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Voltammetric Detection of Hydrogen Peroxide at Prussian Blue Modified Glassy Carbon Electrode

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Abstract

Prussian Blue electrochemicaly modified onto a glassy carbon electrode surface to prepare a chemically modified electrode which acts as a good electrocatalyst for the reduction of hydrogen peroxide. This study was carried out using different pH ranges (3-8) of 0.05M phosphate buffer solution. H_2O_2 was determined over range of 1 μM- 1 mM . Different electroanalytic techniques such as cyclic voltammetry, differential pulse and square wave voltammetry were used. The result showed that the solution pH affects the stability of the PB film. The cathodic peak current response for the reduction of 1mM H_2O_2 at pH 8 decreased by 55.7 % compared to the response at pH 3.

Key words, Glassy carbon electrode, Prussian Blue, Cyclic votammetry (CV), Differential pulse voltammetry (DPV), Square wave voltammetry (SWV), Hydrogen peroxide.

1. INTRODUCTION

1.1. Hydrogen Peroxide

Hydrogen peroxide is an important analyte. H_2O_2 is a chemical threat agent; its excessive concentration as a product of industry and atomic power stations affects the environment. On the other hand, H_2O_2 is the most valuable marker for oxidative stress, recognized as one of the major risk factors in progression of disease-related pathophysiological complications in diabetes, atherosclerosis, renal disease, cancer, aging and other conditions. Hydrogen peroxide is also a side product of oxidases, the enzymes used as terminal ones in the majority of analytical kits [1].

Hydrogen peroxide in the atmosphere performs an important role in the transformation of atmospheric pollutants because it can act as a source of reactive oxygen radicals such as OH., HO_2 . and RO_2 . (important oxidizing species). Moreover, hydrogen peroxide can efficiently oxidize sulfur oxide to sulfuric acid in acid liquid phase (upto pH 5.5) and the availability of H_2O_2 in the atmosphere can lead to important information on the relationship between sulfur emissions and wet deposition of sulfate. For this reason, the knowledge of H_2O_2 concentration in the atmosphere is necessary to understand H_2O_2 sources and sinks. The main pathway in the formation of atmospheric hydrogen peroxide, H_2O_2, is the combination of two hydroperoxyl radicals (HO_2 .), that are produced by photochemical reactions of atmospheric trace gases such as ozone and volatile organic compounds. Because of the high solubility of hydrogen peroxide in water, gaseous H_2O_2 is the major source of the aqueous-phase H_2O_2. Hence, gaseous H_2O_2 can be conveniently monitored in rainwater [2-4].

The monitoring of hydrogen peroxide (H_2O_2) is of great importance for environmental control, various branches of industry, and modern medicine. Hydrogen peroxide is present in rain and groundwater as waste product of industry. Additionally, hydrogen peroxide is also used for disinfections of pools, food and beverage packages, which is why it is important to measure its residual concentrations [5].

Hydrogen peroxide is present in countless biological reactions as the main product of several oxidases, and is an important parameter for the monitoring of these bioprocesses [6].

The amperometric detection of H_2O_2 has been an important topic for sensor research for decades. However, direct amperometric detection at conventional electrode is only possible at the applied potential ca. 0.6 V vs. Ag/AgCl. At this potential, the presence of oxidizable compounds such as ascorbate, bilirubin, urate, etc. can easily interfere in the measurement, being oxidized at the electrode together with hydrogen peroxide. To overcome this difficulty, the trend has been employing the self-assembly technique for the fabrication of amperometric biosensor to detect H_2O_2 [4].

1.2. Chemical Property of Hydrogen Peroxide

Hydrogen peroxide has a marked tendency to decompose to water and oxygen, releasing a large amount of heat.

$$2H_2O_2 \rightarrow 2H_2O + O_2 \qquad \Delta H = -196.2 \text{ kJ} \text{ ------- } (1)$$

At room temperature, it appears metastable, either in solution or in pure state. It is stable because the first step in its thermolysis involves splitting the molecule into two $HO^{\cdot}$ radicals and this requires a large input of energy. Once formed, the radical initiates a chain reaction. The most characteristic property of hydrogen peroxide is its oxidizing action.

$$H_2O_2 \rightarrow H_2O + O \quad \text{or} \quad H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \text{ --------}(2)$$

It is thus like ozone, capable of oxidizing lead sulphide to lead sulphate and iron (II) to iron (III) salts as shown below.

$$PbS + 4O \rightarrow PbSO_4 \text{ ---------------------------------- } (3)$$

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

Since hydrogen peroxide is reduced to water and no other products, it is favored in the laboratory as a clean oxidizing agent.

1.3. Physical Property of Hydrogen Peroxide

Pure, water-free hydrogen peroxide is nearly colorless although a very thick layer appears blue. It is a syrupy liquid due to extensive hydrogen bonding with b.p 150.2^0C and a density at 20^0C of 1.448 gcm^{-3}. It freezes to give crystalline needles, m.p-0.43^0C and it resembles water in many of its physical properties. Physiologically, it is very irritating to the skin, and especially to mucous membranes of the respiratory passages and eyes. If swallowed, H_2O_2, causes internal bleeding [7].

1.4. Electrochemistry of Hydrogen Peroxide

It is well known that the detection of hydrogen peroxide (H_2O_2) plays an important role in many fields including environmental protection, industry and clinical diagnosis. H_2O_2 is electroactive in aqueous solutions, making its electrochemical detection feasible and convenient. However, many substances normally present in biological fluids, such as ascorbic acid (AA), uric acid (UA) etc., are easily cooxidized at similar potentials and may cause interfering responses.

There are mainly two effective protocols in diminishing or inhibiting these interferences, (1) d ecreasing the H_2O_2-detecting potential and sensitizing the reduction of H_2O_2 by using a catalyst immobilized at an electrode, instead of its oxidation; (2) taking a permselective film that diminishes or inhibits the electroactivity of the interferents, while a high permeability for H_2O_2 across the film still allows its high electroactivity at electrode. Two typical kinds of species, peroxidases (or its analogues) and metal hexacyanoferrates, have been commonly used in the electrocatalytic reduction of H_2O_2 at low potentials [8-9].

Since it was announced almost 25 years ago, that Prussian Blue or ferric ferrocyanide, electrodeposited onto an electrode surface, could act as an electrocatalyst for hydrogen peroxide reduction, many attempts were made to achieve a suitable catalytic surface for the amperometric determination of hydrogen peroxide. Both the electrodes material (Pt, Au, graphite, carbon paste, and glassy carbon) and the techniques of immobilization of Prussian Blue (mechanical immobilization, electrodeposition etc.) were tested.

The main advantage of electrodeposited Prussian Blue relies on the fact that hydrogen peroxide can be detected selectively through electrocatalytic reduction in the presence of molecular oxygen, at a low electrode potential (-0.05 V vs. SCE), where the influence of the so-called reductants (ascorbate, urate, acetaminophen) on the electrochemical response can be largely avoided, which is always a common problem for systems based on the electrochemical oxidation of hydrogen peroxide. Moreover, it is known that the detection of hydrogen peroxide plays a very important role for the construction of many electrochemical biosensors, since the hydrogen peroxide producing oxidases such as, glucose, lactate, alcohol, glutamate oxidase, etc., commonly used for biosensor construction, in their reaction sequence starting with the oxidation of their substrate produce hydrogen peroxide as an end product that in turn is measured.

Glucose biosensor, using glassy carbon as basic electrode material, can thus be obtained by initially electrodepositing Prussian Blue followed by immobilizing glucose oxidase. These kinds of glucose biosensors have been found to exhibit high sensitivity and linear behavior in a broad concentration range. Similarly ethanol, glutamate, oxalate, choline, alanine, and lactate biosensors have been produced [10-12]].

Various analytical techniques to determine hydrogen peroxide in rainwater are reported in the literature, including methods based on spectrophotometry, fluorometry, amperometry, potentiometry and chemiluminescence. On the other hand, rapid, accurate and simple method are continuously sought, justifying the attention attracted by electrochemical techniques because they possess quick response, low cost, simplicity of instrumentation, high sensitivity and possibility of miniaturization. However, the direct amperometric detection of H_2O_2 has a relatively large anodic potential (0.6 V vs. Ag/AgCl). Hence, the development of electroanalytical methods for the selective determination of hydrogen peroxide at less positive potentials has been a major target, especially in the case of complex matrices. Numerous attempts have been made to reduce the large over potential by depositing chemical mediators to circumvent the higher energy required in the electrochemical reduction of hydrogen peroxide[2].

The problem of monitoring of low levels of hydrogen peroxide is required in medicine, environmental control and in industry. There are known different methods of such detection, but electrochemical analysis is the most sensitive, selective and the cheapest method [13].

1.5. Prussian Blue

Prussian Blue, or ferric hexacyanoferrate is definitely one of the most ancient coordination materials known. The earliest announcements found are from the very beginning of the eighteenth century. However, a quite recent investigation by Neff[15] , that Prussian Blue forms electroactive layers after electrochemical or chemical deposition onto the electrode surface, has opened a new area in fundamental investigation of this unique inorganic polycrystal. PB can be synthesized chemically by mixing of ferric (ferrous) and hexacyanoferrate ions with different oxidation states of the iron atoms, either Fe^{3+} + $[Fe^{II}(CN)_6]^{4-}$ or Fe^{2+} +$[Fe^{III}(CN)_6]^{3-}$, or electrochemically from aqueous solutions containing a mixture of ferric (Fe^{3+}) and hexacyanoferrate ($[Fe^{III}(CN)_6]^{3-}$) ions on various conductive surfaces.

PB is deep blue in color at ground state. However, under a suitable applied potential it can be reduced to colorless Prussian White (PW) known as Everitt Salt (ES), while by oxidation the yellow form, PrussianYellow (PY) also known as Berlin Green is obtained. Chemically deposited Prussian blue is in the form of soluble, $K_4Fe^{II}[Fe^{II}(CN)_6]_3$, whereas initially electrochemically deposited PB is in the form of insoluble, $Fe^{III}_4[Fe^{II}(CN)_6]$ i.e., free of potassium ions. However, when it is cycled between the redox states,in a solution containing potassium ions, the insoluble form is converted to the soluble form. The term soluble was introduced by Keggin and Miles and refers to the ease with which potassium ions can be peptized rather than to the real solubility of Prussian blue [14]. In 1978, the favorable electrocatalytic behavior of Prussian Blue (PB) was first revealed attracting the attention of a wide number of research groups [16].

