ADDIS ABABA UNIVERSITY
DEPARTEMENT OF CHEMISTRY

AN ELECTROCHEMICAL SENSOR FOR THE OXIDATIVE DETERMINATION OF 4-NITRO PHENOL AT POLY (BROMO CRESOLPURPLE) MODIFIED GLASSY CARBON ELECTRODE

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February 2020

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DEPARTMENT OF CHEMISTRY

This is to certify that the thesis prepared by Ananiya Abebe entitled: An Electrochemical sensor for the oxidative Determination of 4-Nitro phenol at BCP/GCE Modified Glassy Carbon Electrode. Submitted in 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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Abstract

A glassy carbon electrode was modified with bromocresol purple (BCP) and characterized for the electrochemical oxidation of 4-nitrophenol (4-NP) by cyclic voltammetry and differential pulse voltammetry. The oxidation peak current of 4-NP at the modified electrode was increased (compared to the bare GCE), thus indicating that the BCP exhibits a remarkable enhancement effect on the electrochemical oxidation of 4-NP. The effects of loading with BCP, pH value and scan rate were examined. The oxidation peak current of 4-NP is proportional to its concentration in the range of 4.0 µM to 100 µM, with a correlation coefficient of 0.984. The detection limit is 2.8 µM. The method is simple, selective and sensitive. It was successfully applied for the determination of 4-NP in water samples, with recoveries ranging from 100% to 101%.

Keywords: 4-nitrophenol, polybromocresol purple (BCP), oxidation, cyclic voltammetry (CV), differential pulse voltammetry (DPV), modified electrode, electropolymerization

List of Abbreviations

PBS	Phosphate Buffer Solution
BCP	Bromocresolpurple
CME	Chemical Modification of Electrode
CV	Cyclic Voltammetry
CMS	Chemically modified surface
DPV	Differential Pulse Voltammetry
E_{pa}	Anodic peak potential
E_{pc}	Cathodic peak potential
GCE	Glassy Carbon Electrode
I_{pa}	Anodic peak current
I_{pc}	Cathodic peak current
MWNT	Multi Wall Nanotubes
SWNT	Single wall nanotubes
CPs	Conducting polymers
E	Potential
ΔE	Change in potential
$E_{1/2}$	Half potential
SHE	Standard hydrogen potential
NP	Nitro Phenol
HAP	Hydroxy appatite
μM	Micro Molar
U.S.EPA	United states Environmental Protection Agency
WE	Working Electrode
CE	Counter Electrode
RE	Reference Electrode
CPE	Carbon Paste Electrode

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1. Introduction

4-Nitro phenol (4-NP) is considered to be hazardous waste by the U.S. Environmental Production Agency [1, 2] which has a high environmental impact due to its toxicity and persistence. Unfortunately, 4-NP is widely used as intermediates in the production of drugs such as pesticides and insecticides. Therefore, 4-NP will be inevitably released into environment to cause pollution in the process of fabrication and application. Based on the above description, it is important to develop simple and reliable method for determination of trace amounts of 4-NP in environments.

Up to now various techniques have been investigated to determine 4- NP such as spectro photometry [3], fluorescence [4], high performance liquid chromatography [5], capillary zone electrophoresis [7], fiberoptode [8] and electrochemical method [9-12]. Among them, electrochemical methods have received considerable attention because of the advantage of fast response, cheap instrument, low cost, simple operation, time saving, high sensitivity and selectivity, real time detection in situ condition. However, until now, many electrochemical techniques mainly focus on utilizing the reduction of nitryl in 4-NP, not the oxidation of hydroxide radical. Thus, the determination would be interfered by the oxygen molecule dissolved in solvent. In order to eliminate it, purging nitrogen for a certain time was employed. But this will make the determination more complicated and time consuming. Moreover, the reduction of nitryl is more complicated than the oxidation hydroxide radical. Therefore, electrochemical, determination of 4-NP using the oxidation signal should be an appropriate alternative. Nevertheless, the use of bare electrode has revealed the drawback of weak electrochemical response and low sensitivity. Consequently, chemically modified electrodes [CME_S] have been widely investigated with various modification materials especially nanophase materials, such as MWNT [9], SWNT [10], gold nanoparticles [11], TiO_2 [12], zeolite [13], sodium motmorllonite [14] and hydroxyappatite (HAP) [15].

There are several modified electrodes used in literature for determination of 4-NP. However BCP has not been reported for fabrication of 4-NP sensors.

The aim of this work is to develop a simple, reliable and sensirive electrochemical method for the determination of 4-NP based on unusual properties of BCP modified electrode. Here the electrochemical oxidation of 4-NP on the BCP modified glassy carbon electrode was reported. The modified electrode showed good electrochemical oxidation to 4-NP with an increase of the oxidation peak current. The electrochemical properties of the modified electrode and the responses towards 4-NP have been investigated by using cyclic voltammetry and differential pulse voltammetry in the concentration range of 1-300 µM. Based on the electrochemical oxidation response of 4-NP on the modified electrode a new differential pulse voltammetric technique was developed to determine 4-NP in water samples.

1.1. Objective

1.1.1. General objective

To modify and characterize BCP on glassy carbon electrode and use as a sensor for the voltammetric determination of 4-NP

1.1.2. Specific objectives

1. To examine the electrochemical behavior and electro activity of BCP.

2. To establish the optimum parameters for the sensitive determination of 4-NP

3. To apply the electrodes prepared for the determination of 4-NP in real sample

2. Literature review

2.1. 4-Nitro phenol (4-NP)

4-NP is a phenol in which the hydrogen that is para to the hydroxyl group has been replaced by a nitro group. 4-Nitrophenol, also called p-nitro phenol or 4-hydroxynitrobenzene, is a phenolic compound that has a nitro group at the opposite position of hydroxy group on the benzene ring.

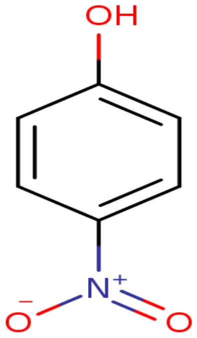


Figure 1 Structure of 4-nitro phenol

2.1.1. Physical and chemical properties of 4- nitro phenol (4-NP)

4-nitrophenol is a colorless to slightly yellow crystal with no odor but has a sweet and burning taste. It is moderately soluble in cold water and carbon disulfide, and soluble in alcohol, chloroform, acetone, pyrimidine, toluene, hot benzene, hot water, ether, fixed alkali hydroxide solutions, and carbonates. It sublimes and is slightly volatile with steam. 4-nitrophenol, if mixed with diethyl phosphite, may explode when heated. When it is heated to decomposition, it emits toxic fumes of nitrogen oxides. Contact may severely irritate skin and eyes by skin contact and ingestion.

2.1.2. Uses of 4-nitro phenol (NP)

4-NP is widely used in the production of pharmaceuticals, pesticides, dyestuffs and insecticides. In addition, 4-NP also be used as leather fungicide and acid-base indicator.

2.1.3. Health and environmental effects of 4-nitro Phenol

2.1.3.1. Health effects

Probable routes of human exposure to 4-nitrophenol are inhalation, ingestion, and dermal contact. Inhalation or ingestion of 4-nitrophenol may cause headache, drowsiness, nausea, and cyanosis. Contact with the eyes may cause irritation.

2.1.3.2. Environmental effects

Phenols-based aromatic nitro compounds, which are widely used in chemical and pharmaceutical industries, are considered anthropogenic toxicants, which can cause irreversible damage to organisms and plants even at very low concentrations. 4-nitro phenol (4-NP), which is predominantly formed through a reversible hydrolysis process during diethyl parathion production is on the United States Environmental Protection Agency's (U.S.EPA) [1, 2] priority pollutant list mainly due to its low degradability and high solubility in most aquatic environments. It is also recognized as a hazardous food chain pollutant if it is found in agricultural crops, vegetables, fruits or water sources when used as an ingredient in fertilizers or pesticides.

2.1.4. Synthesis of 4-nitro phenol

P-nitro phenol is synthesized using p-nitro chloro benzene and sodium hydroxide with phase transfer catalyst. Several reaction factors, such as catalyst type, the amounts of catalysts and sodium hydroxide, the concentration of sodium hydroxide solution as well as reaction time, affect greatly the yield of p-nitro phenol.

The optimum reaction conditions are as follows: A-1 used as phase transfer catalyst whose consumption is 6% (mole fraction) of p-nitro chloro benzene; the molar ratio of sodium hydroxide to p-nitro chloro benzene is 3:1; the concentration of sodium hydroxide solution is 50% (mass fraction). Nitrobenzene, whose molar amount is 2.45 times as much as that of p-nitro chloro benzene; reaction time is 7 h at 140 °C under normal pressure. The final yield of p-nitro phenol is 83.6%.

2.2. Electrochemical sensors

Electrochemical sensors are used in physical and analytical chemistry, material science, biochemistry, solid-state physics, device fabrication, electrical engineering, even statistical-analysis. A typical chemical sensor is a device that transforms chemical information in selective and reversible ways ranging from the concentration of specific sample component to composition analysis into an analytically useful signal. This work focus on an electro chemical sensors from analytical perspective. A huge research effort has taken place over years to achieve electrochemical sensors with attractive qualities including rapid response, low cost, miniaturisable, superior sensitivity and selectivity, appropriate detection limits. Approximately 2000 peer –reviewed papers concerning electrochemical sensors were published in 2011 showing the considerable research effort underway in this field [16, 17].

