"ELECTROANALYTICAL ASSESSMENT OF SOME BIOLOGICALLY IMPORTANT COMPOUNDS BY DEVELOPING BIOSENSORS"

A Thesis submitted to

VISVESVARAYA TECHNOLOGICAL UNIVERSITY, BELAGAVI

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in

CHEMISTRY

By

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JANUARY 2020

CERTIFICATE

This is to certify that the thesis entitled *"Electroanalytical assessment of some biologically important compounds by developing biosensors"* submitted for the award of Doctor of Philosophy in Chemistry is the original research work completed by **Mr. Amit Teradale** under my supervision and it has not been submitted to any other University wholly or in part for any other degree.

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DECLARATION

I hereby declare that the entire work embodied in this doctoral thesis has been carried out by me at Research Centre, Department of Chemistry, BLDEA's V.P.Dr.P.G. Halakatti College of Engineering and Technology, Vijayapur, Karnataka, India under the supervision of Dr. Swastika N. Das, Professor and Head, Department of Chemistry, BLDEA's V. P. Dr. P. G. Halakatti College of Engineering and Technology, Vijayapur, Karnataka, India and under the co-supervision of Dr. S.D. Lamani, Assistant Professor, P.G. Department of Chemistry, S.B.Arts and K.C.P.Science College, Vijayapur, Karnataka, India. This thesis has not been submitted in part or full for the award of any degree /diploma of this or any other University.

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Date:

Place: *Vijayapur* ***Amit Teradale***

I dedicate this thesis to

My Guru Muni Shri 108 Mahima Sagar Maharaji

&

My Beloved Parents

Mr. Balasab B. Teradale

Mrs. Pushpa B. Teradale

&

My Wife

Madhuri A. Teradale

& My Son

Aniket A. Teradale

& My Brother

Kapil. B. Teradale

LIST OF ABBREVIATIONS

SYMBOLS	
A	Area of electrode
ASA	Acetylsalicylic acid
Ipa	Anodic peak current
AA	Ascorbic acid
AE	Auxiliary electrode
BCPE	Bare carbon paste electrode
CZ	Carbamazepine
CC	Catechol
Ipc	Cathodic peak current
CTAB	Cetyltrimethylammonium bromide
CE	Counter electrode
CMC	Critical micelle concentration
CV	Cyclic voltammetry
CV-grams	Cyclic voltammograms
DPV	Differential pulse voltammetry
D_0	Diffusion coefficient
DMSO	Dimethyl sulphoxide
DMA	Dimethylaniline
DMF	Dimethylformamide
DA	Dopamine
EP	Epinephrine
F	Faraday constant
R	Gas constant
G	Gibb's free energy
GCE	Glassy carbon electrode
HPLC	High-performance liquid chromatography
IBD	Inflammatory bowel diseases
ID-MS	Isotope dilution mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MSZ	Mesalazine
MCPE	Modified carbon paste electrode
MCPE'S	Modified carbon paste electrode sensors
n	Number of electrons
NC	Niacin
NA	Niacinamide
PC	Paracetamol
I_P	Peak current
E_P	Peak potential
PBS	Phosphate buffer solution
PASA/MWNT'S	Poly amidosulfonic acid and multi wall carbon nanotubes

RE	Reference electrode
RSD	Relative standard deviation
SAL	Salicylaldehyde
SCE	Saturated calomel electrode
SEM	Scanning electron microscopy
υ	Scan rate
SLC	Sensitive liquid chromatography
SWCNTS	Single walled carbon nanotubes
k^0	Standard heterogeneous rate constant
$\upsilon^{1/2}$	Square root of scan rate
SWV	Square wave voltammetry
T	Temperature
UA	Uric acid
WE	Working electrode

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ABSTRACT

Background: Analytical Chemistry is an important branch of modern chemistry which deals with the separation and determination of the constituents present in the matter of interest. It involves the application of a range of techniques to assess the complete information about the analyte species present in a solution. The qualitative and quantitative assessments as well as structural assessments become very much reliable with the application of analytical techniques. A new branch of analytical chemistry is electroanalytical chemistry which can analyse a species depending upon its electrical properties with the help of an electrochemical cell. The electrical parameters like potential (E), current (I), charge (Q) and resistance (R) are related to the concentration (C) of the analyte. These types of electrochemical measurements for analytical purposes cover a wide range of applications in the field of biomedical analysis, environmental monitoring and industrial quality control. The electroanalysis can be done by a method called cyclic voltammetry which is considered as a sensitive, selective and versatile technique for the investigation of the redox process in the electrochemical system. In this system, the current is produced due to electron transfer between the solution and the electrode surface. A voltammogram gives information about adsorption or diffusion-controlled processes in chemical reactions, the thermodynamics, and kinetics of heterogeneous electrochemical reactions. In our present study, we have focused only on the cyclic voltammetry technique for the determination of biologically active compounds by the use of different modified carbon paste electrodes.

Objectives: To design electrochemical (bio) sensors by modifying bare carbon paste electrodes using different techniques so that a more sensitive, selective and simple electrochemical method can be developed to assess analytic species (drugs) in the solution. The effect of different modifier amount, pH, scan rate and concentration for the selected drugs are to be analyzed. Also the electrochemical behavior of the analytes (reversible/irreversible/quasi-reversible) and electrode processes (adsorption controlled/diffusion-controlled) are to be explored by using CV technique. The probable reaction mechanisms on the electrode surface are to be understood and to be illustrated.

Method: All the investigations were done using an electrochemical workstation (model CH-Instrument-660c electrochemical analyzer USA) and connected to IBM PC and

printer. The electrochemical cell, potentiostat and the recorder are the most important components of the workstation.

Three-electrode cell system is as follows:

- Reference Electrode- A saturated calomel electrode
- Counter/Auxiliary Electrode- A platinum wire
- Working Electrode- Various modified electrodes were prepared by using Niacinamide, Carbamazepine, Niacin and surfactant like CTAB.

The electrochemical reactions were studied for different drugs by using BCPE and all the modified electrodes. The voltammograms were recorded at temperature 25±0.2 °C.

Results:

(i) *Poly-Niacinamide/MCPE Sensor for Catechol (CC)*

The cyclic voltammetry (CV) behavior of Catechol was investigated with BCPE and poly-NA/MCPE. The effect of different scan rate, pH and concentration were studied. The modified electrode has shown higher sensitivity and better selectivity when compared to the BCPE, and a better lower detection limits of 1.49 μM. The study established that the electrochemical process is reversible, diffusion-controlled with involvement of equal number of protons and electrons. The probable electropolymerisation mechanism of niacinamide was proposed.

(ii) CTAB-Immobilized/MCPE Sensor for Mesalazine(MSZ)

The electro-oxidation behavior of MSZ was studied at CTAB/MCPE using cyclic voltammetry technique. The investigations included the study of the effect of pH variation, scan rate, and concentration of MSZ. The investigation revealed that the type of electrode reaction was quasi-reversible; adsorption controlled and involves equal number of protons and electrons transfer. Under optimal conditions, the lower limit of detection with CTAB immobilized MCPE was found to be 1.9×10^{-9} M. Thus, the present study was very impressive on account of used modifiers due to its affectability, selectivity, reproducibility and low LOD value.

(iii) Carbamazepine (CZ)/MCPE Sensor for Paracetamol (PC)

The electrochemical behaviour of paracetamol was investigated with modified carbamazepine film coated carbon paste electrode (CZ/MCPE) by CV technique. Under optimum conditions, the new modified electrode sensor showed good voltammetric responses of PC over varying concentration range and lower LOD was obtained at 0.24×10^{-6} M. Further parameters like scan rate and pH variation were optimized for the

analysis of PC. The overall study reveals that the PC electrochemical process is reversible; adsorption controlled and involves two proton-electron exchanges. The recovery test of paracetamol drug in tablet analysis was also done to conclude that the CZ/MCPE sensor can be effectively chosen for the selective resolve of PC in pharmaceutical samples.

(iv) (Poly)-NC/CPE Sensor for Epinephrine (EP) with Uric Acid (UA)

The oxidation of EP and UA were studied at (poly)-NC/CPE using a CV technique. The investigations included the study of the effect of pH variation, scan rate, and concentration of EP. The obtained results displayed the type of electrode reaction was irreversible, adsorption controlled and involves equal number of protons and electrons transfer. Under optimal conditions, the lower limit of detection of EP with (poly)-NC/CPE was found at 11.3×10^{-9} M. The applicability of the current methodology was successfully estimated by the quantification of EP in injection. Thus, the present study was very remarkable on account of used modifiers due to its affectability, selectivity and reproducibility.

Conclusion: The present study has been carried out to give an explanation of the electrochemical behavior of four biologically active drugs such as; catechol, mesalazine, paracetamol, and epinephrine by preparing the modified electrodes. For each of the drugs we could establish the reaction mechanisms on the modified electrode surface and it has been observed that the electrode processes were either adsorption controlled or diffusion controlled and electrochemical behaviour of the analytes were reversible/irreversible/quasi-reversible. All the modified electrodes prepared were shown better selectivity and sensitivity as compared to BCPE. The lower detection limit for every drug was found to be enhanced when compared with the other modified electrodes reported earlier. The interference study was carried out and established a validated method for tablets and injections analysis.

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Chapter 1

INTRODUCTION

1.1. Electrochemistry

Electrochemistry is the branch of Chemistry that is used to study the relationship between electrical and chemical effects. The progressive research in the field of electrochemistry has helped to develop fast, sensitive, inexpensive and reliable applied techniques to study industrial electrolysis, electroplating, batteries, fuel cells, electrochemical matching, bio-electrochemistry and biosensor [1]. Electrochemical science studies the electron transfer efficacy of the electrochemical species between the solution and electrode surface. The current produced during the electron transfer can be used to analyze the species and mechanism of electrochemical reaction. Scientific research has come a long way using electroanalytical techniques for the study of relationship between current, voltage or potential and other chemical parameters involved in the reaction. Such kind of analysis as found applications in developing sensors for pollution control in water environment, biomedical analysis and industrial quality control [2].

Different electroanalytical methods can be used to study to develop microelectrodes, to design monolayer's, receptors containing cavities of molecular size, the synthesis of ionophores, the coupling of biological components, electrochemical transducers, the micro-fabrication of molecular devices or efficient flow detectors, and in environmental pollution control developing chemical and biosensors. The classifications of electroanalytical techniques are given in Fig. 1.1[3].

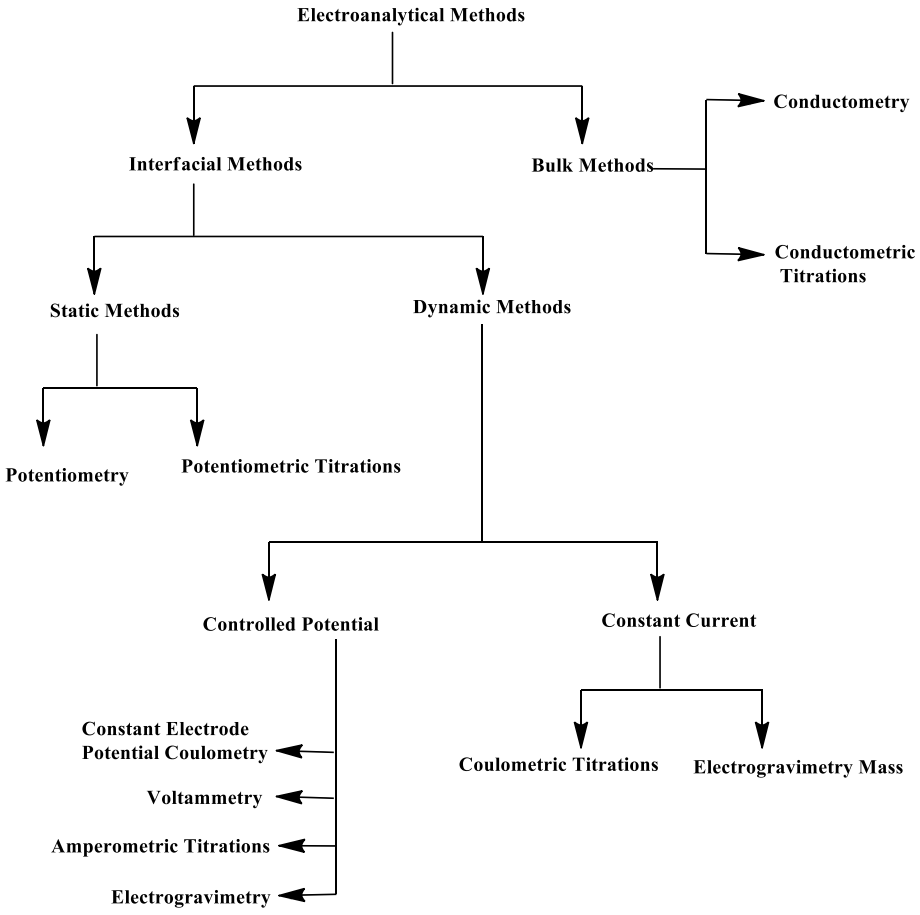


Fig. 1.1- Classification of common electroanalytical methods

Among the different techniques mentioned in Fig.1.1, voltammetry, conductometry, and potentiometry are the most popular techniques with a variety of applications. In this chapter, brief literature of review about voltammetry, development of electrode sensors and their uses for the resolve of some biologically active compounds are discussed.

1.2. Voltammetry

The voltammetry method has been developed from "polarography" in the year 1922 by the "Czech Chemist Jaroslav Heyrovsky" [4]. Earlier, this technique had some limitations which caused difficulties during routine analytical processes. However, significant advances in the methodology and instrumentation of this technique during the sixties and seventies, the chemists and biologists were given attention to voltammetry to investigate the redox reaction process (oxidation-reduction), investigate the adsorption phenomenon on various surfaces, reaction mechanism, qualitative and quantitative determination of metal ions, organic compounds and pharmaceutical compounds [5, 6]. There are different categories of voltammetric techniques which are mentioned below:

- Cyclic voltammetry
- Linear Sweep Voltammetry
- Differential Pulse Voltammetry
- Potential Step Voltammetry
- Adsorptive Stripping Voltammetry
- Normal pulse polarography
- Alternative Current Voltammetry
- Polarography
- Anodic Stripping Voltammetry
- Cathodic Stripping Voltammetry

1.2.1. Cyclic Voltammetry (CV)

The CV is considered as a sensitive, selective and versatile technique for investigating the redox (oxidation and reduction) process in the electrochemical system as described theoretically by *Randles* and it was first reported in 1938 [7]. In this system, the current is produced due to electron transfer between the solution and the electrode surface. The amount of current produced can be measured by a pair of working electrode (WE) and a counter electrode (CE) which are attached to a potentiostat. The voltammogram is recorded on a recorder which determines the peak potentials (Ep) and peaks current (Ip) [8]. A voltammogram gives information about adsorption or diffusion-controlled processes and coupled chemical reactions, the thermodynamics, and kinetics of heterogeneous electrochemical reactions [9, 10]. In our present study, we

have focused only on the cyclic voltammetry technique for determination of biologically active compounds by the use of different modified carbon paste electrodes.

1.2.1(a). Principle and Theory of Cyclic Voltammetry

Principle: CV is based on the sweeping of the electrode potential in a known range of initial and final values maintaining a known scan rate. In the beginning of the experiment, the WE is set at a potential ($E_{initial}$) at which no electrode reaction takes place. The potential is swept in an increasing order at a fixed scan rate between two limiting potentials ($E_{initial}$) and (E_{final}) during the measurement (Fig. 1.2) of forward and reverse reaction. As the applied potential changes the current also changes and are to be noted down. When the applied potential is decreasing, a cathodic scan represents negative sign while the applied potential is increasing, anodic scan shows positive sign [11]. From the voltammogram the nature of the electrode reactions can be predicted [12].

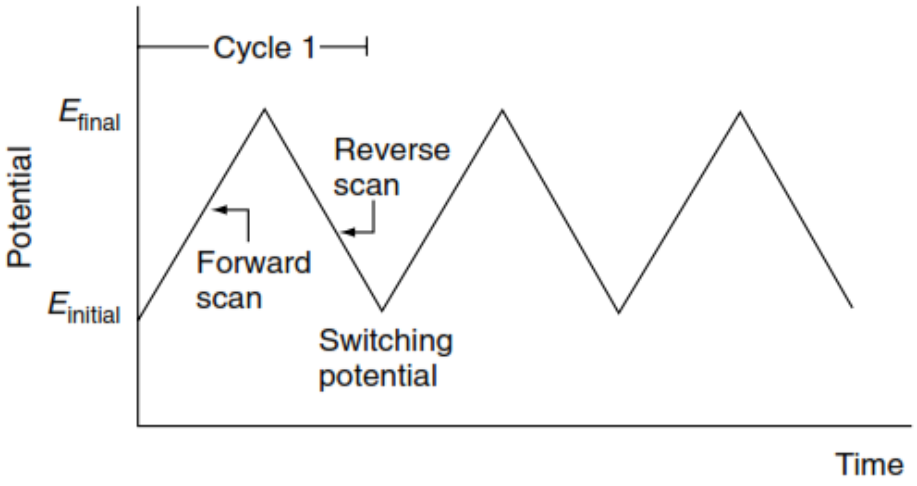


Fig.1.2- Variation of the applied potential as a function of time in a cyclic voltammetry experiment.

1.2.1(b). Electrochemical Process by Cyclic Voltammetry

In CV technique a potential is applied using a three electrode system and a redox reaction takes place. The concentration of oxidized (O) and reduced (R) species differ on the surface of the electrode and in the bulk solution. Hence a diffusion process starts with the analyte molecules moving from the bulk solution to the electrode surface to equalize both concentrations [13]. If the electrode process is reversible (electron transfer is fast) then the current is determined by the rate of mass

transfer of analyte to the electrode. The difference in concentration of the solution on the electrode surface and in the bulk is determined by the value of the applied potential via the "Nernst equation" (Eq. (1.1)) [14].

$$O + ne \longrightarrow R$$

$$E = E^o + RT/nF \ln [Oxi/Red] \text{----------(1.1)}$$

E = Potential (V)

E^0 = Standard electrode potential (V)

n = Number of electron

R = Gas constant (J/K/mol)

T = Absolute temperature (K)

F = Faraday constant (C/mol)

Oxi/Red = [Oxidize species]/[Reduce species]

By putting the values of

$R=8.314\ JK^{-1}mol^{-1}$

$F=96500\ Cmol^{-1}$

T=298 K, equation (1.1) turns into:

$$E = E^o + (0.0591/n) \log [Oxi/Red] \text{ ----------(1.2)}$$

"The volume of solution in which the concentration gradient develops is called a diffusion layer". By using "First Fick's Law" (Eq 1.3), the rate of diffusion can be calculated [15].

$$J = - D_{ox} \delta C_{ox}(x,t) / \delta x \text{----------(1.3)}$$

Where,

J=flux

D_{ox}=diffusion coefficient for oxidized species (in cm^2s^{-1}).

By using "Fick's Second Law", (Eq. 1.4), the rate of change of concentration with time can be calculated [16].

$$\delta C_{ox}(x,t) / \delta x = D_{ox} \delta^2 C_{ox}(x,t) / \delta x^2 \text{----------(1.4)}$$

"Where, $C_{ox}(x,t)$ = the concentration of oxidized species whose value depends on distance (x) from the planar interface and time (t). A similar equation can be derived for

the concentration of the reduced species (C_R). The value of D varies from system to system"[17].

1.2.2. Study of Electron Transfer Process Using Cyclic Voltammetry

"Nicholson and Shain" have introduced the field of electrochemistry in a different approach and carried out quantitative simulations of cyclic voltammetry in the year of 1964. From the shape of the cyclic voltammogram, the nature of the electrochemical reaction can be predicted. There are three kinds of electrochemical reactions-

- Reversible process
- Irreversible process
- Quasi-reversible process

1.2.2(a). Reversible Process

Fig. 1.3 depicts a cyclic voltammogram for a reversible process. The oxidation (O) and reduction (R) peaks are sharp and stable. In the reversible process, the electron transfer occurs at a fast rate if compared with an irreversible and quasi reversible process. The applied potentials and the scan rate of the e^- transfer process on the surface are in equilibrium. The surface concentration follows the "Nernst equation" as shown in (Eq. 1.1).

For a "diffusion-controlled" system the "Fick's Law of Diffusion" holds good for both oxidation and reduction reactions. Under these conditions, the peak current(i_p) is given by "Randles-Sevcik Equation" (Eq. 1.5);

$$i_p = (2.69 \times 10^5)\, n^{3/2}\, A\, D^{1/2}\, C_0\, \upsilon^{1/2} \quad \text{----------} \quad (1.5)$$

where,

n- number of electrons involved in the electrode process

A- area of electrode in cm^2

D_o- diffusion coefficient of the species O in cm^2s^{-1}

C_o - concentration of the species O in mol/cm^3

υ - scan rate in Vs^{-1}.

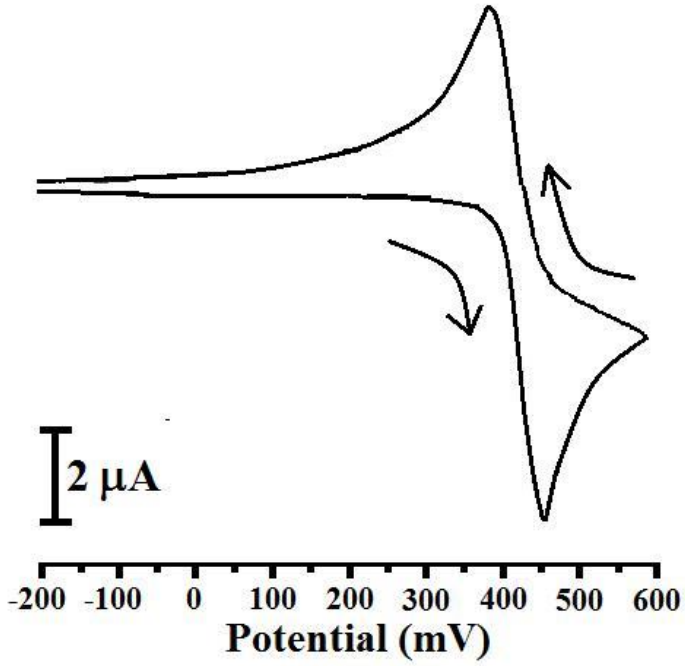


Fig. 1.3- Typical voltammogram for a reversible process.

A diagnostic test can be used to study the cyclic voltammogram to predict the nature of the reaction. For a reversible process the diagnostic test is mentioned below

Diagnostic test for the reversible process at 25ºC.

