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RESEARCH ARTICLE

A cyclic voltammetric study on the electrochemical behavior and endurance of pyridoxine at cobalt hexacyanoferrate based carbon paste sensor in local available vitamin-B_6 medications.

Alemu Mekonnen*[1], R.C.Saini*, AbrahaTadese and Rishi Pal[2]

1. Department of Chemistry, Arba Minch University, Arba Minch, Ethiopia.

2. SBMN Inst. of Pharma.Sci. & Res., Baba Mastnath University, Asthal Bohr, India

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Corresponding Author

AlemuMekonnen

Abstract

Cyclic voltammetric technique was used to study the electrochemical behavior of pyridoxine on surface ofcobalt hexacyanoferrate based carbon paste electrode in phosphate buffer solution of pH 6 at a scan rate 100mV/s. The voltametric parameters and experimental conditions were optimized for present study. On application of this sensor instead of bare carbon paste sensor, the oxidation peak current signal of pyridoxine indicated significant increase from 82.96 μA to 343.2 μA. The effects of pH and impurities of solutions on electrochemical characteristics of chemically improved sensor were studied. The magnitude of electrochemical parameters like current density ratio (i_{pa}/i_{pc}=1.31) and increasing peak potential's gap (E_{pa}- E_{pc}) with rising analyte concentration as well as applied scan rate, predicted the irreversibility of the redox process, involving transfer of two electrons (pH effect) at interface. The effect of scan rate showed that the electrode process was both diffusion and adsorption controlled. The relationship between anodic peak current density and analyte concentration (5.0 μM to 26.0 μM) was found linear with a correlation coefficient, 'R'=0.9998 (n =10) and a standard deviation 'S' = 0.01535. The linear regression equation was found as "i_p (μA) = 0.09668C (μM) + 1.04561".The detection and quantification limits were found 4.8 × 10^{-7}M and 1.56 x 10^{-6} M by this method, respectively. The recovery results determined by the application of analytical standard curve using the non-spiked and the spiked sample solutions of locally available vitamine-B_6 medicines were found in good agreement to that of recent reported literature values for pyridoxine, cyclicvoltametrically. Interference studies suggested an excellent selectivity of the proposed method toward vitamine-B_6.

INTRODUCTION

Vitamin B_6 (VB_6) plays a vital role in the activities of many enzymes. It is essential for the breakdown and use of proteins, carbohydrates and fats from food and the release of stored carbohydrates as energy for body functions of several hormonal and nervous systems. Its deficiency may cause weakness, depression, anemia, skin disorders and sometimes cause permanent nerve damage. Therefore, development of suitable analytical sensor and viable analysis method for its detection is essential, in food and pharmaceutical industries [1-5]. Several analytical methods have been described in literature [6-10] for determination of pyridoxine, including spectrophotometry, chromatography, (liquid, HPLC, GC) and electrochemical method. A few papers on the determination of pyridoxine have used

voltammetry techniques. The electrocatalytic oxidation of pyridoxine was demonstrated on a Nafion/lead ruthenate-pyrochlore modified electrode by cyclic voltammetry [11]. The voltammetric behavior of pyridoxine was studied at a glassy carbon electrode in phosphate buffers using cyclic, linear sweep and differential pulse voltammetry [12]. It was also reported that pyridoxine was studied in pharmaceutical preparations by cyclic voltammetry using carbon paste electrodes modified with copper(II) hexacyanoferrate(III) using an acetate buffer [13]. There are reports, where chromium(III) hexacyanoferrate(II) modified glassy carbon electrode was used to determine pyridoxine in drugs by cyclic voltammetry [14]. Furthermore, the electrochemical behavior of pyridoxine was investigated using Multiwall Carbon Nanotube Modified Carbon-Ceramic electrode in phosphate buffer solution [9]. Recently, electrocatalytic oxidation and Voltammetric determination of pyridoxine by assDNA-modified electrode [10] and electrochemical analysis on compounds of the pyridoxine family using glassy carbon electrodes has been reported [15].

A sensitive and easy available new sensor based on modifier cobalt hexacyanoferrate and carbon paste was fabricated and deployed in our recent work [16], for quantification of pyridoxine in different medicines using square wave voltametric technique. Our inclination in the present study, thus, lies in the direction of application of this fabricated sensor as an economical, easy operable and quick responsive tool for analysis of pyridoxine in different pharmaceutical formulations available in local market, using cyclic voltammetric technique.

