/MGCE. **B.** Plot of Ipa of 5-HT v/s $v^{1/2}$. **C.** Plot of log Ipa of 5-HT v/s logv.

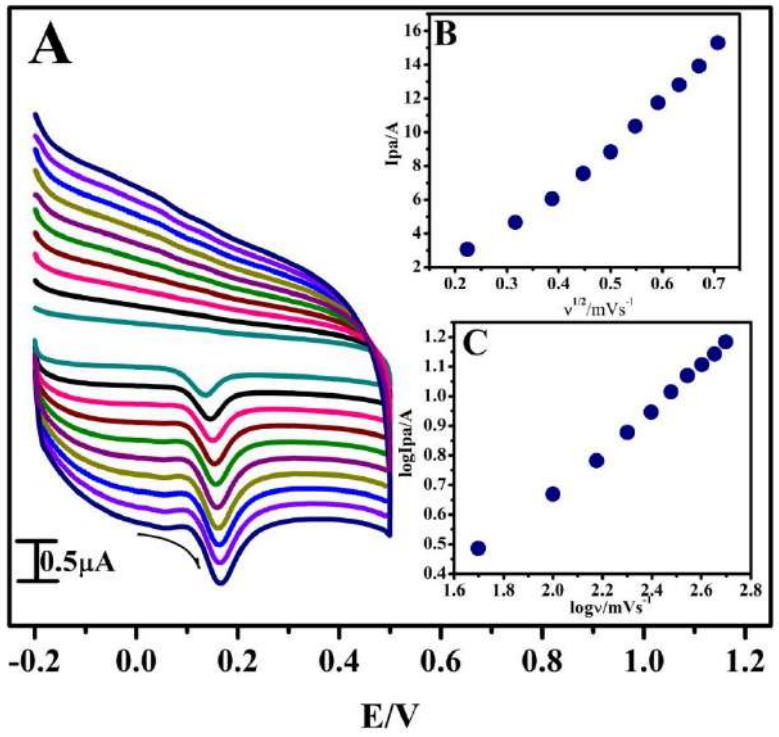

Fig. 5.8A. CVs of 0.1×10^{-4} M EP in assistance with 0.2M PBS of pH 7.0 for distinctive sweep rates (50-500 mVs^{-1}) at p-NGB/MGCE. **B.** Plot of Ipa of EP v/s $v^{1/2}$. **C.** Plot of log Ipa of EP v/s logv.

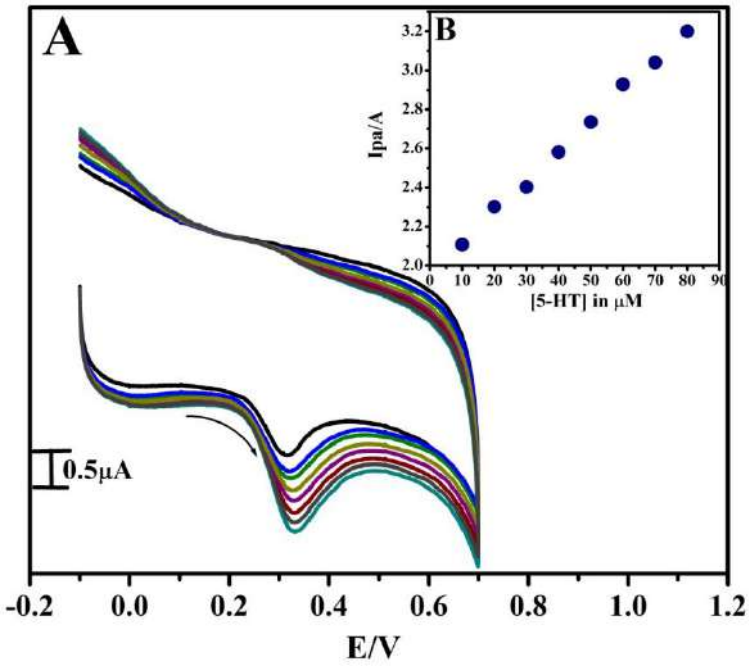

Fig. 5.9A. CVs curves for uneven concentration of 5-HT (0.1×10^{-4} M to 0.8×10^{-4} M) at p-NGB/MGCE in 0.2 M PBS of pH 7.0 along with the sweep rate of 50mVs^{-1}.
B. Plot of Ipa of 5-HT with differed concentration.

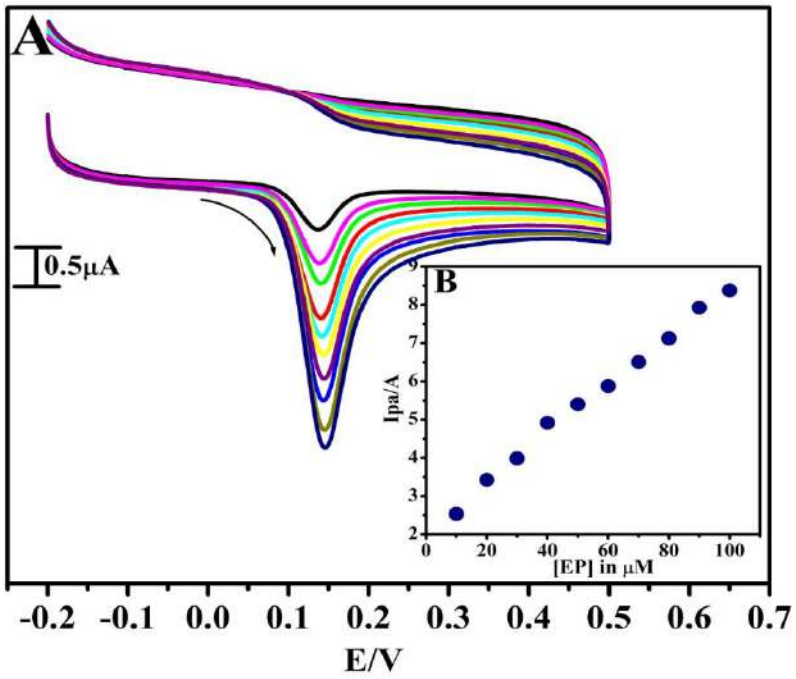

Fig. 5.10A. CVs curves for uneven concentration of EP (0.1×10^{-4} M to 1×10^{-4} M) at p-NGB/MGCE in 0.2M PBS of pH 7.0 along with the sweep rate of 50mVs^{-1}. **B.** Plot of Ipa of EP with diverse concentration.

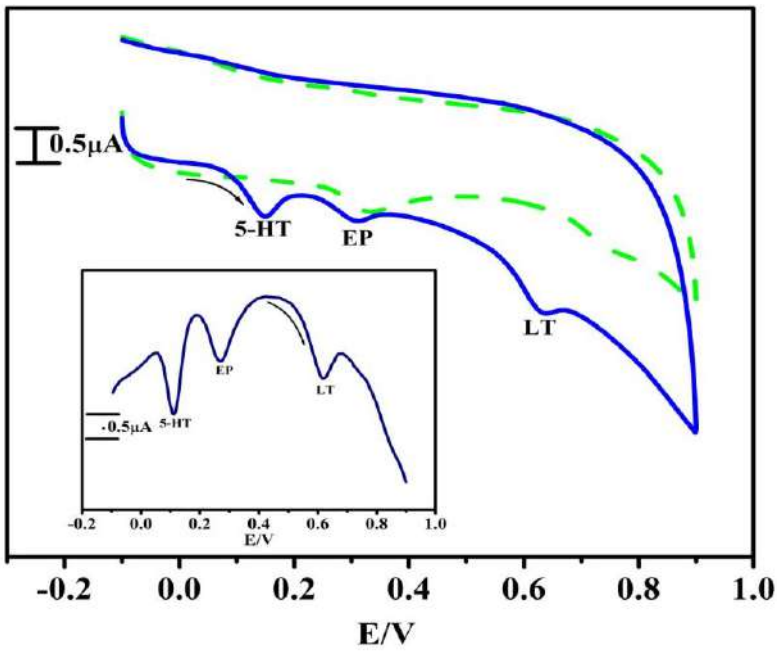

Fig. 11A and B. CVs and DPVs logged for a ternary solution of 5-HT, EP and LT of same concentration (0.1×10^{-4} M) at p-NGB/MGCE in presence of 0.2 M PBS of neutral pH with the speed rate of $50mVs^{-1}$.

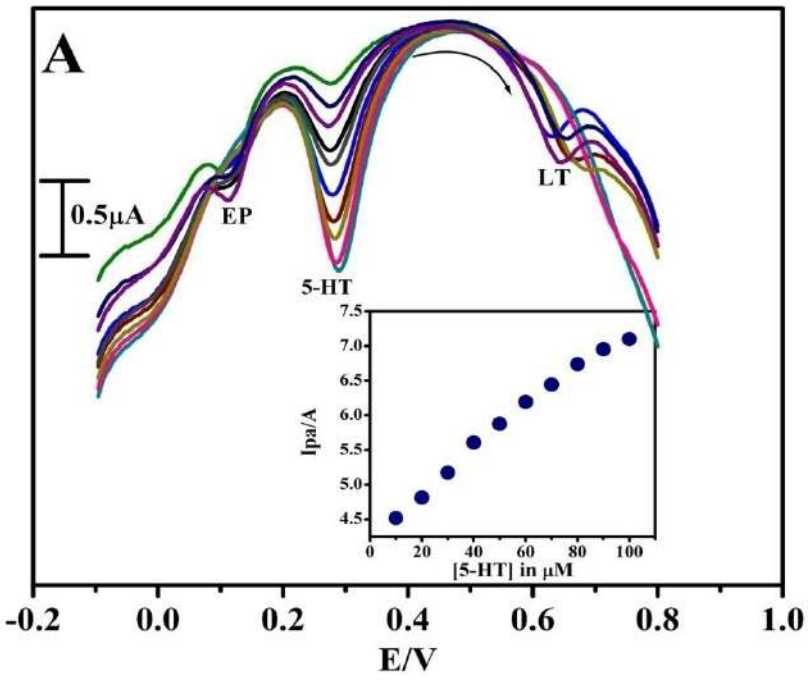

Fig. 5.12A. DPVs for variation of 5-HT concentration from 0.1×10^{-4} M to 1×10^{-4} M in 0.2 M PBS of pH 7.0 with 0.1×10^{-4}M EP, 0.1×10^{-4} M LT at p-NGB/MGCE at sweep rate of $50mVs^{-1}$.

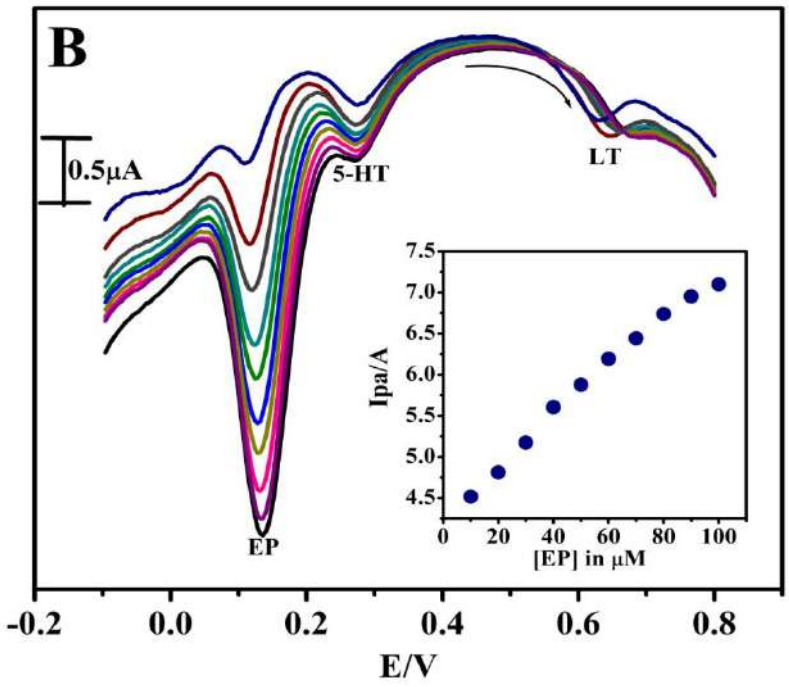

Fig. 5.12B. DPVs for variation of EP concentration from 0.1×10^{-4} M to 1×10^{-4} M in 0.2 M PBS of pH 7.0 with 0.1×10^{-4} M 5-HT, 0.1×10^{-4} M LT at p-NGB/MGCE at sweep rate of $50mVs^{-1}$.

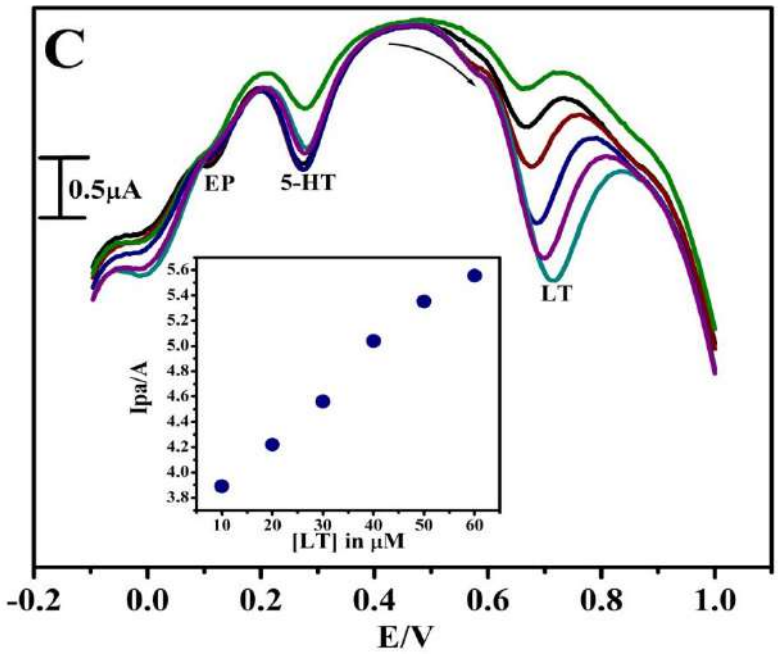

Fig. 12C. DPVs for variation of LT concentration from 0.1×10^{-4} M to 0.6×10^{-4} M in 0.2 M PBS of pH 7.0 with 0.1×10^{-4} M 5-HT, 0.1×10^{-4} M EP at p-NGB/MGCE at sweep rate of $50mVs^{-1}$.

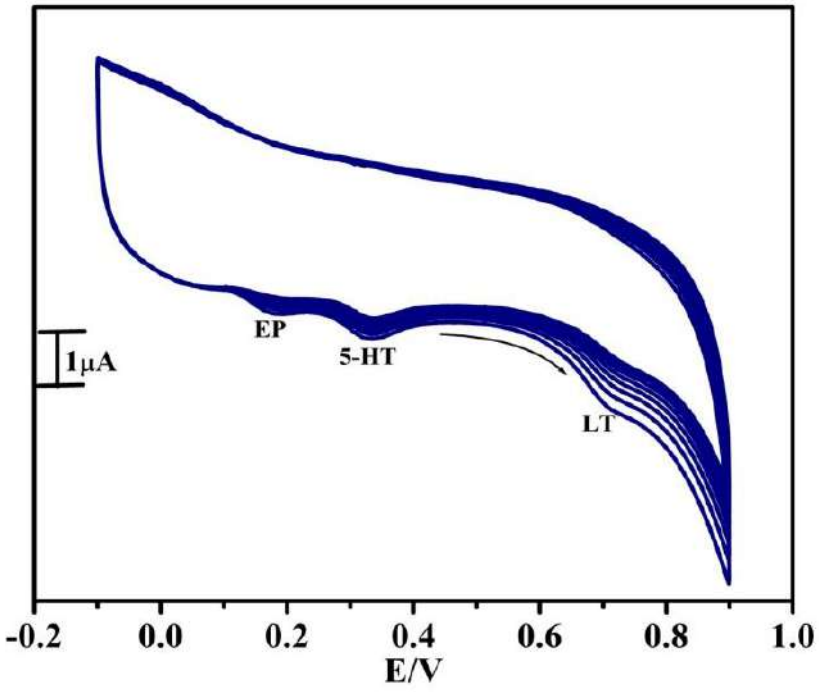

Fig. 5.13. CVs documented for the mixed 5-HT, EP and LT (0.1×10^{-4} M) in 0.2 M PBS of pH 7.0 with speed rate of $50mVs^{-1}$ at p-NGB/MGCE.

Table.5.1. Comparison of different modified electrodes for 5-HT detection.

SI No	Electrode	Detection limit(μM)	Method	Reference
01	AuNPs@PPy/GSPE	33.2	SWV	[35]
02	MWCNT/Nafion/MAO-A	20	DPV	[36]
03	f-MWCNTs/BR9/GCE	9	DPV	[37]
04	3D-ITO	7.5	DPV	[38]
05	IL-DC-CNT/GE	2.0	DPV	[39]
06	Poly(ser)/MWCNT/GCE	2	DPV	[40]
07	p-NGB/MGCE	1.83	CV	**Present study**

Table.5.2. Comparison of different modified electrodes for EP detection.

SI No	Electrode	Detection limit(μM)	Method	Reference
01	Poly(caffeic acid)/GCE	20	CV	[41]
02	Poly(1-Methylpyrrole)/MGCE	16.8	CV	[42]
03	f-MWCNTs/BR9/GCE	9	DPV	[37]
04	CAP/MWCNT/GCE	7.20	CV	[43]
05	GNPs/GCE	5.0	SWV	[44]
06	MWCNT/ GCE	0.9	DPV	[45]
07	Poly(ser)/MWCNT/GCE	2	DPV	[40]
08	Ty/MWCNTs/GCE	0.51	DPV	[46]
09	p-NGB/MGCE	1.83	CV	**Present study**

Table. 5.3. Detection of 5-HT and EP in real sample (n=3)

Sample	5-HT added(µM)	Found (µM)	Recovery (%)
5-HT	20	18.81	94.05
	30	29.30	97.66
	40	38.04	95.12
EP	20	19.9	99.5
	30	28.9	96.33
	40	38.21	95.52

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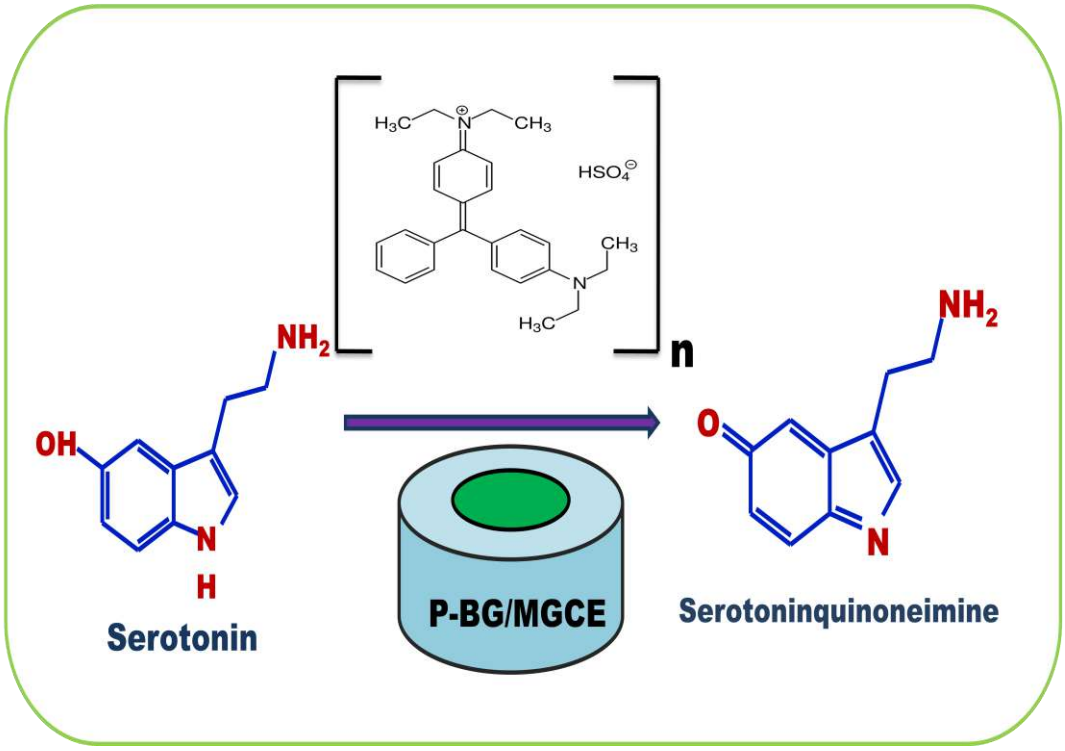
Chapter-V

PART-B

A SELECTIVE ELECTROCHEMICAL SENSING OF SEROTONIN AND EPINEPHRINE AT GLASSY CARBON ELECTRODE MODULATED WITH BRILLIANT GREEN: A VOLTAMMETRIC STUDY



Communicated to Current Analytical Chemistry (2022)

5.6. Introduction

Serotonin (SE) and Epinephrine (EP), vasopressors are of important catecholamine class acts as a neuromediators and also as hormones and collaborating in shipping of chemical signals among biological units. They are embroiled in a wide spectrum of biological as well as physiological duties in mammals [1]. SE is a neurochemical mainly appears in brain and intestines. Most of the SE of around 90% broadly identified in the skin cells of gastrointestinal tract where it has been increasingly esteemed for its hormonal, endocrine paracrine and autocrine functions [2-5]. SE often known as "feel good" chemical and portrays the key responsibilities in controlling and normalizing the emotional status and several body activities such as sleep, digestion, wound healing, bone health and blood clotting. Too small or too high serotonin ranges can leads to numerous physical as well as mental sicknesses like panic attacks, irritabilility and serotonin toxicity[6-10].

EP is a neural tissue exists in central nervous system and in bodily fluids as organic cation. EP serves as hormone and also as neuromodulator and their by involved in fixing the visceral activities of the body [11-13]. It can also be employed as a medicine because of its miscellaneous functions and to medicate the allergic reactions, to recover cardiac rhythm, to manage mucosal congestion, asthma and glaucoma [14-16]. EP associated with multiple life phenomenon and any abnormalities in its level in blood approaches a way to many afflictions such as hypoglycemia, myocardial infarction and orthostatic hypotension [17-18]. Usually SE and EP co-occur in biological fluids. Hence, it is of paramount significance to develop precise and simple analytical proceedings for concurrently determined SE and EP in the evaluations of therapeutic impacts.

The isolation of oxidation signals of SE and EP has a great deal of research initiatives because of their less difference of oxidation potentials. However, there are plenty of analytical proceedings are known for their determination like capillary electrophoresis[19], mass spectrometry[20] and liquid chromatography[21]. Despite the fact that, Most of these techniques experience some drawbacks comprising elaborated treating process, time taking, low sensitivity and excessive price. Among all the methods electrochemical approach rewarded much more consideration because

of its attractive traits such as ease of use, comparatively minimal cost, short response time and practicality. The electropolymerisation of dyes have been the focus of intense research concerns in the area of electrochemical sensors because of their phenomenal electrocatalytic qualities like chemical constancy, easy charge transfer and interpenetration [22].

In this regard, In current investigation, a facile and easy brilliant green amplified glassy carbon electrode was cultivated and served for specific and sensible electroanalysis of SE in presence of EP.

5.7. Experimental section

5.7.1. Instrumentations and Reagents

The voltammetric measurements of SE and EP were examined at the surface of bare and brilliant green modified glassy carbon working electrode (BGCE and p-BG/MGCE). The generated current was assessed through a platinum wire as auxiliary electrode and saturated calomel reference electrode. The three electrodes were joined to an electrochemical analyser (CH-instrument-660c electrochemical workstation). All the measurements were executed at room temperature.

SE in its pure form was procured from Himedia to prepare the stock solution of 25×10^{-4} M in deionised water. EP was brought from sigma Aldrich and its stock solution was prepared in 0.1M perchloric acid. Brilliant green dye was purchased from himedia laboratories. The pH of the supporting solution was maintained in the range of 6.2 to 7.8 using phosphate buffer solution of ionic strength 0.2 M. Analytical range chemicals and doubly demineralised water were employed in the measurements.

5.7.2. Electrode modification:

Before each quantification, a glassy carbon electrode (GCE) surface was smoothened over micro cloth pads using 0.05μM alumina powder then rinse off carefully using distilled water to get shiny and clean surface. After polishing, the electrode was dipped in a solution mixture containing 1mM Brilliant green monomer in 0.1M NaOH as supporting medium to form p-BG/MGCE. Later, the

electropolymerisation was carried out by applying the cyclic sweeps in the potential range of -1.0V to +1.4V having the speed rate of 100mV/s for continuous 10 sweep cycles (Fig. 5.14A). The electrode deposition was regulated by restricting the sweep numbers (5- 20). However, the current signals of SE and EP started to decrease above the 10 cycles (Fig. 5.14B). This was because of the fact that thickness of the polymer membrane increases, which hinders the rate of electron transfer. Subsequently, the electrode surface was purified with doubly deionised water to eradicate the physically adsorbed unreacted monomer species and was employed for further electroanalysis.

