Addis Ababa University

College of Natural and Computational Sciences

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Development of electrochemical sensor based on polybromocresol purple for the determination of o-aminophenol

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DEPARTMENT OF CHEMISTRY

Development of electrochemical sensor based on polybromocresol purple for the determination of o-aminophenol.

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

By: Mekonnen Mulugeta

Advisor: Dr. Negussie Negash

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This is to certify that the thesis prepared by Mekonnen Mulugeta entitled "***Development of electrochemical sensor based on polybromocresol purple for the determination of o-aminophenol"*** and submitted in partial fulfilment of requirments the degree of master in chemistry compiles with the regulation of the university and meets the accepted standards with respect to originality and quality.

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List of abbreviations

ABS	Acetate Buffer Solution
APZ.	2-amino phenoxazine-3-one
BCP	Bromocresolpurple
CME	Chemical modification of electrode
CPE	Carbon Paste Electrode
CV	Cyclic Voltammetry
CPs	Conductive polymers
DPV	Differential pulse voltammetry
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
ECME	Electrochemical modification of electrode
EIS	Electrochemical impedance spectroscopy
Epa	Anodic peak potential
Epc	Cathodic peak potential
FDP	Faculty development program
GCE	Glassy Carbon Electrode
ipa	Anodic peak current
ipc	Cathodic peak current
LSV	Linear sweep voltammetry

MWCNT	Multi-Wall Carbon Nanotubes
NPV	Normal Pulse Voltammetry
PBS	Phosphate Buffer Solution
PBCP	Poly Bromocresol purple
POAP	poly (ortho-aminophenol)
OAP	Ortho-aminophenol
SCE	Saturated calomel electrode

Abstract

The aim of this work was to study the effect of conducting properties of polybromocresol purple (PBCP) in the determination of o-aminophenol. Cyclic Voltammetry (CV) and Differential pulse Voltammetry (DPV) are employed in this study. The electrochemical oxidation of ortho-amino phenol was carried out using a PBCP/GC electrode in aqueous acid medium. A glassy carbon electrode is modified with polybromocresol purple (PBCP) over potential range of -1.5 V - 1.6 V. O-aminophenol was studied by using cyclic voltammetry at different scan rates in the potential range of -0.1 V to 0.7 V. The results indicated that the oxidation reaction of the o-aminophenol at the modified glassy carbon electrode is irreversible and controlled by diffusion. Under the optimum conditions, the differential pulse voltammetric current of the sensor was linear in the concentration range of 1-100 µM with a detection limit of 0.21 µM. The modified electrode was capable to quantify o-aminophenol in the presence of common interferences and of detection in the tap water.

Key words: o-Aminophenol, Poly bromocresol purple (PBCPs), electropolymerization, cyclic voltammetry, differential pulse voltammetry

1. Introduction

1.1. A brief description about the o-aminophenol

Ortho- aminophenol is aromatic compound that contains amino and hydroxyl group adjacent to each other on benzene ring. It is intermediate in photographic, chemical dye and an organic compound with the formula C_6H_7NO [1, 2]. Its isomer is 4-aminophenol, which is an amphoteric molecule (behaves as either weak acid or weak base), however, the basic character is predominantly good reducing agent and chemically reactive. It is a useful reagent for the synthesis of dyes, heterocyclic compounds and pharmaceutical industries [2].

The oxidation of o-aminophenol (OAP) on different electrode materials such as (gold, platinum, carbon, indium-tin oxide, etc.) in aqueous medium to form poly-o-aminophenol (POAP) [1, 3]. O-AP can be polymerized electrochemically in acidic, neutral and alkaline solutions. While a conducting film only formed in acidic media, POAP synthesized in neutral and alkaline media leads to a non-conducting film [4]. O-aminophenol is interesting electrochemical materials since, unlike aniline and other substituted anilines, which have two groups ($–NH_2$ and OH), which could be oxidized [4, 5, 6 and 7]. Therefore, it could show that the electro chemical behavior resembling anilines and/or phenols. An important factor would be the relative position of the amino and hydroxyl group in the aromatic ring. Accordingly, the reported electrochemical properties of the three positional isomers (ortho, meta, and para) are strongly defferent.

P-aminophenol is a well-known compound, which, in its simple form, or derivative, has been used as redox agent in photography. In neutral media, it is oxidized to complex oligomer dyes that could be used in enzymatic activity [8]. The electro polymerization of p-aminophenol on a platinum electrode yields a soluble electro active polymer in organic solvents.

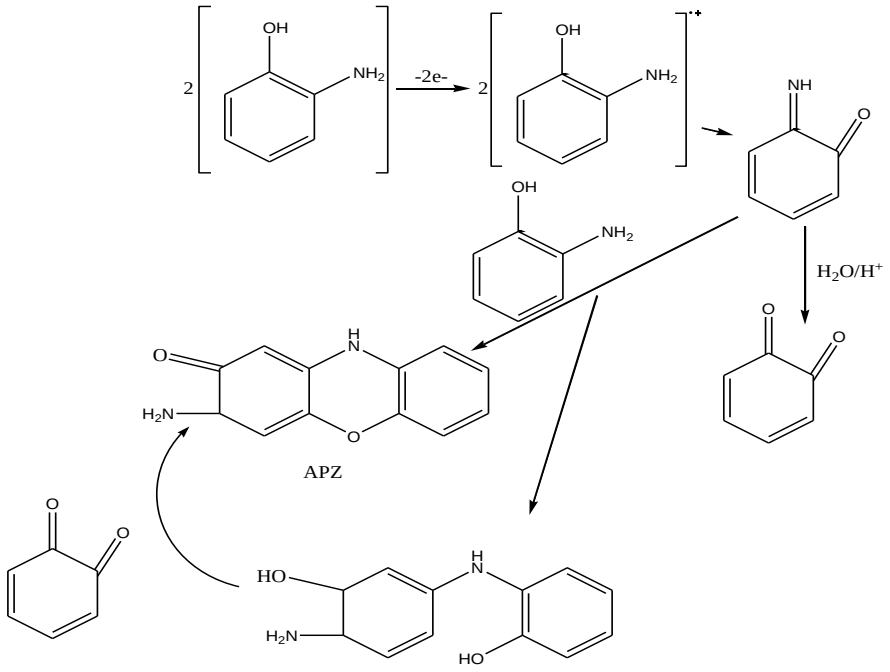
In the oxidation of m-aminophenol in aqueous solution on SnO_2 electrodes, only the amino group of m-aminophenol undergoes oxidation while the hydroxyl group remains unchanged [9].

On the other hand, the oxidation of o-aminophenol (OAP) on different electrode materials (platinum, glassy carbon electrode, gold, etc.) in aqueous acid medium to form P0AP [8].

1.2. Oxidation mechanism of o-aminophenol

OAP involves numerous reactions particularly condensation and cyclization due to ortho effect of hydroxyl and amino groups on benzene ring to give 2-amino phenoxazine-3-one [2]. Electro determination of OAP in acid medium occurred within the potential range of $-0.1\,V < E < 0.7\,V$ (versus Ag/AgCl) at pH 5 and scan rate 100 mV/s. The electro activity of OAP is explained by a redox mechanism that involves an addition/elimination of protons coupled with a reversible electron transfer.

The first oxidation step of OAP is an electron transfer to form radical cation followed by chemical coupling of radical cation or radical monomer species [10]. Radical cation formed in the region peak I react quickly near the electrode surface, and after this first step involving two electrons. Then the soluble products are easily formed by hydrolysis (schem1). Oxidation of OAP produces intermediate benzoquinone monoimines, when the solution becomes yellowish and brown after successive cycling. Particularly at less controlled conditions such as at higher final potentials and lower scan rates, monoimines react with neutral OAP giving an intermediate (2-amino-o-indophenol) prior to cyclization to APZ [10].



2-amino –o-indophenol

Scheme 1.mechanism of OAP electro oxidation in aqueous acidic media **[10]**

1.3. Physical and chemical properties of o-aminophenol

1.3.1. Physical properties

At room temperature, o- aminophenol is white solid crystalline compounds [11]. It is well soluble in ethanol and hot water, very soluble in acetonitrile, ethyl acetate, acetone, dimethylsulfoxide (DMSO) and in dimethylformamide (DMF). It is also slightly soluble in benzene, toluene, chloroform and cold water: It is soluble in water: 17 g/L (20 °C). As compared to other compounds with a similar molecular mass; for example, 2-methylphenol melts at 31 °C. O-aminophenol (OAP) forms white orthorambic bipyramidal needles having eight molecules to the elementary cell. It has hydrophilic character.

Table 1. Some physical property of o- aminophenol

Molecular formula	C_6H_7NO
Molar mass	109.13 g/mol
Melting point	174 °C
Boling point	164 °C
Density	1.328 g/cm^3
Solubility in water	17 g/L at (20 °C)
Acidity (pKa)	4.78 at (20 °C)

1.3.2. Chemical properties

O-Aminophenol appears as colorless needles or as white crystalline substance turning to brown on exposure to air. The compound exhibits intra- and intermolecular hydrogen bonding involving the neighboring amine and hydroxyl groups. i.e. the molecules are hydrogen bonded from OH to N to form chain [11].

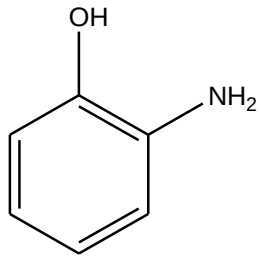


Figure 1 : Structural formula of o-aminophenol

1.4. Applications and use of o-aminophenol

Ortho-aminophenol has a variety of uses. As a reducing agent, o-Aminophenol is an intermediate in the synthesis of dyes. It is particularly useful in yielding metal-complex dyes when diazotized and coupled to a phenol, naphthol, or other aromatic or resonant dye species. The simultaneous presence of hydroxyl and amino groups on the POAP backbone should allow the polymer to coordinate with most transition metal cations. In this connection, some metal

cations increase the catalytic activity of POAP through their incorporation into the POAP matrix. The metal complex formed due to the interaction between POAP and metal cations [12].