The development of all voltammetric techniques has been based predominantly on the use of mercury, carbonaceous materials and noble metals as working electrodes. However, toxicity of mercury, and its limited range of potentials for mercury anodic reactions cause inconvenience in working with liquid mercury electrode, carbon based working electrode materials include all allotropic forms of carbons graphite, glassy carbon, amorphous carbon. fullerenes, and nano tubes are all used as important electrode materials in electro analytical chemistry [16,17].

Two branches of electrochemical sensors are developing:

I .Sensors with increased specificity, and

ii. Sensors capable of simultaneous/ multiple determinations [16].

The analytical and physical properties that must be considered when developing and commercializing chemical and biological sensors include (but are not limited to): cost, miniaturization, sensitivity, sensor reproducibility, selectivity/specificity, multi-analyte detection, and stability [16]

2.2.1. Chemically modified electrodes

Chemically modified electrodes are different from other types of sensors as they are a molecular monolayers or micrometer- thick layers or films made from a certain chemical depending on the function of the electrode. The film is coated on the surface of the electrode (here in on a glassy carbon electrode). The outcome would be a modified electrode with special new properties in terms of physical, chemical, electrochemical, optical, electrical (electron transport) and other useful properties [18], chemically modified electrodes depend on electron transport that is a general term for electrochemical process where the charge transports through the chemical film to the electrode. The term coverage is used to express the area-normalized in mol/m^2 of a specific type of chemical site in the thin chemical film on the surface of chemically modified electrode [17, 19]

2.2.2. Purpose of developing chemically modified electrodes

Advancement in the field of electrochemical sensors kept getting more through until chemists in this field found no use of bare surface to continue their investigations. The reason behind that is researchers that involved electrodes required certain chemical and physical properties that did not naturally exist in the materials used as electrical conductors (e.g, bare glassy carbon electrode). To solve their problem, they used chemical modification to tailor the materials they used. Atoms, molecules, and nano particles are attached to the surface of materials and modify their electronic and structural properties, leading to change their functionality [19].

2.2.3. Applications of chemically modified electrodes

In the first stage, CMSs were merely applied in technologies they were initially made for tuning surface for electrochemical investigations. After that, CMSs provided powerful routes to tune the performance of electrodes. The modification of sensors facilitated the following processes in electro analytical chemistry: providing selectivity of electrodes, resist fouling concentrating species, improving electro catalytic properties, and limiting of interference in samples [17].

2.3. Conducting polymers (CPs)

In general, 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 and double bonds and the optical electro chemical, and electrical properties of the conducting polymers is due to their pi conjugated systems. It is known that the parameters that most affect physical properties of conducting polymers are their conjugation length, degree of crystallinity, and intra and inter chain interactions.

Conducting polymers 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. However the development of the properties of conducting polymers has not been completely commensurate with those of their metallic and inorganic semiconductor counter parts. Consequently, conducting polymers have been modified or hybridized with other heterogeneous material components to overcome their inherent limitations in terms of solubility, conductivity, and long term stability. Judicious coupling of conducting polymers with other materials can result in materials with attractive properties and new applications 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. A rich spectrum of conductivities has been achieved, ranging from insulating to metallic.

Successful preparations of CP composites with high stabilities, flexibilities and conductivities have proven that CPs can serve as key material components in light emitting diodes transistors, electro chromic devices actuators, electrochemical capacitors, photovoltaic cells and sensors [20]. 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.

2.4. Bromocresol purple

IUPAC name is 4-(1,1-Dioxido-3H-2,1-benzoxathiol-3,3-diyl)-bis(2-bromo-6-methyl phenol or it can be named as 5.5-Dibromo-o-cresolsulfonephthalein or bromocresol purple has a molecular formula of $C_{21}H_{16}Br_2O_5S$ and structural formula as shown in the Fig. 2 below

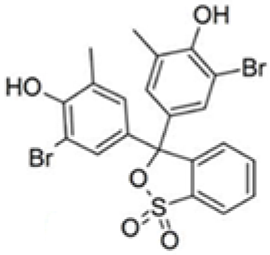


Figure 2: Molecular structure of bromocresol purple

Bromocresol purple (BCP), 5,5-dibromo-o-cresolsulfoneophthalein is a pH indicator [21]. It is also used as dye to measure albumin in medical laboratories. The mechanism for the color change in bromocresol purple bound to human serum albumin is a reversible redox reaction as shown in Fig.3 below [22]

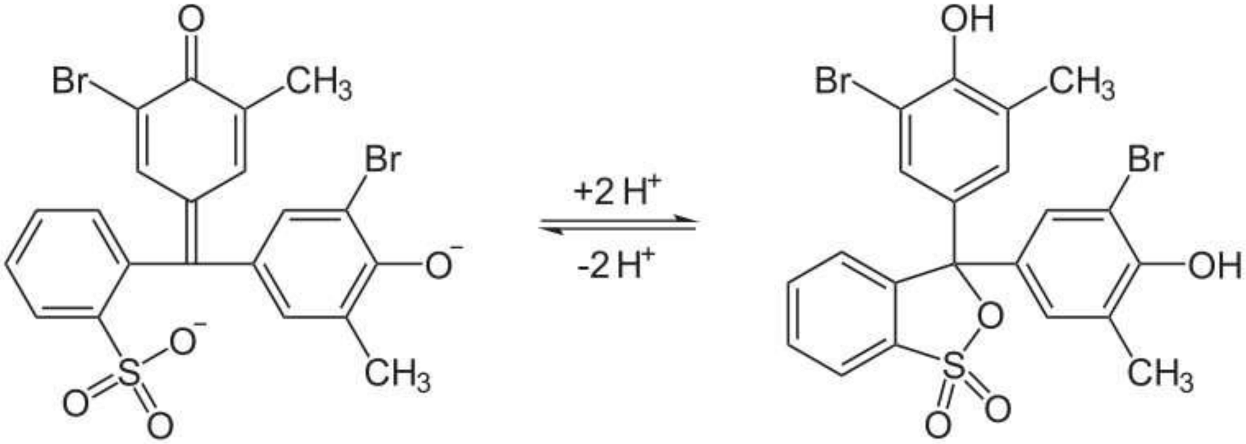


Figure 3 Reversible redox reaction of BCP

Its electro polymerization, at the electrode surface and its function as an electro catalyst, has been reported in some literature for some phenolic compounds [23]. A polymer film of BCP is used to modify a glassy carbon electrode (GCE) and described the electro chemical behaviors of 4-NP at the poly (BCP) modified carbon electrode. The poly (BCP) modified electrode possessed conjugated bonds, and a great deal of active sites and better conductivity, which led to the dissimilar conjugation effect of the purine derivatives with the electrode interface. Therefore, the electro chemical reversibility of the oxidation of 4-NP may be greatly improved in the presence of the poly BCP film by accelerating the rate of electron transfer, which indicated that the poly BCP film is first reduced by oxidizing 4-NP and then oxidized when 4-NP is reduced [22, 23].

2.5. Electro analytical technique

Electro analytical techniques encompasses a group of quantitative analytical methods that are based upon 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 [24].

2.5.1. Advantages of electrochemical techniques

Electro analytical 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, kinetic data. Electro analytical 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 electro chemical, spectrum of electro active species in solution, analogous to probing the energy states of a molecule with light via spectroscopy [24]

2.5.2. Voltammetry

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. The potential is varied in some systematic manner and the resulting current-potential plot is called a voltammogram. Although an electrochemical cell needs only two electrodes to operate, it is very important to introduce a third electrode for voltammetric techniques. One of these electrodes is a reference that is an electrode which has a known and fixed electrode potential. No current should ever pass this electrode as a current would change its potential, and possible damage this electrode. The reference electrode is used to control the potential of the working electrode by measuring the voltage between these two electrodes.

Voltammetry provides an electrochemical method, the promise of which is that current is linearly dependent upon the concentration of the electro active species (analyte) involved in a chemical or biological determination process(at a scanned or fixed potential). It can be used to analyze electro active species that is, 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. Conversely, as the potential becomes more positive, it becomes more strongly oxidizing. Therefore, the redox reaction taking place on the electrode can be controlled by controlling the electrode potential.

The current, on the other hand, 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 faradic. The current due to a reduction (cathodic current) is, by convention, assigned a positive sign. The current due to an oxidation (anodic current) is assigned a negative sign [25].

2.5.2.1. Cyclic voltammety (CV)

Cyclic voltammetry is the most widely used technique for acquiring qualitative information about electrochemical reactions. The power of cyclic voltammetry results from its ability to rapidly provide considerable information on the thermodynamics of 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 electrochemical study of a compound, a biological material, or an electrode surface. The effectiveness of CV results from its capability for rapidly observing the redox behavior over a wide potential range. The resulting voltammogram is an analogous to a conventional spectrum in that it conveys information as a function of an energy scan [26].

