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Modification of Glassy Carbon Electrode by Polybromocresol using Cyclic Voltammetry as a Sensor and its Analytical Applications in Determination of Pyridoxine Hydrochloride in Commercial Drinks

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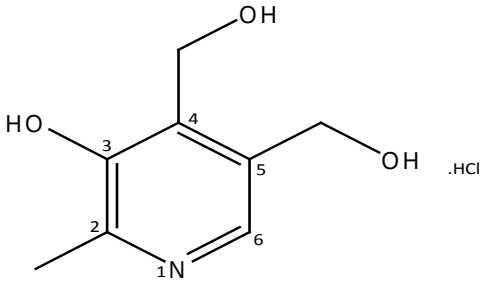
Abstract- Voltammetric behaviour of Pyridoxine hydrochloride (PHC) at bromocresol purple modified glassy carbon electrode (Poly BCP-GC) in phosphate buffers was used successfully for the analysis of PHC. Morphology of the electrode was characterised by atomic force microscopy. The effects of various parameters on the sensitivity of the method were investigated. Owing to the enhanced area and extraordinary characteristic surface of the electrode, the Poly BCP-modified glassy carbon electrode showed obvious electro-catalytic activity to the oxidation of PHC. The oxidation process was shown to be irreversible over the entire pH range studied (3.0-9.0) and was diffusion controlled. A possible electrooxidation mechanism was proposed. An intense peak was obtained with a pH value of 3.0, scan rate of 100mV/s, initial and final potential values were 0.4 V and 1.3 V. A sensitive linear voltammetric response for PHC was obtained in the concentration range of 2.0×10^{-8}–8.0×10^{-8} M and 2.0×10^{-8}–9.0×10^{-8} M with the detection limits of 6.28×10^{-9} M and 1.16×10^{-9} M for square wave voltammetric and differential pulse voltammetric techniques respectively. Analytical applications of this electrode have been studied for the determination of PHC in various pharmaceutical samples, commercial energy drinks and human blood serum. Due to its low detection limit, fast response, low cost and simplicity, this method proves to be advantageous over other methods.

Keywords- Pyridoxine hydrochloride, Poly bromocresol purple modified glassy carbon electrode, Differential pulse voltammetry , Square wave voltammetry

1. INTRODUCTION

Vitamins are the large group of essential organic compounds, required in minor quantities for the normal growth and functioning of human and animal bodies [1]. Pyridoxine Hydrochloride also known as Vitamin B6 or 4, 5-bis(hydroxymethyl)-2-methylpyridin-3-ol. HCl (PHC) and it's structure is as shown in Scheme 1.



Scheme 1. Structure of Pyridoxine Hydrochloride

PHC is a water soluble vitamin, which also acts as coenzyme for the transfer of amino acids [2], helps in metabolism of glycogen and in the syntheses of neurotransmitters serotonin, dopamine and norepinephrine [3-5]. Deficiency of Vitamin B_6 leads to many skin related problems, certain types of anaemia, nervous system changes [6]. A higher dose of PHC helps to reduce the risk of kidney stone formation in women [7]. Hence the assessment of PHC status in biological, environmental and pharmaceutical compounds, gains more attention and importance. Since it is difficult to determine the water soluble vitamins in various samples due to their chemical instability and complexity of matrices [8], a suitable method to overcome such difficulties has to be developed which would be advantageously simple, sensitive, stable, fast and less time consuming.

Greater attention is being given to the construction and application of modified electrodes in relation to their enhanced sensitivity and selectivity in electrochemical techniques. Electro-polymerisation has been an efficient technique for depositing polymers as it offers direct control over the thickness of the film, permeation and charge transport characteristics [9]. Such modified electrodes show more enhanced reproducibility, stability, homogenous deposition, rise in number of active sites and strong adherence to the surface of electrode [10]. Bromocresol purple (BCP) forms a polymer film (poly (bromocresol purple)) on the surface of the glassy carbon electrode by electrochemical method. This modified electrode was used to establish a novel electrochemical method for the determination of PHC. Already some methods have been developed for the determination of PHC, which include chemiluminescence [11], flow injection spectrophotometry [6] and high performance liquid chromatography [12]. In addition to these costlier methods, voltammetric methods have

proved to be more efficient, economical and reliable. A few voltammetric studies based on modified electrodes were also studied for the determination of PHC [13-16].

The present work aims to study the oxidative property and mechanism of PHC, influence of various parameters such as, effect of pH, scan rate, interferents study and assay of PHC at Poly BCP-GC electrode. When compared with earlier methods, the present work has many advantages such as lower detection limit, better reproducibility and simplicity. The proposed method has been applied successfully for the determination of PHC in pharmaceutical tablets and energy drinks.

2. EXPERIMENTAL

2.1. Reagents and chemicals

All the solutions used during the course of experiments were prepared using double distilled (DD) water and chemicals were of analytical grade reagents. The standard stock solution of PHC (1.0 mM) (Sigma Aldrich) was prepared carefully by dissolving 20.56 mg in 100 mL of DD water. This solution was further diluted as and when required. Phosphate buffer solutions (PBS) ranging from 3.0-9.0 pH were prepared by dissolving appropriate amount of KH_2PO_4, Na_2HPO_4, Na_3PO_4, H_3PO_4 [17,18]. Poly BCP-GC electrode was prepared by dissolving BCP in pH 6.0 PBS. Tablets, energy drinks containing PHC were commercially available and were purchased from local markets.

2.2. Instrumentation

All voltammetric measurements were performed with a CHI 630D electrochemical analyzer (CH Instrument Inc., USA). The experiment was carried out using a conventional three electrode system (glass electrochemical cell, 10 mL), using poly BCP-GC modified electrode as a working electrode, Ag/AgCl/3.0 M KCl as reference electrode, a platinum wire as auxiliary electrode. All potentials were given against the Ag/AgCl/3.0 M KCl. Characterisation and surface morphologies were studied using atomic force microscopy (AFM) images using Nanosurf® Easyscan 2, Switzerland. pH measurements were carried out with Elico LI120 pH meter (Elico Ltd., India).