- $\Delta E_p = E_{pa} - E_{pc} = 59/n$ mV, at all sweep rates where, 'n' is the electrons transferred
- $i_{pc}/i_{pa} = 1$(at all sweeps rates)
- $i_p \alpha \nu^{1/2}$ (υ= sweep rate)
- E_p is independent of ν

1.2.2(b). Irreversible Process

In an irreversible process, the surface concentration of "O" and "R" changes more slowly with applied potential and no reverse peak is observed. In this case, either the peak of oxidized species or peak of reduced species is obtained (Fig. 1.4). For an irreversible process the chemical reaction becomes very slow due to a slow electron exchange on the

electrode surface [18]. For an irreversible electrode process, the mass transfer is much faster as than the charge transfer.

The peak current i_p for an Irreversible reaction can be obtained by following equation (1.6) [19]

$$i_p = 2.99 \times 10^5 \, n \, (\alpha n)^{1/2} \, A \, D_0^{1/2} \, \nu^{1/2} \, C_o \text{----------(1.6)}$$

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

The diagnostic tests justify the irreversible process at 25ºC.

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

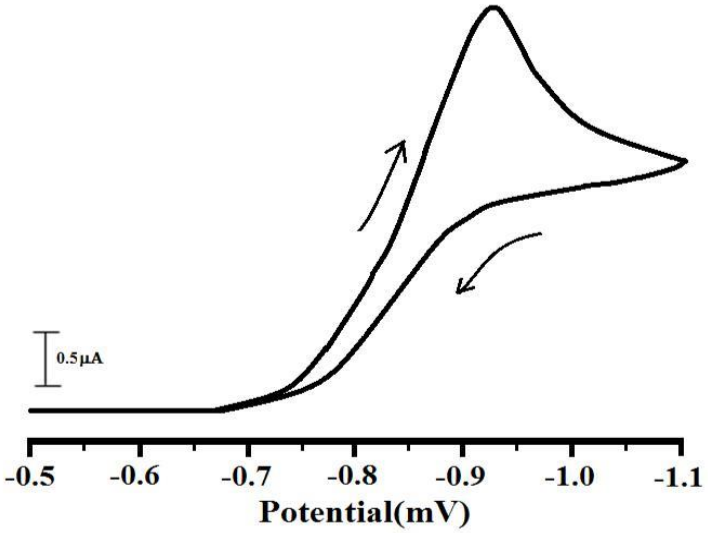


Fig. 1.4- Typical voltammogram for an irreversible process.

1.2.2(c). Quasi-reversible Process

Fig. 1.5 shows the CV profile of the quasi-reversible process. In a quasi reversible process the rate of electron transfer falls between reversible and irreversible process that is the rate is neither as fast as a reversible process nor as slow as irreversible process. It is clearly visible that the peak to peak separation is a larger than that of the reversible process. In this process both electron and mass transfer take place [2, 12]. If the difference in peak potential (ΔEp) is greater than 59/n mV, then it is understood to be a quasi-reversible process. The diagnostic test for this process is described below.

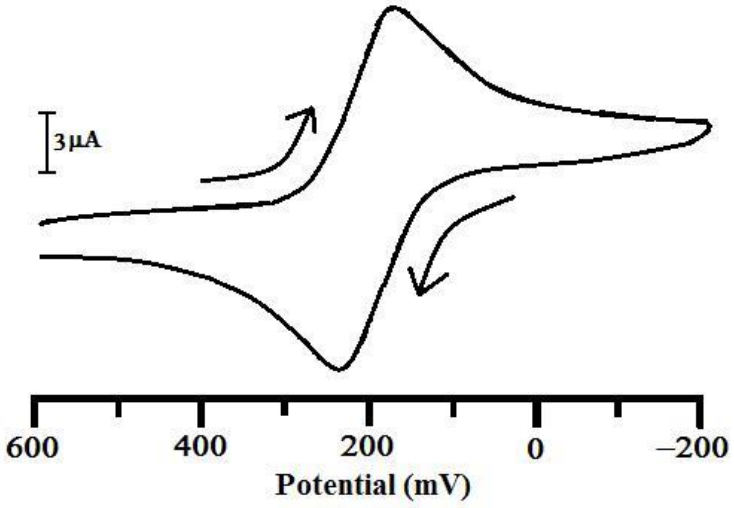


Fig. 1.5- Typical voltammogram for a quasi-reversible process.

Diagnostic tests for the quasi-reversible process at 25ºC are as follows:

- i_p increases with scan rate, but is not proportional to scan rate.
- $i_{pc}/i_{pa} = 1$, provided $\alpha_c=\alpha_a = 0.5$
- ΔE_p is greater than 59/n mV and its increases with increasing scan rate
- E_{pc} shifts negatively with increasing ν

1. 3. The electrode assembly:

In CV techniques, the basic requirement is a proper electrode system which is capable of studying the electron transfer and the current produced during the same. The Fig.1.6 shown a set up of three different electrodes in an electrochemical cell which is also used for the current work.

- The working electrode (WE).
- Reference electrode (RE).
- Auxiliary electrode(AE) or counter electrode(CE)

1. 3.1. Working Electrode

The WE which is also called an indicator electrode exhibits greater polarizing effect due to smaller electrode surface area [20]. The redox reaction of molecular species occurs at the working electrode surface and as the reaction proceeds the potential also varies with time. The most widely used working electrodes are -Platinum (Pt), Gold (Au), Silver (Ag), Glassy carbon electrode (GCE) (Fig. 1.7) as they have a wide range of sensing application, high conductivity and reactivity, low background current and good surface area [21]. The solid electrodes, when electrochemically modified have effective applications to study the dosage forms of biologically active compounds or drugs. In addition, these electrodes are also used for the qualitative and quantitative determination of pharmaceutical formulations [22].

1. 3.2. Reference Electrode

In the voltammetric technique, the most widely used reference electrodes are saturated calomel electrode(SCE) and silver-silver chloride electrode(Ag/AgCl). The reference electrodes are of constant potentials and are used to determine the change in the potential of the working electrode.

1. 3.3. Auxiliary Electrode

The AE is usually made up of inert materials - Pt and gold. It has a relatively large surface area. The process which takes place in electrochemical cell is not affected by this electrode and it only provides the current required by working electrode. In this experiment platinum wire has been used as a counter electrode (Fig. 1.7).

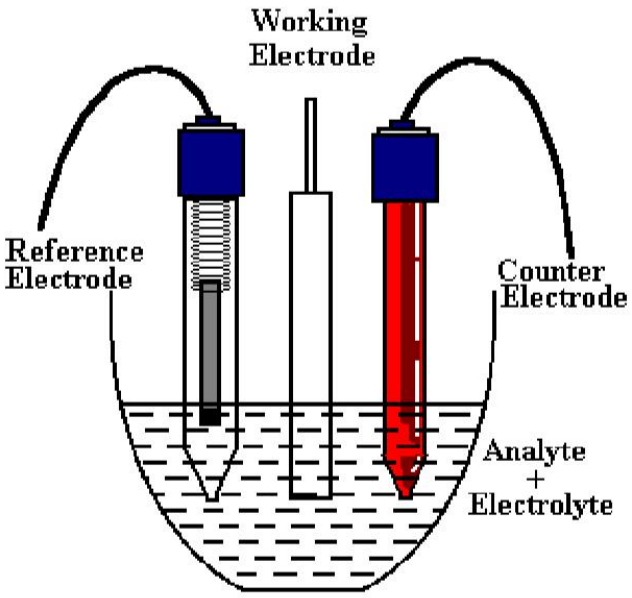


Fig.1.6- Schematic representation of an assembled electrochemical cell containing an electrolyte solution and the three electrodes (WE, RE, and CE) for cyclic voltammetric experiments.

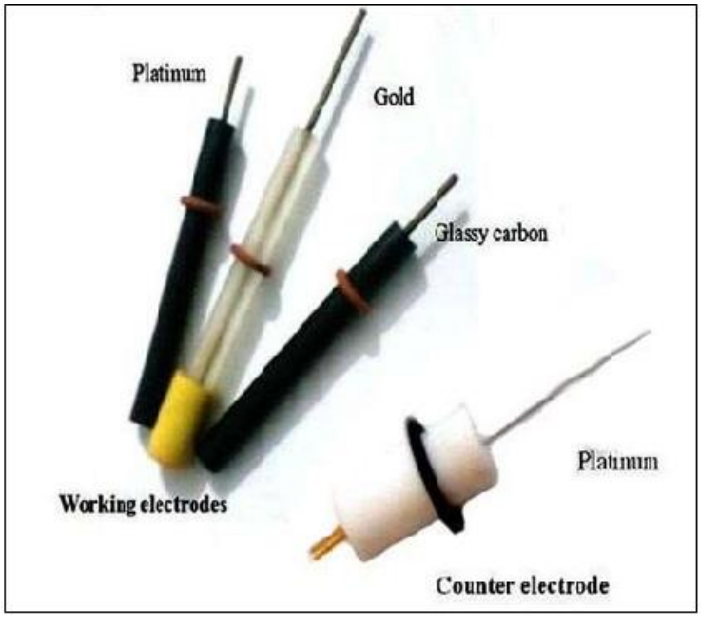


Fig.1.7- Working and counter electrodes

1. 4. Electrode Process

The electrochemical reaction takes place between the electrode and solution interface and can proceed through different steps involving the conversion of the oxidized species into the reduced species (Fig. 1.8). The rate of electrode reaction depends the following factors:

- Rate of mass transfer
- Rate of electron transfer of non-adsorbing species
- Rate of hetero or homogeneous electron transfer
- Rate of other surface reactions like crystallization, desorption, and adsorption, etc.

In an electrochemical process, the charge exchange takes place at the electrode surface, the electro-active substance gets exhausted and concentration gradient established. Under such conditions, reactant species diffuse moves towards an electrode surface and the final formed product, which allows the new reactant to the surface by moving away from the electrode surface. The end result current is essentially depending on two parts;

1) The rate of electron transfer,

2) From the bulk, the rate of movement of the species to the electrode surface. Here, three varieties of mass transport which is able to influence an electrolysis reaction (Fig. 1.9).

- Diffusion: "A Spontaneous movement of the molecules under the influence of concentration gradient that is from a region of high concentration to region of lower concentration aimed at minimizing concentration differences". After a detailed study of the geometry of the electrodes, diffusion process is named by plane, spherical and wire electrode is known as planar, spherical and cylindrical diffusion. The diffusion is governed and characterized by "Flick's Laws" of diffusion [23, 24].
- Migration: "Movement of charged particles along an electrical field".
- Convection: "Transport to the electrode by a gross physical movement. Such fluid flow occurs with stirring or flow of the solution and with rotation or vibration of the electrode (forced-convection) or due to density gradients" (natural-convection).

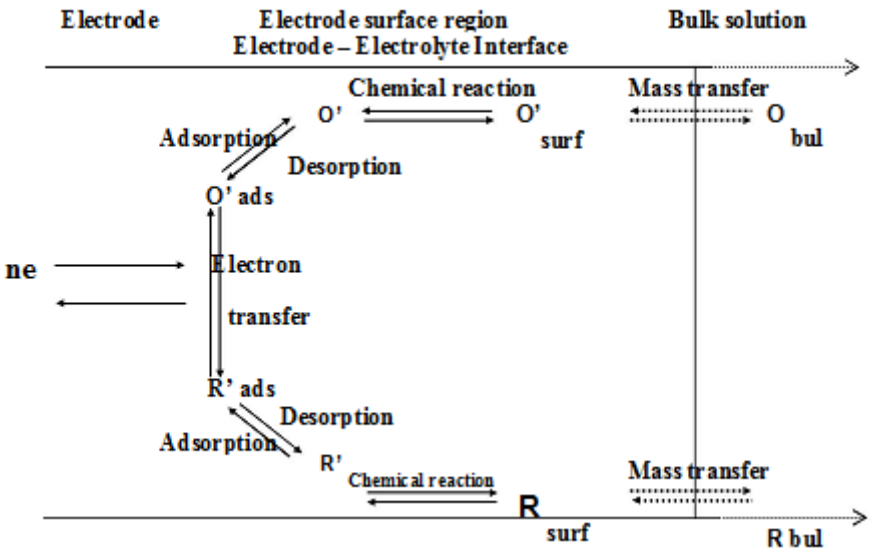


Fig. 1.8- General pathway of electrode-mediated processes of oxidized (O) and reduced (R) electroactive species.

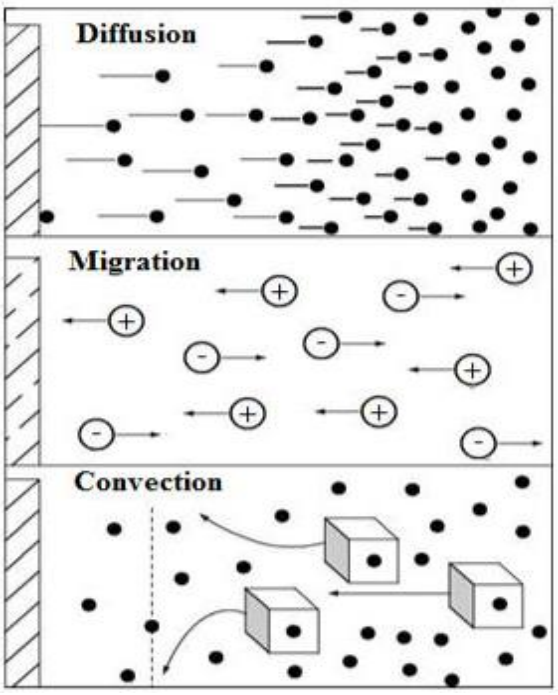


Fig. 1.9- Modes of mass transport.

1.5. Solvent

For electrochemical work choosing a solvent is important and should be considered on the basis of the number of physicochemical properties [25]. A good solvent has the following characteristics:

- The solvent is liquid at room temperatures.
- It must be able to dissolve the biomolecules or analytes and supporting electrolyte completely.
- It must have a wide potential range in the case of redox reaction study.
- The solvent must not help in producing any toxic reactions with the biomolecules or supporting electrolyte.
- It can be purified easily.
- It should have enough solubility for ionic species.
- Crucial parameters for the solvent are its dielectric constant [26].
- Water is considered as the cheapest solvent and is most widely used. It can dissolve many biomolecules and supporting electrolytes. But some molecular species are insoluble in water. In this case, other solvents like methanol, acetonitrile, ethanol, dimethyl sulphoxide (DMSO) and dimethylformamide (DMF) are used.

1. 6. Supporting Electrolyte

- The selected solvent must be able to dissolve the electrolyte.
- For an effective voltammetric technique, it is essential to record the well-developed limiting currents and voltammetric curves. To obtain such outcomes a suitable supporting electrolyte must be preferred and employed.
- The choice of supporting electrolytes depends on the existing potential range, type and degree of interaction with molecular species.
- It must have a very high concentration.
- In the condition of experiments, the supporting electrolyte should be electrochemically and chemically inert.
- It plays an important role in maintaining constant pH, increasing conductivity, and ionic strength.
- It should not get adsorbed on the surface.
- H_2SO_4, $HClO_4$, and HCl are used to study in acidic media and NaOH or KOH are used for alkaline media. To study in the neutral medium a suitable buffer solution is needed. Acetate, citrate and phosphate buffers are normally used [27].

1. 7. Validation Method in Electroanalysis

For the development of new electrochemical procedures, method validation is necessary for the study of application of new biomolecules, the study of bio-equivalence, drug detection, and bio-availability. Types of procedures of method validation for voltammetric studies are as follows:

1. 7.1. Sensitivity

It is a measure of lower limit detection method and has the ability and significant to enhancement associated with the interfacial accumulation. Sensitivity can be stated as "the minimum amount of an analyte that can be reliability measured". In cyclic voltammetry sensitivity and detection limit are similar terms.

1. 7.2. Specificity and Selectivity

In analytical chemistry, the term selectivity and specificity are often used interchangeably [28]. Selectivity and specificity are defined as follows:

- Selectivity: "the ability to differentiate and quantifying the analyte in the presence of other components from its matrix".
- Specificity: "the ability to assess the analyte clearly in the presence of components which might be expected to be present."

1. 7.3. Linearity

An electroanalytical instrument generates the analytical response for the concentration of the analyte (i.e., the quantity of interest) in a solution. So, the quantity of interest is often estimated from a "calibration line". The calibration line is generated from multiple responses from known quantities of multiple standard samples. The plot analytical response versus the quantity of interest gives a straight line and refers to linearity. To get good linearity; at least five concentrations are recommended for the study.

1. 7.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

- **LOD:** It may be defined as "the lowest amount of an analyte in a sample which can be detected but not necessarily quantified as an exact value". Several approaches for determining the detection limit exist. LOD is usually assessed using the calculation of the signal to noise ratio. A signal-to-noise ratio of 3:1 or 2:1 is usually considered acceptable for estimating the detection limit. During the method validation, determination of the LOD is not essential, because the assay may have high variability in that level [29, 30]. In general, LOD is expressed as an analyte concentration in the sample, such as μg mL^{-1}, M, ppb, and ppm.

$$LOD = 3S/m \text{----------} (1.7)$$

- **LOQ:** It is stated as "a characteristic of a quantitative assay for a low level of an analyte in a sample matrix." It can be shown as the lowest concentration of the compound in a sample, the end result which can be accurately quantified. In general, the LOQ is all the time higher than the LOD [31].

$$LOQ = 10S/m \text{----------} (1.8)$$

1. 8. Cyclic Voltammetry-Applications

- The CV is a much more admired technique as compared to other techniques for electroanalytical investigation of new methods.
- It is also confirmed as a sensitive device for studies of systems and getting accurate results from complicated electrode processes [32, 33].
- "CV method can be applied for the evaluation of various thermodynamic and kinetic parameters like the number of electrons transferred (n), heterogeneous rate constant (k_0), entropy (S), Gibb's free energy (G) and diffusion coefficient (D_0)", etc [18].
- The CV methods are used in brain studies of rats [34], *in vivo* studies [35], in bacteria [36] and in plants [37, 38].
- The CV method is applicable for the investigation of a number of inorganic and organic compounds. Rotating disk electrodes can be used to examine industrial corrosion. The voltammetric method can be applied to detect and monitor heavy metal like lead levels in the bloodstreams [39].

Chapter 2

LITERATURE SURVEY

The CV is the powerful electroanalytical tool which has many important applications for the study of electroactive biomolecules. The CV is often used to investigate the oxidation and reduction (redox) processes. Nowadays, many different chemically modified carbon electrode sensors are being used to study important biomolecules, due to their unique electrode surface properties, i.e., development of high sensitivity, rapid response, reliable and simple procedures. The modified sensors can not only boost the reaction rate but also can be sensitive and selective to different biologically active molecules.

- Santos *et al.,* [40] investigated the electro-catalytic behavior of electrodeposited film of glutamic acid on glassy carbon (GC) electrode under neutral conditions (pH 5.6) using a square wave voltammetric scan. They observed a high electrocatalytic activity of the poly (glutamic acid) modified GC electrode towards the oxidation of caffeic acid in red wine samples without interference from other hydroxycinnamic acids or ascorbic acid.
- Wang *et al.,* [41] assessed a poly(taurine) MGCE for the determination of dopamine (DA), epinephrine (EP) and simultaneously in their mixture by using the voltammetric technique. Under the optimum conditions, well peak separation was observed between two compounds and the potential difference shown 390 mV in the CV.
- Dopamine (DA), L-Tryptophan, ascorbic acid (AA), and uric acid (UA) were determined simultaneously at poly(glutamic acid)CNTs/MCPE by using CV by Xiao Liu et al., [42]. The modified carbon paste electrode (MCPE) shows selectivity, sensitivity, good stability, reproducibility, and pharmaceutical formulation towards L-Tryptophan.
- Pablo R. Dalmasso et al., [43] studied the GCE/MWCNT-Polyhistidine electrode towards the oxidation and simultaneous study of AA and PA in their sample mixture in 0.050M PBS at pH 7.4. The GCE/MWCNT-Polyhistidine modified sensor showed an outstanding electro-catalytical action on the oxidation of AA and PA by cyclic voltammetry and differential pulse voltammetry technique.
- S. Chitravathi et al., [44] developed a poly(naphthol green B) MCPE for the electrochemical studies of DA in presence of UA in B-R (Britton-Robinson) buffer solution by an electro-fabrication technique using CV. The result from the fabrication of MCPE revealed the high electro-oxidation activity of DA.
- Aslanoglu *et al.,* [45] reported the study of electrooxidation for DA with AA using at poly(3-Acetythiophene) MGCE by CV and DPV. The result shows that the poly(3-Acetythiophne) MGCE enhanced the rate of the e^- transfer process of DA.

- Simultaneous study of AA and DA by thin film-coated N, N, dimethylaniline (DMA) on glassy carbon electrode at pH 7.0 of 0.2 M PBS by CV was reported by Roy *et al.*, [46]. The good oxidation peak separation was observed at 300 mV for AA and DA at a MGCE and which was large enough for the simultaneous determination of these two molecular species in their mixture solution.
- Zhang *et al* [47] developed the poly(styrene sulphonic acid) sodium salt/single-wall carbon nanotube film MGCE for the determination of AA and DA.
- Chen *et al* [48] have been reported poly(toluidine blue) MGCE for the simultaneous study of DA in the presence of AA. The resulting MGCE can catalyze the electrooxidation DA and AA.
- Maofang He et al., [49] developed a poly(folic acid) MCPE characterized by scanning electron microscopy(SEM) to study electrochemical oxidation of DA by CV technique.
- Electrooxidation of DA in presence AA and UA at poly(eriochrome black T) MCPE in PBS of 0.05 M at pH 4.0 by cyclic voltammetry technique was developed by Yao *et al.*, [50]. The resulting poly(folic acid)MCPE showed excellent electrocatalytic activity and enhanced the redox current towards the simultaneous oxidation of DA, AA, and UA.
- Electrochemical determination of acetylsalicylic acid (ASA) at a platinum electrode in aqueous solutions by CV and DPV techniques was developed by Edyta Wudarska et al., [51]. The reaction of oxidation and its kinetics has been investigated. They observed that the resulting rate constant, diffusion coefficients and electron transfer coefficient were determined for ASA electrooxidation.
- M. Madej et al., [52] conducted the oxidation of viloxazine by DPV with a boron-doped diamond electrode. The best response was observed in phosphate buffer among different buffers tested. The LOD value for DPV was found to be 0.04 μmol dm^{-3}. Further, the method was adopted for the analysis of viloxazine in the spiked river and tap water samples with a revival of 95.8-98.8%.
- C. González-Vargas et al., [53] studied the detailed determination of anti-hypertensive drug hydrochlorothiazide at the polymer film of L- and D- glutamic acids modified electrode by DPV technique. The analysis was carried out in Britton-Robinson buffer. Further DPV techniques were developed for the trace determination of HCTZ from pharmaceutical forms.
- The antihistamine drug pheniramine was investigated in solubilized systems by a modified glassy carbon electrode with a multi-walled carbon nanotubes sensor by R.