Metal complex dyes using copper or chromium commonly used for producing dull colors. Tridentate ligand dyes are useful because they are more stable than their bi- or mono-dentate counterparts due to the adjacency of the amino and hydroxyl groups, o-aminophenol readily forms heterocycles. These heterocycles, such as benzoxazoles, can be biologically active and useful in the pharmaceutical industry; o-Aminophenol is also used in oxidative hair dye formulations at a maximum concentration of 1.2 %, which, after mixing 1:1 with H_2O_2 just prior to usage, corresponds to an on-head concentration of 0.6 % up

1.5. Environmental effect of ortho-aminophenol

O-aminophenol is a combustible material and it can burn but does not ignite readily. At this time, the containers may explode when heated. The runoff may pollute waterways. This substance may transport in a molten form [13].

1.6. Health effects of ortho-aminophenol

Ortho-aminophenols are possessing toxic effects because of their easy penetrating property into the skin sensitization [13, 14], Mucous membrane irritation, and membranes of plants and animals cause genotoxic, mutagenic and hepatotoxic effects. Further, in presence of metal ions it causes DNA damage. These effects are due to their wide use as important industrial raw materials, synthetic intermediates, chemical inhibitors and petroleum additives [2, 14]. It is contained in hair dyes and can cause contact dermatitis in hairdressers and consumers.

2. Literature Review

2.1. Chemical modification of electrodes (CMEs)

Chemical modification of electrode an electrode made of a conducting or semiconducting material that is coated with a selected monomolecular, multimolecular, ionic, metallic or polymeric film of a chemical modifier and that by means of faradaic (charge-transfer) reactions or interfacial potential differences (no net charge transfer) exhibits chemical, electrochemical, and/or optical properties of the film. CME is an electrical conductor (material that has the ability to transfer electricity) that has surface modified for different functions. Chemically modification of electrodes are made using advanced approach to electrode system by adding thin film or layer of a certain chemical to change property of conductor according to the targeted function [15,16]. On a modified electrode, an oxidation-reduction substance accomplishes electro catalysis by transferring electrons from the electrode to a reactant or reaction substrate. (Fig. 2)

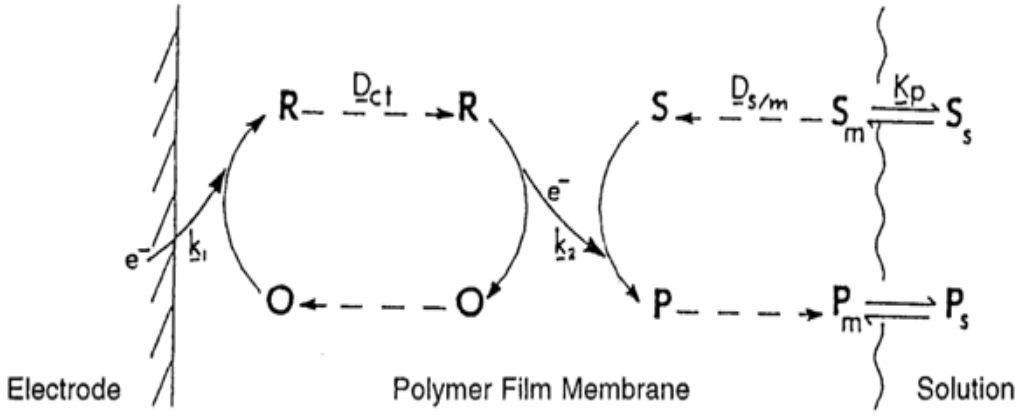


Figure 2: Chemical modification of electrode

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 electro catalyst 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 mediator undergoes homogeneous electron transfer (rate constant = K_2) with the substrate in the polymer film. The overall process at the electrode surface can be described as:

$M_{ox} + A_{Red} \rightarrow M_{Red} + A_{Ox}$

$M_{Red} - ne \rightarrow M_{Ox}$

The transfer of electrons takes place between the electrode and mediator and not directly between the electrode and the analyte. In essence, then, the mediator can be considered to function simply as an electron shuttle between the electrode and the analyte. The net results of this electron shuttling are a lowering of the overvoltage to the formal potential of the mediator and increase in current density [17]. However, electrochemical modification of electrode attracts great attention. Currently, electrochemical modifications of electrode dose not involve intermediate that accomplish the transfer of electrons between the electrode and the analyte.

2.2. Conducting polymers (CPs)

Conducting polymers have unique chemical structures and are easy to assemble or deposited into many electronic devices [18]. Conducting materials have received significant scientific and technological interest in recent years. Aniline based compounds and polymers have grabbed special attention as a base material for the synthesis of conducting devices.

CPs provides the advantage of chemical diversity, low density, flexibility, corrosion resistance, easy-to-control shape and morphology, and tunable conductivity over their existing inorganic counter parts. However, the development of the properties of CPs has not been completely commensurate with those of their metallic and inorganic semiconductor counterparts. Consequently, CPs ‘has been modified with other heterogeneous material components to overcome their inherent limitations in terms of solubility, conductivity, and long-term stability.

Conducting polymers such as polythiophene, polyfuran, polypyrrole, poly (phenylene), poly fluorene [19] and Polyaniline (PAN) [20], Polyvinylenes [21], polyarylenes [22] and polyheterocycles [23] are the major classes. Polyvinylenes well has known polymers that

possess good thermal stabilities and appreciably high electrical conductivities. Poly (p-phenylene) and poly (phenylenevinylene) belong to the class of polyarylenes or poly-aromatics. Poly-(p doping that was reported in 1980 [24]. Polythiophene [25], Polypyrrole [26], polyfuran [27] and their derivatives having a five Polymerization are formed by reacting monomer molecules together in a chemical reaction to form polymer chains for coating electrode. It is used to modify electrode in acidic media. 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.

2.2.1.. Polybromocresol purple (PBCP)

The common feature of conducting polymers is the existence of an extended π – conjugated system. Many polymers have excellent conductivity to make suitable for electron transfer, ion recognition and electroanalysis. Bromocresolpurple (BCP) is a pH indicator that can be used as a dye.The poly bromocresol purple film has proved to possess abundant active sites, π-π conjugated bonds, large real surface area and good conductivity (fig.3). The electro-poly (BCP) film modified electrode showed good electrocatalytic activity towards some compounds such as uric acid, xanthine and hydrazine [28]

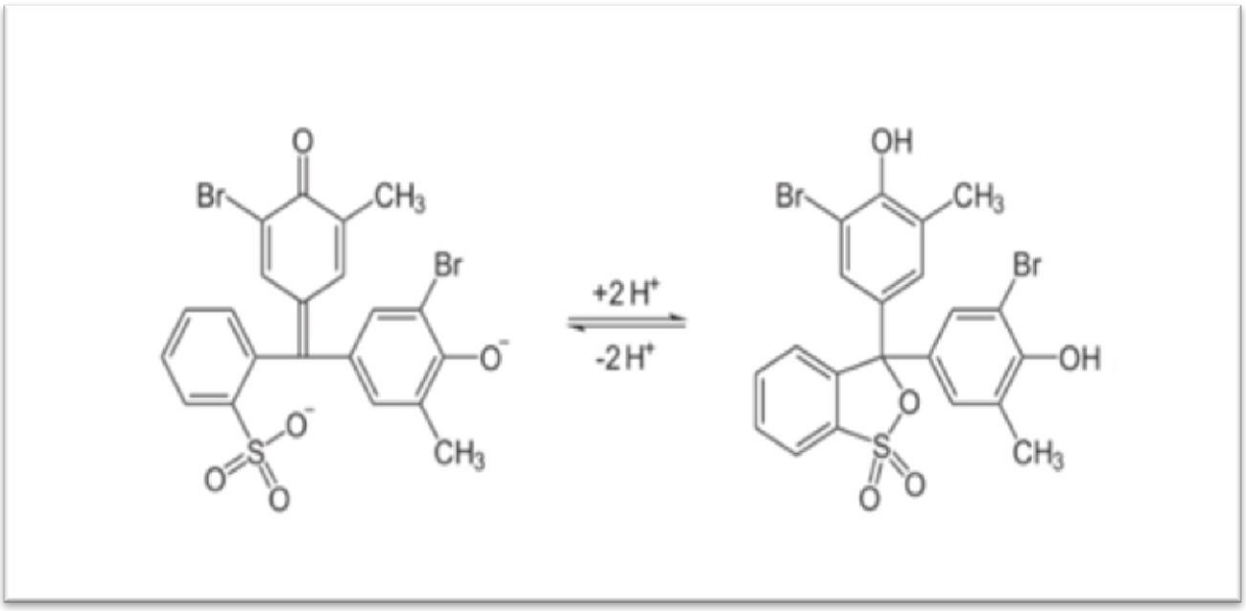


Figure 3: Redox reaction of bromocresol purple

2.3. Electrochemical techniques

Electro analytical techniques are concerned with the interplay between electricity and chemistry, namely the measurements of electrical quantities, such as current, potential, or charge and their relationship to chemical parameters. Such use of electrical measurements for analytical purposes has found a vast range of applications, including environmental monitoring, industrial quality control, and biomedical. 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 methods are extensively applied in sensing and biosensing [29]. There are many different voltammetric techniques. Among these, potentiometry, amperometry, cyclic voltammetry, linear voltammetry, differential pulse voltammetry, square-wave voltammetry, and electrochemical impedance spectroscopy (EIS) represent the most used electrochemical techniques for sensor and biosensor fabrication and detection of analyte

Table 2. Classifications of electrochemical techniques

Electrochemical Techniques					
Static techniques		Dynamic Techniques			
Type	measured	Pulse techniques		Sweep techniques	
Potentiometry	potential	Type	measured	Type	measured
		DPV	Current	CV	Current
		SWV	Current	LSV	Current
		NPV	Current	EIS	Current

Interfacial electrochemical techniques are classified into **static** and **dynamic techniques,**

In static techniques, do not allow current to pass through the electrochemical cell and, as a result, the concentrations of all species remain constant. Potentiometry, measures the potential of an electrochemical cell under static conditions, and one of the most important quantitative electrochemical methods.