CV consists of cycling the potential of an electrode, which is immersed in an unstirred solution, and measuring the resulting current. The potential of this working electrode is controlled versus a reference electrode such as a silver/silver chloride (Ag/AgCl). The controlling potential which is applied across these two electrodes can be considered an excitation signal. The excitation signal for CV is a linear potential scan with a triangular wave form as shown in Fig.4.This triangular potential excitation signal sweeps the potential of the electrode between two values, sometimes called the switching potentials [27].The excitation signal in Fig.4 causes the potential first to scan negatively from +0.8 to -0.2 V versus Ag/AgCl at which point the scan direction is reversed, causing a positive scan back to the original potential of +0.8 V. A second cycle is indicated by the dashed line. Single or multiple cycles can be used.

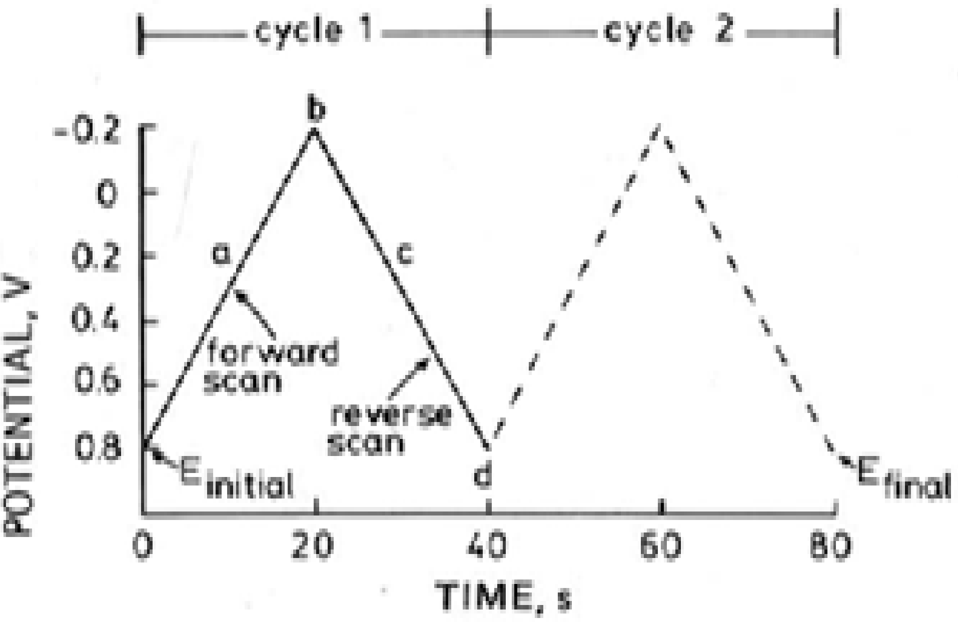


Figure 4 Typical excitation for cyclic voltammetry

A cyclic voltammogram is obtained by measuring the current at the working electrode during the potential scan. The current can be considered as the response signal to the potential excitation signal. The voltammogram is a display of current (vertical axis) versus potential (horizontal axis). Because the potential varies linearly with time, the horizontal axis can also be thought of as a time axis.

A typical cyclic voltammogram is shown in Fig.5 for a platinum working electrode in a solution containing 6.0 mM $K_3Fe(CN)_6$ as the electro active species in 1.0 M KNO_3 in water as the supporting electrolyte. The potential excitation signal used to obtain this voltammogram is that shown in Fig. 4 but with in a negative switching potential of -0.15 V. Thus, the vertical axis in Fig.4 is now the horizontal axis for Fig.5. The initial potential (E_i) of 0.8 V applied at (a) is chosen to avoid any electrolysis of $Fe(CN)_6^{3-}$ when the electrode is switched on. The potential is then scanned negatively, forward scan, as indicated by the arrow. When the potential is sufficiently negative to reduce $Fe^{III}(CN)_6^{3-}$, cathodic current is indicated at (b) due to the electrode process.

$$Fe^{III}(CN)_6^{3-} + e^- \rightarrow Fe^{II}(CN)_6^{4-} \text{------------------------------------} 1$$

The electrode is now sufficiently strong reductant to reduce $Fe^{III}(CN)_6^{3-}$. The cathodic current increases rapidly (b→d) until the concentration of $Fe^{III}(CN)_6^{3-}$ at the electrode surface is substantially diminished causing the current to peak (d). The current then decays (d→g) as the solution surrounding the electrode is depleted of $Fe^{III}(CN)_6^{3-}$ due to its electro catalytic conversion to $Fe^{II}(CN)_6^{4-}$. The scan direction is switched to positive at -0.15 V (f) for the reverse scan. The potential is still sufficiently negative to reduce $Fe^{III}(CN)_6^{3-}$, so cathodic current continues even though the potential is now scanning in the positive direction. When the electrode becomes a sufficiently strong oxidant, $Fe^{II}(CN)_6^{4-}$, which has been accumulating adjacent to the electrode, can now be oxidized by the electrode process.

$$Fe^{II}(CN)_6^{4-} \rightarrow Fe(CN)_6^{3-} + e \text{--------------------------------} 2$$

This causes anodic current (i→k). The anodic current rapidly increases until the surface concentration of $Fe^{II}(CN)_6^{4-}$ is diminished causing the current to peak (j). The current then decays (j→k) as the solution surrounding the electrode is depleted of $Fe^{II}(CN)_6^{4-}$. The first cycle is completed when the potential reaches +0.8 V. Simply stated, in the forward scan $Fe^{II}(CN)_6^{4-}$ is electrochemically generated from $Fe^{III}(CN)_6^{3-}$ as indicated by the cathodic current. In the reverse scan this Fe^{II} $(CN)_6^{4-}$ is oxidized back to Fe $(CN)_6^{3-}$as indicated by the cathodic current.

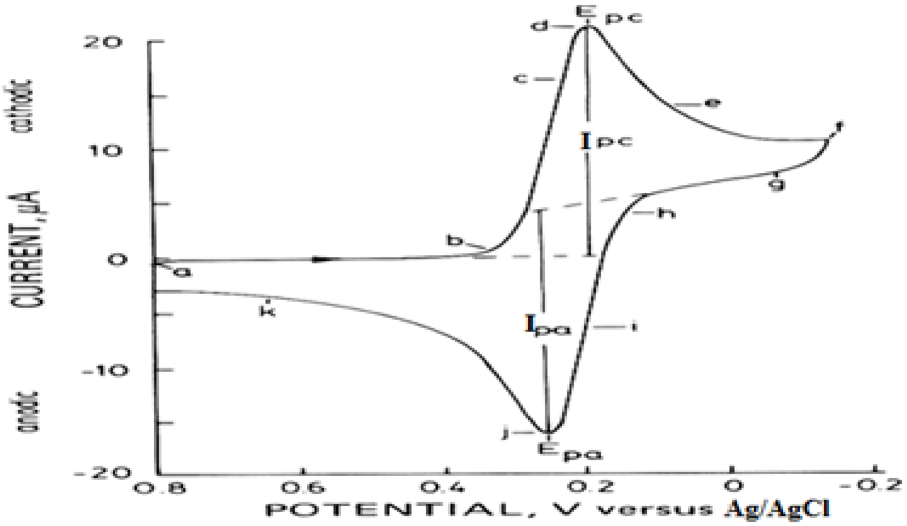


Figure 5 Cyclic voltammogram of 6 mM $K_3Fe(CN)_6$ in 1 M KNO_3

The important parameters of cyclic voltammogram are the magnitudes of the anodic peak current (Ipa) and cathodic peak current (Ipc), and the anodic peak potential (Epa) and cathodic peak potential (Epc). These parameters are labeled in Fig.5 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 easy, particularly for more complicated systems. A redox couple in which both species rapidly exchange electrons with the working electrode is termed an electrochemically reversible couple. The formal reduction potential (E^0) for a reversible couple is centered between Epa and Epc.

$$E^0 = \frac{Epa+Epc}{2} \text{ --------------------------------3}$$

The number of electrons transferred in the electrode reaction (n) for a reversible couple can be determined from the separation between the peak potentials.

$$\Delta E = Epa \_ Epc \sim \frac{0.059}{n} \text{ --------------------------------4}$$

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.

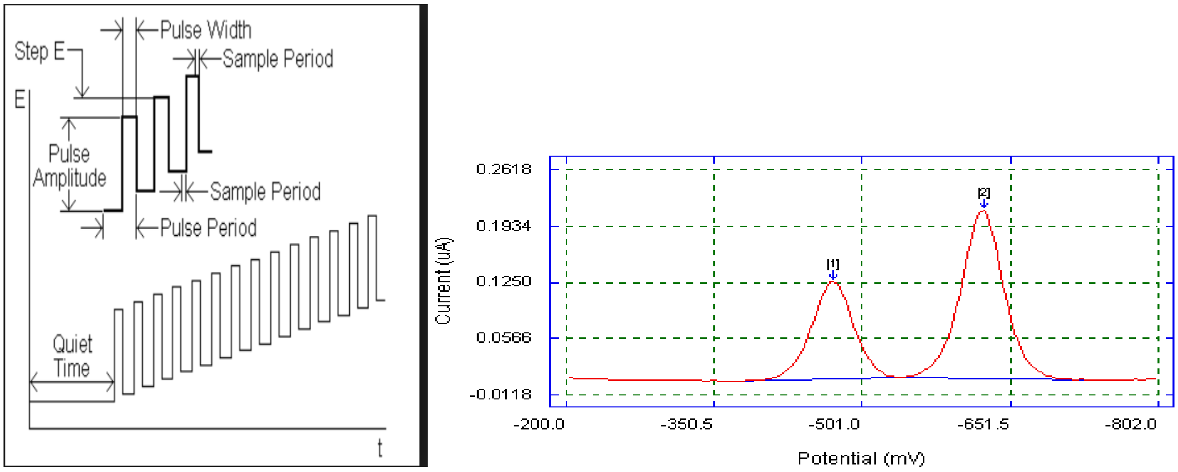
$$Ip = (2.69*10^{5})\, n^{3/2}A\, D^{1/2}C\, V^{1/2} \text{ --------------------------5}$$