5.8. Results and discussion

5.8.1. Effective surface area of the bare and modified working electrode:

A traditional potassium ferrocyanide redox system was employed as standard to examine the electrocatalytic assets of the fabricated sensor. The voltammograms logged for 1mM $K_4[Fe(CN)_6]$ solution using BGCE(dashed curve) and p-BG/MGCE (hard curve) at the sweep rate of 50mV/s along with supporting electrolyte(1M KCl) are presented in Fig. 5.15. It was noticed from the Figure, the peak potential difference (ΔEp) at BGCE was 137mV. But for the customised p-BG/MGCE ΔEp is 64mV. The electron transfer rate increases with a decrease in ΔEp value and the augmented peak signals was sighted at modified sensor [23]. These results concluded that the p-BG/MGCE shows better redox characteristics as compared to BGCE and this improvement are due to the increase in effective vicinity of the modified sensor. The active area of the working electrode was computed via Randles-Sevicks equation (1) [24]. The area of p-BG/MGCE was found to be 0.0352 cm^2 which is comparatively higher than BGCE (0.0241 cm^2).

$$Ip = (2.69\times10^5)\, n^{3/2}\, A\, D_0^{1/2}\, C_0\, \nu^{1/2} \quad \text{..... (5.4)}$$

Where, A is area (cm^2) of working electrode, C_0 is the concentration (mol/cm^3) of the electro active substance, ν is sweep rate and D_0 is diffusion coefficient (cm^2s^{-1}).

5.8.2. Impact of pH variation on SE and EP oxidation at p-BG/MGCE:

The pH of the supporting buffer for the sensing of analyte molecule has a leading role because it impacts the electron transfer rate and the electrochemical character of SE and EP. The outcome of the fluctuation of pH of the 0.2 M PBS(6.2-8.0) and the corresponding electrocatalytic nature of 0.1×10^{-4} M SE and 0.1×10^{-4} M EP at p-BG/MGCE along with sweep rate of 50mV/s are shown in Fig. 5.16A and 5.17A. Deviation in the oxidation currents and anodic potentials were perceived as the pH of the buffer was changed. Peak signals of SE and EP rises progressively till a pH value of 7.0, and consequently dropped with further raise in supporting pH and the potential tends to fall towards less positive values. This results implying that the electrode process at the modified sensor involves the protons. The slope values(60mV/pH for SE and 71mV/pH for EP) obtained by plotting the Epa of SE and Epa of EP versus varied pH (Fig. 5.15B and 5.16B) suggested that the equal number of electrons and protons are contributed in the electrochemical oxidation of SE and EP[25]. Most sensible oxidation peaks for SE and EP with a highest peak current were noticed at pH 7.0, this is considered for remaining electro analysis.

5.8.3. p-BG/MGCE sensor for SE and EP oxidation

The usability of bare and modified sensor for the exposition of electroanalysis of SE and EP were studied by CV process. CVs performed for 0.1×10^{-4} M SE and 0.1×10^{-4} M EP in 0.2 M PBS of pH 7.0 having the speed rate of 50mV/s at BGCE(dashed line) and p-BG/MGCE(solid line) as displayed in Fig. 5.18 and Fig. 5.19 respectively. At BGCE, both SE and EP are seems to have the feeble and broad oxidation signals. While at functionalized p-BG/MGCE, the voltammetric signals of SE and EP discloses the significant augmentation in their peak intensities. This astounding analytical response may possibly due to the massive surface area and improved electrocatalytic productiveness offered the rapid electron transport at the surface of modified sensor toward SE and EP.

5.8.4. Scan rate implications on SE and EP at p-BG/MGCE

The influence of scan rate was performed to explore the electrooxidation mechanism and kinetic specifications of SE and EP at modified electrode. Fig. 5.20A and 5.21A demonstrates the CVs profile for 0.1×10^{-4} M SE and 0.1×10^{-4} M EP at p-BG/MGCE for a disparate sweep rate from the range of 50-500 mV/s in the presence of 0.2M PBS of pH 7.0. it is visible from the figures that upon raising the sweep rate, the oxidation signals of SE and EP were also regularly upraised and switching the potential to the positive verge. The linearity exhibited with the sweep rate in the studied range as appeared in the Fig. 5.20B and Fig. 5.21B by plotting the anodic currents (Ipa) of 5-HT and EP with square root of scan rate ($\nu^{1/2}$) with a regression factor (R^2) value of 0.9895 & 0.9931 respectively. Furthermore, the slope values collected from the plot of log Ipa of SE and EP versus log of scan rate (Fig. 7c and Fig. 8c) were found to be 0.7105 for SE and 1.4916 for EP. This outcome depicts that the reaction on the electrode surface is diffusion controlled [26] for SE and adsorption controlled for EP [27].

5.8.5. Concentration variation and detection limit

The most impressive benefits of the presented sensor were its ability to minimize the detection and quantification limits towards SE and EP determination. Fig. 5.22A and 5.23A manifests the CVs logged for SE and EP in occurrence with 0.2 M PBS of neutral pH having the speed rate of 50mV/s at p-BG/MGCE for uneven concentration of respective analytes. The recorded result reveals that, as extending the SE and EP concentrations boosts up the current intensities about the range of 0.1×10^{-4} M to 0.7×10^{-4} M for SE and 0.1×10^{-4} M to 1×10^{-4} M for EP. The calibration curves are plotted between the Ipa of SE and EP against concentration as illustrated in Fig. 5.22B and Fig. 5.23B respectively. The plots established a finer linearity with a correlation factor (R^2) of 0.9980 for SE 0.9967 for EP. Equation (2) & (3) are implemented to quantify the LOD and LOQ [28]. The estimated detection LOD was found to be 0.74×10^{-6} M for SE and 0.58×10^{-6} M for EP and the LOQ values are 2.49×10^{-6} M for SE and 1.95×10^{-6} M for EP. Further, the LOD values of working electrode is comparable with previously reported methods (Table.5.4 and Table 5.5), implicates the potency of the work.

$$LOD = 3\sigma/M \quad \text{..... (5.5)}$$

$$LOQ = 10\sigma/M \quad \text{..... (5.6)}$$

Where, σ denotes standard deviation and M is slope from calibration plots.

5.8.6. Simultaneous resolution of SE and EP at p-BG/MGCE

The capability of the p-BG/MGCE to instigate the concurrent analysis of the targeted analytes in mixed solution was explored by using CV procedures. Fig. 5.24 resembles the CVs recorded for the simultaneous examination of equimolar solution of 0.1×10^{-4} M SE and 0.1×10^{-4} M EP in occurrence with 0.2M PBS of pH 7.0 along with the speed rate of 50mV/s. It is observed from the recorded Figure, the less sensible and concurred oxidation potentials were noticed at BGCE (Scattered line). Meanwhile, the distinct and well resolved oxidation signals of 5-HT and EP were observed at p-BG/MGCE (Hard line) and the two separate oxidation potentials noticed at 287 mV and 125mV for 5-HT and EP respectively. This result confirmed that the customised p-BG/MGCE has remarkable selectivity towards simultaneous resolution of SE and EP.

5.8.7. Selective study of SE and EP at p-BG/MGCE

To demonstrate the electroanalytical versatility of the designed sensor, the selective investigation of SE and EP in the mixture using p-BG/MGCE has been performed by the application of highly susceptible DPV procedure. The analysis was done by altering the concentration of each individual and by retaining the constant concentration of other species. Fig. 5.25A represents the DPVs of steady concentration of EP (0.1×10^{-4} M) and unlike SE concentrations from 0.1×10^{-4} M to 0.8×10^{-4} M. Likewise, the Fig. 5.26A illustrates the differing the concentration of EP with fixed SE concentration (0.1×10^{-4} M). The Fig. 5.25B and 5.26B depicts the plots of anodic peak current against the distinct concentration of SE and EP respectively. The linear connection between the oxidation peak current with concentration and the corresponding regression factor for SE and EP found to be 0.9983 and 0.9978 for SE and EP respectively withought fluctuating the peak potential as well as current of fixed analytes concentration. This outcome resembles the good specificity of the

fabricated sensor towards simultaneous investigation of respective analytes in their sample mixture.

5.9. Conclusion

This report deputed the discrimination of a simple, sensible and a rapid electrochemical biosensor towards the evaluation of SE and EP. The sensor amplification was based on the electropolymerization of brilliant green dye on the BGCE surface. The distinct experimental confines were attributed and successfully demonstrated the voltammetric particularities of the tailored electrode. The augmentation of surface area after modification was established. The kinetic etiquette of the fabricated sensor was noticed by examining the scan rate variation study. The p-BG/MGCE depicts the excellent linear dynamic range with a reduced detection limit. The amplified electrode was imported as a susceptible senor for selective detection of SE in presence of EP with good sensitivity and fast response.

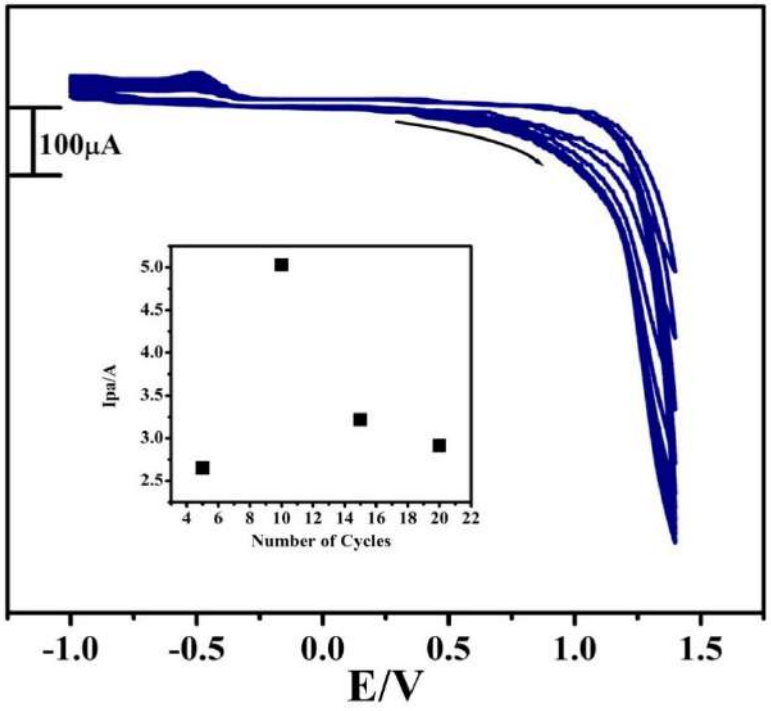


Fig. 5.14A. CVs of developing of p-BG/MGCE using 1mM brilliant green solution in 0.1 M NaOH at 10 cycles. **B.** Display of Ipa of 5-HT v/s number of polymerization cycles.

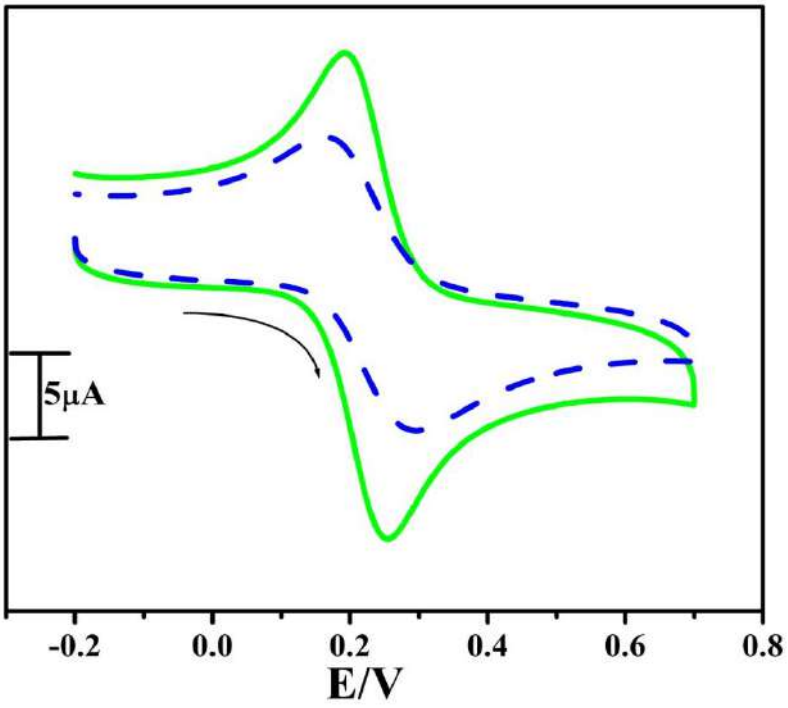


Fig. 5.15. CV plots of 1mM potassium ferrocyanide system at BGCE(dashed line) and p-BG/MGCE(hard line) with the sweep rate of 50mV/s in 1M KCl.

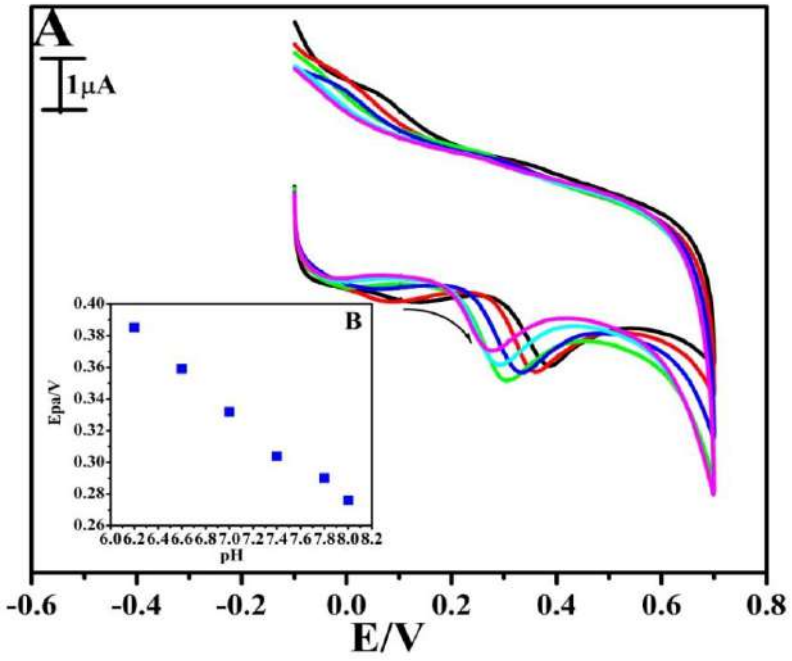


Fig. 5.16A. CVs of 0.1×10^{-4} M SE at the surface of p-BG/MGCE in 0.2 M supporting electrolyte at distinct pH values (6.2-8.0) having speed rate of 50mV/s. **B.** Display of plot drawn between Epa of SE against pH.

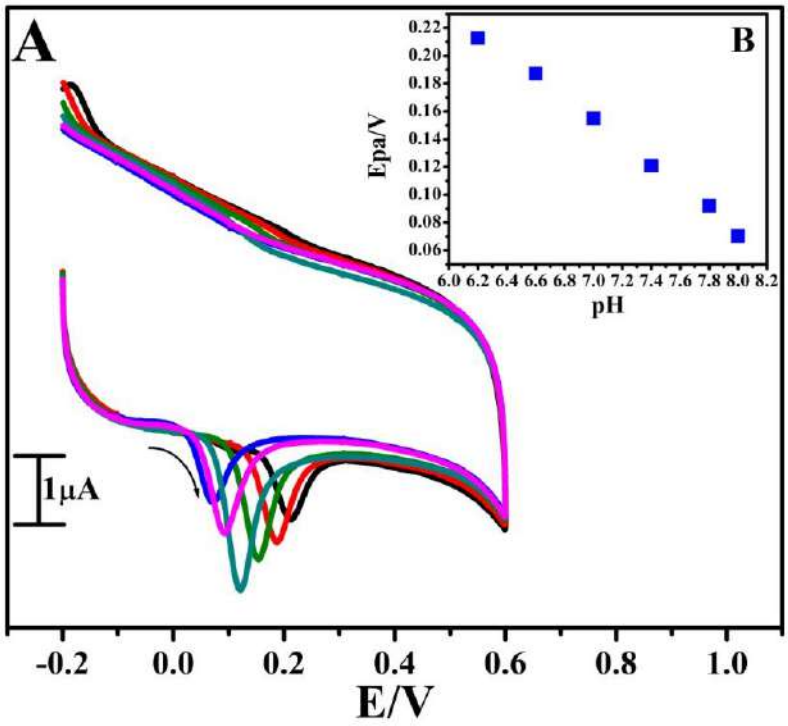


Fig. 5.17A. CVs of 0.1×10^{-4} M EP at the surface of p-BG/MGCE in 0.2 M supporting electrolyte at separate pH values (6.2-8.0) having speed rate of 50mV/s. **B.** Display of plot drawn between Epa of EP against pH.

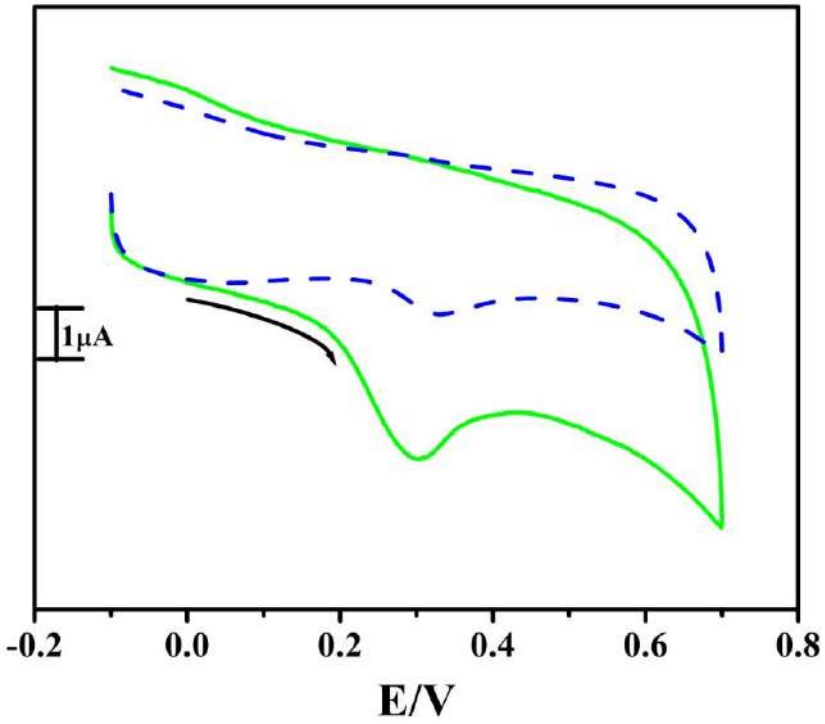


Fig. 5.18. CVs for SE (0.1×10^{-4} M) response at BGCE (scattered line) and p-BG/MGCE(solid line) in 0.2M PBS of pH 7.4 with speed rate of 50 mV/s.

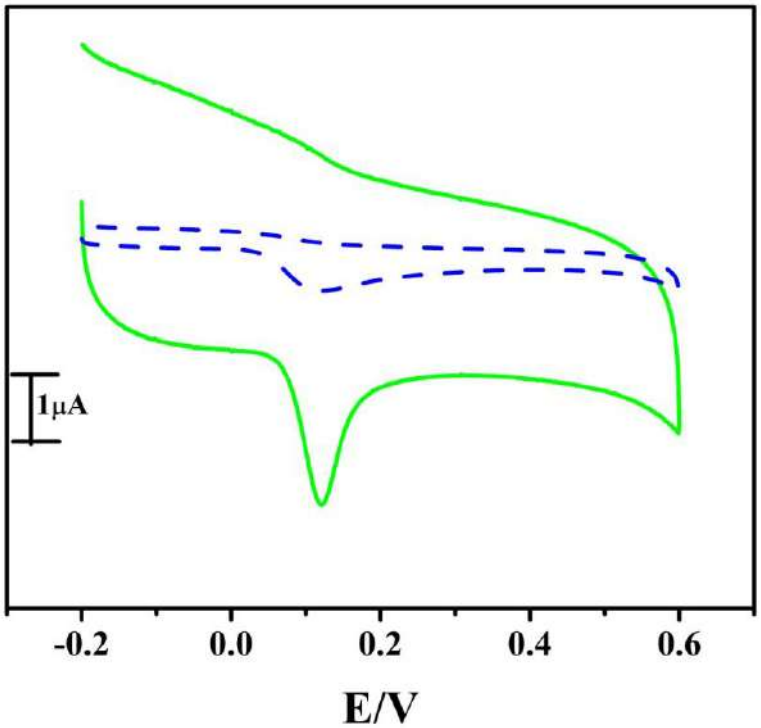


Fig. 5.19. CVs for EP (0.1×10^{-4}M) response at BGCE(scattered line) and p-BG/MGCE (solid line) in 0.2 M PBS of pH 7.4 with speed rate of 50 mV/s.

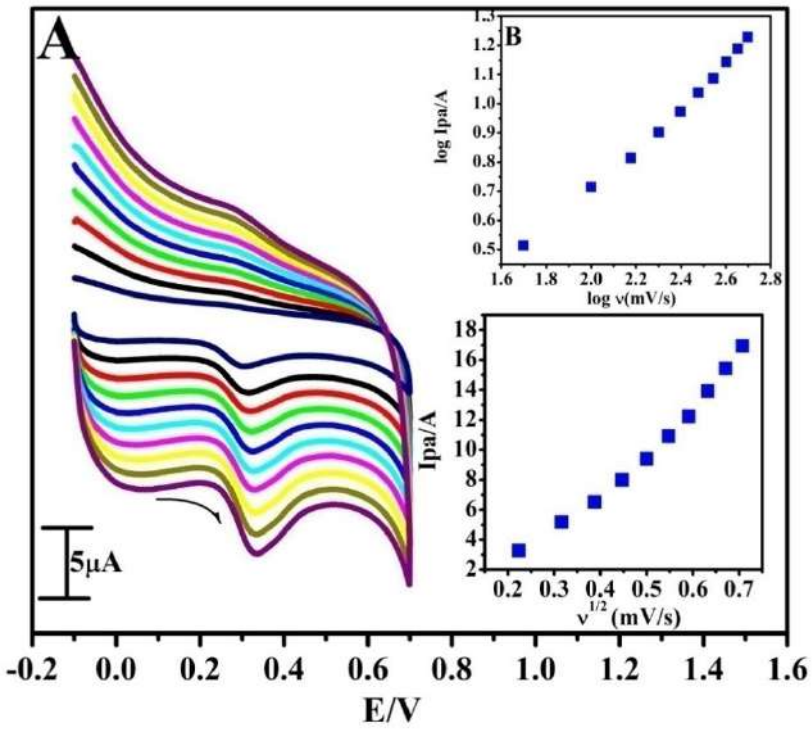


Fig. 5.20A. CVs of 0.1×10^{-4} M SE at p-BG/MGCE at various scan rates (50 to 500mV/s) in PBS of pH 7.4. **B.** Plot of log Ipa of SE versus log ν. **C)** Plot of Ipa of SE with $\nu^{1/2}$.

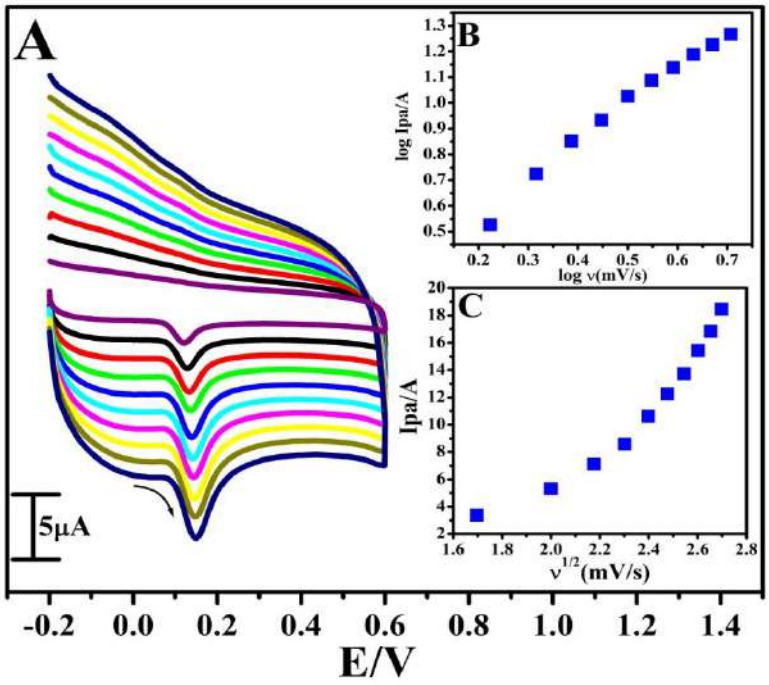


Fig. 5.21A. CVs of 0.1×10^{-4}M EP at p-BG/MGCE at disparate scan rates (50 to 500mV/s) in PBS of pH 7.4. **B.** Plot of log Ipa of EP versus log ν. **C.**Plot of Ipa of EP with $\nu^{1/2}$.

