ADDIS ABABA UNIVERISTY

DEPARTMENT OF CHEMIISTRY

ELECTROCHEMICAL DETERMINATION OF FOLIC ACID AT POLYBROMOCRESOL PURPLE MODIFED GLASSY CARBON ELELCTRODE

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February 2020

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A Graduate Thesis Submitted to the Department of Chemistry in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

Addis Ababa University

Addis Ababa, Ethiopia

February 2020

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DEPARTMENT OF CHEMISTRY

This is to certify that the thesis prepared by Eshetu Amdie entitled: electrochemical determination of folic acid at polybromocresol purple modified glassy carbon electrode. Submitted to the partial fulfillment of the requirements for Degree of Master (Analytical Chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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February 2020

Abstract

Polybromocresolpurple (PBCP) was used for the modification of glassy carbon electrode (GCE) to determine the electrochemical behavior of folic acid (FA) in 0.1 M PBS at pH 7.0 at the scan rate of 100 mV/s. The modified electrode was characterized and used to electrochemically determine FA using cyclic and differential pulse voltammetry. The oxidation peak current of FA at the modified electrode increased compared to the bare GCE, hence indicating that the BCP displays a significant improvement on the electrochemical oxidation of FA. The oxidation peak current of FA is proportional to its concentrations in the range of 20-100 µM with 0.987 correlation coefficient. The detection limit was found to be 1.772 µM. Based on the relationship between the oxidation peak current and square root of scan rate, the overall electrode process was found to be diffusion controlled at BCP/GCE. The modified electrode exhibited good sensitivity, and reproducibility for the determination of FA, indicating the capable applications of the modified electrode in real sample analysis.

Key words: Folic acid, modified electrode, Polybromocresolpurple (BCP), electropolymerization, cyclic voltammetry, differential pulse voltammetry

Acknowledgements

First, I have to thank God for giving me the gift that he did and for everyday for everything that happens to me.

My Sincere gratitude goes to my advisor, Dr. Negussie Negash for his professional advice, encouragement and strong support. Dr. Negussie continuously supported this work from its initial stage. He gave me a good insight from the very beginning. He patiently read the first draft of the thesis and gave me very valuable comments, corrections and suggestions. I have a high regard for his interest in this work and am grateful for all clues in my studies, which were always very valuable.

I honestly thank Tesfu Hailu (Analytical chemistry PhD student), for his sincere guidance, technical aid and assistance in every day laboratory problems facing me in the research direction.

I would like to express my thanks to the Department of Chemistry, Addis Ababa University, for providing me this scholarship, for the financial support and giving me access to laboratory facilities for this study.

I would like to express my gratitude to the ministry of educaton sponsoring me for this program

I wouid like to appreciate my friends Ananiya Abebe and Mekonnen Mulugeta for helpful discussions and helping me by giving valuable advice during the difficult times. Besides to this my deepest and heart full thank goes to my brother Kassahun Amdie for his moral, material and finacial support.

Finally, my heartfelt gratitude shall go to my family, Mahlet Nigussie and my sons Mikiyas and Abel for their understanding, tolerance and inspiring support during this program.

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List of Abbreviations

AA	ascorbic acid
BCP	Bromocresol Purple
CE	Counter electrode
CME	Chemical Modifiedof Electrode
CPs	conducting polymers
CV	Cyclic Voltammetry
D	Diffusion coefficient
DNA	Deoxyribonucleic Acid
DPV	Differential Pulse Voltammetry
E	Potential
$E_{1/2}$	Half wave potential
E_{pa}	Anodic Peak Potential
E_{pc}	cathodic peak potential
FA	folic acid
GCE	glassy carbon electrode
HPLC	high performance liquid chromatography

I_{pa}	anodic peak current
I_{pc}	cathodic peak currentI
ISEs	Ion Selective Electrodes
K_1	Rate Constant For Heterogeneous Electron Transfer
k_2	Rate Constant For Homogenous Electron Transfer
K_p	Partition Coefficient
LC-MS	liquid chromatography- mass spectroscopy
m	Membrane Phase
MEEKC	microemulsionelectrokinetic chromatography
mRNA	Massager Ribonucleic Acid
MWCNTs	Multi Wall Carbon Nanotubes
NPV	Normal Pulse Voltammetry
PBS	Phosphate Buffer Solution
Ph	phenol
Pm	Polymer Film Membrane
Ps	polymer film Solution
R	Regression Coefficient
RE	Reference electrode

RNA	Ribonucleic Acid
S	Substrate
SWCNT	Single Wall Carbon Nano Tube
WE	working electrode
ΔE	Change in potential
μA	Microampere
μL	Micro liter
μM	Micro molar

1. Introduction

1.1. General description of folic acid

Vitamins are a group of organic compounds, essential in small amount for the normal functioning of the body and regulate the metabolic activity [1]. Deficiency of vitamins may result in often painful and potentially harmful diseases. As a result vitamins play an important role in our body.

Folic acid belongs to the B-vitamin group also referred as vitamin M, vitamin B9 (commonly called folate), vitamin Bc (or folacin). As it cannot be synthesized in body it must be supplied daily from the foods such as fruits, vegetables, mushrooms, algae, fortified grains, etc.[2]. In food FA predominantly exist as polyglutamates, which have to be hydrolyzed in body to monoglutamates in order to be transported. Folate, as conjugate base of FA, represents active form which is incorporated in many metabolic path-ways, mainly in carbon transfer reactions, such as amino acid inter-conversions and purine and pyrimidine biosynthesis [3]. Also, it has important role in homocysteine metabolism. However, synthetic B9-vitamin (in dietary supplements or in fortified food) is in the form of folic acid, which is most stable form [4] and in human body FA is transformed into its active form by the enzyme dihydrofolate reductase.

Folic acid (FA) is a slightly water soluble vitamin and was first discovered in Spinach [5]. FA is a nutrient of great importance, especially for women planning for pregnancy. To reduce significantly the incidence and reoccurrence of neural tube defects per conceptual supplementation of folic acid has been demonstrated [6]. Moreover FA is usually employed in the treatment or prevention of megaloblastic anemia during pregnancy [7]. The US Food and Drug Administration introduced mandatory reinforcement of cereal grain products with folic acid at a concentration of 140 mg/100 g in January 1998 [8]. The Department of Health in the UK proposed fortification of flour with folic acid at 240 mg/100 g [9]. A lack of FA in our body fluids gives rise to several complications including gigantocytic anaemia, leucopoenia, devolution of mentality, psychosis and increasing possibility of heart attack and stroke [10]. Therefore, the accurate determination of FA is an important and significant work in practice. Some of the analytical methods including liquid chromatography/tandem mass spectrometry (LC/MS/MS) [11], high performance liquid chromatography(HPLC) [12], capillary electrophoresis [13], micro emulsion electro kinetic chromatography (MEEKC)

[14] , enzyme linked immunosorbent assay (ELISAs) [15], UV-vis spectrophotometry [16], flow-injection chemiluminescence [17], ion chromatography [18], quartz crystal microbalance [19], voltammetry [20-24] and electrochemical methods [25] have been applied to determine FA. Nevertheless, most of these methods required complicated sample preparation, suffered from low sensitivities or strong acidic analysis system. Thereby, it is of primary importance to develop an alternative method for FA determination with high sensitivity and simplicity under physiological conditions. Among those reported different methods, electrochemical methods have attracted considerable attention in recent years, especially in determination of biological and environmental analysis [26,27], as a result they are more selective and sensitive, less expensive and more convenient. FA (Fig 1) is one of the electro active species and accordingly we employed electrochemical method for the determination and some of the electrochemical methods for other electro-active species have been reported [28-32].

In this work, the electrochemical method for the determination of FA using poly bromocresol purple modified glassy carbon electrode (BCP/GCE) was developed. An electrochemical technique such as cyclic voltammetry (CV) was used for characterization and differential pulse voltammetry (DPV) for quantification. Using this methods, the electro- analytical parameters for the determination of FA in the concentration range of 1-300 μM are determined.

1.2. Occurrence of folic acid

Folic acid is largely extended in nature and it should be ingested by the consumption of mushroom, spinach, green leaves, avocado, asparagus, meat, eggs and yeast.

1.3. Physical and chemical properties of folic acid

1.3.1. Physical properties

Folic acid is a yellow to orange, tasteless, needles crystal. The melting point of FA is 250 ºC and above this temperature it decomposes. Folic acid is slightly soluble in water, ethanol, methanol and butanol. It is insoluble in acetone, chloroform, ether and benzene. Solutions of hydroxides and carbonates are the best solvents, together hot and dilute solutions of hydrochloric acid and sulfuric acid.

Folic acid has a molecular formula of $C_{19}H_{19}N_7O_6$ with a relative molecular mass of 441.4 g/mol and its structural formula is shown in Fig1.

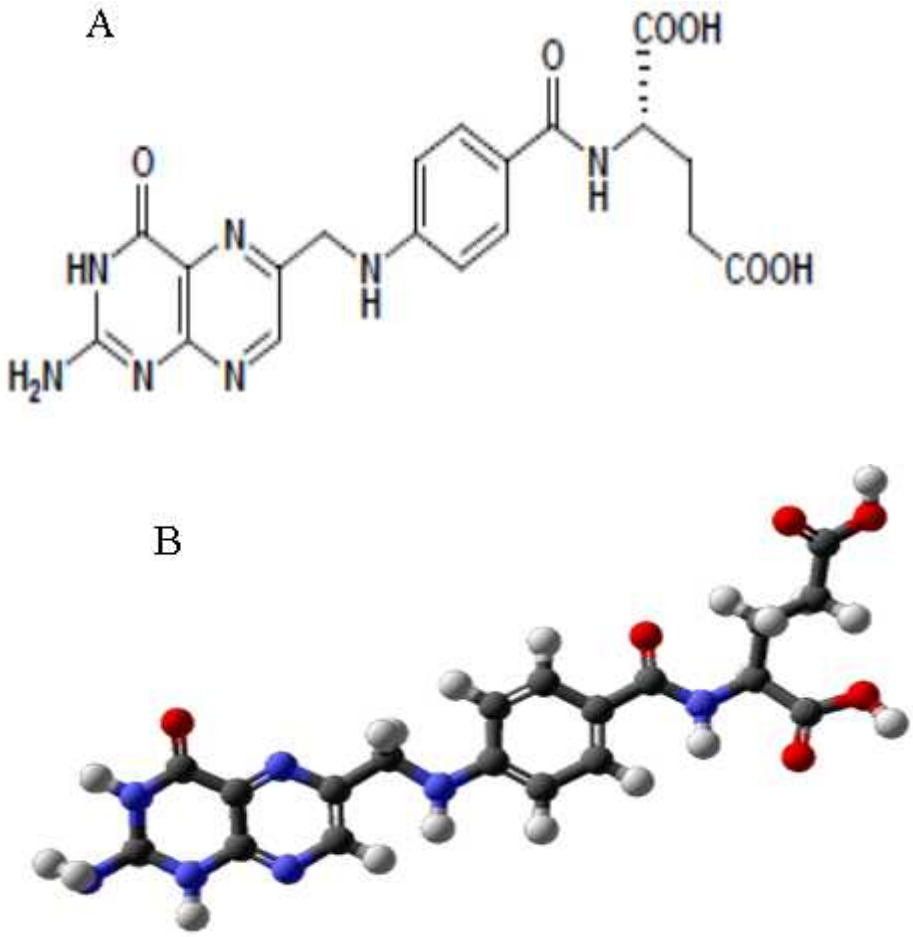


Figure 1: A) structure of folic acid B) 3D structure of folic acid

1.3.2. Chemical properties

Folic acid in nature is involved in reactions of transference of methyl groups to organic compounds in the amino acids metabolism.

1.4. Uses of folic acid

Folic acid is an essential vitamin for our body, and similar to other vitamin it should be ingested through a diet containing the right quantity of vegetables. Folate is especially important during periods of frequent cell division and growth, such as infancy and pregnancy. It is essential for red blood cell production, DNA replications and amino acids synthesis.

1.5. Health effect of folic acid

As consequence of low folates intake, numbers of health disorders were reported: neural tube defect, coronary heart diseases and osteoporosis, increased risk of breast and colorectal cancer, poor cognitive performance, hearing loss, anemia. Especially during the pregnancy, the diets low in folic acid is critical due to responsible for some diseases on newborns such as neural tube defects. Folate deficiency hinders DNA synthesis and cell division, affecting hematopoietic cells and neoplasms the most because of their greater frequency of cell division. RNA transcription and subsequent protein synthesis are less affected by folate deficiency. Moreover, a poor ingestion of folic acid can cause decrease on the fertility of women and men. as the mRNA can be recycled and used again (as opposed to DNA synthesis, where a new genomic copy must be created

1.6. Synthesis of folic acid

Folic acid is produced synthetically through the condensation of 2, 5, 6-triamino-4(3H)-pyrimidinone, p-aminobenzoyl-L-glutamic acid and 2,3-dibromopropanal.

2. Literature Review

2.1. Chemical Modification of electrodes (CMEs)

Chemically modified electrode (CME) is an electrode made of a conducting or semiconducting material that is coated with a selected monomolecular, multimolecular, ionic, or polymeric film of a chemical modifier and that by means of faradic (charge-transfer) reactions or interfacial potential differences (no net charge transfer) exhibits chemical, electrochemical, and/or optical properties of the film.

Chemically modified electrodes (CMEs) over the last decade has continued to spark considerable interest in analytical chemistry with respect to electrocatalysis, development of new electrochemical and spectroelectrochemical sensors, and electrochromic displays, just to name a few. Since the novel work by Lane and Hubbard involving chemisorbed metals on platinum electrode surfaces, numerous methods have been developed to immobilize species onto a variety of electrode surfaces. These modification techniques include covalent attachment, spin coating, electropolymerization, and others.

Chemically modified electrodes (CMEs) comprise a relatively modem approach to electrode systems [33] that finds utility in 1) a wide spectrum of basic electrochemical investigations, including the relationship of heterogeneous electron transfer and chemical reactivity to electrode surface chemistry, electrostatic phenomena at electrode surfaces, and electron and

ionic transport phenomena in polymers, and 2) the design of electrochemical devices and systems for applications in chemical sensing, energy conversion and storage, molecular electronics, electrochromic displays, corrosion protection, and electro-organic syntheses.

Compared with other electrode concepts in electrochemistry, the distinguishing feature of a CME is that a generally quite thin film (from a molecular monolayer to perhaps a few micrometers-thick multilayer) of a selected chemical is bonded to or coated on the electrode surface to endow the electrode with the chemical, electrochemical, optical, electrical, transport, and other desirable properties of the film in a rational, chemically designed manner. The range of electrode surface properties includes, but is more diverse than, that of ion-selective electrodes (ISEs) which also involve, in their highest forms, rational design of the phase-boundary, partition and transport properties of membranes on or between electrodes. While CMEs can operate both amperometrically (or voltammetrically) and potentiometrically, they are generally used amperometrically, a faradic (charge transfer) reaction being the basis of experimental measurement or study, whereas ISEs are generally used in potentiometric formats where a phase-boundary potential (interfacial potential difference) is the measured quantity [34]. Gas-sensing electrodes (e.g., for CO_2, NH_3, NOx) are also potentiometrically based [35] although the oxygen electrode, which functions amperometrically, is an exception. Chemically sensitive field effect transistors are basically non-faradaic electrode systems in which electric field variations in the semiconductor gate region control the magnitude of the source-drain current **[**36**].** Enzyme-based electrodes detect the product(s) of a reaction between an immobilized enzyme layer and a reaction substrate in many ways, including both amperometric and potentiometric means. The distinction between CMEs and amperometric enzyme-modified electrodes is thus very narrow, the latter being based on a natural biological catalyst, but also with a rational biomolecular electrode design.