There are two sets of peaks in the cyclic voltammogram of a Prussian blue modified electrode. The cathodic one corresponds to the Prussian Blue/Prussian White redox system with potassium as the counter cation to compensate the transfer of electrons in the film according to the equation

$$Fe^{III}_4[Fe^{II}(CN)_6] + 4e^- + K^+ \leftrightarrow K_4Fe^{II}[Fe^{II}(CN)_6]_3 \text{ ---------- } (5)$$

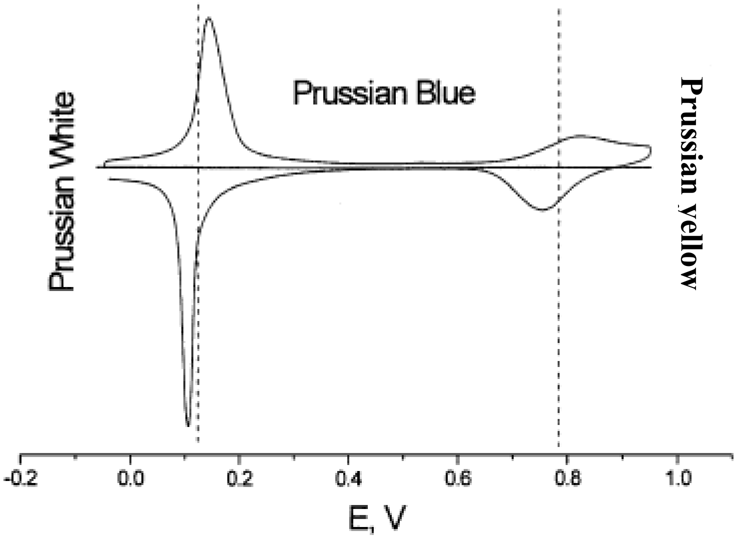


Fig.1 Typical cyclic voltammogram of Prussian Blue modified glassy carbon electrode.

At high anodic potential, the Prussian Blue fully oxidized redox state is denoted as Berlin green or Prussian yellow. The only possible mechanism for charge compensation is the enterapment of an ion in course of oxidative reaction [17-19].

$$\mathrm{Fe^{III}_4[Fe^{II}(CN)_6]_3\ -\ 3e^-\ +\ 3A^-\ \leftrightarrow\ Fe^{III}_4[Fe^{III}(CN)_6A]_3}\ \text{-----}\ (6)$$

The most important feature of PB when it comes to analytical applications is the electrocatalytic activity for the reduction of hydrogen peroxide and oxygen which was initially demonstrated by Itaya et al [17]. Later it was shown that the electrodeposition of PB under certain conditions led to synthesis of an electrocatalyst selective for hydrogen peroxide reduction in the presence of oxygen. Due to both its high activity and selectivity, which are commonly the properties of biological catalysts, Prussian blue has been denoted as an artificial peroxidase.

In fact, the reduced form of Prussian blue, i.e., Prussian white, which is the catalytically active form for the reduction of hydrogen peroxide, is thermodynamically unstable on the electrode surface and sensitive to OH^-, the product of the electrocatalytic reduction of hydrogen peroxide in neutral media, which is able to solubilize the inorganic polycrystals. Many efforts on increasing the stability of PB has been performed either by improving the crystalline structure of the deposited PB or by additional post treatment [14]. Nanostructured Prussian blue modified electrodes have better performances, lowering the overpotential, and increasing the signal/noise ratio if compared with those obtained working with the microstructured bulk films or micro/macro particles directly deposited (for casting procedure) on the electrode surfaces [20].

1.6. Prussian Blue Modified Glassy Carbon Electrode

Glassy carbon electrode has been very popular because of its excellent mechanical and electrical properties, wide potential window, chemical inertness (solvent resistance), and relatively reproducible performance. But to enhance its activity, modifying with electroactive species is the vital one [21].

Chemically modified electrodes (CMEs) have great significance as important analytical tools for the electrochemical determination of various analytes with the benefits of electrode modification. The modification of electrode results in efficient detection of electro-active biomolecules at very lower potential without its major interferences. Generally, modification of electrodes surface was achieved by using inorganic and organic materials. Among them polymer-modified electrodes (PMEs) are widely used [22-23].

Chemically modified electrodes have received extensive attention owing to their evident advantages such as high sensitivity, high selectivity, and high stability over a wide range of solution composition, less prone to surface fouling and low over potential at which the electron transfer process occurs, compared with inert substrate electrodes. At present, various inorganic materials including clay, zeolites, metal oxides, transition metal nanoparticles, polyoxo-metallate and transition metal hexacyanoferrates (MHCFs) (have been used to fabricate the inorganic film modified electrode. The inert modified electrodes involve glassy carbon, platinum, aurum, and graphite [5]. Recently, among these modified electrodes, Prussian blue (PB) iron (III) hexacyanoferrate (II) thin film modified electrodes are particularly attractive due to their ease of preparation by using a simple electrochemical or non-electrolytic chemical procedure and their wide range of applications such as electroanalysis, electrochromic applications, solid-state batteries, biosensors and optical sensors. These applications of PB-modified electrodes are based on reversible redox characteristics (PB/PW and PB/PY transition) in appropriate potential ranges [24].

The present investigation of a novel electrochemical transducer is defined by the requirements of the one of the most significant fields of modern analytical biotechnology, i.e., electrochemical biosensors. Prussian Blue, which has been deposited on the surface of glassy carbon electrode under certain conditions, was found to be a selective electrocatalyst for H_2O_2 reduction in the presence of O_2 [25].

1.7. Prussian Blue as an Advanced Transducer for Hydrogen Peroxide

The PB film, which features facile electrochemical deposition on various electrodes, low cost, high sensitivity for H_2O_2 detection and acceptable stability, has been widely employed in the development of various H_2O_2- detecting electrodes for detection of many substrates in recent years [26].

The requirements for selective detection of hydrogen peroxide rise from the following main reasons. Hydrogen peroxide itself is a chemical threat agent present in rain and ground waters as waste product of industry and atomic power stations. In addition, hydrogen peroxide is used for disinfection of water pools, food and beverage packages, which makes it important to measure its residual concentration. On the other hand, hydrogen peroxide is a side product of oxidases, the enzymes, which are included as terminal ones in more than 90% of the existing enzyme-based biosensors and analytical kits. The low-potential detection of hydrogen peroxide was found to be the most progressive procedure for operation of the oxidase-based biosensors providing both high sensitivity and high selectivity in the presence of easily oxidizable compounds [17].

Recent progress in biosensor development has attracted a lot of attention in monitoring several biologically significant compounds such as glucose, lactate, alcohol, phenol, and cholesterol. In the enzyme-based approaches, cholesterol can be detected with a better specificity through the use of hydrogen peroxide, or through an enzymatic reaction of a redox enzyme [27].

The biosensors were made by enzyme immobilization on the top of the Prussian Blue modified electrodes. The electrochemical transducer and biosensors were suitable for detection of low analyte levels and were practically independent of potentially interfering reductants. A cholesterol biosensor prepared by immobilization of ChOx in silicic sol-gel membranes on top of PB modified electrodes is described below [28].

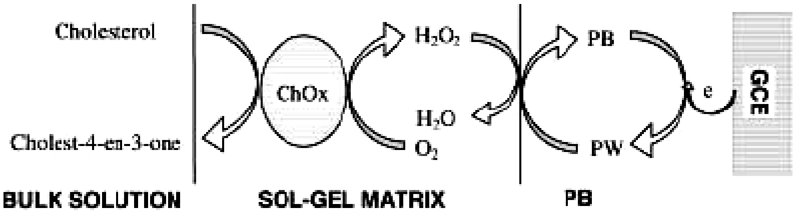


Fig.2 Scheme of multi-step reactions between cholesterol/ enzyme/ electrode at the biosensor

Prussian Blue (PB) has been considered as a class of interesting materials for H_2O_2 electrocatalyst for years. The fast electron transfer rate of PB makes PB-modified electrode very rapid and versatile for various electrochemical methods, which always provide very good analytical performances with respect to H_2O_2 detection. Moreover, the peculiar cubic geometry of the PB molecules seems to be the cause for an effective electrochemical selectivity due to the fact that molecules with molecular weight higher than H_2O_2, such as ascorbic acid and uric acid, can not penetrate the PB lattice and give a catalytic redox reaction. This is probably the main advantage of using PB as a mediator for H_2O_2 reduction.

Karyakin [29] found that the catalytic activity of PB/GCE towards H_2O_2 was about 100 times greater than towards oxygen and that the rate constant for H_2O_2 reduction was 500-$molL^{-1}$ s^{-1}. More advantages such as low cost and easy deposition with a PB layer grown either with a chemical or electrochemical synthesis are achieved. These promising advantages have been used to obtain a sensitive and interference free probe for H_2O_2 detection. Despite the advantages reported, the operational instability of PB has represented the main shortcoming to the use of PB modified electrode.

The operational stability of PB modified electrode seems to be dependent on the method of deposition of the PB layer. An enhanced stability of the PB layer at alkaline pH was observed by adopting a chemical deposition method, which was a useful alternative to the most used electrochemical approach for the modification of the electrode surface with PB. The greatly enhanced stability of PB layer made possible the practical application of H_2O_2 sensor, which was no loss of signal for H_2O_2 detection after 50 h even at alkaline pH. When H_2O_2 was added to the solution, an obvious increase in cathodic catalytic current and little decrease in anodic peak were observed [30].

The performance of the PB modified gold electrode (PB/Au) demonstrated that this electrode was more suitable for hydrogen peroxide monitoring than the equivalently prepared PB modified platinum electrode (PB/Pt). In fact, when these PB modified electrodes were employed for H_2O_2 monitoring, the current response for the PB/Au electrode was found to be around 7000 nA, a value at least twice higher than that observed for PB/Pt [31].