2.3. Preparation of poly(bromocresol purple) modified glassy carbon electrode (Poly BCP-GC)

Bare glassy carbon electrode (Ø=3 mm) was polished on smooth polishing pad using 0.3 µm Alumina powder to get a shiny mirror surface. This was followed by ultrasonication in 0.5 M H_2SO_4. After pretreatment, the GC was subjected to 20-cycles by cyclic voltammetry, between the potential range of -0.4 V to 1.8 V at a scan rate of 100 mV/s in solution

containing 0.5 mM of bromocresol purple in 0.2 M phosphate buffer (6.0 pH). Hence poly (BCP) film was electrochemically deposited on the GC.

2.4. Area of electrode

The active surface area of the electrode, was obtained by cyclic voltammetric method using 1.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl by recording current voltage curves at different scan rates. The active surface area of modified and unmodified electrodes was determined with the help of Randles-Sevcik equation(1) [19]

$$I_{pa} = 0.4463\ (F^3/RT)^{1/2} n^{3/2} A D_0^{1/2} C_0 v^{1/2} \quad (1)$$

where 'I_{pa}' refers to the anodic peak current, 'n' is the number of electrons transferred, 'A' is the surface area of the electrode, 'D_0' (7.6×10^{-6} cm^2s^{-1}) is diffusion coefficient, 'F' (96,480 $Cmol^{-1}$) is Faraday constant, 'R' (8.314 $JK^{-1}mol^{-1}$) is gas constant, 'υ' is the scan rate, and 'C_0' is the concentration of $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte, 'T'=298 K, n=1. The area of modified electrode was calculated to be 0.937 cm^2, which is much higher than the area of bare GC 0.0462 cm^2. This indicates the enhanced electrocatalytic nature of modified electrode.

2.5. Sample preparation

Five tablets of three different brands (Supradyn®, Becosules capsules, Neurobion Forte) were weighed and crushed to fine powder and 100 mg of this was dissolved in 100 mL of double distilled water and sonicated to prepare a homogenous stock solution. This stock solution was further diluted to the extent where the concentration of PHC falls in the range of our calibration plot.

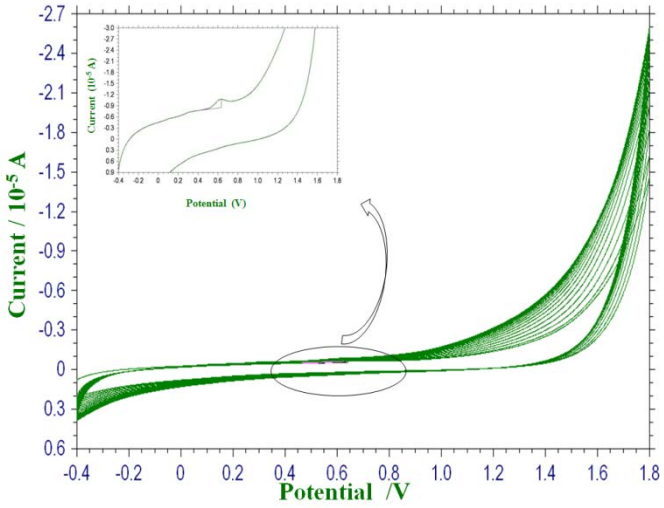
Bourn Vita (health drink), Tang lemon (instant mix drink), Protinex powder (health drink) and PediaSure (health drink for children) packets were purchased from local stores. The powder was dissolved in water and centrifuged for 10 min at 1500 rpm. Then the supernatant liquid was extracted and diluted with DD water. This solution was transferred into the voltammetric cell and analysed without any further pretreatment.

Human blood samples were collected from healthy volunteers in dry evacuated tubes. The blood is kept undisturbed for clotting and then centrifuged for 10 min at 1500 rpm to separate the clot. Carefully the supernatant liquid (2.5 mL) is extracted and diluted to 25 mL using phosphate buffer solution (pH 3.0). This diluted sample solution was further transferred to the voltammetric cell for the analytical studies.

3. RESULTS AND DISCUSSION

3.1. Growth of poly(bromocresol purple) film on glassy carbon electrode

Fig. 1 shows the cyclic voltammograms obtained during the electrochemical growth of (0.5 mM) poly(bromocresol purple) film in 0.1 M phosphate buffer solution (pH 6.0) on glassy carbon electrode. During the first cycle, an oxidation peak was observed at 0.62 V which reduces with subsequent peaks indicating that oxidation of BCP is nonreversible and self-limiting [20]. Decreasing anodic peak current shows the formation of polymer film on the surface of the electrode [21,22]. Fig. 1B shows the modification steps of the electrode.



(a)

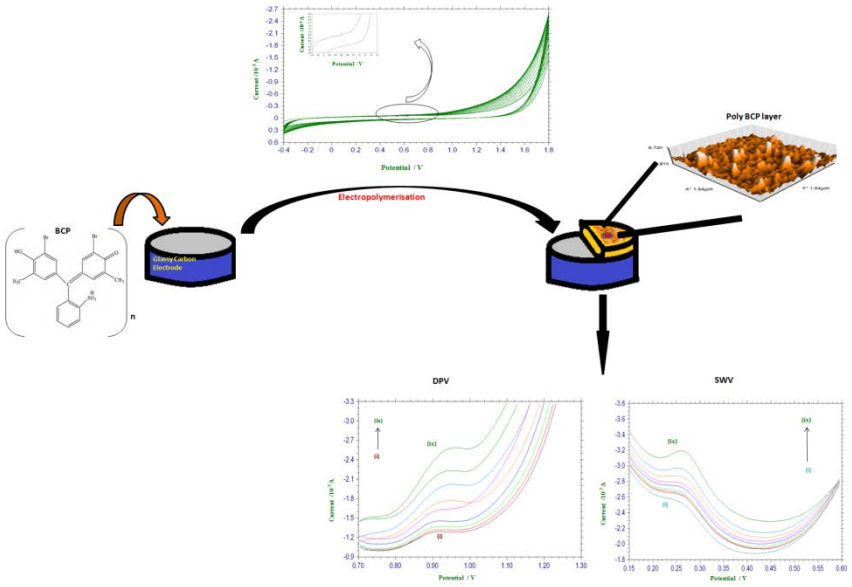


Fig. 1. A Cyclic voltammograms obtained during polymerisation of BCP on glassy carbon electrode with 20 cycling number in the potential range of -0.4 to 1.8 V at a scan rate of 100 mVs^{-1}.Inset: The cyclic voltammogram of the first cycle is zoomed; **B** Schematic representation of the modification of electrode

3.2. Optimization of number of cycles

The response of poly BCP-GC modified electrode was found to be increased with the increase in the number of cycles (Fig. 1). The highest peak current was observed by applying 20 cycles in the electropolymerisation. Therefore the optimum number of polymerisation cycles was found to be 20.