Where Ip is peak current (A), n is electron, A is electrode surface area (cm^2), D is diffusion coefficient (cm^2/s), C is concentration ($mole/cm^3$), and V is scan rate (V/A). Accordingly,Ip increases with $V^{1/2}$ 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 Ipa and Ipc should be identical for a simple reversible (fast) couple [28]. That is

$$\frac{Ipa}{Ipc} = 1$$

2.5.2.2. Differential pulse voltammetry (DPV)

The 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 to100 mV), 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.6) [29].



a) b)

Figure 6 Differential pulse voltammetry a) an anodic scanning of the potential *vs* time: b) plot of voltammogram

2.6. Electrodes

The advent of modem electrochemistry created the need for new electrodes and electrode setups. The most common arrangement today is the electrochemical cell with three different electrodes:

a) Working electrode (WE)
b) Reference electrode (RE)
c) Counter electrode (CE)

2.6.1. Working electrode

Working electrode is an electrode in an electrochemical system on which the reaction of interest is occurring. Micro electrode whose potential is varied with time: a) most common is a small sphere, small disc or a short wire, but it could also be metal foil, a single crystal of metal or semiconductor or evaporated thin film, b) has to have useful working potential range, c) can be large or small usually < 0.25 cm^2, d) smooth with well defined geometry for even current and potential distribution. 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) [30].

2.6.1.1. Glassy carbon electrode (GCE)

Glassy carbon electrode (Fig.7) 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 [31]

Figure 7 Glassy carbon electrode

2.6.2. Reference electrode

Reference electrode is an electrode which has a stable and well known electrode potential. The role of the reference electrode is to: a) provide a fixed potential which does not vary during the experiment and b) maintain a constant potential if a few micro-amps are passed through its surface.

2.6.2.1. Silver-silver chloride reference electrode

Ag wire coated with AgCl(s), immersed in NaCl or KCl solution.

$$Ag^+ + e^- \leftrightarrow Ag(s)$$

Advantages: a) chemical processing industry has standardized on this electrode b) convenient c) rugged/durable.

Disadvantages: a) solubility of KCl/ NaCl temperature dependent.

Figure 8 A typical silver-silver chloride reference electrode

2.6.3. Counter electrode

Auxiliary electrode is an electrode which is used to close the current circuit in the electrochemical cell:

a) Serve to supply the current required by the W.E. without limiting the measured response.

b) Current should flow readily without the need for a large over potential.

c) Products of C.E. reaction should not interfere with the reaction being studied.

d) It should have a large area compared to the W.E. and should ensure equipotentiality of the W.E.

e) Usually long Pt wire (straight or coiled) or Pt mesh (large surface area).

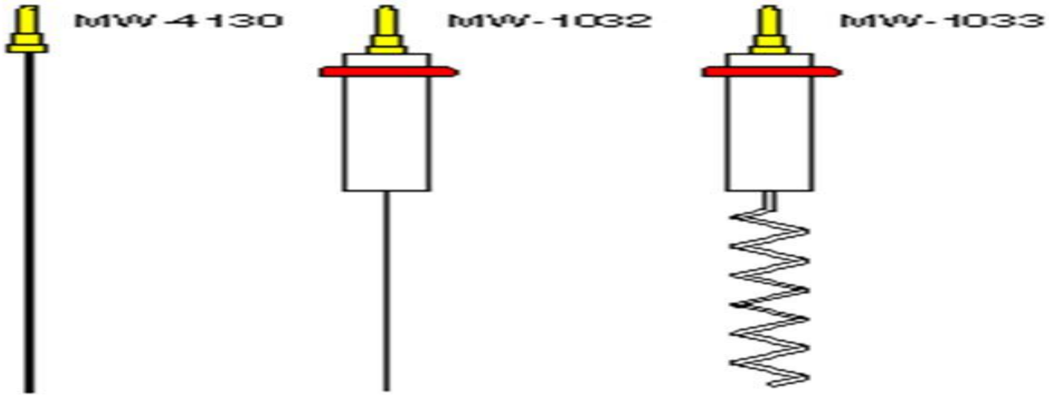


Figure 9 Counter electrodes

2.7. Methods of 4-nitro phenol detection

Up to now various techniques have been investigated to determine 4-NP such as spectro photometry [3], fluorescence [4], high performance liquid chromatography [5], capillary zone electrophoresis [7], fiber optode [8] and electrochemical method [9-12]. Among them, electrochemical methods have received considerable attention because of the advantage of fast response, cheap instrument, low cost, simple operation, time saving, high sensitivity and selectivity, real time detection in situ condition

3. Experimental parts

3.1. Reagents and chemicals

4-nitrophenol ($C_6H_5NO_3$), hydroquinone ($C_6H_6O_2$), bromocresolpurple ($C_{21}H_{16}Br_2O_2S$), potassium ferrichexacyanide ($K_3Fe(CN)_6$, and alumina (Al_2O_3) are from SIGMA ALDICH, monosodium hydrogen phosphate (NaH_2PO_4), and disodium hydrogen phosphate (Na_2HPO4) are from Northampton, KCl, and HCl are from Riedel-deHaen-France, absolute ethanol (CH_3CH_2OH), and NaOH are from Labmark chemicals, India, ascorbic acid from Nice-India, 2-amino phenol, distilled and tap water were used for this experiment. All chemicals used were analytical grade and were used without any further purification.

3.2. Instruments and apparatus

The voltammetry experiments were performed using BAS CV-100 W voltammetric analyzer (USA) and CHI760D model instrument coupled with personnel computer (Fig.10). A conventional three-electrode cell (Fig.11) was used for measurements with a bare glassy carbon electrode (GCE) and/or BCP/GCE as the working electrode, Ag/AgCl (3M KCl) as reference electrode, and a platinum wire as counter electrode

Figure 10 BAS CV-100W voltammetric analyzer coupled with a personnel computer

Figure 11 Setup of an electrochemical cell

An electronic digital balance (Model: Scientech: ZSA 120) was used to measure the weights of solid chemicals during solution preparation. The pH of the buffer solution was measured with a digital pH meter (senses ion TM + MM150). Ultrasonic cleaner was used to clean the working electrode. Labopettes, single channel with 2-20 µL and 100-1000 µL volume configuration were used with tip injection and calibration options.

3.3. Preparation of buffer solution

3.3.1. Phosphate buffer (Na_2HPO_4-NaH_2PO_4) solution

Phosphate buffer solution (PBS) from pH 4-11 was prepared by mixing solutions of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 and adjusting the pH with 0.1M HCl or 0.1 M NaOH using pH meter.

3.4. Preparation of bromocresol purple solution

First 0.1 M phosphate buffer solution (PBS, pH 6) was prepared from 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4 solutions. Then the bromocresol purple (BCP) suspension was prepared by dissolving 3 mg BCP into 100 mL phosphate solution.

3.5. Preparation of 4-nitro phenol solution

5 mM stock solution of 4-NP was prepared by dissolving 70 mg 4-NP into 100 mL distilled water, and then stored at 4 ℃ in a refrigerator. 0.1mM 4-NP working solutions were freshly prepared by appropriate dilution of the stock solution with 25mL phosphate buffer solution (PBS, pH=7.0). All the solutions were prepared with distilled water and all the measurements were carried out at room temperature (25±5℃).

3.6. Modification of the glassy carbon electrode with BCP film

Prior to modification, the bare GCE was polished on a polishing cloth with alumina slurry, washed successively with distilled water and absolute ethanol in an ultrasonic bath for 5 min and dried in air. Then 0.05 mM bromocresol purple suspension was deposited on the surface of GCE and dried in air. The obtained modified electrode was noted as BCP/GCE.

3.7. Analytical procedure

Unless otherwise stated, 0.1 M PBS (pH=7.0) was used as the supporting electrolyte for 4-NP determination. A 500 µL volume of 4-NP working solution and 25 ml of 0.1M PBS were transferred into an electrochemical cell, and then the three electrode system was installed on it. After an accumulation of 7 min at open circuit under stirring and following quiet for 2s, the cyclic voltammograms were recorded from -0.7 V to 0.8 V at scan rate 100 mV/s. The differential pulse voltammograms were recorded from 0 V to 0.4 V. The same procedure was carried out for the water sample analysis.

4. Results and discussion

4.1. Characterization of BCP/GCE

The BCP/GCE was characterized by cyclic voltammetry in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl as shown in Fig.12, the polymer film was made by applying 15 cycles (which was optimized) in the range of -1.5 to 1.6 V at scan rate of 100 mV/s. The peak potential was observed at 1.168V with peak current $2.56x10^{-4}$ A. The film thickness was controlled by the number of cycles [35].