Jain et al., [54]. Linearity was exhibited in the range of 200-1500 mg/mL with a correlation coefficient of 0.9987. The limit of detection was found to be 8.31mg/mL. Good reproducibility and repeatability were shown by the modified electrode.

- Ensafi et al., [55], studied the determination of azithromycin based on multiwalled carbon nanotubes modified with $MgCr_2O_4$ by voltammetry. The amelioration in peak current was observed at pH 7.0 of PBS. Lower LOD value and excellent stability of the electrode were accounted for. Furthermore, the process was applied for the practical detection of the drug in real matrix samples.
- Zayed et al., [56] reported the determination of amiloride hydrochloride at MCPE by using the voltammetric technique. The modified electrode exhibits an enhanced peak current with decreased potential. Under the optimal concentration range of AmilCl (0.60-4.23 µg/ml), the LOD and LOQ were found to be 0.26 and 0.87 µg/ml respectively.
- A newly constructed poly-L-methionine-gold-nanocomposite/MWCNT MGCE for the electrochemical oxidation of amlodipine was studied by Emami et al., [57]. Under the optimal conditions, the linear range of amlodipine covering from 5 nM to 2.5µM along with 1 nM LOD was obtained.
- Sanati et al., [58] studied a voltammetric sensor for two drugs i.e. morphine and diclofenac. The modification was achieved via NiO/CNTs ionic liquid CPE. The modified electrode exhibits an enhanced peak current with decreased potential. The LOD value was found to be 0.01µM in morphine using the square wave voltammetry (SWV) method. The electrode was successfully employed for these drug analyses in tablet samples and human plasma samples.

Chapter 3

AIM AND OBJECTIVES

3.1. Aim

"To develop new bio-sensors to assess electroanalysis of biological compounds including drugs in the very minute concentration".

3.2. Objectives

1. To design electrochemical (bio) sensors based on the modification of bare electrodes surface by fabrication and polymerization method.
2. To develop a more sensitive, selective and simple electrochemical method using modified carbon paste electrode sensors (MCPE's).
3. To examine the effect of modifier amount, pH effect, scan rate variation, and concentration-effect for the selected drugs using cyclic voltammetry technique to characterize the redox (oxidation and reduction) behavior.
4. To explore the electrochemical behavior of the analytes (reversible/irreversible/quasi-reversible) and electrode processes (adsorption controlled/diffusion-controlled) by CV technique.
5. To illustrate the probable reaction mechanism on the electrode surface.
6. To check the interference in the examination and to validate the method for the pharmaceutical analysis in tablets and injections.

Chapter 4

EXPERIMENTAL PART

In the present study electrochemical investigation of some important selected biologically active molecular species has been performed out cyclic voltammetry technique. The details of chemicals instrumentation and methodology are specified below;

4.1. Instrumentation:

4.1.1. Cyclic Voltammetry

All the investigations were done using an electrochemical workstation (model CH-Instrument-660c electrochemical analyzer USA) and connected to IBM PC and printer (Fig. 4.1). This electrochemical work station is functional for six electroanalytical techniques. The electrochemical cell, potentiostat, and the recorder are the most important components of the workstation.

Three-electrode cell connected to the analyzer was used for the entire study. The electrode cell system is as follows:

- RE- A saturated calomel electrode
- CE- A platinum wire
- WE- Poly-NA/MCPE, CTAB/Modified, CZ/MCPE and (poly)-NC/CPE.

All the voltammograms were recorded at temperature 25±0.2 °C. The oxidation potential of the resultant biomolecules was recorded with saturated calomel electrode, counter electrode, and working electrode assembly.

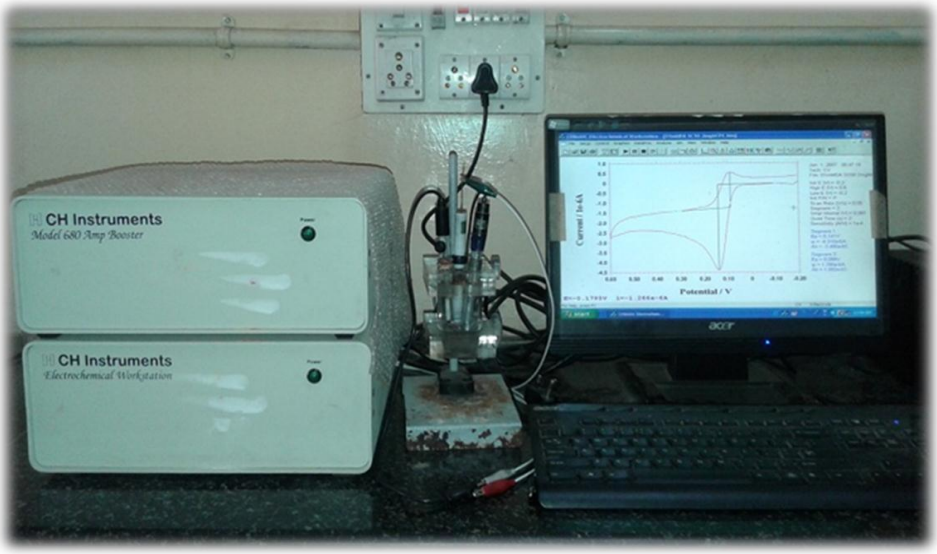


Fig. 4.1- Experimental set-up used to record all electrochemical measurement.

4.1.2.pH Meter

A pH meter Systronics made MK-IV was used for checking the pH of different solutions.

4.1.3. Apparatus

Beakers, volumetric flasks, pipettes measuring cylinders all were used in this experiment with Borosil grade.

4.2. Reagents and Chemicals

All the chemicals, biologically active compounds (i.e., catechol, epinephrine, mesalazine, paracetamol, niacinamide, niacin, carbamazepine and uric acid etc), surfactant Cetyltrimethylammonium bromide (CTAB), phosphate buffer ($NaH_2PO_4 \cdot H_2O$ and Na_2HPO_4), silicon oil and other chemicals and reagents used throughout this study were purchased from "Himedia" chemicals (Mangalore-India) and "Sigma-Aldrich" (Mumbai-India). Graphite powder of particle dimension 50.0μM was acquired from "Merck".

4.3. Preparation of Solutions

All the stock solutions of biologically active compounds and buffer were prepared freshly before each experiment with double distilled water at 25±0.2 ℃.

4.3.1. Preparation of Buffer

0.2 M of phosphate buffer solution (PBS) was prepared by mixing a proper ratio of $NaH_2PO_4 \cdot H_2O$ and Na_2HPO_4, using double-distilled water.

4.3.2. Preparation of Biologically Active Compounds

The stock solutions of catechol, epinephrine, mesalazine, paracetamol, and uric acid about concentration (25×10^{-4} M) were prepared by using double distilled water at temperature 25±0.2 ℃.

4.3.3. Preparation of Other Chemicals

The stock solutions of other chemicals such as niacinamide, niacin, and carbamazepine (25×10^{-3} M), CTAB surfactant (10×10^{-3} M) were prepared by using double distilled water at temperature 25±0.2 ℃. These solutions were used as modifiers on the surface of the electrodes.

4.4. Preparation of Bare Carbon Paste Electrode (BCPE)

"Graphite powder and silicone oil" were mixed in the ratio of 70:30(%) an agate mortar and then homogenized. This prepared homogenized mixture is the required carbon paste for the experiments. A portion of the carbon paste was packed firmly in the cavity of a PVC tube of internal diameter 3.0 mm and the surface was made smooth by rubbing

against the weighing rinse paper. Then the surface is washed with water. The paste was removed after every measurement, to get a new surface. The resulting electrode was used as a carbon paste electrode. A copper wire was connected to the end of the tube to make external electrical contact (Fig.4.2).

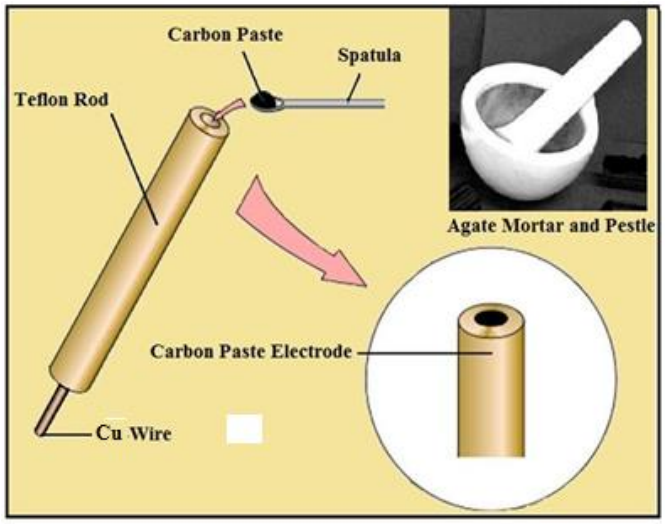


Fig. 4.2- Carbon paste electrode, preparation and filling.

4.5. Preparation of Modified Carbon Paste Electrode (MCPE)

The development of MCPE's has got attention in recent years. The MCPE can be prepared by polymerization, attaching specific molecules, or film coating on the surface of the electrodes. These new modified electrodes show interesting properties that may form the basis of new applications and novel devices. In general, the most important cause for changing an electrode surface is to get qualitatively new biosensors which may show superior catalytic effects, diminish the overpotential, enhance the rate of reaction along with improvement in the sensitivity and selectivity of bio-molecules, etc.

In the present work, various modifiers were adopted like Niacinamide, Carbamazepine, Niacin, and surfactant like Cetyltrimethylammonium bromide (CTAB) and the modified electrodes were prepared by several different techniques like:

- Spontaneous adsorption
- Electropolymerization
- Grind modification
- Surface modification

4.6. Determination of Drugs in Tablets

All the drugs used in the experiment in their tablet form were purchased from the local pharmacy. Each tablet was finely powdered by using mortar-pestle. A stock solution of concentration 1.0×10^{-3} M was prepared for each tablet by taking the required calculated amount dissolved in 100 mL double distilled water in volumetric flask. The clear supernatant solution was obtained, and then it was transferred to a cell containing relevant pH solutions. The proper amount of this solution was analyzed using cyclic voltammetry in optimal conditions and voltammograms were recorded in a potential range of -0.2 V and 0.8 V maintaining the scan rate of 0.05 V/s. A recovery test was performed to check the interferences. In case higher percentage recovery obtained, that indicates no interferents and additives interfere during the tablet analysis and resulted in low SD±RSD values implying the reproducibility of the method.

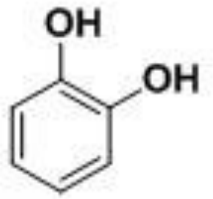
Chapter 5

RESULTS AND DISCUSSION

5.1. Poly-Niacinamide/MCPE Sensor for Catechol

5.1.1. Preamble

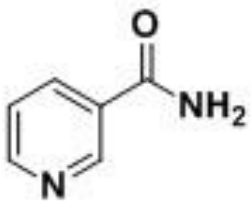
Catechol (CC) or 1,2-dihydroxy benzene is a simple organic compound having two hydroxyl groups bonded to a benzene ring (Scheme 5.1.1). It is found that catechol is a component present in many vegetables and tea. During the manufacture and processing of these natural products, catechol is released to the atmosphere [59]. CC shows antioxidant and antiviral activities along with the power of stimulating flowers and affecting some enzymes. Hence it is biologically significant [60-62]. It is also an environmental pollutant as it is toxic and does not degrade easily [63].



Scheme 5.1.1- Structure of catechol.

From the earlier studies, it has been understood that catechol can be estimated by using various analytical methods. A few methods like iodine-coated poly-crystalline platinum electrode [64], clay-modified electrode [65], bare indium tin oxide electrodes [66], poly(brilliant blue)MCPE [67], MWCN/MGCE [68], anthraquinone MCPE [69], screen printed graphite electrode [70] electro-chemiluminescence [71], p-aminosalicylic acid and MWCN composite film MGCE [72] and DL-norvaline MGCE [73] method were lengthy, complicated and incapable to detect very low concentration. Our study was focused to develop a more accurate and low-cost method.

Niacinamide (NA) (pyridine-3-carboxamide) is the active form of vitamin B_3 which is readily soluble in water. It is also known as nicotinamide. It is used as a dietary supplement and medication. Its chemical formula is $C_6H_6N_2O$ and molar mass is 122-127 g/mol. Due to its important biological action, it is broadly examined far more than the last few decades (Scheme 5.1.2) [74]. In the present work modified electrode sensor is developed by electropolymerization of niacinamide and this sensor is used for the study of CC by CV method.



Scheme 5.1.2- Structure of niacinamide (NA).

5.1.2. Experimental

The instrumentation, chemicals-reagents, and preparation of BCPE discussed in (section from 4.1 to 4.4; page no. 25-27).

5.1.3. Observations and Analysis

5.1.3(a). Electrochemical Polymerisation and Preparation of Poly Niacinamide MCPE

The poly-NA/MCPE was made-up by the cyclic voltammetry technique. The electrochemical cell containing aqueous solution of 1.0×10^{-3} M niacinamide monomer in 0.2 M phosphate buffer solution of pH 7.4 (physiological pH). The potential window was maintained between -0.8 V to +1.8 V at a scan rate of 0.1 V/s by applying 10 multiple cycles. The potentials cycle was repeatedly applied until a stable voltammogram was obtained. By the application of multiple cycles, the voltammogram was slowly descended with an increase in the number of cycles as shown in Fig. 5.1.1. This result indicates the deposition of a thin layer of niacinamide on the BCPE and hence the preparation of the poly-NA/MCPE was confirmed. The probable reaction mechanism for CC was predicted and is described in Scheme 5.1.3 which is well corroborated with earlier reports [73].

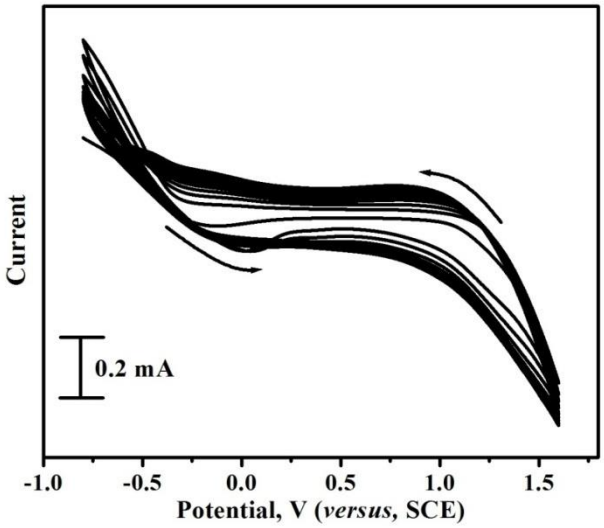
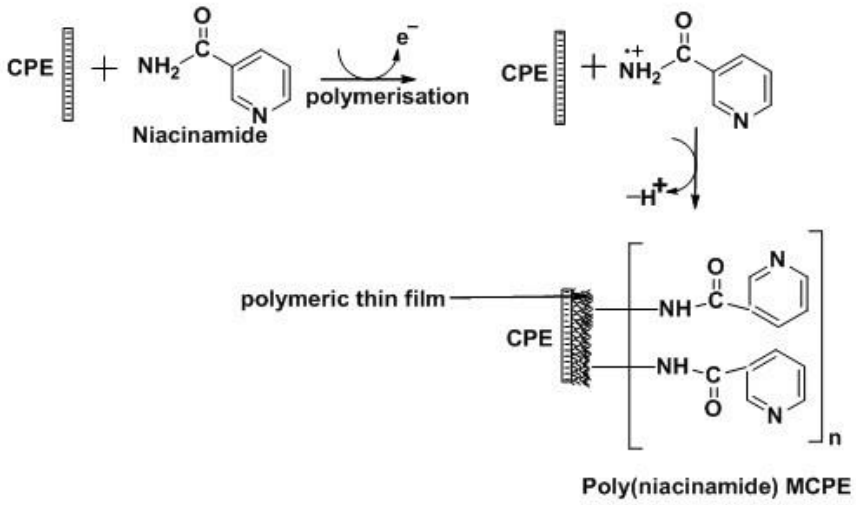


Fig. 5.1.1- Cyclic voltammograms of preparation of poly-NA/MCPE, 1.0×10^{-3} M aqueous solution of niacinamide in 0.2 M PBS of pH 7.4 at 10 cycles with scan rate of 0.1 V/s.



Scheme 5.1.3- Mechanism of electropolymerisation of niacinamide on the surface of BCPE.

5.1.3(b). Characterization of Poly-NA/MCPE

The CV response of 1.0×10^{-3} M "potassium ferrocyanide ($K_4[Fe(CN)_6]\cdot3H_2O$)" in 1.0 M KCl as a electrolyte was recorded by maintaining a scan rate of 0.05 V/s (Fig.5.1.2). From the figure, it is clear that the CV response with BCPE (dashed line) is much less sensitive than that of poly-NA/MCPE (solid line). This indicates that the surface morphology of poly-NA/MCPE is remarkably changed and this modified electrode has better electrocatalytic activity than BCPE. The total surface area available for the reaction can be calculated by using the "Randles-Sevcik Equation" [75] as discussed in chapter-1(section 1.2.2(a); page no. 7-8). The calculated electroactive surface area for poly-NA/MCPE was found to be 0.0412 cm^2 as against the surface area of BCPE 0.0288 cm^2.

"Approximate surface coverage area of poly-niacinamide on the surface of CPE was computed by the following equation (5.1.1) [76] and it was calculated to be 0.0476×10^{-10} M/cm^2".

$$Ip = n^2F^2A\Gamma\upsilon/4RT \text{ ----------(5.1.1)}$$

Where,

- Γ (M/cm^2)-represents the surface coverage concentration which is proportional to the peak current (Ip),
- υ-is the scan rate,
- A-is the geometric surface area of the electrode,
- n-is the number of electrons involved in the reaction and
- R, F and T have their standard significance.

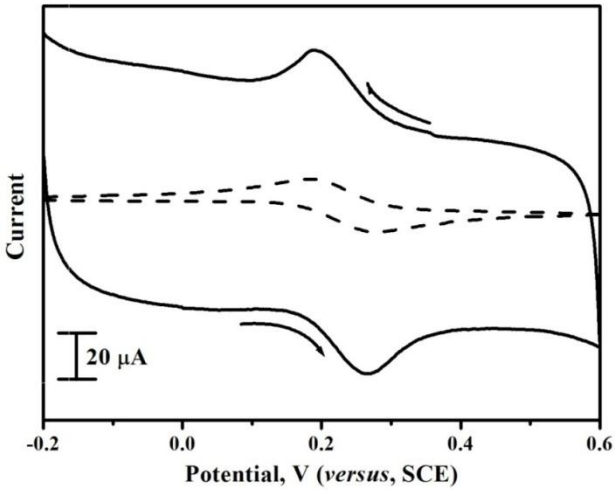


Fig. 5.1.2- Cyclic voltammograms of 1.0×10^{-3} M potassium ferrocyanide at BCPE (dashed line) and poly-NA/MCPE (solid line) at scan rate of 0.05 V/s.

5.1.3(c). Electrochemical Behavior of Catechol at Poly-NA/MCPE

The CV behavior of CC was investigated with BCPE and poly-NA/MCPE (Fig. 5.1.3). From Fig.5.1.3 it is evident that with BCPE, oxidation peak potential of CC was 0.322 V and the current signal was poor (dashed line). Whereas with poly-NA/MCPE the current signal is improved significantly and oxidation peak potential was observed at 0.204 V (solid line). The enhanced current signal with poly-NA/MCPE indicates fast electron transfer phenomenon as compared to BCPE. Also the minimization of overpotential and enhancement of current behavior proves that the poly-NA/MCPE is electrocatalytically active towards oxidation of catechol [67].

The proposed method of oxidation of catechol and its electrocatalytic response towards the modified electrode sensor is shown in Scheme 5.1.4 and this type of mechanism has been proposed in earlier works [77, 78].

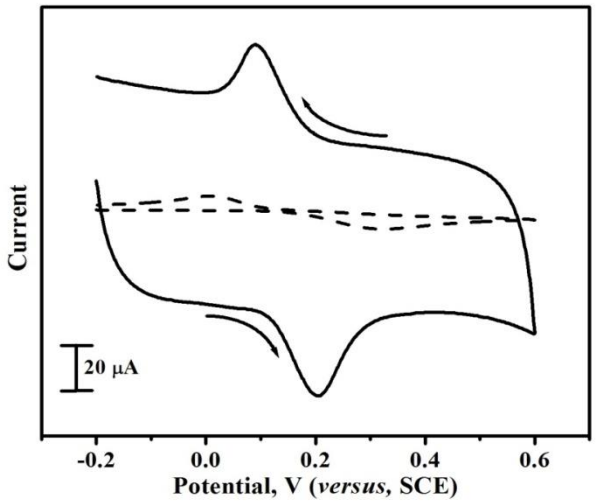
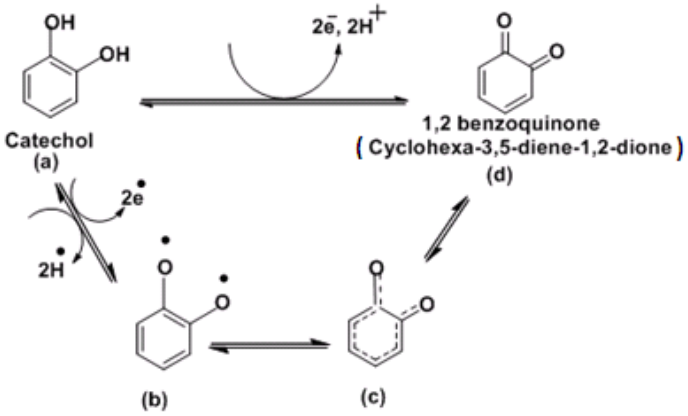


Fig. 5.1.3- Cyclic voltammograms for 0.2×10^{-3} M CC at BCPE (dashed line) and poly-NA/MCPE (solid line) in 0.2M PBS of pH 7.4 at scan rate 0.05 V/s.



Scheme 5.1.4- Oxidation mechanism of catechol.

5.1.3(d). Variation of Scan Rate

The effect of varying scan rate on the CV behavior of CC was investigated to realize the nature of the electrode reaction. Hence the cyclic voltammograms were recorded (Fig.5.1.4(a)) with 0.2×10^{-3} M CC in physiological pH of 0.2 M PBS with poly-NA/MCPE by varying the scan rate from **0.025 V/s - 0.2 V/s**. The plot Ip versus υ was obtained as shown in Fig.5.1.4(b). The peak current is directly proportional to the scan rate satisfying "Randles-Sevcik Equation". Another graph was plotted between Ip versus $\upsilon^{1/2}$ as shown in Fig. 5.1.4(c). Both the plots are linear with a satisfactory correlation coefficient (r^2). The values of r^2 are 0.9936 and 0.9960 for the graph Ip versus υ and the r^2 0.9974 and 0.9966 for the graph Ip versus $\upsilon^{1/2}$. This signified that the electrode process was "diffusion-controlled" [79, 80].