Nanosized Prussian blue (PB) particles were synthesized with a chemical reduction method and then the PB nanoparticles were assembled on the surface of multiwall carbon nanotubes modified glassy carbon electrode (PB/ MWNTs/GCE). The results showed that the PB/MWNTs nanocomposite exhibits a remarkably improved catalytic activity towards the reduction of hydrogen peroxide [32].

The use of PB as for hydrogen peroxide reduction is still a matter of concern because of its poor operational stability limited to a range of hours and to a very high decrease in sensitivity in neutral and alkaline pH[31].

1.8. The Objective of the Project

The objective of the project is to get an understanding of the use of the Prussian Blue modified glassy carbon electrode as a sensor for hydrogen peroxide in aqueous media of different pH's.

2. ELECTROCHEMICAL TECHNIQUES

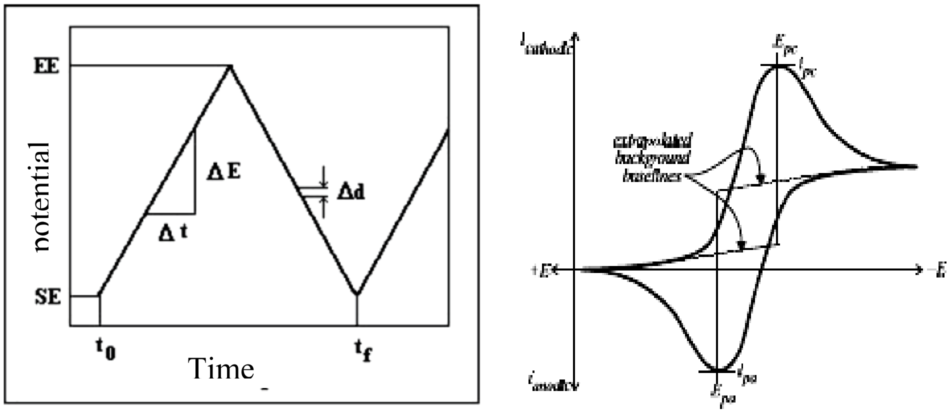
Electrochemical techniques have a wide range of application. The electrochemical techniques give information on the processes taking place when an electric potential is applied to the system under study. Their use tends to be concerned with trying to find out about properties of the metal-solution interface: for example, the rate of reactions at the surface, the nature of films on the surface or the morphology of the surface [33].

The common characteristic of all voltammetric techniques is that they involve the application of a potential (E) to an electrode and the monitoring of the resulting current (i) flowing through the electrochemical cell. In many cases the applied potential is varied and the current is monitored over a period of time (t). Thus, all voltammetric techniques can be described as some function of E, i, and t. They are considered active techniques (as opposed to passive techniques such as potentiometry) because the applied potential forces a change in the concentration of an electroactive species at the electrode surface by electrochemically reducing or oxidizing it.

The analytical advantages of the various voltammetric techniques include excellent sensitivity with a very large useful linear concentration range for both inorganic and organic species (10^{-12} to 10^{-1} M), a large number of useful solvents and electrolytes, a wide range of temperatures, rapid analysis times (seconds), simultaneous determination of several analytes, the ability to determine kinetic and mechanistic parameters, a well-developed theory and thus the ability to reasonably estimate the values of unknown parameters, and the ease with which different potential waveforms can be generated and small currents measured[22].

2.1. Cyclic Voltammetry, CV

Cyclic Voltammetry is a particular linear sweep voltammetry among different voltammetric electrochemical techniques. In this way a redox couple in solution is exposed before to an oxidation and afterwards to a reduction (or vice and versa). This technique is based on varying the applied potential at a working electrode in both forward and reverse directions (at some scan rate) while monitoring the current. Depending on the analysis, one full cycle, a partial cycle, or a series of cycles can be performed. The applied triangular waveform is shown in fig (3A). When the electrode reaction is reversible, the cyclic voltammogram exhibits peaks fig. (3B).



A B

Fig.3 Cyclic voltammogram

A- Potential scanning starting from an anodic sense;

B- Cyclic voltammogram of a reversible redox system:

where E_{pa}= anodic peak potential (mV) E_{pc}= cathodic peak potential.

Generally, this technique is not used for quantitative analysis because of its poor sensitivity. The important parameters in a cyclic voltammogram are the peak potentials (Epc , Epa) and peak currents ($i$pc , $i$pa) of the cathodic and anodic peaks, respectively. If the electron transfer process is fast compared with other processes (such as diffusion), the reaction is said to be electrochemically reversible, and the peak separation is

$$\Delta Ep = / Epa - Epc / = 2.303\ RT/nF \text{ ----------------- } (7)$$

Thus, for a reversible redox reaction at 25 °C with *n* electrons ΔEp should be $0.0592/n$ V or about 60 mV for one electron. In practice this value is difficult to attain because of such factors as cell resistance.

Irreversibility due to a slow electron transfer rate results in $\Delta E\text{p} > 0.0592/n$ V, greater, say, than 70 mV for a one-electron reaction.

The formal reduction potential (*Eo*) for a reversible couple is given by

$$E^{\circ} = \frac{E_{p,a} + E_{p,c}}{2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (8)$$

For a reversible reaction, the concentration is related to peak current by the Randles–Sevcik expression (at 25 °C):

$$i_p = 2.686\ x\ 10^5 n^{3/2} A C^0 D^{1/2} \nu^{1/2} \text{ --------------------- } (9)$$

where i_p is the peak current, in amps *A is* the electrode area (cm^2), *D* is the diffusion coefficient ($cm^2\ s^{-1}$), c^0 is the concentration in mol cm^{-3}, and ν is the scan rate in V s^{-1}.

Cyclic voltammetry is carried out in quiescent solution to ensure diffusion control. A three-electrode arrangement is used. Working electrodes include glassy carbon, platinum, gold, graphite, and carbon paste [34, 22].

For irreversible process (those with sluggish electron transfer), the individual peaks are reduced in size and widely separated. Irreversible systems are characterized by a shift of the peak potential with scan rate. The dependency of Ep on scan rate is given by

$$Ep = E^0 - (RT/\alpha n_a F)[0.78 - \ln(k^0/D)^{1/2}] + \ln(\alpha n_a F\nu/RT)^{1/2} \text{ ----------- } (10)$$

where α is the transfer coefficient, n_a is the number of electrons involved in the charge – transfer step, k^0 is the standard rate constant and ν is the scan rate.

The peak current given by

$$i_p = (2.99 \text{ x}.10^5)\, n\, (\alpha n)^{1/2}\, ACD^{1/2}\nu^{1/2} \text{ -------------------------- } (11)$$

is still proportional to the bulk concentration and to $\nu^{1/2}$[35].

2.2. Differential Pulse Voltammetry, DPV

Differential- pulse voltammetry is an extremely useful technique for measuring trace levels of organic and inorganic species [34]. If a series of periodical constant pulse of potential is superimposed to a linear scanning, a consistent enhancement of the signal is achieved. Moreover, if the difference between the current just before and at the end of the pulse is measured, a reading less influenced by the capacitive current can be performed. In this way this differential reading of the current generates a peak shaped voltammogram.

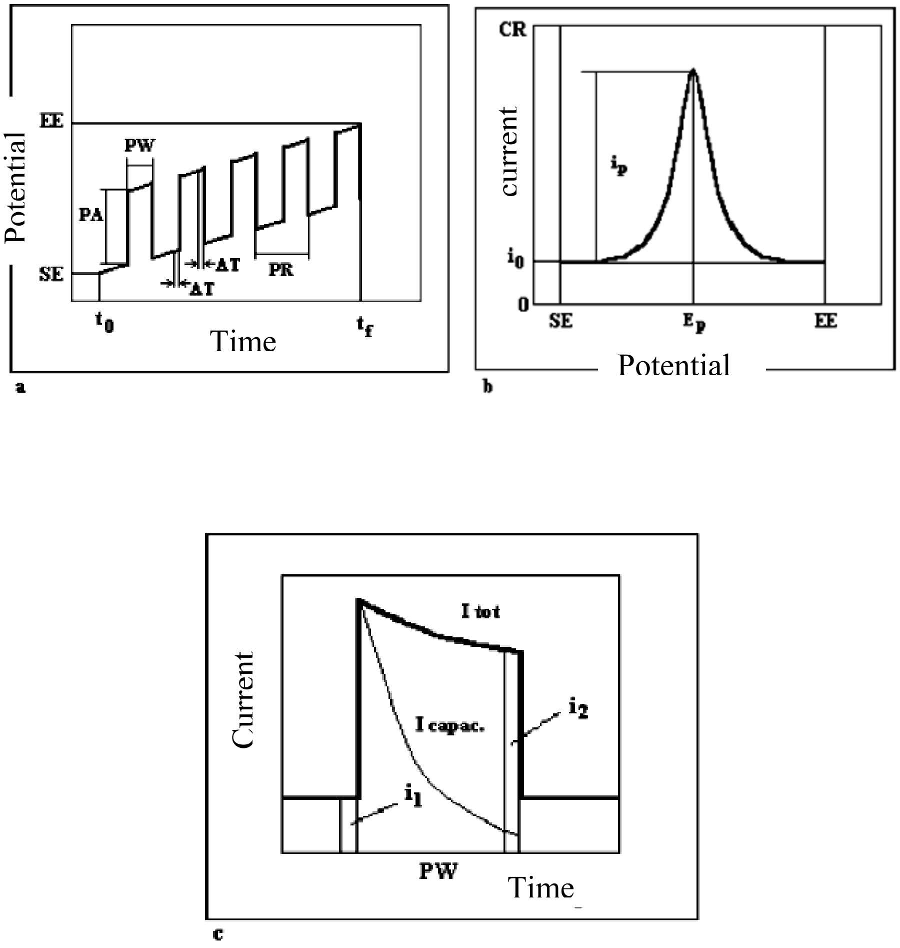


Fig. 4 Differential Pulse Voltammetry

a- Anodic scanning of the potential;

b- Plot of voltammogram; i_o =current at the beginning of scanning

c- Progress of the current during a pulse; i_p=peak current

SE = Start potential; EE= End potential; E_p = peak potential

$t_{0/F}$= starting and final time temp. of the scanning, pA = potential pulse amplitude

ΔT = sampling time; CR = Current range; pR = pulse repetition time

PW = Pulse Time; i_2 = current at the end of the pulse

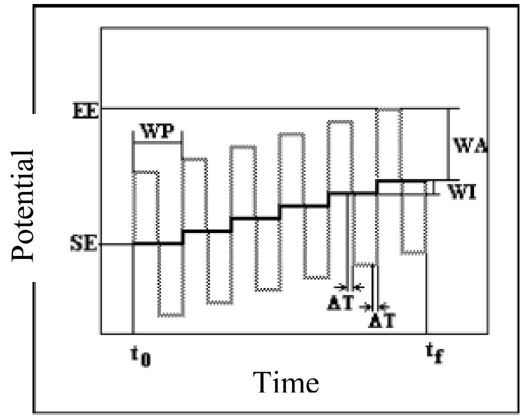
i_1= Current before the pulse; I_{tot}= Total current;

I_{capac}= capacitive current.