3.3. Morphological Characterisation of poly BCP-GC

Atomic Force Microscopy (AFM) was used for the characterisation of poly BCP-GC. Fig. 2 (a,b,c) show AFM of BCP and Fig. 2 (d,e,f) show AFM of poly BCP film. All the AFM measurements were made with the scan range of 5 µm in x-y directions. From AFM topographs, it was observed that roughness of poly BCP film was more enhanced compared to BCP. Fig. 2(d) showed better surface texture than Fig. 2(a), surface texture includes waviness, roughness, lay and flaws.

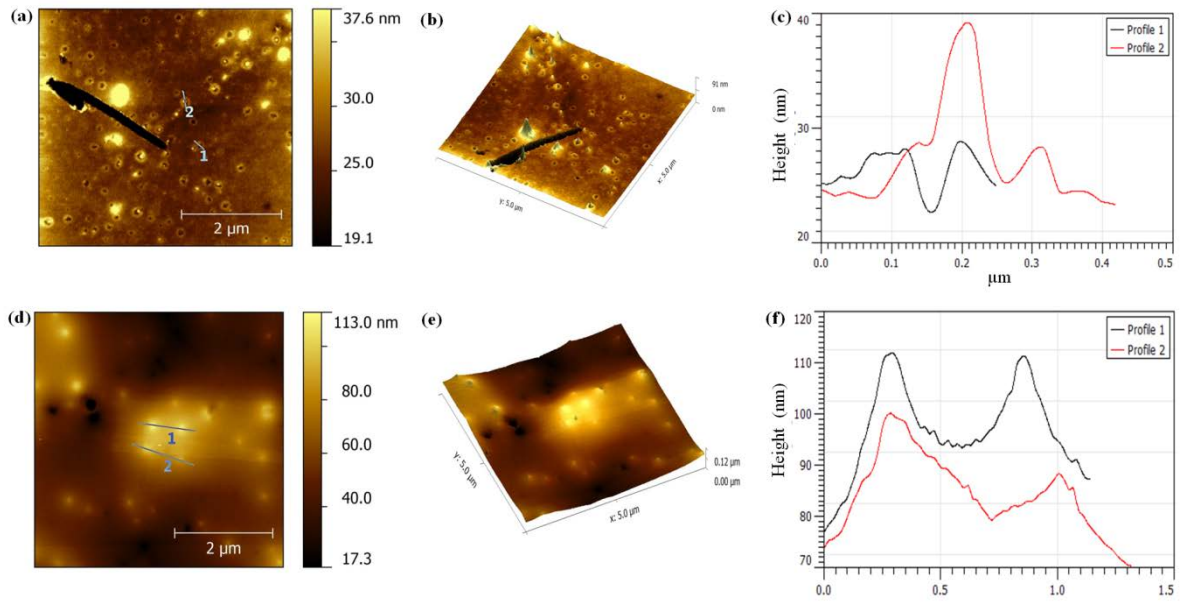


Fig. 2. AFM topographical images: **(a,d)** 2D images of BCP and Poly BCP; **(b,e)** 3D images of BCP and Poly BCP; **(c,f)** 2D graphs . **(a,b,d,e)** scan size 5 µm X 5 µm; **(a,b)** z range 91 nm and **(d,e)** z range 120 nm

The AFM images were used to calculate the root mean square roughness (R.M.S. roughness) which represents the standard deviation of the heights of different topographical images; it was calculated using equation (2) [23]:

$$\text{R. M. S. roughness} = \sqrt{\frac{1}{n-1}\sum_{selection}\left(Z_i - Z_{average}\right)^2} \qquad (2)$$

where 'Z_i' is the height at the 'i' point in an image, '$Z_{average}$' is the average of the 'Z' values at various 'i' points and it was calculated using equation (3):

$$Z_{aveargе} = \frac{1}{n}\sum_{selection} Z_i \quad (3)$$

'n' is the number of 'i' points in the image. R.M.S. roughness value for BCP was 5.28 nm, which was almost half the roughness value of 11.24 nm for poly-BCP. Greater roughness of active surface area of corresponding electrode promises higher sensitivity and lower detection limit.

3.4. Cyclic Voltammetric behaviour of PHC

The cyclic voltammetric behaviour of PHC in pH 3.0 phosphate buffer solution has been represented diagrammatically in Fig. 3, which unveils the electro-catalytic effect of poly BCP-GC. The modified electrode showed no electrochemical activity in blank buffer solution (supporting electrolyte) (Fig. 3 (curve a)), whereas Fig. 3(curve b), (curve c) show the cyclic voltammetric profile of 1.0 mM PHC at bare GC and poly BCP modified GC electrodes, respectively.

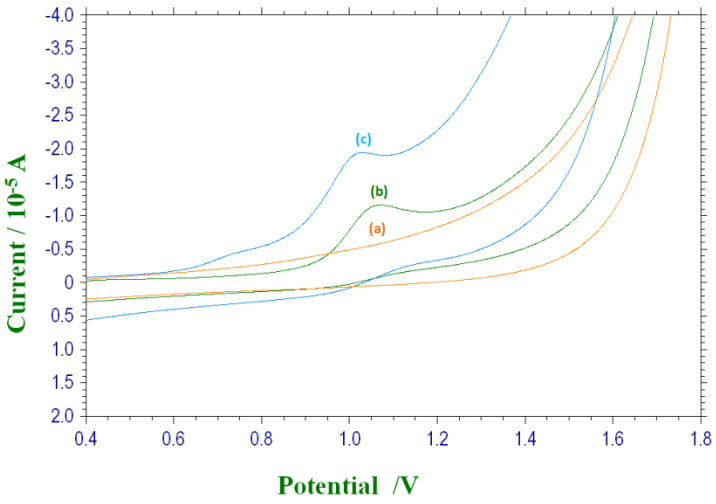


Fig. 3. Cyclic voltammograms with pH 3, 0.2 M PBS, at scan rate of 100 mV s^{-1} **(a)** Poly BCP-GC with Buffer solution only **(b)** bare-GC with 1.0 mM PHC **(c)** Poly BCP-GC with 1.0 mM PHC