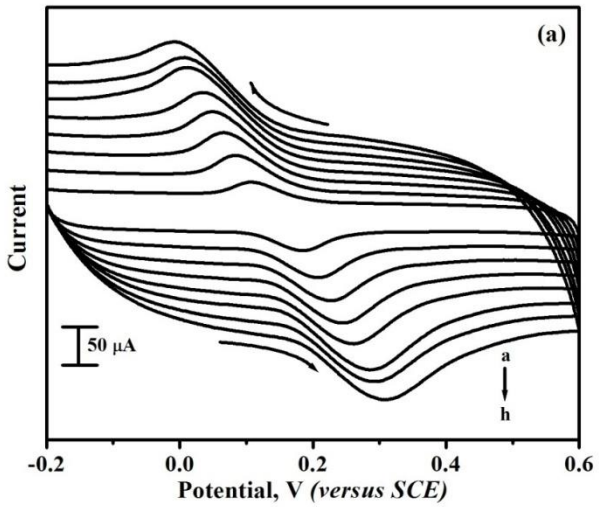


Fig. 5.1.4(a)- Cyclic voltammograms for 0.2×10^{-3} M CC at poly-NA/MCPE in 0.2 M PBS of pH 7.4 at different scan rate (a-h; 0.025 V/s, 0.05 V/s, 0.075 V/s, 0.1 V/s, 0.125 V/s,0.15 V/s, 0.175 V/s and 0.2 V/s).

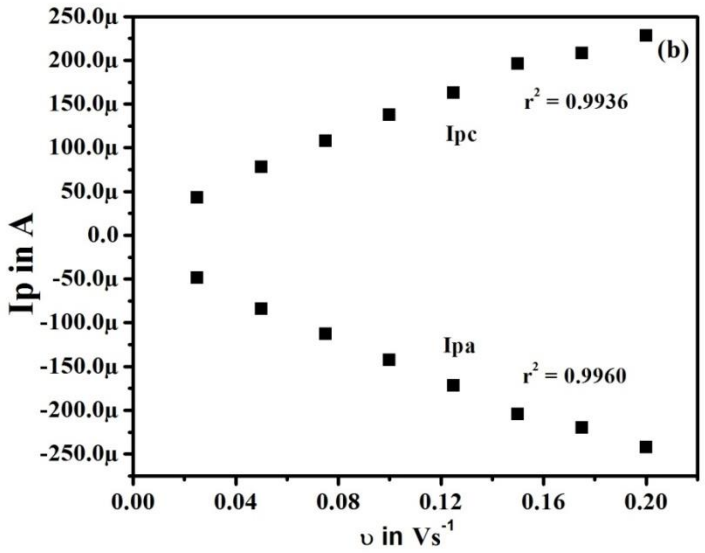


Fig. 5.1.4(b)- The Graph of peak current (Ip) versus scan rate (υ).

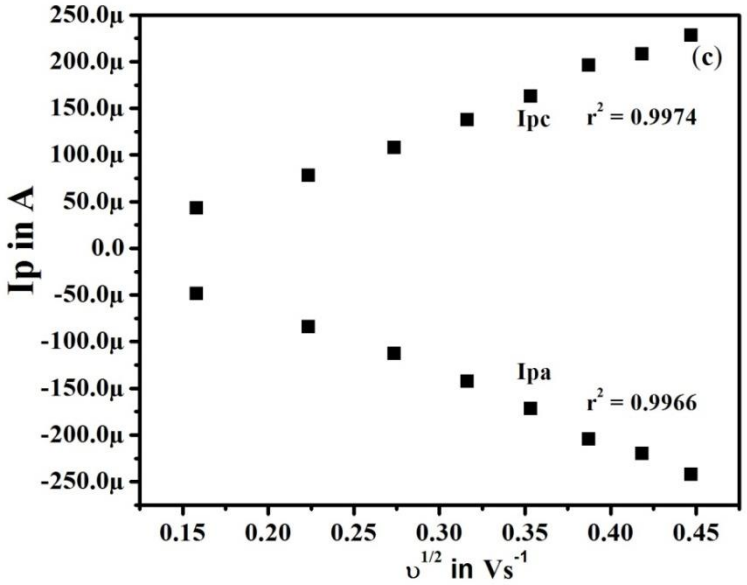


Fig. 5.1.4(c)- The Graph of peak current (Ip) versus square root of scan rate ($\upsilon^{1/2}$).

5.1.3(e). Variation of pH

The CV profile of catechol was observed by varying the pH of the solution in the range of 6.0-8.0 and the observations were recorded (Fig. 5.1.5(a)). It was observed that when the pH was increased the oxidation potential was shifted to the negative side. A graph between Epa versus pH was plotted to understand the number of electrons and

protons involved in the reaction. The graph Fig. 5.1.5(b) indicates that the Epa decreases linearly with increase in pH. The slope of graph was found to be 0.0558 which suggests that there is an involvement of "equal number of electrons and protons" in the electrode reaction mechanism obeying the "Nernst equation". The earlier information [67, 78] supported our present findings.

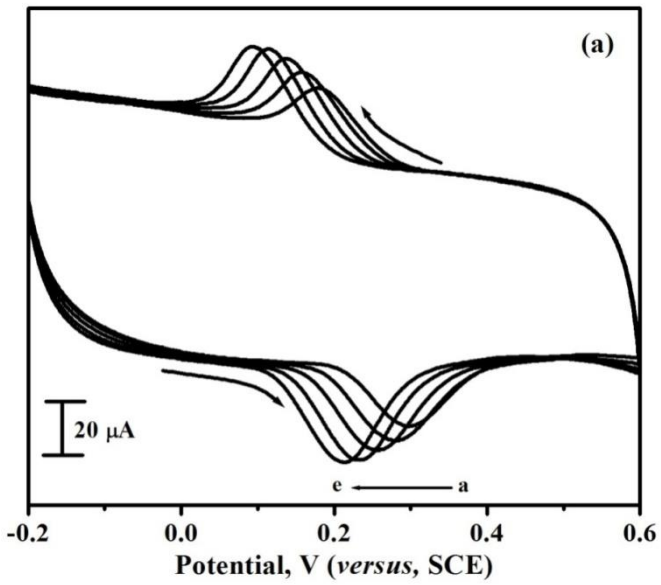


Fig. 5.1.5(a)- Cyclic voltammograms of 0.2×10^{-3} M CC at poly-NA/MCPE in 0.2 M PBS solution of different pH values (a-e: 6.0 to 8.0) at scan rate of 0.05 V/s.

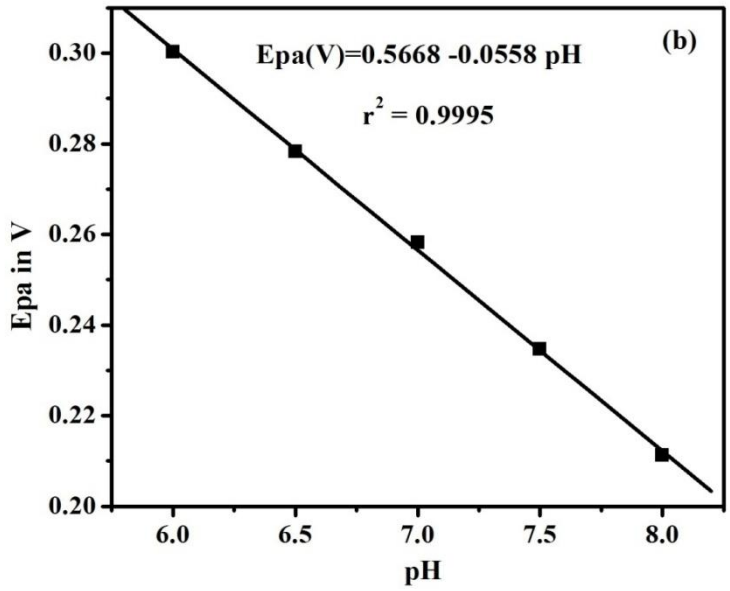


Fig. 5.1.5(b)- The effect of pH on the peak potential response of CC.

5.1.3(f). Variation of Concentration

The concentration of CC was varied in the range of 20.6 μM to 229.0 μM to study the oxidation behavior of catechol by CV techniques. The cyclic voltammograms obtained are as shown in Fig.5.1.6(a). The Ipa increases with the increase in concentration, with a very slight shift in Epa. A graph of Ipa versus [CC] which is a straight line also supports the fact that concentration and Ipa are linearly dependent (Fig.5.1.6(b)). The LOD was found to be 1.49 μM and the LOQ was found to be 4.99 μM. Further, the LOD values were compared with those obtained from other methods as tabulated in Table 5.1.1. From the table it is clear that the technique used in the present work produced better result compared to other reported methods [66, 73, 81-83].

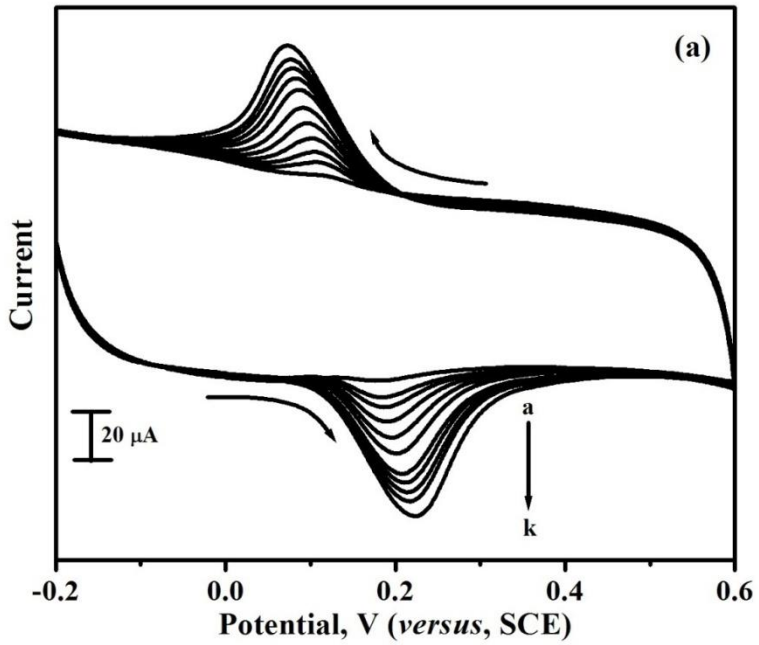


Fig. 5.1.6(a)- Cyclic voltammograms of CC in 0.2 M PBS solution of pH 7.4 at poly-NA-MCPE at scan rate of 0.05 V/s with different concentrations (a-k: 20.6 μM, 40.9 μM, 60.9 μM, 80.6 μM, 100.0 μM, 119.0 μM, 137.7 μM, 156.2 μM, 174.4 μM, 192.3 μM and 229.0 μM).

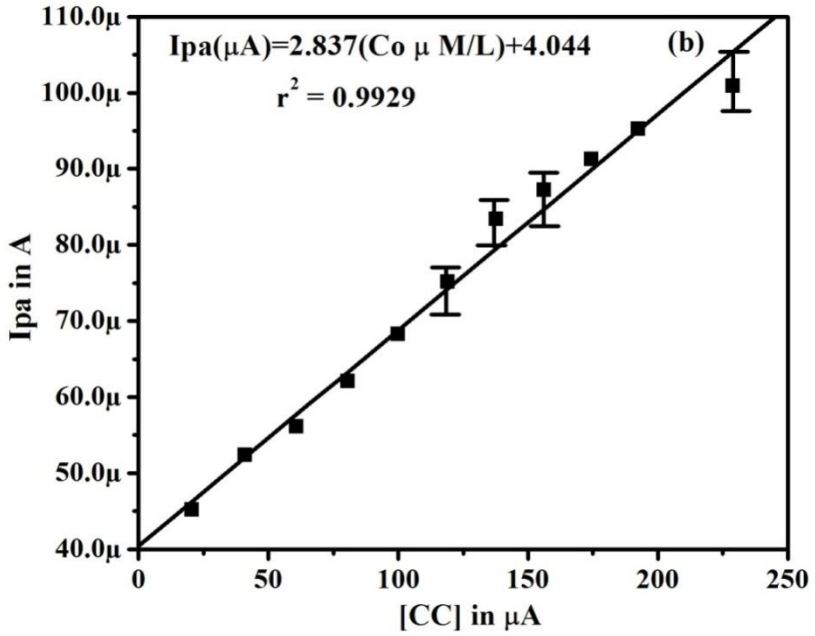


Fig. 5.1.6(b)- Graph of anodic peak current versus concentration of CC.

Table 5.1.1- Comparison of linear range and detection limits for catechol with different classical methods and electrodes.

Classical methods	Electrode/modifier biosensors	Detection limits (μM)	Detection Potential (V)	Refs.
DPV	Bare indium tin oxide electrode	1.0	0.26	[66]
DPV	DL-norvaline modified GCE	0.8	0.37	[73]
DPV	[Cu(Sal-β-Ala)(3,5-DMPz)2]/SWCNTs/GCE	1.46	0.07	[81]
DPV	GCE/with a composite consisting of silver nanoparticles (AgNPs), polydopamine, and graphene	0.1	-	[82]
DPV	PASA/MWNTs composite film modified GCE	1.0	-	[83]
CV	Poly-NA modified CPE	1.49	0.20	Present work

DPV - Differential pulse voltammetry
CV-Cyclic voltammetry
GCE-Glassy carbon electrode
Sal-Salicylaldehyde
β-Ala - β-alanine
3,5-DMPz - 3,5-dimethylpyrazole
SWCNTs - Single-walled carbon nanotubes
PASA/MWNTs - Poly-amidosulfonic acid and multi-wall carbon nanotubes

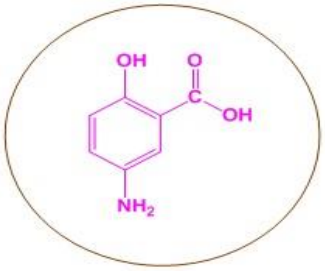
5.1.4. Conclusion

A poly-NA/MCPE was used for the determination of CC in physiological pH of 7.4 by CV technique. The poly-NA/MCPE showed higher sensitivity, better selectivity, and lower detection limits when compared to the BCPE. The study established that the electrochemical process is reversible, diffusion-controlled with involvement of equal number of protons and electrons. Under most selective conditions, the cyclic voltammetry response of CC in the concentration range from 20.6 μM to 229.0 μM, the LOD and LOQ were found at 1.49 μM and LOQ 4.99 μM respectively which are better than other reported methods.

5.2. CTAB-Immobilized/MCPE Sensor for Mesalazine

5.2.1. Preamble

Mesalazine (MSZ), (Scheme 5.2.1) or (5-aminosalicylic acid [5-ASA]) is used to treat inflammatory bowel diseases (IBD) like “Ulcerative Colitis and Crohn’s Disease”. These diseases can be treated with mesalazine if the condition is mild to moderately severe. The action of Mesalazine is believed to be topical that is at the site of the inflammation, especially at colon as it acts on the colonic mucosa and reduces inflammation. Not only that, mesalazine can also prevent the onset of colorectal cancer which may arise due to chronic inflammation [84-86]. Mesalazine is available in various oral and rectal formulations like tablets, micro pellets, suppositories, and enema.



Scheme 5.2.1- Structure of mesalazine.

MSZ works on the cell line and reduced the swelling of cell mucosa by removing reactive oxygen metabolites, thus limiting their toxicity. Moreover, it is found that when MSZ is administered orally maintaining mild to moderate doses, the small intestine cell wall can absorb this drug rapidly and reduces inflammation [87]. This drug should not be used in children suffering from aspirin allergy and kidney disease. Adverse effects are mainly “gastrointestinal” including headache, nausea, abdominal pain, and diarrhea. Oral consumption of MSZ may cause “agranulocytosis, leukopenia, and neutropenia” as well as hair loss, liver problems [88].

As per the literature, different analytical methods like HPLC [89], RP-HPLC [90], fluorescence spectroscopy [91], spectrophotometry [92, 93], ultra-performance-LC [94], LC-MS/MS [95], and HPLC-ESI-MS/MS [96] were used to estimate Mesalazine. These processes involve complicated derivatization procedures, use of expensive equipment, tedious extraction procedures and less sensitivity, etc. At the same time, the study of MSZ by CV techniques is more credible as it has a number of advantages like a rapid response, low price, accuracy, specificity, simplicity and more sensitivity [97-100].

Surfactants are widely used for electrochemical investigation as these amphiphilic molecules are easily adsorbed in the interfaces and surfaces. A few studies have reported that the formation of micellar aggregates on electrode surfaces affected the redox

potential, diffusion coefficients of electrode processes and charge transfer coefficients [101-103]. Generally, adsorption of surfactant molecules on the interface and on the surfaces begins much below the critical micelle concentration (CMC) [104, 105]. Hence to prepare a surfactant immobilized modified electrode low surfactant concentrations (below CMC) can be used. If the surfactant is cationic by nature then, on adsorption of surfactant, the electrode surface becomes positively charged. Similarly, the anionic surfactant makes the electrode surface negatively charged. "The charged electrodes affect the charge transferring rates and subsequently affect the oxidation potential in electrochemical measurements". "The use of the surfactant modified electrodes for the determination of biological molecules is reported earlier [106-111]. The determinations of MSZ by different MCPE were reported earlier [112-116] but no work involving a surfactant modified carbon paste electrode to determine Mesalazine was reported. Hence, the present work is adopted to develop a simple voltammetric sensor by immobilizing CTAB on the surface of CPE for the determination of MSZ.

5.2.2. Experimental

The chemical, instrumentation part, and preparation of BCPE have been discussed in (section from 4.1 to 4.4; page no.25-27).

5.2.2(a). Preparation of CTAB-immobilized MCPE

The "cetyltrimethyl ammonium bromide" (CTAB) immobilized MCPE (i.e., CTAB/MCPE) was prepared by immobilizing a CTAB solution of concentration ranging from 5.0-35.0 µl (below CMC of CTAB) on the bare electrode surface. The CTAB/MCPE was left for 15 min at room temperature to have the maximum adsorption of CTAB on the bare electrode surface. The electrode was then washed with double distilled water to remove unabsorbed CTAB from the electrode surface. The peak current for MSZ was observed for each concentration of CTAB as shown in figure 5.2.1. The peak current response of the electrode increased slowly with an increase in immobilization of CTAB concentration at 0.2 M PBS of physiological pH 7.4 for the electrochemical oxidation of 0.1×10^{-3} M mesalazine. It can be seen from fig 5.2.1 that a saturation level (at 20.0 µL) is reached when a further increase in the concentration of the CTAB solution decreased the peak current response. Thus, 20.0 µL of an active agent (CTAB molecule) was chosen for the diffusion into the porous CPE and for further studies 20.0 µl CTAB concentration was maintained [117, 118]. The probable immobilization mechanism of active agents with electro-catalytic interaction of mesalazine is illustrated in Scheme 5.2.2. This is well corroborated with earlier reports [119, 120].

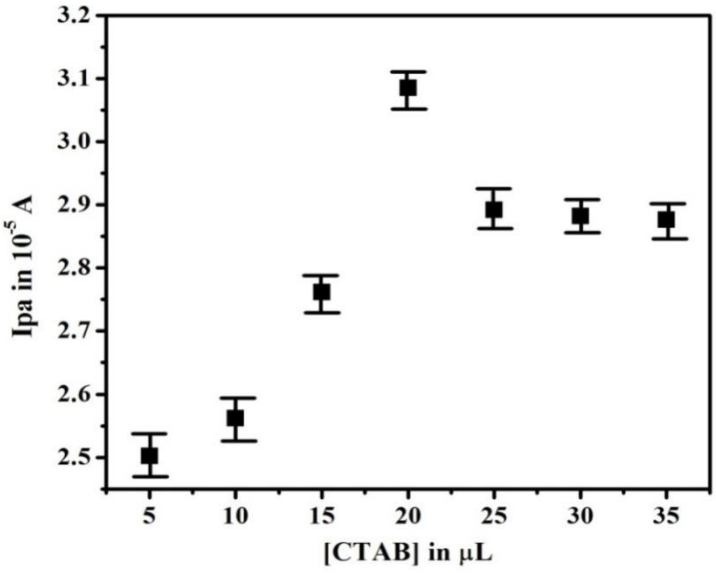
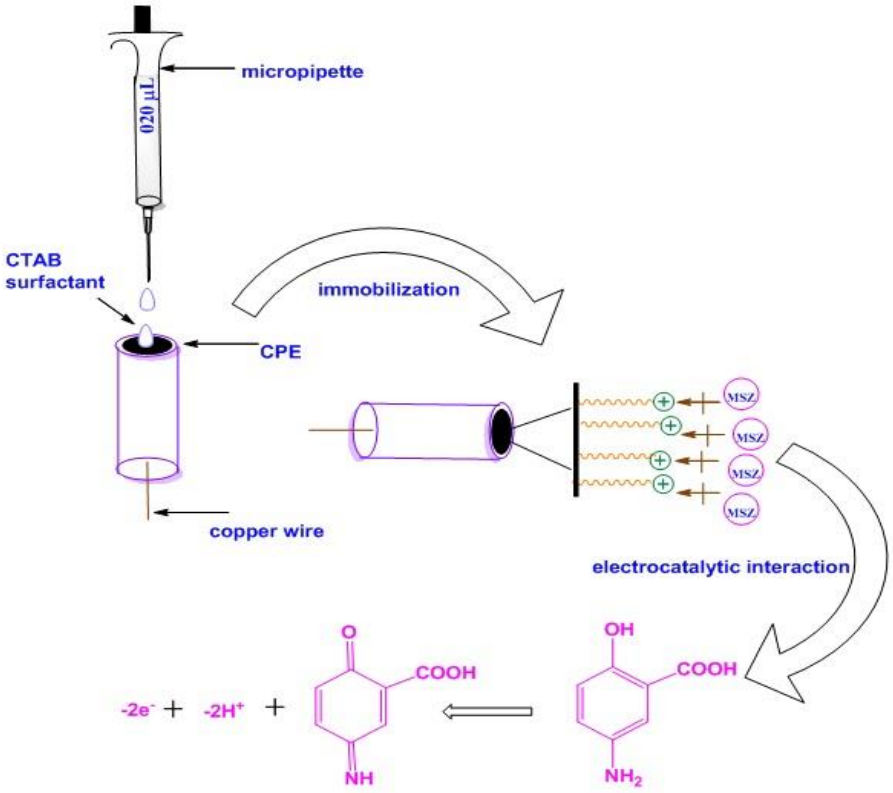


Fig. 5.2.1- Graph of anodic peak current obtained for the oxidation of 0.1×10^{-3} M MSZ in 0.2 M PBS of pH 7.4 versus different concentration of CTAB/MCPE.