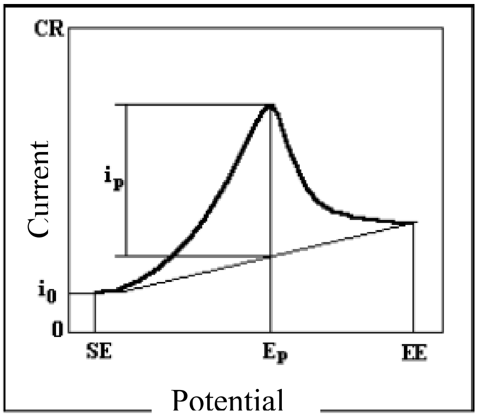
Note how capacitive current reaches the maximum at the beginning of the pulse and how it decreases rapidly after the pulse. Practically, at the end of the pulse only the faradic current is sampled. The technique is very sensitive and detection limits range near 10 – 100 µg/l.

2.3. Square Wave Voltammetry, SWV

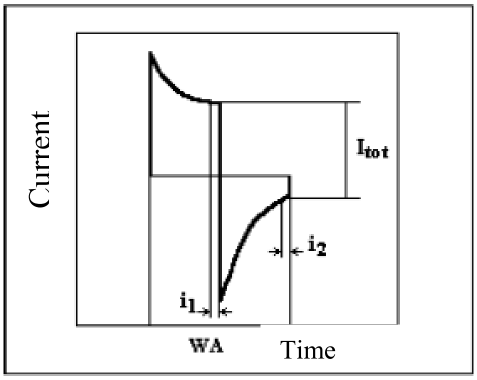
This technique represents a further development of the preceding one. A rapid step scanning of potential is applied to the electrode and on each step is superimposed a high frequency square wave (20 – 100 Hz). The current is sampled two times at the end of the two half waves. If the amplitude of the wave is very little and the redox system is reversible, during the first half wave the electroactive compound can be reduced (or oxidized), while, in the second half wave, at the contrary, it can be oxidized (or reduced). The two current are then summed up and so, the sensitivity is increased.



a



b



c

Fig.5 Square wave voltammetry
a- Anodic scanning of the potential; b- Plot of voltammogram;
c- Progress of the current during a square pulse, SE = Start potential;
other terms have similar meaning as used in Fig.3.

Note in c that the measured total current represent the algebraic sum of the anodic and cathodic current. The sensitivity of this technique can be increased by enhancing the amplitude of the square wave or the frequency. The limit of the enhancing is strictly related to the kinetics aspects of the redox system: it has not to be slower than the velocity of the scanning of potential. The interference due to capacitive current are lowered to minimum because the current is sampled just at the end of the half waves, when the current of the double electrical layer is the least.

Square-wave voltammetry has several advantages. Among these are its excellent sensitivity and the rejection of background currents. Another is the speed (for example, its ability to scan the voltage range over one drop during polarography with the DME). This speed, coupled with computer control and signal averaging, allows for experiments to be performed repetitively and increases the signal to- noise ratio. Applications of square-wave voltammetry include the study of electrode kinetics with regard to preceding, following, or catalytic homogeneous chemical reactions, and determination of some species at trace levels [35].

3. EXPERMENTAL PART

3.1. Reagents and Chemicals

The reagents and chemicals used were 30% hydrogen peroxide (Scharlau chemi S.A), potassium hydrogen phosphate and dipotassium hydrogen phosphate (Riedel-deHaen AG), phosphoric acid and tris-potassium phosphate salt (Pharmacus LTD, South end-England), KCl (Techo Pharma Chem., BAHADVRAGARH, India), HCl (BDH Limited Poole, Englnd), and alumina (BDH chemicals LTD Poole, England). All experiments were carried out with double distilled water

3.2. Instrumentation

The conventional three electrode cell with a Ag/AgCl (3MKCl) electrode as a reference electrode, a platinum wire as a counter electrode, bare and Prussian Blue modified glassy carbon as working electrode was used for all measurements. The electrochemical experiments were carried out with BAS 100B electroanalyzer, which was connected to a Dell computer of Pentium 4 model. The pH of the buffer solution was measured with a Hannan digital pH 301 meter model. All the cyclic voltammetric, square wave voltammetric and differential pulse voltammetric were recorded in the potential range from 350 to -50 mV/s with scan rate 50 mV/s. The net current of the different voltammetric measurements were recorded.

3.3. Procedures

The GC electrode was polished with alumina paste (Al_2O_3, 1 mm, Struers, Copenhagen, Denmark) to obtain a mirror like surface followed by electrochemical deposition of PB on the GC electrodes. The deposition was performed in three steps according to the procedure described by De Mattos [10].

1. Deposition: A solution containing 2.5 mM $FeCl_3$ and 2.5 mM $K_3[Fe(CN)_6]$ in 0.1M HCl and 0.1M KCl was deaerated by purging with nitrogen for 20 min. PB was then deposited on the GC electrodes by applying a potential of + 400 mV for 60 s.

2. Activation: The potential of the electrode was cycled 25 times from -50 to +350 mV at a scan rate of 50 mVs^{-1}. The supporting electrolyte used was 0.1M KCl and 0.1M HCl. The electrode was then dried at 100^0C for 60 min and then allowed to cool to room temperature.

3. Conditioning: A potential of -50 mV was applied for 600 s in 0.05 M phosphate buffer solution and 0.1M KCl at different pH which was adjusted by 0.5 M phosphoric acid and then the potential of the electrode was cycled 10 times from - 50 to + 350 mV at a scan rate of 50 mV s^{-1} in the same electrolyte solution.

4. RESULT AND DISCUSSION

4.1. Cyclic Voltammetry Investigations

The cyclic voltammograms were recorded on a bare glassy carbon electrode in 0.1KCl + 0.1HCl supporting electrolyte solution before (a) and after (b) addition of 1mM H_2O_2. these are shown in Fig. 6. It is clearly seen that H_2O_2 is not electroactive at potentials less negative than those corresponding to the onset of the oxygen cathodic reduction at bare GCE.

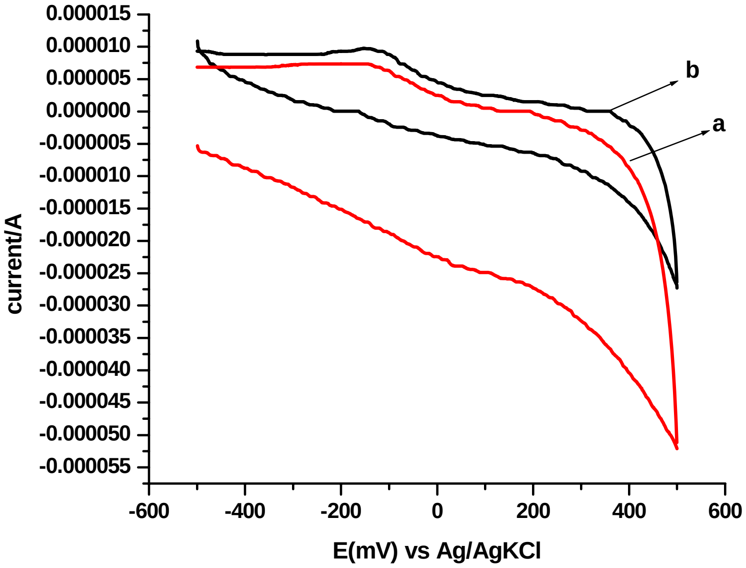


Fig.6. Cyclic voltammogram of bare GC (a) and bare GC +1mM H_2O_2 (b) in 0.1MKCl +0.1 M HCl

Fig.7. (a) shows cyclic voltamogram of the Prussian Blue modified glassy carbon electrode registered between -50 and 350 mV in a supporting electrolyte, 0.1MKCl + 0.1MHCl. One clear cathodic peak at158 mV and one clear anodic peak at 209 mV wave were shown. These peaks were attributed to the reversible redox interconversion of Prussian blue and Prussian white or the oxidized and reduced forms respectively. The ΔEp value was found 51 mV.

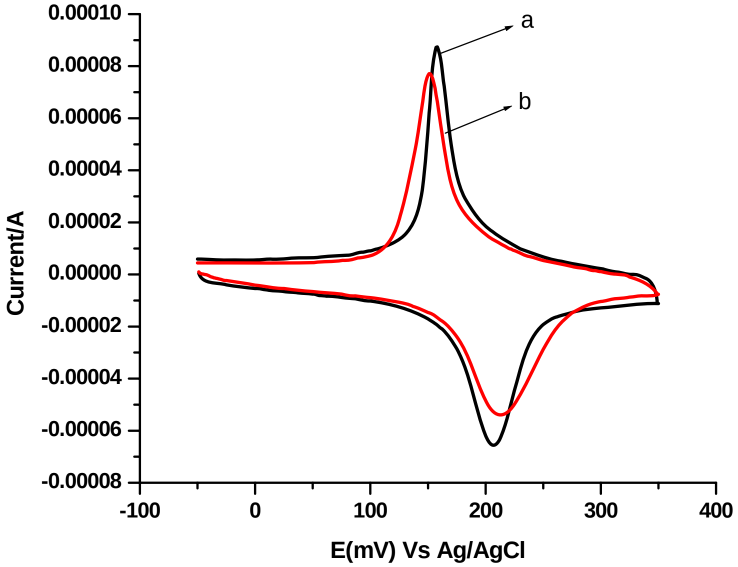


Fig.7. Cyclic voltammogram of a GC- electrode after electrodeposition of Prussian Blue registered between 350 and-50 mV (vs. Ag/AgCl) (a) in 0.1MKCl + 0.1MHCl and (b) in 0.05M phosphate buffer, pH=3, and 0.1M KCl: scan rate 50 mV/s

Fig7. (b) Shows the cyclic voltammogram run in the presence of 0.05M phosphate buffer, pH=3, and 0.1M KCl. Compared to (a), the half width of the wave became larger and the peak currents were significantly lower both for anodic (-64.76 µA to -57.55 µA) and cathodic (87.9 µA to 78.6 µA) waves. Additionally, the cathodic peak potential and anodic peak potential appears at 154mV and 216 mV respectively. As a result ΔEp was slightly increased from 51mV to 62 mV Vs Ag/AgCl (3MKCl). Despite these observations, when the PB film was cycled in this buffer medium an increase in stability were observed.