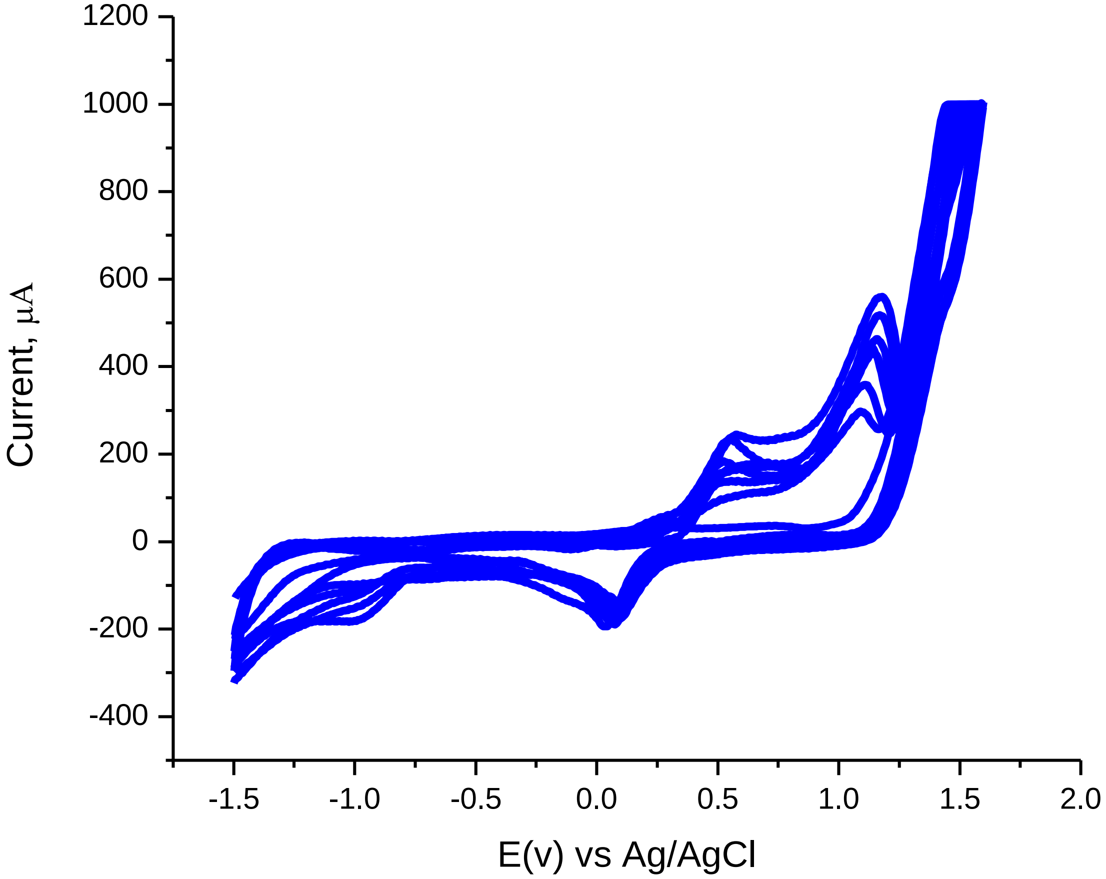


Figure 12 Cyclic voltammograms of BCP /GCE in the electro-polymerization process

4.2. Electro chemical behaviors of 4-NP at the modified electrode

The electrochemical oxidation behaviors of 4-NP were investigated at GCE and BCP/GCE by cyclic voltammetry. The results were shown in Fig.13. No oxidation peak was observed in PBS without 4-NP at GCE and BCP/GCE, suggesting that BCP is an electrochemically inactive material in the selected potential range. After 4-NP was added into PBS (pH=7.0), an oxidation peak was obtained at both electrodes. It is no doubt that the oxidation peak should be attributed to the oxidation of 4-NP. However, no corresponding reduction peak was observed in the following reverse scan from 0.8 V to -0.7 V, implying that the oxidation of 4-NP is a totally irreversible electrode process under the above experimental conditions. For the bare GCE, oxidation peak current (Ipa=1.33×10^{-5} A) was observed at +0.238 V (Fig.13a). At BCP/GCE, the oxidation peak current of the 4-NP (Ipa=4.69×10^{-5} A) increased with negatively shifted peak potential to +0.232 V (Fig.13b) which indicated that the electrochemical oxidation of 4-NP was substantially improved.

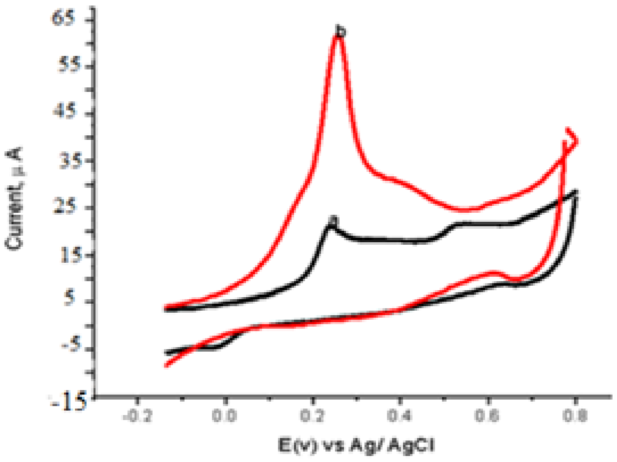


Figure 13 Cyclic voltammograms of 100 μM 4-NP in 0.1M PBS (pH 7) at different electrodes: (a) bare GCE; (b) BCP/GCE; at scan rate: 100 mV/s.

There were two reasons for this improvement. First, 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 4-NP and the BCP/GCE interface. Second, the BCP film contained a lot of negatively charged functional groups e.g (SO_3^- and electron - rich O atom) capable of interacting with 4-NP. The BCP film produced a strong electro catalytic mediation effect, which greatly enhanced the current and lowered the over potential [32]. Therefore, BCP was chosen as the working electrode.

In order to determine the electro active surface area of both GCE and BCP modified GC electrodes the electrochemical behavior of potassium ferric cyanide $K_3Fe(CN)_6$ 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 was increased in the modified GCE as shown in Fig.14.

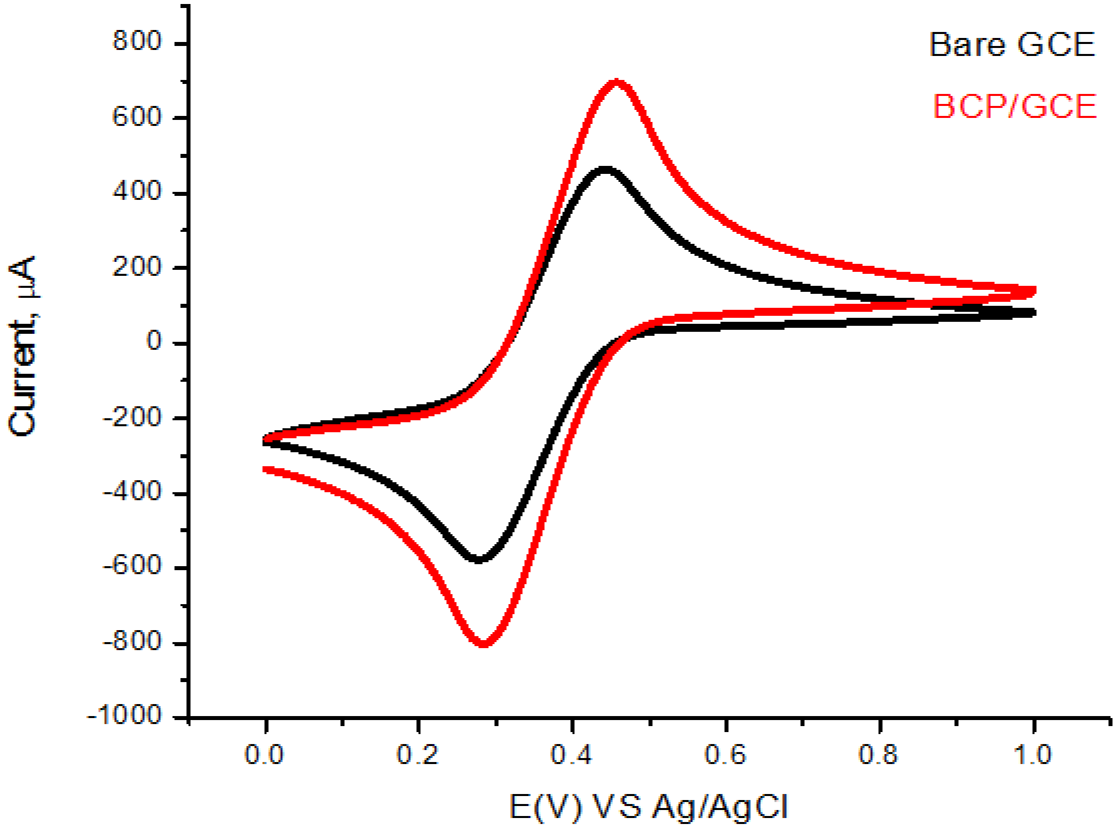


Figure 14 Cyclic voltammogram of 5 mM potassium ferric cyanide in 0.1M KCl at bare GCE and BCPs/GCE with scan rate 100 mV/s.