Electrocatalysis at a modified electrode is usually an electron transfer reaction between the electrode and some solution substrate which, when mediated by an immobilized redox couple (i-e., the mediator) proceeds at a lower over potential than would otherwise occur at the bare electrode. This type of mediated electrocatalysis process can be represented by the Fig 2.

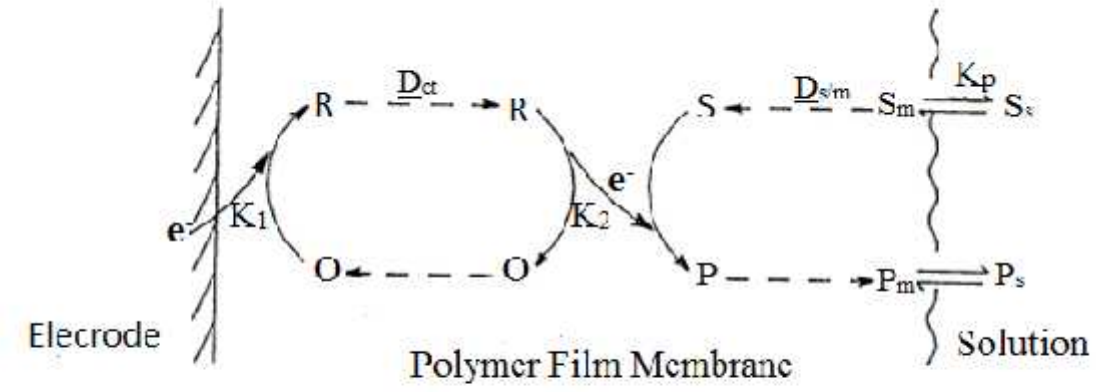


Figure 2: Electrocatalytic reactions on chemically modified electrode [33]

In Fig 2, the subscripts s and m designate the solution and membrane phases, respectively. The substrate, **S,** which is irreversibly (or quasi-reversibly) reduced at the bare electrode, is transported across the polymer film-solution interface (partition coefficient, Kp) and diffuses into the polymer film membrane (diffusion coefficient, Ds/m). The electrocatalyst or mediator, R/O, undergoes heterogeneous electron transfer (rate constant = k*l*) at the electrode surface and charge propagation through the polymer film is described by a rate given by the charge-transport diffusion coefficient, Dct (the symbol De is not recommended). The mediator undergoes homogeneous electron transfer (rate constant = K2*)* with the substrate in the polymer film.

2.2. Conducting Polymers (CPs)

Polymers are the insulating materials that are often used to cover cables and electrical devices. However, there are also a number of polymers that are electrically conductive.

A few intrinsically conducting polymers(ICPs) exist that have alternating single and double bonds along the polymer backbone (conjugated bonds) or that are composed of aromatic rings such as phenylene, naphthalene, anthracene, pyrrole, and thiophene which are connected to one another through carbon-carbon single bonds[37].

Generally, conducting polymers include electronically conducting polymers and Ionically conducting polymers. Ionically conducting polymers are usually called polymer electrolytes. Electronically conducting polymers can also include conjugated conducting polymers and the insulating polymers blending with conducting materials.

Conducting polymers possess alternating single (δ) and double (π) bonds, and these π-conjugated systems lend the CPs their inherent optical, electrochemical, and electrical/electronic properties. The parameters that most affect the physical properties of CPs are their conjugation length, degree of crystallinity, and intra- and inter- chain interactions. CPs provide the advantages of chemical diversity, low density, flexibility, corrosion resistance, easy to control shape and morphology, and tunable conductivity over their existing inorganic counter parts [38,39]. However, the development of the properties of CPs has not been completely commensurate with those of their metallic and inorganic semiconductor counterparts.

Accordingly, CPs have been modified or hybridized with other heterogeneous material components to overcome their inherent limitations in terms of solubility, conductivity, and long term stability. Careful coupling of CPs with other materials can result in materials with attractive properties and new application opportunities in diverse fields ranging from electronics to energy devices. Researchers in this field have reported a variety of strategies to obtain CP-based composites and hybrids with novel structures such as graphene, carbon nano fibers, and carbon nano tubes have been developed. These carbon nano-species improved the structural ordering of the CP chains and facilitated delocalization of the charge carriers, resulting in enhanced conductivity. An affluent spectrum of conductivities has been achieved, ranging from insulating to metallic.

Successful preparations of CP with high stabilities, flexibilities and conductivities have proven that CPs can serve as key material components in light emitting diodes, transistors, electrochromic devices, actuators, electrochemical capacitors, photovoltaic cells and sensors [40].

Polymer modified electrodes have received global attention due to their unique physical, chemical and catalytic properties. Furthermore, electro polymerization has been successfully used for the immobilization of polymers to control their thickness and uniformity. The electro polymer modified electrodes are highly selective and sensitive to a wide range of electro active substances.

2.3. Bromocresolpurple (BCP)

Bromocresol purple (BCP) is a dye of the triophenylmethane family (triarylmethane). It is colored yellow below pH 5.2, and violet above pH 6.8. Its IUPAC name is 4,4-(1,1-Dioxido-3H-2,1-benzoxathiol-3,3-diyl)-bis(2-bromo-6-methylphenol).The molecular formula of bromocresol purple is $C_{21}H_{16}Br_2O_5S$, and its structural formula is as shown in Fig 3.

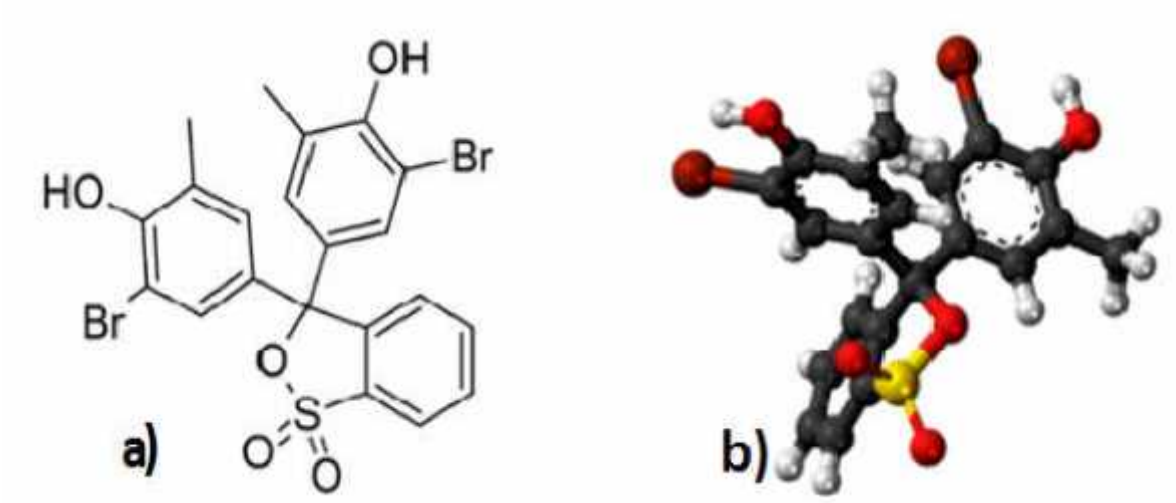


Figure 3:a) structure of bromocresol purple b) 3D structure of bromocresol purple

Bromocresol purple (BCP), 5, 5-dibromo-o-cresolsulfophthalein is used as a pH indicator [41].

Below pH 5.2 Above pH 6.8

It is also used as dye to measure albumin in medical laboratories. Recently, the electro-poly bromocresolpurple film has been proved to possess abundant active sites, π-π conjugated bonds, large real surface area and good conductivity Fig 4. The electro - poly (BCP) film modified electrode showed good electro catalytic activity towards some compounds such as uric acid, xanthine and hydrazine [42].

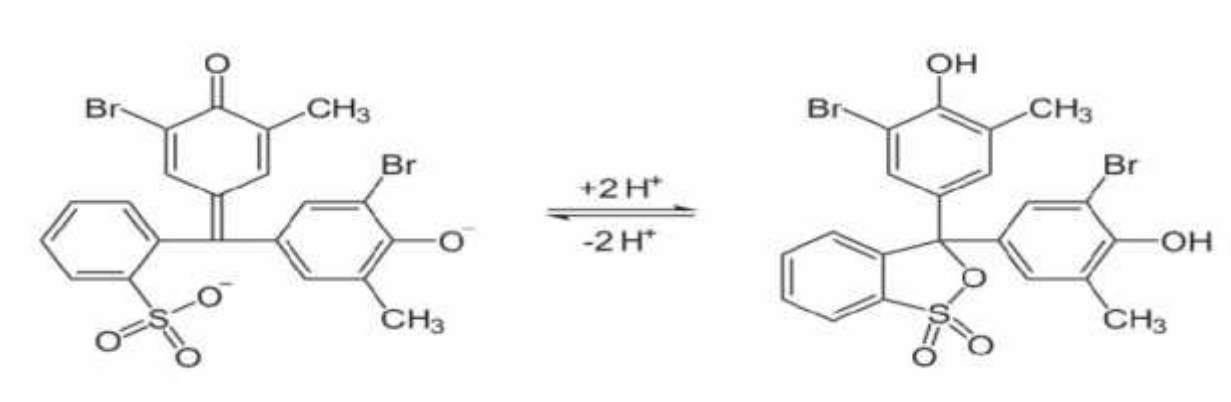


Figure 4: Redox reaction of bromocresolpurple

2.4. Electroanalytical Technique

An Electroanalytical technique encompasses a group of quantitative analytical methods that are based up on the electrical properties of a solution of the analyte when it is made a part of an electrochemical cell. These techniques are capable of producing exceptionally low detection limits and wealth of characterization information describing electrochemical addressable systems. Electrochemical techniques are powerful and versatile analytical techniques that offer high sensitivity, accuracy and precision as well as a large linear dynamic range. Electro analytical measurements offer a number of important benefits:

a. specificity
b. selectivity resulting from the choice of electrode material
c. high sensitivity and low detection limit
d. possibility of furnishing results in real time or close to real time
e. application as miniaturized sensors where other sensors may not be useful [43]

2.4.1. Advantage of Electrochemical Techniques

Electroanalytical methods are well established and use relatively inexpensive equipment to produce unique characterization information for molecules and chemical systems: qualitative and quantitative analytical data, thermodynamic data, and kinetic data. Electroanalytical methods are sensitive; they are able to detect sub micro molar concentrations and sub Pico mole amounts of electro active material. Therefore, the methods are selective; they are able to control the potential of an electrode, which makes it possible to determine the electrochemical "spectrum" of electro active species in solution, analogous to probing the energy states of a molecule with light via spectroscopy [43]

2.4.2. Voltammetry

Historically, the branch of electrochemistry we now call voltammetry developed from the discovery of polarography in 1922 by the Czech chemist Jaroslav Heyrovsky [44], for which he received the 1959 Nobel Prize in Chemistry. Voltammetry is the electrochemical technique in which the current at an electrode is measured as a function of the potential, or voltage, applied to the electrode. Potentiometric measurements which employ only two electrodes while voltammetric measurements utilize a three electrode electrochemical cell. The use of three electrodes (working, auxiliary and reference) along with the potentiostat

instrument allows accurate application of potential functions and measurement of the resultant current.

The potential is varied in some systematic manner and the resulting current-potential plot is called a voltammogram. The most common application of voltammetry is for analytical purposes.

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

Voltammetry can also be used to analyze any chemical species that is electro active, i.e., that can be made to oxidize or reduce. The potential of the electrode is the controlled parameter that causes the chemical species to be oxidized or reduced. The potential can be thought of as "electron pressure" which either forces a species in solution to gain an electron (reduction) or lose an electron (oxidation). As the potential of the electrode becomes more negative, it becomes more strongly reducing. On the other hand, as the potential becomes more positive, it becomes more strongly oxidizing. Thus, the redox reaction taking place on the electrode can be controlled by controlling the electrode potential.

The current, alternatively, is simply a measure of electron flow. The current is due to electron transfer which takes place when an oxidation or reduction occurs on the electrode surface.

This type of current is termed as faradic. In voltammetry, the faradic current is proportional to concentration. The current due to a reduction (cathodic current) is assigned a negative sign. The current due to an oxidation (anodic current) is assigned a positive sign [45].

2.4.2.1.Cyclic Voltammetry (CV)

Cyclic voltammetry is a method for investigating the electrochemical behavior of a system. It was first reported in 1938 and described theoretically by Randies [46]. Cyclic voltammetry is very frequently used because it offers a wealth of experimental information and insights into both the kinetic and thermodynamic details of many chemical systems [47].

Cyclic voltammetry is the most widely used technique for acquiring qualitative information about electrochemical reactions. In cyclic voltammetry, one sweeps the potential of the working electrode at a specific sweep rate (volts/sec), and measures the resulting current *vs* time curve. Usually the sweep is reversed at a specific switching potential, hence the name cyclic voltammetry [48]. The influence of cyclic voltammetry results from its ability to quickly provide substantial information on the thermodynamics of redox processes, on the kinetics of heterogeneous electron transfer reactions, and on coupled chemical reactions or adsorption processes. Cyclic voltammetry is often the first experimental approach performed in an electro analytical study, since it offers rapid location of redox potentials of the electro active species and convenient evaluation of the effect of media up on the redox process [49]. Cyclic voltammetry is a potential sweep technique which involves sweeping the electrode potential between potential limits E1 and E2 at a known sweep rate. On reaching limit E2 the sweep is reversed to E1 to obtain a cyclic scan. The CV scan is a plot of current verses potential and indicates the potential at which redox process occur. The potential axis is also a time axis that is related to scan rate [50]. The excitation signal for CV is a linear potential scan with triangular wave form as shown in Fig 5. This triangular potential excitation signal sweeps the potential of an electrode between two values, sometimes called the switching potential.