MATERIAL AND METHOD

Reagents and Solutions

The reagents and chemicals used were vitamin B_6 {Univial-China}; graphite powder and di-sodium hydrogen orthophosphate anhydrous {BDH-England}; paraffin oil,sodium dihydrogen orthophosphate, hydrochloric acid, potassium chloride, cobalt chloride {Nice-India}; potassium hexacyanoferrate(III) {KiraLight-India}; sodium hydroxide {Scharlau- Spain} and five different brands of vitamin- B_6tablets i.e. Benadon, B-Long, Pyridoxine, Neurobin&Neurovit,were purchased and used in the present investigations. All the chemicals were of analytical grades, so used without further purification. Deionized water was used for the preparation of all solutions.

Supporting electrolyte of phosphate buffers of different pH in the range (2 to 8) was prepared from 0.1M NaH_2PO_4 and 0.1M Na_2HPO_4 in deionized water. The pH of the solutions was adjusted by adding 0.1M HCl and 0.1M NaOH dropswise. Stock solution of pyridoxine was prepared by dissolving 0.103g of it in 100 ml of the supporting electrolyte. The required concentrations of pyridoxine solution were prepared by diluting the stock solution with the supporting electrolyte solution of required pH.

Apparatus

All voltammetric measurements and processing of data were carried out in glass voltammetric cell, using BAS 50W Cyclicvoltammetric analyzer, which was connected to Dell Pentium personal Computer containing three electrode systems, carbon paste (CPE)/Cobalt hexacyanoferrate (CoHCFE) electrode as working electrode, silver-silver chloride electrode as a reference electrode and platinum wire as an auxiliary electrode. The pH of the buffer solution was measured with a 353 ATC digital pH meter using glass electrode. One milliliter syringe {Plastipak-Spain} andWhatman filter paper {Whatman-England} was used for the preparation of the working electrode in this experiment.

Synthesis of cobalt(II) hexacyanoferrate(III)

The pathway for the synthesis of cobalt(II) hexacyanoferrate(III) was described in our earlier communication [16].

Preparation of working electrodes

The procedure adopted for the preparation of unmodified carbon paste and the cobalt(II) hexacyanoferrate(III) modified carbon paste electrodes has been described elsewhere [16].

Sample preparation of vitamin-B_6 tablets

To carry out the present investigations the sample solutions of different pharmaceutical preparations were prepared using phosphate buffer. Ten tablets of each brands of vitamin B_6 were accurately weighed and powdered by hand crushing with mortar and pestle, separately. The weights of the powder of each brand proportional to that of its one tablet were transferred to 100 ml volumetric flasks to prepare the solutions. Cyclic voltammetry (CV), in conjunction with standard addition technique was applied for the determination of the pyridoxine content and also to

examine the effect of the tablet matrix on its determination in the samples.Th applied potential range was between -200 and +1200 mV vs Ag/AgCl/KCl_{satd} electrode.

RESULTS AND DISCUSSION

Electrochemical behavior of pyridoxine

Electrochemical characterization of CoHCFE using CV in 0.1 M phosphate buffer solution of pH 6.0 at a scan rate 100 mV/s was conducted in potential range -200 to 1200 mV (a) in absence and (b) in presence of 1.0 mM pyridoxine and displayed in Figure 1. The appearance of anodic and cathodic peak signals (Figure 1a), indicated some interference effects of the modifier CoHCF to that with redox signals of the pyridoxine. This was expected because of the redox charactaritics of CoHCF. In the presence of pyridoxine, the oxidation peak current signal indicated significant increase from 82.96 µA to 343.2µA using CoHCFE. The electrochemical properties of pyridoxine at the CPE and CoHCFE were examined through CV and the results presented in Figure 2. A poor signal of oxidation peak current density (-21.1µA) was noted from the Figure 2a at oxidation peak potential 1036 mVusing the CPE. As compared to CPE, the electrochemical response of pyridoxine at CoHCFE responded an improved enhancement of peak current density during oxidation (3.43×10^{-4} A) and reduction (2.62×10^{-4}A), respectively. The oxidation peak potential at 689 mV and reduction peak potential at 155 mV vs. Ag/AgCl/KCl_{satd} could be seen from Figure 2b. The ratio of redox peak current density (I_{pa}/I_{pc}) obtained 1.31 and the peak to peak separation (ΔE_P) was found 534 mV. This observed high magnitude of ΔE_p indicating a limitation due to electron transfer kinetics at interface. These characteristics were specifying an irreversible nature of redox electrode process. The remarkable peak current enhancement and the fall of oxidation over-potential testified that the CoHCFE accelerated the electron transfer process of the redox couple noticeably, at the electrode surface. Thus, the prepared electrochemical sensor was characterized for higher sensitivity and reproducibility than that of CPE in electrochemical investigations and determination of pyridoxine in real samples. The low detection limit responding the insignificant matrix effects in solutions of low concentrations.