5 mM $K_3Fe(CN)_6$ in 0.1 M KCl was used as a probe to measure the effective surface area of the poly BCP/GC modified electrode and bare GCE using cyclic voltammetry. Based on the voltammogram obtained the effective surface area was calculated using, the peak current Ip for a reversible system as described by the Randles Sevcik equation [33];

$$Ip=(2.69x10^5)\ n^{3/2}\ A\ D^{1/2}\ V^{1/2}\ C$$

For the forward reaction: $[Fe(CN)_6]^{3-} + e^- \rightarrow [Fe(CN)_6]^{4-}$

Where Ip is current in A, n number of electrons in the redox reaction, A is the electrode surface area in cm^2, D (in cm^2/s) is the diffusion coefficient for the reduction (forward sweep) C is the concentration in mol/cm^3, and V is the scan rate in Vs^{-1}. The calculated electrochemical active surface area of the bare GCE was 0.039 cm^2 and that of the poly BCP/GC modified electrode was 0.05 cm^2, indicating that the electrode effective surface area was increased obviously after electrode modification, which would increase the electrochemical response, and decrease the detection limit.

4.3. Effect of pH

The effect of pH on the oxidation of 100 µM 4-NP at BCP/GCE was investigated by cyclic voltammetry in the pH range from 4 to 11. As shown in Fig.15, the oxidation peak current of 4-NP increased gradually with increasing pH from 4 to 7, and the maximum current was achieved at pH 7. With further increasing pH, the oxidation peak current conversly decreased. Therefore, pH 7 was chosen for subsequent analytical experiments.

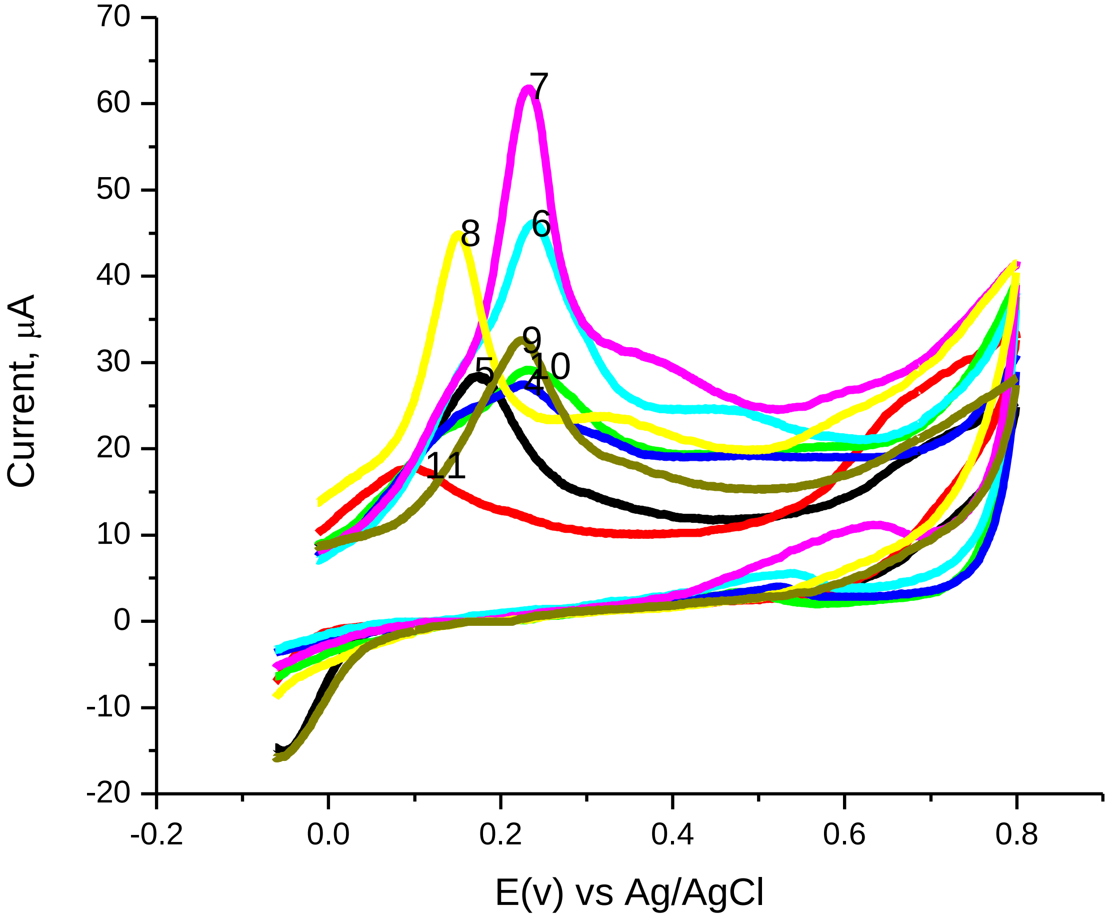


Figure 15 Effect of pH on CV response of modified GC electrode of 100 µM 4-NP in 0.1 Mphosphate buffer at BCP/GCE (pH range of 4 - 11)

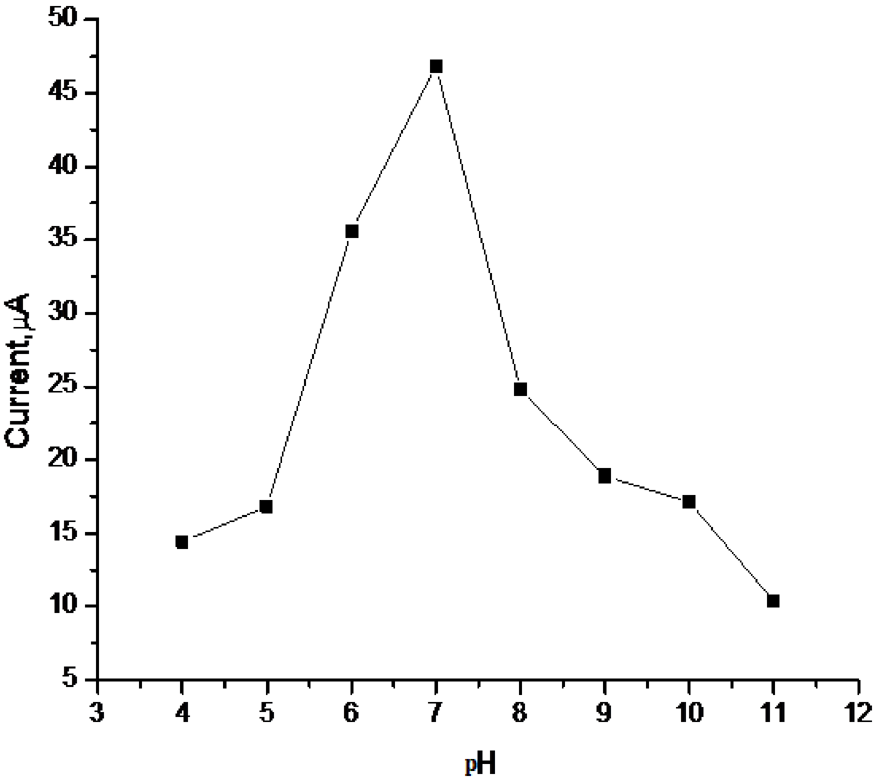


Figure 16 Plot of CV anodic peak current as a function of pH for 100 μM 4-NP in 0.1 M PBS

4.4. Effect of scan rate

Figure 17 shows the influence of scan rate on the electrochemical oxidation behavior of 4-NP. The oxidation current increased with increasing the scan rate. As can be seen from the insert of Fig.17, the oxidation peak current increased linearly with the square root of scan rate in the range 25 to 500 mV/s, indicating that the oxidation of 4-NP on BCP/GCE is a diffusion controlled process [34].