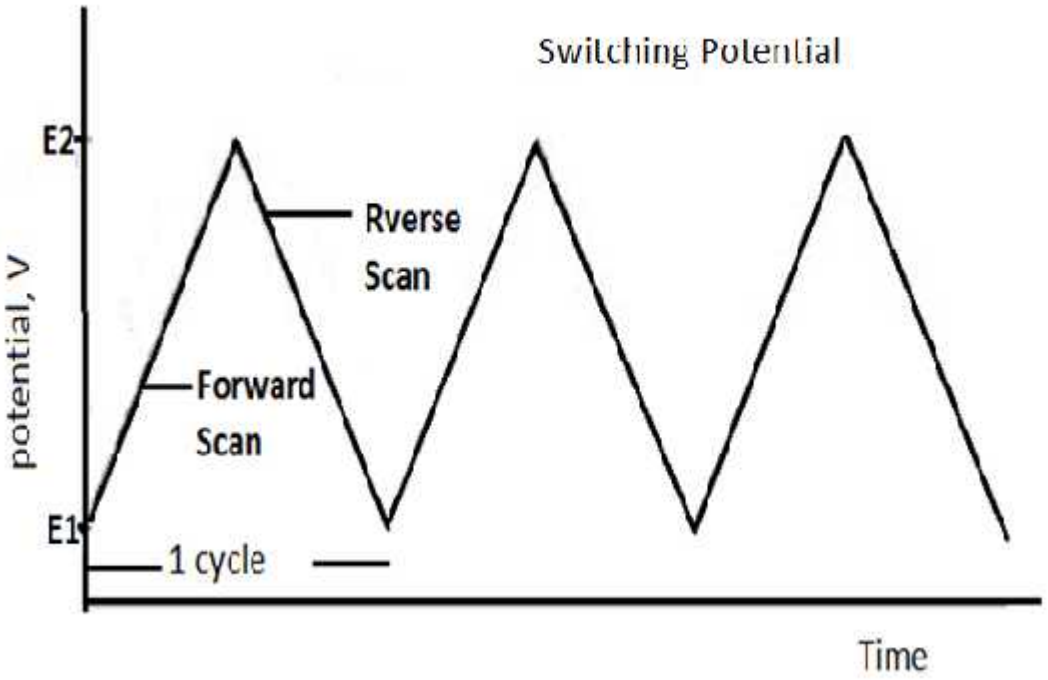


Figure 5: Variation of the applied potential as a function of time in a cyclic voltammetry experiment

To perform an oxidation process, a positive potential ramp is applied and the electro active species loses an electron at the electrode giving rise to an anodic peak current (I_{pa}) which usually gives an oxidation peak at a given potential (E_{pa}). Cathodic currents (I_{pc}) are observed when the potential is applied in the negative direction leading to a reduction process, typically giving a reduction peak at a given potential (E_{pc}). The CV is usually initiated at a potential where species are not electro active. The basic shape of the current verses potential response for a cyclic voltammetry experiment is shown below Fig.6. At the start of the experiment, the bulk solution contains only the reduced form of the redox couple (R) so that at potentials lower than the redox potential, i.e. the initial potential, there is no net conversion of R into O the oxidized form (point A). As the redox potential is approached, there is a net anodic current which increases exponentially with potential. As R is converted in to O, concentration gradients are set up for both R and O, and diffusion occurs down these concentration gradients. At the anodic peak (point B), the redox potential is sufficiently positive that any R that reaches the electrode surface is immediately oxidized to O. Therefore, the current now depends upon the rate of mass transfer to the electrode surface and so the time dependence is qt resulting in an asymmetric peak shape. Upon reversal of the scan (point C), the current continues to decay with a qt until the potential nears the redox

potential. At this point, a net reduction of O to R occurs which causes a cathodic current which eventually produces a peak shaped response (point D). If a redox system remains in equilibrium throughout the potential scan, the electrochemical reaction is said to be reversible. In other words, equilibrium requires that the surface concentrations of O and R are maintained at the values required by the Nernst Equation. Under these conditions, the following parameters characterize the cyclic voltammogram of the redox process. The peak potential separation (Epa - Epc) is equal to 59/n mV for all scan rates where n is the number of electron equivalents transferred during the redox process. The situation is very different when the redox reaction is not reversible.

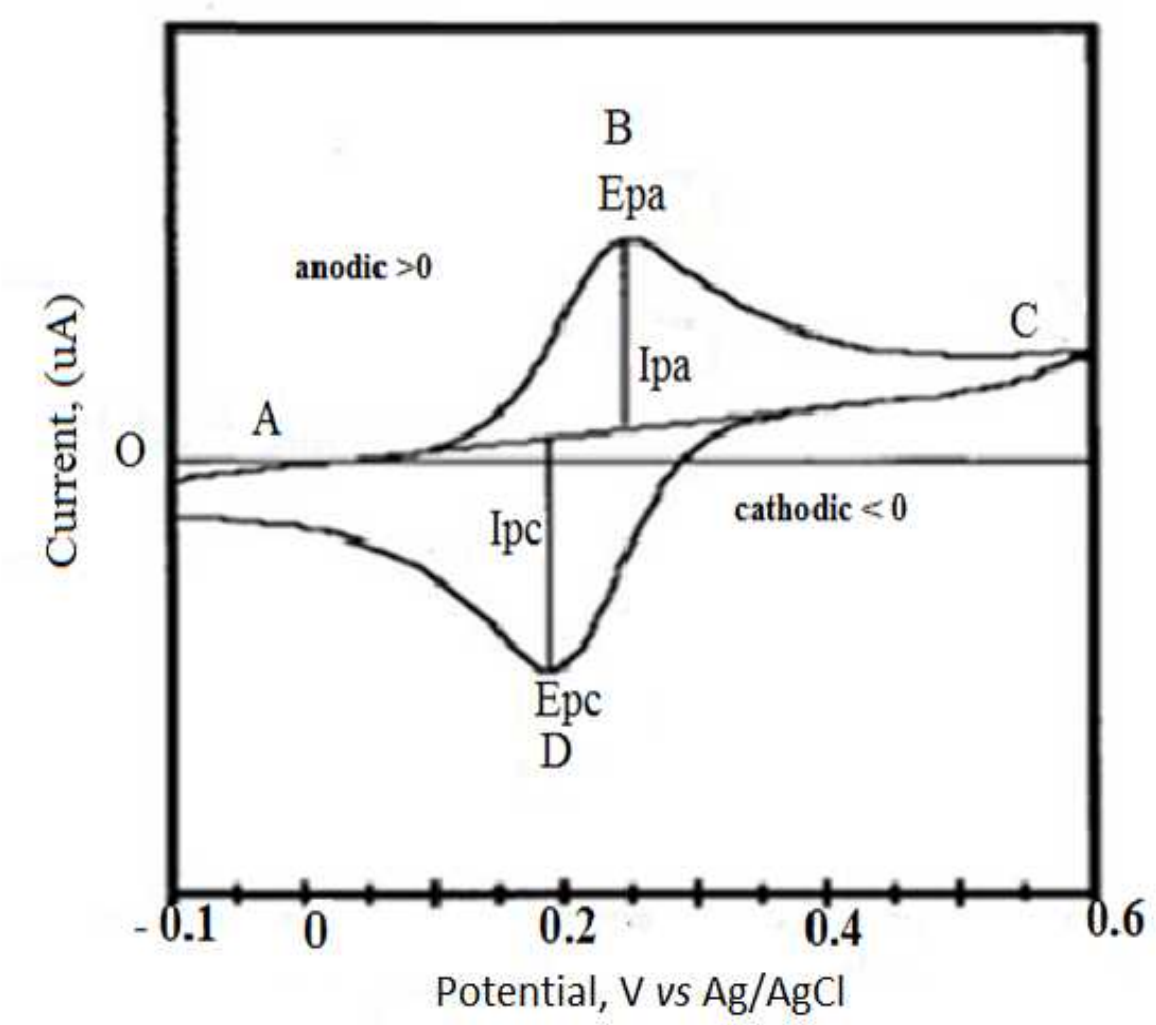


Figure 6: A typical cyclic voltammogram

The important parameters of cyclic voltammogram are the magnitudes of the anodic peak current (I_{pa}) and cathodic peak current (I_{pc}), and the anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}). These parameters are labeled in Fig 6 one method for measuring Ip involves extrapolation of a base line current . The establishment of a correct base line is essential for the accurate measurement of peak currents. This is not always simple, particularly for more complicated systems. A redox couple in which both specious rapidly exchange electrons with the working electrode is termed an electrochemically reversible couple. The half - wave potential (E1/2) for a reversible couple is centered between E_{pa} and E_{pc}

$$E_{1/2} = \frac{E \ +E}{2} = E^0 + \frac{R}{n} \ln(D_R^{1/2}/D_O^{1/2}) \quad \text{---------- 1}$$

The formal potential (E°) can be described with the formula below

$$E° = E_{1/2} + E_{ref} \quad \text{---------- 2}$$

Where the Eref is the formal potential of the reference electrode ($E_{Ag/AgCl}$ = 0.210 V, I= 3M KCl). The number of electrons transferred in the electrode reaction (n) for a reversible couple can be determined from the separation between the peak potentials.

$$\Delta E = \left| E_{pa} - E_{pc} \right| = 2.3\frac{R}{n} \sim 0.059/n \quad \text{---------- 3}$$

Thus, a one electron process such as the reduction of $[Fe^{3+}(CN)_6]^{3-}$ to $[Fe^{2+}(CN)_6]^{4-}$ exhibits a ΔE = 0.059 V. Slow electron transfer at the electrode surface, "irreversibility", causes the peak separation to increase.

The peak current for a reversible system is described by the Randles-Sevcik equation for the forward sweep of the first cycle.

$$I_p = (2.69 \times 10^5)\ n^{3/2} A D^{1/2} C \nu^{1/2} \quad \text{---------- 4}$$

Where I_p is peak current (A), n is electron stoichiometry, A is electrode area (cm^2), D is diffusion coefficient (cm^2/s), C is concentration ($mole/cm^3$), and ν is scan rate (V/s). Accordingly, I_p increases with $\nu^{1/2}$ and is directly proportional to concentration. The relationship to concentration is particularly important in analytical applications and in studies of electrode mechanisms. The values of I_{pa} and I_{pc} should be identical for a simple reversible (rapid) couple [51]. That is

$$\frac{I_{pa}}{I_{pc}} = 1 \quad \text{---------- 5}$$

2.4.2.2. Differential Pulse Voltammetry (DPV)

This technique was proposed by Barker and Gardner [49]. DPV can provide greater sensitivity and more efficient resolution and differentiation of various species. This technique differs from NPV because each potential pulse is fixed, of small amplitude (0.01 to 0.1V), and is superimposed on a slowly changing base potential. Current is measured at two points for each pulse, the first point just before the application of the pulse and the second at the end of the pulse. These sampling points are selected to allow for the decay of the non-faradic (charging) current. The difference between current measurements at these points for each pulse is determined and plotted against the base potential (Fig.7) [52]

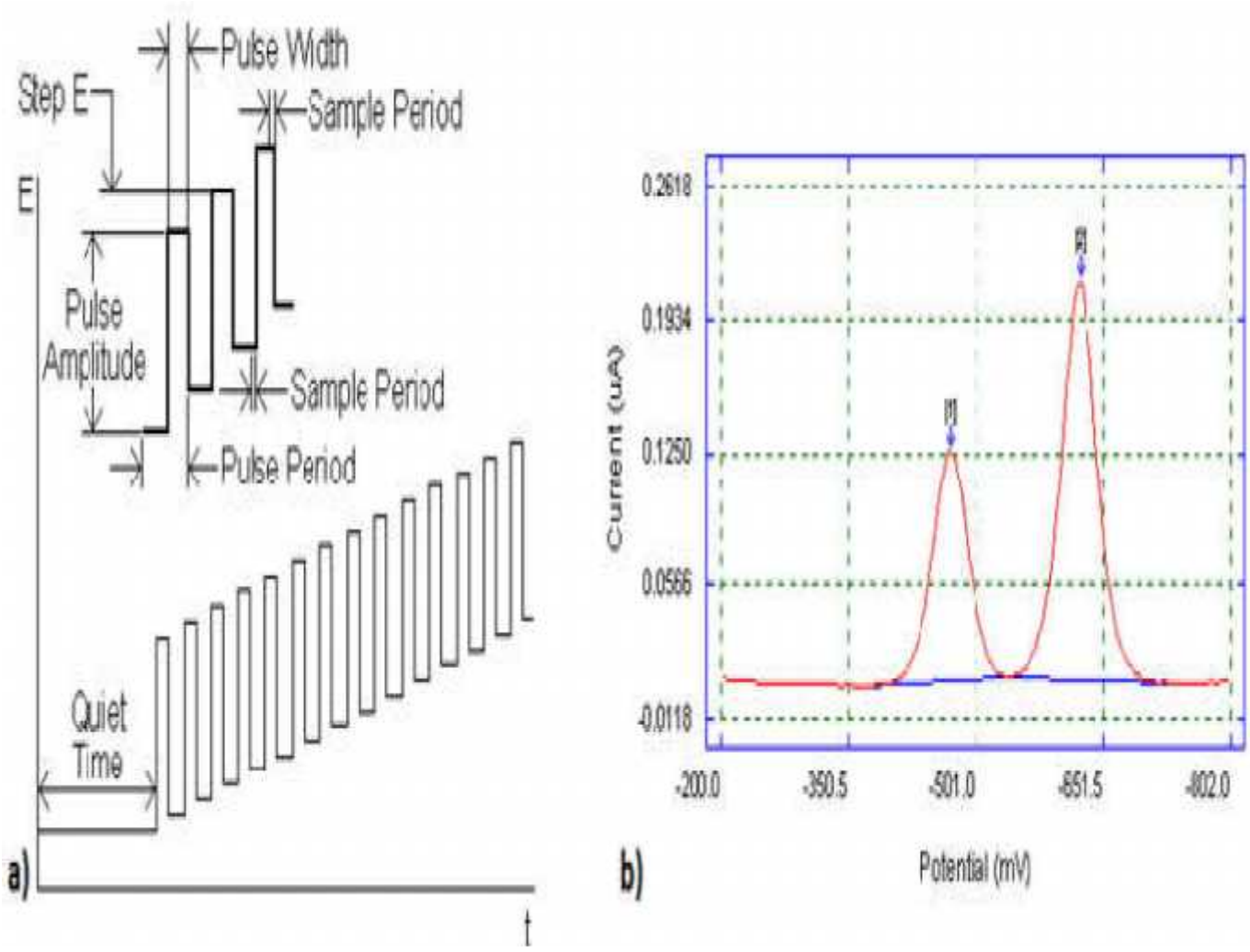


Figure 7: Differential pulse voltammetry, a) an anodic scanning of potential *vs* time b) plot of voltammograms

2.5. Electrodes

2.5.1. Working electrode

The working electrode is the electrode in an electrochemical system on which the reaction of interest is occurring. The working electrode is often used in conjunction with an auxiliary electrode, and a reference electrode in a three electrode system. Depending on whether the reaction on the electrode is a reduction or an oxidation, the working electrode is called cathodic or anodic, respectively.

The working electrode represents the most important components of an electrochemical cell. It is at the interface between the WE and the solution that electron transfers of greatest interest occur. The selection of a working electrode material is critical to experimental

success. Several important factors should be considered. Firstly, the material should exhibit favorable redox with the analyte, ideally fast, reproducible electron transfer without electrode fouling. Secondly, the potential window over which the electrode performs in a given electrolyte solution should be as wide as possible to allow for the greatest degree of analyte characterization. Additional considerations include the cost of the material, its ability to machined or formed in to useful geometries, the ease of surface renewal following a measurement, and toxicity. There are different kind of working electrode those are platinum electrode (Pt), Gold electrode (Au), Glassy carbon electrode (GCE), Silver electrode (Ag), Mercury electrode (Hg) and Carbon Paste electrode (CPE) [53].

2.5.1.1. Glassy carbon electrodes (GCEs)

Due to excellent physical and chemical properties, glassy carbon electrodes have been widely used in Electroanalytical chemistry. Glassy Carbon electrode (Fig.8) has been widely used in the preparation of modified electrodes, such electrodes offer the advantages of versatile bulk modification, fast response, renewability and a low back ground current, high temperature resistance, hardness, low density, low electrical resistance, low friction, low thermal resistance, extreme resistance to chemical attack and impermeability to gases and liquids. Glassy carbon is widely used as an electrode material in electrochemistry [54].

Figure 8: Glassy Carbon working electrode

2.5.2. Reference electrode

Reference electrodes, as their name suggests, are used to give a value of potential to which other potentials can be referred in terms of a potential difference potentials can only be registered as differences with respect to a chosen reference value. Thus, a good reference electrode[55] needs to have a potential that is stable with time and with temperature and

which is not altered by small perturbations to the system that is, by the passage of a small current. The potential of a working electrode in a voltammetry experiment is always controlled with respect to some standard, and that standard is the reference electrode. While the thermodynamic scale of half-reaction potentials found in most textbooks measures electrode potentials against the "standard hydrogen" reference electrode (SHE), in actual practice the SHE is much too cumbersome to use. For this reason, a number of other reference electrodes have been developed.