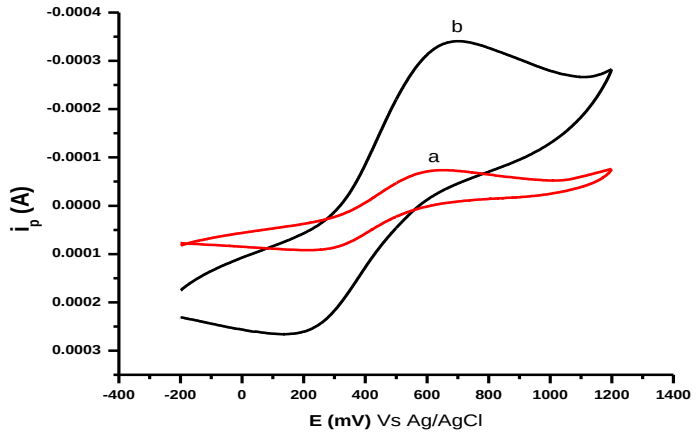

Figure1: Typical cyclic voltammograms of CoHCFE in 0.1 M phosphate buffer solution of pH = 6.0 at a scan rate of 100 mV/s in the absence (a) and presence (b) of 1.0 mM pyridoxine

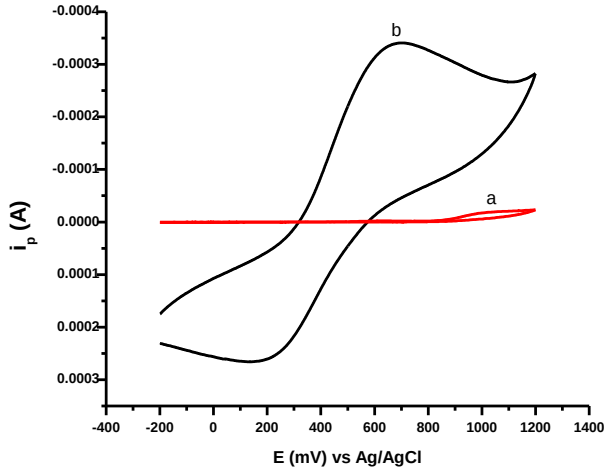

Figure 2: Cyclic voltammograms of 1.0 mM pyridoxine obtained at CPE (a) and CoHCFE (b) in 0.1M phosphate buffer solution of pH 6.0 at scan rate 100mV/s.

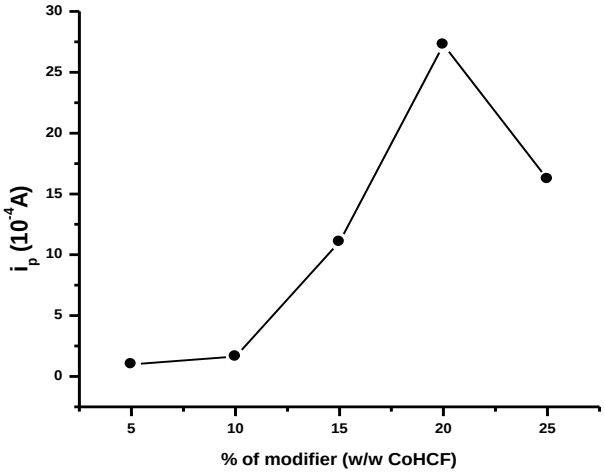

Figure 3: Effect of electrode composition on anodic peak current in 1.0mM pyridoxine at 0.1 M PBS of pH 6.0 between 5 to 25% CoHCF modifier at a scan rate 100 mV/s.

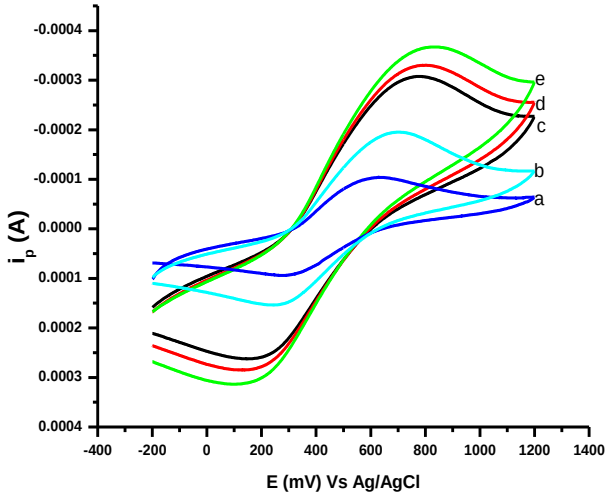

Figure 4: Cyclic voltammograms for 1.0mM pyridoxine in 0.1 M PBS of pH 6.0 at modified electrode at different scan rates (a) 20; (b) 40; (c) 60; (d) 80; (e) 100 mV/s.