The cyclic voltammogram of the Prussian Blue modified glassy carbon electrode without H_2O_2 gave two peaks (cathodic and anodic) as is seen in Fig.8. In this potential range, there are no peaks on the bare GC electrode (with out H_2O_2).

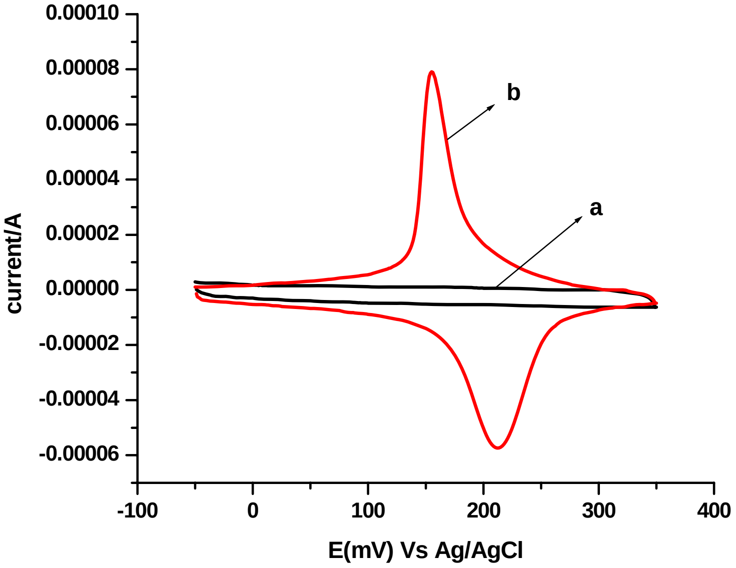


Fig.8. Cyclic voltammogram of PB-modified glassy carbon in 0.05 phosphate buffer + 0.1 MKCl, pH =3: between + 350 and -50mV (b) and bare GCE (a) with scan rate 50mV/s.

4.1.1. Dependence on pH

The effect of pH on the response of PB/GCE was investigated in the pH range 3-8 in the presence of 1mM H_2O_2 using cyclic voltammetry. As clearly shown in fig.9, when the pH varies from 3 to 8 the cathodic peaks of the CVs moves towards more negative potentials as the pH increases. The peak current decreases with a broadening of the peak and increase of the ΔEp. The cathodic peak current at pH 8 was decreased by 55.7% compared to the one at pH 3. This confirms that maximum current response is obtained in acidic media.

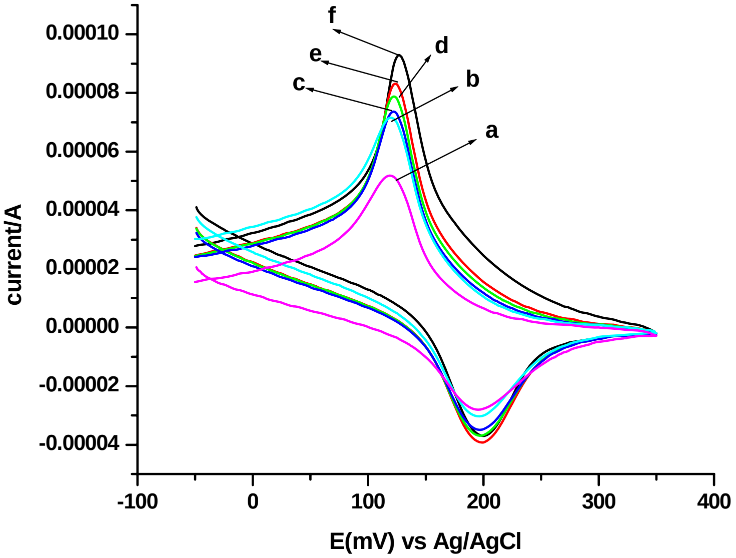


Fig.9. Cyclic voltammogram of 1mM H_2O_2 at PB/GCE in 0.05 M phosphate buffer + 0.1 M KCl , : between + 350 and -50 mV, as a function of pH a) 8, b) 7, c) 6, d) 5, e) 4. f) 3 with scan rate 50mV/s.

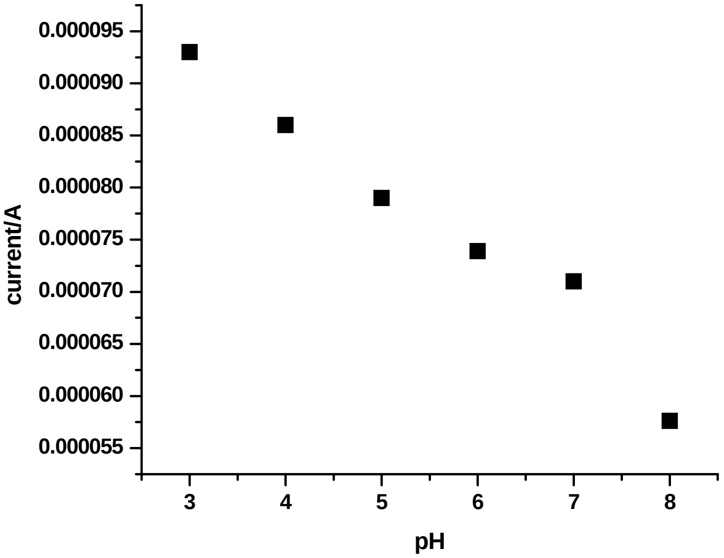


Fig.10 Plot of $i_{p,c}$ of 1mM H_2O_2 as a function of pH in 0.05 M phosphate buffer + 0.1 M KCl solution.

The graph of the cathodic peak current as a function of different pH at PB/GCE in 0.05M phosphate buffer + 0.1M KCl solution is shown in Fig.10. The cathodic peak current of 1 mM H_2O_2 has a high value in acidic media and a low value in basic media. Since the maximum current is achieved at pH =3, this pH was chosen through out this work.

4.1.2. Dependence on Concentration

The efficiency of the Prussian Blue deposited on the electrode was also tested in terms of hydrogen peroxide detection. As the concentration of hydrogen peroxide changed from 1μM to1mM, See Fig. 11, the cathodic peak current increases. But the reverse is true for the anodic peak current. More over, the cathodic peak potential decreased from160 mV to 150 mV.

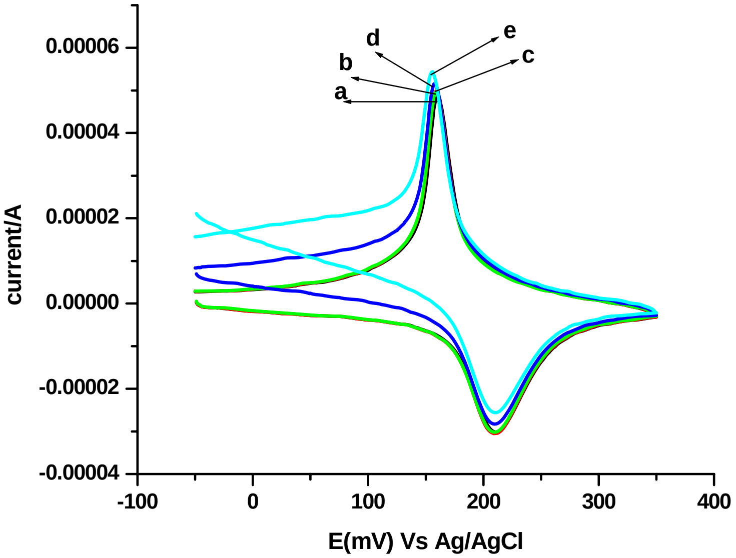


Fig.11 Cyclic voltammogram of PB-modified glassy carbon in 0.05 phosphate buffer + 0.1 MKCl, pH =3: between + 350 and -50mV. Different concentration of H_2O_2 a) 0 M, b) 10^{-6} M, c) 10^{-5} M, d) 10^{-4} M, e) 10^{-3} with scan rate 50mV/s.

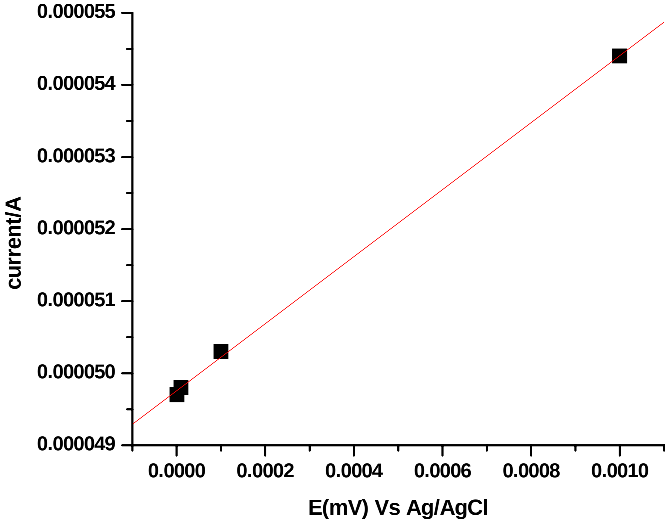


Fig.12. Plot of $i_{p,c}$ as a function of change in concentration at PB/GCE in 0.05M phosphate buffer solution (r = 0.99976).