An intense and well-resolved peak with a strong increased peak current was observed at 1.046 V for poly BCP-GC (Fig. 3(curve c)) in the potential range of 0.4 V to 1.8 V. In the same potential range, a less intense oxidation peak with lesser peak current was observed for bare GC. However a weak oxidation peak cannot be used for quantitative determination [24]. The remarkable increase in the peak current for poly BCP-GC undoubtedly testifies the

electrocatalytic behaviour of the modified electrode which indicates the improved sensitivity on account of its unusual structure and properties such as larger surface area, subtle electronic properties. In the reverse scan, no reduction peak was observed, showing that the oxidation of PHC is an irreversible process.

3.5. Influence of pH

The influence of pH on the electrochemical responses of PHC was investigated in the pH range of 3.0-9.0 (Fig. 4(a)). The pH of the solution influenced the peak current. The results indicated pH 3.0 as optimum pH value (Fig. 4(b)). Henceforth all the experimental measurements were carried out in this pH. Optimizing the pH value facilitates the determination of number of electrons and protons involved in the electrooxidation of PHC.

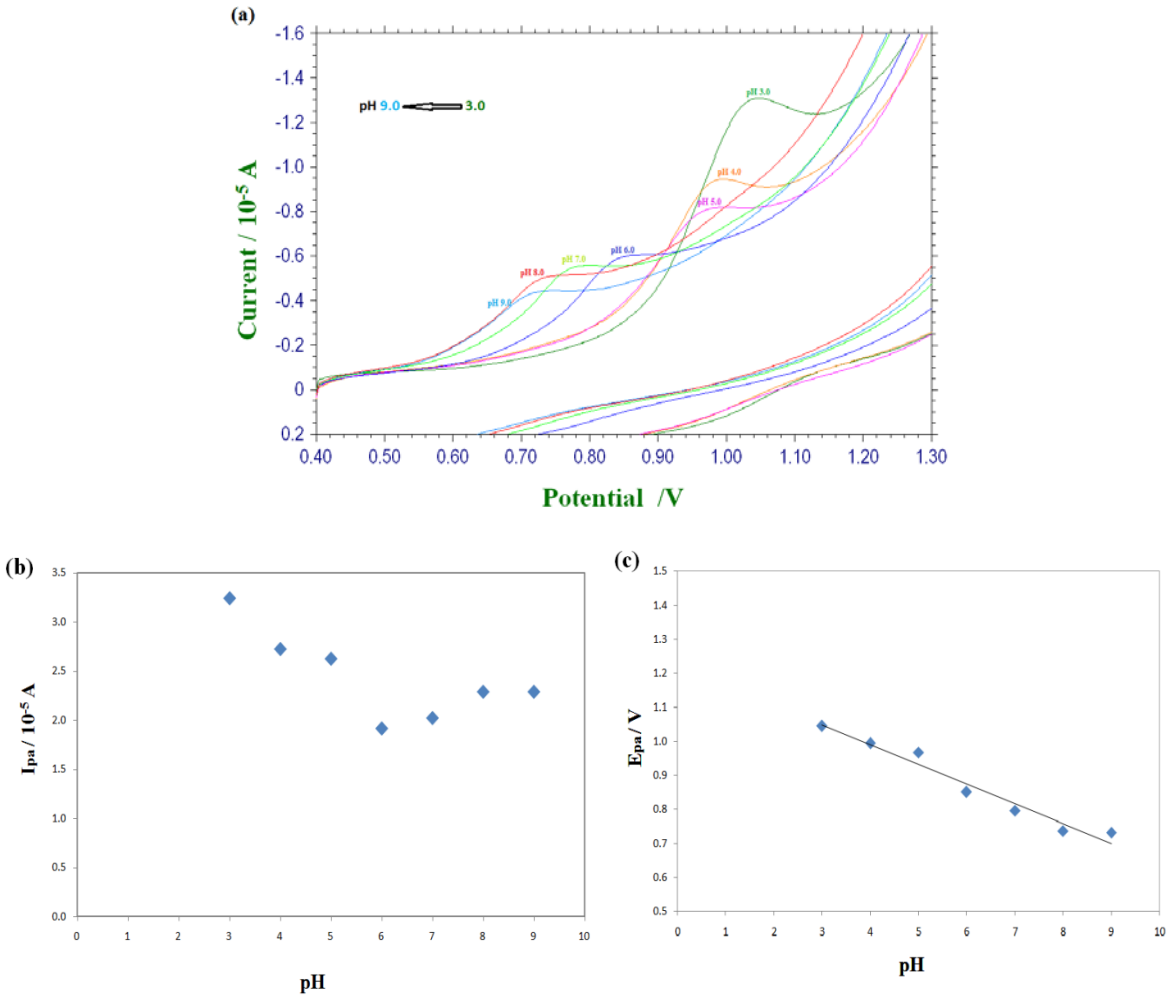


Fig. 4. (a) Effect of pH on the shape of the peaks in phosphate buffer solution at pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 with potential scan rate 100 mV s^{-1} for 1.0 mM PHC; **(b)** Variation of oxidation peak current with pH; **(c)** Effect of pH on the peak potential of 1.0 mM PHC

The peak potential (E_{pa}) shifted linearly towards less positive values with the increase in pH of supporting electrolyte and the linear relation between E_{pa} and pH (Fig.4(c)) is expressed as,

$E_{pa}(V)$= -0.058pH+1.224 r=0.961

The slope value 58.0 mV/pH obtained is nearer to the theoretical value of 59 mV/pH indicated that the number of electrons and protons involved in the reaction are equal according to Nernst equation [25,26].

3.6. Influence of scan rate

In order to characterise the modified electrode, some kinetic parameters such as number of electron transfer (n), electron transfer coefficient (α), and heterogeneous rate constant (k^0) were evaluated by varying the scan rates from 50-450 mVs^{-1}. The electrochemical behaviour of PHC at poly BCP-GC at different scan rates was studied using cyclic voltammetry (Fig 5(a)).