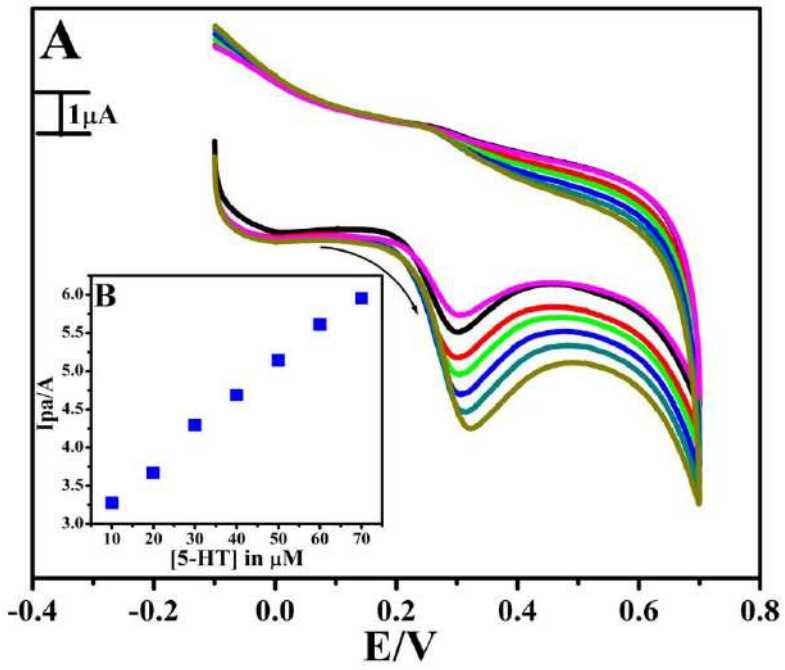


Fig. 5.22A. CVs logged for SE with differed concentration (0.1×10^{-4} M to 0.7×10^{-4} M) employing 0.2 M PBS of pH 7.4 at p-BG/MGCE along the sweep rate 50 mV/s.
B. Graph of Ipa of SE against concentration.

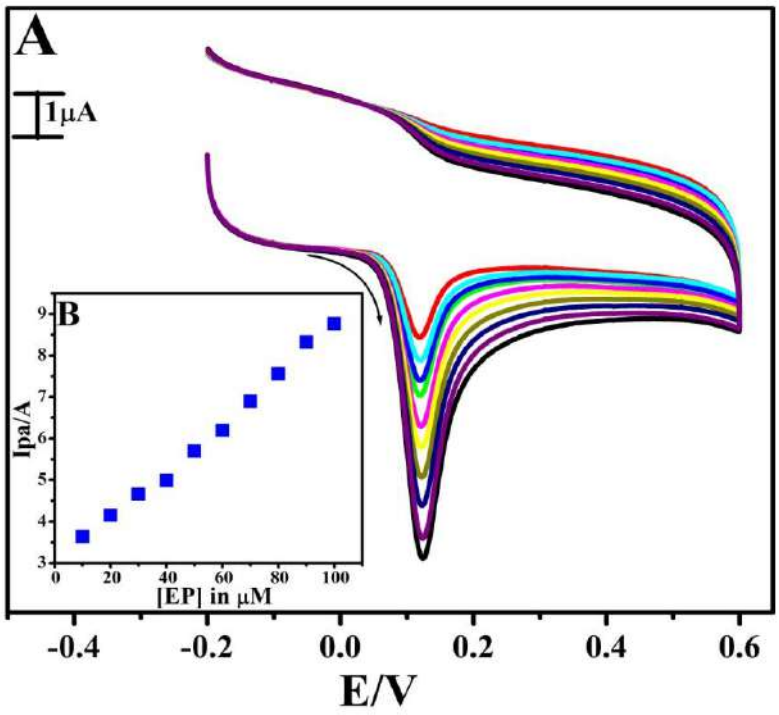


Fig. 5.23A. CVs logged for SE with differed concentration (0.1×10^{-4} M to 0.7×10^{-4} M) employing 0.2 M PBS of pH 7.4 at p-BG/MGCE along the sweep rate 50 mV/s.
B. Graph of Ipa of SE against concentration.

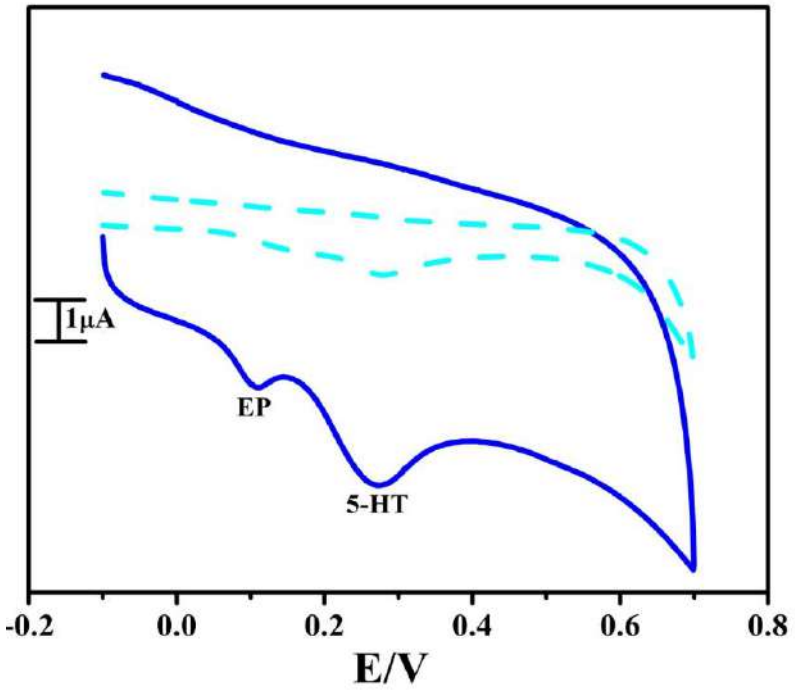


Fig. 5.24. CVs for selectivity analysis of SE & EP with speed rate of 50 mV/s in 0.2M PBS of pH 7.4 at BGCE (dashed curve) and p-BG/MGCE (hard curve).

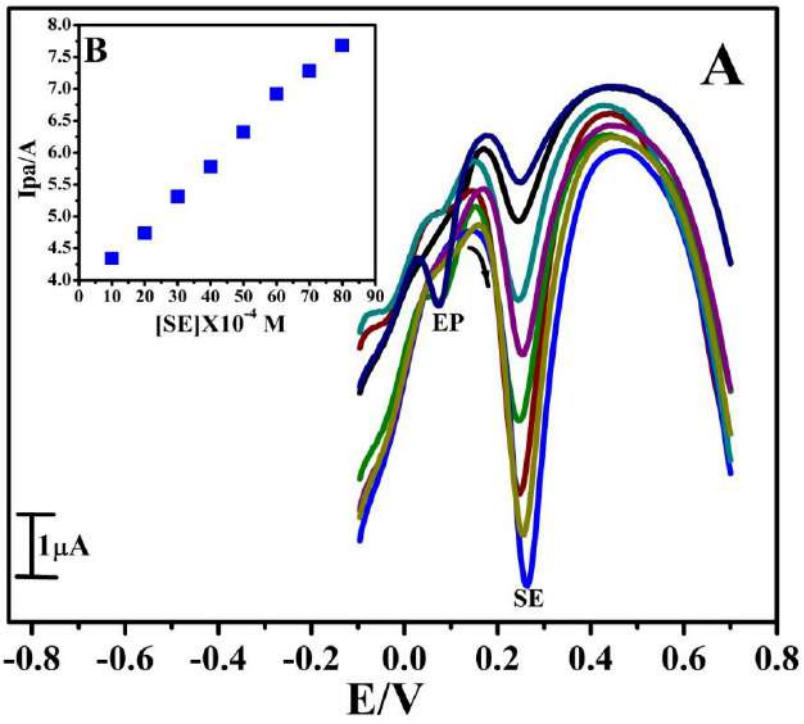


Fig. 5.25A. DPVs got for varied of concentration SE (0.1×10^{-4} M to 0.8×10^{-4} M) with EP (0.1×10^{-4} M) at p-BG/MGCE with speed rate of 50 mV/s in 0.2M PBS of pH 7.4. **B.** Display of Ipa of SE against concentration.

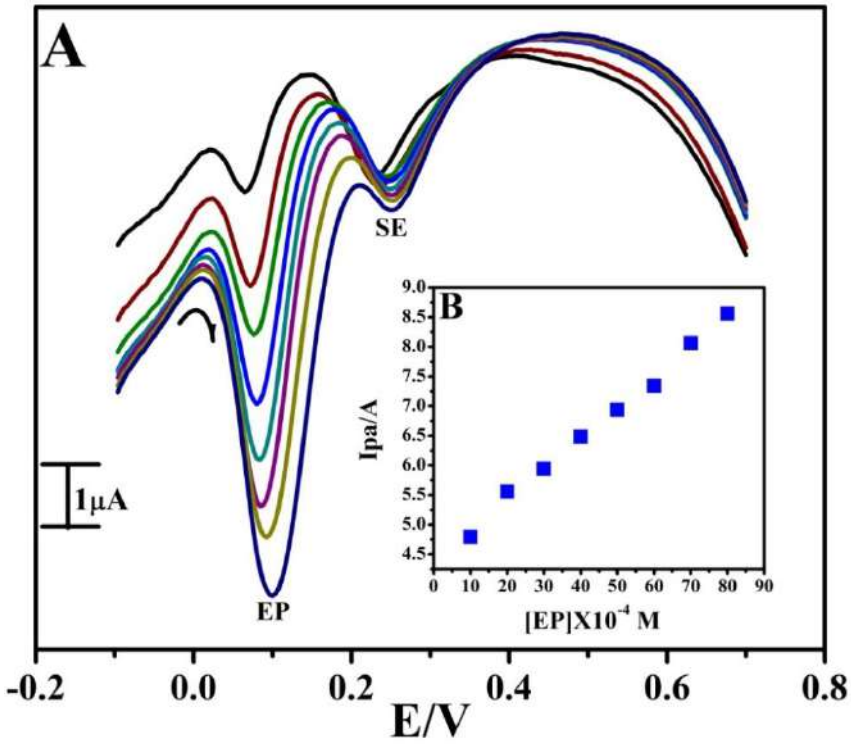


Fig. 5.26A. DPVs got for divergent concentration EP (0.1×10^{-4} M to 0.8×10^{-4} M) with SE (0.1×10^{-4} M) at p-BG/MGCE with speed rate of 50 mV/s in 0.2M PBS of pH 7.4. **B.** Display of Ipa of EP against concentration.

Table.5.4. Comparison of different modified electrodes for SE detection.

Sl No.	Electrode	LOD(μM)	Method	Reference
1	AuNPs@PPy/GSPE	33.2	SWV	[29]
2	MWCNT/Nafion/MAO-A	20	DPV	[30]
3	f-MWCNTs/BR9/GCE	9	DPV	[31]
4	3D – ITO	7.5	DPV	[32]
5	IL-DC-CNT/GC	2	DPV	[33]
6	Poly(ser)/MWCNT/GCE	2	DPV	[34]
7	p-NGB/MGCE	1.83	CV	[35]
8	p-BG/MGCE	0.78	CV	**This study**

Table.5.5. Comparison of different modified electrodes for EP detection.

Sl No.	Electrode	LOD(μM)	Method	Reference
1	Poly(caffeic acid)/GCE	20	CV	[36]
2	Poly(1Methylpyrrole)/MGCE	16.8	CV	[37]
3	f-MWCNTs/BR9/GCE	9	DPV	[32]
4	CAP/MWCNT/GCE	7.2	CV	[38]
5	GNPs/GCE	5.0	SWV	[39]
6	Poly(ser)/MWCNT/GCE	0.9	DPV	[34]
8	p-BG/MGCE	0.58	CV	**This study**

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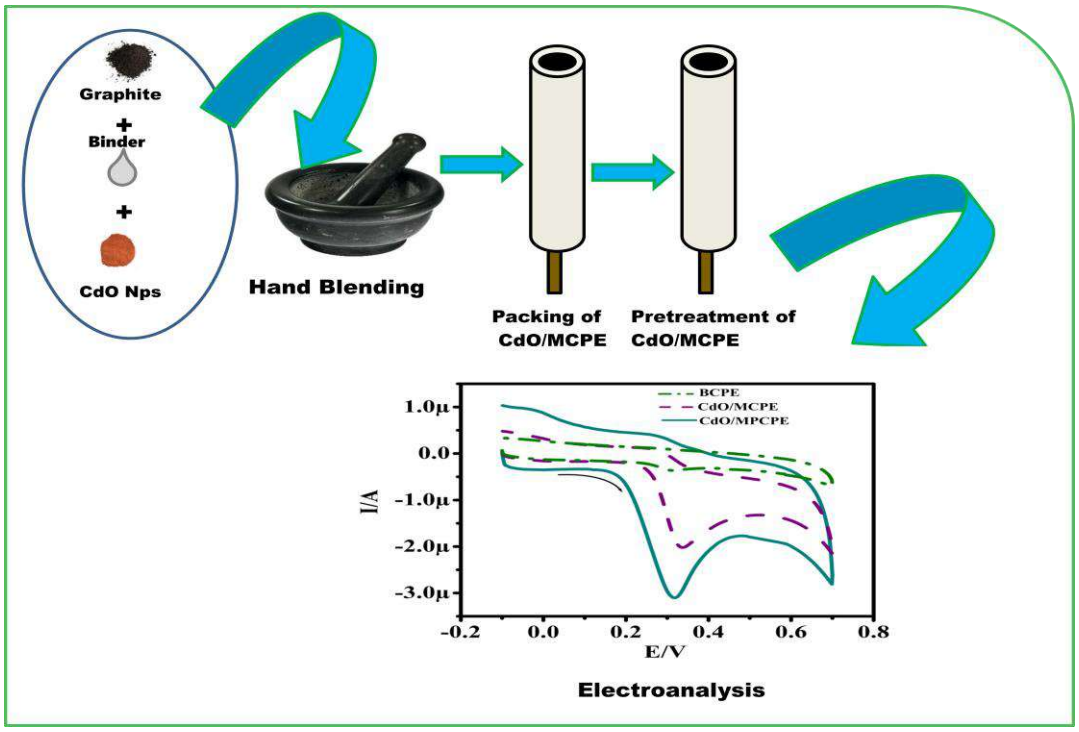
Chapter-VI

ELECTROCHEMICAL SENSOR FACILITATED BY SYNTHESIS OF CdO NANOPARTICLES AMPLIFIED PRE-TREATED CARBON PASTE ELECTRODE FOR QUANTIFICATION OF SEROTONIN IN THE PRESENCE OF EPINEPHRINE



Communicated to Analytical and Bioanalytical Electrochemistry (2022)

6.1. Introduction

The current growth and impression of nanoscience and nanotechnology and their applicabilities in the field of analytical chemistry has led to the development of new approaches in the area of biosensing applications. Metal oxide nanoparticles extensively adopted in electrochemical sensing of bio essential materials and matters [1-3]. Nanoparticles flourishes a special physiological and catalytic functionalities and properties like optimal electronic and optical characteristics, improved surface area of sensing interaction, excellent ability to promote direct electron transmission between the electrode and the reactive sites of the targeted molecules, biocompatibility, chemical constancy and enhanced signal responses [4-7]. CdONPs are familiar to be extremely reactive n-type semiconductor and have been exploited in energy storage devices [8], magnetoresistive systems [9], heterogeneous catalysis and other optoelectronic devices [10]. CdONPs has beneficial demand due to its supreme electrocatalytic actions towards bio-related compounds such as catecholamine molecules [11].

SE and EP are catecholamine neurochemicals immeasurably crucial for forwarding of neuronal indications between nerve cells in the brain as well to and from other regions of the body [12]. SE serves multifarious range of duties across the distinct biological processes, including influencing learning, memory, hunger, happiness and adjusting the temperature of livings. SE ranges that are too low or too high can triggers multiple physical and neurological health challenges such as the scarcity of serotonin thought to perform a character in depression and phobias. The upper ranges of SE bring on a serotonin toxicity, restlessness and confusions [13-17].

EP is a neuromediator andneuroendocrine harmone generated by a small number of neurons in the medulla oblongata and adrenal gland. EP works on vast array of biological responsibilities and on adrenergic and adrenergic receptors in disparate organs [18-20]. EP acts as a first-line treatment for analphylaxis and involved in manifold clinical solicitations like heart failure and asthma by relaxing the muscles in the airways and tightening the blood veins. Abnormal EP magnitudes can lead to arrhythmias, pulmonary oedema and thyroid hormone deficiency [21, 22]. SE

and EP are associated with mental status and body functions, their early determination being an important goal for clinical diagnosis.

This study focuses on the development of electrochemical sensor facilitated by the modification of prepared CdONPs followed by pretreatment and the designed sensor was employed to concurrent and specific discrimination of SE in the occurrence with EP.

6.2. Experimental Segment:

6.2.1. Equipments and Reagents:

Electroanalytical experiments were accomplished with the facilitation of CH analyser of model CHI 660c. The electrolytic cell comprised of three electrode pattern includes bare and cadmium oxide modified pre-treated carbon paste (BCPE and CdO/MPCPE) working electrode, saturated calomel reference electrode to measure cell potential and platinum wire counter electrode. The structural analyses, elemental composition and morphological features of the prepared CdO nanoparticles were interpreted by adopting XRD, SEM and EDX approaches.

Analytically pure SE, KCl, $K_4[Fe(CN)_6]$, $Cd(NO_3)_3{\cdot}4H_2O$ and NaOH were brought from Himedia laboratories. EP was gotten from Sigma Aldrich. The silicone oil and pure graphite powder were from Fluka and Merck chemicals. Every solution was freshly procured form doubly demineralised water and the solution of EP was made by employing 0.1M perchloric acid. Supportive buffer was acquired from Na_2HPO_4 and NaH_2PO_4 in a pH ranges from 5.8 to 7.8.

6.2.2. Preparation of CdO nanoparticles:

The CdO/NPs were synthesized through co-precipitation practice by dissolving 0.1M $Cd(NO_3)_3{\cdot}4H_2O$ in 50ml of demineralised water and stirred magnetically for 1 h at room temperature. The precipitating reagent, 1M NaOH was mixed drop wise to the precursor solution and stirred vigorously until the pH value of the solution reaches 10. The building up of white precipitate was started instantaneously. The mixture was continually stirred for 4 h. Then the formed precipitate was filtered and purified

numerous times. The filtered solid was heated at 80 °C for 5 h and the gotten powder was then calcinated at 600 °C for about 3 h.

$$Cd(NO_3)_2{\cdot}4H_2O + NaOH \rightarrow Cd(OH)_2 + 2NaNO_3 + 4H_2O\uparrow$$

$$Cd(OH)_2 \rightarrow CdO + H_2O$$

6.2.3. Setting up of the working electrodes:

The bare carbon paste was framed according to the literature [23]. The CdO/MCPE was fashioned by plodding the prepared CdO/NPs in a varied amount (2mg, 4mg, 6mg and 8mg) with the commixture of mineral oil and graphite powder. The identical blending and stuffing procedures were repeated. Then the amplified CdO/MCPE was employed towards the study of SE and EP. However the greater current responsiveness was accomplished at 6mg (Fig. 6.1B) hence it was chosen for electrode amplification. The designed CdO/MCPE was electrochemically pre-treated in order to enhance the electrocatalytic sensitivity of the sensor. The pre-treatment of the sensor was done by continual sweeping of the potential between -0.4V to 1.2V in being of 0.1M NaOH along the speed rate of 0.05 V/s (Fig. 6.1A). Afterward, the tailored electrode designated as CdO/MPCPE and employed for electrochemical analysis of SE in the presence of EP.

6.3. Result and Discussion:

6.3.1. Characterisation of synthesised CdO/NPs:

The crystalline nature and structure of the as-prepared CdO/NPs were scrutinised by XRD interpretations. Fig. 6.2A displays the XRD patterns of CdO/NPs. The typical diffraction peaks indexed that cubic structure and the reference pattern closely mathes with the JCPDS file No. 05-0640. The enhancement in the peak sharpness and nonappearance of the impurity peaks attributed to the purity and crystallanity of the NPs. The prominent intensive peaks have been employed to estimate the size of the particles through Debye-Scherer formulae (1) [24] and the average grain size was found to be 35 nm.

$$D = \frac{K\lambda}{\beta Cos\theta} \quad \text{..... (1)}$$

Where, β is the full width half maximum intensity, θ is the diffraction angle (in radian) of the considered diffraction peak, λ is the wavelength (λ = 0.1543 nm) and K is a constant(0.90). The superficial surface specificities of the prepared CdO/NPs were examined using SEM measurements. Fig 6.2B depicts the SEM pictures of CdO/NPs. The images clearly evidenced that the synthesized CdO/NPs has agglomerated structures in which the particles are irregularly fashioned. Fig 6.2C significantly stipulates the EDAX exploration and the emergence of well-defined signals approved the presence of elements, Cadmium and oxygen in the as-prepared CdO/NPs.

6.3.2. Electrochemical depiction of CdO/MPCPE:

The electron transfer approaches of CdO/MPCPE were monitored by the application of conventional redox probe ($K_4[Fe(CN)_6]$). Fig. 6.3 presents the CVs developed for the 1mM $K_4[Fe(CN)_6]$ in bearing with supporting solution of 1M KCl having the speed rate of 0.05V/s at BCPE(dashed curve), CdO/MCPE(dashed dotted curve) and CdO/MPCPE (solid curve). The BCPE offered the reduced current signals with wider overvoltage and a trifling increment of peak intensity was observed at CdO/MCPE. The substantial hike in the peak current with the lessening of peak potential was perceived at the composite CdO/MPCPE sensor. This outcome testifies that the magnified surface and redox properties along with the rapidity in electron transportation after pretreatment of the electrode. The availability of effective surface area of the series of working electrodes were enumerated via Randles-Sevicks prescriptions (2) [25]. The composite CdO/MPCPE gave higher electroactive area (0.0358 cm^2) in contrast to BCPE (0.024 cm^2) and CdO/MCPE (0.0281 cm^2).

$$Ip = (2.69\times10^5)\, n^{3/2}\, A\, D_0^{1/2}\, C_0\, \nu^{1/2} \quad \text{..... (2)}$$

Where, A is area (cm^2) of working electrode, C_0 is the concentration (mol/cm^3) of the electro active substance, ν is sweep rate and D_0 is diffusion co-efficient (cm^2s^{-1}).

6.3.3. Consequences of solution pH

The optimization of the pH of the supportive medium was found to have influential consequences on the detection sensibility and peak resolution of respective electroactive species. The CV method was accustomed to study the interrelation of the oxidation of SE and EP on pH. Fig. 6.4A and Fig. 6.5A pretences the CVs logged separately for the 10μM SE and 10μM EP at varied pH values (5.8 to 7.8) being with 0.2M PBS supporting solution at CdO/MPCPE along with 0.05V/s sweep rate. The peak potential moved to less positive side with increasing buffer pH. This implies the electro-oxidation of SE and EP was governed by proton and electrons. Fig. 6.4B and Fig. 6.5B presents the correlation between peak potential and differed buffer pH and the respective regression equations, Epa(pH 5.8-7.8) = −0.0678pH + 0.7963(r^2=0.9912) for SE and Epa (pH 5.8-7.8) = −0.0778 pH+ 0.8963(r^2=0.9895) for EP respectively. The respective slope value with good linearity confirms the equal number of electron and proton participation [26]. However, the greater peak resolution and sensitivity for SE and EP was witnessed at pH 7.4. So, this pH was elected as optimal for entire electroanalysis.

6.3.4. Sensing of SE and EP at different working electrode

The comparative analytical response of SE and EP were appraised by CV process to monitor the electrocatalytic implementation of bare and chemically modified working electrodes. The oxidation signals were recorded for 10μM SE and 10μM EP at the surface of BCPE(dashed line), CdO/MCPE(dashed dotted line) and CdO/MPCPE(solid line) in appearance with 0.2 M PBS of pH 7.4 together with 0.05V/s scan rate as revealed in Fig. 6.6 and Fig. 6.7 respectively. Contrasted with BCPE, CdO/MCPE displays the minute increment in the anodic peak current. Whereas, after pretreatment of CdO/MCPE with NaOH, the astronomical hike in the current densities were ascertained with minimisation of over potential. This favourable result authenticates the uplifted surface behaviours, easy electron transfer and higher conductive nature of composite sensor which makes it as appropriate choice for the formulation of susceptible SE and EP sensing element.

6.3.5. Study of potential scan rate

The kinetic particularities and oxidation mechanism were explored for SE and EP at CdO/MPCPE by conducting the study of scan rate variation. CVs registered for10μM SE and 10μM EP in existence with 0.2M PBS of pH 7.4 with the discrete sweep rate ranging from 0.05V/s to 0.5V/s at CdO/MPCPE are provided in Fig. 6.8A and Fig. 6.9A. As perceived from Figures, the anodic current signals were eventually intensified for hike in each sweep rate with positive alteration of peak potential. To assess the electrode kinetics, the graph was plotted between the log Ipa of SE and EP against log ν as illustrated in Fig. 6.8B and 6.9B. The attained graph shows appreciable linearity with the slope values of 1.241(r^2= 0.98827) for SE and 1.063 (r^2= 0.9894) for EP. This finding affirms that the electrode proceedings were governed by adsorption controlled phenomenon for both SE and EP [27]. The Fig. 6.8C and 6.9C portrays the plots of Ipa of SE and EP versus Square root of speed rate ($\nu^{1/2}$). The offered plots shows marvellous linearity for SE and EP with a correlation factor r^2= 0.9841 and r^2= 0.9877 respectively, which further accredits the kinetics of the formulated electrode.