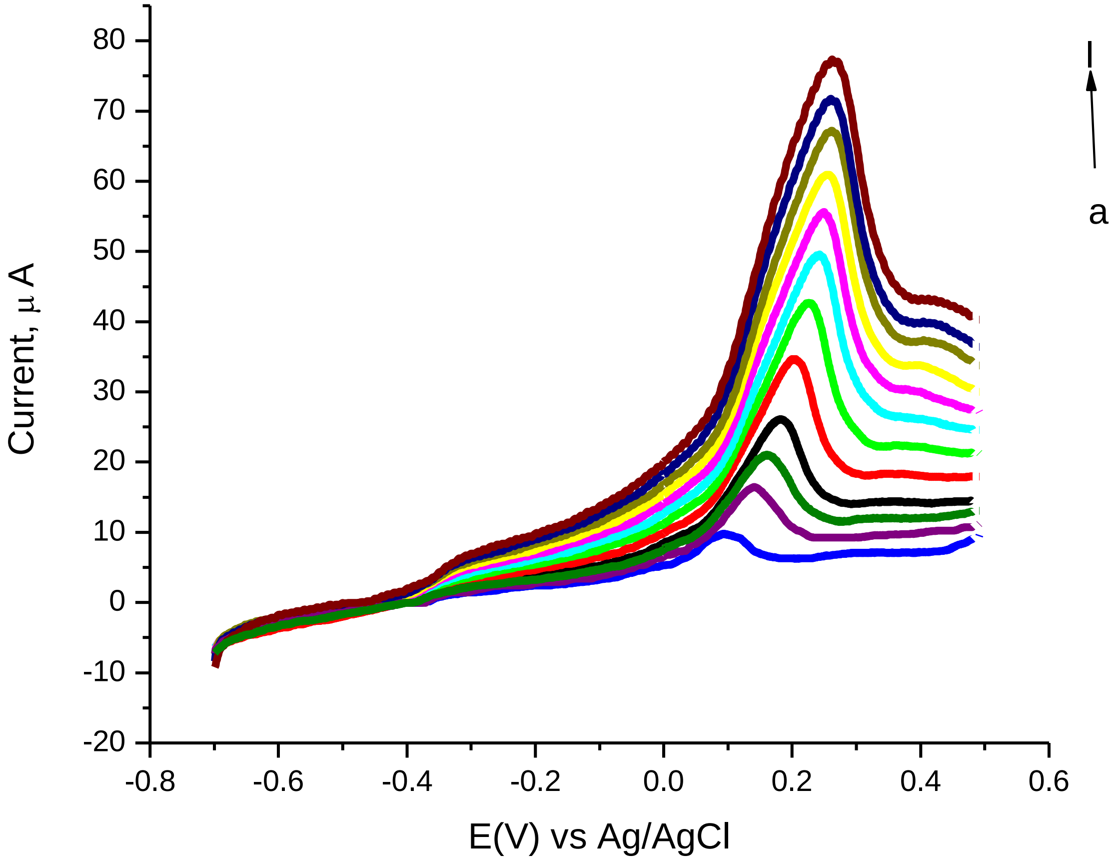


Figure 17 Cyclic voltammograms recorded at BCPs /GCE for 100 µM 4-NP in 0.1M (NaH_2PO_4 – Na_2HPO_4) (pH 7.0) at different scan rates: 0.025, 0.050, 0.075, 0.100, 0.150, 0.200, 0.250, 0.300, 0.350, 0.400, 0.450, 0.500 V/s

Figure 18 shows that oxidative peak current of 4-NP increased with scan rate (25 to 500 mV/s). Also it shows that the oxidation peak current of 4-NP exhibited a linear relation to the square root of scan rate ranging from 25 to 500 mV/s. The linear regression equations of the Ipa for the scan rates are expressed as Ipa (µA) = -7.57 +2.39*$V^{1/2}$, R = 0.996.

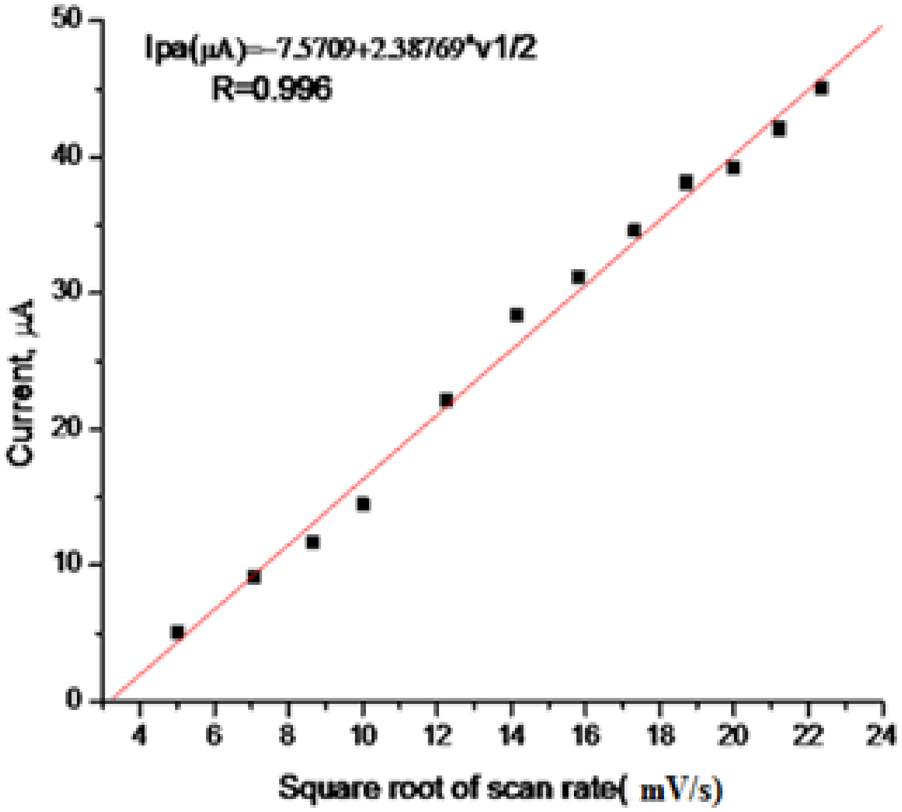


Figure 18 The dependence of the peak current on square root of scan rate

It can be seen that due to the increase of the scan rate, the anodic peak currents increased and the anodic peak potential shifted to more positive potential values when increasing scan rate, indicating that the electro oxidation process of 4-NP is irreversible [36] and shown by Fig.19.

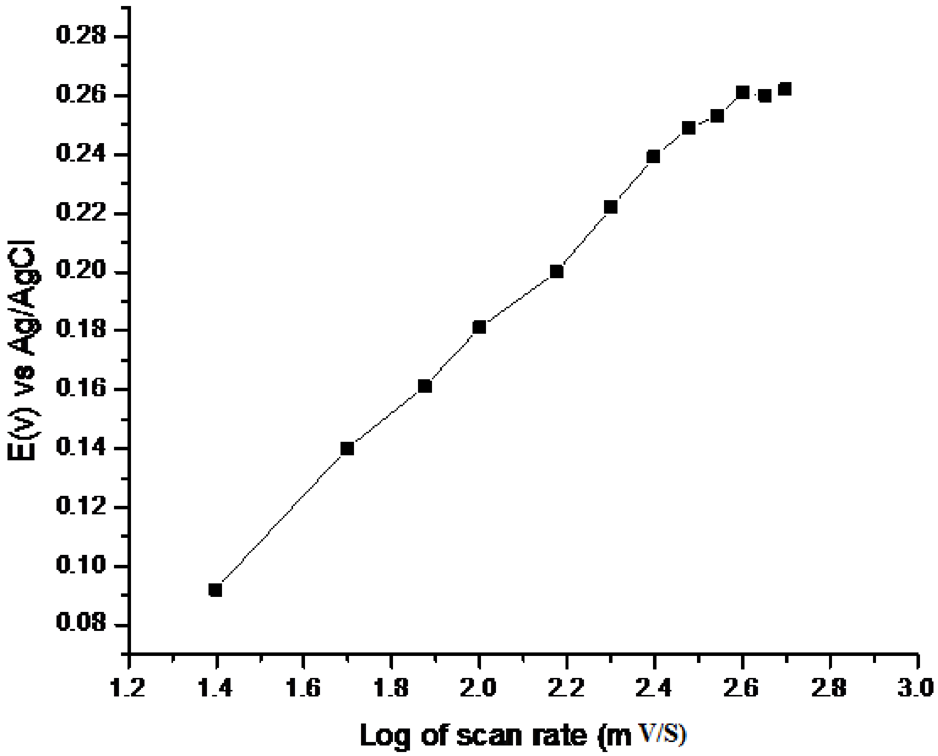


Figure 19 The dependence of the peak potential Ep on log v

4.5. Differential pulse voltammetric analysis

4.5.1. Optimized experimental conditions

The effect of pulse amplitude, pulse width, sample width, pulse period and increment were studied to obtain the optimized experimental conditions for differential pulse voltammetric determination of 4-NP at BCPs/GC modified electrode. The optimum parameters identified for the determination of the analyte for plotting the calibration curve are summarized in Table1

Table 1 optimization value of DPV parameters

Parameters	Optimum value
Pulse width	0.05 s
Pulse amplitude	0.05 V
Sample width	0.017 s
Pulse period	0.2 s
Increment	0.004 V

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 BCP/GCE electrode for the determination of concentration effect of 4-NP on current peak. The effects of DPV scan parameters on the response of the BCP/GCE modified electrode have been studied and optimized. Based on the optimum experimental conditions, the dependence of voltammetric signal on the concentration of 4-NP and the sensitivity of the method are illustrated by differential pulse voltammetry for different concentration of 4-NP. Figure 20 shows DPV voltammograms recorded at the BCP/GC modified electrode for different 4-NP concentrations.