A plot of $i_{p,c}$ values as a function of concentration of hydrogen peroxide in Fig.12 showed a linear dependence of $i_{p,c}$ on concentration. The detection limit was cal.4.5 X 10^{-6}.

4.1.3. The Combined Effect of pH and Concentration Change

Three different pHs'of 0.05M phosphate buffer solution and three different concentrations of hydrogen peroxide were tested to investigate any possible difference between their electrochemical, electrocatalytical and stability characteristics after being modified with electrodeposited Prussian Blue. The two important parameters compared were the difference between the anodic and cathodic peak potentials, ΔEp and cathodic current peaks, $i_{p,c}$.

Fig.13. represents cyclic voltammograms obtained from three different pHs' and three different concentration of hydrogen peroxide at the PB/GC electrode. The comparative investigation given below was performed based on glassy carbon electrode modified with PB through steps described in the procedure part (p. 20). At pH = 3, there was the significant increment of cathodic peak current, $i_{p,c}$ and ΔEp for change in concentration of hydrogen peroxide compared to pH = 5 and pH = 7 as clearly seen in the table. 1 below.

Table.1Varition of $i_{p,c}$ and ΔEp as a function of pH and concentration change

pH	Concentration					
	1 mM H_2O_2		3 mM H_2O_2		6 mM H_2O_2	
	$i_{p,c}$ (μA)	ΔEp(mV)	$i_{p,c}$(μA)	ΔEp(mV)	$i_{p,c}$(μA)	ΔEp(mV)
3	84.6	88	124.3	98	173.1	124
5	58.1	86 mV	67.9	92	74.6	94
7	54.2	61 mV	60.4	63	69.0	64

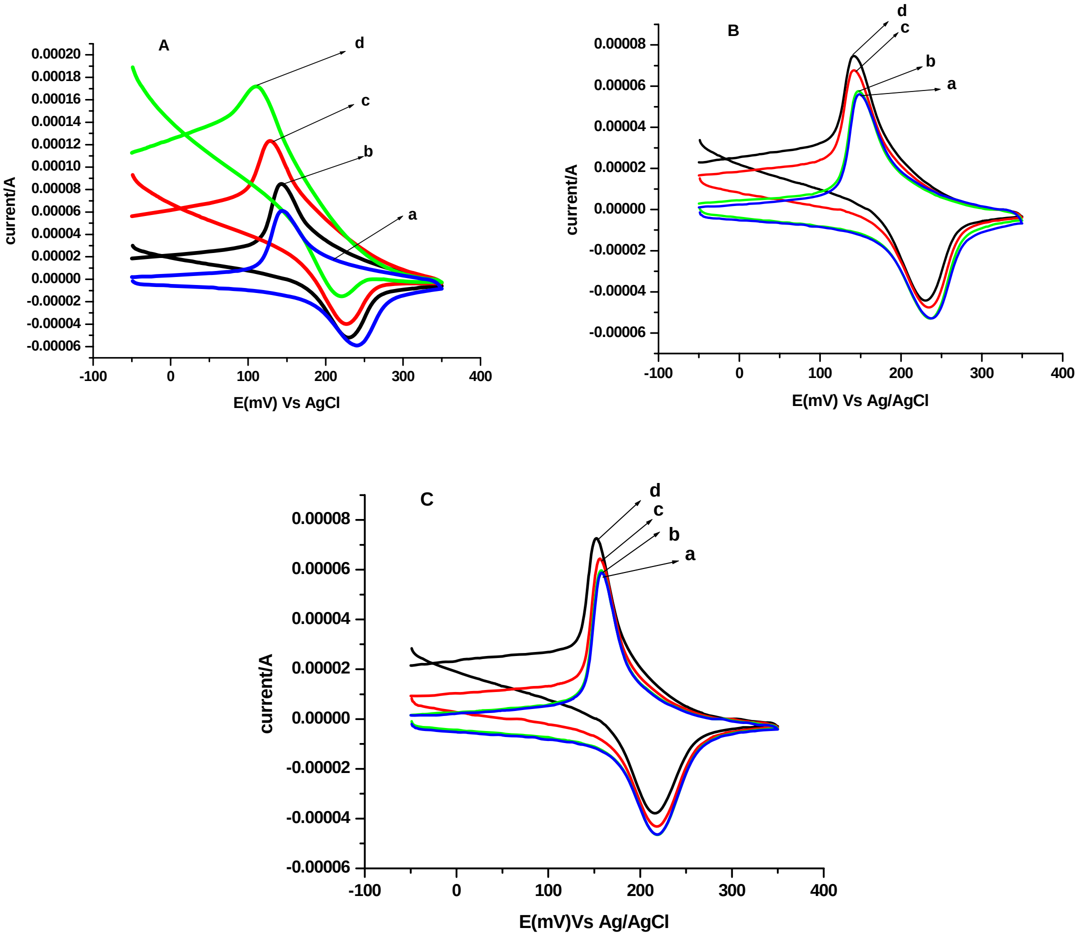


Fig.13. Cyclic voltammograms of PB-modified glassy carbon in 0.05 phosphate buffer, A) pH=3, B) pH=5 C) pH = 7 + 0.1 MKCl, a) 0 mM H_2O_2, b) 1 mM H_2O_2 c) 3 mM H_2O_2 d) 6 mM H_2O_2: between + -50 and 350mV with scan rate 50mV/s.

Even if the current obtained and the stability of PB/GCE at pH = 5 is less compared to that at pH = 3, it is better relative to pH = 7. At pH = 7, the appearance of lower $i_{p,c}$ describes that the PB layer is still well bound on the electrode surface. At all pHs' (pH = 3,5 and 7), when the concentration of added hydrogen peroxide increases an obvious increase in cathodic catalytic current and little decrease in $i_{p,a}$ were observed. The catalytic reduction mechanism of hydrogen peroxide has been described as follows in acidic media.

$$Fe^{III}_4[Fe^{II}(CN)_6]_3 + 4K^+ + 4e^- \leftrightarrow K_4Fe^{II}_4[Fe^{II}(CN)_6]_3\text{--------(5)}$$

$K_4Fe^{II}_4[Fe^{II}(CN)_6]_3 + 2H_2O_2 + 4H^+ \rightarrow Fe^{III}_4[(CN)_6]_3 + 4H_2O + 4K^+$ ---(12)

By combining Eq(5) and (12)

$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$ --------- (13)

In neutral solutions (pH = 7), how ever the over all mechanisms of hydrogen peroxide reduction has been given as [37].

$Fe^{III}_4[Fe^{II}(CN)_6]_3 + 4K^+ + 4e^- \leftrightarrow K_4Fe^{II}_4[Fe^{II}(CN)_6]_3$--------(5)

$K_4Fe^{II}_4[Fe^{II}(CN)_6]_3 + 2H_2O_2 \rightarrow Fe^{III}_4[Fe^{II}(CN)_6]_3 + 4OH^- + 4K^+$ …(14)

By combining Eq (5) and (14)

$H_2O_2 + 2e- \leftrightarrow 2OH^-$ ----------- (14)

The hydroxide ion dissolved the electroctalyst, Prussian white. As a result the film thickness and the stability of Prussian Blue decreased in basic media.

One possible reason for the decrease in electroactivity of PB and the increased ΔEp could additionally be caused by the less accessible K^+ needed for the redox reaction of PB. The electroactivity of PB on glassy carbon not only depends on the electron transfer rate between the electrode deposited layer of PB but also on the transfer, back and forth of K^+ ions needed to set the electroneutrality at the electrode surface and also taking an active part of the electrodeposited layer.

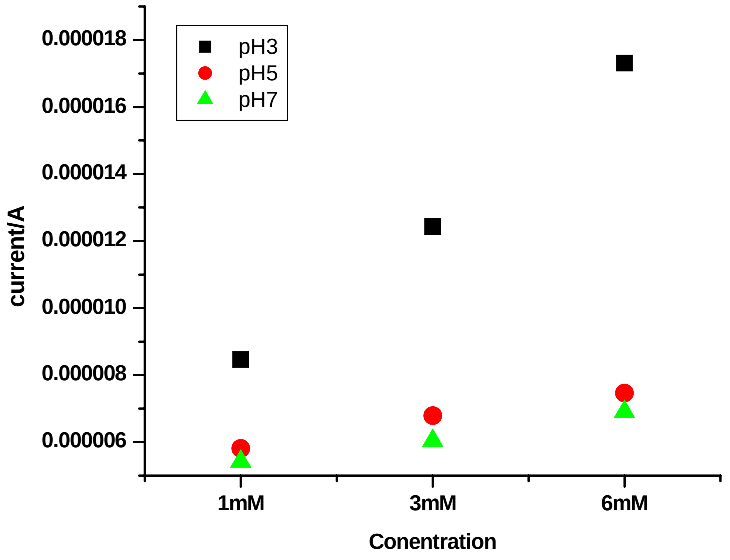


Fig.14. Plot of $i_{p,c}$ as a function of change in both pH and concentration at PB/GCE in 0.05M phosphate buffer solution.

Fig.14. Shows calibration data demonstrating the performance of PB/GCE for the reduction of different concentration of hydrogen peroxide at different pH of 0.05M phosphate buffer solution with 0.1M KCl. The loss of linearity for 6mM H_2O_2 at pH = 5 and pH=7 explains decreasing of the response of the deposited PB film as the pH of the solution increases.

4.1.4. The Effect of Scan Rate

As shown below in Fig.15 and table. 2 the cyclic voltammogram of 1mM H_2O_2 at the PB/GCE was ran at different scan rate. With increment of scan rates, the anodic and cathodic peak potentials shifted systematically resulting in a constant value of E^0.

Table.2 Relationship of scan rate and formal potential for 1mM H_2O_2 at PB/GCE in 0.05 M phosphate buffer solution, pH = 3.

Potential (mV)	50mV/s	75mV/s	100mV/s	125mV/s	150mV/s	175mV/s	200 mV/s
Ep,a	222	227	233	238	243	248	253
p,c	150	145	139	134	129	124	119
E^0	186	186	186	186	186	186	186

Although the formal potentials were independent of the scan rate, the peak potential separation (ΔEp) increased with increments of potential scan rates indicating a possible influence of the charge transfer kinetics.