6.3.6. Calibration of CdO/MPCPE towards SE and EP

Under the appropriate test conditions, the sensing efficacy and linear range for the desired analytes were investigated at the surface of amplified sensor by oscillating the corresponding anayte concentration. CV peaks entered for SE and EP being with 0.2 M PBS of pH 7.4 along with the speed rate 0.05V/s for unfamiliar concentration at CdO/MPCPE using CV technique as indicated in Fig. 6.10A and 6.11A respectively. The gathered outcomes evidenced for that the progressive hike in current signals with boosting concentration of SE (10μM-80μM) and EP (10μM-100μM) with diminutive switching of positive peak potential. The calibration graph was plotted for anodic peak current of SE and EP contrary to the analyte concentration as given in the Fig. 10B and 11B respectively. The depicted plots reflects the straight line with the correlation expressions, Ipa (μA) = 0.1101 (μM) + 4.83 (r^2 = 0.9953) for SE and Ipa (μA) = 0.0536(μM) + 2.12 (r^2 = 0.9979) for EP. The LOD values found to be 0.88μM for SE and 1.82μM for EP and LOQ were approximated to be 2.95μM for SE and 6.07μM for EP in accordance with the following equalities (2) and (3)[28,29].

$$LOD = 3S/M \quad \text{.....(2)}$$

$$LOQ = 10S/M \quad \text{.....(3)}$$

Where, M is the slope of the calibration curve and S is the standard deviation.

6.3.7. Concurrent eletroanalysis of SE and EP at CdO/MPCPE

The competencies to consider the specificities and sensitivity of the customised electrode is its ability to resolve the electroanalytical response of SE in concurred with probable interfering neurochemical like EP. CV method employed to measure the selectiveness of the modified sensor. Fig. 6.12 displays the CVs documented for a mixed solution of 10μM SE and 10μM EP in bearing with 0.2M PBS of pH 7.4 having the scan rate of 0.05 V/s at BCPE(dashed dotted curve), CdO/MCPE(dashed curve) and CdO/MPCPE(solid curve). The Figures depicted that the BCPE and CdO/MCPE was produce less sensitive and failed to resolve the peak potentials of the separate analytes. Whereas, the formulated CdO/MPCPE consequently resolute and distinctly separated peaks of SE and EP were established. The individual peak potential for SE and EP were sited at 0.285 V and 0.1413 V respectively. This outcome signifies the potentiality of the CdO/MPCPE sensor for concurrent and specific discrimination in mixture of analytes.

6.3.8. Selective examination of SE and EP at CdO/MPCPE

The isolation of voltammetric signals of the specific analyte in the sample mixture is a key factor to decide the sufficiency of the tailored sensor. The immensely responsive DPV approach was employed to explore the selectivity of SE and EP by differing concentration and holing constant concentration of other. DPV response tracked for the SE in the linear range of 10μM-80μM by keeping the invariable EP (10μM) concentration in assistant with 0.2 M PBS of biological pH at CdO/MPCPE as shown in Fig. 6.13A. As noticed, the signal density of SE elevated with rise in its concentration. Identically, to examine the EP, the SE (10μM) concentration was fixed and the concentration of EP was altered in the range of 10μM-80μM as resembled in Fig. 6.14A. Fig. 6.13B and Fig. 6.14B reflects the linearity plot between the Ipa of SE and EP with fluctuated concentration. It can be notable from the above outcome, the oxidation peak currents of SE and EP are positively proportional to their concentration

and deviating the concentration of one species does not affect the peak current and peak potential of another species. It signifies the greater selectivity of the formulated sensor.

6.4. Conclusion

Herein, the CdO nanoparticles were prepped with the help of chemical co-precipitation fashions and the morphological and the other qualities of the developed material was performed using XRD, SEM and EDX analysis. The electrocatalytic properties were scrutinised by applying the CV and DPV procedures. The as-prepared nanoparticles were utilised for the fabrication of CdO/MCPE and then followed by pretreatment with NaOH. The customised CdO/MPCPE portrays the augmented voltammetric reactivity towards the identification of SE and EP in physiological pH(7.4). the kinetic experiments were operated to acknowledge the type of electrode process and it was recognised to be adsorption controlled for both SE and EP. The formed CdO/MPCPE electrode yielded lower LOD (0.88μM for SE and 1.82μM for EP) and LOQ (2.95μM for SE and 6.07μM for EP) values and specific in the isolation of SE from a commixture consisting both SE and EP. The designed sensor protects the surface of electrode from fouling and presents favourable sensibility and efficaciously applicable as biosensor in neurochemistry applications and the quantification of the like bioactive elements.

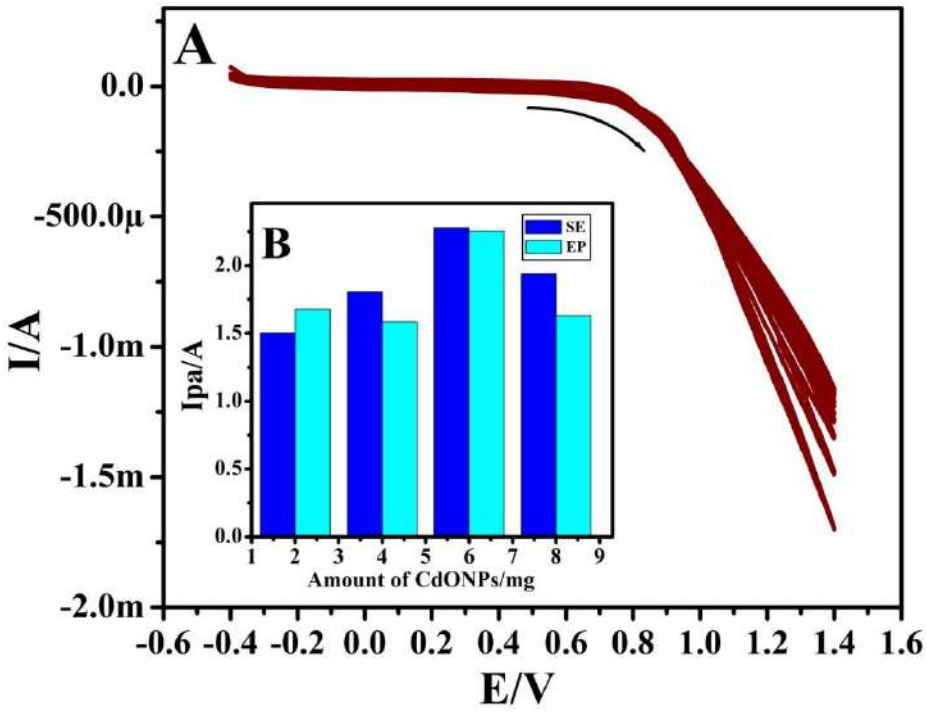
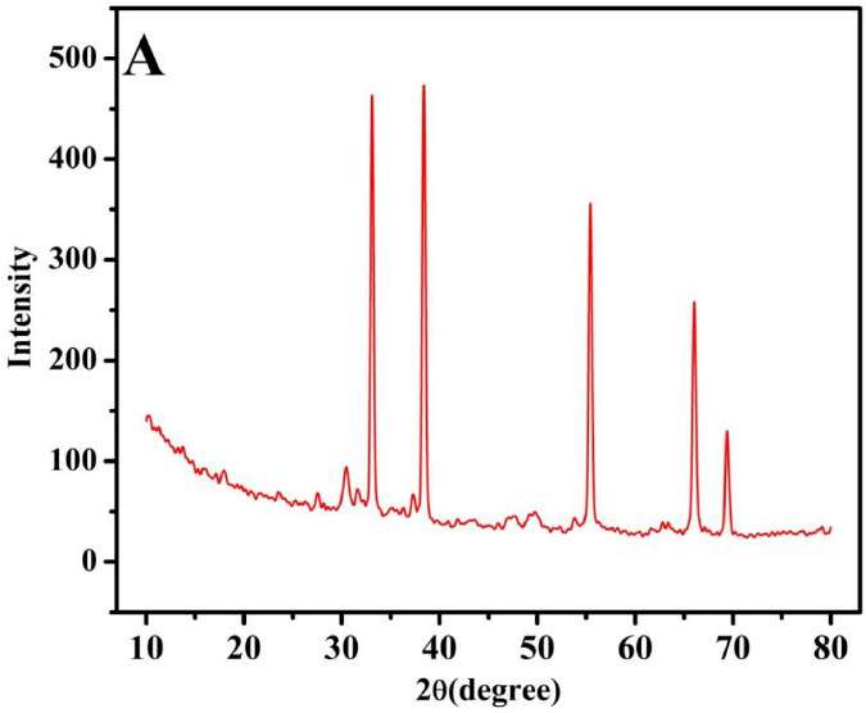
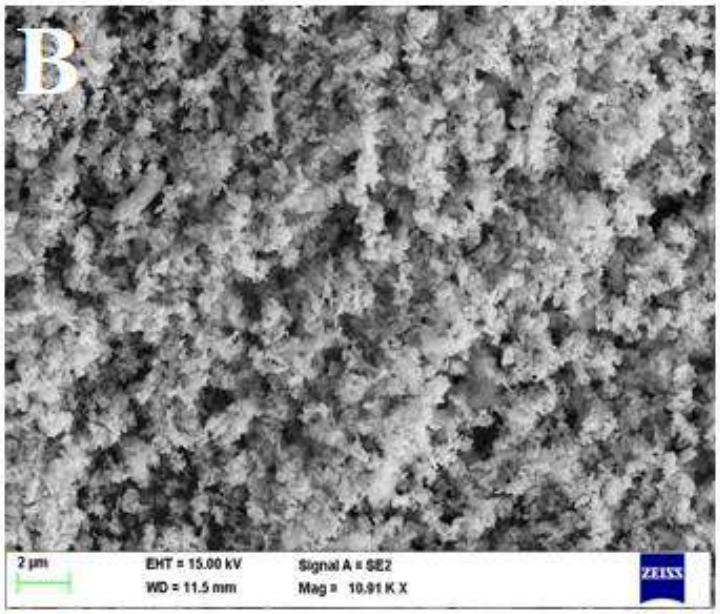


Fig. 6.1A. Electrochemcial pre-treatement of CdO/MCPE in 0.1M NaOH with the sweep rate 0.05V/s. **B.** Plot of Ipa of SE and EP versus amount of synthesised CdONPs.





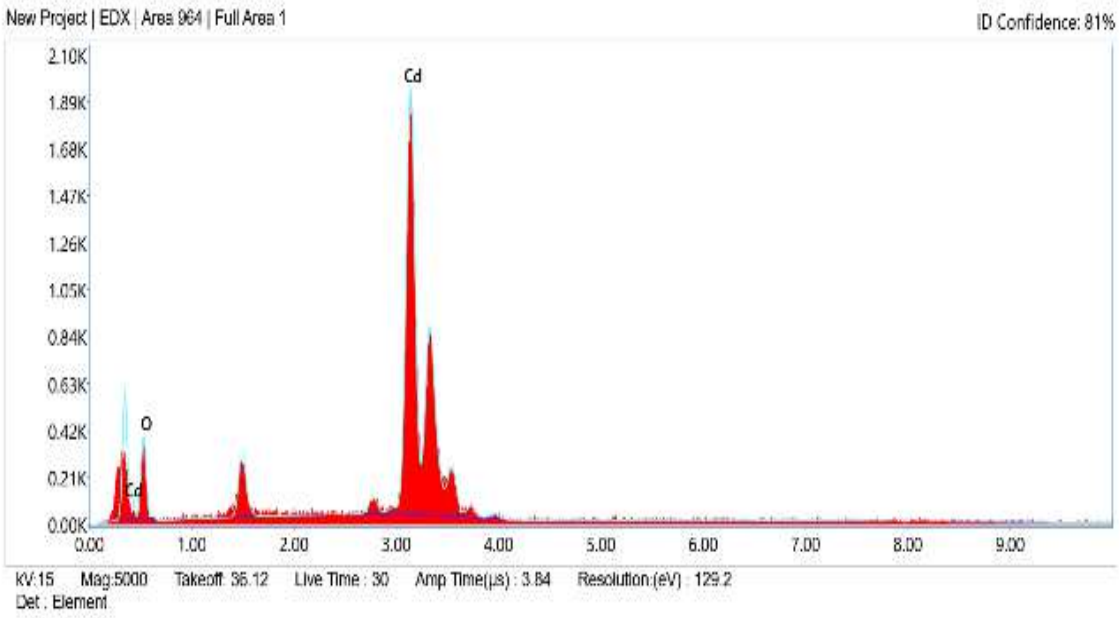


Fig. 6.2A. XRD pattern for prepared CdO nanoparticle. **B.** SEM image of CdO nanoparticle. **C.** EDAX pattern for as-prepared CdO nanoparticles.

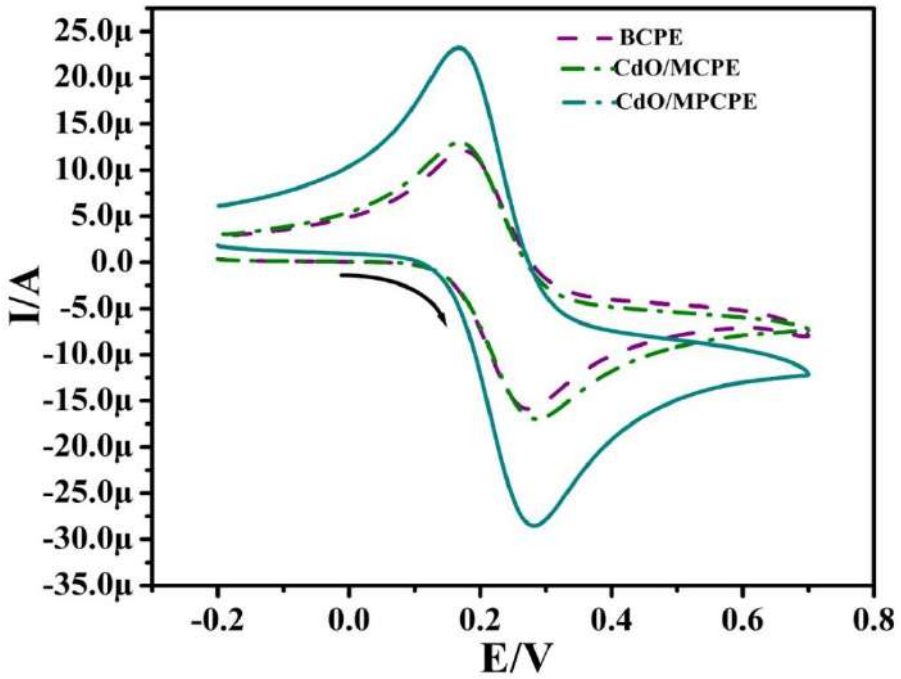


Fig. 6.3. CVs curve for 1 mM $K_4[Fe(CN)_6]$ at BCPE (dashed line), CdO/MCPE (dashed dotted line) and CdO/MPCPE (solid line) with sweep rate of 0.05V/s using 1M KCl (supporting electrolyte).

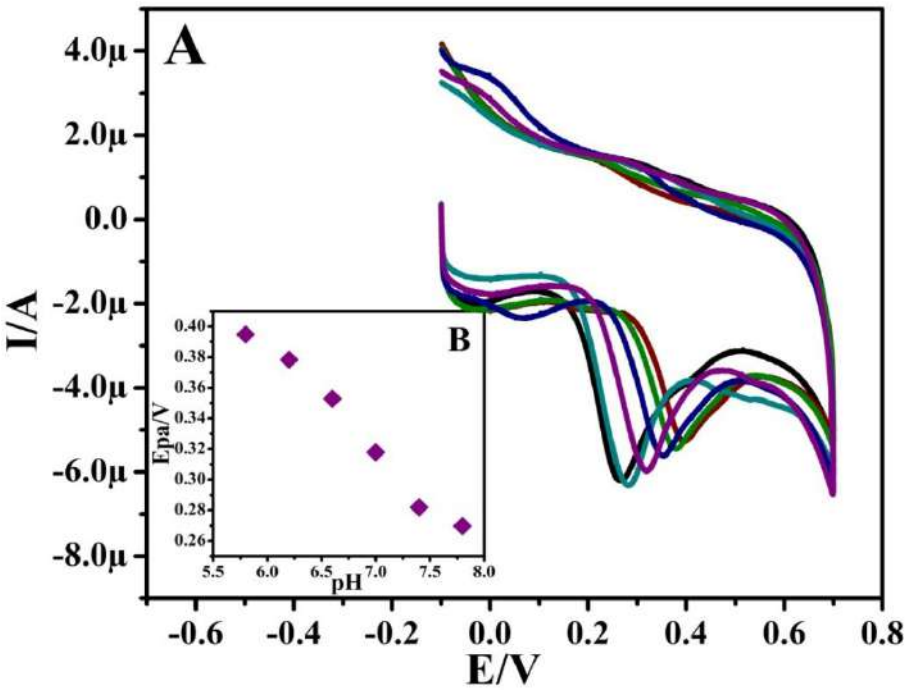


Fig. 6.4A. CVs curve of 10 µM SE at CdO/MPCPE in the presence of varied pH (5.8 to 7.8) with sweep rate 0.05 V/s. **B.** Graph of Epa of SE versus pH.

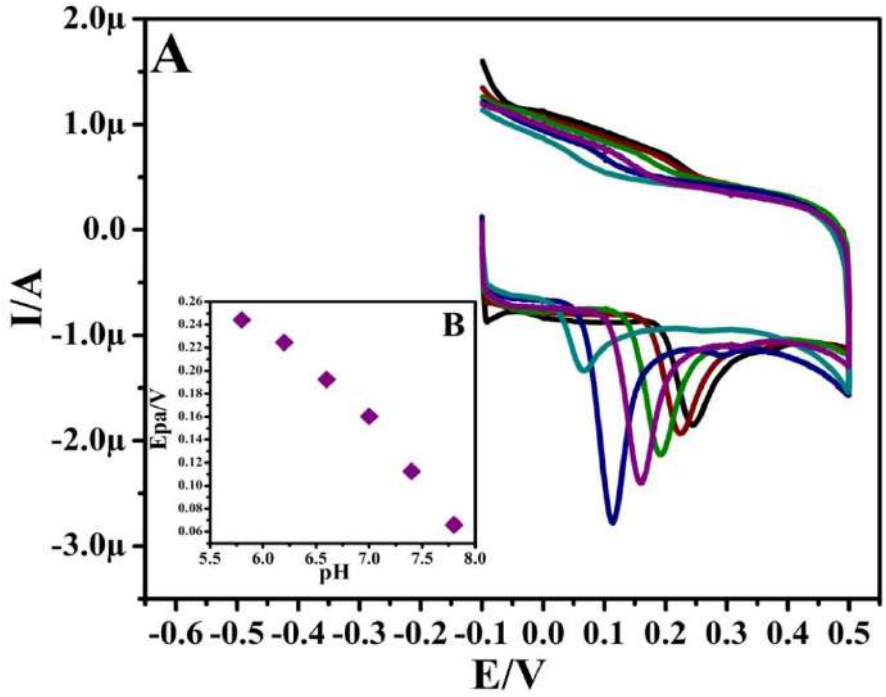


Fig. 6.5A. CVs curve of 10 µM EP at CdO/MPCPE in the presence of varied pH (5.8 to 7.8) with sweep rate 0.05 V/s. **B.** Graph of Epa of EP versus pH.

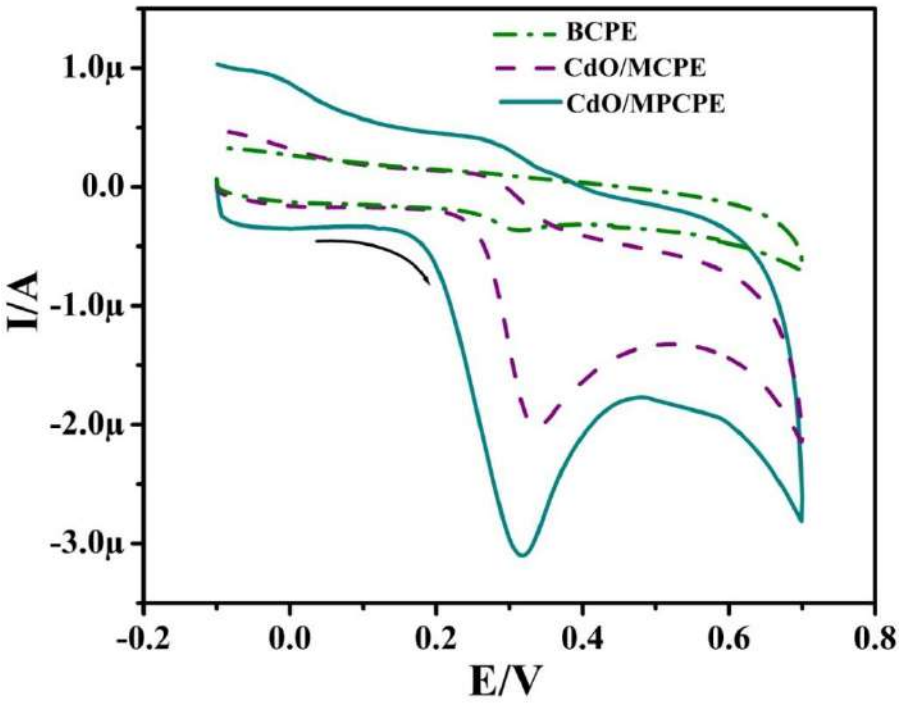


Fig. 6.6. CVs curve for 10 µM SE at BCPE (dashed dotted line), CdO/MCPE (dashed line), and CdO/MPCPE (solid line) in 0.2 M PBS of pH 7.4 with sweep rate 0.05 V/s.

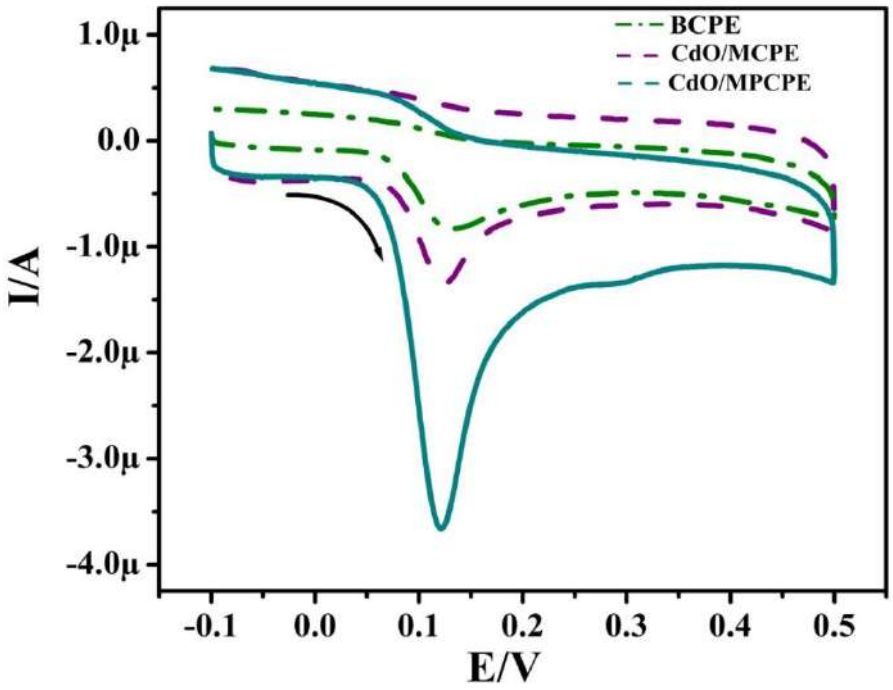


Fig. 6.7. CVs curve for 10 µM EP at BCPE (dashed dotted line), CdO-MCPE (dashed line), and CdO-MPCPE (solid line) in 0.2 M PBS of pH 7.4 with sweep rate 0.05 V/s.