Fig. 5(b) shows a proportional increase in the anodic peak current with the square root of scan rate at lower sweep potentials suggesting diffusion controlled mechanism [27,28] in the present study. Whereas at higher scan rates, the plots curve off indicating that the electron transfer becomes rate determining [29]. The diffusion controlled mechanism is further confirmed by a double logarithmic plot of logarithm of peak current *vs* logarithm of scan rate (Fig 5(c)) with the linear equation given as,

$\log I_p = 0.561 \log v - 0.933$

The slope value of 0.561 was nearly equal to the theoretical value of 0.5 which indicated that the electrooxidation of PHC was diffusion controlled [27,30,31].

The peak potentials increased linearly with increase in logarithm of scan rates as shown in the Fig. 5(d). According to the Laviron equation [32], for an irreversible process E_{pa} is expressed by equation (4):

$$E_{pa} = E^0 + \left(\frac{2.303RT}{\alpha nF}\right)\log\left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right)\log v \quad (4)$$

where, 'n' the number of electrons transferred, 'α' is the transfer coefficient, '*v*' is the scan rate, 'k^0' is standard heterogeneous rate constant of the reaction and 'E^0' is the formal standard redox potential. Other symbols have their usual meaning. Thus the value of αn was calculated from the slope of the graph E_{pa} *vs.* log *v* (Fig. 6(d)) as 1.792 by taking R=8.314 $JK^{-1}mol^{-1}$, T=298 K, and F=96480 C mol^{-1}. According to Bard and Faulkner [33], α value was calculated using equation (5):

$$\alpha = \frac{47.7}{E_{pa} - E_{pa/2}} \text{mV} \quad (5)$$

where '$E_{pa/2}$' is the potential obtained when the current is half the peak value, thus the 'α' value obtained was 0.468 mV.

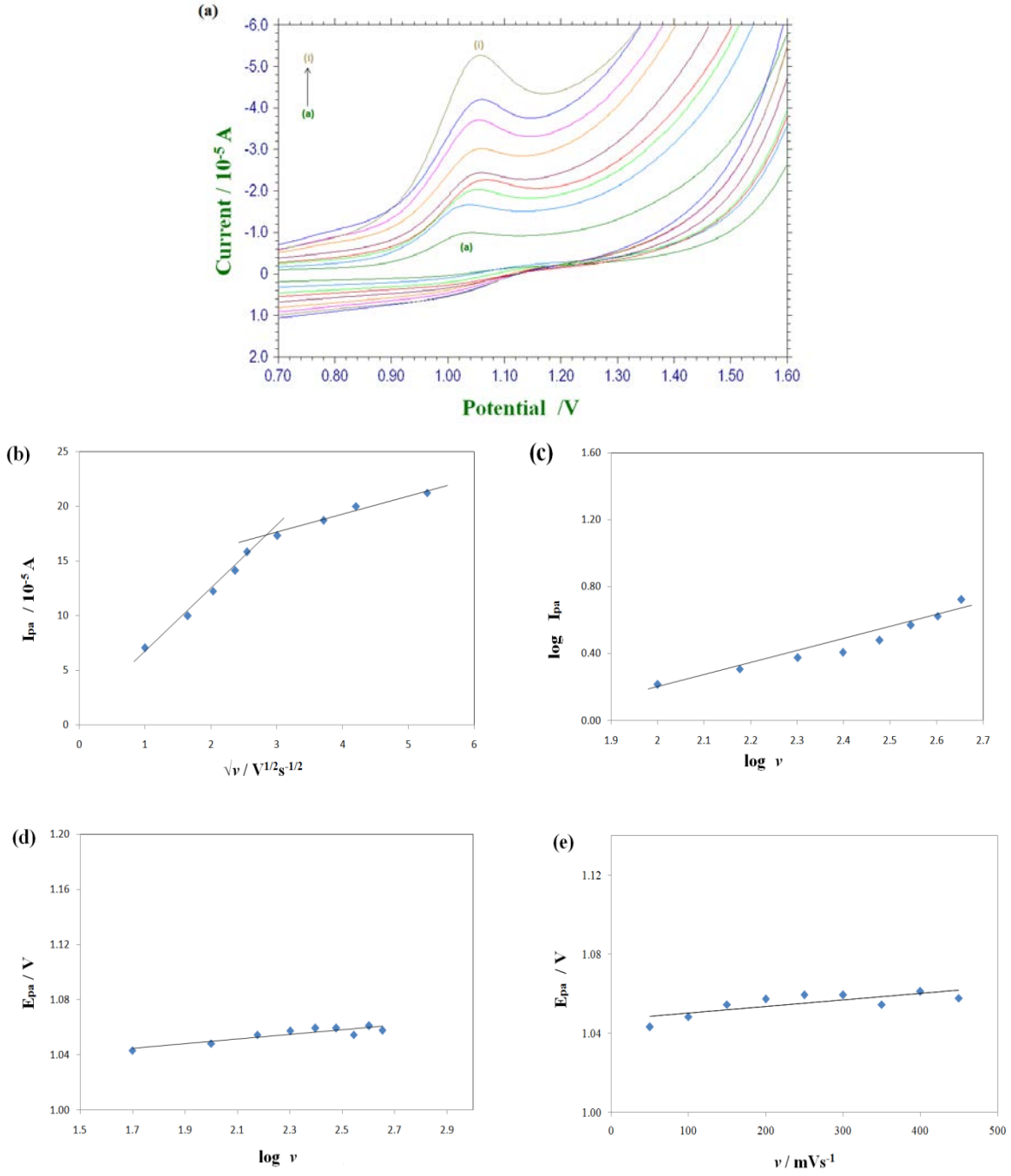
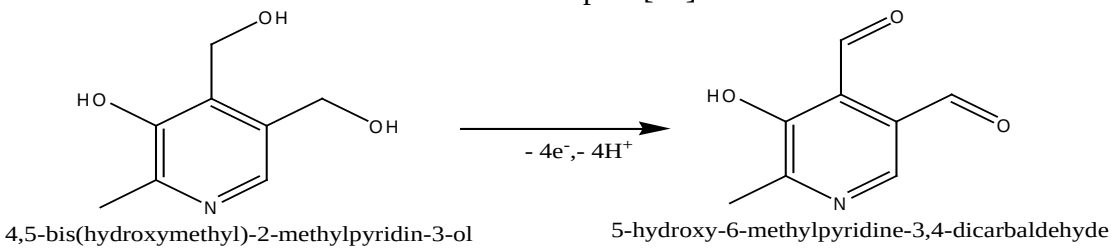