Dynamic techniques allow current to flow and force a change in the concentration of species in the electrochemical cell.

2.3.1. Potentiometric Methods

Potentiometer is used to determine the difference between the potential of two electrodes. The potential of one electrode the working or indicator electrode responds to the analyses' activity and the other electrode the reference electrode has a known, fixed potential. The electrochemical cell consists of two half-cells, each of which contains an electrode immersed in a solution of ions whose activities determine the electrode's potential. A salt bridge that contains an inert electrolyte, such as KCl, connects the two half-cells. The ends of the salt bridge fixed with porous frits, which allow the electrolyte's ions to move freely between the half-cells and the salt bridge. This movement of ions in the salt bridge completes the electrical circuit.

Potentiometry, measure the potential of an electrochemical cell under static conditions. Because no current or only a negligible current flows through the electrochemical cell, its composition remains unchanged. For this reason, potentiometry is a useful quantitative method of analysis. The first quantitative potentiometric applications appeared soon after the formulation, in 1889, of the Nernst equation, which relates an electrochemical cell's potential to the concentration of electro active species in the cell [29.30].

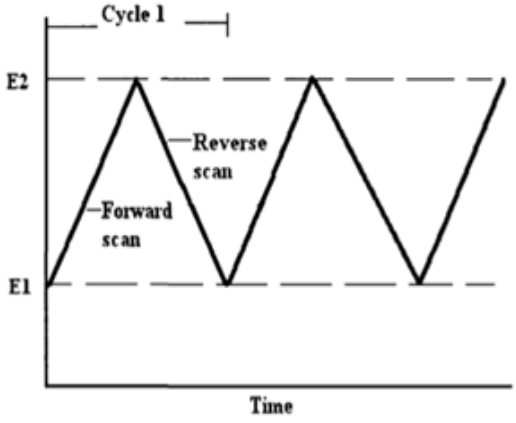
2.3.2. Voltammetric Technique

Voltammetry has been used for the quantitative analysis of a wide variety of samples, including environmental samples, clinical samples, pharmaceutical formulations, steels, gasoline, and oil. Voltammetry is a category of electro analytical methods used in analytical chemistry and various industrial processes. In voltammetry, information about an analyte will be obtained by measuring the current as the potential is varied. The analytical data for a voltammetric experiment comes in the form of a voltammogram, which plots the current produced by the analyte versus the potential of the working electrode. Voltammetric measurements are carried out using an electrochemical cell made up of three electrodes immersed in a solution containing the analyte and an excess of a nonreactive electrolyte called the supporting electrolyte. There are different voltammetric techniques [29], which can be shortly discussed as follows.

2.3.2.1. Cyclic Voltammetry

Cyclic voltammetry (CV) is the most widely used technique for acquiring qualitative information about electrochemical reactions. CV is a widely used electrochemical technique and involves the cyclical scanning of the potential (i.e., scanning from a starting to an ending potential and back again at a constant rate) and measuring the resultant current. The resulting "cyclic voltammagram" passes information about the redox potential of the analyte, the number of electrons transferred in the reduction or oxidation, the reversibility and speed of the electrochemical process, the stability of the oxidized or reduced form of the analyte. Cyclic voltammetry is one of the major techniques for experimental study of electro active polymers. The method uses an electrochemical cell with three electrodes; a reference electrode, working electrode and counter electrode. CV is a type of potentiodynamic electrochemical measurement in which the working electrode potential is ramped linearly versus time [31, 32]. This ramping

is known as the potential scan rate (Vs^{-1}). The potential is measured between the reference electrode and the working electrode and the current is measured between the working electrode and the counter electrode. Experimental data are plotted as current (i) versus potential (E). During a typical cyclic voltammetry experiment, a component of the solution is electrolyzed (oxidized or reduced) by placing the solution in contact with an electrode, and then applying a potential to that electrode that is sufficiently positive or negative with respect to a reference half-cell (e.g., calomel or Ag|AgCl). The electrode's voltage is adjusted higher or lower linearly, and finally, the voltage is returned to the original value at the same linear rate. As the potential is swept back and forth past the formal potential, E°, of an analyte, current flows through the electrode that either oxidizes or reduces the analyte. Electron-transfer is a measurable current in the electrode's circuitry. The magnitude of this current is proportional to the concentration of the analyte in solution, which allows cyclic voltammetry to be used in an analytical determination of concentration. The result is a cyclic voltammogram (or CV), where potential is plotted on the x-axis, and current is plotted y-axis



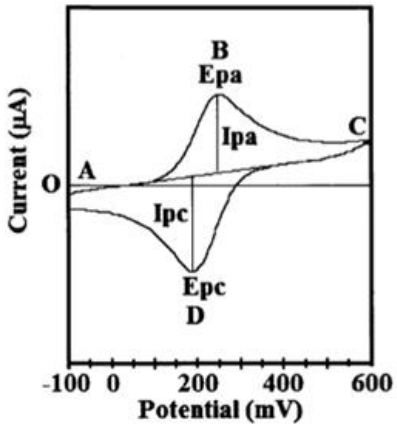


Figure 4: Excitation Signal for Cyclic voltammetry **Figure 5**: Typical cyclic voltammogram

Currents generated by the oxidation and reduction reactions at the working electrode are measured and plotted against applied potential on a cyclic voltammogram (CV). Fig.5 shows the shape of typical CV for a single electron transfer process. Cyclic voltammogram is characterized by peak potentials (Ep), at which the current reaches a local maximum or minimum and the value of the peak current (Ip), at these point. The formal reduction potential is

centered between the anodic peak potential, *E*pa and cathodic peak potential, *E*pc, and is mathematically given by:

$$E^0 = \frac{E\,pa + E\,pc}{2} \text{ ---------- [1]}$$

The number of electrons transferred in the reversible reaction at the electrode is given by:

$$\Delta E = /Epa - Epc/ \approx \frac{0.059}{n} \text{ ---------- [2]}$$

Thus, a one electron process such as the reduction of $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$ exhibits a ΔE = 0.059 V. The peak current for a reversible system is described by the Randles-Sevcik equation for the forward sweep of the first cycle [33]

$$ip = 2.69\times10^{-5} n^{3/2} A\, D^{1/2}\, C\, v^{1/2} \text{ ---------- [3]}$$

Where *n* is the number of electrons, *A* is electrode area (cm^2), *D* is the diffusion coefficient (cm^2/s, *C* is the concentration (mol/cm^3) and ν is the scan rate (in V/s) Thus, a great deal of information learned about the system by varying known parameters, such as concentration and scan rate.

The i_p is linearly proportional to the bulk concentration, C, of the electro active species, and the square root of the scan rate, $\upsilon^{1/2}$. Thus, an important diagnostic is a plot of the ip vs. $\upsilon^{1/2}$. If the plot is linear, it is reasonably safe to say that the electrode reaction is controlled by diffusion, which is the mass transport rate of the electro active species to the surface of the electrode across a concentration gradient. The thickness δ, of the "diffusion" layer can be approximated by $\delta \sim [Dt]^{1/2}$ where D is the diffusion coefficient and t is time in seconds. A quiet (i.e. unstirred solution) is required. The anodic peak current i_{pa} is equal to the cathodic peak current i_{pc}, so that the relationship,

$$\frac{ipa}{ipc} = 1 \text{ ---------- (4)}$$

2.3.3. Pulse voltammetric techniques

The primary advantage of the pulse voltammetric techniques, such as normal pulse or differential pulse voltammetry, is their ability to discriminate against charging (capacitance) current. As a result, the pulse techniques are more sensitive to oxidation or reduction currents (faradaic currents) than conventional dc voltammetry. Differential pulse voltammetry yields peaks for faradaic currents rather than the sigmoidal waveform obtained with dc or normal pulse techniques. This results in improved resolution for multiple analyte systems and more convenient quantitation

2.3.3.1. Normal pulse voltammetry (NPV)

This technique uses a series of potential pulses of increasing amplitude. The current measurement is made near the end of each pulse, which allows time for the charging current to decay. It is usually carried out in an unstirred solution at either DME (called normal pulse polarography) or solid electrodes. The potential is pulsed from an initial potential Ei. The duration of the pulse, τ, is usually 1 to 100 msec and the interval between pulses typically 0.1 to 5 sec. The resulting voltammagram displays the sampled current on the vertical axis and the potential to which the pulse is stepped on the horizontal axis [29, 30].

2.3.3.2. Differential pulse voltammetry (DPV)

Differential pulse voltammetry (DPV) is a voltammetric technique, similar to SWV, with an enhanced discrimination of Faradaic currents (electron transfer to and from an electrode) that can be obtained using DPV, where the potential perturbation, which consists of small pulses, is superimposed upon a staircase waveform. This technique is comparable to normal pulse voltammetry in that the potential is also scanned with a series of pulses. However, it differs from NPV because each potential pulse is fixed, of small amplitude (10 to 100 mV), and is superimposed on a slowly changing base potential [29]. Current is measured at two points for each pulse, the first point (1) just before the application of the pulse and the second (2) at the end of the pulse. These sampling points are selected to allow for the decay of the non-faradaic (charging) current. The difference between current measurements at these points for each pulse is determined and plotted against the base potential. Fig. 6 shows the excitation waveform.

The system of this measurement is usually the same as that of standard voltammetry. The potential between the working electrode and the reference electrode is changed as a pulse from an initial potential to an inter level potential and remains at the inter level potential for about 5 to 100 milliseconds; then it changes to the final potential, which is different from the initial potential. The pulse is repeated, changing the final potential, and a constant difference is kept between the initial and inter level potential that shows current. The value of the current between the working electrode and auxiliary electrode before and after the pulse are sampled and their differences are plotted versus potential.