Scheme 5.2.2- Mechanism of immobilization of 20.0 µL of 10×10^{-3} M CTAB solution on the surface of CPE and its electrocatalytic interaction with mesalazine.

5.2.3. Observations and Analysis

5.2.3(a). Electrochemical Characterization of CTAB/MCPE

The Voltammograms of 1.0×10^{-3} M "potassium ferrocyanide ($K_4[Fe(CN)_6]\cdot3H_2O$)" in 1.0 M KCl for both BCPE (dashed line) and CTAB/MCPE (solid line) was obtained with the scan rate of 0.05 V/s by CV technique (Fig. 5.2.2). It can be seen from the voltammogram that the CV response with CTAB/MCPE is better than that with the BCPE. A similar observation was noted also with poly NA-MCPE for the determination of Catechol and explained in chapter-5 (section 5.1.3(b); page no.33).

The electroactive surface area was found out by using the "Randles-Sevcik Equation" and was found to be 0.03622 cm^2 for CTAB/MCPE and with BCPE the electrode active surface area was found to be 0.02842 cm^2. The use of the "Randles-Sevcik Equation" to calculate electrode active surface area was already discussed in chapter-1**(**section 1.2.2(a); page no.7-8).

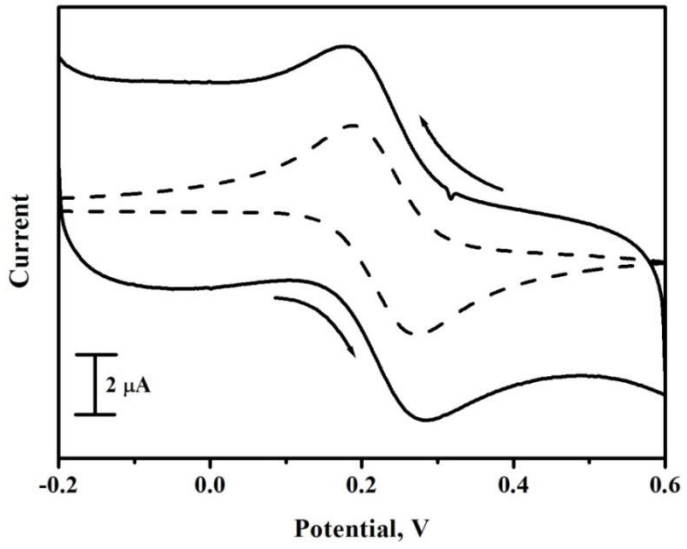


Fig. 5.2.2- Cyclic voltammograms of 1.0×10^{-3} M potassium ferrocyanide at BCPE (dashed line) and CTAB/MCPE (solid line) at the scan rate of 0.05 V/s.

5.2.3(b). CV Analysis of Mesalazine at CTAB/MCPE

The cyclic voltammetric behavior of MSZ was explored with BCPE and CTAB/MCPE (Fig.5.2.3). The peak potential is with BCPE was observed at 0.222 V (dashed line) with low current (-4.454×10^{-6}A) and a broad voltammetric response. However, under similar condition, with CTAB/MCPE, the peak potential of MSZ was obtained at 0.180 V with a considerable increase in peak current signal (-3.085×10^{-5}A). Hence, there is a significant increase (almost 10 folds) in current signal with CTAB/MCPE. From this observation, it can be concluded that the CTAB/MCPE is electro catalytically active for electrochemical oxidation of MSZ.

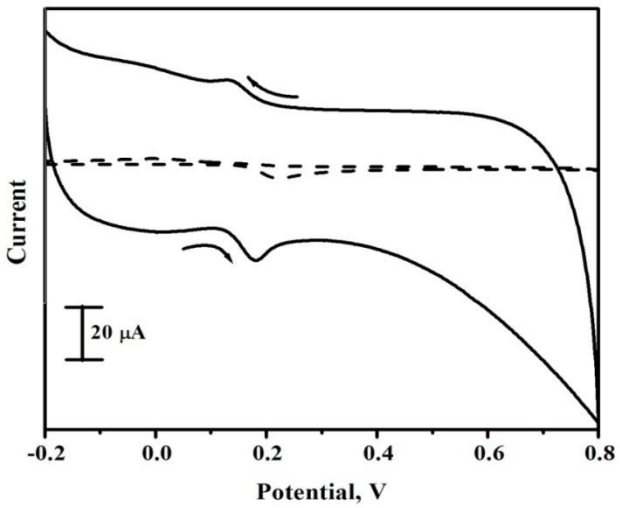


Fig. 5.2.3- Cyclic voltammograms for 0.1×10^{-3} M MSZ at BCPE (dashed line) and CTAB/MCPE (solid line) in 0.2 M PBS of pH 7.4 at scan rate 0.05 V/s.

5.2.3(c). Variation of Scan Rate

The electrochemical oxidation of MSZ was studied by the CV technique by varying the scan rate in the range (0.02-0.3 V/s) as shown in Fig. 5.2.4(a). It has been observed that, as the scan rate is increased the redox peak current is also increased with a slight positive shift of the peak potential. The graph was plotted between Ip versus υ (Fig.5.2.4(b)) and the correlation coefficients (r^2) of the straight lines were obtained. Another graph was plotted between Ip versus $\upsilon^{1/2}$ and the correlation coefficients (r^2) of the straight lines were obtained as shown in Fig. 5.2.4(c). From the correlation coefficients of both the plots, we could conclude that the electrochemical process was "adsorption controlled phenomenon" [121, 122].

The heterogeneous rate constant (k^0) values for the MSZ oxidation were calculated by using Equation (5.2.1) and given in (Table 5.2.1). The voltammograms whose ΔEp values are greater than 10 mV [123] are used to calculate k^0. The values of k^0

$$\Delta Ep = 201.39 \log(\upsilon/k^0) - 301.78 \text{----------(5.2.1)}$$

obtained at the scan rate of 240mV/s exhibits a larger heterogeneous rate constant when compared with those determined in other scan rate variation studies.

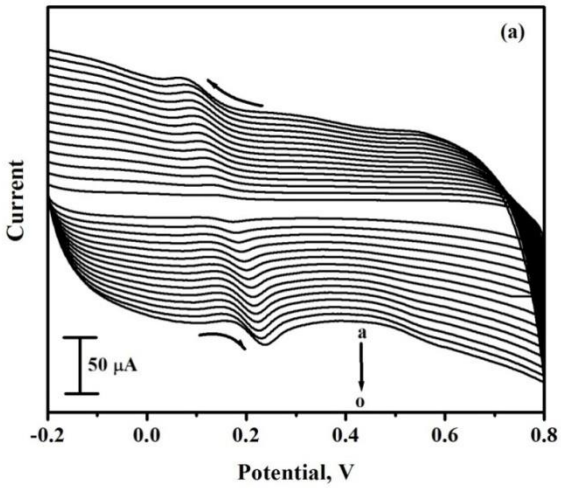


Fig. 5.2.4(a)- Cyclic voltammograms for 0.1×10^{-3} M MSZ at CTAB/MCPE in 0.2 M PBS of pH 7.4 at different scan rate (a-o; 0.02 V/s, 0.04 V/s, 0.06 V/s, 0.08 V/s, 0.1 V/s, 0.12 V/s, 0.14 V/s, 0.16 V/s, 0.18 V/s, 0.2 V/s, 0.22 V/s, 0.24 V/s, 0.26 V/s, 0.28 V/s and 0.3 V/s).

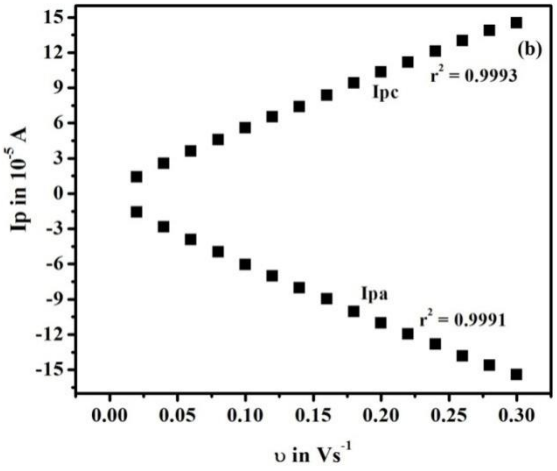


Fig. 5.2.4(b)- The Graph of peak current (Ip) versus scan rate (υ).

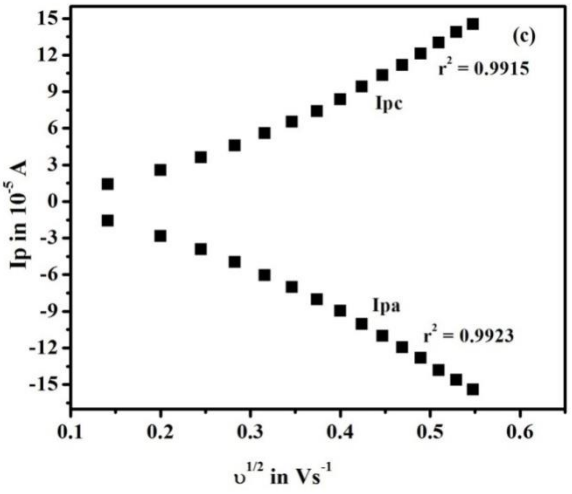


Fig. 5.2.4(c)- The Graph of peak current (Ip) versus square root of scan rate.

Table 5.2.1- Variation of the voltammetric parameters from the plots shown in Figs. 5.2.4(a), 5.2.4(b) and 5.2.4(c) as a function of the potential scan rate.

υ (mV/s)	ΔE_p (mV)	k^0 (s^{-1})
20	29.4	0.453
40	46.2	0.746
60	60.8	0.950
80	69.2	1.150
100	81.7	1.247
120	92.3	1.325
140	98.6	1.439
160	113.2	1.391
180	119.6	1.455
200	123.6	1.544
220	132.1	1.541
240	**136.3**	**1.603**
260	146.8	1.539
280	155.0	1.510
300	165.6	1.433

5.2.3(d). Variation of pH:

The peak current response of 0.2×10^{-3} M MSZ was studied by varying pH within the range 5.5-8.0 and the voltammograms are recorded as in Fig.5.2.5(a). It can be observed that when the pH of the 0.2 M PBS is increased, the peak potential was shifted gradually towards the negative side. The graph of Epa and Ipa versus pH was plotted as shown in Fig. 5.2.5(b). The graph of Epa vs pH is a straight line with (r^2=0.9571) and slope of 0.0838V/pH. The linear dependency confirmed the involvement of an "equal number of protons and electrons" in the redox reaction which is also supported by the earlier reports [124, 125].

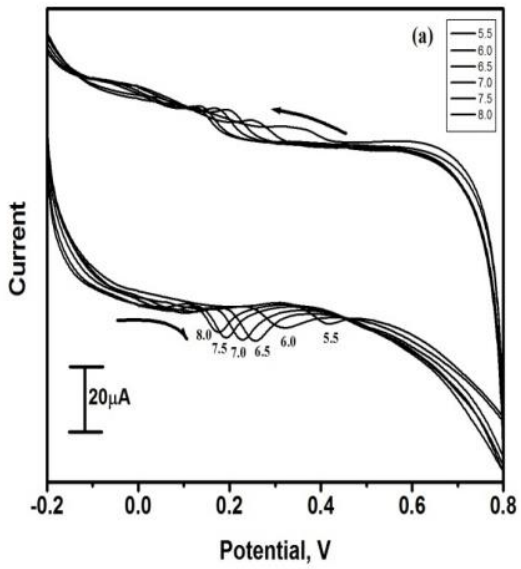


Fig. 5.2.5(a)- Cyclic voltammograms of 0.1×10^{-3} M MSZ at CTAB/MCPE in 0.2 M PBS solution of different pH values (5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) at scan rate of 0.05 V/s.

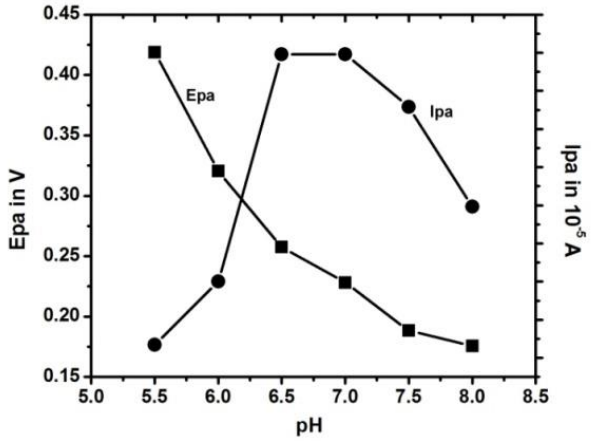


Fig. 5.2.5(b)- The effect of anodic peak current and anodic peak potential versus pH.

5.2.3(e). Variation of Concentration:

The CV profile of MSZ at different concentrations (60.0 μM - 140.0 μM) with the CTAB/MCPE sensor was studied. The scan rate was maintained at 0.05 V/s, pH of the solution was maintained at 7.4 with 0.2M PBS buffer. The cyclic voltammograms are shown in Fig.5.2.6(a). There is a slight positive shift in Epa with increase in MSZ concentration. Further, a graph was plotted between [MSZ] and Ipa Fig.5.2.6(b) and a straight line was obtained with the linear regression equation-

$$Ipa\ (10^{-5}A) = 0.0167(C_o10^{-4}M/L)+2.2443$$

The LOD was calculated as is described in chapter-1(section 1.7.4; page no.16) [112-116] and found to be 1.9×10^{-9} M of MSZ at CTAB/MCPE. Further, the LOD value was compared with the reported techniques in Table 5.2.2. It can be seen that the present work could found out the best technique to detect mesalazine with CTAB modified CPE.

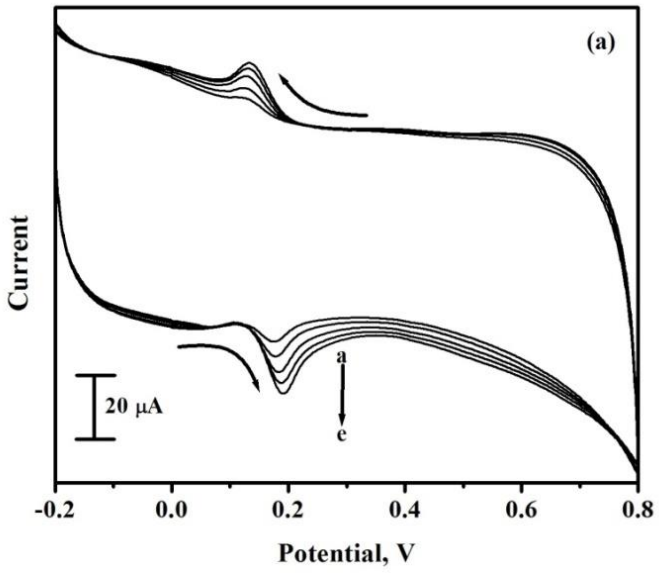


Fig. 5.2.6(a)- Cyclic voltammograms of MSZ in 0.2 M PBS solution of pH 7.4 at CTAB/MCPE at scan rate of 0.05 V/s with different concentrations (a-e: 60.0 μM, 80.0 μM, 100.0 μM, 120.0 μM and 140.0 μM).

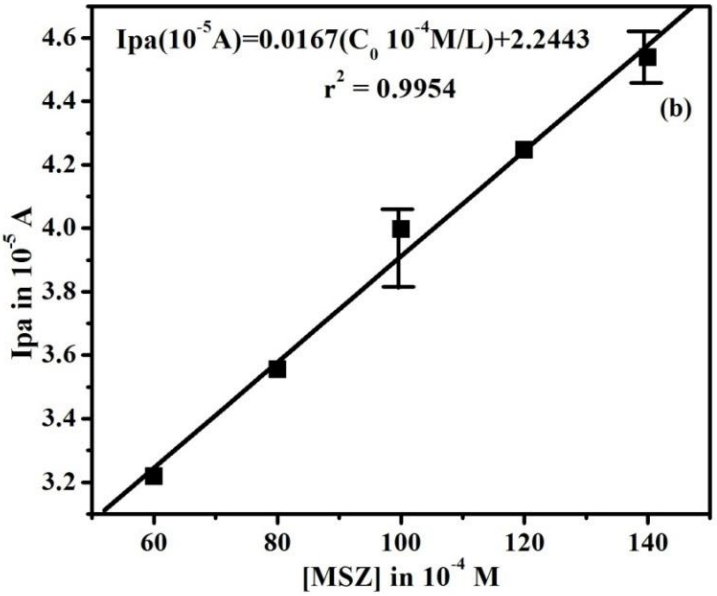


Fig. 5.2.6(b)- Graph of anodic peak current versus concentration of MSZ.

Table 5.2.2- Comparison of linear range and detection limits for MSZ with different classical methods and electrodes.

Classical methods	Electrode/modifier biosensors	Linear working range	Detection limits (M)	Refs.
LSV	CNT/PPY doped by 1,5-napthalenedisulphonic acid	$1.0x10^{-8}$- $1.0x10^{-6}$	$3.0x10^{-9}$	[112]
SWV	Pencil graphite electrode	$9.8x10^{-7}$-$7.3x10^{-5}$	$2.1x10^{-8}$	[113]
LSV	Glassy carbon electrode	$1.0x10^{-6}$- $5.7x10^{-5}$	$3.0x10^{-7}$	[114]
CV	Poly(Glutammic acid) MGCE	$50.0x10^{-6}$-$0.5x10^{-3}$	$23.9x10^{-9}$	[115]
DPV	Glassy carbon electrode	$2.0x10^{-6}$- $1.0x10^{-4}$	$8.2x10^{-7}$	[116]
CV	CTAB immobilized modified CPE	$60x10^{-6}$-$140x10^{-6}$	$1.9x10^{-9}$ (nM)	Present work

5.2.3(f). Tablet Analysis and Recovery Test of MSZ

The tablet analysis was done as described in chapter-4(section 4.6.; page no.28). The recovery test of MSZ was studied and evaluated in the concentration range between 1.0×10^{-6} M to 2.0×10^{-5} M and maintaining pH= 7.4. The peak current values were obtained for each solution by CV technique. The recovery percentage was calculated and listed in Table 5.2.3. The recovery percentage shows that the CTAB/MCPE has a good recovery with SD±RSD (< 1.0% to 1.5%) for the electrochemical resolve of mesalazine in pharmaceutical samples.

Table 5.2.3- Determination of MSZ in commercial pharmaceutical sample.

Formulation Sample	MSZ added	Detected[a]	Recovery (%)	SD±RSD (%)
Tablet (Mesacol)	1.0×10^{-6}	1.05×10^{-6}	105	0.0353±0.0252
	3.0×10^{-6}	3.03×10^{-6}	101	0.0212±0.0151
	5.0×10^{-6}	4.99×10^{-6}	99.8	0.0070±0.0050
	7.0×10^{-6}	7.06×10^{-6}	100.8	0.0424±0.0303
	9.0×10^{-6}	8.99×10^{-6}	99.8	0.0141±0.0101
	2.0×10^{-5}	1.97×10^{-5}	98.5	0.0212±0.0151

"[a]Average of three trials for each concentration of MSZ"

5.2.3(g). Interference Study

Effects of interferents like NH_4Cl (ammonium chloride), $CaCl_2$ (calcium chloride), $CaSO_4$ (calcium sulfate), $MgCl_2$ (magnesium chloride), $C_2K_2O_4$ (potassium oxalate), NaCl (sodium chloride) starch, and sucrose ($C_{12}H_{22}O_{11}$) were inspected in the determination of MSZ in order to judge the selectivity. The results of the analysis are recorded in Table 5.2.4. It was observed that ten-fold excess of $CaSO_4$, $MgCl_2$, $C_2K_2O_4$, NaCl and $C_{12}H_{22}O_{11}$ did not interfere with the voltammetric signal of MSZ. However, ten-fold excess of NH_4Cl, $CaCl_2$ and starch had an apparent influence on the voltammetric signal of 1.0×10^{-3} M MSZ. This confirmed that the electrode fabricated was highly selective for the assay of MSZ.

Table 5.2.4- Influence of potential interferents on the cyclic voltammetric response of 1.0×10^{-3} M MSZ. Scan rate 0.05 V/s at CTAB/modified CPE.

Interferents	Concentration (10^{-2}M)	Signal Change (%)
Ammonium chloride	1.0	4.46%
Calcium chloride	1.0	2.28%
Calcium sulphate	1.0	1.16%
Magnesium chloride	1.0	1.40%
Potassium oxalate	1.0	1.10%
Sodium chloride	1.0	1.32%
Starch	1.0	2.01%
Sucrose	1.0	1.38%

5.2.4. Conclusion

The electro-oxidation behavior of MSZ was studied at CTAB/MCPE using a cyclic voltammetry technique. The investigations included the study of the effect of pH variation, scan rate, and concentration of MSZ. The obtained results displayed the type of electrode reaction was quasi-reversible; adsorption controlled and involves equal number of protons and electrons transfer. Under optimal conditions, the lower limit of detection with CTAB immobilized MCPE was found at 1.9×10^{-9} M while MSZ concentration range was maintained from 60 µM to 140 µM. Thus, the present study was very impressive on account of used modifiers due to its affectability, selectivity, reproducibility and low LOD value.

5.3. Carbamazepine/MCPE Sensor for Paracetamol

5.3.1. Preamble

Paracetamol (PC) (or acetaminophen) is the most widely used drug as antipyretic analgesics [126, 127]. It has good bioavailability (63-89%) with molecular weight (151.163 g/mol) and the molecular formula ($C_8H_9NO_2$).

For medication, the PC is used to treat fever, cold and cough, muscular pain, toothache, backache, headache, postoperative pain, arthritic pain and aspirin addiction [128-131]. An overdose of paracetamol may cause high toxicity of liver leading to acute liver failure [132].

Literature review reveals that, a few investigative techniques are used for the study of paracetamol in pharmaceutical analysis such as; GC-MS-HPLC [133], FT-infra red Raman spectrometry [134], MEKC-method [135], spectro-fluorometry [136], chemiluminescence[137], GC-MS [138], MS-LC [139], automatic sequential injection analysis [140], titrimetry [141], micellar electrokinetic chromatography [142] TLC [143] and FI-spectrophotometry [144]. But, these methods are expensive, time-consuming, tedious and less accurate. Hence it is necessary to develop effective electroanalytical techniques for the determination of PC [145].