2.5.2.1. Silver-silver chloride reference electrodes

The silver-silver chloride or "Ag/AgCl" reference electrode is quite popular. The half reaction for this reference electrode is as follows:

$$AgCl(s) + e \leftrightarrow Ag(s) + Cl^-(aq)$$

The actual potential assumed by an Ag/AgCl reference depends only on the activity of the chloride anion. (The other two species appearing in the half reaction are solids which always have unit activity). To serve as a reference, the chloride activity needs to be held constant. To accomplish this, a silver wire (coated with a layer of silver chloride) is immersed in an internal solution saturated with potassium chloride. The chloride ion concentration remains fixed at the saturation limit. The short hand notation for this reference electrode half-cell is given below:

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

Electrical contact is made by direct connection to the silver wire, and the internal electrode solution is placed in ionic contact with the test solution through a salt bridge or porous glass frit Fig 9.

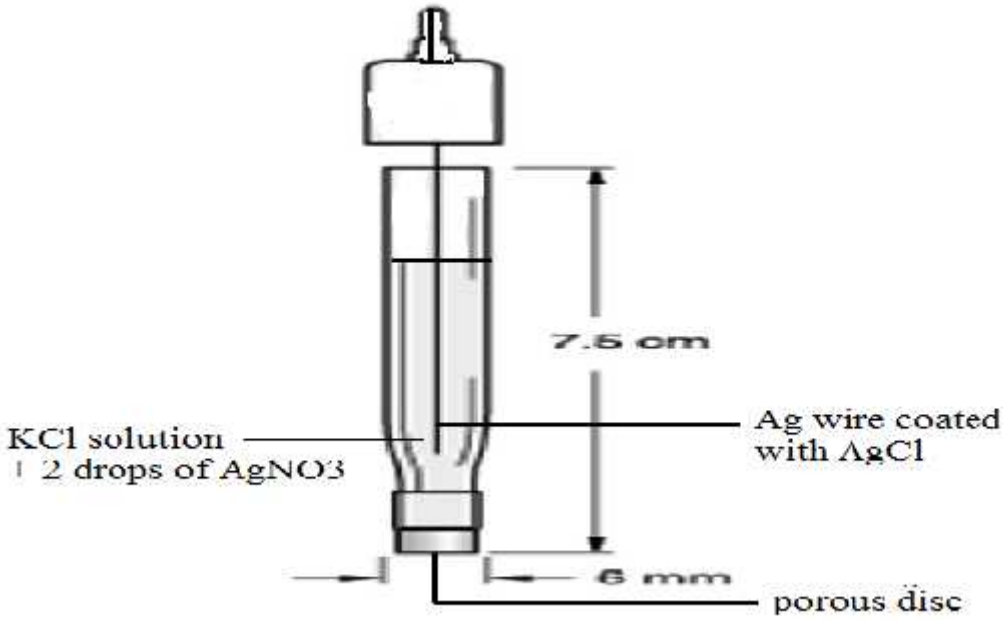


Figure 9: A typical silver-silver chloride reference electrodes

2.5.2.1.1. Advantages of Ag /AgCl Reference Electrode

Silver-silver chloride reference electrode has various advantages:

a) chemical processing industry has standardized on this electrode
b) convenient
c) rugged / durable
d) most widely used
e) easily prepared
f) works in all aprotic solvents

2.5.2.1.2. Disadvantages of Ag/AgCl Reference Electrode

The disadvantages of silver-silver chloride reference electrode are:

a) potential depends on solvents and electrolytes
b) care must be taken to minimize junction potentials

2.5.3. Auxiliary Electrode

The auxiliary electrode, often also called the counter electrode, is an electrode used in a three electrode electrochemical cell for voltammetric analysis or other reactions in which an electric current is expected to flow [54]. The auxiliary electrode is distinct from the reference electrode, which establishes the electrical potential against which other potentials may be measured, and the working electrode, at which the cell reaction takes place.

In traditional two electrode cells that have only a working electrode and a reference electrode, current is necessarily forced to flow through the reference electrode whenever a

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

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

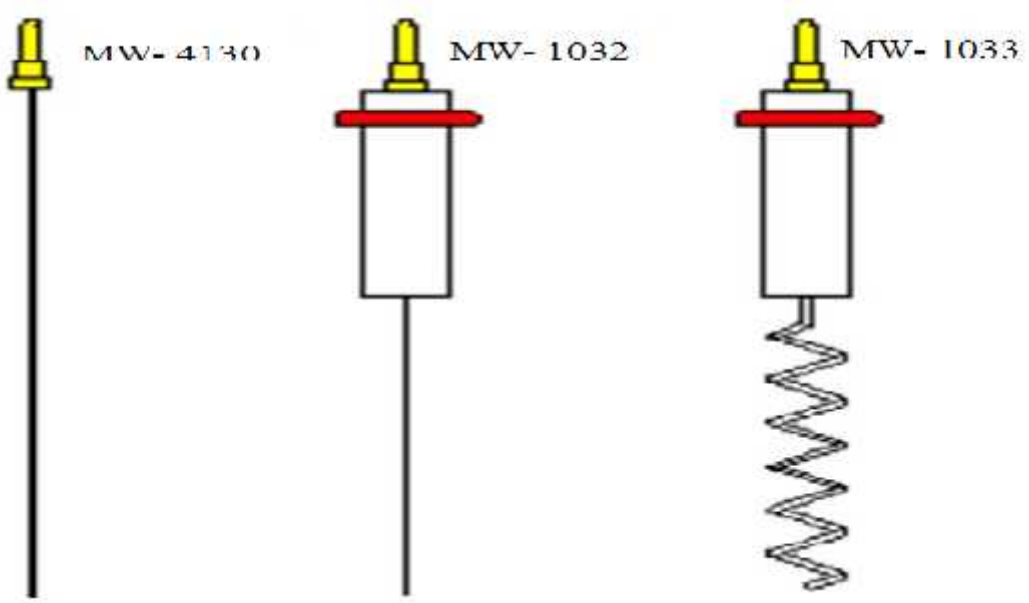


Figure 10: Auxiliary electrodes

2.6. Methods of Determination of folic acid

A various methods have been developed to detect FA in given samples, most of them are based on liquid chromatography/tandem mass spectrometry (LC/MS/MS), microemulsion electrokinetic chromatography (MEEKC), enzyme linked immunosorbent assay (ELISAs), UV-vis spectrophotometry, flow-injection chemiluminescence, ion chromatography, high performance liquid chromatography, capillary electrophoresis, quartz crystal microbalance and voltammetry . Among these, the electrochemical methods have attracted substantial attention because of proven simplicity, sensitivity, selectivity, fast response, and low-cost production.

In this work, the electrochemical method for the determination of FA in aqueous solution via bromocresolpurple modified glassy carbon electrode (BCPs/GC) and electrochemical techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) are employed to determine FA.

2.7. Objective of the study

2.7.1. General objective

To synthesize and characterize poly bromocresol purple on glassy carbon electrode and utilize as an electrochemical sensor for the voltammetric determination of folic acid.

2.7.2. Specific objective

a) To study the electrochemical behavior and the electrocatalytic activity of BCP using cyclic voltammetry for characterization and differential pulse voltammetry for quantitative determination of folic acid.
b) To establish the optimum parameters for the sensitive determination of FA i.e. to study the basic electroanalytical parameters required to determine FA at the specified condition.
c) To apply the electrodes prepared for the determination of FA in real sample.

3. Experimental Parts

3.1. Reagents and Chemicals

Folic acid was purchased from sigma (USA), Bromocresolpurple (BCP) is from Fluka, Na_2HPO_4 and NaH_2PO_4 are from Himedia chemicals, NaOH (Labmerk chemicals, lndia) , potassium ferric hexa cyanide, $K_3[Fe(CN)_6]$, alumunium oxide a 0.05 µM, pyridoxine hydrochloride , iron(III) chloride six hydrate, are from Sigma Aldrich, phenol and potassium chloride are from Riedel-de Haën-France, ascorbic acid is from (Nice, India), commercial

folic acid table and distilled water . All chemicals used were analytical grade and were used without any further purification:

3.2. Apparatus and Instruments

3.2.1. Apparatus

The electrochemical cell consisted of a bare glassy carbon working or poly BCP/ERGO/GCE modified electrodes, Ag/AgCl 3M KCl reference electrode, and platinum counter electrode. The cyclic voltammetry apparatus used was CV-100W electrochemical analyzer [Bio-analytical systems (BAS), USA], coupled to a Dell computer (Pentium 4). Voltammetric analyzers CH instruments, (model CHI 760D), ultrasonic cleaner power-80W, labopette, single channel with 0.5-10 µL, 2-20 µL, and100-1000 µL volume configuration, certified conformity with tip injection and calibration options(variable volume single channel manual pipettes), pH meter (senses ion TM+MM150) and electronic Balance (Model: Scientech: ZSA 120) were also used.

3.2.2. Instruments

The voltammetric experiments were performed using BAS CV 100W and CHI 760D voltammetric analyzer coupled with personal computer Fig 11.

Figure 11: BAS CV-100 W Voltammetric Analyzer Coupled with Personnel Computer

A conventional three-electrode cell (Fig 12) was used for measurements with a bare glassy carbon electrode (GCE) and/or BCPs/GCE as the working electrode, Ag/AgCl (3 M KCl) as reference electrode, and a platinum wire as counter electrode. Fig 12 below illustrates the instrumentation of the electrochemical cell for voltammetric determination.

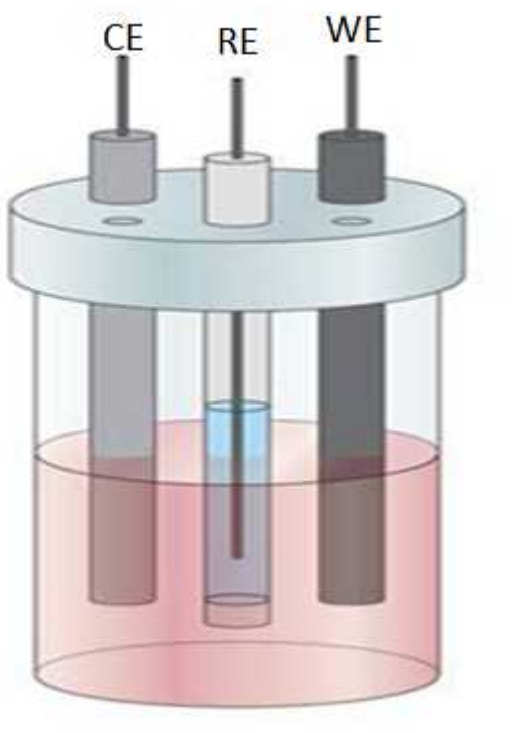


Figure 12: Setup of an Electrochemical Cell

3.3. Preparation of Supporting electrolyte

Supporting electrolyte of phosphate buffer (NaH_2PO_4 – Na_2HPO_4) in the pH range 4 - 11 was prepared from 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 in distilled water. The pH of the solutions was calculated by using the Henderson–Hasselbalch equation proportion, and adjusted using pH meter.

Phosphate buffer (Na_2HPO_4/NaH_2PO_4) of pH 6 used as the background (supporting) electrolyte in the electro polymerization of monomer BCP. Phosphate buffer was prepared from 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 in distilled water and the pH was adjusted using pH meter.

3.4. Preparation of Bromocresolpurple

Initially 0.1 M of phosphate buffer solution (PBS, pH 6) was prepared from 0.1 M Na_2HPO_4 and 0.1 M $NaHPO_4$. A 0.05 mM bromocresolpurple was prepared using PBS as a supporting electrolyte.

3.5. Preparation of folic acid

A stock solution of 5 mM FA was prepared in trace amount of 0.1 M NaOH, and then distilled water was added to 100mL volumetric flask and diluted to the required concentrations using 0.1 M Phosphate buffer pH 7.0**.** Experiments were carried out at room temperature and all solutions were prepared from distilled water. The stock solution of FA prepared was stored in the refrigerator to avoid exposure to air and light to keep its stability.

3.6. The modification of the glassy carbon electrode

Before modification, the bare GCE was polished with 0.3 and 0.05 μM Al_2O_3 slurry on a polishing kit which is in a lapping cloth to obtain a mirror like electrode surface and washed with distilled water. Then rinsed with ethanol and distilled water in an ultrasonic bath for 5 minutes. The polymer was electrochemically deposited from 0.05 mM bromocresolpurple (BCP) monomer in 0.1M phosphate buffer solution (PBS ,pH 6.0). These processes were done before every modification of GCE.

3.7. Analytical Procedure

Unless otherwise stated, 0.1 M PBS(pH= 7.0) was used as the supporting electrolyte for the determination of FA. A 500 μL volume of FA working solution and 25mL of 0.1M PBS were transferred into an electrochemical cell, and the three electrode system was installed on it. After accumulation of 7 minutes at an open circuit under stirring and following for 2 s, the cyclic voltammogram were recorded from -0.1 V to 1.3 V at scan rate of 100 mV/s. The differential pulse voltammogram were recorded from 0.2 V to 1.0 V. The same procedure was performed for the real sample analysis(commercial FA tablet)

4. Results and discussion

4.1. Characterization of the GC electrode with poly BCP

The BCP/GC modified electrode was characterized using cyclic voltammetry. As shown in Fig 13 the polymer was made by applying 15 potential cycles (which was optimized) in the range of -1.5 to 1.6 V at scan rate of 100 mV/s. In the first scanning cycle the peak potential was observed at 0.634 V with peak current of 6.990×10^{-5}A. In the subsequent scanning cycles a falling of oxidation potential was observed. This change has been attributed to

lengthening of the oligomer chain, its oxidation potential decreases due to an increase in the conjugation, and that accounts for the formation of a deposit on the glassy carbon electrode. The layer thickness was controlled by the number of cycles [56]

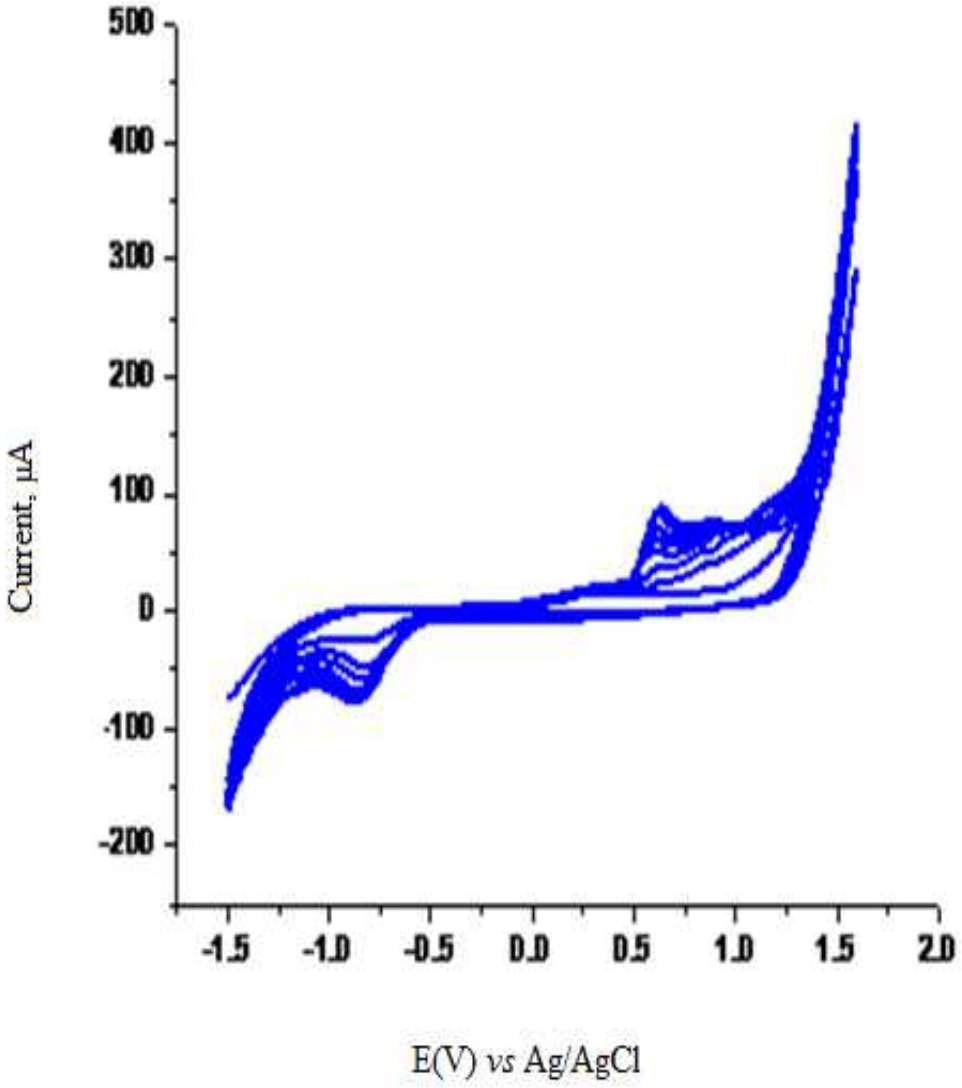


Figure 13: Cyclic voltammograms of BCP on the GCE in the electro polymerization process