Fig. 5. (a) Cyclic voltammograms obtained for 1.0 mM PHC in PBS of pH 3.0 at scan rates of (a) 0.05, (b) 0.1, (c) 0.15, (d) 0.2, (e) 0.25, (f) 0.3, (g) 0.35, (h) 0.4 and (i) 0.45 Vs^{-1}; **(b)** Dependence of the oxidation peak current on the square root of scan rate; **(c)** Dependence of the logarithm of peak current on logarithm of scan rate; **(d)** Relationship between peak potential and logarithm of scan rate; **(e)** Relationship between peak potential and scan rate

By knowing the 'αn' and 'α' values, the value of 'n' was calculated as 3.829≈4. k^0 value was calculated as 1.615 s^{-1} from the intercept of Fig. 5(d) by knowing the 'E^0' value. 'E^0' value was obtained from the intercept of the graph of peak potential *vs* scan rate (Fig. 5(e)), extrapolated to *v*=0 [33,34].

3.7. Mechanism

As evidenced from the above results, the number electrons and protons transferred during the electrochemical oxidation were found to be equal, and the number of electrons was calculated as four. Hence the mechanism is proposed as depicted in Scheme 2. Two primary alcoholic groups attached to 4th and 5th carbon atoms of pyridoxine molecule get oxidised to aldehyde giving 5-hydroxy-6-methylpyridine-3,4-dicarbaldehyde as oxidation product. This observation was in accordance with the earlier report [35].



Scheme 2. Probable mechanism for the electrooxidation of PHC

3.8. Linear range and detection limits

In order to develop a voltammetric method for determining PHC, the Differential pulse voltammetric (DPV) and Square wave voltammetric (SWV) techniques were selected as the peaks obtained were sharper and well defined at lower concentrations of PHC than those obtained by CV, with the lower background current, resulting in improved resolution. Fig. 7(a) and (b) represent the voltammograms obtained in DPV and SWV techniques respectively for different concentrations of PHC at poly BCP-GC electrode.

Peak current *vs* [PHC] calibration plots (Fig 6(c), (d)) showed good linearity in the range of 2.0×10^{-8}- 9.0×10^{-8} M and 2.0×10^{-8}-8.0×10^{-8} M for DPV and SWV respectively. The linear regression equations for the calibration curves were given as

I_{pa} (10^{-7} A) = 0.069 ([PHC]×10^{-8}) + 1.043 for DPV and
I_{pa} (10^{-7} A) = 0.047 ([PHC]×10^{-8}) + 2.422 for SWV.

Characteristics of the calibration curves obtained for PHC at poly BCP-GC using DPV and SWV have been shown in Table 1. The deviation of the linearity at higher concentrations was attributed to adsorption of either PHC or its oxidation product on to the surface of

electrode. The values of limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following equations

$$\mathrm{LOD} = 3\,\mathrm{s/m} \qquad \mathrm{LOQ} = 10\,\mathrm{s/m}$$

Here, 's' is the standard deviation of intercept of the calibration curves and 'm' is the slope of the calibration curves [36].