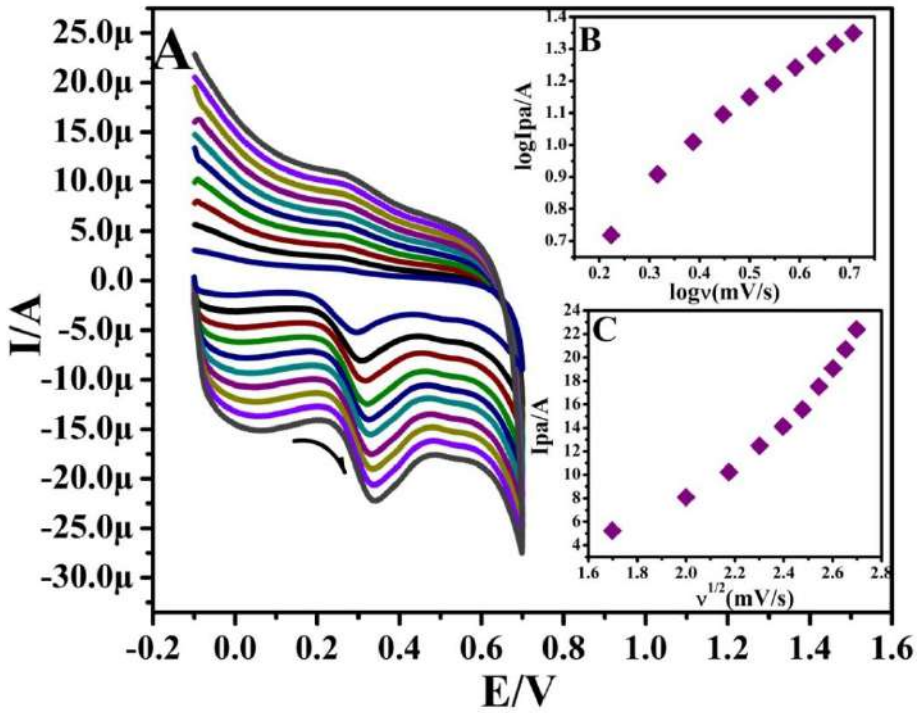


Fig. 6.8A. CVs curve for 10 µM SE at CdO/MPCPE with varied sweep rates (0.05-0.5V/s) using 0.2 M PBS of pH 7.4. **B.** Graph of log Ipa versus log of sweep rate. **C.** Graph of Ipa versus square root of sweep rate.

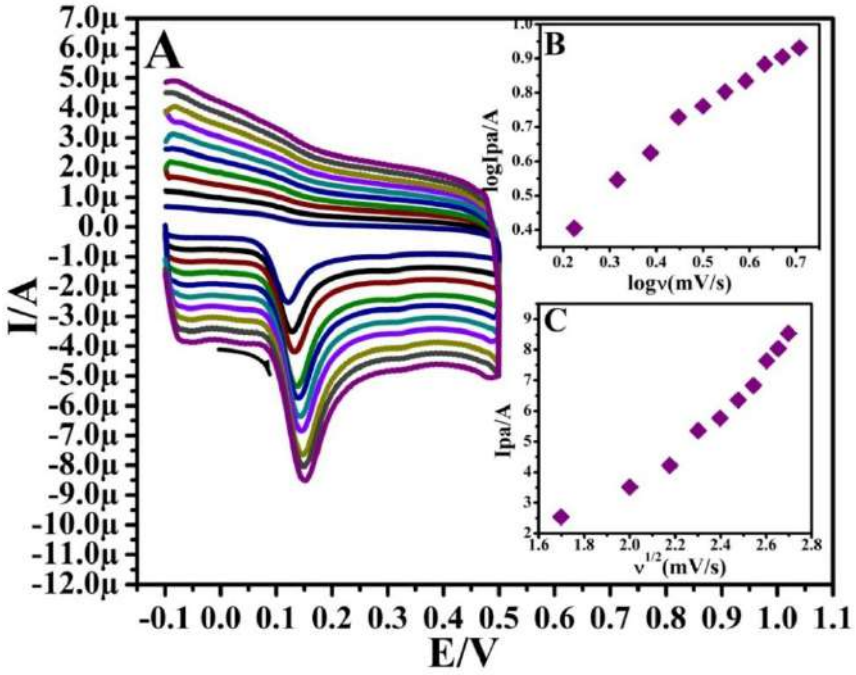


Fig. 6.9A. CVs curve for 10 µM EP at CdO/MPCPE with varied sweep rates (0.05-0.5V/s) using 0.2 M PBS of pH 7.4. **B.** Graph of log Ipa versus log of sweep rate. **C.** Graph of Ipa versus square root of sweep rate.

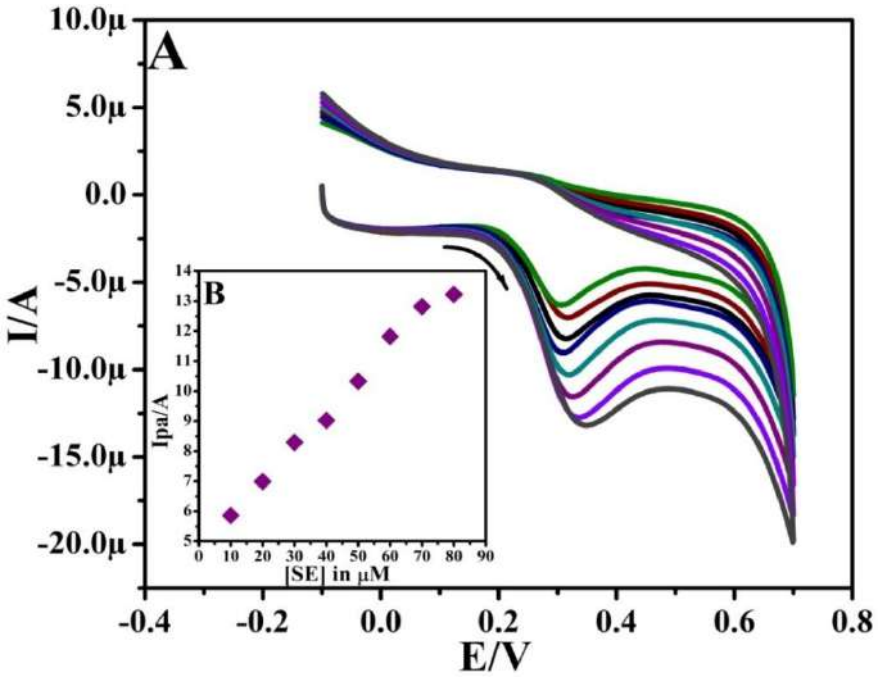


Fig. 6.10A. CVs curve for SE at CdO/MPCPE with varied concentrations (10-80µM) using 0.2 M PBS (pH 7.4) with sweep rate of 0.05 V/s. **B.** Graph of Ipa versus concentration of SE.

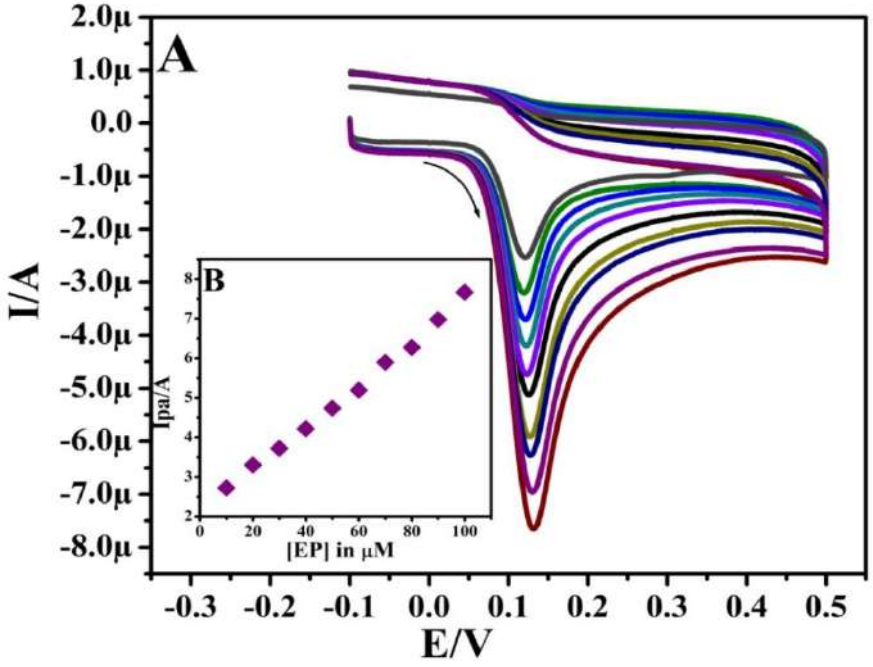


Fig. 6.11A. CVs curve for EP at CdO/MPCPE with varied concentrations (10-80µM) using 0.2 M PBS (pH 7.4) with sweep rate of 0.05 V/s. **B.** Graph of Ipa versus concentration of SE.

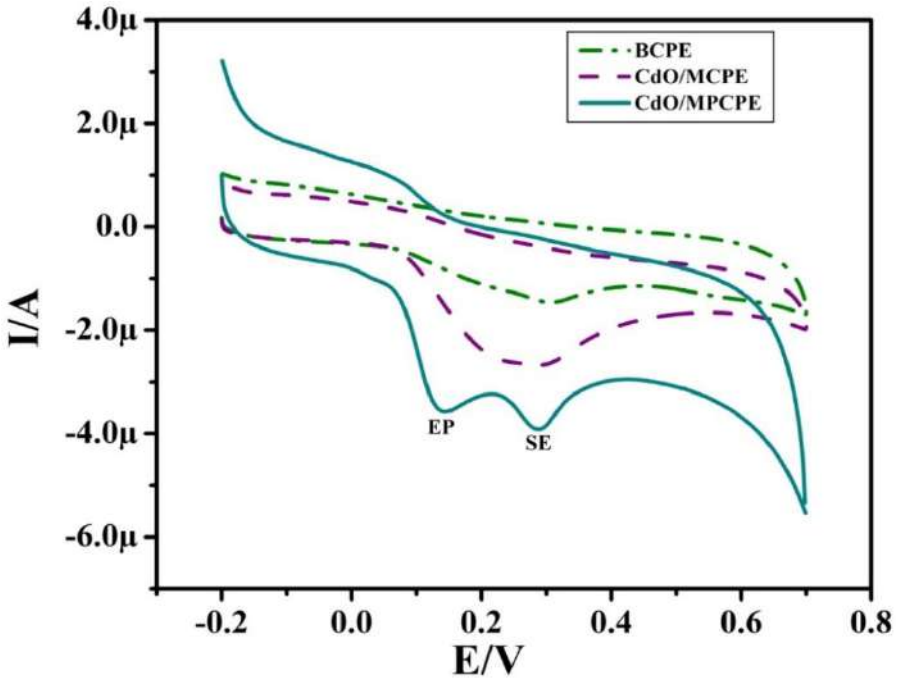


Fig. 6.12. CVs for concurrent detection of SE and EP (10 µM) at BCPE (dashed line) CdO/MCPE (dashed line), and CdO/MPCPE (solid line) in 0.2 M PBS of pH 7.4 with sweep rate 0.05 V/s.

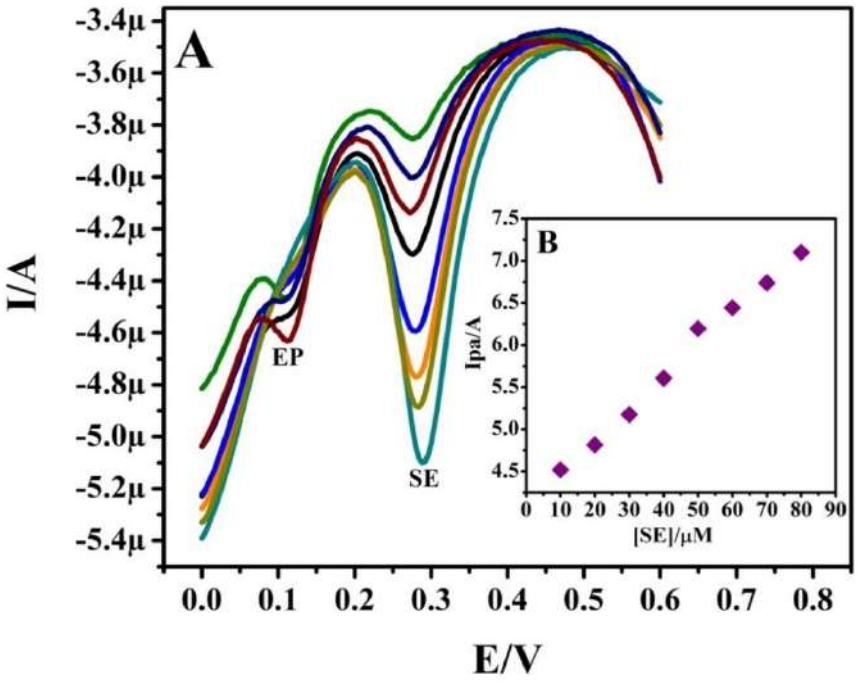


Fig. 6.13A. DPVs got for varied of concentration SE (10-80μM) with EP (10μM) at CdO/MPCPE with speed rate of 0.05 mV/s in 0.2 M PBS of pH 7.4. **B.** Display of Ipa of SE against concentration.

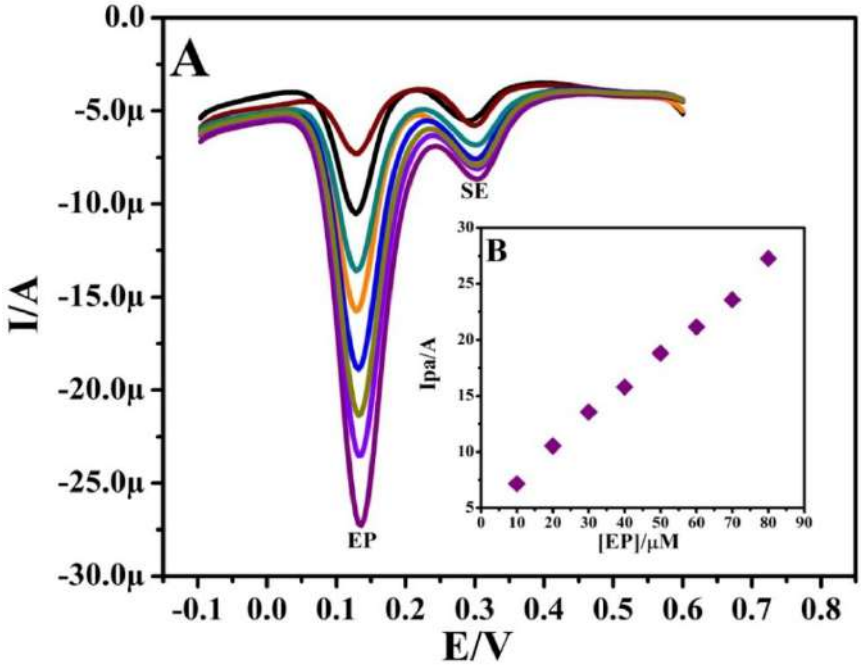


Fig. 6.14A. DPVs got for varied of concentration EP (10-80μM) with SE (10μM) at CdO/MPCPE with speed rate of 0.05mV/s in 0.2 M PBS of pH 7.4. **B.** Display of Ipa of EP against concentration.

Table.1. Comparison of analytical performance of different modified electrodes for SE detection.

SI No	Electrode	Detection limit(μM)	Method	Reference
01	AuNPs@PPy/GSPE	33.2	SWV	[30]
02	CDP-Choline/MCPE	5.81	CV	[31]
03	3D-ITO	7.5	DPV	[32]
04	IL-DC-CNT/GE	2.0	DPV	[33]
05	P-VBB/MCPE	0.89	CV	[26]
06	CdO/MPCPE	0.88	CV	**Present study**

Table.2. Comparison of analytical performance of different modified electrodes for EP detection.

SI No	Electrode	Detection limit(μM)	Method	Reference
01	Au 4MpyAuNPs	4.5	CV	[34]
02	Gold film electrode	19	CV	[35]
03	TiO_2/MCPE	4.2	CV	[36]
04	TiO_2/RGO-MCPE	2.0	DPV	[37]
05	CAP/MWCNT/GCE	7.2	CV	[38]
06	MoS_2-MW/CNTs	3.0	CV	[18]
07	Cu-ZnO/TX-100/MCPE	3.9	CV	[39]
08	CdO/MPCPE	1.82	CV	**Present study**

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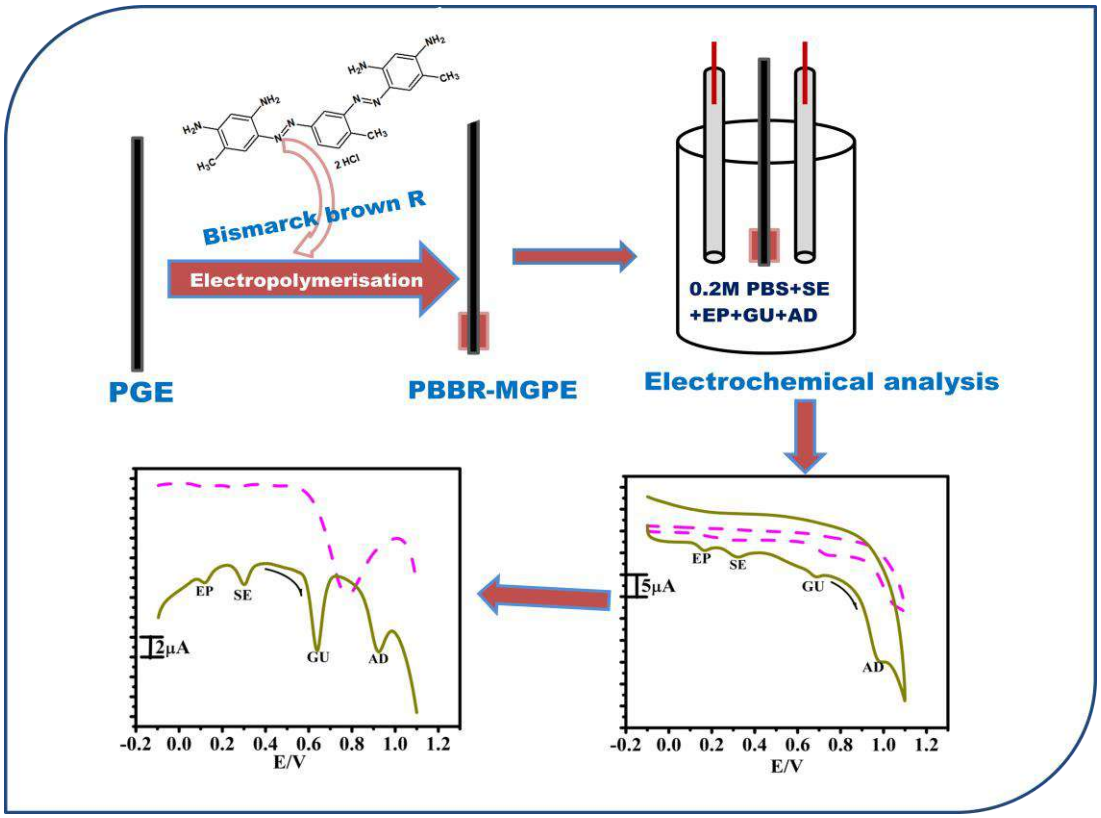
Chapter-VII

VOLTAMMETRIC ANALYSIS OF SEROTONIN AND EPINEPHRINE IN THE PRESENCE OF GUANINE AND ADENINE AT BISMARCK BROWN R AMPLIFIED GRAPHITE PENCIL ELECTRODE.



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7.1. Introduction

The adoptability of graphite pencil leads as an electrode materials are largely increased in recent eras in the fields of electroanalytical methods for the quantification of various types of bio significant matrices. When compared to other carbon based electrodes, GPEs posses a wide range of profitable characteristics such as lower background currents, better sensibility and reproducibility [1-5]. GPEs are more convenient to use, less expensive and having an adjustable surface area and granting the evaluation of samples in lower concentrations without any preconcentration stages and also GPEs are renewable and easily disposable [6-8]. SE and EP are biogenetic catecholamine presumably serves as neuromediaters and chemical channels that relays the indications between the nerve cells. The mood stabilizing agent, SE is an eminent inhibitory neurochemical broadly distributed in the biosystems [9-11]. The usual concentration level of SE in human body is 0.25-0.74μM [12]. SE plays prominent role in controlling the multifarious behavioral and emotional status together with other neurochemicals such as sleep, emesis and appetite. Any variations in the serotonin level leads to multiple psychiatric illnesses such as deficit SE levels causes depression, suicidal tendencies and increased level associated with serotoninergic malfunctions [13-16].

EP is an organic cation assists both as neorutransmitter as well as harmone. EP specifically secreted in the adrenal medulla and the body releases it during acute stress. Its stimulatory effects energies and instruct the body for 'fight or flight' response and raises the breathing and heart rate also helps the brain to take quick actions in the face of danger. The deviated levels of EP inflames the variety of life threatening illness such as myocardial infarction, hypoglycemia and pheochromocytoma [17-22].

AD and GU are purine bases imperatively acts as constituents of the nucleic acids [23]. They play influential roles in storing genetic data and participated in the numerous mechanisms like supplying energies, coenzyme formation and metabolic managements. Also, they are vital in cerebral and coronary transmissions, managing the blood flow, neurotransmitter secretion and regulating the functions of adenylate cyclase [24-26]. Any anomalous variations in the AD and GU ascribed to deficiencies

in the immune mechanisms in the human systems and some disorders like cancers, epilepsy, renal calculi and AIDS [27, 28]. SE, EP, AD and GU are bio essential molecules co-occurred in living organisms. The precise and rapid measuring of these molecules are requisite in clinical interpretations as their quantities acts as crucial markers for the diagnosis of various diseases [29, 30]. Several technical methods are employed for analysis of these molecules including calorimetry [31], HPLC [32], electrophoresis[33], photokinetics and isotope dilution mass spectrometry[34]. The described techniques are absolutely accredited for the analysis of biological and environmental substances. But they exhibits inadequate selectivity, high cost instrumentation and longer time of analysis. Among these, the electrochemical techniques have more qualities compared with other traditional methods such as higher sensibility, rapid response, real-time application, inexpensive equipments and potential miniaturization, green nature, short duration for sample preparation and detection and easily modifiable electrode surfaces [35-39]. In reported study, we customized a pencil graphite electrode with a Bismarck brown R by electropolymerisation to examine its electrocatalytic action towards oxidation of SE, EP, AD and GU specifically and simultaneously.

7.2. Experimental

7.2.1. Materials and Measurements

Cyclic and differential pulse voltammetric measurements were functioned by employing electrochemical analyzer of a model CHI-660c potentiostat. A traditional three electrode cell assembly incorporated with platinum wire, saturated calomel electrode (SCE), bare pencil graphite and poly(BBR)modified graphite pencil electrode (PBBR-MGPE) as counter, reference and working electrodes respectively. The morphology of modified electrode surface was categorised by SEM determinations and conducted by applying ZEISS Ultra-55. All the estimations were performed at room temperature and all oxidation potentials were recorded versus SCE.

All analytical grade chemicals were utilized withought any treatment. The graphite pencil leads (0.7mm) were purchased from local book stores. SE, AD, GU, KCl, $K_4[Fe(CN)_6]$ and NaOH were procured from Himedia laboratories, Bangalore, India. L-epinephrine was obtained from sigma Aldrich, Bangalore, India. The stock

solutions of EP (25×10^{-4} M) was made by dissolution in 0.1M perchloric acid solution and the phosphate buffer solution(PBS) was prepared by using equimolar(0.2M) mixture of disodium hydrogen orthophosphate and dihydrogen sodium phosphate solutions and all the aqueous stock solutions were made by employing doubly demineralised water.

7.3. Result and discussion

7.3.1. Fabrication and optimization of working electrode

The graphite pencil lead was exploited as bare working electrode and the PBBR films were electrochemically polymerized on BGPE by placing 1mM aqueous BBR monomer by CV procedure in assistance with 0.1 M NaOH as supportive medium at the speed rate of 100mv/s. Fig.7.1A reflects the electropolymerisation of BBR on BGPE surface for 20 uninterrupted cycles. The effective polymeric layer was achieved by repeatedly cycled the potential between +0.0V to +1.8V. The film deposited electrode was purified with demineralised water to wipe out the physically adsorbed unreacted materials from the surface. The thickness of the coated p-BBR membrane has huge impact on the electrocatalytic properties of modified sensor. However, the thickness of the layer was restricted by altering the sweep cycles and the corresponding electrochemical performance of SE and EP were tested (Fig.7.1B). The electrocatalytic functions of SE and EP enhanced with increase in the polymerization sweeps varied from 5 to 25 cycles. In that, the striking catalytic properties for SE and EP were noted at 20 multiple sweeps. Therefore, the 20 sweep segments were considered for further modification.