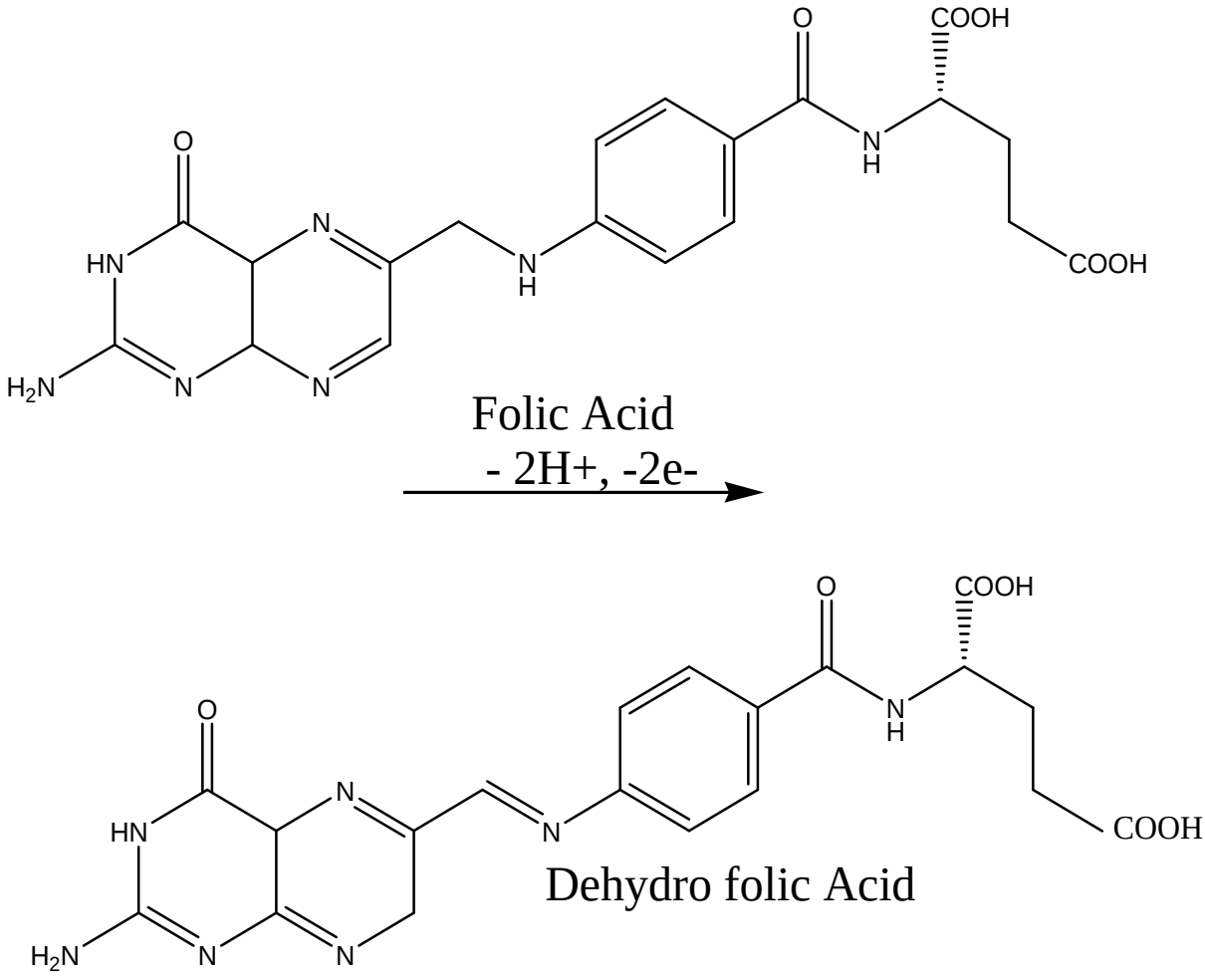
4.2. Electrochemical behaviors of folic acid at modified electrode

Fig 14 compares the cyclic voltammograms response of bare GCE (curve a) and BCP/GCE (curve b) of 100 µM FA in 0.1 M phosphate buffer solution (PBS, pH 7.0). From curve a, it can be seen that the oxidation peak potential of FA appeared at +0.793 V, suggesting that an irreversible electrochemical process of FA at bare GCE, while at the BCP/GCE modified electrode curve b , the oxidation peak potential value of FA negatively shift from +0.793 V

to +0.781V and the oxidation peak current of FA increases slightly. The reasons for this improvement are the BCP possessed a large real surface area, π-π conjugated bond, abundant active sites, and high conductivity. This led to a dissimilar conjugation between FA and the BCPs/GCE boundary.

The BCPs/GCE contained a lot of negatively charged functional groups that are capable of interacting with FA. The BCP layer produced a strong electrocatalytic effect which greatly enhanced the current and lowered the over potential [57]. As shown in Fig 14 much more pronounced current responses were obtained at the modified electrode.

Folic acid at the poly bromocresol purple modified glassy carbon electrode exhibited a well-defined oxidation peak resulting from the oxidation of folic acid to dehydro folic acid [58], which can be ascribed to a two-electrons and two-proton process (the reaction mechanism of folic acid) as shown below in scheme 1



Scheme 1: Electrochemical reaction of folic acid.

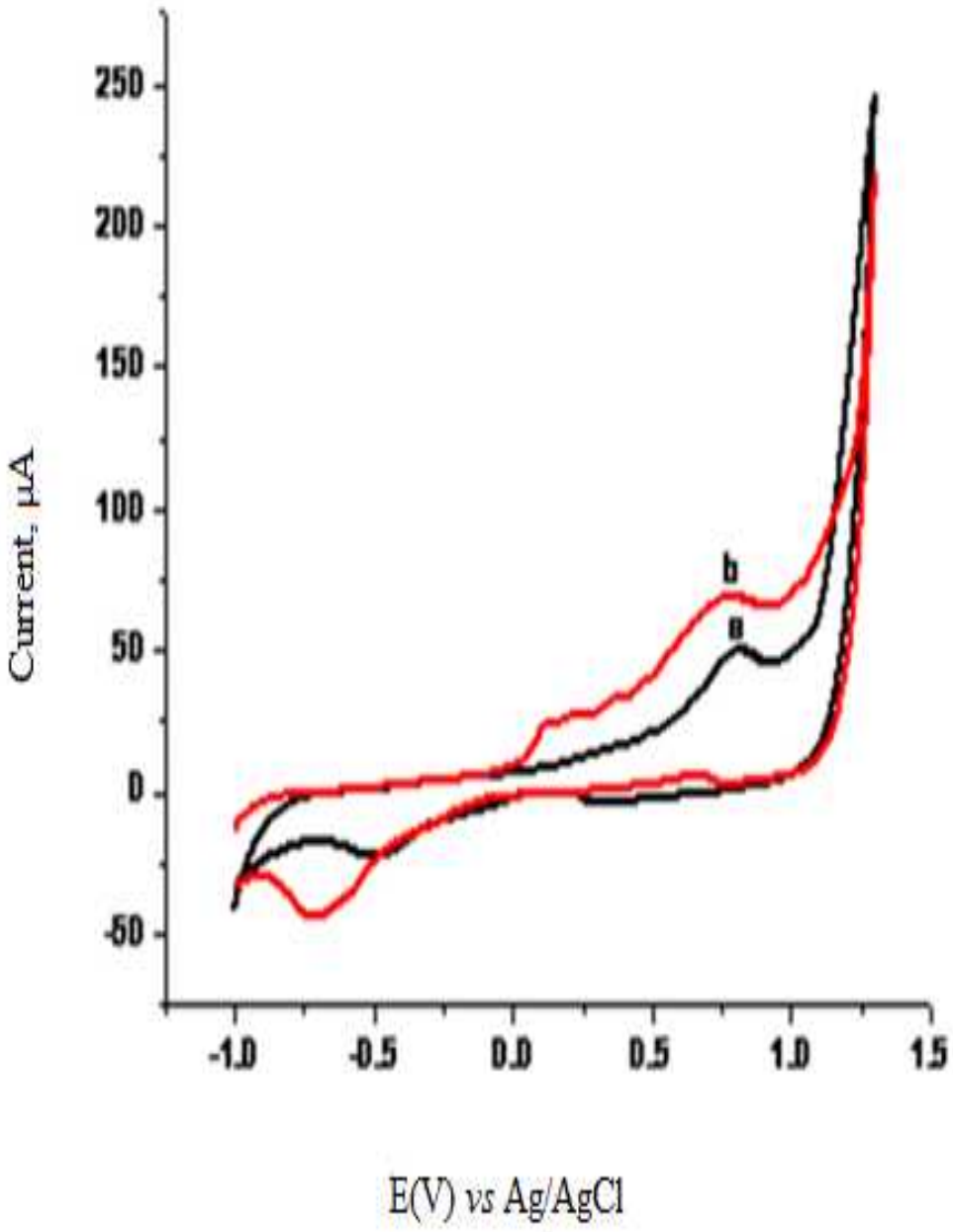


Figure 14: Cyclic voltammograms of 100 μM FA in 0.1 M PBS (pH 7.0) at different electrodes: (a) bare GCE; (b) BCP/GCE at scan rate 100 mV/s

In order to determine the electroactive surface area of both bare GCE and BCPs/GC modified electrodes the electrochemical behavior of potassium ferriccyanide, $K_3Fe(CN)_6$ in 0.1 M KCl supporting electrolyte was studied using CV recorded at scan rate 100 mV/s with bare and modified GCE. It was found that the peak current increased in the modified GCE as shown in Fig 15.

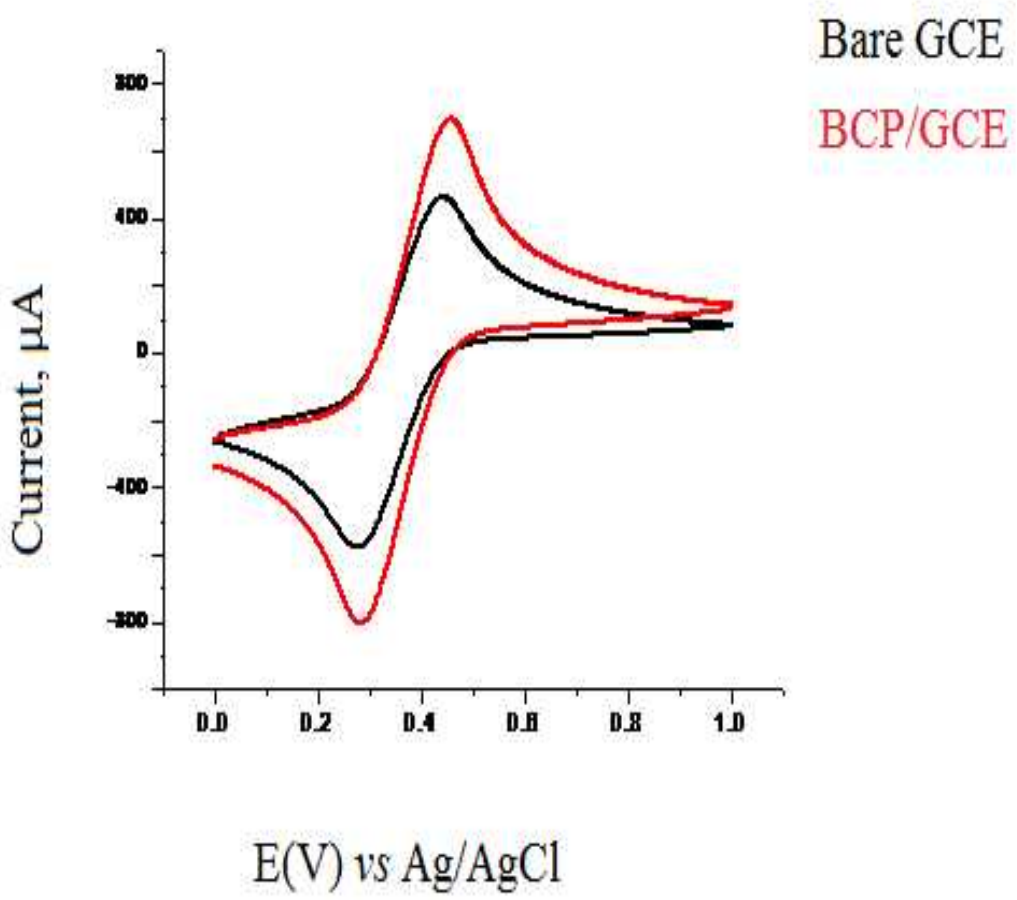


Figure 15: cyclic voltammogram of 5 mM potassium ferric cyanide in 0.1M KCl at bare GCE and BCPs/GCE with scan rate 100 mV/s

5 mM $K_3Fe(CN)_6$ in 0.1 M KCl was used as a probe to measure the electrochemical effective surface area of the BCPs/GCE modified electrode and the bare GCE using cyclic voltammetry (fig 15). Based on the voltammogram obtained the electrochemical effective surface area was calculated using the peak current, I_p for the reversible system as describe by Randles Sevcik equation:

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

for the forward reaction: $[Fe(CN)6]^{3-} + e^- \leftrightarrow [Fe(CN)6]^{4-}$

Where I_p is current in A, n number of electrons in the redox reaction, A is the electrode surface area in cm^2, D (in cm^3/s) is the diffusion coefficient, C is the concentration in mol/cm^3 and ν is the scan rate in vs^{-1}. The diffusion coefficients D_O and D_R of the species $[Fe(CN)_6]^{-3}$ and $[Fe(CN)_6]^{-4}$ in 0.1 M KCl found to be 0.73 $x10^{-5}$ and 0.67 $x10^{-5}$ cm^2/s respectively [59]. Thus the surface area was 0.05 cm^2 and 0.039 cm^2 for the BCP/GCE and

the bare GCE respectively. The result indicates that the electrochemical effective surface area increased after modification of the glassy carbon electrode by poly bromocresol purple.

4.3. Effect of pH

The pH of the supporting electrolyte plays an important role in the electrochemical oxidation of folic acid, that's why the influence of peak current and peak potential was carefully examined over pH range because it affects the rate, equilibrium state and the electrode reaction. For this reason the parameter was studied using CV as illustrated in Fig 16 for FA determination at the BCP/GC modified electrode in the pH range 4 to 11.