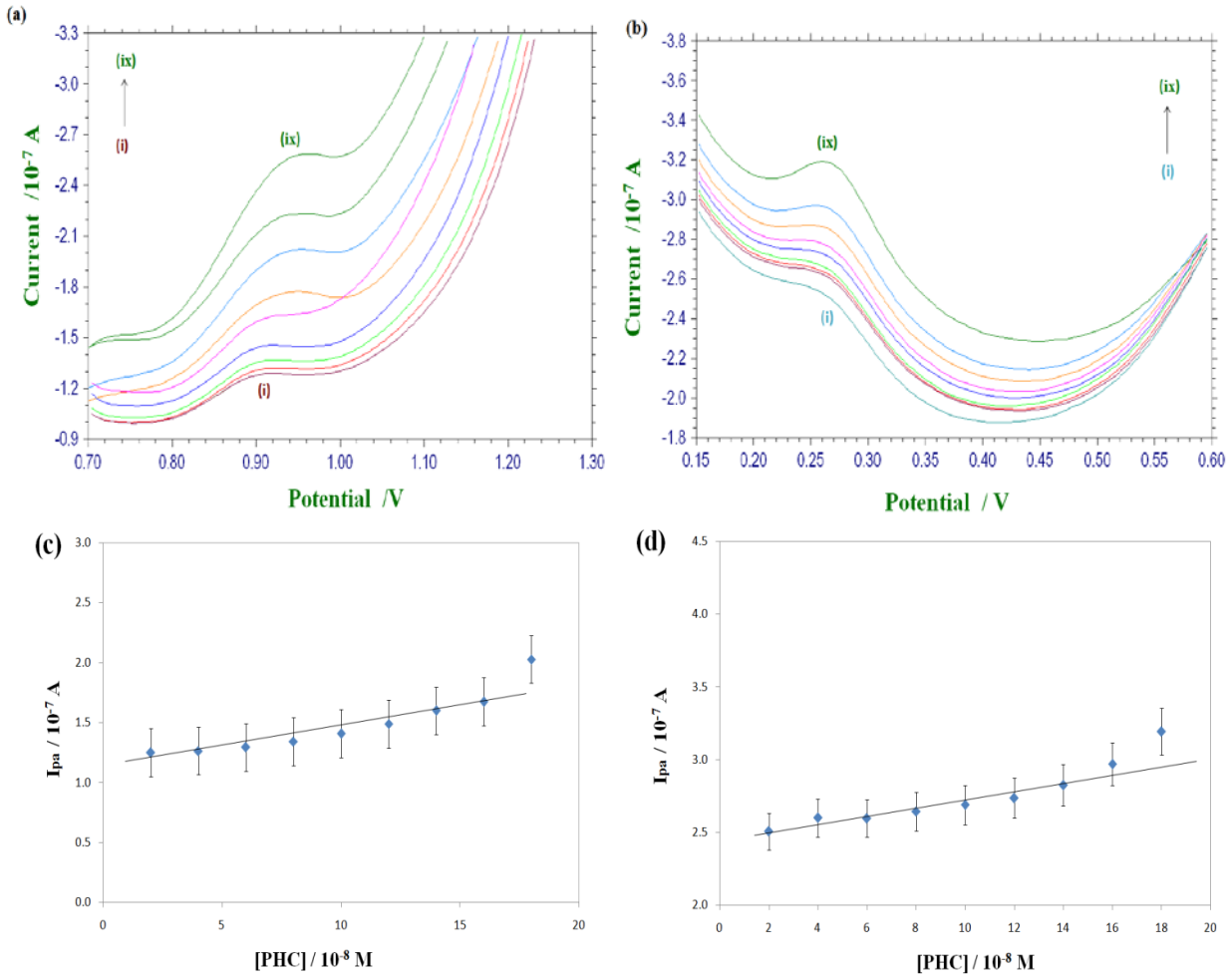


Fig. 6. **(a)** Differential pulse voltammograms of poly BCP-GC modified electrode recorded at various concentrations of PHC (i-ix): 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0 nM; **(b)** Square wave voltammograms of poly BCP-GC modified electrode recorded at various concentrations of PHC(i-ix): 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0 nM; **(c)** Calibration plot (I_{pa} *vs*. concentration of PHC) obtained using DPV; **(d)** Calibration plot (I_{pa} *vs*. concentration of PHC) obtained using SWV

Table 1. Characteristics calibration plots for PHC

	DPV	**SWV**
Linearity range (M)	2.0×10^{-8}- 9.0×10^{-8}	2.0×10^{-8}- 8.0×10^{-8}
Slope of the calibration plot	0.069	0.047
Intercept	1.043	2.422
Correlation coefficient (r)	0.917	0.953
RSD of slope (%)	0.841	4.778
RSD of intercept (%)	0.253	0.418
Number of Data points	5.0	5.0
LOD (M)	1.16×10^{-9}	6.28×10^{-9}
LOQ (M)	3.85×10^{-9}	2.09×10^{-8}

The LOD and LOQ values calculated for DPV and SWV were 1.16×10^{-9} M and 3.85×10^{-9} M, 6.28×10^{-9} M and 2.09×10^{-8} M, respectively. Greater linearity and lower limit of detection was shown by DPV among both the techniques. Hence all the analytical measurements were taken with this technique. The LOD values obtained from the proposed method using poly BCP-GC electrode were lower compared with the earlier reported methods as shown in Table 2. Hence the proposed method is better than reported methods.

In order to check the precision of the method, the experiment was repeated five times in the same day (repeatability) and after two days for different standard solutions (reproducibility). It was found that good repeatability and reproducibility were observed in the proposed method.

Table 2. Comparison of linear range and detection limits for PHC to different voltammetric methods

SLNO.	**Sensors used**	**Concentration Range (M)**	**LOD (M)**	**Ref.**
1	Chromium(III) Hexacyanoferrate(II) modified GC electrode	1.33×10^{-6}-1.32×10^{-5}	3.46×10^{-7}	[37]
2	Copper(II) Hexacyanoferrate(III) modified GC electrode	1.2×10^{-6}-6.9×10^{-4}	4.1×10^{-7}	[14]
3	Glassy Carbon Electrode	3.0×10^{-7}-2.0×10^{-4}	1.0×10^{-7}	[38]
4	ssDNA- modified GC electrode	0.1×10^{-3}-6.0×10^{-3}	4.0×10^{-5}	[39]
5	Nanocrystalline metallosillicate modified GC	1.2×10^{-7}-6.0×10^{-4}	3.0×10^{-8}	[40]

6	Au-nano particles/MWCNT/modified GC electrode	1.38×10^{-6}-4.99×10^{-4}	2.57×10^{-6}	[41]
7	poly(methylene blue) film modified GC electrode	4.9×10^{-5}-5.008×10^{-3}	6.52×10^{-3}	[42]
8	MWNT-modified GC electrode	5.0×10^{-7}–1.0×10^{-4}	2.0×10^{-7}	[13]
9	Au-CuO/MWCNTs/ modified GC electrode	7.9×10^{-7}–1.84×10^{-5}	1.5×10^{-7}	[43]
10	Poly Bromocresol purple modified GC electrode			**Present work**
	DPV Technique	2.0×10^{-8}-9.0×10^{-8}	1.16×10^{-9}	
	SWV Technique	2.0×10^{-8}-8.0×10^{-8}	6.28×10^{-9}	

3.9. Effect of Interferents

The potential interference for the determination of PHC was also studied. In the present work, the interferences of several chemical species were investigated. The determination of PHC at the level of 1.0×10^{-5} M in presence of hundred-fold excess (1.0×10^{-3} M) of interferents was studied using DPV, the results are given in Table 3 and Fig. 7.

Table 3. Influence of various potential interferents on the DPV response for 10.0 μM PHC

Interferents, 10^{-3} M	**E_{pa}**	**Signal change, %**
Tartaric acid	0.9719	2.04
Gum acacia	0.9446	0.78
Lactose	0.9717	2.04
Glucose	0.9771	2.57
Starch	0.9729	2.15
Citric acid	0.9593	0.76
Sucrose	0.9866	3.50
D-Mannitol	0.8771	8.53
Sodium citrate	1.0211	6.77
Caffeine	0.9708	1.17
Ascorbic acid	0.9834	0.11
NH_4Cl	0.9688	1.73
$CuCl_2$	1.0012	4.91
$FeCl_3$	1.0002	4.82
$CaCl_2$	0.9750	2.35
NaCl	0.9803	2.88
$MgCl_2$	0.9688	1.73
K_2SO_4	0.9530	0.10

It was found that poly BCP-GC could tolerate interferences of certain organic as well as inorganic species such as glucose, sucrose, tartaric acid, lactose, citric acid, gum acacia, starch, ascorbic acid, caffeine, Cu^{2+}, NH_4^+, Ca^{2+}, Fe^{3+}, Na^+, Mg^{2+}, K^+, Cl^- and SO_4^{2-} since these excipients have the current response of PHC with signal change below 5%, whereas a few compounds like mannitol and sodium citrate showed potential interference when used in 100 fold excess with the signal percentage of more than 5%. Hence the proposed method has excellent sensitivity towards PHC.