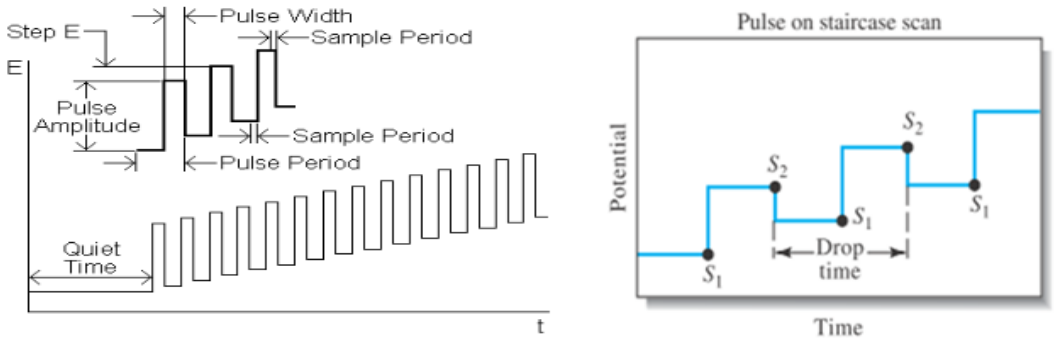


Figure 6**:** Excitation waveform of differential pulse voltammetry

2.3.3.3. Square wave voltammetric (SWV)

Square wave voltammetry has received growing attention as a voltammetric technique for routine quantitative analyses. Square wave voltammetry reported in 1957 by Barker, the utility of the technique limited by electronic technology. Square wave voltammetry is used to perform an experiment much faster than normal and differential pulse techniques, which typically run at scan rates of 1 - 10 mV/s. Square wave voltammetry employs scan rates up to 1 V/sec or faster, allowing much faster determinations. A typical experiment requiring three minutes by normal or differential pulse technique can be performed in a matter of seconds by square wave voltammetry [34, 35].

The waveform used for square wave voltammetry is shown in Fig.7. A symmetrical square wave is superimposed on a staircase waveform where the forward pulse of the square wave (pulse direction same as the scan direction) is coincident with the staircase step. The reverse

pulse of the square wave occurs half way through the staircase step. The excitation signal in SWV consists of a symmetrical square-wave pulse of amplitude ΔE shows superimposed on a staircase waveform of step height ΔE, where the forward pulse of the square wave coincides with the staircase step [36]. The net current, I_{net}, is obtained by taking the difference between the forward and reverse currents ($I_{for} - I_{rev}$) and is centered on the redox potential. The peak height is directly proportional to the concentration of the electro active species and direct detection limits as low as 10^{-8} M is possible [29].

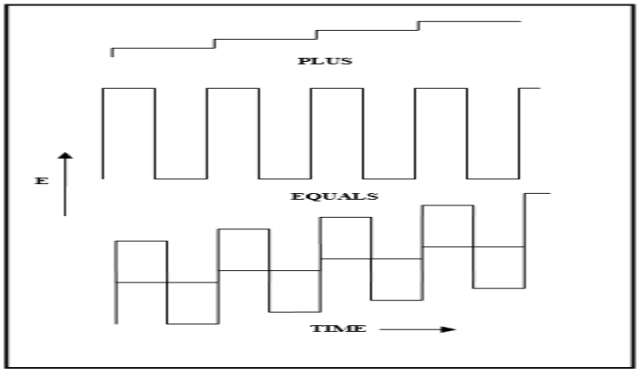


Figure 7: Applied excitation in square wave voltammetry.

2.4. Three electrodes systems

In this work, the three electrodes are used to determine OAP based on PBCP/GCE.

2.4.1. Working electrode (WE)

WE form an ideal phase interface for electron transfer reaction. They have a large potential range suitable for both anodic and cathodic reactions. WE should possess long-term stability and an easily reproducible surface for their excellent analytical application. At the working electrode redox process occurs. Many kinds of materials can be used as WEs. Generally, they are often made of unreactive metals or carbon and broadly classified as solid type and liquid type. Carbon based (glassy carbon, carbon paste, graphite, graphene, etc.) and metal-based (chromium, silver, gold, nickel, copper, palladium, titanium, platinum and stainless steel) electrodes are included in the category of solid electrodes. Example for liquid electrodes is dropping mercury electrode, hanging mercury drop electrode, static mercury drop electrode etc. Glassy carbon, platinum and gold electrodes with chemical modifiers are chosen as the working

electrodes for the determination of various pharmaceuticals analyte. The electrochemical techniques and fabrication of working electrodes have many advantages such as low priced instruments, simple operation, fast response, time saving, good sensitivity, and good selectivity towards the determination of different analyte.

2.4.1.1. Glassy Carbon Electrode (GCE)

GCE possess the properties of both glassy and ceramic materials combined with graphite [37]. Their specific surface orientation; 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 renders them suitable as an electrode material for sensor fabrication. The advantage of GCE over other electrodes is that they can operate in positive as well as negative potentials. Glassy carbon is widely used as an electrode material in electrochemistry [38, 39].

Figure 8: Glassy Carbon working electrode

2.4.2. Reference electrodes,

The reference electrode is an electrode, which has a stable and well-known electrode potential. They should have a well–defined, stable equilibrium electrode potential unaffected by the electrolyte species. Most common primary reference electrode is Standard Hydrogen Electrode (SHE). However, due to the difficulty in constructing and maintaining SHE a secondary reference electrode (Ag/AgCl, Calomel electrode (Hg/Hg_2Cl_2)) is often preferred in voltammetric measurements. Ag/AgCl electrode is composed of Ag wire immersed in a solution saturated with AgCl and KCl.

2.4.2.1. standard Calomel reference electrodes

A calomel reference electrode is mercury/mercury chloride electrode based on the following redox couple between Hg_2Cl_2 and Hg

$$Hg_2Cl_2(s) + 2e^- \rightleftharpoons 2Hg(l) + 2Cl^-(aq)$$

For which the potential is $E = E^o_{Hg2Cl2/Hg} - \frac{0.0592}{2}\log (Q_{Cl-})^2 = 0.02682V - \frac{0.0592}{2}\log (Q_{Cl-})^2$.

The potential of a calomel electrode, therefore, depends on the activity of Cl^- in equilibrium with Hg and Hg_2Cl_2.

As shown in fig. 9, in saturated calomel electrode (SCE) the concentration of Cl^- is determined by the solubility of KCl. The electrode consists of an inner tube packed with a paste of Hg, Hg_2Cl_2, and KCl, situated within a second tube that contains a saturated solution of KCl. A small hole connects the two tubes and a porous wick serves as a salt bridge to the solution in which the SCE is immersed. A stopper in the outer tube provides an opening for adding addition of saturated KCl. The concentration of Cl^- is fixed by the solubility of KCl, the potential of an SCE remains constant even if the lose some of the inner solution to evaporation. A significant disadvantage of the SCE is that the solubility of KCl is sensitive to a change in temperature. At higher temperatures, the solubility of KCl increases and the electrode potential decreases. For example, the potential of the SCE is +0.244 V at 25 ^oC and +0.237 V at 35 ^oC

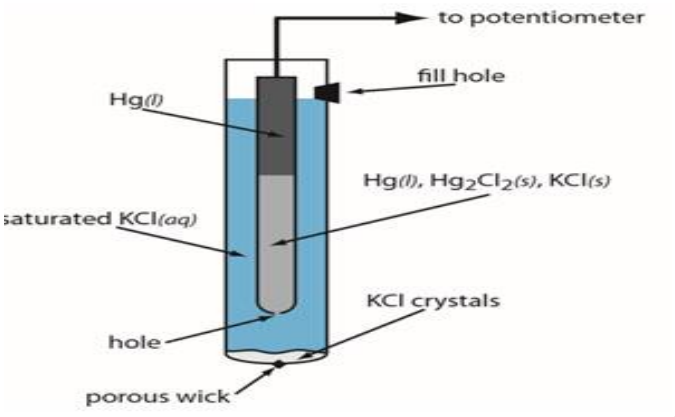


Figure 9: Schematic diagrams showing the saturated calomel electrode

2.4.2.2. Silver/silver chloride reference electrodes

Another common reference electrode is the silver/silver chloride electrode, which is based on the reduction of AgCl to Ag.

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

Like that of calomel electrode, the activity of Cl^- determines the potential of the Ag/AgCl electrode; thus

$E = Eo_{AgCl/Ag} - 00592 \log Q_{Cl^-} = 02223V - 00592\ Log\ (Q_{Cl^-})$

Typical Ag/AgCl electrode is shown in Fig. 10 and consist of a silver wire, the end of which are coated with a thin film of AgCl, immersed in a solution that contains the desired concentration of KCl. A porous plug serves as the salt bridge.

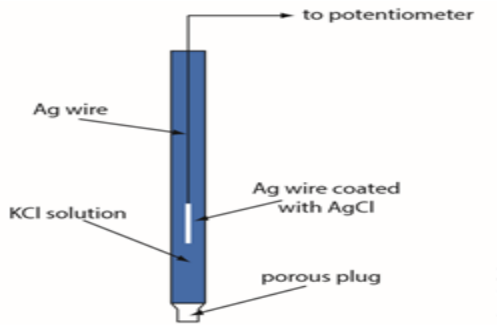


Figure 10: Schematic diagrams showing a Ag/AgCl electrode.

2.4.3. The counter electrode

In potentiometry two electrode cells that have only a working electrode and a reference electrode were used, current is necessarily forced to flow through the reference electrode whenever a measurement is made. If enough current flow through a reference electrode, its internal chemical compositions may be significantly altered, causing a potential to drift away from the expected standard value. For this problem, it is advisable to make electrochemical measurement without current flowing through the reference electrode. Modern three-electrode potentiostats use a feedback circuit to prevent this from happening but this feedback circuit requires that an additional auxiliary (counter) electrode be introduced into the electrochemical cell.