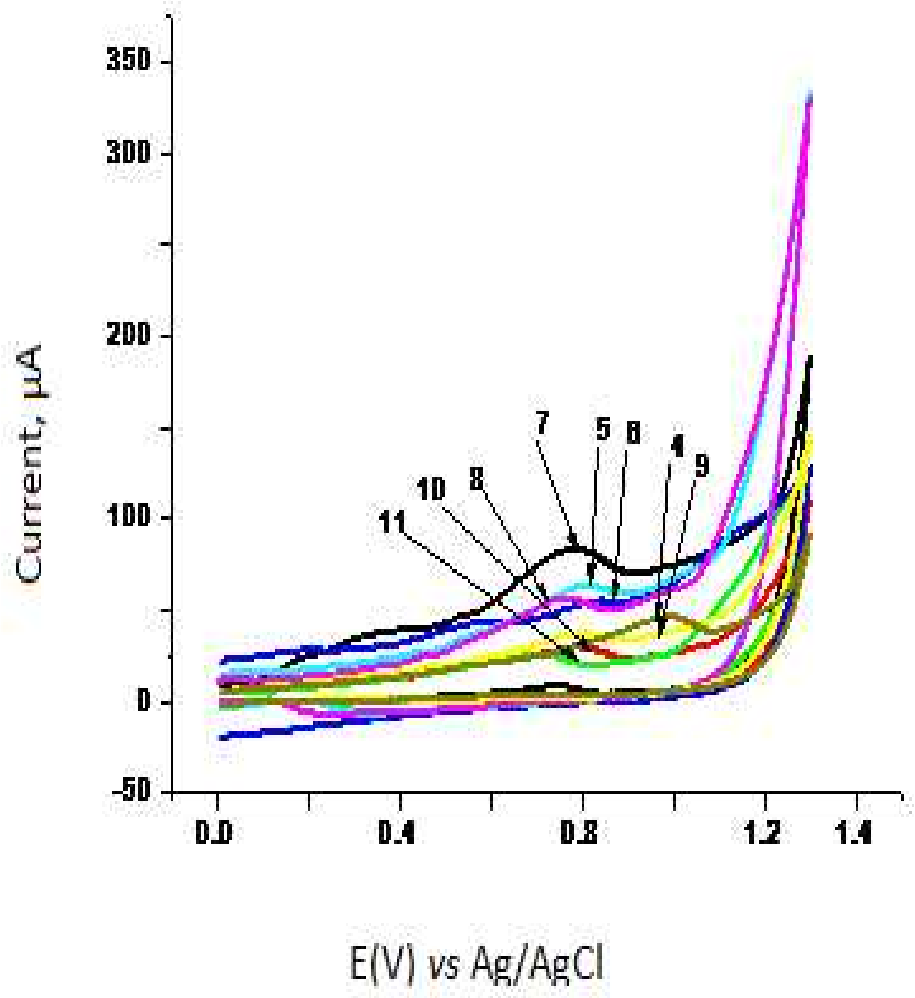


Figure 16: Effect of pH on CV response of modified GC electrode of 100 µM FA in 0.1 M PBS at BCPs/GCE (pH range of 4 - 11)

As shown in Fig 17 the anodic peak currents of FA were greatly influenced by solution of pH. According to the observed results the amount of accumulated FA at BCP/GC modified electrode is largest at pH 7.0. The anodic peak current increased as the pH increased and reaches the highest peak at pH 7.0 and then decrease. When pH equals to 7 the anodic peak currents raise up to 3.825 x 10^{-5} A. The optimum pH required to investigate the electrochemical oxidation of FA at BCP/GC modified electrode was determined to be pH 7.0.

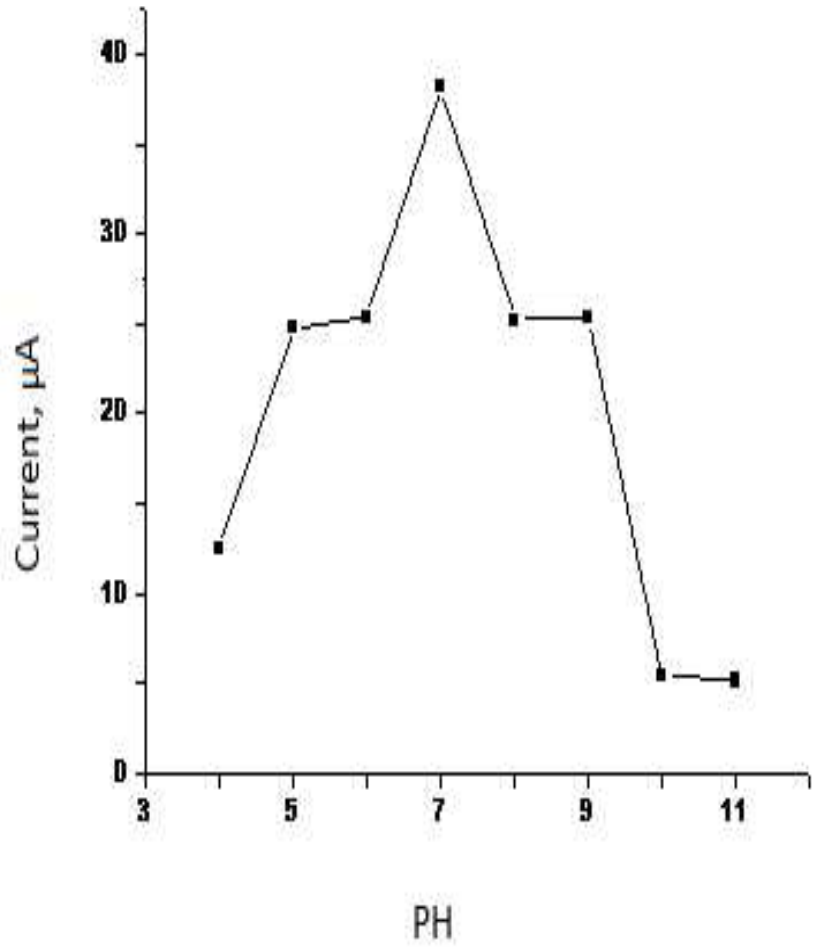


Figure 17: Plot of CV anodic peak current as a function of pH for 100 µM FA in 0.1M PBS(PH 7.0)

4.4. Effect of scan rate

The effect of diverse scan rates for the oxidation of folic acid was studied to inspect the kinetics of electrode reactions. The oxidative current of 100 µM folic acid at the poly

BCP/GC modified electrode in a 0.1 M phosphate buffer (PBS, pH 7.0) electrolyte solution was studied. As show in Fig 18 the cyclic voltammogram obtained in the potential range of -1.0 – 1.3 V. To investigate the diffusion behavior CVs were recorded for the scan rate range from 25 to 500 mV/s and a shift in the anodic peak potentials, and change in the magnitude of the anodic peak currents were observed. Thus, it was found that the electrode reaction of folic acid at the surface of poly BCP/GC modified electrode depends on the scan rate Fig 18.

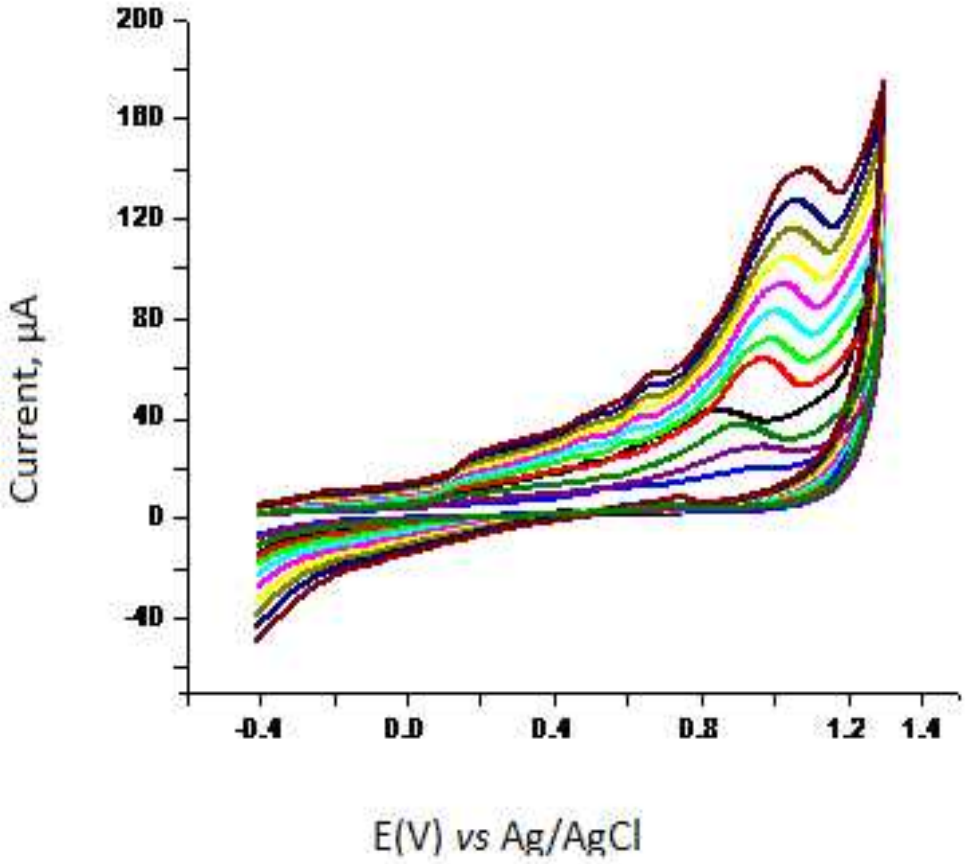


Figure 18: Cyclic voltammograms recorded at BCPs /GCE for 100 µM FA in 0.1M PBS (pH 7.0) at different scan rates: 0.025, 0.05, 0.075, 0.100, 0.150, 0.200, 0.250, 0.300, 0.350, 0.400, 0.450 and 0.500V/s

To investigate the oxidation behavior of folic acid, the effect of scan rate at the oxidative peak current of 100 µM FA at BCPs/GCE in 0.1 M PBS (pH 7.0) were studied. As shown in Fig 19 the oxidative peak current of FA increased with scan rate (75- 400 mV/s). The oxidation peak current of FA exhibited a linear relation to the square root of scan rate in the range of 75 - 400 mV/s. The linear regression equations of the I_{pa} for the scan rates are expressed as $I_{pa}(\mu A) = -6.1588 + 2.4963*\nu^{1/2}$, R = 0.997. The relationship between the oxidation peak current and square root of the scan rate indicates that the oxidation of FA at the modified electrode is a diffusion controlled process [60]. The dependence of the peak current on square root of scan rate is summarized in Table 1.

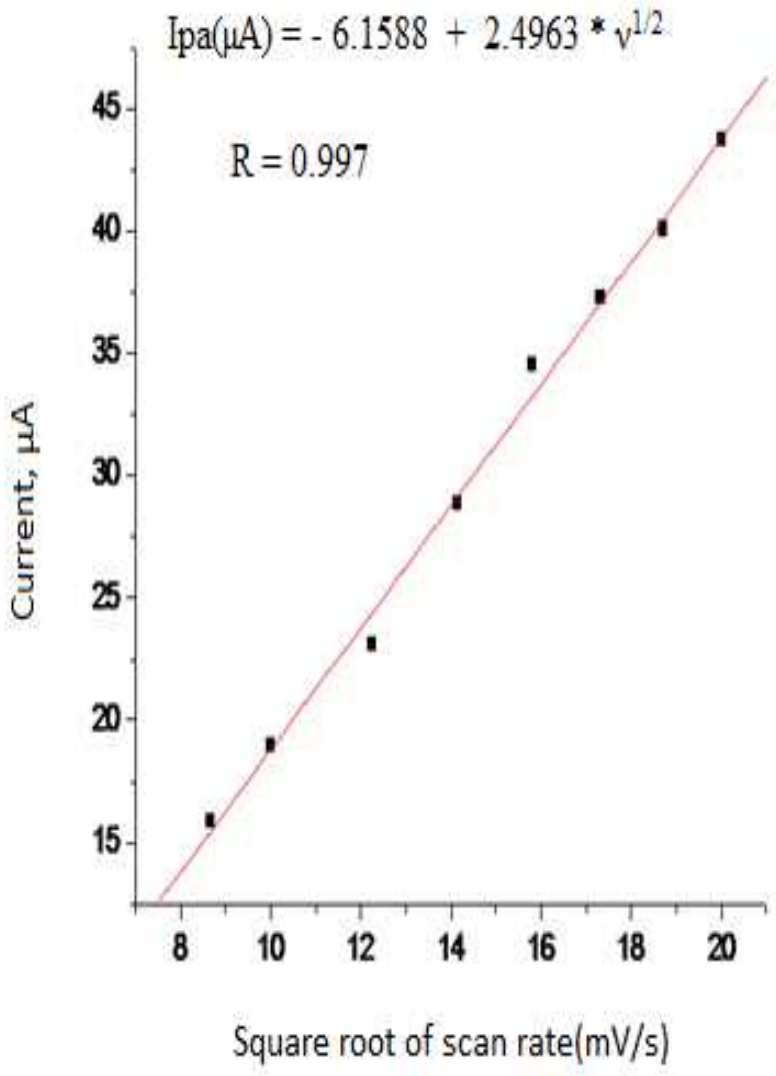


Figure 19: The dependence of the peak current on square root of scan rate

Table 1 The dependence of the peak current on the square root of the scan rate

Scan rate(mv/s)	Square root of scan rate (mv/s)	I_{pa}(μA)
25	5	4.467
50	7.07	5.08
75	8.66	15.86
100	10	19
150	12.25	23.09
200	14.14	28.9
250	15.81	34.56
300	17.32	37.27
350	18.71	40.08
400	20	43.74
450	21.21	50.17
500	22.36	64.8

It can also be seen that with increasing the scan rate, the anodic peak potential is shifted to more positive potential values as shown in Fig 20. This implies that increasing scan rate indicating that the electroxidation process of FA is irreversible [45]. The dependence of the peak potentials on the logarithm of scan rates are summarized in Table 2

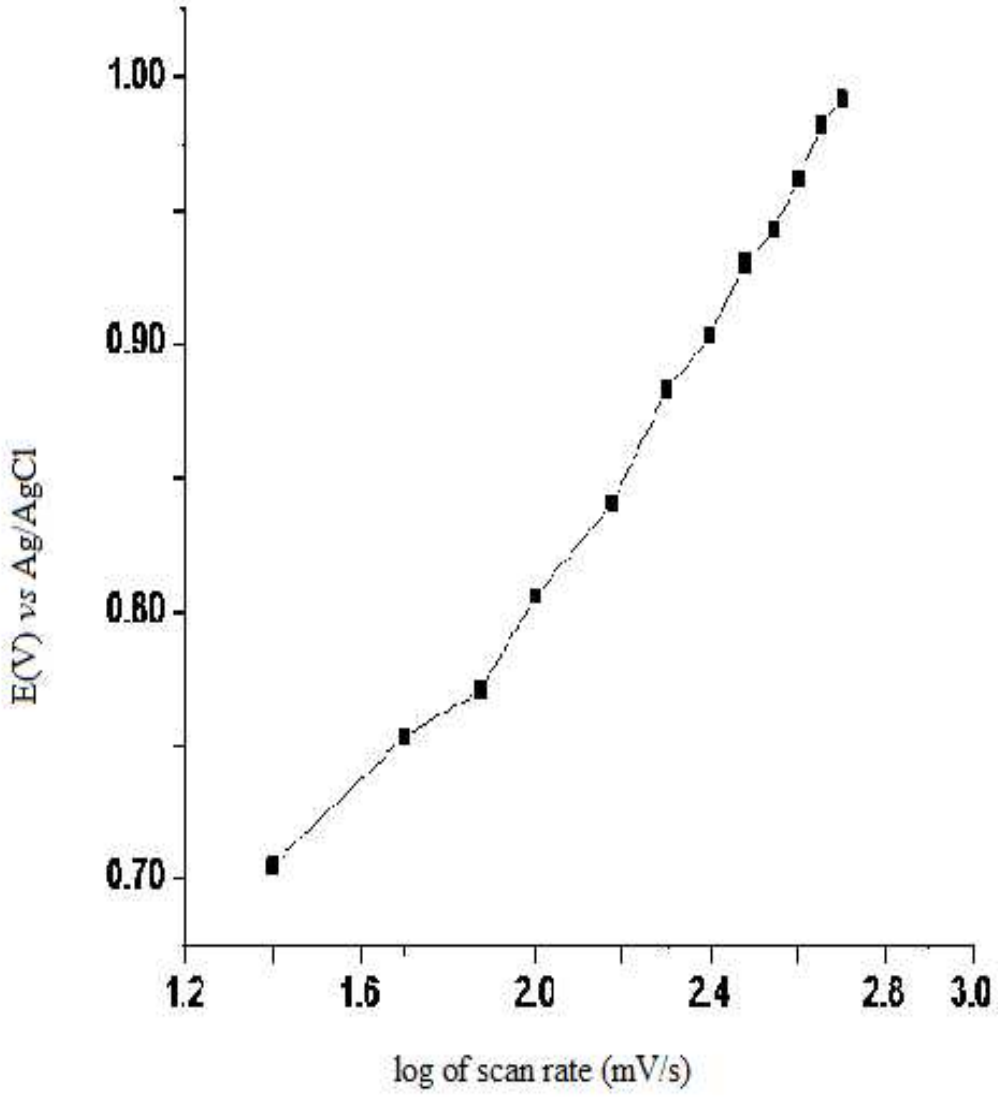


Figure 20: the dependence of peak potential, Ep on log v

Table 2.The dependence of peak potentials on the logarithm of scan rate

Scan Rate (mv/s)	Log of scan rate (mv/s)	E_{pa} (v)
25	1.398	0.700
50	1.699	0.753
75	1.875	0.771
100	2	0.793
150	2.176	0.831
200	2.301	0.867
250	2.398	0.890
300	2.477	0.922
350	2.544	0.943
400	2.602	0.959
450	2.653	0.982
500	2.699	0.999