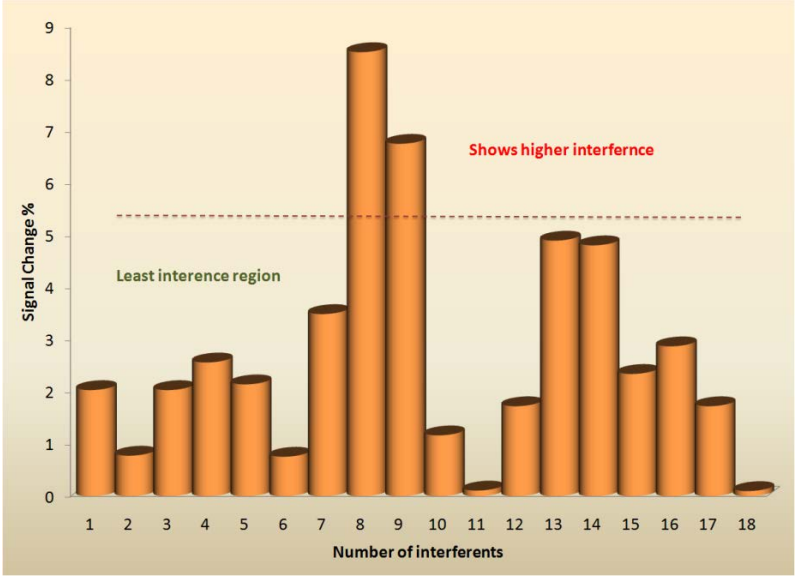


Fig. 7. Bar diagram showing the interence study in the analysis of PHC

3.10. Tablet analysis and recovery test

In order to check the applicability of the proposed method, the concentration of PHC in three different types of tablets (Supradyn®, Becosules capsules, Neurobion Forte) was investigated. 5.0 mL of the sample solution prepared as mentioned earlier was added in the voltammetric cell containing 10 mL of 0.2 M PBS and differential pulse voltammograms were recorded for recovery studies as DPV has more sensitivity towards PHC. The results obtained were in good agreement with the content marked in the label (Table 4). Hence the proposed method was successfully applied for the assay of PHC.

Recovery studies were carried out after the addition of known amounts of PHC present in the tablets to various pre-analysed preparations of PHC. The results indicated good recoveries that is 99.67% for Supradyn, 100.26% for Becosules capsules and 100.83% for Neurobion Forte with RSD 0.320%, 0.331% and 0.209% respectively.

Table 4. Assay results of tablets by DPV and mean recoveries

	Supradyn®	**Becosules capsules**	**Neurobion Forte**
Labelled claim (mg)	3.0	3.0	3.0
Amount found (mg) [(a)]	3.010	2.991	0.297
RSD (%)	0.731	0.146	0.673
Added (mg)	0.311	0.761	0.962
Found (mg) [(a)]	0.312	0.759	0.954
Recovered (%)	99.67	100.26	100.83
RSD (%)	0.320	0.331	0.209
Bias (%)	-0.321	0.263	0.832

(a) Average of five determinations

3.11. Detection of PHC in Energy drinks

The developed DPV method for the determination of PHC was applied for determination of PHC in a few health and energy drinks. Bourn Vita, Tang, Protinex and PediaSure sample solutions were prepared as mentioned in sample preparation. The standard addition method was followed [44] for the determination of PHC in samples. The results are shown in Table 5. The results gave good recovery values up to 99.0%, 101.8%, 101.2% and 99.9% and the resultant %RSD was equivalent to 0.181%, 0.673%, 0.15% and 0.16% for bourn vita, tang, protinex and pediasure respectively. Since the recovery results obtained were reproducible, the method can be applied for the analysis of real samples. The results show the efficiency of the modified electrode.

Table 5. Application of DPV to the determination of PHC in energy drinks

	Bourn Vita	**Tang**	**Protinex**	**PediaSure**
Labelled claim (mg)	25.0	0.4	1.7	1.0
Found (mg)[(a)]	25.24	0.393	1.72	0.99
Added (µg)	250.0	40.0	34.0	20.0
Found (µg) [(a)]	252.4	39.3	34.4	19.9
Recovered (%)	99.0	101.8	101.2	99.9
RSD (%)	0.181	0.673	0.15	0.16
Bias (%)	-0.96	1.75	-0.01	0.01

(a) Average of five determinations

3.12. Determination of PHC in blood serum sample

Another analytical application of the sensor was also demonstrated by determining the concentration of PHC in spiked human blood serum samples. The serum samples were

prepared as mentioned under sample preparation heading (section 2.5). The recovery studies was performed by spiking the PHC free serum sample with known amount of PHC and the differential pulse voltammograms were recorded. The amounts of PHC were calculated using the calibration graph. The recovery results obtained for three different concentrations of PHC in serum samples are listed in Table 6. The lesser RSD values and greater recovery results bring considerable importance to the work.

Table 6. Determination of PHC in human blood serum samples

Serum Samples	**Spiked (10^{-8} M)**	**Detected (10^{-8} M)**	**Recovery (%)**	**SD $\pm$ RSD (%)**
Sample 1	3.0	3.098	103.25	0.0018$\pm$0.058
Sample 2	5.0	5.031	100.61	0.0514$\pm$1.023
Sample 3	7.0	7.002	100.02	0.0111$\pm$0.159

4. CONCLUSION

In the present work, the electrochemical behaviour of PHC at poly BCP-GC was investigated using cyclic voltammetry, DPV and SWV. The study revealed that the electrode reaction was irreversible and diffusion controlled at lower scan rates. A probable mechanism was proposed. From the calibration graph, it was concluded that the present proposed method showed the lowest detection limit than any of the earlier methods. In order to show the applicability of the study, analytical work was carried out to determine the concentration of PHC in tablets, energy drinks and blood serum samples. The proposed method ensures low cost, short time for analysis and accuracy which makes it advantageous for analytical studies in quality control laboratories.

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