Counter (auxiliary) electrode permits the passage of current in an electrochemical reaction through it without disturbing the potential of the reference electrode. Platinum electrode, fig. 11 is often the best choice in this regard due to its inertness and speed with which most electrode reactions occur at its surfac**e.** A wire that is simply serves to conduct electricity from the signal source through the solution to the other electrodes. This counter electrode provides an alternate route for the current to follow, so that only a very small current flows through the reference electrode [40]

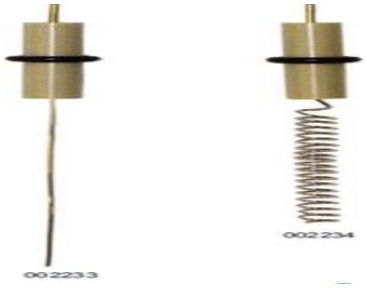


Figure 11: Schematic diagrams showing of counter electrode

2.5. Objective of the study

2.5.1. General objectives of the study

1. To develop an electrochemical sensor based on polybromocresol purple for the determination of o-aminophenol

2.5.2. Specific objectives

1. To prepare and characterize PBCP/GCE for the determination of o-aminophenol.
2. To examine the polymerization of BCP/GC electrode for determination of o-aminophenol
3. To describe the conductivity and the electrocatalytic activity of PBCP using cyclic voltammetry and differential pulse voltammetry for the determination of OAP
4. To establish the optimum working parameters for the sensitive determination of OAP i.e. to study the basic electro analytical parameters required to determine OAP at the specified condition

3. Experiment

3.1. Reagent and chemicals

The chemicals used,o-aminophenol is from Reidle-Dehein, Bromocresol purple (BCP), CH_3COOH and CH_3COONa are from ALDRICH, Na_2HPO_4 and NaH_2PO_4 are from North Ampton, hydroquinone, catechol, ascorbic acid, p-aminophenol, KCl, HCl, NaOH are from Lambert chemical, India, alumina powder are from USA. All reagents were used as received without any further purification.

3.2. Instruments used

Voltammetric measurements are conducted in a three-electrode system, which offers the advantage of maintaining a stable reference potential. The three electrodes are working electrode (GCE and PBCP/GCE), Ag/AgCl reference electrode in (3 M KCl) and platinum auxiliary/counter electrode. Electrode cleaning was carried out in an ultrasonicator (ultrasonic power 80W, Model 1-35130-B), the voltammetry experiments were performed using BAS CV-100A voltammetric analyzer and Optiplex GX280 desktop computer. Fig.12: shows typical BAS CV-100A from USA

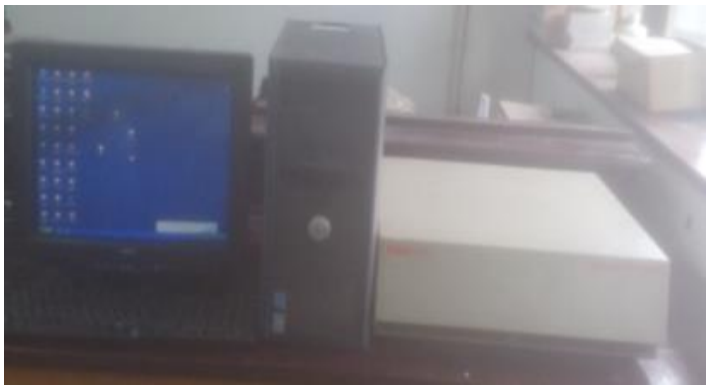


Figure 12: BAS CV-100A Voltammetric analyzer coupled with personal computer

3.3. Three electrode setup

The three-electrode cell setup is the most common electrochemical cell setup used in electrochemistry. Fig. 13 indicates that the current flows between the CE and the WE. The potential difference is measured between the WE and the RE.

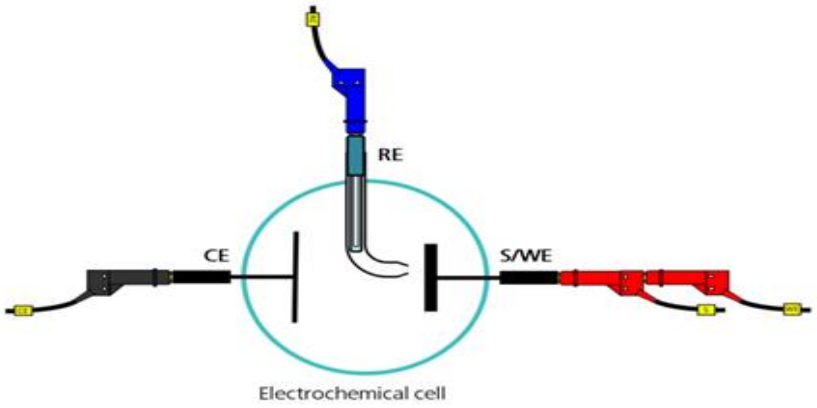


Figure 13: Schematic diagram of three-electrode setup of electrochemical cell

3.4. Preparation of buffer solution

3.4.1. Preparation of Acetate buffer

Supporting electrolyte of acetate buffer (CH_3COOH-CH_3COONa) in pH 5 was prepared from 0.1 M CH_3COOH and 0.1 M CH_3COONa in distilled water. The pH of the solution was adjusted by adding drops of 0.1 M NaOH and measured using a pH-meter

3.4.2. Preparation of Phosphate buffer

By calculating their concentration, 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 were prepared separately indistlled water. By using ion meter the required pH of buffer was measured and pH 6.0 was prepared by mixing the two solution.

3.5. Preparation of ortho-aminophenol

The stock solution of 5 mM OAP was prepared by dissolving 0.055 g of OAP in 100 mL distilled water. By suitably diluting the stock solution using PBS as supporting electrolyte,

solutions of various concentration of the analyte were prepared. This experiment was carried out at room temperature

3.6. Preparation of bromocresol purple

First 0.1 M of phosphate buffer solution (PBS) at pH 6 prepared from 0.1M KH_2PO_4 and 0.1 M K_2HPO_4. Then 0.05 mM bromocresolpurple was prepared by using 0.1 MPBS as a supporting electrolyte.

3.7. Electrochemichal coating of GCE

GC electrode was pretreated before electro polymerization with BCP. The bare GCE was polished carefully with 0.3 and 0.05 µM Al_2O_3 powders to a mirror finish. The electrode surfaces were polished and then cleaned ultrasonically with distilled water and ethanol alcohol for 5 min, and dried at room temperature.

4. Results and discussion

4.1. Electrochemical Modification of GCE

As Figure 14 shows that GCE was electrochemically coated in a0.1 M PBS solution (at pH 6.0) by scanning with cyclic voltammetry from -1.5 V to 1.6 V at scan rate 100 mV/s for 15 cycles (which was the optimum). The cyclic voltammagram obtained shows a single cathodic current at apotential -0.0106 V with current peak -0.17 mA during the first scanning cycle and the peak current decrease in the following scanning cycle. In addition, the positive sweep two peaks are well defined. The first peak was observed at 0.549 V with a current of 0.28 mA and the other peak was at potential 1.029 V with current 0. 38 mA. That indicated the π-π the transition of electronic hopping. Fig. 15 illustrated that the polymerization BCP/GCE at pH with scan rate 100 mV/s for 15 cycles.

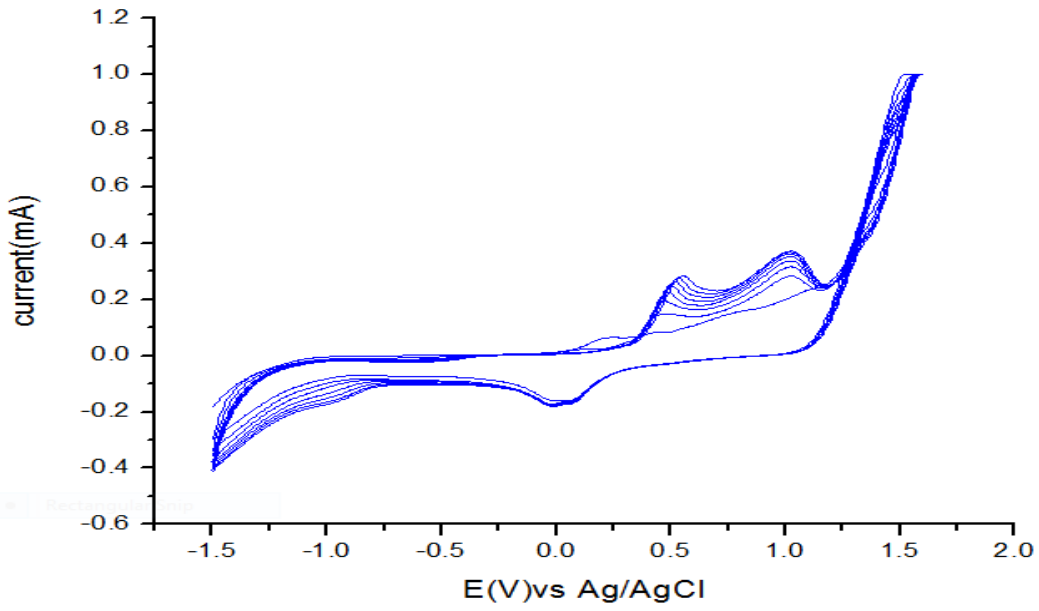


Figure 14: Cyclic Voltammogram of BCP on GCE in the electropolymerization process.

In this experiment, the basics of CV illustrated using a one-electron reduction of ferricyanide to ferrocyanide. This redox couple exhibits nearly a reversible electrode reaction without any complications of proceeding or post chemical reactions. Experiments gave strong evidence that

ferricyanide/ferrocyanide couple has been a popular choice through the years to use as a standard to demonstrate CV and the following redox couple is investigated.

During a sufficiently negative potential scan, the reduction of ferricyanide (as shown below) takes place and this reduction reaction results in a cathodic current. At the same time, oxidation of ferrous cyanide will occur in the reverse direction as the potential is scanned towards a more and more positive potential. The redox reaction is summarized as follow

$$[Fe(CN)_6]^{3-} + e^- \rightleftharpoons [Fe(CN)_6]^{4-}$$

To study the effect of electro-active surface area of both GCE and PBCP/ GC electrodes the electrochemical behavior of potassium ferrocyanide ($K_3Fe(CN)_6$) in 0.1 M KCl supporting electrolyte was studied using CV, and recorded at different scan rates with bare and coated GCE. It was found that the peak current increased, as the scan rate was increased.