4.5. Differential Pulse Voltammetric Investigation

The electrochemical oxidation of folic acid at different concentration using poly BCP/GCE was studied. To evaluate the analytical performance of BCPs/GC modified glassy carbon electrodes for FA analysis require optimization of many parameters. These include pulse amplitude, pulse width, sample width, pulse period, increment and pH. According to this study, a sequence of experiment was conducted to attain suitable experimental condition for determination of FA. The effect of particular variable was studied under identified condition by keeping all variables constant except one under study

4.5.1. Optimized Experimental Conditions

The effect of the parameters for differential pulse voltammetric determination of folic acid at poly BCP/GC modified electrode was optimized as revealed in Table 3. Optimum conditions for the electrochemical response were established by measuring the peak current on dependence on all parameters increment, amplitude, pulse width, sample width, pulse period, and quiet time. The optimum parameters identified for the determination of the analyte for plotting the calibration curve are summarized in Table 3.

Table 3 Optimum Experimental Conditions for the determination of FA by DPV at BCPs/GC modified electrode.

Parameters	Optimum Value
Pulse Amplitude	0.05v
Pulse Width	0.05s
Sample Width	0.017s
Pulse period	0.2s
Increment	0.004v
Quiet time	2s
sensitivity	10^{-4} M
pH	7.0

4.6. Linear Range and Detection Limit

Fig 21 shows the cyclic voltammogram response for different concentrations of folic acid from 20 to 100 μM at BCP/GC modified electrode in 0.1M PBS at pH 7.0 with scan rate of 100mV/s were studied using DPV. The results reveal that anodic peak current increases linearly with increase in the concentration of folic acid along with anodic potential shifts towards positive side. Based on the optimum experimental conditions, the reliance of voltammetric signal on the concentration of folic acid and the sensitivity of the method are illustrated by differential pulse voltammetry for different concentration of FA (Fig 21).

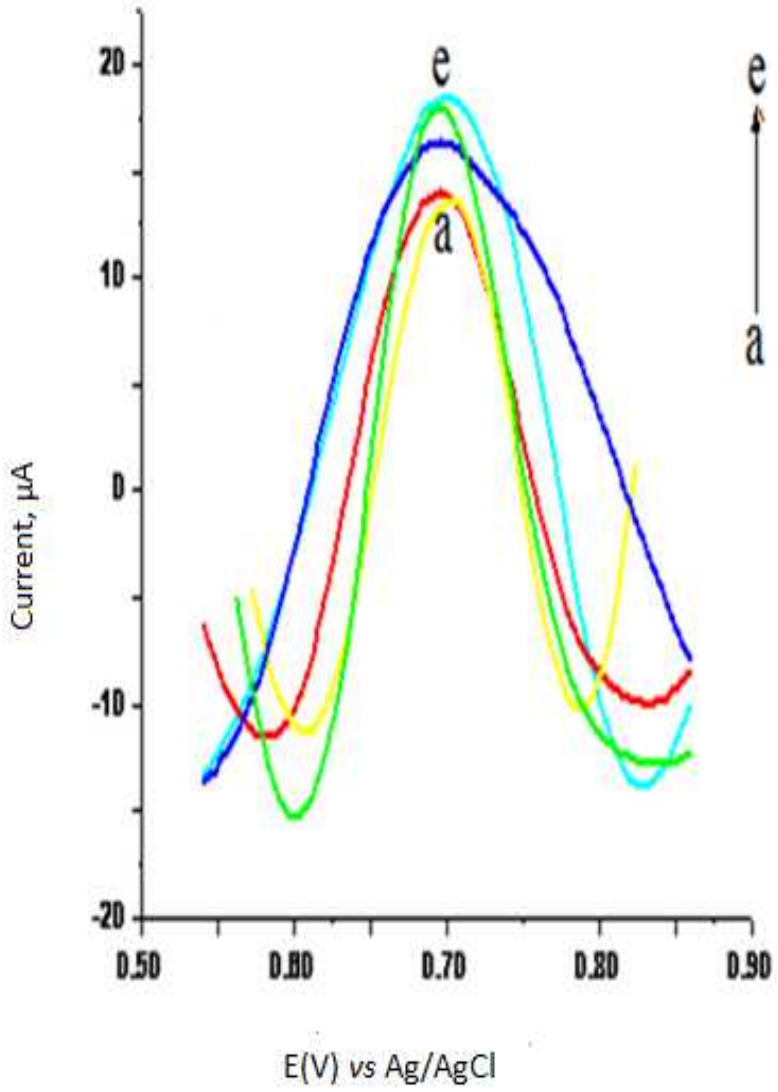


Figure 21: Differential pulse voltammograms for BCPs/GCE modified electrode for different concentration of folic acid. Pulse amplitude 50 mV, pulse width 50 ms, and pulse period 200 mV (a = 20 µM e= 100 µM)

The peak height for folic acid was found to increase with an increase in concentration from 20 to100 µM. The calibration curves for five data points was found to be linear with R = 0.987 and with the regression equation, I_{pa} (µA) = 2.1441 + 0.0123 * C (µM).The numerical value of regression coefficient for this experiment showed a good linear fit because the value of R is close to one. The detection limit for FA (at an S/N= 3) was found to be 1.772 µM.

LOD= $\frac{3S}{m}$

m - Slope of the regression line
SD_b - Standard deviation of blank sample

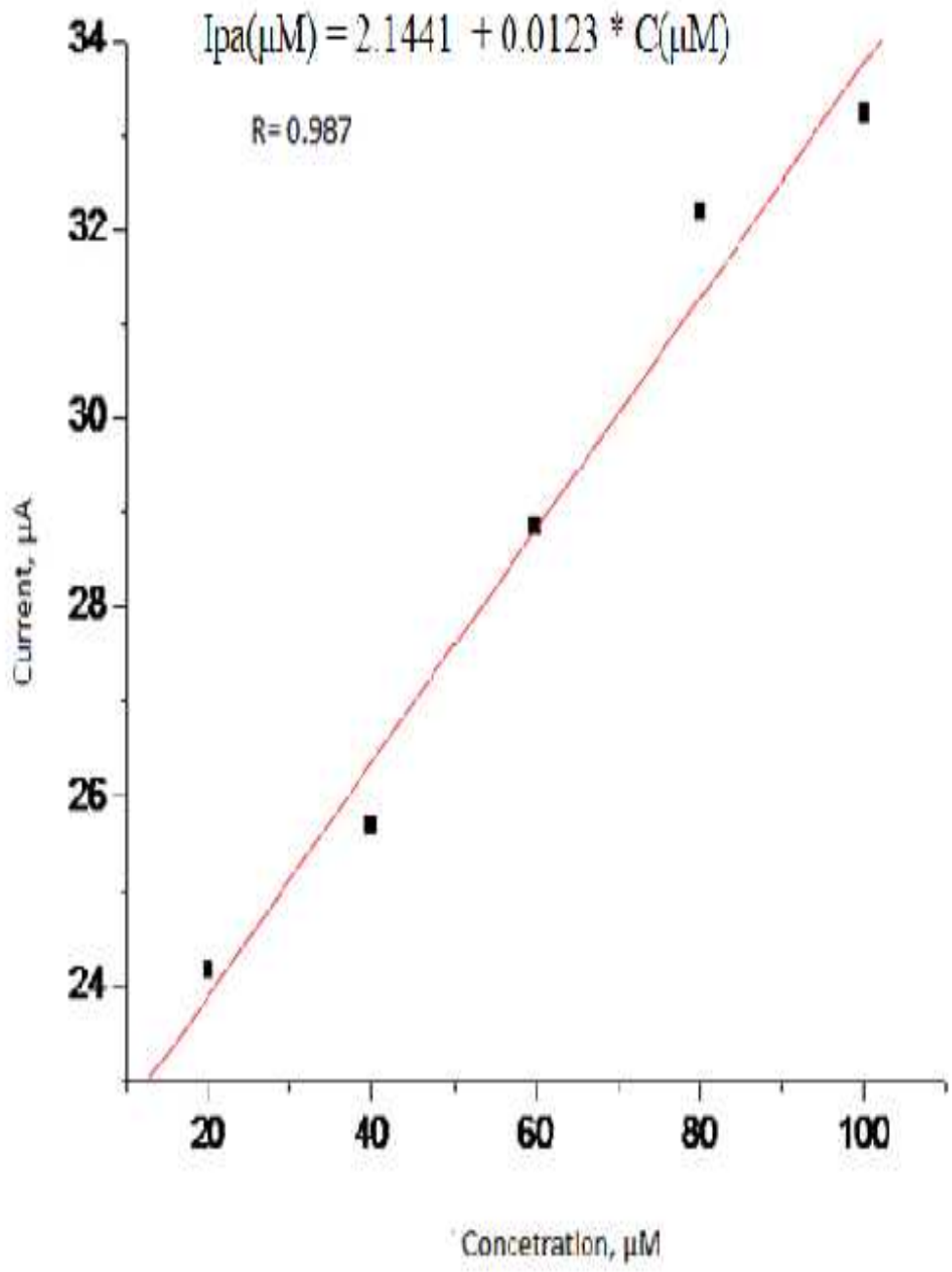


Figure 22: Plot of DPV anodic peaks current as a function of FA concentration from 20 to 100µM.

The analytical performance of the BCPs/ GCE electrode was compared with that of different electrodes and the results are summarized in Table 4. It can be noted that the BCPs/GCE has comparable linear response range and satisfactory limit of detection.

Table 4 Comparison of characteristics values obtained from some literatures and this work

Electrodes	Linear Range (μM)	LOD (μM)	Reference
Poly(5-amino-2-mercapto-1,3,4-thiadiazole)	0.1- 800	0.00023	[11]
SWCNTs-ionic liquid (OMIMPF6)	0.002- 4.0	0.001	[20]
ZrO2 nanoparticles	20-250	9.86	[21]
Poly(3-amino-5-mercapto-1,2,4-triazole)	20-180	0.25	[22]
MWCNT and ferrocenedicarboxyic acid	4.6- 152	1.1	[23]
SWCNTs film modified glassy carbon electrode	0.01-1.0	0.001	[61]
BCPs/GCE	20-100	1.772	This work

4.7. Interference Study

Possible interferon's in the detection of 100 μM FA such as various inorganic ions, ascorbic acid, iron (III) chloride. six hydrate, pyridoxine hydrochloride and phenol at poly BCPs/GCE modified electrode were studied. To a constant concentration of FA the same, two times, five times, and ten times concentration of FA of these compounds were spiked and recorded using DPV between 0.2 V and 1.0 V. The addition of common inorganic ions such as Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Cl^{-}, NO_3^{-}, and SO_4^{2-} have no effect on the response of the designed BCP/GC modified electrode.

a) The effect of Ascorbic acid

Bare GC electrode fails to determine the concentration of FA in the presence of AA due to the surface fouling caused by the oxidized products of AA and FA. However, the BCPs/GCE film modified electrode not only separates the voltammetric signals of AA and FA with potential difference of 360 mV between AA-FA, but also shows higher oxidation current for these analytes. As shown in Figure 23 the effect of different concentrations of AA on the peak height of 100 µM FA were studied. The result shows in the addition of the 100 µM, 200 µM, 500 µM and 1000 µM of AA linearly increase the peak current of AA and greatly affect the peak current of FA. Thus, the peak current of folic acid was significantly increasing as the concentration of AA was increased.

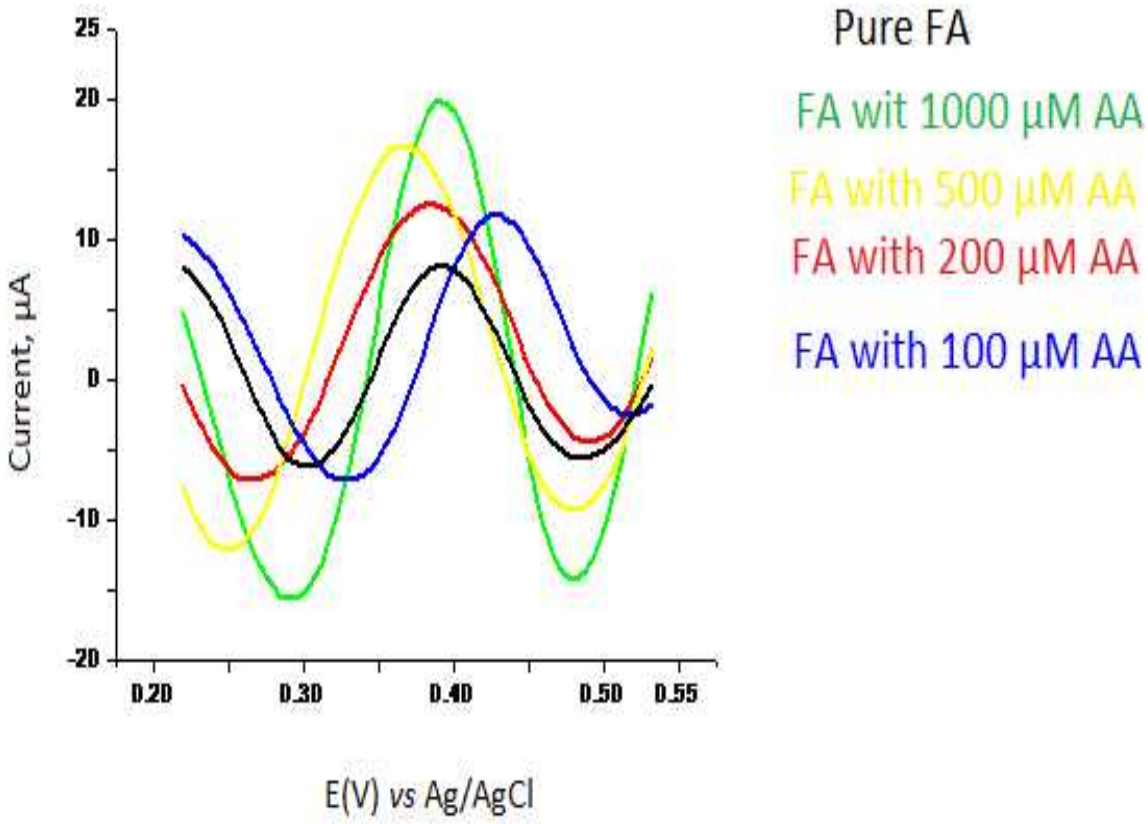


Figure23:Differential pulse voltammogram of 100 µM FA at BCPs/GCE in the presence of Ascorbic Acid; mix ratio: 1:1, 1:2, 1:5 and 1:10 in 0.1 M PBS (pH 7.0).

b) The effect of Iron (III) Chloride. six hydrate

The effects of the addition of different concentration of $FeCl_3H_{12}O_6$ on the electrochemical determination of 100 µM folic acid in 0.1M PBS at modified electrode were studied. To examine this 100µM, 200 µM, 500 µM and 1000 µM $FeCl_3H_{12}O_6$ were taken. The voltammetric current responses of folic acid at modified electrode are shown in Fig 24. It was generally seen that there is a remarkable decrease in the peak current of folic acid as the concentration of $FeCl_3H_{12}O_6$ was increased. Indicating that folic acid cannot be detected in the presence of iron (III) chloride six hydrate.