7.3.2. Surface characterization and morphology of BGPE and PBBR-MGPE

The SEM images of BGPE and PBBR-MGPE were picturised to examine their surface morphologies as illustrated in Fig.7.2A and Fig.7.2B respectively. After modification with BBR, It was found that the electrode surface was fully deposited with uniformly distributed polymeric layer of BBR molecules and posses a relatively higher active sites toward SE and EP electrolysis. The newly prepared $K_4[Fe(CN)_6]$ was practiced as electrochemical probe to explore the electrocatalytic activities of the

designed sensor by the application of CV technique. Fig.7.3 demonstrates the descriptive CVs of 1mM $K_4[Fe(CN)_6]$ at BGPE(dashed curve) and PBBR-MGPE(solid line) in 1M KCl supporting electrolyte at scan rate of 50mV/s. The reversible redox signal with reduced capacitive current sensitivity was observed at BGPE due to sluggish electron transport with extensive peak potential separations. Reciprocally, the trivial and eminent improvement in the peak signal was noted at PBBR-MGPE with decrement in potential separation could be authorized to the change in morphologies and increased electrical conductivity as well as the reaction sites due to electropolymerisation. The total reaction surface area of the formulated sensor was computed through the Randles-Sevick's expression (1)[34]. The surface area of higher value was found for PBBR-MGPE (0.055cm^2) as compared to the BGPE (0.019 cm^2) signifies that the finer electro-catalytic properties of the sensor. By Equation (2) [40], the approximate surface coverage concentration of Bismarck brown R film was quantified to be

$$Ip = (2.69\times10^5)\, n^{3/2}\, A\, D_0^{1/2}\, C_0\, \nu^{1/2} \quad \text{..... (7.1)}$$

$$Ip = n^2F^2A\Gamma\upsilon/4RT \quad \text{……..(7.2)}$$

Where, Γ (M/cm^2) is the surface average concentration, n is transported electrons, A is area (cm^2) of tailored electrode, C_0 is the concentration (mol/cm^3) of the electro active substance, ν is sweep rate and D_0 is diffusion coefficient (cm^2s^{-1})

7.3.3 Importance of supporting electrolyte pH on oxidation of SE and EP at PBBR-MGPE

The distinct pH value of the buffer solutions simulates the electrocatalytic reactions and the shape of the peaks. In order to get better sensibility, the optimisation of pH of supporting medium was highly recommended. The interdependency of SE and EP oxidation on pH was inspected through CV method. The diversified pH values of the 0.2 M phosphate buffer as supporting solution in presence of 10μM SE and 10μM EP was examined with the speed rate of 50mV/s at PBBR-MGPE. With increasing the pH of the solution the anodic peak potentials of SE and EP switched to negative side from 5.8 to 7.8 as displayed in Fig.7.4A and Fig.7.5A respectively. This observed result accredits the direct proton involvement in the oxidation of SE and EP

at PBBR-MGPE. The potential diagram was framed between Epa of SE and Epa of EP against differed pH values as showed in Fig. 7.4A and Fig.7.5B respectively. The established linear relationship was expressed by the regression equation, Epa(V)= 0.0548-8.692(pH), (r^2 = 0.9898) for SE and Epa(V)= 0.075-8.696(pH), (r^2 = 0.9947) for EP. The obtained slope value suggested that the overall electrode reaction was comprised by same number of electrons and protons [41]. Although, the maximal peak currents were noticed at pH 7.4. Hence, pH 7.4 has been selected as adequate for the study of other parameters.

7.3.4 Electrochemical oxidation of SE and EP at BGPE and PBBR-MGPE

The comparative voltammetric response of SE and EP were tested to prove the proficiency of the modified sensor. In Fig.7.6 and Fig.7.7 the scattered line and solid line depicts the CVs interpreted for 10μM SE and 10μM EP at BGPE and PBBR-MGPE in the presence of 0.2M PBS of pH 7.4 at the sweep rate of 50mV/s. Both SE and EP are readily oxidisable electroactive molecules shows irreversible peak nature with broad and very weak peak current of SE at unmodified electrode. At BGPE, no peak was noticed for EP. Whereas, the drastic enhancement in the oxidation peak current densities and sharp peak signals were sighted at PBBR-MGPE and the peak potentials minutely shifted to negative direction from 0.325 V to 0.288V for SE and the oxidation peak potential for EP was appeared at 0.103V. This outcome suggested that the more facile electron transfer, increased electrical conductivity and greater surface area of developed PBBR-MGPE.

7.3.5 Influence of scan rate on SE and EP at PBBR-MGPE

The scan rate variation greatly influenced the electrode reaction. The peak current and peak potentials of the electroactive species are catalytically depends on the scan rate. Fig.7.8A displays the CVs reported for 10μM SE at PBBR-MGPE in assistance with 0.2 M PBS of pH 7.4 for unlike sweep rates. As illustrated in figure, the anodic peaks have continuously elevated and the peak potentials were moved to negative directions over the studied range from 50mV/s to 500mV/s. The linearity was ascertained by plotting the graph of log of scan rate (logν) against the log of anodic peak current of SE (log Ipa) as shown in Fig.7.8B which yielded the slope value of

0.618(r^2= 0.9679). This finding confirms the electrode kinetics was maintained by diffusion process [42]. Fig.7.8C depicts the plot of square root of scan rate ($\nu^{1/2}$) versus the oxidation peak current (Ipa) of SE. the established plot provide good linearity which further authenticates the electrode reaction. In the same manner, the CVs were accounted for 10μM EP at differed sweep rates in 0.2M PBS of pH7.4 (Fig.7.9A). The peak currents enhanced with increase in scan rate (50mV/s to 500mV/s). Fig.7.9B represents the plots of log Ipa of EP versus log ν gives excellent linearity with slope value of 0.79 (r^2= 0.9978) for adsorption controlled electrode phenomenon. Fig.7.9C provides the plot of Ipa of EP against $\nu^{1/2}$ also shows linearity with regression factor of r^2= 0.9981.

7.3.6 Consequences of SE and EP concentration and detection limit

PBBR-MGPE was used to estimate the linear range and detection limit of SE and EP under adequate experimental conditions. Fig.7.10A and Fig7.11B portraits the recorded CVs of SE and EP in presence of 0.2 M PBS of pH 7.4 at PBBR-MGPE at their dissimilar concentrations with the sweep rate of 50mV/s. The gotten CVs proved that the oxidation peak currents of SE and EP were upgraded with hiking the concentration of respective analytes in the range of 10-80μM with the slight switching of peak potentials (0.289V to 0.276V for SE and 0.100V to 0.115V for EP) to negative sides. The graphs were plotted between the oxidation peak currents of SE and EP with altered concentrations as displayed in Fig.7.10B and Fig.7.11B respectively. The plot gives excellent linearity with the corresponding regression expressions, Ipa (μA) = 0.1908 (μM) + 2.27 (r^2 = 0.9969) for SE and Ipa (μA) = 0.2182 (μM) + 3.64 (r^2 = 0.9963) for EP. By utilizing the slope (M) and standard deviation(S) acquired from the plots LOD and LOQ [43] were valuated by equation (3) and (4).

$$LOD = 3S/M \quad \text{..... (7.3)}$$

$$LOQ = 10S/M \quad \text{..... (7.4)}$$

The LOD values were found to be 0.31μM & 0.27μM and LOQ values for SE and EP were obtained to be 1.04μM & 0.91 μM respectively. The PBBR-MGPE ascribes the lower LOD values for SE and EP as compared to many other reported methods as provided in Table.7.1 and Table. 7.2.

7.3.7 Sensibility of PBBR-MGPE towards simultaneous resolution of SE and EP in presence of AD and GU

The predominant parameter to validate the sensitiveness and selectiveness of the designed sensor is its ability to resolve the oxidation peaks of individual analytes in the sample mixture. The catalytic specificity of the modified sensor was inspected by employing CV and DPV in a quaternary mixture consisted equimolar samples of SE, EP, AD and GU. Fig.7.12A exhibits the CVs verified for the mixture of SE, EP, AD and GU (10 μM) in existence with 0.2 MPBS of pH 7.4 with the speed rate of 50mV/s at BGPE (scattered curve) and PBBR-MGPE (hard curve). The Unmodified sensor was incapable to individualise the peak potentials of quaternary mixture. However, the fabricated PBBR-MGPE sorted out the four clearly differentiated peak signals and specific oxidation peak potentials were positioned at 0.29V, 0.105V, 0671V and 0.963V for SE, EP, AD and GU respectively. Similarly the inset Fig. 7.12B describes the DPVs obtained for concurrent analysis of mixture containing four analyte samples in bearing with 0.2 M PBS of pH 7.4. By figure, it was noticed that the bare electrode insufficient to distinguish the peak potentials. For instance, the customised PBBR-MGPE was able to gives the separate oxidation peaks with superior increment in their peak currents. Therefore it can be concluded that the developed sensor shows excellent potentiality for the concurrent revealing of SE and EP in assistance with AD and GU.

7.3.8 Selective study

The efficacy of the developed electrode for isolating the voltammetric response of individual analyte in their uniform mixture was investigated by using higher sensitive DPV system. Fig.7.13A presents the DPV curves accounted for the admixture of SE, EP, AD and GU in that the concentration of EP was modified in the linear range over 10-80μM and the concentration of three other analytes were kept same(10μM) at PBBR-MGPE in 0.2M PBS of pH 7.4. The inset Fig.7.13B depicts the voltammogram obtained by altering the SE concentration in presence of stable concentration of EP(10μM), AD(10μM) and GU (10μM). Likewise, in the Fig.7.14A the GU concentration was varied and other species concentration was retained (SE, EP and AD). The same process was followed by changing the concentration of AD and

holding the steady concentration of SE, EP and GU (Fig.7.14B). By noticing the above reported figures, the anodic peak current of analyte is directly proportional to their concentration in the range of 10-80μM and there was no change in peak potential and current of constant analytes. This result signifies that estimable selectivity and the anti-interference ability of the tailored sensor.

7.3.9 Success of stability of PBBR-MGPE

The constancy of the fabricated PBBR-MGPE was traced by using the combined solution of constant concentration of SE, EP, AD and GU in 0.2M PBS of biological pH with the scan rate of 50mV/s. The Fig.7.15 represents the CVs recorded for 50 continuous scans. The minute decrement in peak current and the fixed peak potentials was observed and the degradation percentage was quantified by the formulae, % degradation = Ip_n/Ip_1. Where, $Ip_1$and Ip_n determines the first and n^{th} anodic peak currents respectively. The steadiness retained by PBBR-MGPE was found to be 92.5%, 88.8%, 89.4% and 95.4% for SE, EP, AD and GU this outcome assured that the sensor was highly stable.

7.4. Conclusion

A new and simplistic graphite pencil electrode sensor was customised by electropolymerisation with Bismarck brown R. The sensor offers appealing characteristics like high stability, effortless electrode fabrication, less expensive and relatively higher reaction surface area and better selectivity for the direct investigation of SE and EP in the presence of AD and GU at biological pH. The kinetic analysis was conducted to complement the nature of electrode process and it was found to be both adsorption and diffusion controlled. The modulated sensor successfully reduces the detection and quantification limits and efficiently resolved the overlapped peaks into four well isolated anodic peaks for SE, EP, AD and GU. The developed sensor prevents the fouling of the electrode surface and shows good reproducibility. All this outcomes suggested that the proposed method was precise and effectually employed as biosensor in the field of neurochemistry and for the detection of bioactive molecules.

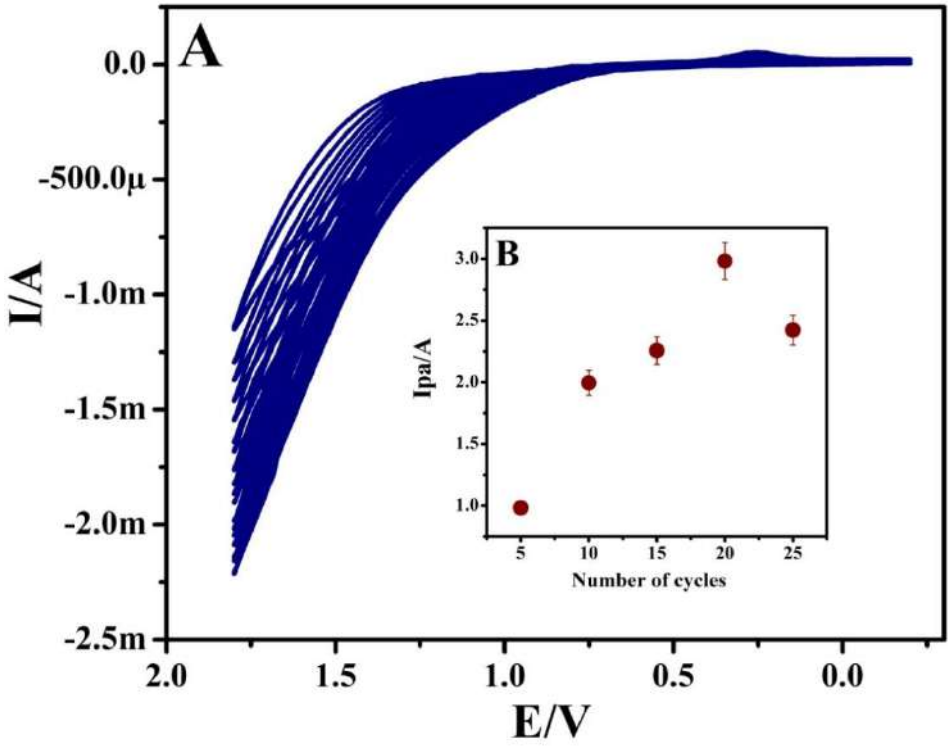

Fig. 7.1A. CVs of formation of PBBR(1mM BBR) film deposited BGPE in presence of 0.1M NaOH at sweep rate of 100 mVs^{-1}. **B.** Plot of Ipa of SE versus number of polymerization sweeps.

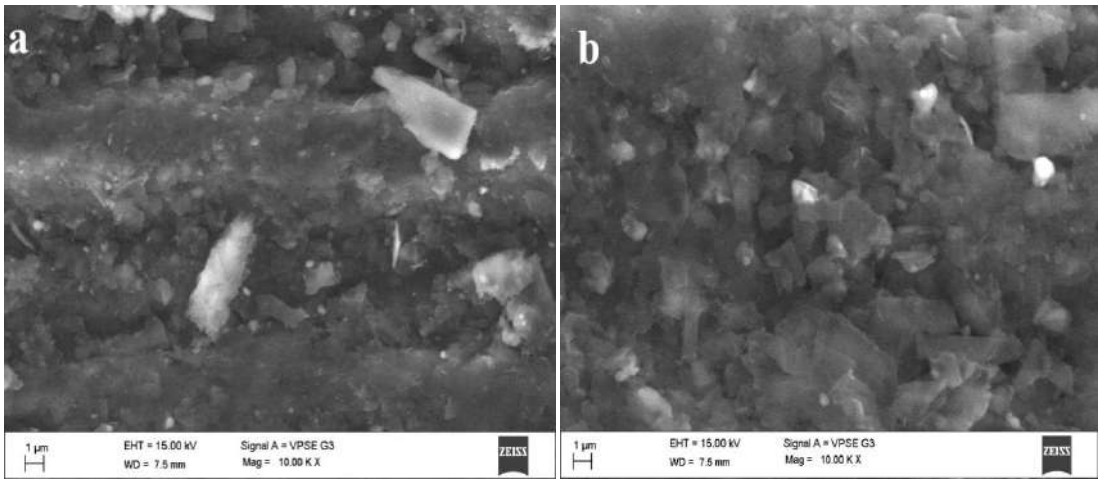

Fig. 7.2A and 2B. The SEM images captured for BGPE and PBBR-MGPE

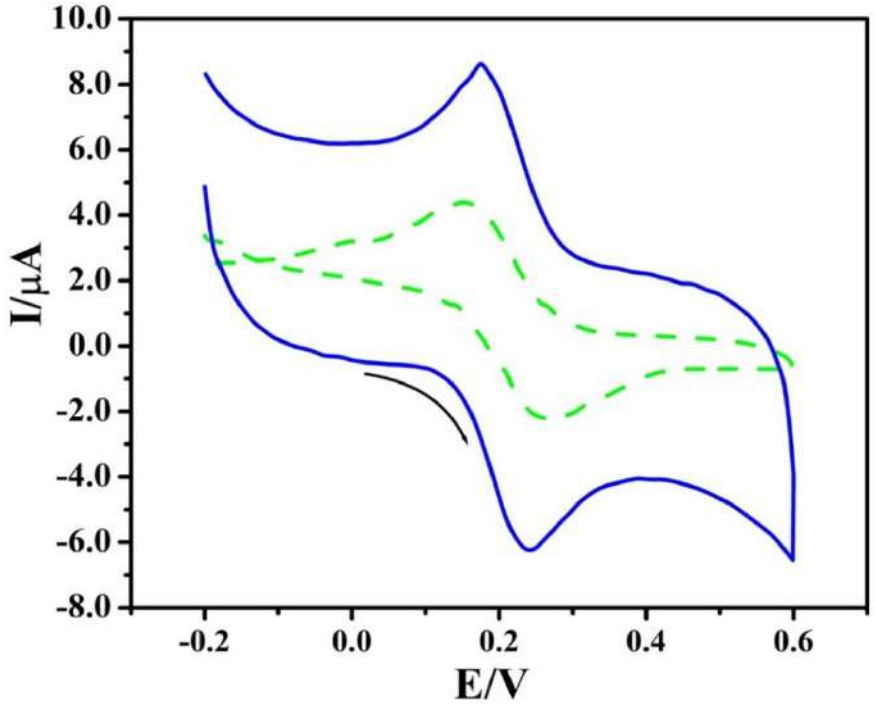

Fig. 7.3. CVs recorded for 1mM $K_4[Fe(CN)_6]$ in 1M KCl at BGPE(scatterd line) and PBBR-MGPE (solid line) with the scan rate of 50 mVs^{-1}.

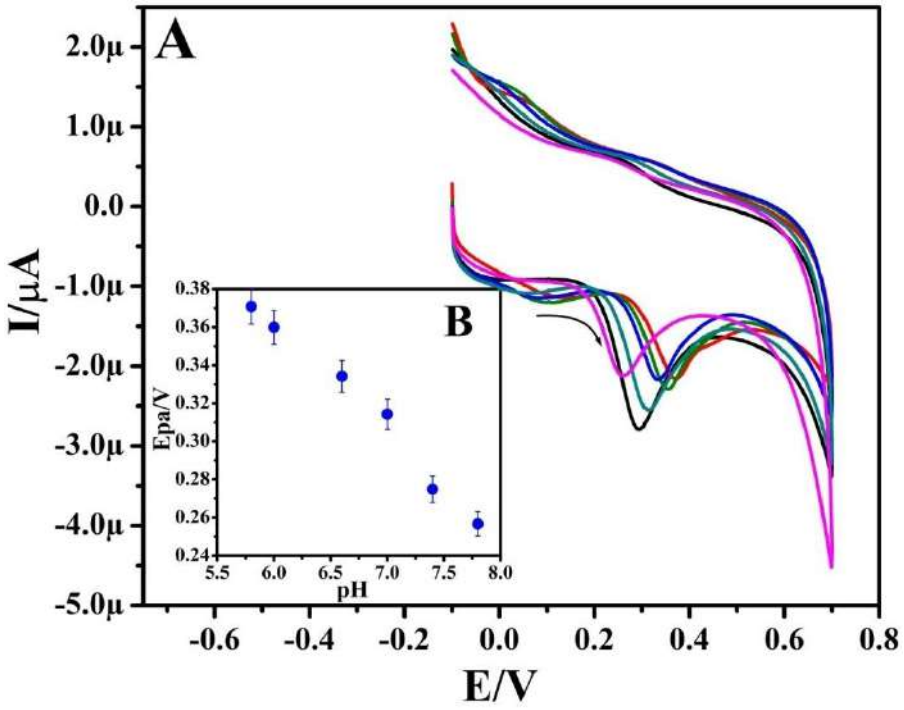

Fig. 7.4A. CVs for influence of buffer pH on the oxidation peak for 10μM SE at distinct pH (5.8-7.8) at PBBR-MGPE. **B.** plot of Epa of SE versus varied pH.

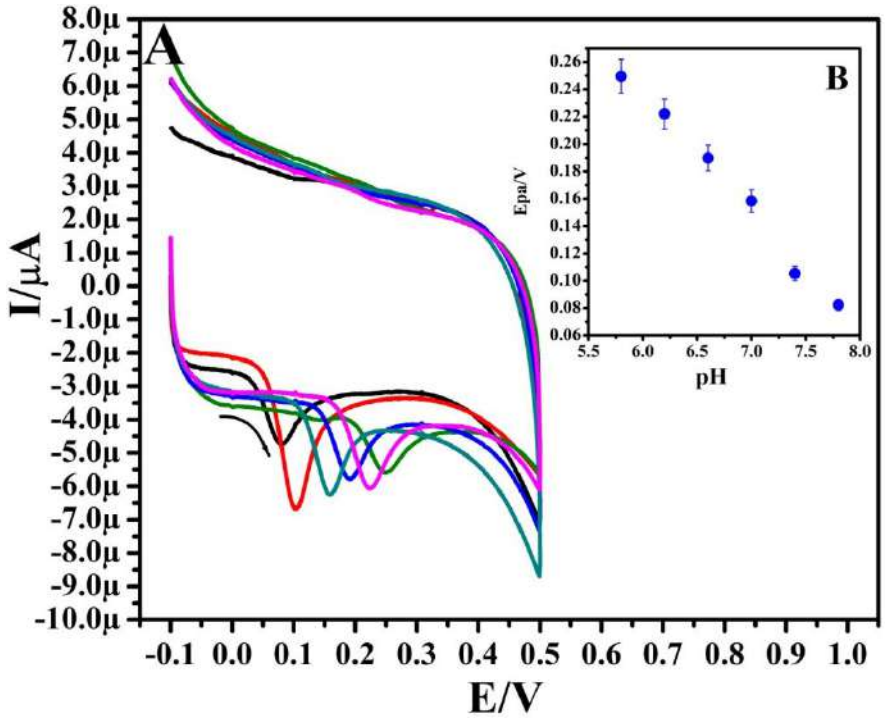

Fig. 7.5A. CVs for influence of buffer pH on the oxidation peak for 10μM EP at distinct pH (5.8-7.8) at PBBR-MGPE. **B.** Plot of Epa of EP versus varied pH.

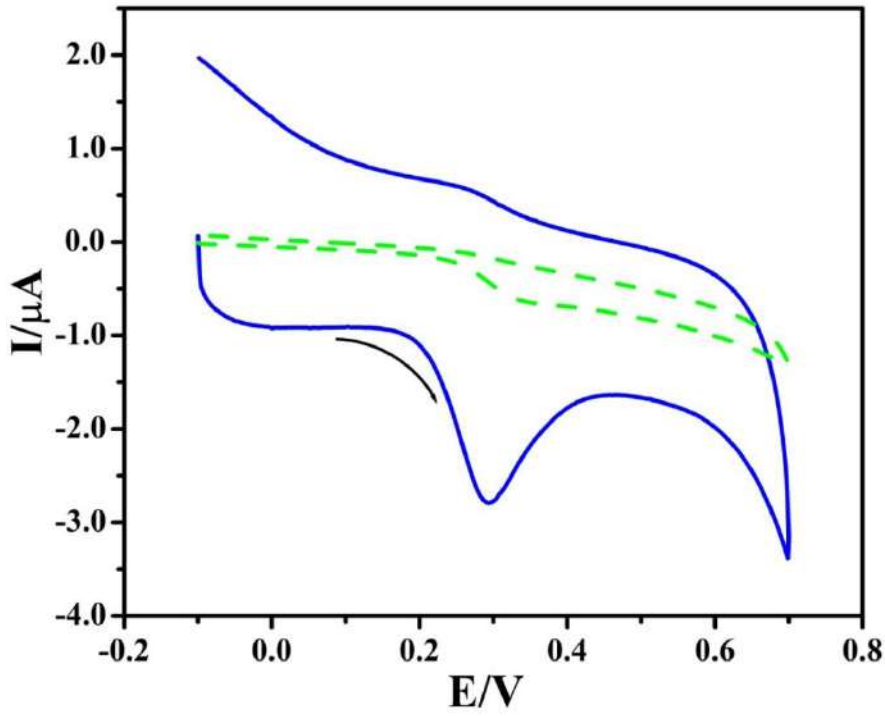

Fig. 7.6. CVs of 10 μM SE in 0.2M PBS of pH 7.4 at BGPE(scattered line) and PBBR-MGPE (solid line) using 50 mVs^{-1}.

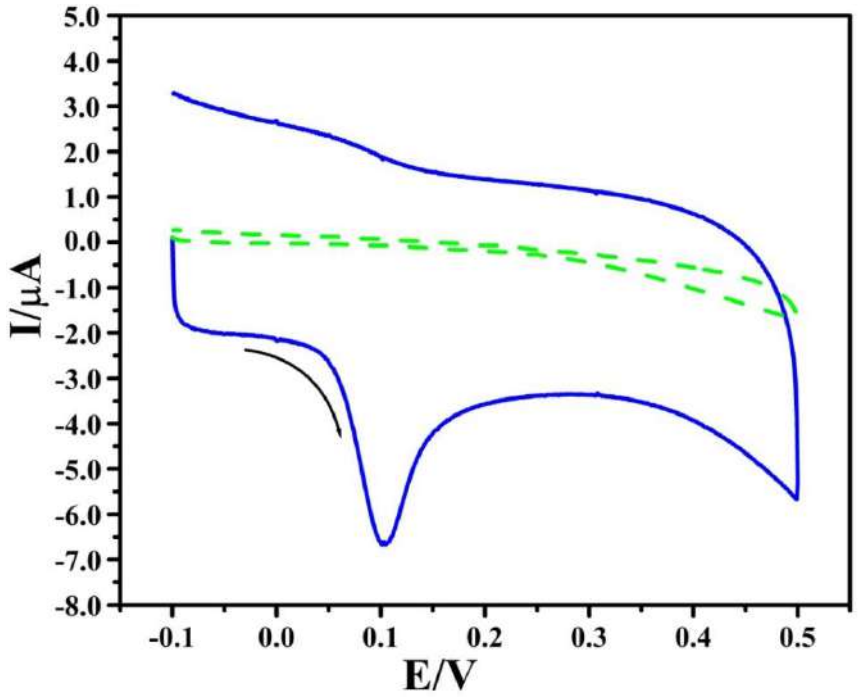

Fig. 7.7. CVs 10 μM EP in 0.2M PBS of pH 7.4 at BGPE(scattered curve) and PBBR-MGPE (solid curve) using 50 mVs^{-1}.