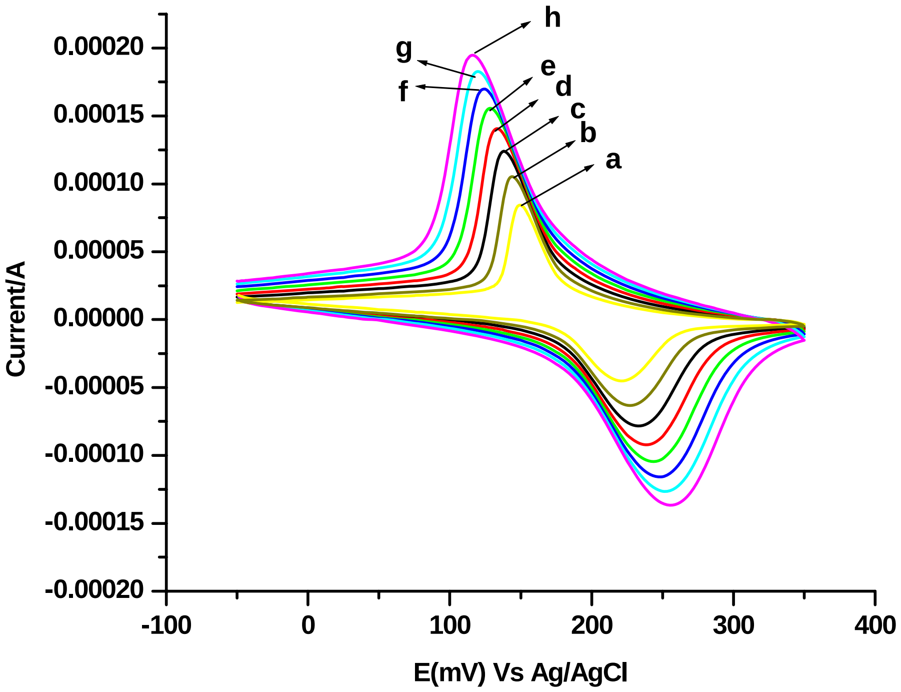


Fig.15. Cyclic voltammogram of PB-modified glassy carbon of 1mM H_2O_2 in 0.05 phosphate buffer + 0.1MKCl , pH =3: between + 350 and -50mV with scan rate a) 50, b) 75, c) 100, d) 125, e) 150, F) 175, g) 200, h) 225mV/s.

The linear increment of the $i_{p,c}$ with the square root of the scan rate describes that the reaction of hydrogen peroxide with Prussian Blue at the surface of PB/GCE is accurately coincided with the Randal-Sevick equation Eq.(9).

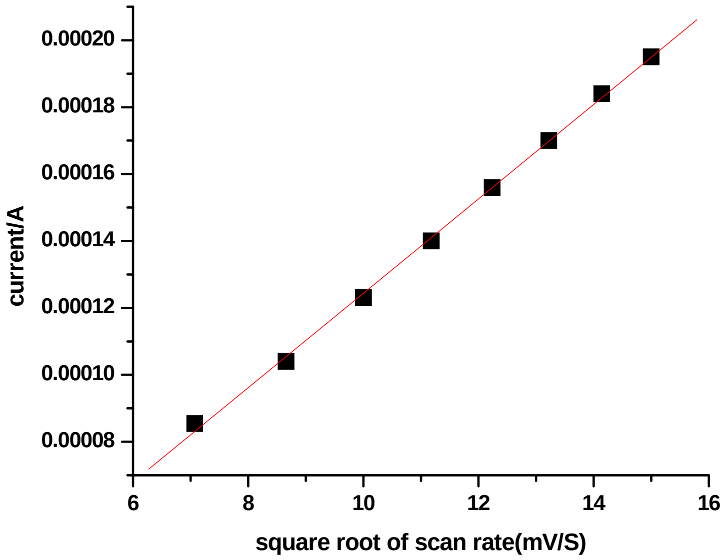


Fig.16. Plot of the cyclicvoltammogram of the reduction of 1mM H_2O_2 at the PB/GCE cathode peak current as a function of scan rate change in 0.05M phosphate buffer + 0.1MKCl solution. (r = 0.99942)

A plot of I_{pc} values as a function of the square root of scan rate in Fig.16 yielded a linear dependence up to 225mV/s with a slope of $1.41x10^{-5}$. Assuming Eq. (9) is applicable the diffusion coefficient of H_2O_2 was ca. 1.5×10^{-6} cm^2/s (n = 2) which compares with the literature value of 3.7×10^{-6} cm^2/s [38].

4.2. Optimization of Instrumental Parameters For DPV and SWV

Detection of analytes using electrochemical techniques depends on the parameters of the technique used. With regard to differential pulse voltammetry and square wave voltammetry detection of the analyte depend on parameters such as amplitude pulse and repeat time for differential pulse voltammetry and square wave amplitude, frequency and step potentials for square wave voltammetry.

4.2.1. Effect of Differential Pulse Parameters

4.2.1.1. Differential Pulse Amplitude

The effect of pulse amplitude was observed in Fig.17 as it varied from 20 mV up to 60 mV. Peak current increased with increasing of pulse amplitude. Because of instability of peak current above 60 mV, 50 mV was chosen for investigation.

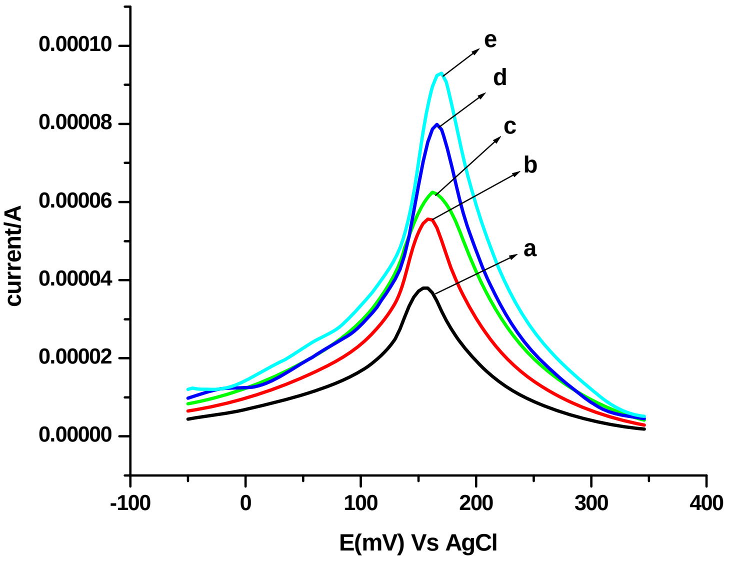


Fig.17. Plot of differential pulse voltammogram of 1mM H_2O_2 at PB/GCE in 0.05M phosphate buffer + 0.1M KCl, pH=3, at different amplitude a) 20, b) 30, c) 40, d) 50, e) 60.

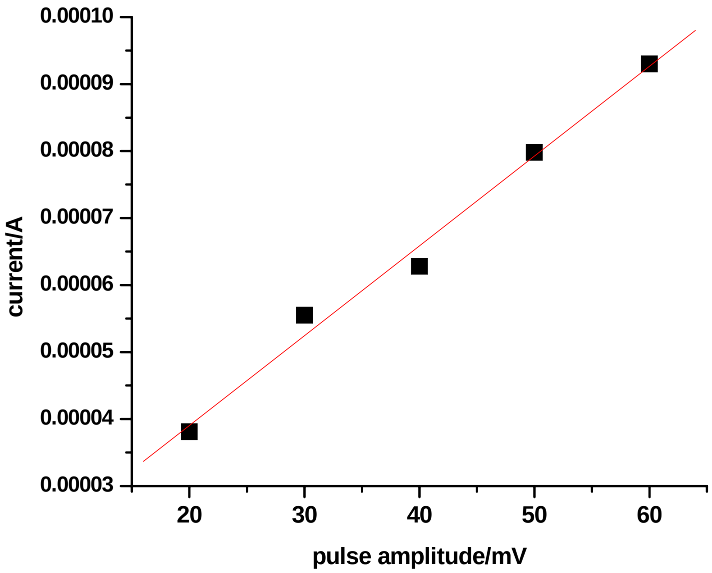


Fig.18. Plot differential pulse voltammetric of peak current as a function of pulse amplitude 1mM H_2O_2 at PB/GCE in 0.05 M phosphate of pH = 3 (r = 0.9945)

4.2.1.2. Pulse Repeat Time

As the pulse, time increases the peak current decreases. On the other hand a very low pulse time, the peak current increases accompanied by peak broadening. When the pulse repeat time is less than 0.5 s the peak current increases. As a result, a pulse repeat time of 0.5 s was chosen.

4. 2.1.3. Step Potential

The net current increases as the step potential increases. Thus, the optimum step potential chosen to maximize sensitivity and to obtain better peak symmetry was 4 mV.

4.2.1.4 Differential Pulse Voltammetry Investigation

The differential pulse voltammogram of 1mM H_2O_2 at Prussian Blue modified glassy carbon electrode as shown in Fig.19. The cathodic peak potential occurred at 182 mV. This was found to be greater than the peak observed using cyclic voltammetry, which was150 mV.

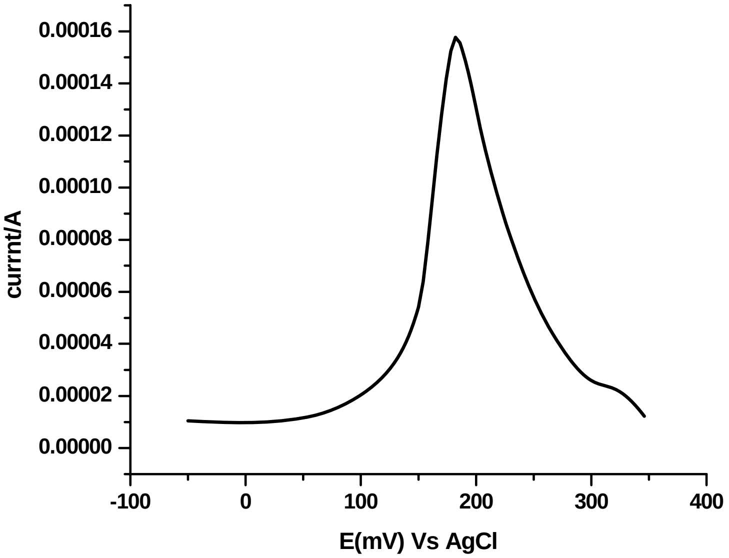


Fig.19. Plot of differential pulse voltammogramof 1mM H_2O_2 at PB/GCE in 0.05M phosphate buffer + 0.1 MKCl, pH=3.