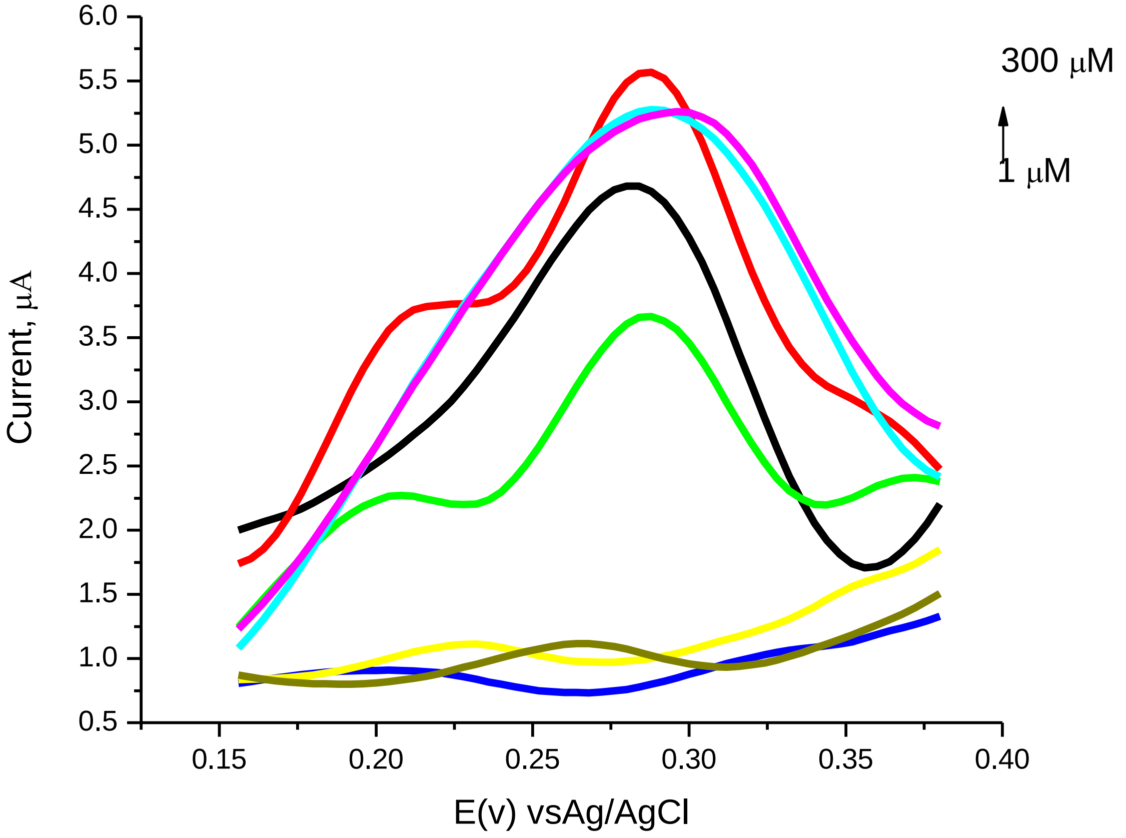


Figure 20 Differential pulse voltammograms for BCP/GC modified electrode for different concentrations of 4-nitrophenol. Pulse amplitude 50 mV, pulse width 50 ms, and pulse period 200 ms (1µM → 300 µM)

Figure 21 shows the differential pulse voltammograms of 4-NP with the concentration ranging from 4 µM to 100 µM under the optimized experimental conditions. The linear regression equation was Ipa (µA) =2.13+0.017*C (µM) with a correlation coefficient of 0.984. The detection limit was estimated to be 2.8 µM. The comparison of BCP/GCE with other modified electrodes for 4-NP determination was listed in Table 2. It can be seen that the BCP/GCE offered reasonable linear range for 4-NP detection and the detection limit was lower than one of the previous reports. These results indicated that BCP/GCE is an appropriate platform for the determination of 4-NP.

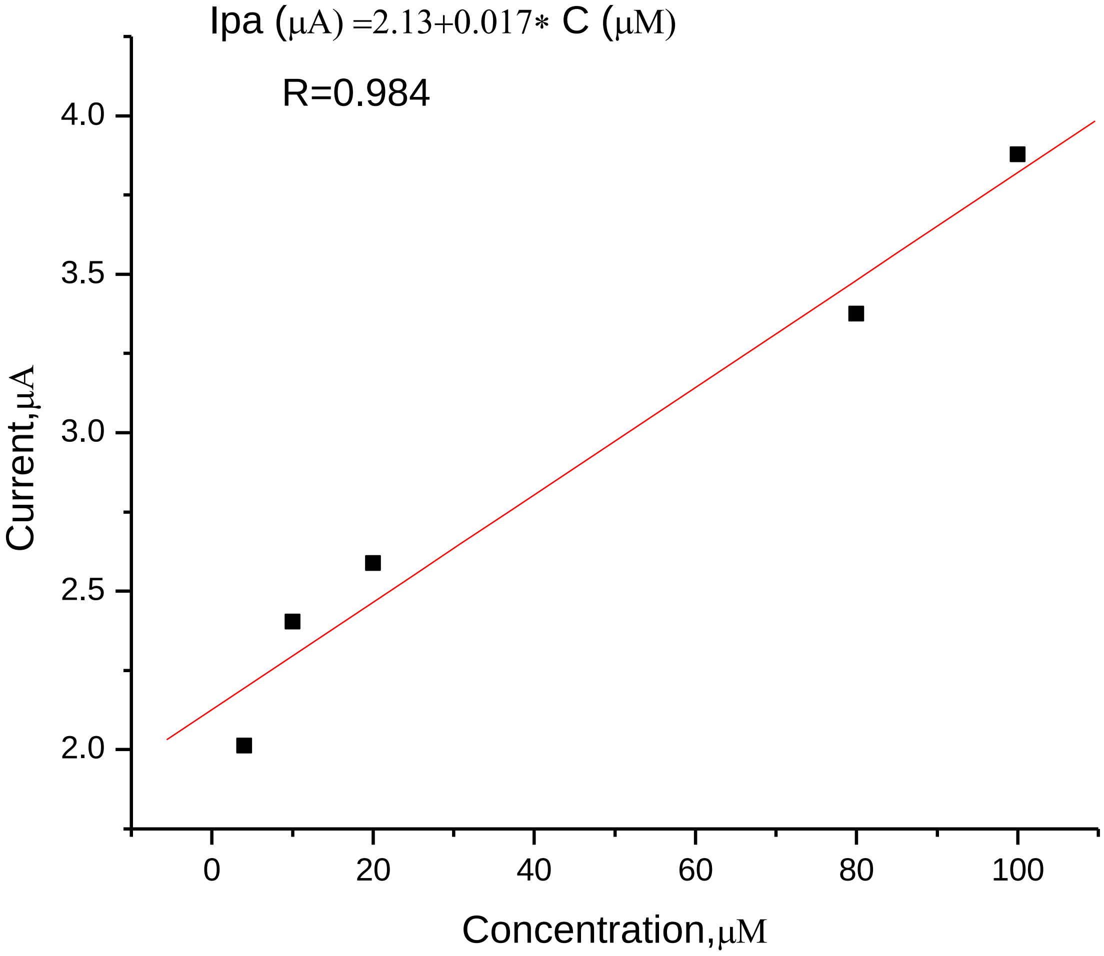


Figure 21 Plot of DPV anodic peaks current as a function of 4-NP concentration from 4 µM to 100 µM.

The analytical performance of the BCPs/ GCE electrode was compared with different modified electrodes and recently published works and the results are summarized in Table 2. It can be noted that the BCPs//GCE has comparable linear response range and better limit of detection. Table 2 Comparison of characteristics obtained from some literatures and this work

Modified electrode	Linear range(μM)	Detection limit(μM)	References
DTD/AgNP/CPE	1-100	0.25	[37]
Nano-gold/GCE	10-1000	8	[38]
HA-NP/GCE	1.0-300	0.6	[39]
MIP/Au	0.1-140	0.1	[40]
GE/SPE	25-620	0.6	[41]
MWNT/SPE	10-620	1.3	[41]
BCP/GCE	4-100	2.8	This work

4.7. Interference study

Possible interferents in the detection of 4-NP by various ions and compounds such as catechol, hydroquinone, 2-amino phenol and ascorbic acid at poly BCP/GC modified electrode were studied. Different μM of the interferon were added to constant concentration (100 μM) 4-NP and differential pulse voltammograms were recorded between 0 V and 0.4 V. The same, twofold, fivefold and tenfold addition of common ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Cl^-, NO_3^-, and SO_4^{2-} do not interfere with the oxidation signal of 100 μM 4-NP. However it is found that the same, twofold, fivefold and tenfold addition of catechol, hydroquinone, 2-amino phenol and ascorbic acid interfere the determination. Nevertheless, these results indicated that BCP/GCE has an excellent selectivity for 4-NP and the fabricated modified electrode might be applied to determine 4-NP in real samples.

4.8. Determination of 4-NP in tap water

To verify the analytical applicability of the sensor, the 4-NP in tap water sample was determined at the poly BCP/GC modified electrode.

DPV method was used to detect 4-NP in the tap water, in the absence of 4-NP for 4ml water (real) samples and 6ml PBS (0.1 M, pH 7) peak current was not observed. However the modified electrode gave correspondingly higher peak currents for increasing concentration of 4-NP spiked to the pH 7 PBS and fixed amount of tap water.

Known concentration of 4-NP containing tap water samples were spiked into the pH 7 PBS solution. The recovery values were calculated in terms of concentration added and founded for pure 4-NP and spiked respectively in the tap water samples as summarized in Table 3.

Table 3 Recovery study

Sample number	4-NP added(μM)	4-NP founded(μM)	Recovery (%)
1	4.8	4.85	101
2	9.1	9.15	101
3	13.0	13.02	100

5. Conclusions

A sensitive and reliable electrochemical oxidation method was investigated for the determination of 4-NP in water samples using BCP/GCE. The polymer film was electrochemically deposited from 0.05 mM bromocresolpurple (BCP) monomer in 0.1 M phosphate buffer solution (pH 6 PBS) using cyclic voltammetry. Differential pulse voltammetric parameters and pH were optimized to study their effects on the peak current and the peak potential. Due to its unique properties of high specific surface area and subtle electronic properties, BCP could effectively enhance the electrochemical response of 4-NP, increase the determination sensitivity and decrease the limit of detection. In addition, the fabrication of BCP/GCE is very simple, cheap and easy to prepare. Based on this a promising electrochemical method was developed for 4-NP determination and applied to water samples using BCP/GCE.

6. References

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