Carbamazepine (CBZ, or CZ) is an anticonvulsant drugs. It is used to treat epilepsy, bipolar disorder, and neuralgic pain. It is metabolized by the CYP-3A enzyme and widely used as an anti-epileptic substance [146]. CZ is recommended more in pharmaceutical studies with variety of doses because it possesses good catalytic activity, fast response and high sensitivity towards biological activities [147]. However, carbamazepine was not used for the development of MCPE to determine PC by CV method. Hence, in the present work we have tried to develop a biosensor by coating carbamazepine on the BCPE and using the same for the determination of paracetamol.

5.3.2. Experimental

Preparation of reagents, instrumentation, and preparation of BCPE has already been discussed in the section from 4.1 to 4.4; (page no. 25-27).

5.3.3. Observations and Analysis

5.3.3(a). Electrochemical Polymerization and Preparation of Carbamazepine MCPE

MCPE with carbamazepine (CZ) was prepared by electropolymerization of carbamazepine monomer on BCPE. The cyclic voltammetric technique of electropolymerization of CZ involves the scanning of BCPE for 5 multiple cycles through the solution of 1.0×10^{-3} M CZ monomer at pH of 7.4. The electropolymerization was achieved by successive CV sweep in a potential window of -0.6 V to +1.8 V with a scan

rate of 0.1 V/s (Fig. 5.3.1). After the electropolymerization of the electrode was done, the modified electrode was rinsed carefully using distilled water and used for the investigation of paracetamol.

The probable electro polymerization mechanism of the CZ/MCPE sensor and electro catalytical interaction with PC is shown in scheme 5.3.1. This is well agreed with earlier report [115].The electroactive surface area was calculated by using the "Randles-Sevcik Equation" (chapter-1**,** section 1.2.2(a); page no.7-8). The electrode active surface area for CZ/MCPE was found to be 0.0415 cm^2 which is greater than that with BCPE (0.0290 $cm^{2)}$. Hence the modified electrode with carbamazepine is better than the BCPE to study paracetamol.

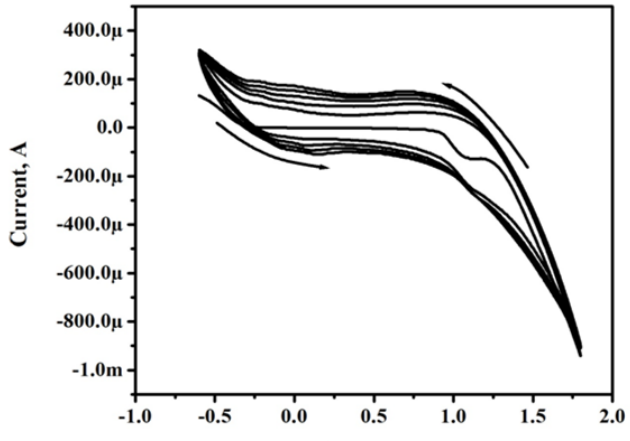
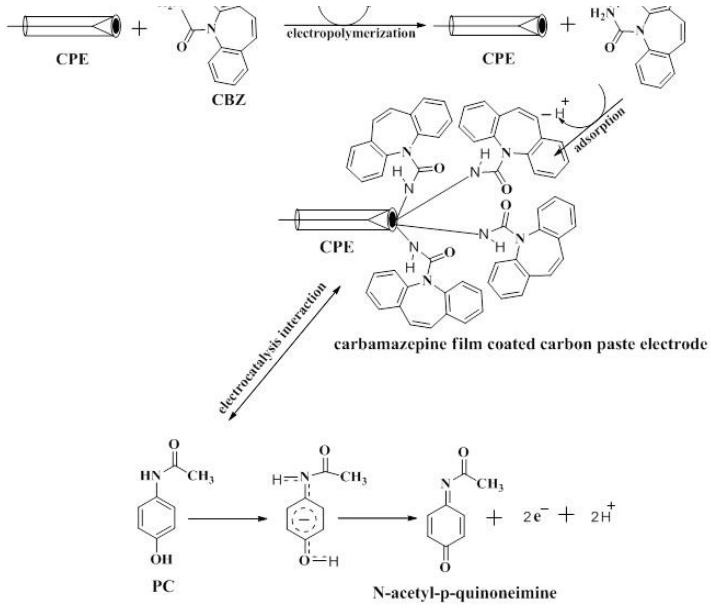


Fig. 5.3.1- Cyclic voltammograms of preparation of carbamazepine film coated carbon paste electrode in 1.0×10^{-3} M solution of carbamazepine in 0.2 M PBS of pH 7.4 at 5 cycles with scan rate of 0.1 V/s.



Scheme 5.3.1- Mechanism of electropolymerisation of carbamazepine on the surface of BCPE and electrocatalysis interaction of paracetamol with carbamazepine film coated carbon paste electrode.

5.3.3(b). Electrochemical Behavior of Paracetamol at CZ/MCPE

The electrochemical responses of paracetamol (0.1×10^{-3} M) were recorded by the CV method by maintaining a scan rate of 0.05 V/s at BCPE and CZ/MCPE in 0.2 M PBS of pH-7.4 as shown in Fig.5.3.2. The oxidation of PC shows a poor voltammetric response with BCPE and the peak potential was observed at 0.3424 V. This can be attributed to slow electron transfer phenomenon. On the other hand, under the same condition the oxidation of PC showed better voltammetric response with the peak potential obtained at 0.3504 V. Fig 5.3.2 further reveals that the electrochemical process is reversible and there is a significant improves in the current signal with a modified electrode sensor. From these observations it is clear that the CZ/MCPE sensor showed clear evidence of electro-catalytic sensitivity towards the electrochemical resolve of PC.

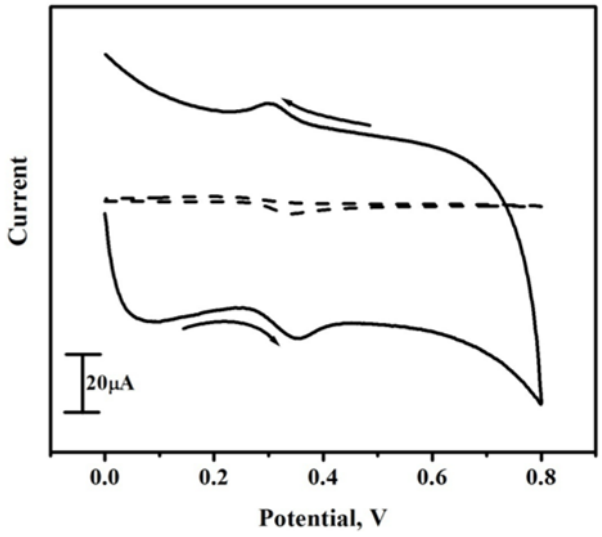


Fig. 5.3.2- Cyclic voltammograms for 0.1×10^{-3} M PC at bare CPE (dashed line) and carbamazepine film coated carbon paste electrode (solid line) in 0.2M PBS of pH 7.4 at scan rate 0.05 V/s.

5.3.3(c). Variation of Scan Rate

The effect of the scan rate on reversible peak current for 0.1×10^{-3} M PC at CZ/MCPE of pH-7.4 at 0.2 M PBS solution was investigated by the CV method. The voltammograms with different scan rates from 0.025 V/s to 0.2 V/s are shown in Fig.5.3.3(a). The peak potential shifted to less positive side with an increase in redox peak current signal with the increase in the scan rate. The plots of Ip versus υ for oxidation-reduction were plotted which reveals that the oxidation and reduction of peak current are directly proportional to the scan rate satisfying "Randles-Sevcik Equation" (Fig. 5.3.3(b)). Another linear curve was obtained when a graph was plotted between Ip versus $\upsilon^{1/2}$ (Fig.

5.3.3(c)). From the correlation coefficients of both plots Fig. 5.3.3(b) and Fig. 5.3.3(c) it can be concluded that the electrode process was "adsorption controlled" [78, 148].

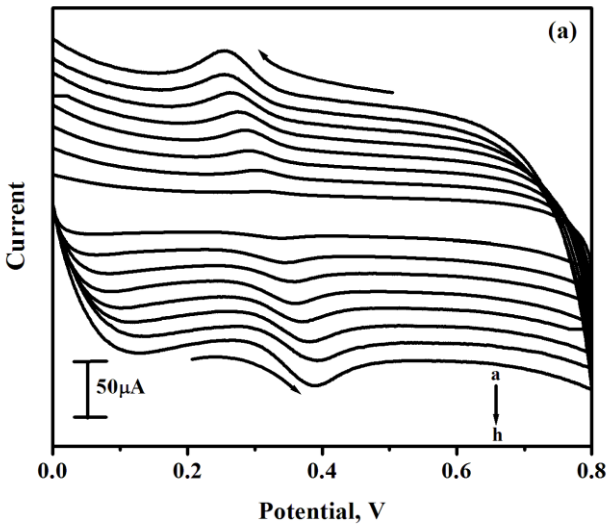


Fig. 5.3.3(a)- Cyclic voltammograms for 0.1×10^{-3} M PC at CZ/MCPE in 0.2 M PBS of pH 7.4 at different scan rate (a-h; 0.025 V/s, 0.05 V/s, 0.075 V/s, 0.1 V/s, 0.125 V/s,0.15 V/s, 0.175 V/s and 0.2 V/s).

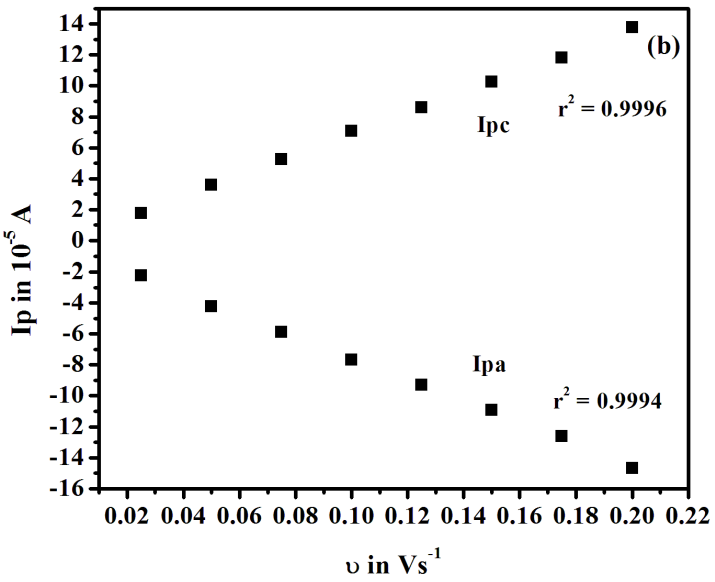


Fig. 5.3.3(b)- Graph of peak current (Ip) versus scan rate (υ).

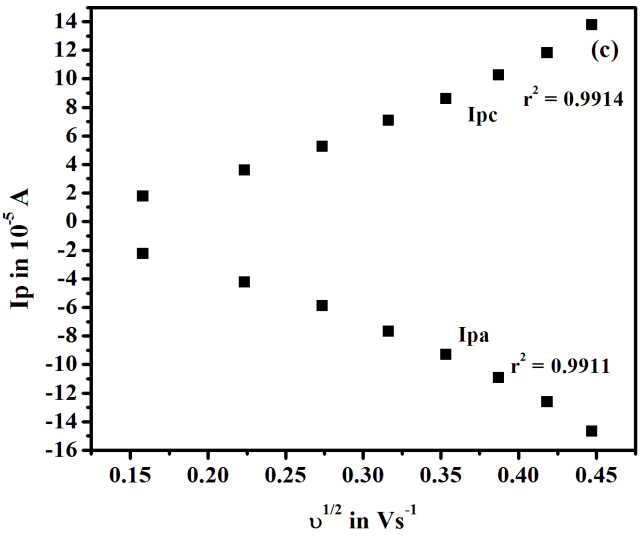


Fig. 5.3.3(c)- Graph of peak current (Ip) versus square root of scan rate ($\upsilon^{1/2}$).

5.3.3(d). Variation of pH

The cyclic voltammograms of the oxidation behavior of PC at CZ/MCPE in the pH range 5.5-8.0 are shown in Fig. 5.3.4(a). It can be observed that the peak potentials are shifted to more negative direction with increase in pH. Further, a graph between pH versus Epa was plotted and a linear curve with the slope of 0.0732 V/pH was obtained as shown in Fig. 5.3.4(b). Hence we can conclude that in the electrochemical process "equal number of electrons and protons" transfer is took place which supports the earlier reports [119, 120].

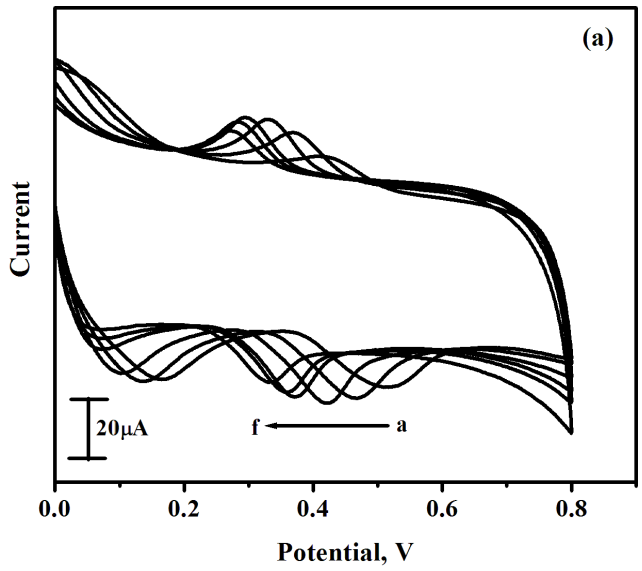


Fig. 5.3.4(a)- Cyclic voltammograms obtained for the oxidation of PC at CZ/MCPE in 0.2 M PBS solution at different pH values (a-e: 5.5 to 8.0) at scan rate of 0.05 V/s.

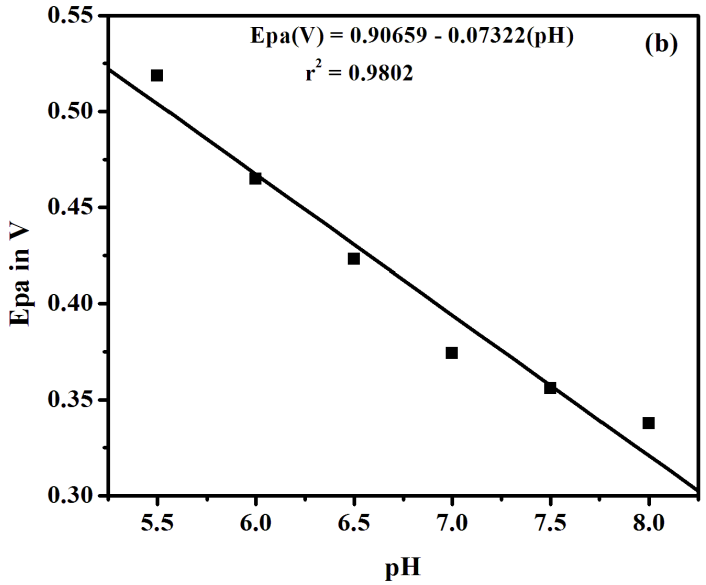


Fig. 5.3.4(b)- The effect of pH on the peak potential of PC in 0.2 M PBS solution.

5.3.3(e). Variation of Concentration

The effect of varying concentrations of PC in the range of 1.0×10^{-4} M to 3.94×10^{-4} M at CZ/MCPE sensor was investigated and recorded with scan rate of 0.05 V/s as shown in Fig. 5.3.5(a). The pH of the solution was maintained at 7.4. The Epa was shifted to positive side with increasing PC concentration and an increase in Ipa was also observed. A graph is plotted between concentration of PC and Ipa, a linear curve (Fig. 5.3.5(b)) is obtained with following linear regression equation

$$Ipa\ (10^{-5}A)=1.4172(C_o10^{-4}M/L)+2.4507$$

The LOD value for PC at CZ/MCPE was found to be 0.24×10^{-6} M which is much lower than the detection limit reported by earlier investigators [149-154]. The comparison of the detection limit of PC with different methods and different modified electrodes is shown in Table 5.3.2. It is clear that a CZ/MCP electrode is better than the other electrodes in the determination of PC.

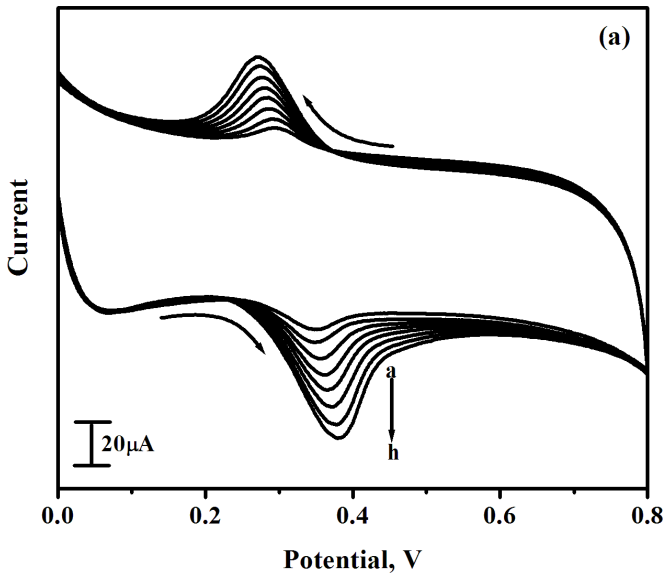


Fig. 5.3.5(a)- Cyclic voltammograms of PC in 0.2 M PBS solution of pH 7.4 at CZ/MCPE at scan rate of 0.05 V/s with different concentrations (a-h: 1.0×10^{-4} M, 1.47×10^{-4} M, 1.92×10^{-4} M, 2.35×10^{-4} M, 2.77×10^{-4} M, 3.18×10^{-4} M, 3.51×10^{-4} M, 3.94×10^{-4} M).

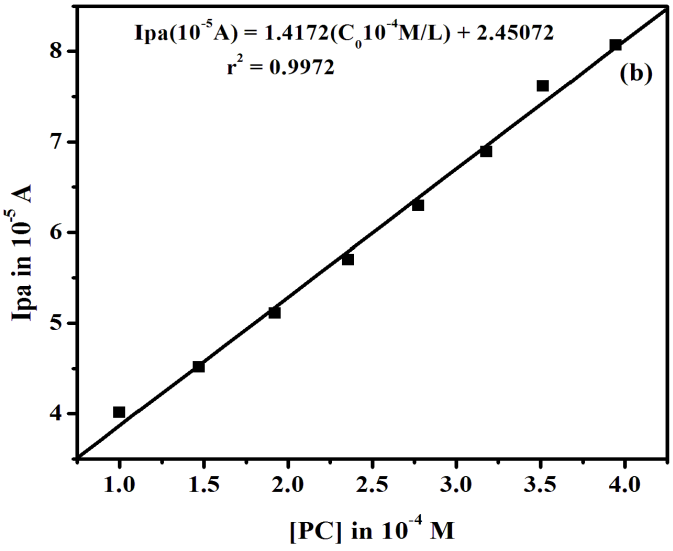


Fig. 5.3.5(b)- Graph of anodic peak current versus concentration of PC.

Table 5.3.1- Comparison of linear range and detection limits for PC with different classical methods and electrodes.

Classical methods	Electrode/modifier biosensors	Linear working range (µM)	Detection limits (M)	Refs.
ATSDPV	ETPGE	0.05-2.5	2.5×10^{-3}	[149]
AdSSWV	D50wx2/GNP/GCPE	0.0334-42	4.7×10^{-3}	[150]
DPV	N-(3,4-dihydroxyphenethyl)-3,5-dinitrobenzamide - MWCNT/CPE	15-270	1.0×10^{-5}	[151]
CV	C_{60}/GCE	50-1500	0.5×10^{-5}	[152]
Multi-commutated flow system	Nafion-modified glassy carbon tubular electrode	50-500	1.7×10^{-5}	[153]
CV	GCE/Cu complex	20-5000	0.5×10^{-5}	[154]
CV	carbamazepine film coated carbon paste electrode	100-394	0.24×10^{-6}	Present work

ETPGE: Electrochemically treated pencil graphite electrode.
ATSDPV: Adsorptive transfer stripping differential pulse voltammetry
D50wx2/GNP/GCPE: A cation exchanger resin, Dowex 50wx2 and gold nanoparticles modified glassy carbon paste electrode.
AdSSWV : Adsorptive stripping square wave voltammetry

5.3.3(f).Tablet Analysis and Recovery Test of PC

The recovery test of paracetamol drug in tablet analysis was done in the concentration range of 3.0×10^{-6} M to 4.0×10^{-5} M using modified carbamazepine CPE sensor maintaining pH=7.4 and the results are summarized in Table 5.3.2. The percentage of recovery was ranged from 95% to 103% with good SD±RSD values (<1.0% to 1.5%). Hence it can be concluded that the CZ/MCPE sensor can be effectively chosen for the selective resolve of PC in pharmaceutical samples.

Table 5.3.2- Determination of PC in commercial pharmaceutical sample.

Formulation Sample	PC-added	Detected[a]	Recovery(%)	SD±RSD (%)
Tablet (Calpol)	-	Not detected	-	-
	3.0×10^{-6}	3.105×10^{-6}	103.5	0.0741 ± 0.0530
	5.0×10^{-6}	4.970×10^{-6}	99.4	0.0212 ± 0.0151
	7.0×10^{-6}	7.116×10^{-6}	101.6	0.0819 ± 0.0585
	9.0×10^{-6}	9.044×10^{-6}	100.4	0.0311 ± 0.0222
	2.0×10^{-5}	1.901×10^{-5}	95.0	0.0700 ± 0.0500
	4.0×10^{-5}	4.102×10^{-5}	102.5	0.0721 ± 0.0515

"[a]Average of three trials for each concentration of PC"

5.3.4. Conclusion

In this procedure, we could develop a more sensitive method for the direct determination of PC using CZ/MCPE by CV technique. Under optimum conditions, the new modified electrode sensor showed good voltammetric responses of PC over varying concentration range of 1.0×10^{-4} M to 3.94×10^{-4} M and lower LOD was obtained at 0.24×10^{-6} M. Further parameters like scan rate and pH variation were optimized for the analysis of PC. The overall study reveals that the PC electrochemical process is reversible; adsorption controlled and involves two proton-electron exchanges. The developed CZ/MCPE sensor showed a quick response, stability and sensitivity for the electrochemical resolve of paracetamol using cyclic voltammetry.