4.2.2. Effect of Square Wave Parameters

4.2.2.1. Square Wave Frequency

By fixing a step potential of 4mV and an amplitude 50 mV in the 0.05M phosphate buffer solution of pH = 3 the effect of square wave frequency was studied and the results are shown in Fig.20 and 21.

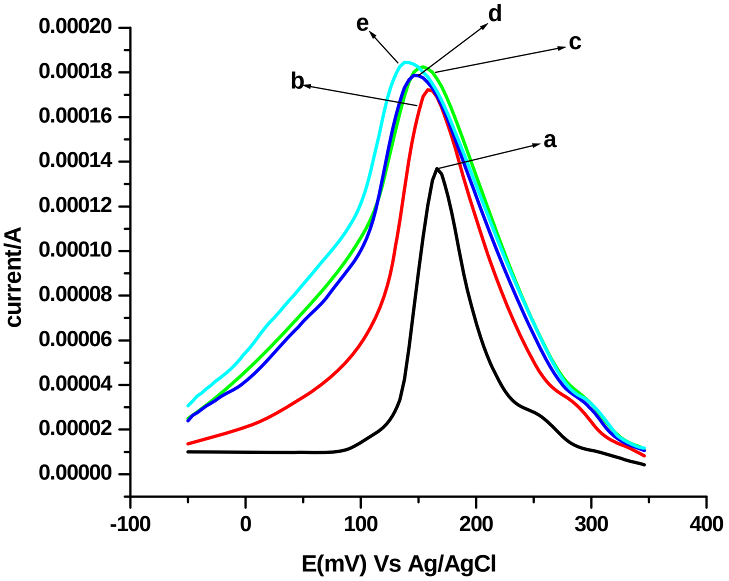


Fig.20 Plot of square wave voltammogram of 1mM H_2O_2 at PB/GCE in 0.05M phosphate buffer + 0.1MKCl, pH=3, at different frequency a) 5, b) 10, c) 15, d) 20, e) 25.

Even though the $i_{p,c}$ increased as the frequency increased from 5 HZ to 10 HZ, the value of peak current at 15 HZ is greater than at 20 HZ. The value of the peak current at 25 HZ was also greater than at 15 HZ. This illustrates that the peak current was unstable at higher frequency and influences the reproducibility of the measurement. Due to this, the selected square wave frequency for this experiment was 15 HZ.

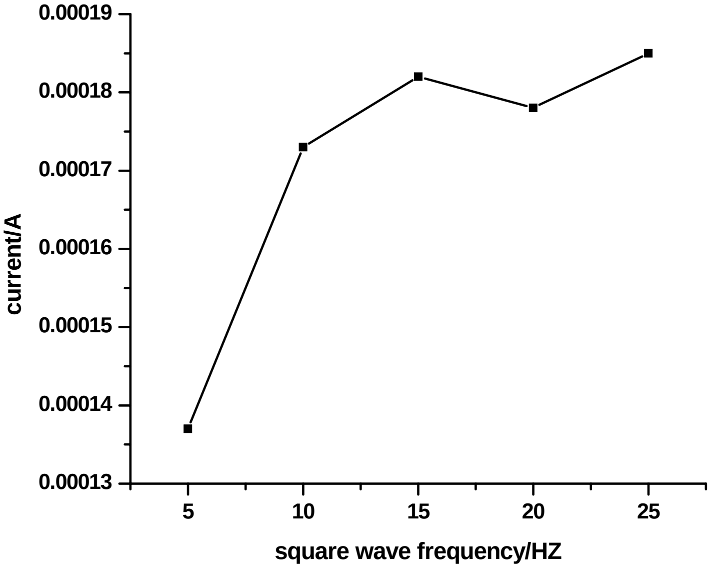


Fig.21. Plot of square wave voltammogram of peak current as a function of square wave frequency of 1mM H_2O_2 at PB/GCE in 0.05M phosphate buffer + 0.05M KCl, pH = 3.

4.2.2.2. Square Wave Amplitude

The effect of square wave amplitude on the peak current of 1mM H_2O_2 was studied by varying the square wave amplitude from 50 mV to 150 mV at frequency of 15 HZ and step potential of 4 mV.

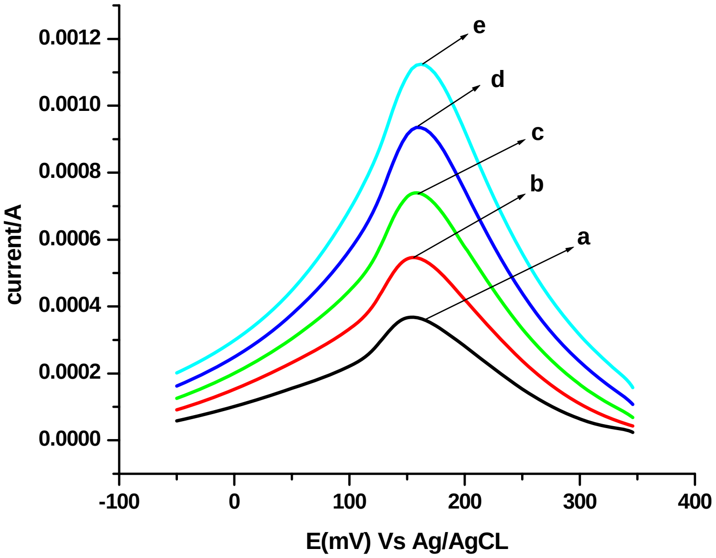


Fig.22. Plot of square wave voltammogram of 1Mm H_2O_2 at PB/GCE in 0.05M phosphate buffer + 0.1 M KCl, pH=3,at different amplitude a) 50, b) 75, c) 100, d) 125, e) 150

As shown in Fig.22 the peak current increased linearly with increasing of square wave amplitude. Thus, 50 mV chosen to perform the experiment.

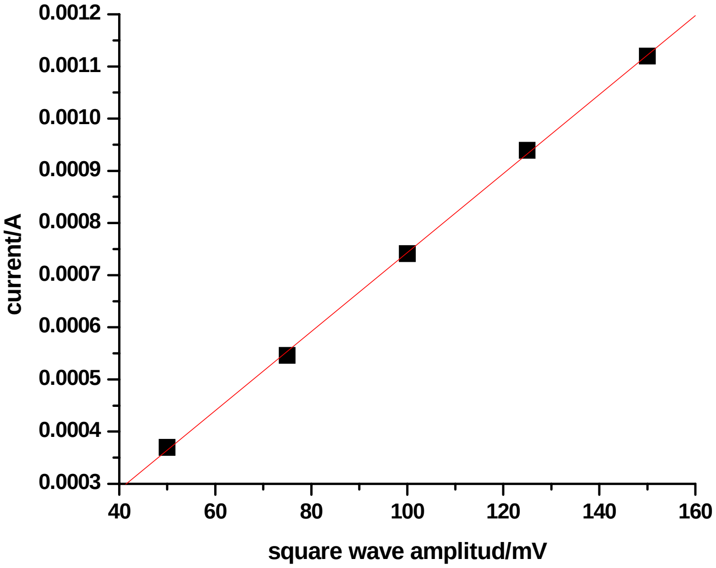


Fig.23. Plot of square wave voltammogram of peak current as a function of square wave amplitude of 1mM H_2O_2 at PB/GCE in 0.05M phosphate buffer + 0.1M KCl, pH =3 (r = 0.9998).

4.2.2.3 Square Wave Voltammetry Investigation

The square wave voltammogram observation Fig.24 of 1mM H_2O_2 at the modified PB/GCE in 0.05 M phosphate buffer and 0.1 MKCl, pH 3 gave a cathodic peak of 154 mV. This was investigated based on the parameters chosen already.

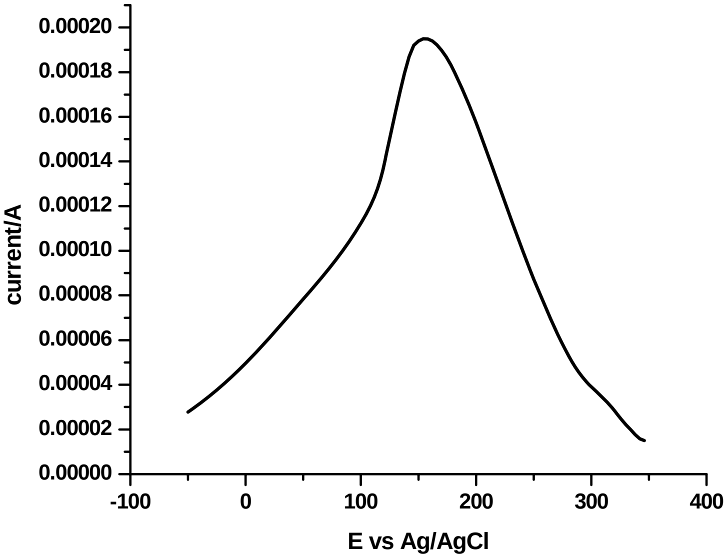


Fig.24. Plot of square wave voltammogramof 1mM H_2O_2 at PB/GCE in 0.05M phosphate buffer + 0.1 MKCl, pH=3.

5. CONCLUSION

Layers of PB were successfully electrodeposited on to glassy carbon electrode surfaces. The applicability of PB/GCE for detection of hydrogen peroxide was examined in different pH of 0.05M phosphate buffer solution. The results shown that PB gave better response for the detection of different concentration (1µM up to1mM) of H_2O_2 in acidic media than the neutral and basic ones. This gave a hint that the detection of hydrogen peroxide using PB in neutral and basic media was better to be replaced by other electrocatalysts, those were more stable than PB in neutral and basic media.

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