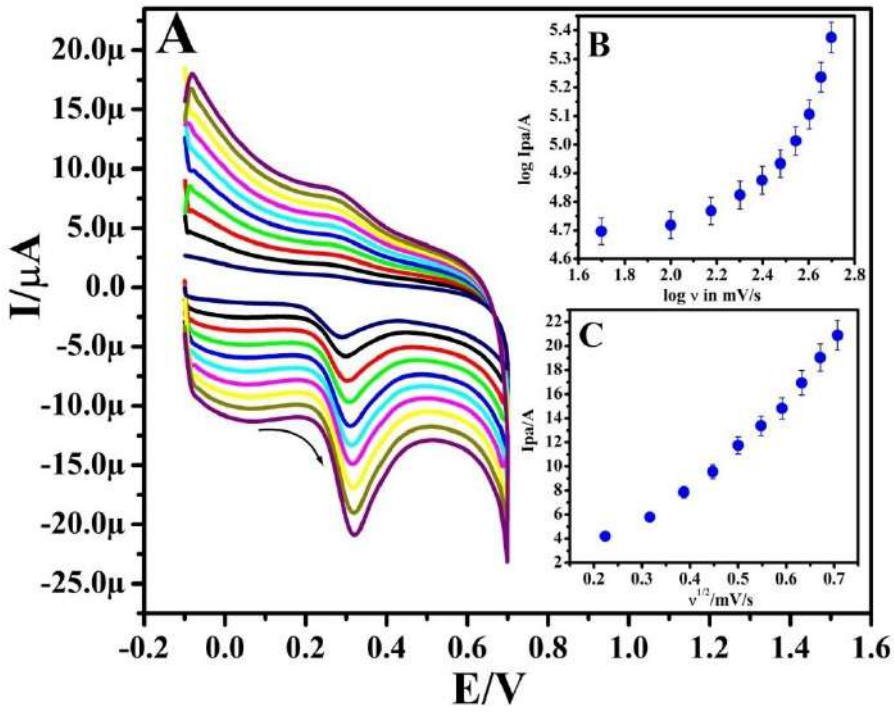

Fig. 7.8A. CVs of PBBR-MGPE in 0.2M PBS of pH7.4 in 10 μM SE at differed sweep rates (50-500 mVs^{-1}). **B.** Plot of log Ipa of SE with log ν. **C.** Graph of Ipa of SE versus square root of scan rate**(**$\nu^{1/2}$).

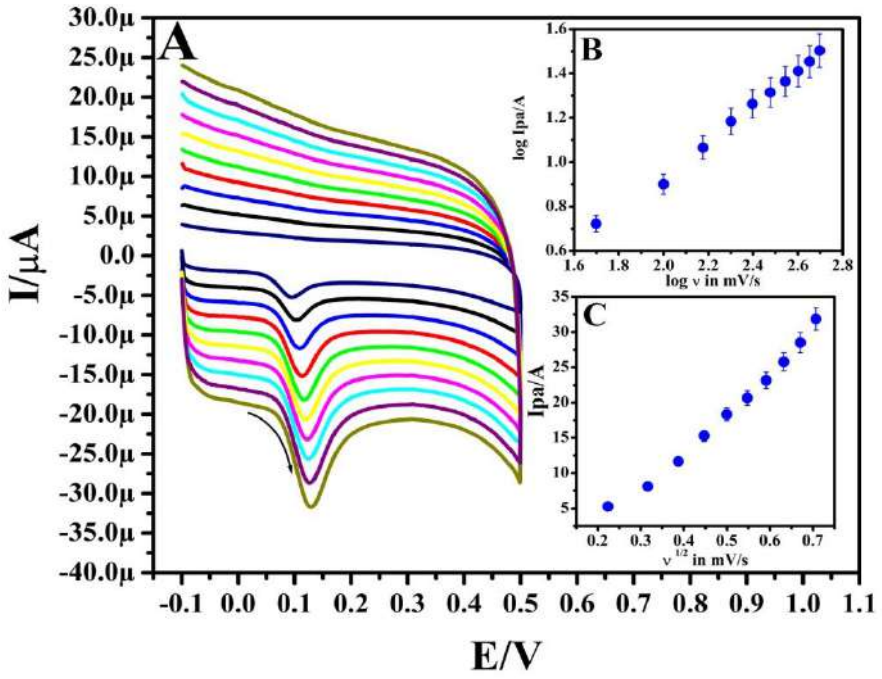

Fig. 7.9A. CVs of 10 μ M EP at PBBR-MGPE in 0.2M PBS of pH7.4 at differed sweep rates (50-500 mVs^{-1}). **B.** Plot of log Ipa of EP with log ν. **C.** Graph of Ipa of EP versus square root of scan rate **(**$\nu^{1/2}$).

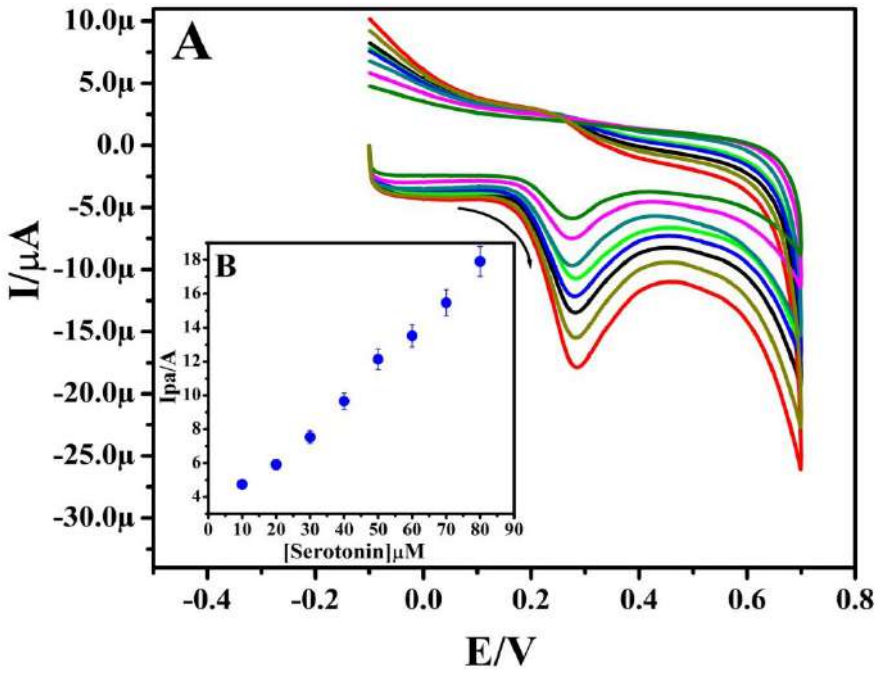

Fig. 7.10A. CVs of SE in 0.2 M PBS of pH 7.4 at sweep rate of 50mVs^{-1} for dissimilar concentration form 10μM - 80μM at PBBR-MGPE. **B.** plot of Ipa versus concentration of SE at PBBR-MGPE.

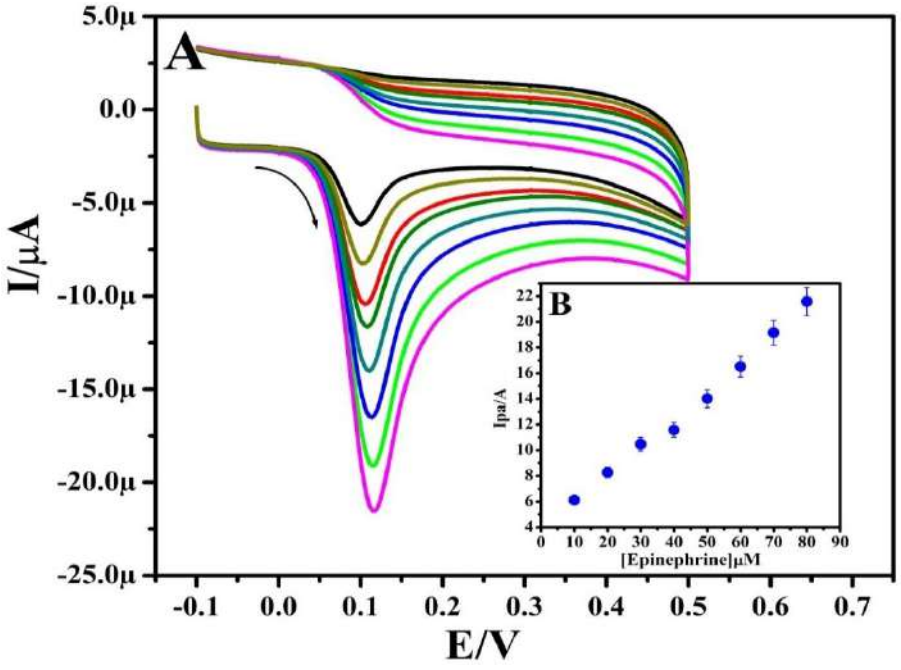

Fig. 7.11A. CVs of EP in 0.2M PBS of pH 7.4 at sweep rate of 50mVs^{-1} for dissimilar concentration form 10 μM - 80 μM. **B.** Plot of Ipa versus concentration of Ep at PBBR-MGPE.

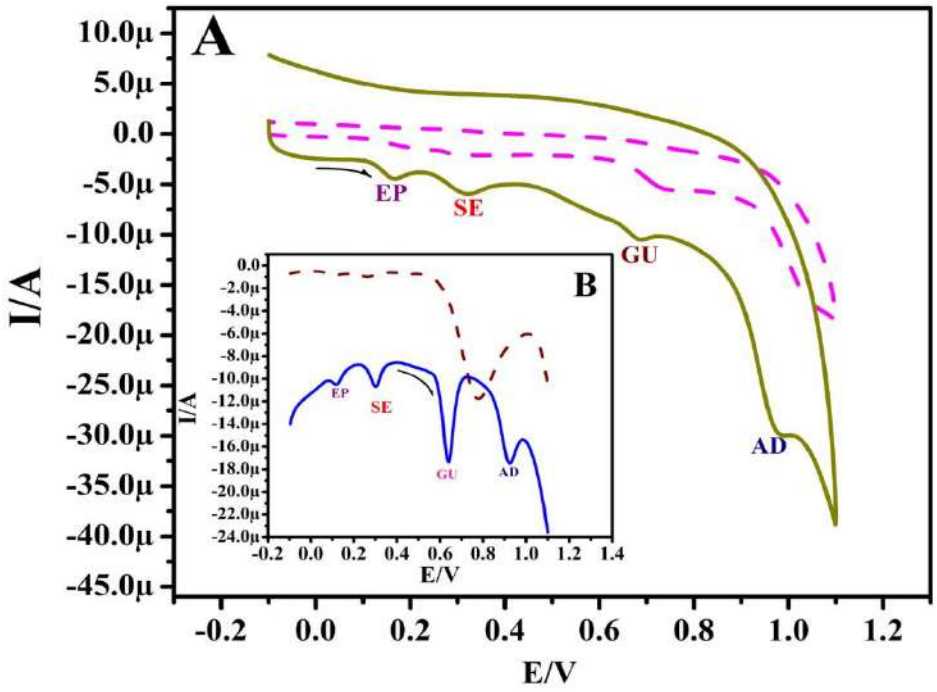

Fig. 7.12A. and B. CVs and DPVs recorded for concurrent resolution of 10 μM SE and 10 μM EP in presence of 10 μM AD and 10 μM GU with the scan rate of 50mVs^{-1} using 0.2 M PBS of ph 7.4 at BGPE (dashed line) and PBBR-MGPE (solid line).

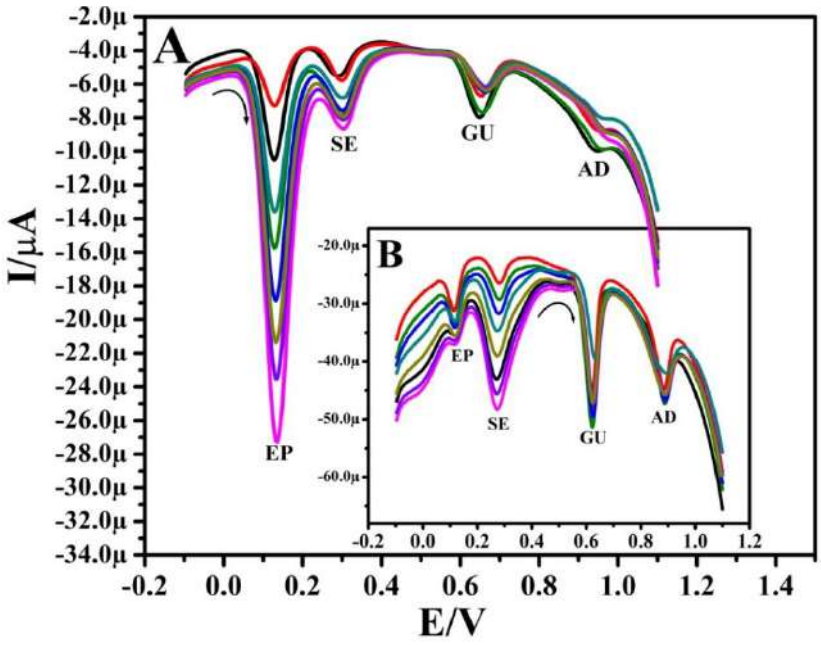

Fig. 7.13A. DPVs for variation of EP concentration from 10 μM - 80μM in PBS of pH 7.4 with 10 μM SE, 10 μM AD and 10 μM GU at PBBR-MGPE and 50mVs^{-1} sweep rate.
B. DPVs for variation of SE concentration from 10 μM - 80μM in PBS of pH 7.4 with 10 μM EP, 10 μM AD and 10 μM GU at PBBR-MGPE and 50mVs^{-1} sweep rate.

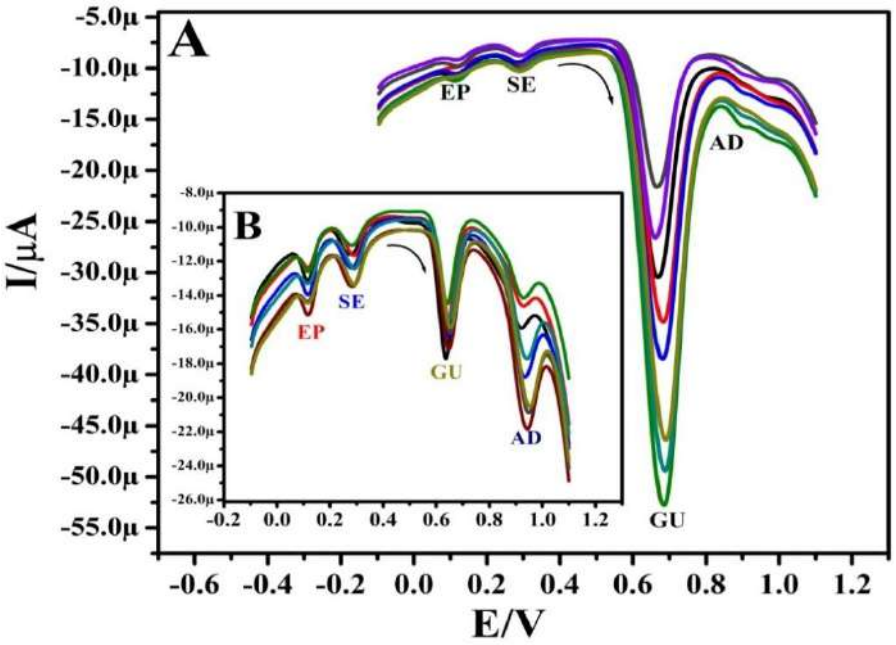

Fig. 7.14A. DPVs for altering GU concentration from 10 μM - 80μ M in PBS of pH 7.4 with 10 μM SE, 10 μM EP and 10 μM AD at PBBR-MGPE and 50mVs^{-1} sweep rate.
B. DPVs for altering AD concentration from 10 μM - 80μ M in PBS of pH 7.4 with 10 μM SE, 10 μM EP and 10 μM GU at PBBR-MGPE and 50mVs^{-1} sweep rate.

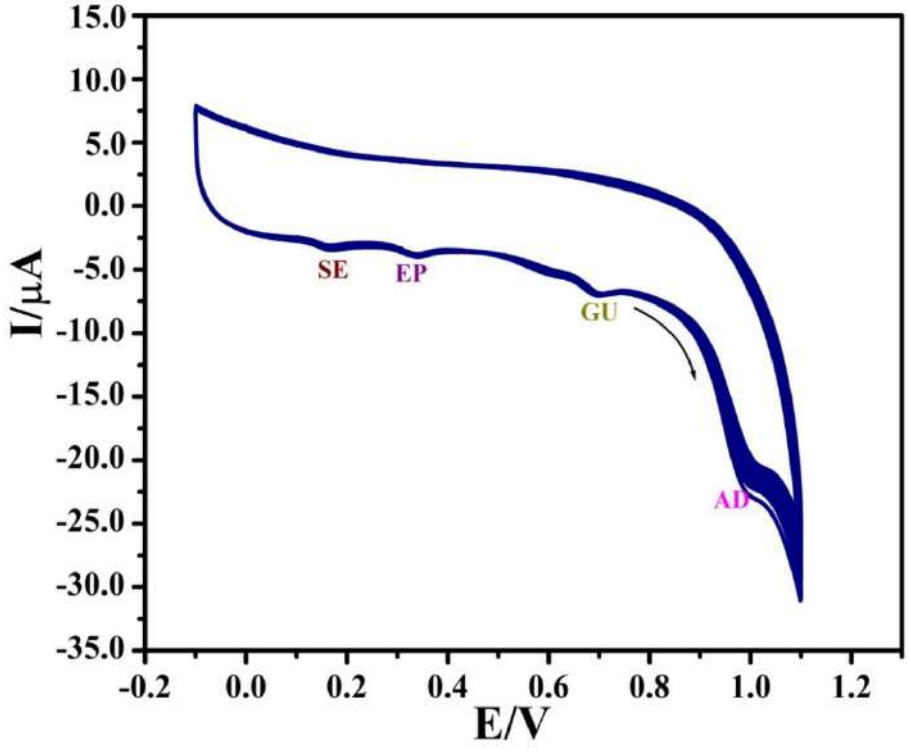

Fig. 7.15. CVs noted for the mixed SE, EP, AD and GU (10 μM) in 0.2M PBS of pH7.4 with speed rate of 50mV/s at PBBR-MGPE.

Table.1: Comparative analytical behavior of SE at PBBR-MGPE with other reported electrodes.

Working Electrode	**LOD (μM)**	**Electrochemical Techniques**	**Reference**
GPE	4.0	CV	[44]
AuNPs@PPy/GSPE	33.2	SWV	[45]
Poly(FSBF)MPGE	1.7	CV	[46]
t-ZrO_2/MCPE	0.58	DPV	[47]
PBBR-MGPE	0.31	CV	**This Study**

Table.2: Comparative analytical behavior of EP at PBBR-MGPE with other reported electrodes.

Working Electrode	**LOD (μM)**	**Electrochemical Techniques**	**Reference**
PGE*	1.46	SWV	[48]
PGE*	0.83	CSWV	[49]
Au 4MpyAuNPs	4.5	CV	[50]
Pt/P.chrysosporium ME446	1.04	CV	[51]
FePc-ME	0.5	CV	[52]
DWO/SPE	0.5	DPV	[53]
PBBR-MGPE	0.27	CV	**This Study**

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LIST OF PAPERS PUBLISHED/COMMUNICATED

1. **Rukaya Banu,** B. E. Kumara Swamy and S. Deepa, Poly(fast sulphone black F) modified pencil graphite electrode sensor for serotonin, ***Sensors International***, 1(2020)100044.
2. **Rukaya Banu**, B. E. Kumara Swamy, Poly (Bromocresol purple) incorporated pencil graphite electrode for concurrent determination of serotonin and levodopa in presence of L-Tryptophan: A voltammetric study, ***Inorganic Chemistry Communication,* 141(2022)109695.**
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4. **Rukaya Banu**, B. E. Kumara Swamy, Eno Ebenso, Voltammetric Analysis of Serotonin and Epinephrine in Presence of Guanine and Adenine at Bismarck brown R Amplified Graphite Pencil Electrode. ***Inorganic Chemistry Communication,* 144(2022) 109868.**
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6. **Rukaya Banu**, B. E. Kumara Swamy, Poly (Congo Red) functionalised-MWCNT composite electrodes for the simultaneous voltammetric detection of serotonin and levodopa in human serum **(*Communicated to Microchemical Journal (2022)*).**

7. **Rukaya Banu**, B. E. Kumara Swamy, Electrochemical sensor facilitated by synthesis of CdO nanoparticles amplified pre-treated carbon paste electrode for quantification of serotonin in the presence of epinephrine ***(Communicated to Analytical and Electroanalytical Chemistry (2022)*)**.

8. **Rukaya Banu**, B. E. Kumara Swamy, A selective electrochemical sensing of Serotonin and Epinephrine at glassy carbon electrode modulated with brilliant green: A voltammetric study ***(Communicated to Current Analytical Chemistry (2022)*)**.

9. M. Shruthi Vishvanath, B. E. Kumara Swamy, K. A. Vishnumurthy and **Rukaya Banu**, Synthesis, characterization and electrochemical studies of Calcium oxide nanoparticles modified carbon paste electrode for the determination of Uric acid in presence of Dopamine ***(Communicated to Frontiers in Voltammetry as a book Chapter(2022))*.**

CONFERENCES/SEMINARS/ WEBINARS

1. **Rukaya Banu**, National conference on *"Industrial applications of Electrochemistry"* organized by Department of Chemistry, PES institute of technology and Management, Shivamogga on 13th and 14th July 2020.
2. **Rukaya Banu**, One week virtual faculty developement programme on **"**Materials and Medicinnal Chemistry-2020(MMC)" organized by Department of Chemistry, Don Bosco institute of technology, Bangalore on 10-15th August 2020.
3. **Rukaya Banu**, Webinar on *"Plagiarism"* organized by Sri Venkateshwara college of Engineering in association with International Journal of advance Study and Research Work on 16th August 2020.
4. **Rukaya Banu**, Five days faculty development program on "*Insight In to Analytical techniques and its applications"*(IATA-2021) organized by Department of Chemistry, Acharya institute of graduate studies on 21st to 25th June 2021.
5. **Rukaya Banu**, International webinar on "*Synthetic methods of nanoparticles and their interesting properties"* organized by Department of Physics/Chemistry, MGVC arts, commerce and science college, Muddebihal on 4th august 2021.
6. **Rukaya Banu,** B. E. Kumara Swamy Poly(fast sulphone black F) modified pencil graphite electrode sensor for serotonin. *International virtual conference on "Emerging trends in Nanoscience & Nanotechnology"* organized by College of Engineering and Technology, Srinivas University, Mangaluru on 6th and 7th August 2021.

7. **Rukaya Banu**, B. E. Kumara Swamy, Poly (Bromocresol purple) incorporated pencil graphite electrode for concurrent determination of serotonin and levodopa in presence of L-Tryptophan: A voltammetric study. National conference on *"Impact of Chemistry and Biology to the Society and Industry"* organized by Department PG and Research in Industrial Chemistry, Kuvempu University, Shankaraghatta on 20th and 21st May 2022.
8. **Rukaya Banu**, International conference on "*Chemical sciences: Academia, Industry & society interface(ICCS-2022)"* organized by Department of Chemistry, post graduation centre, Jyothi Nivas College autonomous, Bangalore on 23rd to 25th June 2022.
9. **Rukaya Banu**, National conference on "*Recent Advances in Chemical Science*" organized by Department of Chemistry, J.S.S. Arts, Science and Commerce College, Gokak on 23rd July 2022.
10. **Rukaya Banu**, Electrochemical sensor facilitated by synthesis of CdO nanoparticles amplified pre-treated carbon paste electrode for quantification of serotonin in the presence of epinephrine. International conference on " *Recent Trends in Chemistry"*, organized by Department of Chemistry, Field Marshal K M Cariappa College, Madikeri on 23rd November 2021.

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Poly (fast sulphone black F) modified pencil graphite electrode sensor for serotonin

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Keywords:
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ABSTRACT

Development of sensitive and rapid biosensor for the investigation of serotonin has great significance because it is a key neurotransmitter and its unusual concentrations associated with serious mental disorders. In this study, an electrochemically modified serotonin-sensing electrode was fabricated by simple electropolymerisation of fast sulphone black F on pencil graphite electrode (PGE) using cyclic voltammetric technique. This modified pencil graphite electrode was applied for selective determination of serotonin (5-HT) and shows increased current responses of 5-HT in 0.2 M PBS of pH 7.4. The various analytical parameters such as effect of scan rate, concentration of 5-HT and solution pH were investigated. The diffusion controlled electrode process was observed for 5-HT and detection limit was found to be 1.7 μM. Interference study of 5-HT was analysed in presence of dopamine (DA) by cyclic voltammetry (CV) and differential pulse voltammetry (DPV).