Fig. 15 indicated that the oxidation peak current of potassium ferrocyanide at PBCP/ GCE increase as compared with bare GCE.

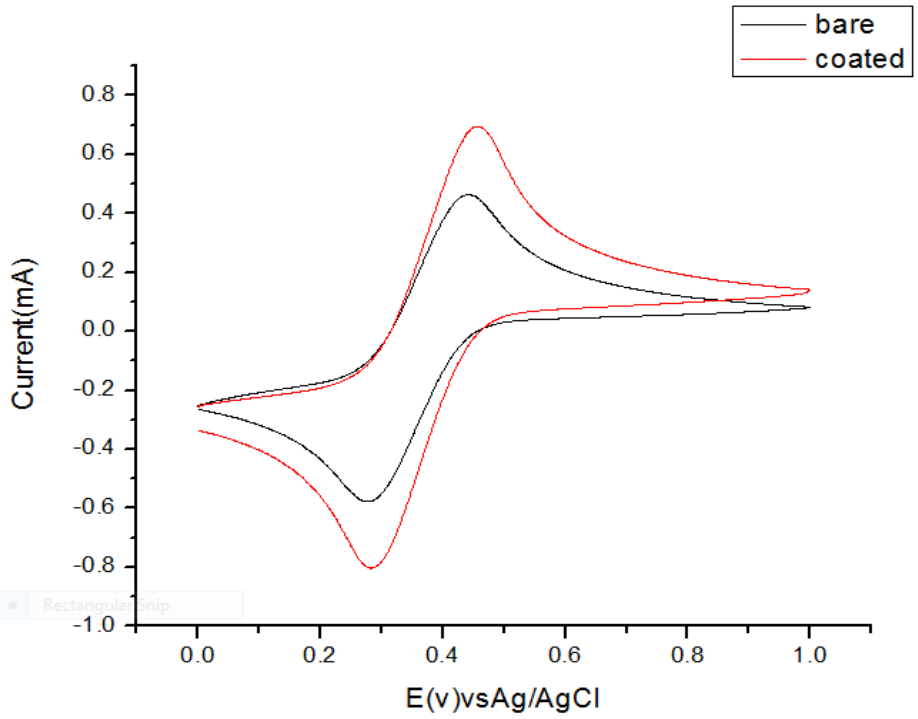


Figure 15**:** Cyclic voltammogram of 0.1 M sodium ferrocyanide in 0.1 M KCl at bare GCE and PBCP/GCE at scan rate 100 mV/s.

The area of the electrode was calculated using Randles-Sevcik equation [33]

$$Ip = (2.69*10^5) *A*D^{1/2}*n^{3/2}*v^{1/2}*C$$

Where Ip is peak current (A), n is electron stoichiometry, which is equal to one. A is electrode area (cm^2), D is diffusion coefficient (cm^2/s), C is concentration (0.1 mol/cm^3), and v is scan rate (V/s)]. From Randles- Sevcik equation, the surface area of the GCE was calculated to be 0.039 cm^2. In the same condition, the area of PBCP/GCE electrode was calculated to be 0.05 cm^2. This illustrated that the effective surface area of the modified electrode increased. Accordingly, Ip increases with $v^{1/2}$ and is directly proportional to concentration.

4.2. Electrochemical Behaviors of o-aminophenol at the Modified Electrode

Cyclic voltammetry was used to investigate the electrochemical behavior of o-aminophenol on GCE and PBCP/GCE, at pH 5.0 ABS containing 100 µM OAP measured at a scan rate of 100 mVs^{-1}. The polymer film was electrochemically deposited from 0.05 mM bromocresol purple (BCP) monomer in 0.1 M phosphate buffer solution at pH 6.0. For the bare GCE, oxidation peak was observed at +0.414 V and with a peak current 4.65 µA (Fig.16a). At PBCP/GCE, the oxidation peak current of the OAP increased with a negatively shifted peak potential to +0.283 V and at peak current 6.19 µA (Fig.16b). The over-potential of o-aminophenol oxidation at PBCP/GCE becomes lower than that of bare GCE with a shift of +0.131 V, and the redox peak currents are higher than that at the bare GCE, which indicated that the electrochemical oxidation of OAP was substantially improved.

There were two reasons for this improvement.

1. The PBCP possessed a large real surface area, π-π conjugated bond, abundant active sites, and high conductivity. This led to a similar conjugation between OAP and the PBCP/GCE interface.
2. The PBCP/GCE film contained many negatively charged functional groups e.g. ($S0_3^-$ and electron- rich oxygne atom) capable of interacting with OAP [28].

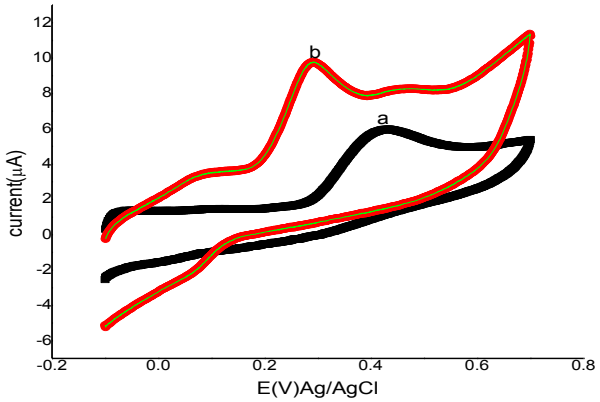


Figure 16: Cyclic voltammogram of 100 µM OAP in 0.1 M ABS (pH 5.0) (a) bare GCE. (b) PBCP/GCE at scan rate 100 m V/s.

4.3. Effect of solution pH

Cyclic voltammetry was used to examined the electrochemical behavior of PBCP/GCE towards the determination of OAP at various pH values with ABS. The effect of the solution pH on the electrochemical behaviors of OAP was investigated in the pH range of 3.5-7 by CV. The corresponding results are shown in Fig. 17.

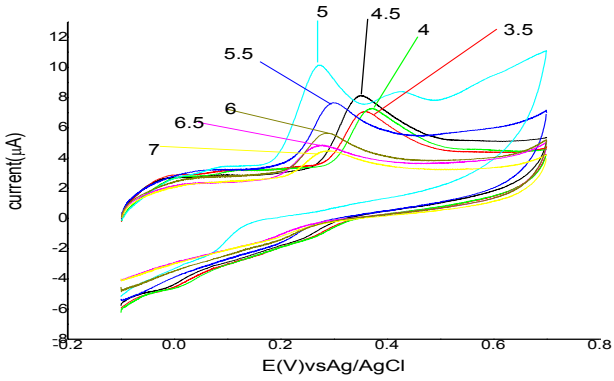


Figure 17: Effect of pH on cyclic voltammetry response of the modified at PBCP/GCE electrode of 100 µM OAP in 0.1 M acetate buffer.

Fig. 18 indicates that, the response of anodic peak current of OAP increases with the increase of the pH value from pH 3.5 – 5. The maximum response observed was at pH 5.0. While it

decreased sharply above pH 5. Therefore, the pH value of 5.0, at which the peak current reach the maximum, was chosen to determine OAP.

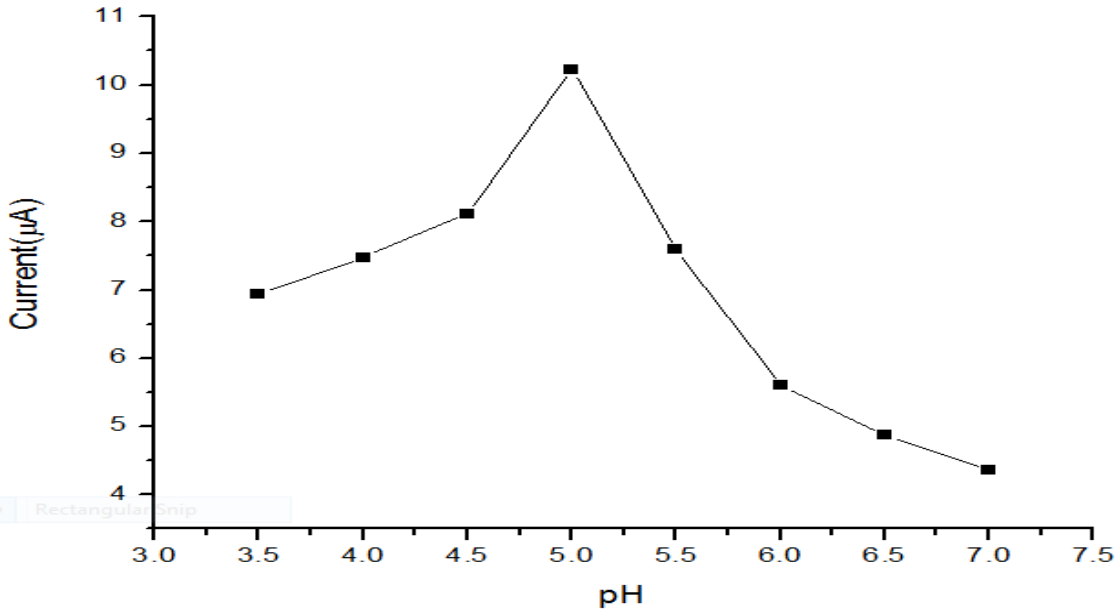


Figure 18: Plot of cyclic voltammetry anodic peak current of 100 µM OAP in 0.1 M acetate buffer as a function of pH.

4.4. The influence of scan rate

The influence of scan rate on the electrochemical behavior of 100 µM OAP at PBCPs/GCE in 0.1 M ABS (pH 5.0) were investigated. Fig. 19 shows the oxidative peak current of OAP in the range of 0.025 to 0.500 V/s. The peak current of OAP increased gradually with an increase in scan rate.