5.4. (Poly)-NC/CPE Sensor for Epinephrine with Uric Acid

5.4.1. Preamble

Epinephrine (EP) or adrenaline is an important catecholamine neurotransmitter present in the mammalian central nervous system and it is used as common emergency healthcare medicine [79]. This drug is derived from and is synthesized as "L-tyrosine" in the adrenal gland in the human body [155]. It is an important drug to treat hypertension, heart disease, and other allergic conditions. The low level of EP may lead to adverse effects like "Parkinson's disease". Hence it is needed to determine EP concentration in urine and plasma in the clinical diagnosis of some diseases [156, 157].

EP can be determined by LC [158], fluorescence [159], FI-electrogenerated chemiluminescence [160], capillary electrophoresis [161], fluorimetry [162, 163] and other detection techniques coupled with chromatography [164, 165]. However, these techniques not only need well-controlled experimental conditions, expensive equipment but also they are time-consuming and follow tedious procedures. Apart from all these tedious methods EP can also be determined by using CV technique which is considered to be the best method as it is economical, highly sensitive, fast process, easy to handle and the results are reproducible.

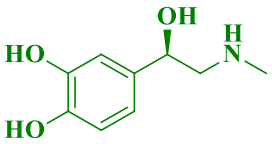
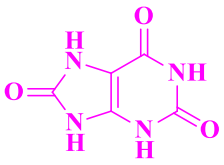
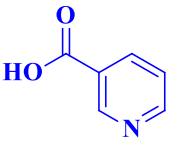
Uric acid (UA) or "2,6,8-trihydroxypurine" is a class of heterocyclic compounds containing C, N, O, and H atoms. It is a strong reducing agent and effective antioxidant [166]. In humans, UA is produced by the oxidation of purine metabolism and excreted through the urine. Normal excretion of UA in the urine is concentration of 250 to 750 mg/L [167, 168]. UA concentration in human blood is 25 to 80 mg/L for men and 15 to 60 mg/L for women.

Niacin (NC) is a biological organic compound and is known as "nicotinic acid", or "vitamin-B_3", or "pyridine-3-carboxylic acid" and the structural formula shown in Table 5.4.1. It is a water-soluble compound and easily loses its strength when dissolved in hot water and NC cannot be accumulated in the human body [169, 170]. The deficiency of NC causes problems such as headache, anemia, and tiredness and UA is also a crucial constituent of "mammalian diet" [171].

The molecular structures of EP, UA and Niacin are given in Table 5.4.1.

In recent years, the development of biosensors technology is the most powerful electroanalytical technique, because of its high accuracy and quick response. So we have developed niacin MCPE for the resolve of EP with UA by CV technique [172].

Table 5.4.1- The structural and molecular formula of epinephrine, uric acid and niacin.

Biomolecules	Molecular formula	Structure
Epinephrine (EP)	$C_9H_{13}NO_3$	
Uric Acid (UA)	$C_5H_4N_4O_3$	
Niacin (NC) (Vitamin B_3)	$C_6NH_5O_2$	

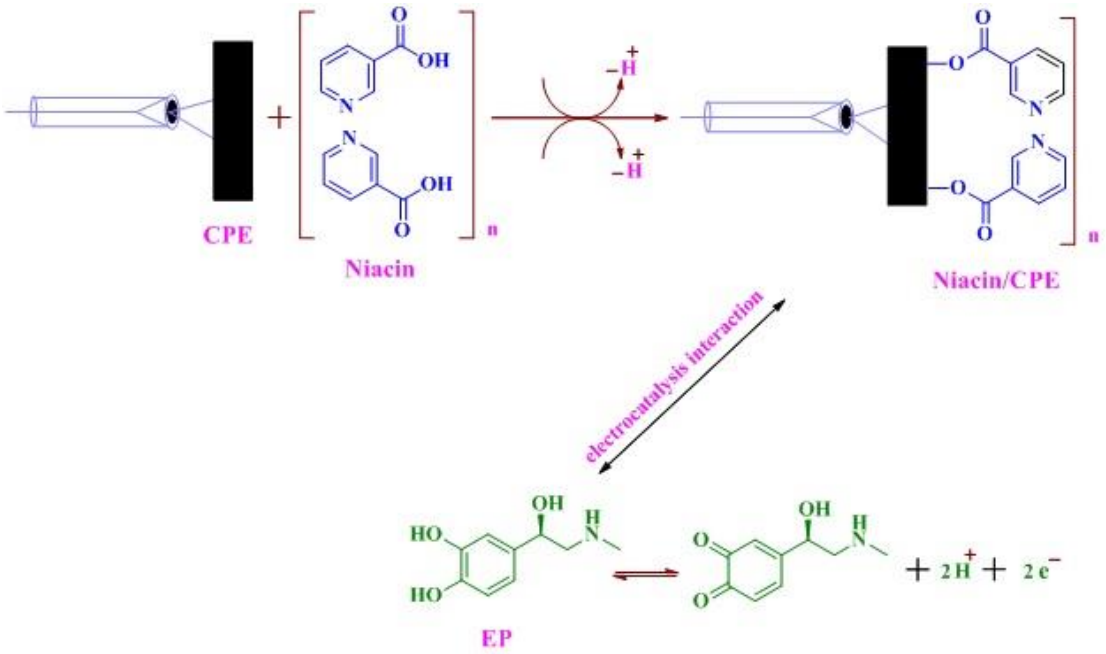
5.4.2. Experimental

The experimental part includes instrument, reagents, chemical and electrode preparation has been discussed in (section from 4.1 to 4.4; page no.25-27).

5.4.3. Observations and Analysis

5.4.3(a). Electrochemical Polymerization and Preparation of Niacin MCPE

In an electrochemical cell containing the 1.0×10^{-3} M niacin monomer was fabricated on BCPE by CV technique. The potential sweep was maintained between -1.0 V and +1.8 V with a scan rate 0.1 Vs^{-1} for 10 multiple cycles. The potentials cycle was repeatedly applied until a stable voltammogram was obtained. By the application of multiple cycles, the voltammogram was slowly descended with an increase in the number of cycles as shown in Fig. 5.4.1. This indicates that the niacin thin film was formed on a surface of bare carbon paste electrode [173, 174]. However, the oxidation peak current increased greatly at the first few multiple cycles and then gradually leveled off. For all electrochemical analysis new MCPE sensor was used. The probable reaction mechanism of (poly)-NC/CPE is described in Scheme 5.4.1 which is well corroborated with earlier reports [175].



Scheme 5.4.1- Mechanism of electropolymerisation of niacin on the surface of BCPE and electrocatalytic interaction of epinephrine with niacin/CPE.

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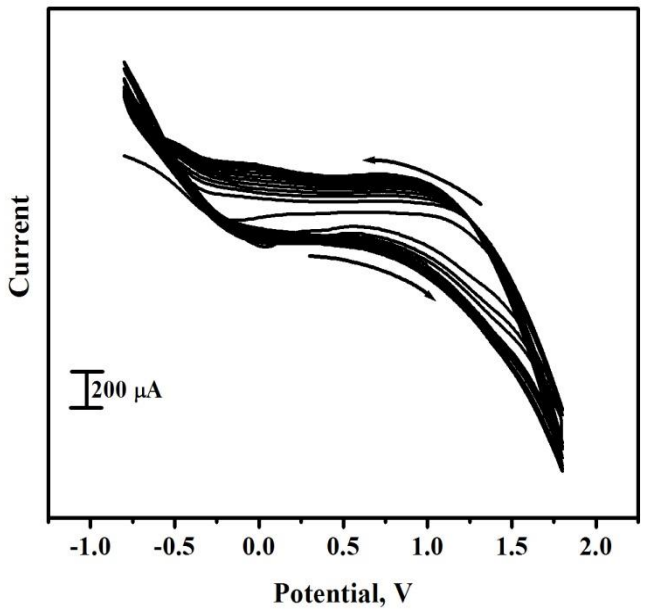

Fig. 5.4.1- Cyclic voltammograms of fabrication of (poly)-NC/CPE. 1.0×10^{-3} M niacin solution in 0.2 M PBS of pH 7.4 at 10 cycles with scan rate of 0.1 V/s.

5.4.3(b). Characterization of (poly)-NC/CPE Sensor

The cyclic voltammetric responses were recorded for the 1.0×10^{-3} M potassium ferrocyanide in 1.0 M KCl applying a scan rate 0.05 V/s. The obtained voltammograms show less sensitivity with BCPE (dashed line). Whereas the (poly)-NC/CPE sensor showed a large improvement with more sensitivity and reproducibility in electrotransfer progression in the same condition (solid line) as shown in Fig. 5.4.2. From the improved result, it is evident that the (poly)-NC/CPE sensor surface property was significantly changed.

The total active surface area can be calculated approximately by using "Randles-Sevcik Equation" [115, 67] discussed in chapter-1 **(**section 1.2.2(a); page no.7-8). "For (poly)-NC/CPE sensor the electroactive surface area is maximum i.e., 0.0457 cm^2 as compared with bare CPE i.e., 0.0287 $cm^{2"}$.

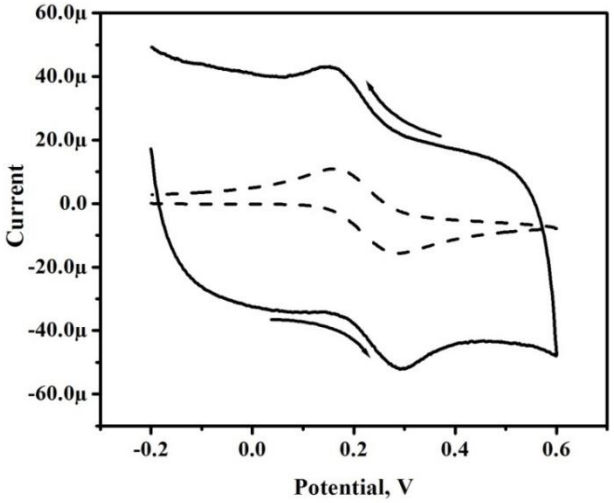

Fig. 5.4.2- Cyclic voltammograms of 1.0×10^{-3} M potassium ferrocyanide at BCPE (dashed line) and (poly)-NC/CPE(solid line) at scan rate of 0.05 V/s.

5.4.3(c). Electrochemical Behavior of Epinephrine at (poly)-NC/CPE Sensor

The voltammetry behavior of EP (0.1×10^{-3} M) were recorded by the CV method by maintaining a scan rate of 0.05 V/s at BCPE and (poly)-NC/CPE in 0.2 M phosphate buffer solution of physiological pH as shown in Fig. 5.4.3. At BCPE, the electro-oxidation behavior of EP reveals a slow electron transfer phenomenon with a peak potential at 0.243 V versus SCE. However, when the electro oxidation of EP was tested with modified (poly)-NC/CPE sensor in identical condition, the voltammograms showed a significant rise in current signal with the peak potential at 0.209 V. Hence, this study reveals the tremendous electrocatalytic activity and reproducibility of (poly)-NC/CPE sensor towards the electrochemical oxidation of EP.

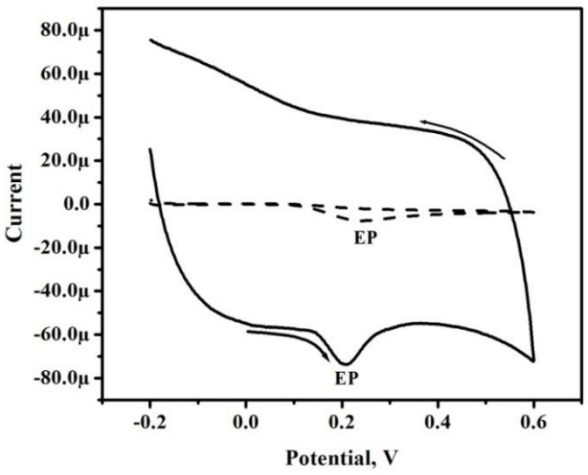

Fig. 5.4.3- Cyclic voltammograms for 0.1×10^{-3} M EP at BCPE (dashed line) and (poly)-NC/CPE (solid line) in 0.2 M PBS of pH 7.4 at scan rate 0.05 V/s.

5.4.3(d). Variation of Scan Rate

The oxidation of EP (0.1×10^{-3} M) in 0.2 M PBS maintaining a pH of 7.4 was studied by varying the scan rate in the range 0.020 V/s to 0.120 V/s [176]. The result showed (Fig. 5.4.4(a)) that with the increase in scan rate, there is a positive shift of peak potential with an increase in peak current. A plot of Ipa versus υ (Fig. 5.4.4(b)) and the plot of Ipa versus $\upsilon^{1/2}$ (Fig. 5.4.4(c)) were obtained. Good linearity for both the graphs was observed with the linear regression values are r^2=0.9964 and r^2=0.9914 respectively. This supports adsorption controlled electrode process [156]. For an "adsorption-controlled" and "irreversible electrode process", the number of electrons transferred and heterogeneous rate constant was calculated by using the "Laviron Equation" [177].

$$E_P = E^{ó} + \frac{2.303RT}{anF}\log\frac{RTk^0}{anF} + \frac{2.303RT}{anF}\log v \qquad \text{----------(5.4.1)}$$

Where,

E^0=formal redox potential

α=transfer coefficient

n=number of electrons transferred

k^0=standard heterogeneous rate constant and other symbols R, T, F have their usual meanings. From the slope value of Ep versus log$v$ plot (data not shown), 'αn' value was calculated. Thus the calculated 'αn' for modified (poly)-NC/CPE was 0.84. Further 'α' was found by the "Bard and Falkner Equation" [178]. For the modified electrode, α value was found to be 0.56, which was calculated by the following equation.

$$\alpha = \frac{47.7}{E_p - E_{p/2}} \text{----------(5.4.2)}$$

The number of electrons transferred was calculated to be one (1.15≈1.0). From the intercept of the plot of Ep versus scan rate by extrapolating to the vertical axis, E^0 value 0.16 was obtained and the intercept of Ep versus logν plot was 0.337. Thus using both intercept values the k^0 was calculated to be $1.304 \times 10^3\ s^{-1}$.

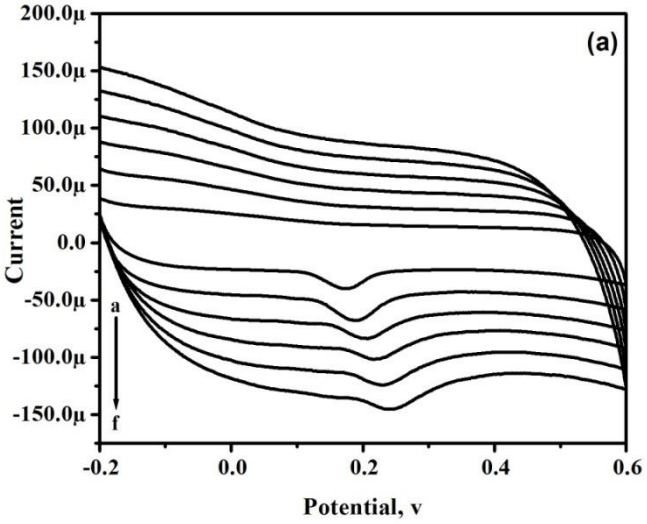

Fig. 5.4.4(a)- Cyclic voltammograms for 0.1×10^{-3} M EP at (poly)-NC/CPE in 0.2 M PBS of pH 7.4 at different scan rate (a-f; 0.020 V/s, 0.040 V/s, 0.060 V/s, 0.080 V/s, 0.100 V/s, 0.120 V/s).

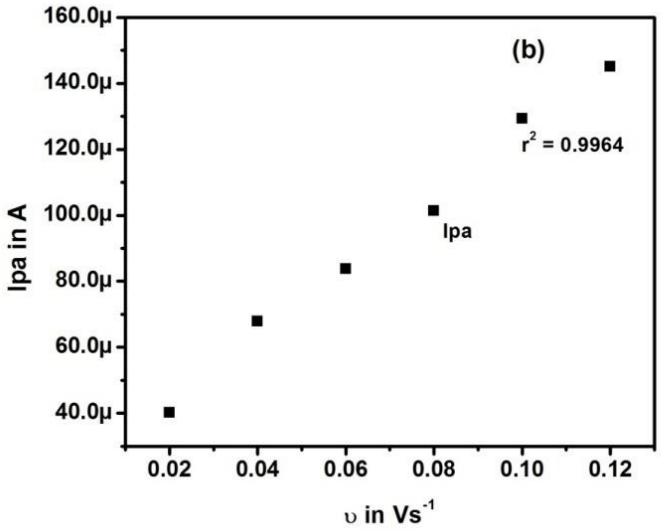

Fig. 5.4.4(b)- Graph of anodic peak current (Ipa) versus scan rate (υ).

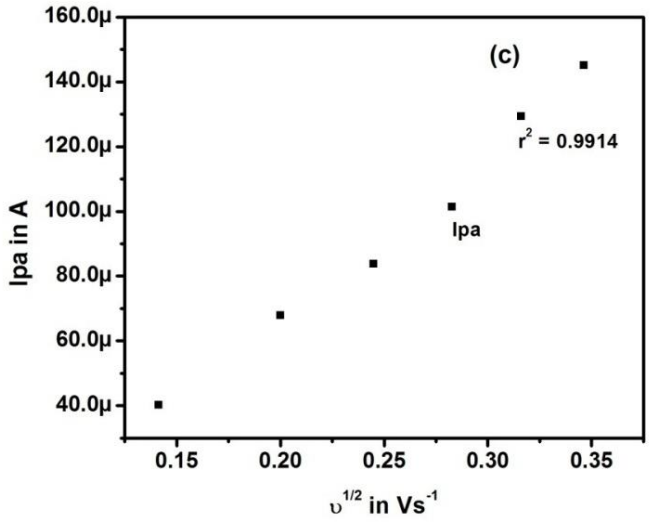

Fig. 5.4.4(c)- Graph of anodic peak current (Ipa) versus square root of scan rate($\upsilon^{1/2}$).

5.4.3(e). Variation of pH

The peak current response of 0.2×10^{-3} M EP was studied by varying pH within the range 5.5-8.0 and the voltammograms are recorded as in Fig. 5.4.5. It can be observed that when the pH of the 0.2 M PBS is increased, the peak potential was shifted gradually towards the negative side [180] and concluding that the electrochemical oxidation of EP depends on pH values.

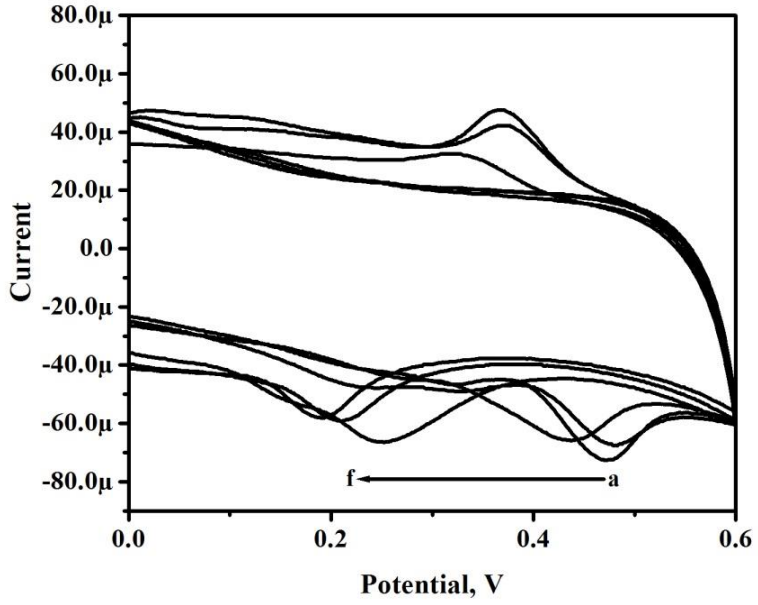

Fig. 5.4.5- Cyclic voltammograms obtained for the oxidation of EP at (poly)-NC/CPE in 0.2 M PBS solution of different pH values (a-f: 5.5 to 8.0) at scan rate of 0.05 V/s.

5.4.3(f). Variation of Concentration

The developed CV profile of EP at different concentrations with (poly)-NC/CPE sensor in 0.2 M PBS of pH 7.4 was studied. The EP concentration range was varied in the range of 20.66 μM-174.4 μM and the scan rate applied was 0.05 V/s. It was observed that with increasing in [EP] the Ipa increases with Epa shifting towards less positive side Fig. 5.4.6(a). A graph was plotted between [EP] and Ipa, a linear curve (Fig. 5.4.6(b)) is obtained with following linear regression equation

$Ipa(10^{-5}A)=0.0196(C_o\mu M/L)+3.8489$, ($r^2=0.9976$)

The LOD was found to be 11.3×10^{-9} M of EP at (poly)-NC/CPE. The calculation method of LOD and its equation is described in chapter-1(section 1.7.4; page no.16). Further, the LOD value was compared with the reported techniques in Table 5.4.2 [176, 181-197]. It can be seen that the present work could found out the superlative method to detect EP at (poly)-NC/CPE sensor.

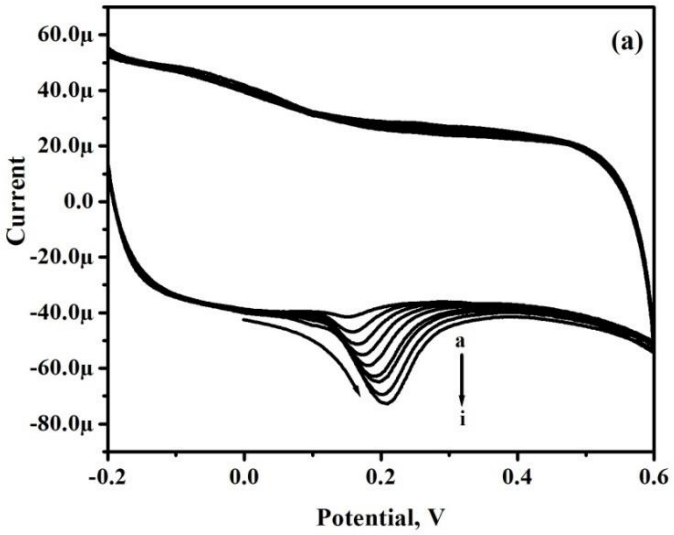

Fig. 5.4.6(a)- Cyclic voltammograms of EP in 0.2 M PBS solution of pH 7.4 at (poly)-NC/CPE with different concentrations (a-i: 20.66 μM, 40.98 μM, 60.97 μM, 80.64 μM, 100.0 μM, 119.04 μM, 137.79 μM, 156.25 μM, 174.4 μM) at scan rate of 0.05 V/s.