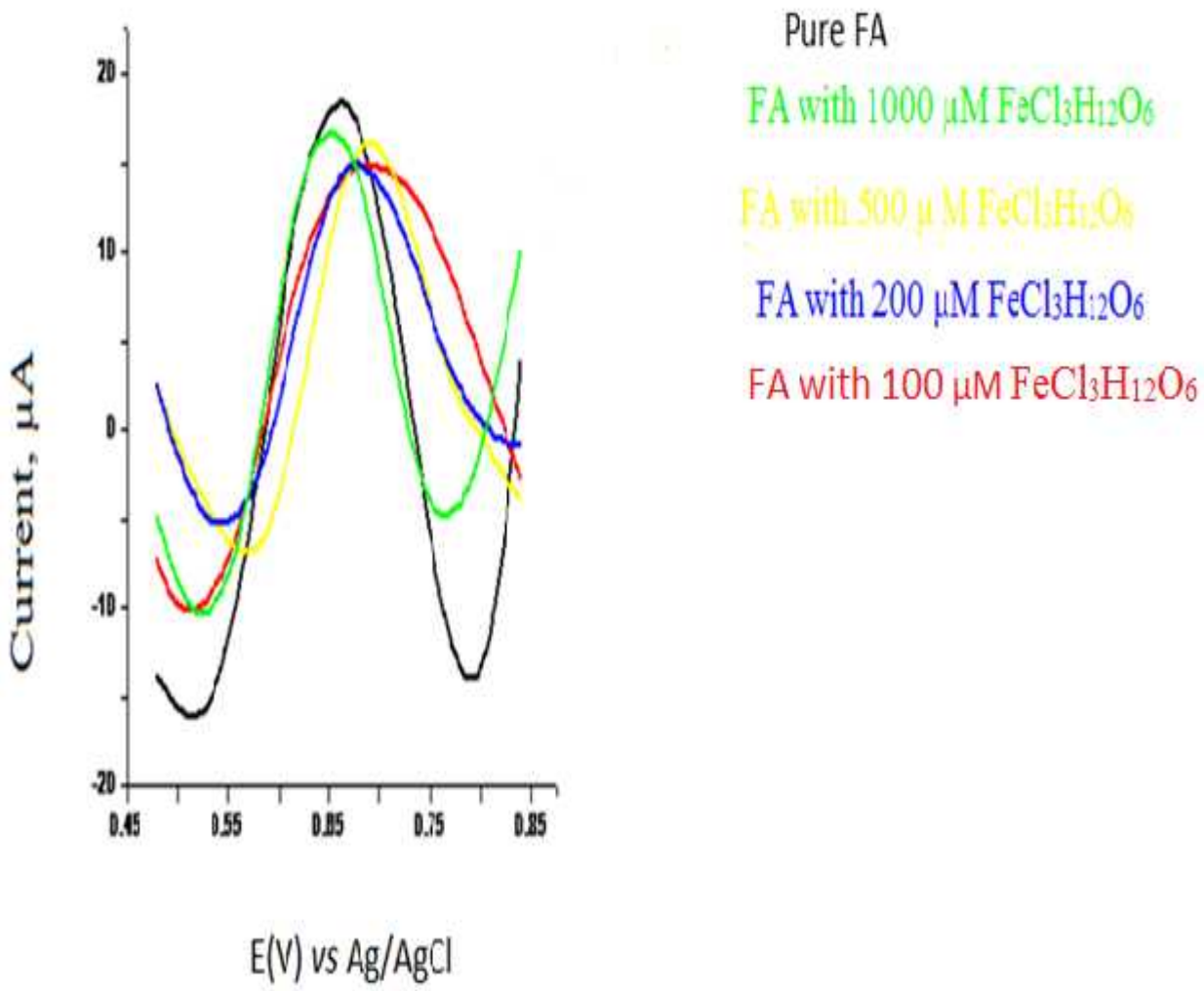


Figure24: Differential pulse voltammogram of 100 µM FA at BCPs/GCE in the presence of iron (III) chloride six hydrate, $FeCl_3.6H_2O$; mix ratio: 1:1, 1:2, 1:5 and 1:10 in 0.1 M PBS (pH 7.0).

c) The effect of pyridoxine hydrochloride

The influence of the addition of different concentration of $C_8H_{11}ClNO_3$ in the electrochemical determination of 100 µM folic acid in 0.1 M PBS at modified electrode was studied. To investigate this 100 µM, 200 µM, 500 µM and 1000 µM of pyridoxine HCl were spiked to 100 µM FA. The oxidation peak current for Pyridoxine HCl increased proportionally to the concentrations from 100 to 500 µM, and it is also clearly seen that the rapid fall of the peak current of Pyridoxine HCl at the concentration of 1000 µM as compared with 500 µM .The result of Pyridoxine HCl on the voltammetric current response of FA at modified electrode is shown in Fig 25. The addition of the 500 µM of Pyridoxine HCl unexpectedly increase the peak current of FA due to electrode enhancement, where as in the addition of 100 µM, 200 µM and 1000 µM of Pyridoxine HCl decrease the peak current of FA as the concentration of pyridoxine HCl was increased. Indicating that folic acid cannot be detected in the presence of iron (III) chloride six hydrate

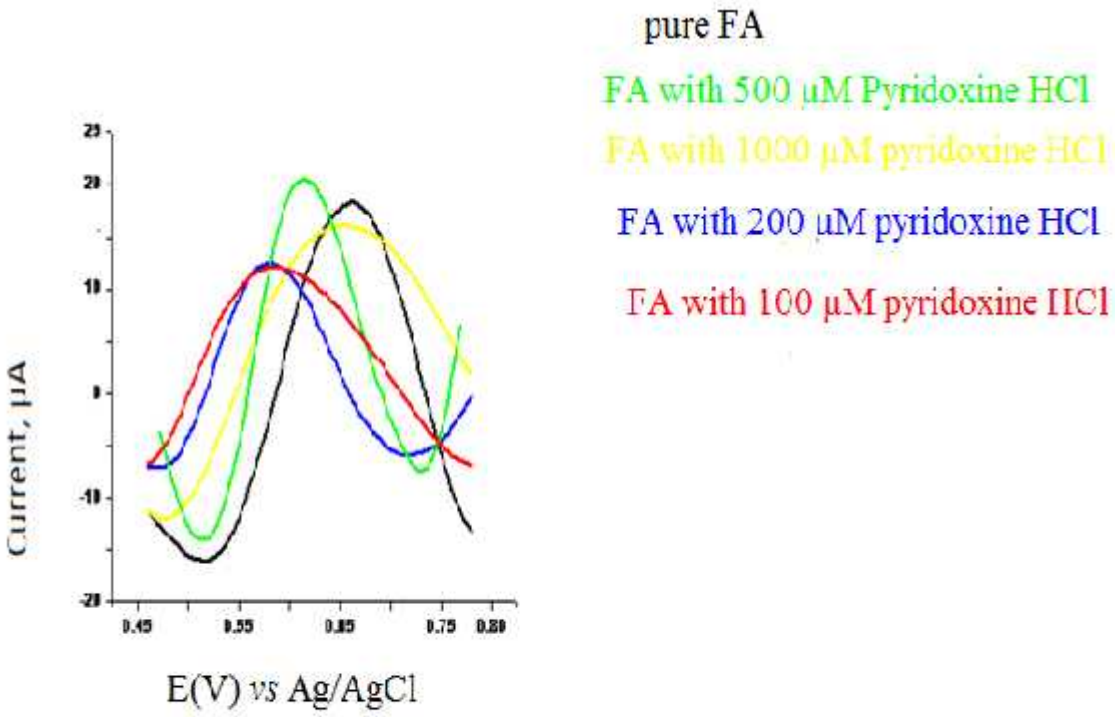


Figure25: Differential pulse voltammogram of 100 µM FA at BCPs/GCE in the presence of pyridoxine hydrochloride, $C_8H_{11}ClNO_3$; mix ratio: 1:1, 1:2, 1:5 and 1:10 in 0.1 M PBS (pH 7.0).

d) The effect of phenol

Phenol which is the most environmental interfering organic compounds was examined as interferon to FA. The effects of phenol in the electrochemical determination of 100 µM FA at modified electrode were studied. To examine this, the 100 µM, 200 µM, 500 µM and 1000 µM phenol were added to 100 µM FA. The voltammogram show the peak current of phenol has marked effect on the response of folic acid at modified electrode as shown in Fig 26. The addition of 200 µM of ph suddenly decrease the peak current of FA due to electrode fouling. While the addition of 100 µM, 500 µM and 1000 µM of ph increase the peak current of FA and shifts its potential positively as the concentrations of phenol was increased. Folic acid cannot be detected in the presence of phenol.

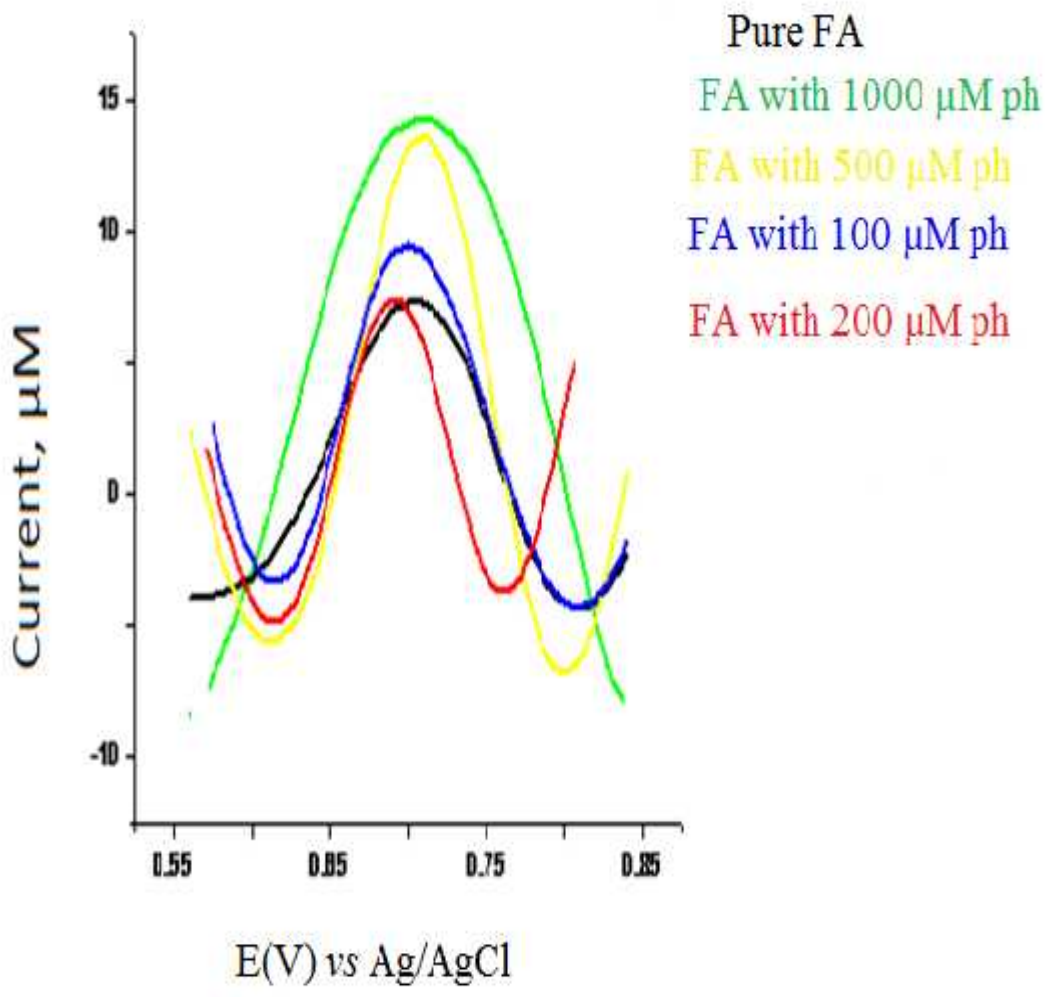


Figure26: Differential pulse voltammogram of 100 µM FA at BCPs/GCE in the presence of phenol, C_6H_6O; mix ratio: 1:1, 1:2, 1:5 and 1:10 in 0.1 M PBS (pH 7.0).

4.8. Real samples analysis

To investigate the capability of the modified electrode, the developed method was applied for determination of FA in commercial tablets. Three pieces of FA tablet were ground up and dissolved in trace amount of 0.1M NaOH and then 100 mL of distilled water was added to the solution . The solutions were acted as the artificial samples without any pretreatment were tested. The detection of FA was performed by the standard addition method. From the stock solution of 100 μM FA, 500, 1000, 1500, and 2000 μL , FA were added to Folacin tablet solution (6 mL Folacin solution and 4 mL PBS) and differential pulse voltammograms were recorded at optimum conditions for each addition then the peak current reading were used to calculate the concentration. The result demonstrated that it is possible to determine the concentration of FA in real sample solutions. The results are summarized in table 5.

When known amounts of FA were added to the artificial samples, quantitative recovery from 96% to 100% was obtained. A feasibility of the BCPs/GCE modified electrode fabricated for determination of FA was therefore evident.

Table 5 Recovery Study

Samples	Content (mg)	FA added (μM)	FA founded (μM)	Recovery (%)
1	5.000	4.8	4.85	101.04
2	5.000	9.1	9.4	103.30
3	5.000	13.0	12.8	98.50
4	5.000	16.7	16.1	96.40

5. Conclusion

In this work, the poly bromocresol purple was prepared by simple and fast electropolymerization method. The GCE was modified with BCPs by electropolymerization technique. The modified electrode was characterized and used as an electrochemical sensor for the determination of FA by CV and DPV respectively. The modified electrode had good sensitivity and selectivity with acceptable recovery. The results demonstrated that the proposed method is rapid, sensitive, and reproducible for the determination of folic acid. Thus, the poly BCP/GC modified electrode can be a useful tool for the assay of folic acid both in research and in detection of FA in real sample (commercial Folacin tablets).

Since the BCP/GC modified electrode showed satisfactory result for the direct determination of FA by differential pulse voltammetry (DPV), it can also be used in several sensor applications for environmental and biological molecules of interest. In the future it is necessary to develop a better modified electrode for determination of FA in the presence of iron(III)chloride six hydrate and phenol

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