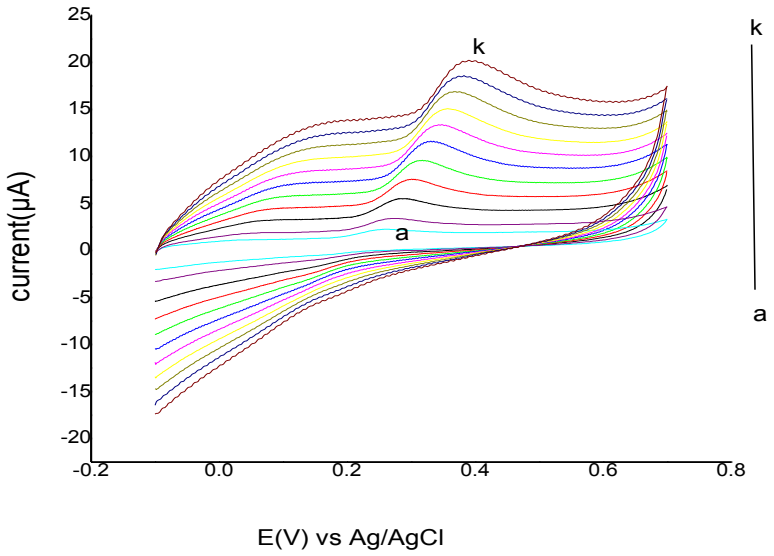


Figure 19: Cyclic voltammogram recorded at PBCPs /GCE for 100 µM OAP in 0.1 M ABS (pH 5.0) at different scan rates: 0.025- 0.500 V/s ,

Fig. 20 shows that oxidation peak current of OAP exhibited a linear relationship to the square root of scan rate range from 0.025 to 0.500 V/s. The linear regression equation of the Ipa for the scan rates are expressed as Ipa (µA) = -0.604 + 0.278 ($v^{1/2}$), R^2= 0.996. The relationship between the oxidation peak current and square root of the scan rate indicated that the oxidation of OAP at the PBCP modified electrode is a diffusion-controlled process [2]

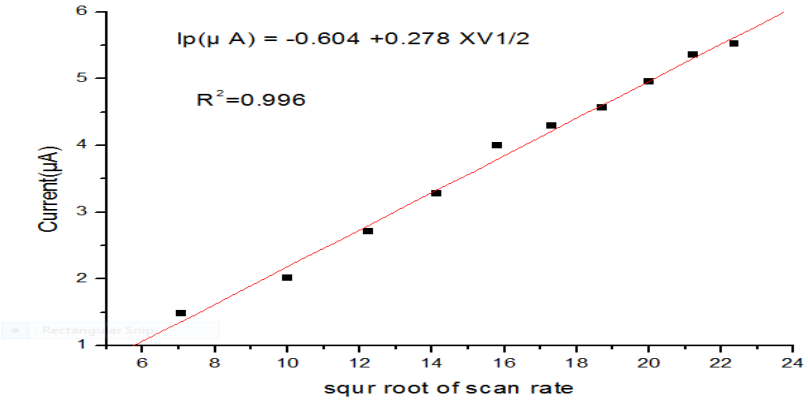


Figure 20: The dependence of the peak current on square root of scan rate

When the scan rate was varied from 0.025 – 0.500 mV/s for 100µM OAP solution it was found that on increasing the scan rate, the anodic peak current increased (Fig. 21). The anodic peak potential shifted to more positive potential values when increasing with potential. This illustrated that the electro-oxidation process of OAP is irreversible [4,5].

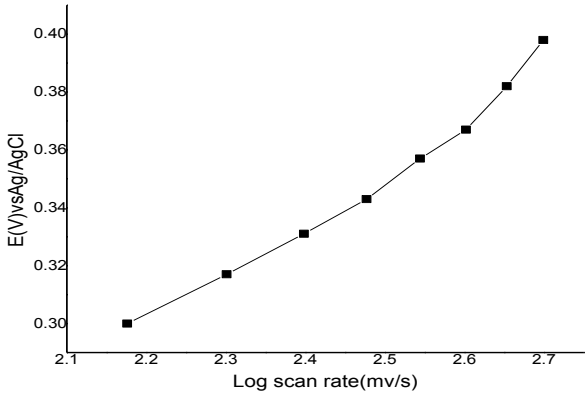


Figure 21: The dependence of the peak potential Ep on log **v**

4.5. Differential Pulse Voltammetric Optimization

Differential pulse voltammetry (DPV) was used to investigate the voltammetric behavior of PBCP/GCE for the determination of OAP. The oxidation peaks for OAP at PBCP/GCE modified electrode was used to evaluate analytical performance of PBCP modified GCE optimizing different parameters. These include pulse amplitude, pulse width, sample width, pulse period, increment and pH value. According to this study, a series of experiment was conducted to attain suitable experimental condition for determination of OAP. The effect of particular variable was studied under identified condition by keeping all variables constant except one under study. The optimum parameters are identified for the determination of the analyte.

Table 3. Optimum Experimental Conditions for the determination of OAP by DPV at PBCPs/GC modified electrode**.**

Parameters	Optimum value
Pulse amplitude	50 mv
Pulse width	50 ms
Sample width	17 ms
Pulse period	200 ms
Increment	350 mv

4.6. Linear Range and Detection Limit

Differential Pulse Voltammetry (DPV) has been employed as a technique for the evaluation of the performance of the PBCP/GCE electrode for the determination of OAP. The modified electrode displayed improved electrocatalytic activity towards o-aminophenol, a lower oxidation potential and a larger peak current when compared to a bare GCE at the optimized analytical conditions. The peak current was linearly related to the concentration of o-aminophenol with range 1–100 μM. Based on the optimum experimental conditions, the dependence of voltammetric signal on the concentration of OAP and the sensitivity of the method are described by differential pulse voltammetry for different concentration of OAP (Fig. 22).

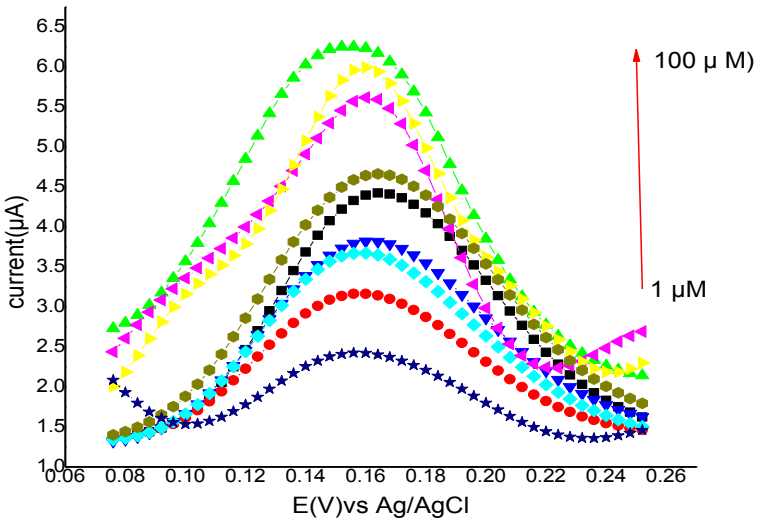


Figure 22: Differential pulse voltammogram at PBCPs/GC modified electrode for different concentration (1 -100 μM) of o-aminophenol. Pulse amplitude 50 mV, pulse width 17 ms, and pulse period 200 ms.

The peak height of OAP increased with increasing concentration. As Fig. 23 shows the calibration curve for 9 data points from 1 to 100 μM was found to be linear with R^2=0.985 with regression equation of Ip (μA)= 3.271+0.03306*C (μM). As it is indicated using the numerical value of regression coefficient for this experiment, the data sets showed a good linear fit because the value of R is close to one. The detection limit of OAP was found to be 0.21 μM. Fig. 23 shows a calibration curve for differential pulse voltammogram at PBCPs/GC electrode for the OAP concentration range 1-100 μM.

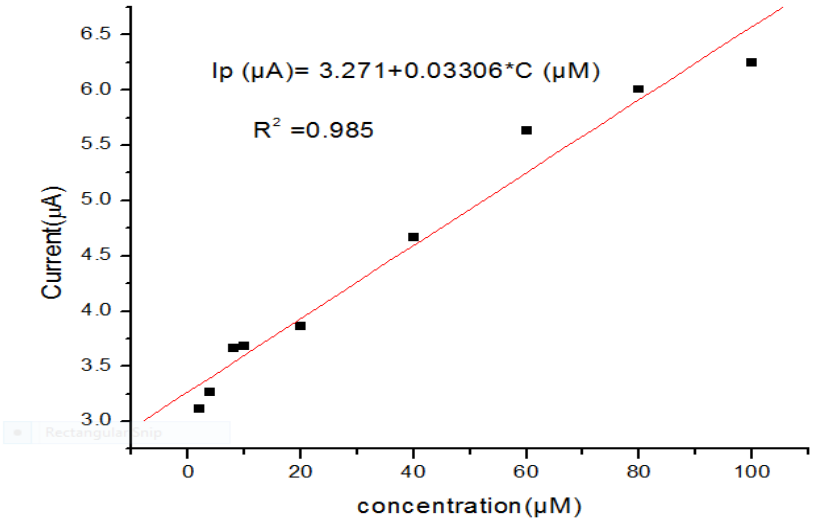


Figure 23: Plot of Differential pulse voltammetry anodic peak current as a function of OAP concentration from 1 to 100 μM

The analytical performance of the PBCPs/GCE electrode was compared with that of different electrodes recently published and the results are summarized in table 4. It can be noted from the table that the PBCPs//GCE has comparable linear response range and better limit of detection.