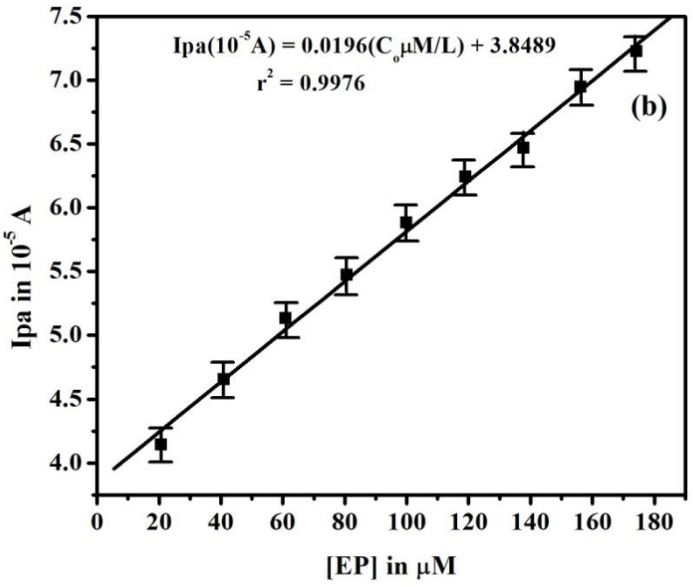

Fig. 5.4.6(b)- Graph of anodic peak current versus concentration of EP.

Table 5.4.2- Comparison of linear range and detection limits for EP with different classical methods and working electrodes.

Working Electrode	Classical methods	Linear working range (µM)	Detection limits (M)	References.
Pen SAM-MAuE	CV	100-0.1	0.1×10^{-6}	[181]
P(1-methylpyrrole)GCE	SWV	0.75-200	0.168×10^{-6}	[182]
p (taurine) ME	DPV	2-600	0.3×10^{-6}	[183]
FePc-ME	CV	1-300	0.5×10^{-6}	[184]
poly(caffeic acid)MGCE	CV	2-300	0.6×10^{-6}	[185]
TTABMCPE	DPV	0.15-30	0.12×10^{-6}	[186]
DH-CN/CPE	DPV	5.0-20 20-600	1.0×10^{-6}	[187]
GCE-MWCNT-CoTSPc	Amp	3.0-15	0.45×10^{-6}	[188]
MnO_2/Nafion/GCE	CV DPV	0.5-100 100-700 0.03-10 10-100	0.100×10^{-6} 0.005×10^{-6}	[189]
Paraffin/MWCNT/CoPc	DPV	1.3-5.5	0.016×10^{-6}	[190]
CNT/GCE	CV	1.0-50	0.100×10^{-6}	[191]
CNT/SSE	DPV	2.0-100	2.000×10^{-6}	[192]
GME/GCE	CV	0.4-13 13-109	0.089×10^{-6}	[193]
GR/Au/GCE	CV	0.05-8.0	0.007×10^{-6}	[194]
PolyCafA/GCE	CV	2.0-80	0.200×10^{-6}	[195]
RuOHCF/MWCNT/GCE	DPV	0.1-10	0.052×10^{-6}	[196]
MWCNT/CFE	DPV	up to 100	0.900×10^{-6}	[197]
2PHCMCNPE	SWV	0.05-550	9.4×10^{-9}	[198]
Niacin/CPE	**CV**	**20.66-192.30**	$\mathbf{11.3 \times 10^{-9}}$	**Present work**

5.4.3(g). Electrochemical Behavior of Uric Acid at (poly)-NC/CPE

Fig.5.4.7 showed electrochemical response for 0.1×10^{-3} M of UA of pH 7.4 in PBS applying scan rate 0.05 V/s for uric acid at BCPE (dashed line) the peak potential observed at 0.286 V with broad peak and less sensitive but on the other hand (poly)-NC/CPE (solid line) the potential peak observed at 0.293 V with enhancement in current signal with sharp peak studied by cyclic voltammetry. The effect of concentration variation in the range 40.98 μM to 137.79 μM of UA was determined at (poly)-NC/CPE as shown in Fig.5.4.8(a). The graph between Ipa vs concentration of uric acid produced a straight line (Fig.5.4.8(b)). The following linear regression equation can obtained,

$Ipa(10^{-5}A)=0.0110(C_o\ \mu M/L)+3.4577$, ($r^2=0.9969$)

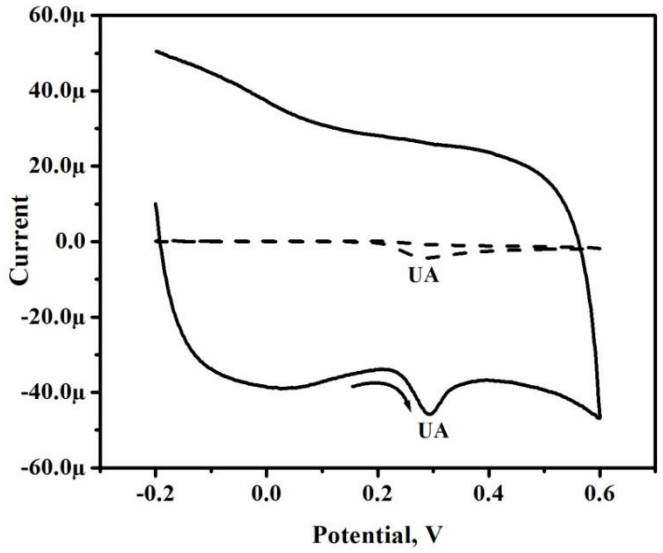

Fig. 5.4.7- Cyclic voltammograms of 0.1×10^{-3} M UA in 0.2 M PBS solution of pH 7.4 at BCPE (dashed line) and (poly)-NC/CPE (solid line) at scan rate of 0.05 V/s.

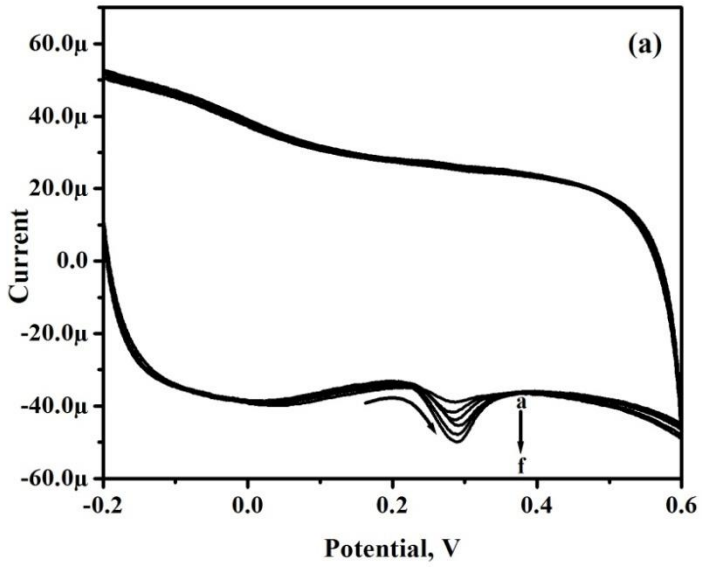

Fig. 5.4.8(a)- Cyclic voltammograms of UA in 0.2 M PBS solution of pH 7.4 at (poly)-NC/CPE with different concentrations (a-f: 40.98 µM, 60.97 µM, 80.64 µM, 100.0 µM, 119.04 µM, 137.79 µM) at scan rate of 0.05 V/s.

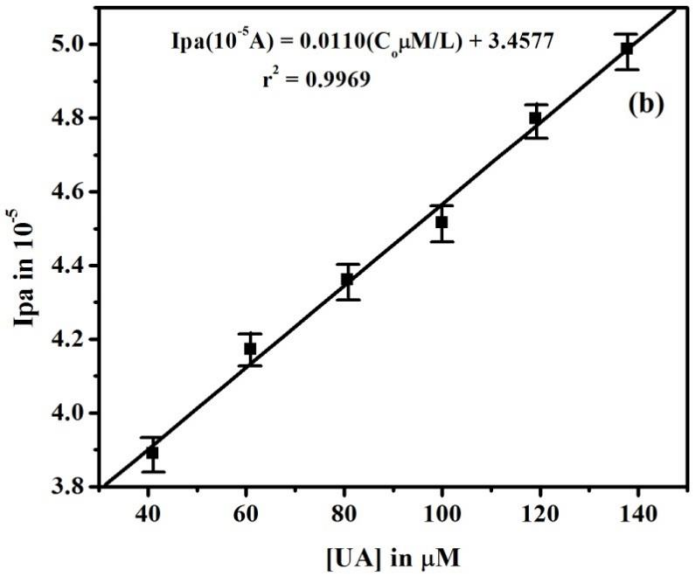

Fig. 5.4.8(b)- Graph of anodic peak current versus concentration of UA.

5.4.3(h). Simultaneous Resolve of Epinephrine and Uric Acid

Simultaneous resolution of 0.1×10^{-3} M EP and 0.1×10^{-3} M UA under optimal condition applying a scan rate of 0.05 V/s was investigated by the CV technique. Fig.5.4.9 showed cyclic voltammograms result of the equimolar combination of the molecular species at BCPE (dashed line) and (poly)-NC/CPE. The peak potential of EP and UA were found to be at 0.171 V and 0.290 V respectively. But, at BCPE, a partially overlapped signal was observed with poor sensitivity and very difficult to identify the peak potential individually. But on the other hand, modified poly-niacin carbon paste electrode sensor was shown sharp sensitivity with good voltammetric signal. Hence, the (poly)-NC/CPE can be used to detect the two analytes in the same solution as it is evident from the Fig.5.4.9 which shows a peak to peak separation of two species. The peak separation is also of significant value of 0.119 V rejecting a chance of overlapping. Hence from the result we can conclude it is an excellent method to determine EP in presence of UA using (poly)-NC/CPE.

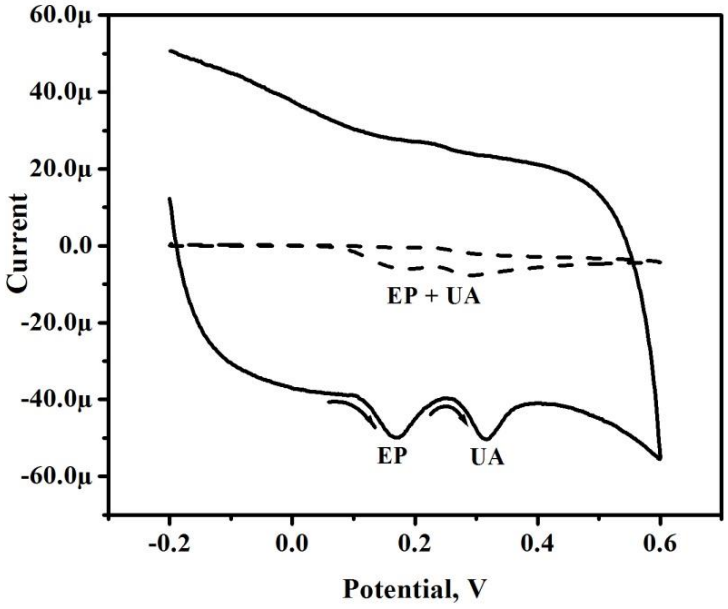

Fig. 5.4.9- Cyclic voltammograms for simultaneous determination of 0.1×10^{-3} M EP and 0.1×10^{-3} M UA at BCPE (dashed line) and (poly)-NC/CPE (solid line) at scan rate of 0.05 V/s.

5.4.3(i). Injection Analysis and Recovery Test of EP

The recovery test of EP in the injection sample was studied and evaluated in the concentration range between 5×10^{-6} M to 30×10^{-6} M and maintaining pH= 7.4. The peak current values were obtained for each solution by CV technique. The recovery percentage was calculated and listed in Table 5.4.3. The recovery percentage shows that the (poly)-NC/CPE has a good recovery with RSD (<1.6%) for the electrochemical resolve of EP in pharmaceutical preparation and in the clinical laboratory to determine the quantity of epinephrine for diagnosing the disease caused by the deficiency of epinephrine [172].

Table 5.4.3- Determination of epinephrine in injection sample by (poly)-NC/CPE.

Sample	Added(μM)	Found(μM)	RSD(%)	Recovery(%)
1	5	4.86	1.41	97.20
2	10	10.28	1.48	102.80
3	15	14.78	1.58	98.54
4	20	19.98	1.38	99.90
5	25	24.68	1.32	98.72
6	30	29.80	1.38	99.34

5.4.4. Conclusion

The oxidation of EP and UA were studied at (poly)-NC/CPE using a CV technique. The investigations included the study of the effect of pH variation, scan rate, and concentration of EP. The obtained results displayed the type of electrode reaction was irreversible; adsorption controlled and involves equal number of protons and electrons transfer. Under optimal conditions, the lower limit of detection with (poly)-NC/CPE was found at 11.3×10^{-9} M while EP concentration range was maintained from 20.66 μM-174.4 μM. The applicability of the current methodology was successfully estimated by the quantification of EP in injection. Thus, the present study was very remarkable on account of used modifiers due to its affectability, selectivity and reproducibility.

Chapter 6

SUMMARY AND CONCLUSION

6.1. Summary

The electroanalytical chemistry takes part in many areas such as “chemistry, biology, environmental science, life science, and food science”. In electroanalytical techniques, the cyclic voltammetry method is accepted and has been proved to be more sensitive and selective for the investigation of drugs including organic, inorganic and bio-molecules. In recent years, the research focus on the progress of electrochemical modifier or biosensors methods to assess some biologically important compounds in very minute concentration is going on due to its time-saving operation and accuracy in the results.

For example catechol, mesalazine, paracetamol, and epinephrine, etc are some biologically important compounds. “These drugs at a very low concentration are very toxic to animals and human beings and they are difficult to degrade. Because of these facts, they are a major problem and cause environmental pollution. To overcome such problems and an assessment of the literature revealed that, there are a few analytical methods used for the determination of” biomolecules in a pharmaceutical analysis like the HPLC method, fluorescence spectroscopy, liquid chromatographic-mass spectroscopy, spectro-photometric method, UV-spectroscopy, sensitive liquid chromatography (SLC). However, some problems have been encountered in utilizing the above-reported methods such as long-time consumption for analysis, high-cost instruments, use of toxic chemicals, lower detection limit incapability and require elaborated extraction procedure.

Whereas, the electrochemical methods are proved as the best challenger for the determination of any biomolecules due to their accuracy, low-priced equipment, lower limit detection, selectivity and sensitivity of sample preparation for the determination of biologically important molecules. Hence the present work was designed to detect these four biomolecules by developing new electrode materials, biosensors, their theory, advantages, limitations, and applications. The work can be summarized as follows;

- Catechol: According to the present study catechol is a class of catecholamine drug showed a reversible electron transfer process by following the maximum criteria of the reversible reaction system with two protons and electrons transfer mechanism. Moreover, this reaction process indicates a diffusion-controlled redox process. The effect of pH, concentration and scan rates, a lower limit of detection (1.49 μM) and limit of quantification (4.99 μM) values in electrochemical reaction has also been observed. Hence, the catechol showed better selectivity and sensitivity at poly-NA/MCPE.
- Mesalazine: It is used to treat a certain bowel disease like “ulcerative colitis” and is represented as a quasi-reversible reaction system by following the maximum criteria

of quasi-reversible diagnostics test. It shows adsorption controlled process on the surface of electrode and influence of pH, variation of scan rate and concentration in electrochemical process has also been studied. The lower limit of detection was found 1.9×10^{-9} M at CTAB/MCPE as compared to other different methods and electrodes. The applicability of the current methodology was successfully estimated by the quantification of mesalazine in tablets.

- Paracetamol: It is an "analgesics (pain relievers) and antipyretic (fever reducers) drug showed reversible oxidation process at physiological pH in 0.2 M of phosphate buffer solution at CZ/MCPE. The end result, during the oxidation process of paracetamol two protons and electrons, were transferred while the electrochemical process is adsorption controlled. The effects of scan rate, concentration, and pH on the electrochemical process have also been noted. The lower limit of detection value was obtained at 0.24×10^{-6} M. Further, the present method was well applied for tablet analysis.
- Epinephrine: It is a class of neurotransmitter drug showed an irreversible reaction system by following the maximum criteria of irreversible diagnostics test. The electrochemical process was found to be adsorption controlled process with the involvement of an equal number of protons and electrons. The parameters such as the effect of scan rate, pH, variation of analytes concentration and heterogeneous rate constant were also studied. The modified electrode sensor showed good electrochemical stability of epinephrine with lower limit of detection 11.3×10^{-9} M.

In general, this electroanalytical technique is suitable for quality control laboratories as well as pharmacokinetic studies.

6.2. Conclusion

The present study has been carried out to give an explanation of the electrochemical behavior of four biologically active drugs such as; catechol, mesalazine, paracetamol, and epinephrine by using various parameters of cyclic voltammetry technique such as variation of scan rate, pH effect and concentration were studied. For each of the drugs we could establish the reaction mechanisms on the modified electrode surface and it has been observed that the electrode processes were either adsorption controlled or diffusion controlled and electrochemical behaviour of the analytes were reversible/irreversible/quasi-reversible. All the modified electrodes prepared were shown better selectivity and sensitivity as compared to BCPE. The lower detection limit for every drug was found to be enhanced when compared with the other modified electrodes reported earlier. The interference study was carried out and established a validated method for tablets and injections analysis.

Therefore the findings of the resulting works from this plan clearly fulfilled all the objectives as desired.

Scope for the future work

- The developed sensors can be applied for some other biomolecules or toxic drugs or pesticides.
- We can evaluate the sensor activity for in-vivo studies.
- The effect of modifier ratio can be evaluated.
- Further, the thickness of the surface, effect of variable modifier concentration and pH of the supporting solution can be evaluated.

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197. M.E, Ghica, C.M. A. Brett, Simple and efficient epinephrine sensor based on carbon nanotube modified carbon film electrodes. *Analytical Letters*., Vol. 46, 2013, pp. 1379-1393.
198. H. Beitollahi, H.K. Maleh, H. Khabazzadeh, Nanomolar and selective determination of epinephrine in the presence of norepinephrine using carbon paste electrode modified with carbon nanotubes and novel 2-(4-Oxo-3-phenyl-3,4-dihydro-quinazolinyl)-N′-phenyl-hydrazinecarbothioamide. *Anal. Chem*., Vol. 80, 2008, pp. 9848-9851.

List of Awards

- Received first best Poster presentation award at in one day National Conference on “Role of Science & Technology in Environmental Protection” (NCRSTEP) organised by Deccan Environmental research Organisation (DERO), Vijayapur, Karnataka, India, in the month of December 2015.

- Received second best Oral presentation award at in one day National Conference on “National Conference on Advance in Biodiversity, Science, Engineering and the Environment” (NCABSEE) organised by Dept. of Chemistry, M.S. Irani Degree College of Arts, Science and Commerce, Kalaburgi (Gulbarga), Karnataka, India, in the month of February-2016.

- Received first best Poster presentation award at UGC sponsored one day state level conference on “Advances in Material Science" organised by Dept. of Chemistry, KLE Society’s, P. C. Jabin Science College Vidyanagar, Hubballi-31, Karnataka, India, in the month of January 2017.

- Awarded “THE BEST RESEARCH SCHOLAR OF THE INSTITUTE” issued in the workshop titled “Organising Research with Reference Management Tools” held on the occasion of Institute Research Day jointly organized by Research and Development Cell and Library and Information Centre at BLDEA’s CET, Vijayapur, Karnataka, India, in the month of May 25-26th, 2018

- Received second best Poster presentation award at in one day Seminar on “Research Trends in Science & Technology” organised by BLDEA’s CET, Vijayapur, Karnataka, India, in the month of May -2019.

Presentations at National / International Conference

- Presented paper entitled "Up growth effect of cetyltrimethyl ammonium bromide with carbon paste electrode for the electrochemical determination of allopurinol and its biological activities" in one day National Conference on "Role of Science & Technology in Environmental Protection" (NCRSTEP) - 2015, held at DERO, Vijayapur, Karnataka, India, India on 29th December 2015.
- Presented paper entitled "Electrochemical investigation of catechol at poly(niacinamide) MCPE A voltammetric study" in one day National Conference on "National Conference on Advance in Biodiversity, Science, Engineering and the Environment" (NCABSEE) held at M.S. Irani Degree College of Arts, Science and Commerce, Kalaburgi (Gulbarga), Karnataka, India, on 17th February 2016.
- Presented paper entitled "Niacin film coated CPEsensor for the determination of epinephrine-A cyclic voltammetric study" in UGC sponsored one day State Level Conference on "Advances in Material Science" held at, KLE Society's, P. C. Jabin Science College Vidyanagar, Hubballi-31, Karnataka, India, on 20th Janauary 2017.
- Presented paper entitled "Electrochemical detection of paracetamol at carbamazepine film coated carbon paste electrode" in one day National Conference on "2nd National Conference on Emerging Trends in Chemistry and Materials Science" (ETCM) held at, KLS Gogte Institute of Technology, Belagavi, Karnataka, India, on 23th Janauary 2016.
- Presented paper entitled "Poly(nile) blue based electrochemical sensor for catechol and hydroquinone" in three days International Conference on "Advanced Functional Materials for Energy, Environment and Health Care" (AFMEEHC) held at, University of Mysore, University with Potential for Excellence (UPE), Mysuru, Karnataka, India, on 18-20th, March 2019.
- Presented paper entitled "Poly(nile) blue based electrochemical sensor for catechol and hydroquinone" in one day Seminar on "Research Trends in Science & Technology" organised by BLDEA's CET, Vijayapur, Karnataka, India, on 25th, May 2019.

List of Publications

- **A.B. Teradale**, S.D. Lamani, B.E. Kumara Swamy, P.S. Ganesh and S.N. Das, "Electrochemical Investigation of Catechol at Poly(niacinamide) Modified Carbon Paste Electrode-A Voltammetric Study". *Advances in Physical Chemistry*, Volume 2016, Article ID 8092860, 8 pages http://dx.doi.org/10.1155/2016/8092860 (HINDAWI).
- **A.B. Teradale**, S.D. Lamani, P.S. Ganesh, B.E. Kumara Swamy and S.N. Das, "CTAB Immobilized Carbon Paste Electrode for the Determination of Mesalazine-A Cyclic Voltammetric Method". *Sensing and Bio-Sensing Research*, Vol. 15:2017, pp. 53-59 (ELSEVIER).
- **A.B. Teradale**, S.D. Lamani, B.E. Kumara Swamy, P.S. Ganesh and S.N. Das, "Electrochemical Sensor for the Determination of Paracetamol at Carbamazepine Film Coated Carbon Paste Electrode". *Z. Phys. Chem.*, 2018, pp. 814-829 (DE GRUYTER).
- **A.B. Teradale**, S.D. Lamani, B.E. Kumara Swamy, P.S. Ganesh and S.N. Das, "Niacin Film Coated Carbon Paste Electrode Sensor for the Determination of Epinephrine in Presence of Uric Acid-A Cyclic Voltammetric Study". *Analytical Chemistry Letters*, Vol. *7*: 2018, pp. 748-764 (TAYLOR & FRANCIS).