1. Introduction

Pencil leads often referred as pencil graphite electrodes (PGEs) gained more prominence in recent days as a working electrode material for various electrochemical applications. PGEs act as a crucial substitution for other carbon electrodes due to their affordable price and thin dimensions. Also shows great potential in designing a disposable biosensing electrode materials [1,2]. A fine particles of graphite is used to produce a pencil leads, which is composed of graphite powder mixing with clay or mica and a high polymeric binders are sometimes added [3]. PGEs are simple and easy to use because of their good adsorption properties, high conductivity, good mechanical strength and easy methods of modifications [4–6]. The renewal of electrode surfaces is simple and faster in case of PGEs among various solid electrodes involving common polishing and cleaning techniques and has larger surface area. Therefore, it is able to detect the analyte in its lower concentration [7–9].

Serotonin and Dopamine are important neurotransmitters belong to catecholamine family and serves as chemical carriers for transporting information between the nerve cells [10]. Both serotonin and dopamine are responsible for various phenomenon occurs in the living organisms. Serotonin, Which is chemically known as 5-hydroxytryptamine(5-HT) (Scheme 1) derived from α−amino acid tryptophan and widely distributed inside and outside of brain tissues. 5-HT together with other neurotransmitter plays a significant role in regulating and controlling the several biological and physiological functions like sleep disturbances, memory, wound healing, appetite, thermoregulation, behaviour and drug dependency [11–14]. Many life functions are depends on the concentration of 5-HT level in blood. The normal range of 5-HT levels in blood is 101–283 ng/mL. Any unbalance in the concentration of 5-HT leads to numerous health issues such as low concentration level causes anxiety, chronic pain, blood clotting. While extremely high concentration leads to potentially fatal effects like serotonin syndrome, carcinoid syndrome, liver regeneration and autism [15–17].

3,4-dihydroxyphenylethylamine commonly known as dopamine (DA) (Scheme 2) is an inhibitory neurotransmitter. It exhibits significant contribution in the proper functioning of hormonal, renal and cardiovascular system [18,33]. The normal level of DA in blood plasma is in the range of 0.04–450 nM [19]. Lower concentration of DA than the normal level causes severe neurological diseases such as Parkinson's disease,

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Short communication

Poly (Bromocresol purple) incorporated pencil graphite electrode for concurrent determination of serotonin and levodopa in presence of L-Tryptophan: A voltammetric study

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ARTICLE INFO

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ABSTRACT

In the present investigation, the new electrochemical sensor was established based on the electropolymerisation of Bromocresol purple on pencil graphite electrode for the sensitive detection of serotonin(5-HT) and Levodopa (LD) in assistance with L- Tryptophan(TRP). The morphological characterisation of developed working electrode was done by Scanning Electron Microscope(SEM). The modified sensor presents admirable electrocatalytic performance towards specific and selective examination of 5-HT, LD and TRP at 0.2 M Phosphate buffer solution of physiological pH having the speed rate of 50 mV/s using cyclic voltammetric(CV) and differential pulse voltammetric(DPV) methods. The distinct experimental conditions like impact of supporting electrolyte, dissimilar concentration and varied sweep rates were optimised to accomplish a better peak current. The designed sensor effectually lowers the detection limits of 5-HT (0.49 μM) and LD (2.3 μM) and it is easy to fabricate, disposable, highly stable and applicable to practical analysis of bioactive molecules.

1. Introduction

The exploitation of pencil graphite leads (PGE) as a sensing element and their great success profoundly influenced the field of biosensors. It opens new approaches of research for the detection of materials and biomatters. Pencil electrodes increasingly earned traction with their superior electrical, mechanical, biocompatible and physical properties. Thanks to exceptional adaptability, portability and easy method of utilizing of pencil electrode in relatively complex samples make them one of the cornerstones of analytical electrochemistry [1–7]. A variety of analytical methods are known to be useful for the discrimination of biologically as well as environmentally beneficial entities such as high performance liquid chromatography, electrophoresis [8], photokinetics [9] and spectrophotometry [10]. Even though the reported methods are completely validated, they possess a lower selectivity, highly expensive equipments and longer time of analysis. Although, the electroanalytical techniques have been considered as promising strategies to overcome all the above mentioned difficulties as it provides superior selectivity, sensibility, easy method of amplification and fast response. Also serves as eco-friendly because of the less consumptions of organic solvents [11–15].

Serotonin (5-Hydroxytryptamine, 5-HT) is an eminent biogenetic neurochemical that mainly identified in blood platelets, bowels, intrinsic and central nervous system of the brain. 5-HT is produced by essential serotonergic neurons and body ulitlises it to relays the information between the nerve cells and regulates their intensity. The biological role of 5-HT is complex and diversified [16–18]. 5-HT along with the other monoamine neuromodulators plays a leading role and contributes in mood modulating, reward, learning, cognition and numerous physiological functions like emesis and vasocontradiction [19–22]. Also serves as precursor for melatonin and thereby regulates the sleep-wake cycle and body clock [23].

L-dopa, also familiar as levodopa (LD) is a peculiar amino acid that produced and utilized as a portion of normal biology of humans, animals as well as in plants [24–26]. LD is a psychotropic drug and naturally derived dietary supplement found in specific type of herbs and foods. It is prepared by a way of biogenesis of nonessential amino acid L-tyrosine in brain and body of mammalian system. LD serves as precursor for dopamine and used as medicine to cure the Parkinson's disorder by raising the dopamine levels in the brain. LD also employed in the medication of identical muscular conditions when they induced by drugs such as fluphenazine, chlorpromazine and others [27–28].

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Short communication

Simultaneous resolution of serotonin and epinephrine at poly (Victoria blue B) amplified carbon paste electrode: A voltammetric study with density functional theory evidences

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Serotonin
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ABSTRACT

Herein, a polymerised film of Victoria blue B monomer was deposited on carbon paste electrode surface and characterised by using scanning electron microscope and electrochemical methods. The polymer film amplified electrode exemplified the elevated electrocatalytic oxidation of 5-HT and EP in presence 0.2 M PBS of physiological pH. The various experimental settings such as effect of supporting electrolyte, scan rate and varying the concentration of targeted analytes were scrutinized and the designed sensor depicts the lower detection limit for 5-HT (0.89 μM) and EP(0.33 μM). The proposed modified electrode also implemented for concurrent resolution and determination of serotonin (5-HT) and epinephrine (EP) by cyclic and differential pulse voltammetry also shows satisfactory steadiness towards 5-HT and EP.

1. Introduction

In recent decades, the formulation of carbon based voltammetric sensors has great deal of interest in electrochemical studies as a sensitive and powerful method to examine the characteristics of the biologically important oxidizable compounds [1,2].

Serotonin(5-HT) and Epinephrine(EP) both are neuromodulators and serves as signalling substances that carries balances and promotes the messages between the neurons[3]. 5-HT is a prominent mood modulating neurochemical extensively secreted by the essential amino acid L-tryptophan and principally found in peripheral and central nervous systems and fulfils the various physiological and biological actions of the body [4–7]. However the highest amount of 5-HT (~90 %) is located in enterochromaffin cells of gastrointestinal tract and involved in multiple gastrointestinal ailments such as irritable bowel syndrome, food hypersensitivity and secretion [8,9]. The insufficiency in 5-HT levels results in distinct neuro problems like Down's syndrome, depression and suicidal inclinations [10–12]. The intensified level of 5-HT leads to toxicity and Huntington's syndrome [13,14]. Also, DNA damage was influenced by 5-HT in presence of copper ions [15].

EP, also referred as adrenaline is a larger organic cation exists in biological fluids and neural tissues[16,17]. EP is an adrenergic hormone and presumably acts as an inhibitory neuromodulator which is released by the adrenal glands and by the neurons in medulla oblongata of mammalian system [18]. It trained the body for action at emergency circumstances by promoting the glucose and oxygen flow to the brain and muscles. EP also serves as a medicant and employed to cure the numerous conditions including superfacial bleeding, cardiac arrest and anaphylaxis. Orthostatic hypotension and parkinsonism are the results of deficit EP concentration. Whereas, the extreme EP levels leads to stress and inadequacy of thyroid hormone [19–24]. Both 5-HT as well as EP plays influential duties in maintaining the human health. In this regard, it is requisite to formulate a sensible method for quantitative determination of these molecules for studies of physiological activities and diagnostics in clinical medicine [25].

At present, Victoria blue B was employed to chemically modifying carbon paste electrode and used for the simultaneous resolution of 5-HT and EP. Victoria blue B is a stain commonly known as basic blue B

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Short communication

Voltammetric analysis of serotonin and epinephrine in the presence of guanine and adenine at Bismarck brown R amplified pencil graphite electrode

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Keywords:
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ABSTRACT

In present study, the novel sensing platform was electrochemically designed by incorporation of Bismarck brown R (BBR) as a enhancing substance onto the graphite pencil electrode(GPE) surface and successfully implemented for the electroanalysis of serotonin(SE) and epinephrine(EP) in assistant with adenine (AD) and guanine(GU) through cyclic and differential pulse voltammetric methods(CV and DPV). The morphology of electrode surface was characterised by scanning electron microscopy (SEM). The test solutions and operating conditions such as scan rate, varied supporting electrolyte pH and concentration of targeted analytes were optimised. The electrode kinetics of a tailored sensor was governed by both adsorption and diffusion phenomenon and gives a reduced detection limits for SE and EP respectively. The configured electrode proffers the potent and persistent specificity and sensibility followed by distinctly separated four anodic signals of SE, EP, AD and GU with appreciable increments in peak currents. Therefore, the offered sensor was cost effective, simple and applied in electrochemical monitoring and biosensing applications.

1. Introduction

The adoptability of graphite pencil leads as an electrode materials are largely increased in recent eras in the fields of electroanalytical methods for the quantification of various types of bio significant matrices. When compared to other carbon based electrodes, GPEs posses a wide range of profitable characteristics such as lower background currents, better sensibility and reproducibility [1–5]. GPEs are more convenient to use, less expensive and having an adjustable surface area and granting the evaluation of samples in lower concentrations without any preconcentration stages and also GPEs are renewable and easily disposable [6–8]. SE and EP are biogenetic chatecholamines presumably serves as neuromediaters and chemical channels that relays the indications between the nerve cells. The mood stabilizing agent, SE is an eminent inhibitory neurochemical broadly distributed in the biosystems [9–11]. The usual concentration level of SE in human body is 0.25–0.74 μM [12]. SE plays prominent role in controlling the multifarious behavioral and emotional status together with other neurochemicals such as sleep, emesis and appetite. Any variations in the serotonin level leads to multiple psychiatric illnesses such as deficit SE levels causes depression, suicidal tendencies and increased level associated with serotoninergic malfunctions [13–16].

EP is an organic cation assists both as neorutransmitter as well as harmone. EP specifically secreted in the adrenal medulla and the body releases it during acute stress. Its stimulatory effects energize and instruct the body for ‘fight or flight’ response and raises the breathing and heart rate also helps the brain to take quick actions in the face of danger. The deviated levels of EP inflames the variety of life threatening illness such as myocardial infarction, hypoglycemia and pheochromocytoma[17–22].

AD and GU are purine bases imperatively acts as constituents of the nucleic acids [23]. They play influential roles in storing genetic data and participated in the numerous mechanisms like supplying energies, coenzyme formation and metabolic managements. Also, they are vital in cerebral and coronary transmissions, managing the blood flow, neurotransmitter secretion and regulating the functions of adenylate cyclase [24–26]. Any anomalous variations in the AD and GU ascribed to deficiencies in the immune mechanisms in the human systems and some disorders like cancers, epilepsy, renal calculi and AIDS [27,28]. SE, EP, AD and GU are bio essential molecules co-occurred in living organisms.

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Short communication

A glassy carbon electrode modulated with Poly (Naphthol green B) for simultaneous electroanalysis of serotonin and Epinephrine in presence of L-tryptophan

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ARTICLE INFO

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ABSTRACT

Present study reports the simplified and efficient method for the quantification of Serotonin(5-HT)and Epinephrine(EP) by using a glassy carbon electrode customised with poly(Naphthol green B) film. The electrochemical measurements were performed through cyclic and differential pulse voltammetry. The experimental outcomes authenticates that the developed sensor accelerates the electrocatalytic current response with reduced overvoltage for 5-HT and EP. The operating system and certain experimental variables includes Sweep rate, differed pH values of supporting medium and the impact of varying concentration of 5-HT and EP were adjusted. The sweep rate study reveals the diffusion controlled kinetics for 5-HT and EP at P-NGB/MGCE. At the optimum reaction conditions, the fabricated electrode exhibits the linear behaviour in the concentration range of 0.1×10^{-4}M to 0.8×10^{-4}M and 0.1×10^{-4}M to 1×10^{-4} M with the lower limit of detection of 2.3 μM & 1.3 μM for 5-HT and EP respectively. The selectivity of the constructed electrode was achieved by concurrent determination of 5-HT and EP in presence of L-Tryptophan(LT). This provides the advantageous of the fabricated sensor as it shows good sensibility, convenient method for fabrication and excellent selectivity for specific and simultaneous study of 5-HT and EP.

1. Introduction:

Electrochemical biosensors are recognised as a striking tool in scientific research for the exposure of the biologically significant substances, which acts as beneficial sources for the disease biomarkers [1–2]. These sensors are portable, less expensive, provides accurate, rapid and real time analysis than other conventional methods and reproducible. Therefore there is a pressing need for the development of electrochemical sensing based biosensors for invasive clinical monitoring from treatment to prevention of diseases [3–4].

5-HT is an electroactive indolamine entity acts as a neurotransmitter and vasoconstrictor [5,6]. It supports in signal transport to regulate various bodily functions as well as mood enhancing chemical in the brain that influences the mood, feelings of well-being and helps to manage hunger. 5-HT positively improves the sleeping patterns for more soothing and rejuvenates sleep [7,8]. It also assists in blood clotting and bowel functions of the body. The deficit 5-HT levels attributed to severe mental sickness such as panic attacks, depression, insomnia, stress and unexplained irritability. The extreme 5-HT concentration is associated with the group of symptoms known as serotonin syndrome and toxicity [9,10].

EP is a fight or flight hormone also an important neurotransmitter of catecholamine class present in the mammalian nervous system [11,12]. EP is a most powerful vasopressor drug and regulates the heart beat, bronchodilation, blood sugar and lipolysis. EP often employed as an emergency medication for the treatment of various conditions such as accidents, cardiac arrest, hypertension and bronchial asthma etc [13,14]. Higher levels of EP are associated with hypoglycaemia, myocardial infarction and stress, while the deficiency in the EP level leads to orthostatic hypotension and Parkinson's disorder [15].

LT is a vital amino acid plays a crucial biochemical and clinical importance. It is needed for the normal growth and positive nitrogen balance in human and herbivores [16,17]. Human body consumes it in the form of dietary supplements and medicines. LT involved in the formation and maintenance of several bio-essential elements like proteins, muscles, enzymes and neurotransmitters. The level of LT in the body

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ELECTROCHEMICAL SENSOR FOR THE DETERMINATION OF SEROTONIN USING DIFFERENT MODIFIED ELECTRODES: A VOLTAMMETRIC STUDY

Thesis to be submitted to the Faculty of Science, Kuvempu University As a partial fulfillment for the requirements of degree of

DOCTOR OF PHILOSOPHY

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Research Guide

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Summary of the Thesis

The main focus of the thesis is to formulate an electrochemical sensing approach for the detection of 5-HT. In present investigation, the different carbon based electrodes (pencil graphite electrode, carbon paste electrode and glassy carbon electrode) are modified by incorporating the various methods (Electropolymerization, mobilization/immobilization, electrochemical pretreatment and hand blending) to examine the electrocatalytic characteristics of serotonin in presence of some biologically important molecules like levodopa, epinephrine, L-tryptophan, adenine and guanine by the application of cyclic and differential voltammetric techniques. The several experimental conditions such as influence of pH of the supporting buffer, sweep rate, analyte concentration and interferences were optimized. The simultaneous analysis and real sample were carried out at different modified electrodes which show excellent performance toward serotonin detection.

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

Chapter-1

Introduction and Overview of Voltammetric Techniques and Cyclic Voltammetry

This chapter explains about voltammetric techniques and brief introduction to cyclic voltammetry and Differential pulse voltammetry. Electrode system, types of electrodes, solvents, electrode processes, electrochemical parameters and various types of voltammograms involved in this technique. A brief literature survey of cyclic voltammetric investigations of serotonin has been reviewed. The importance of serotonin is discussed. Also, objectives and scope of the present thesis were discussed in this chapter.

Chapter-2

Experimental

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

Chapter-3

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

Part-A

Poly (Bromocresol Purple) incorporated Pencil Graphite Electrode for Concurrent Determination of Serotonin and Levodopa in Presence of L-Tryptophan: A Voltammetric Study

In this chapter, the new electrochemical sensor was established based on the electropolymerisation of Bromocresol purple on pencil graphite electrode for the sensitive detection of serotonin (5-HT) and levodopa (LD) in assistance with L-Tryptophan(TRP). The morphological characterisation of developed working electrode was done by Scanning Electron Microscope (SEM). The modified sensor presents admirable electrocatalytic performance towards specific and selective examination of 5-HT, LD and TRP at 0.2 M Phosphate buffer solution of physiological pH having the speed rate of 50mV/s using cyclic voltammetric(CV) and differential pulse voltammetric(DPV) methods. The distinct experimental conditions like impact of supporting electrolyte, dissimilar concentration and varied sweep rates were optimised to accomplish a better peak current. The designed sensor effectually lowers the detection limits of 5-HT (0.49μM) and LD (2.3μM) and it is easy to fabricate, disposable, highly stable and applicable to practical analysis of bioactive molecules.

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Part-B

Poly(Fast Sulphone Black F) Modified Pencil Graphite Electrode Sensor for Serotonin

This chapter involves, the development of sensitive and rapid biosensor for the investigation of serotonin has great significance because it is a key neurotransmitter and its unusual concentrations associated with serious mental disorders. In this study, an electrochemically modified serotonin-sensing electrode was fabricated by simple electropolymerisation of fast sulphone black F on pencil graphite electrode (PGE) using cyclic voltammetric technique. This modified pencil graphite electrode was applied for selective determination of serotonin(5-HT) and shows increased current responses of 5-HT in 0.2M PBS of pH 7.4. The various analytical parameters such as effect of scan rate, concentration of 5-HT and solution pH were investigated. The diffusion controlled electrode process was observed for 5-HT and detection limit was found to be 1.7μM. Interference study of 5-HT was analysed in presence of dopamine(DA) by cyclic voltammetry(CV) and differential pulse voltammetry(DPV).

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Chapter-4

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

Part-A

Simultaneous Resolution of Serotonin and Epinephrine at poly(Victoria blue B) Amplified Carbon Paste Electrode: A Voltammetric Study.

Herein, a polymerised film of Victoria blue B monomer was deposited on carbon paste electrode surface and characterised by using scanning electron microscope and electrochemical methods. The polymer film amplified electrode exemplified the elevated electrocatalytic oxidation of 5-HT and EP in presence 0.2 M PBS of physiological pH. The various experimental settings such as effect of supporting electrolyte, scan rate and varying the concentration of targeted analytes were scrutinized and the designed sensor depicts the lower detection limit for 5-HT (0.89μM) and EP (0.33μM). The proposed modified electrode also implemented for concurrent resolution and determination of serotonin (5-HT) and epinephrine (EP) by cyclic and differential pulse voltammetry.

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Part-B

Poly (Congo Red) Functionalised-MWCNT Composite Electrodes for the Simultaneous Voltammetric Detection of Serotonin and Levodopa in Human Serum

Serotonin (5-HT) and Levodopa (LD) are the imperative biomolecules that concurrently exist in body fluid and show undesirable effects on the functions of one another. LD depletes the levels of 5-HT in the brain in proportions to increase the dopamine level. The reduced 5-HT level can lead to severe neurodegenerative disorders. Hence, the simultaneous detection of 5-HT and LD has great importance in disease diagnosis. In the present study, an electrochemical sensing platform was formulated by amending carbon paste with functionalized multi-walled carbon nanotubes followed by electropolymerization of congo red (p-CR/NH_2-MWCNTs/MCPE). The established electrode shows excellent electrochemical properties for individual and concurrent detection of 5-HT and LD in a phosphate buffer solution of neutral pH at a sweep rate of 50 mV/s through cyclic voltammetric (CV) and differential pulse voltammetric (DPV) approaches. Various analytical variables like scan rate effect, concentration, and effect of pH were investigated. The modified sensor effectively reduces the detection limit values found to be 1.7 μM and 3.0 μM for 5-HT and LD respectively. Moreover, the proposed method offered favourable selectivity, stability, reproducibility and reliable recoveries of molecules in serum samples.

Communicated to Microchemical Journal (2022)

Chapter-5

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

Part-A

A Glassy Carbon Electrode Modulated with poly(Naphthol green B) for Simultaneous Electroanalysis of Serotonin and Epinephrine in Presence of L-Tryptophan.

Present study reports the simplified and efficient method for the quantification of Serotonin and Epinephrine by using a glassy carbon electrode customised with poly(Naphthol green B) film. The electrochemical measurements were performed through Cyclic and differential pulse voltammetry. The experimental outcomes authenticates that the developed sensor accelerates the electrocatalytic current response with reduced overvoltage for SE and EP. The operating system and certain experimental variables includes Sweep rate, differed pH values of supporting medium and the impact of varying concentration of SE and EP were adjusted. The sweep rate study reveals the diffusion controlled kinetics for SE and EP at P-NGB/MGCE. At the optimum reaction conditions, the fabricated electrode exhibits lower limit of detection of 2.3μM & 1.3μM for SE and EP respectively. The selectivity of the constructed electrode was achieved by concurrent determination of SE and EP in presence of L-Tryptophan. This provides the advantageous of the fabricated sensor as it shows good sensibility, convenient method for fabrication and excellent selectivity for specific and simultaneous study of SE and EP.

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Part-B

A Selective Electrochemical Sensing of Serotonin and Epinephrine at Glassy Carbon Electrode Modulated with Brilliant Green: A Voltammetric Study

In the present investigation, a novel electrochemical sensing approach based on the modulation with electropolymerisation of Brilliant green on glassy carbon electrode was introduced to rapid and sensitive identification of SE and EP by Cyclic (CV) and differential (DPV) pulse voltammetric procedures. Under the adequate circumstances, the analytical variable like pH of the supporting solution was performed over the range of 6.2-8.0. Furthermore, the electro-kinetic parameter was surveyed and the electrode depicts the proportionality between the current intensities with the concentration of analytes with a low detection limit. The modulated sensor portrays the supreme electrocatalytic characteristics toward simultaneous quantification of SE and EP in a sample mixture.

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Chapter-6

Electrochemical Sensor Facilitated by Synthesis of CdO Nanoparticles Amplified Pre-treated Carbon Paste Electrode for Quantification of Serotonin in the Presence of Epinephrine

Herein, the modest co-precipitation mode was implemented to fabricate the cadmium oxide nanoparticles (CdO). The size, elemental composition and morphological characteristics of the synthesized nanomaterials were confirmed by XRD, EDS and SEM measurements. The designed CdO nanoparticles were exploited for the amplification followed by pre-treatment of carbon paste electrode (CdO/MPCPE) and successfully utilised for the quantification of serotonin(SE) in the presence of epinephrine(EP) at biological pH. The tailored composite sensor proclaims the rapid electron transport behaviour which results in accretion in the oxidation peak signals for SE and EP. The several experimental conditions includes pH of supportive buffer, speed rate and concentration of analytes species were idealised. The CdO/MPCPE displays better sensing capability towards specific and simultaneous quantifications and lower detection limits were achieved. The customised electrode facilitates the high selectivity, excellent electrocatalytic stability and agreeable results for rapid diagnosis of identical bioactive entities.

Communicated to Analytical and Bioanalytical Electrochemistry (2022)

Chapter-7

Voltammetric Analysis of Serotonin and Epinephrine in Presence of Guanine and Adenine at Bismarck brown R Amplified Graphite Pencil Electrode.

In present study, the novel sensing platform was electrochemically designed by incorporation of Bismarck brown R (BBR) as a enhancing substance onto the graphite pencil electrode(GPE) surface and successfully implemented for the electroanalysis of serotonin(SE) and epinephrine(EP) in assistant with adenine (AD) and guanine(GU) through cyclic and differential pulse voltammetric methods(CV and DPV). The morphology of electrode surface was characterised by scanning electron microscopy (SEM). The test solutions and operating conditions such as scan rate, varied supporting electrolyte pH and concentration of targeted analytes were optimised. The electrode kinetics of a tailored sensor was governed by both adsorption and diffusion phenomenon and gives a reduced detection limits for SE and EP respectively. The configured electrode proffers the potent and persistent specificity and sensibility followed by distinctly separated four anodic signals of SE, EP, AD and GU with appreciable increments in peak currents. Therefore, the offered sensor was cost effective, simple and applied in electrochemical monitoring and biosensing applications.

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