Table 4. Comparison of characteristics of different electrode obtained from some literatures and this work

Biosensor or modified Electrode	Linearity range (μM)	LOD (μM)	Ref
P-β-CD-L-Arg/CNTs/GNRs/GCE	0 .025.0 – 1.300	0.0062	[40]
CoTSAPc-MWCNT-GCE	0.1– 30	0.03	[2]
PBCP/GCE	1-100	0.21	This work

4.7. Interference study

The effect of the foreign species on the determination of 100 µM OAP at PBCP was examined by DPV. At constant concentration of OAP the same, two times, five times, and ten times of these compounds were spiked and recorded using DPV. Catechol, ascorbic acid, p-aminophenol and hydroquinone were selected for the study of selectivity of the proposed method. All these compounds interfere with o-aminophenol analysis at the poly BCP/GCE sensor. The addition of common ions such as Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Cl^{-}, NO_3^{-} and SO_4^{2-} has no effect on the responses OAP.

The effects of catechol in the electrochemical determination of OAP at PBCP/GCE were examined. Fig. 24 shows the effect of different concentrations of catechol on the peak height of 100 µM OAP. The result shows the addition of the same, two, five and ten fold of catechol increased the peak current OAP. However, it was observed that there is an increase in the peak current of OAP with negative shift of potential as the concentration of catechol was increased [4,5].

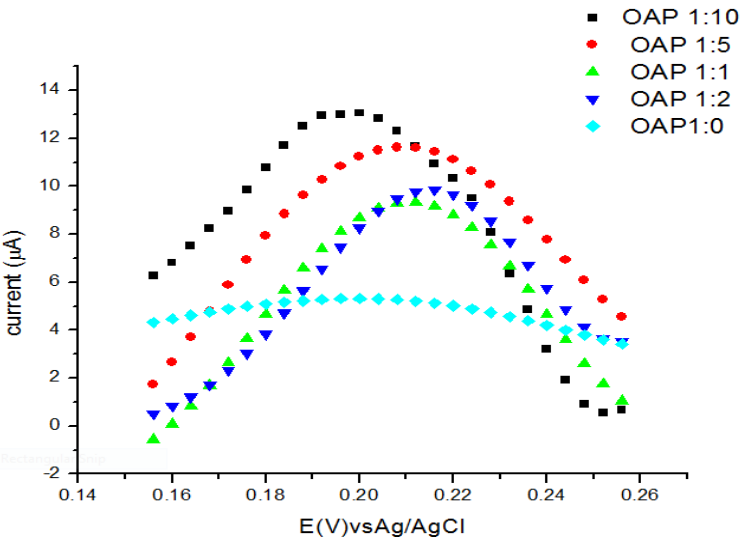


Figure 24: Differential pulse voltammogram of 100 µM OAP at PBCP/GCE in the presence of catechol: mixed ratio: 1:1, 1:2, 1:5, and 1:10 in 0.1 M ABS pH 5

The effect of ascorbic acid in the electrochemical determination of 100 µM of OAP in 0.1 M ABS at PBCPs/GCE was investigated. The result showed with the addition of the same, two, five and ten fold of ascorbic acid the peak current OAP increased as indicate in Fig. 25.

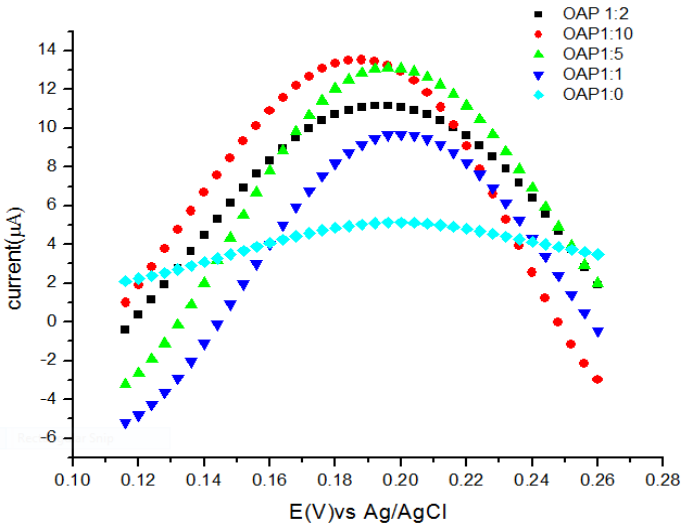


Figure 25: Differential pulse voltammogram of 100 µM OAP at PBCP/GCE in the presence of ascorbic acid mix ratio: 1:1, 1:2, 1:5, and 1:10 in 0.1 M ABS pH 5

The effects of hydroquinone in the electrochemical determination of 100 µM OAP in 0.1 M ABS at PBCPs/GCE also studied. The result of the addition of hydroquinone on the voltammetric current response OAP at PBCP/GCE is shown in Fig. 26. It was observed that there is an increase in the peak current of OAP as the concentrations of hydroquinone increased. However, a positive shift in potential was observed as the concentration of hydroquinone increased.

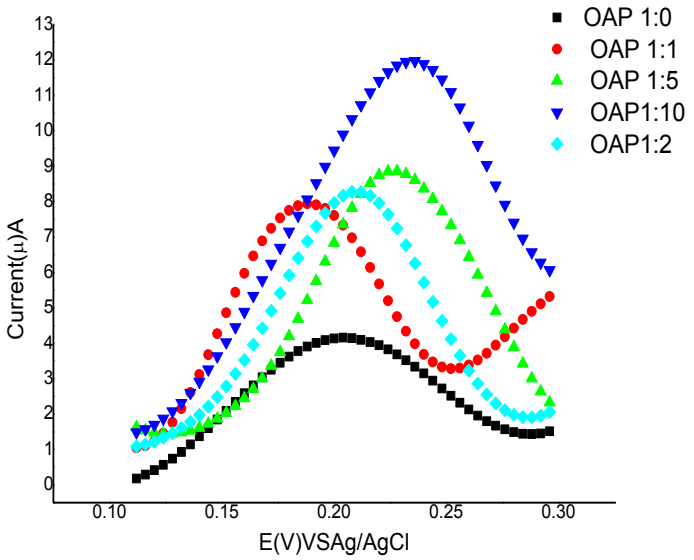


Figure 26: Differential pulse voltammogram of 100 µM OAP at PBCP/GCE in the presence of hydroquinone mix ratio: 1:1, 1:2, 1:5, and 1:10 in 0.1 M ABS pH 5.

The effects of p-aminophenol in the electrochemical determination of OAP at BCPs/GCE were also examined. Fig. 27 shows the effect of different concentrations of p- aminophenol on the peak height of 100 µM OAP. The result shows with the addition of the same, two, five and ten fold of p-aminophenol was increased the peak current of OAP

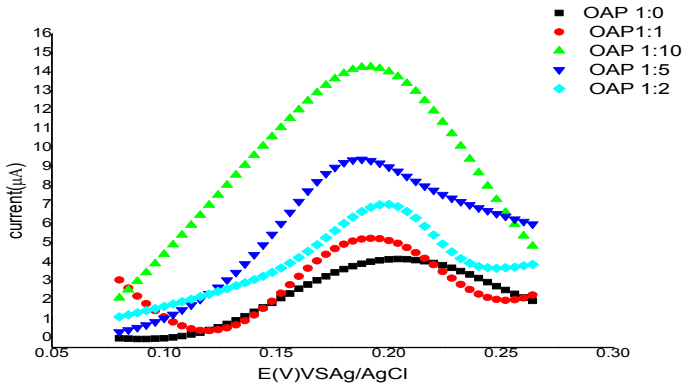


Figure 27: Differential pulse voltammogram of 100 µM OAP at PBCP/GCE in the presence p-aminophenol mix ratio: 1:1, 1:2, 1:5, and 1:10 in 0.1 M ABS pH 5.

In general 1:1, 1:2, 1:5 and 1:10 mix ratio of catechol, ascorbic acid, hydroquinone and para aminophenol increased the response peak current in the determination of OAP using DPV. This is so, because all of them possess the same active site of -OH group.

4.8. Determination of o-aminophenol in real water samples

To further explore practical applications of the obtained electrode, the BCP/GCE was used to detect OAP in real water samples, the concentrations of OAP in each sample were determined by the standard addition method to evaluate the accuracy of this methed. From the stock solution of 100 µM OAP, 500, 1000, 1500, 2500, 3000 (µL) OAP were spiked in to tap water solution (6 ml water and 4 ml ABS) and differential pulse voltammogram 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 OAP in real water sample solutions. The results are summarized in table 5.

Table 5. Recovery Study

Sample added (µM)	OAP conc. added (µM)	OAP conc found (µM)	Recovery %
100	4.8	4.44	108
200	9.1	9.01	101
300	13.04	13.04	100
400	16.7	16.8	99
500	20	20.74	96
600	23.1	22.48	103

$$\% \text{ of Recuvery} = \frac{\text{OAP conc.added (µM)}}{\text{OAP conc found (µM)}} X100$$

5. Conclusion

The electrochemical determination of o-aminophenol was evaluated by voltammetric measurements using PBCP/GCE modified electrode. Glassy carbon electrode was modified with the conducting polymer bromocresol purple (BCP). The PBCP/GCE was electrochemically polymerized in a 0.1 M PBS at pH 6.0 by scanning cyclic voltammetry from -1.5 V to 1.6.0 V at scan rate 100 mV/s for 15 cycles. The BCP solution was prepared from 0.05 mM bromocresol purple (BCP) monomer in 0.1 M phosphate buffer solution (pH 6.0 PBS). The polymerization of bromocresol purple (BCP) at GC electrode showed excellent electrocatalytic property for the oxidation of OAP by significantly lowering the oxidation potential and accelerating the electron transfer rate of the oxidation reaction. Differential pulse voltammetric parameters and pH were optimized to study their effects on the peak current and the peak potential. The proposed electrode was found to be a suitable electrochemical device for o-aminophenol detection. The modified electrode has high sensitivity, good linear range and very low detection limit (0.21 µM) for response to o-aminophenol, and with acceptable recovery. For the future, a better-modified electrode is necessary to determine and characterize OAP in the presence of catechol, hydroquinone, ascorbic acid and p-aminophenol.

6. References

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