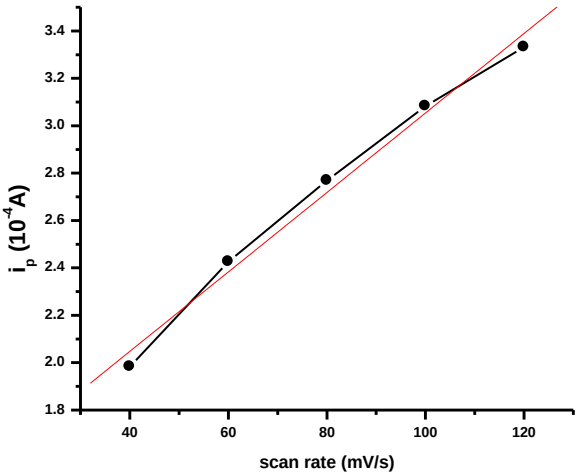

Figure 5: The variation of scan rate on the anodic peak current of 1.0 mM pyridoxine in 0.1 M PBS of pH 6.0.

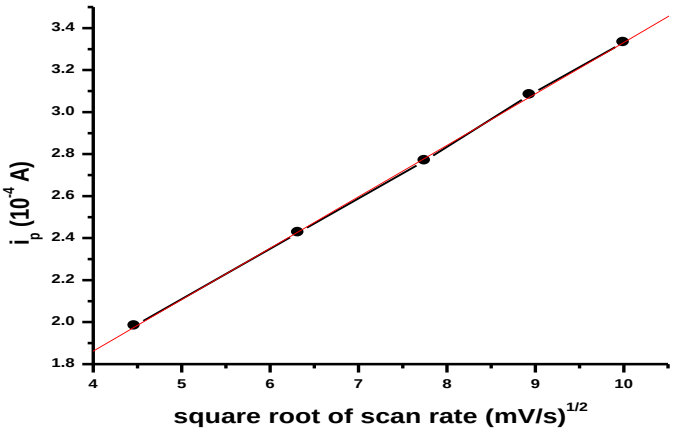

Figure 6: The variation of square root of scan rate on the anodic peak current of 1.0 mM pyridoxine in 0.1 M PBS of pH 6.0.

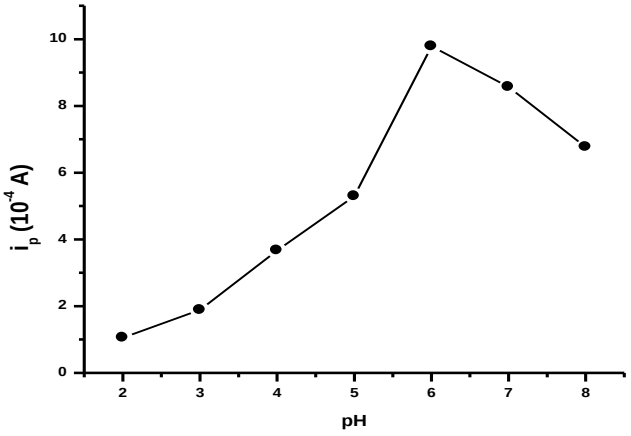

Figure 7: Variation of anodic peak current as function of pH using 1.0 mM pyridoxine in 0.1 M phosphate buffer at CoHCFE at a scan rate 100mV/s.

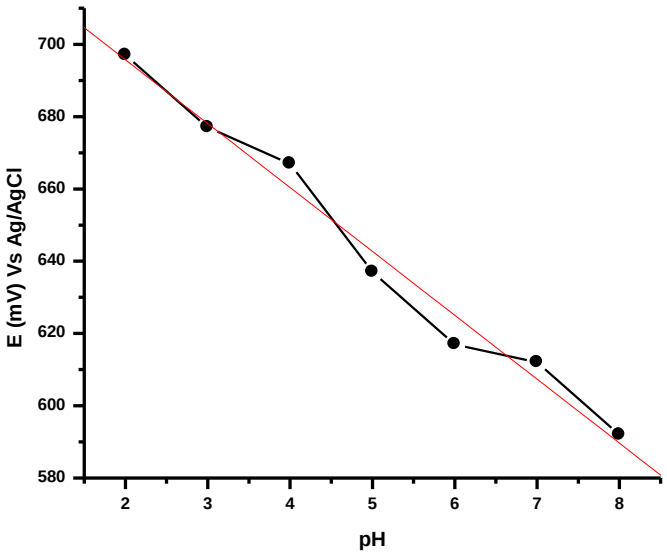

Figure 8: Effect of variation of pH on the anodic peak potential of 1.0 mM pyridoxine in 0.1 M phosphate buffer solution at CoHCFE at a scan rate 100 mV/s.

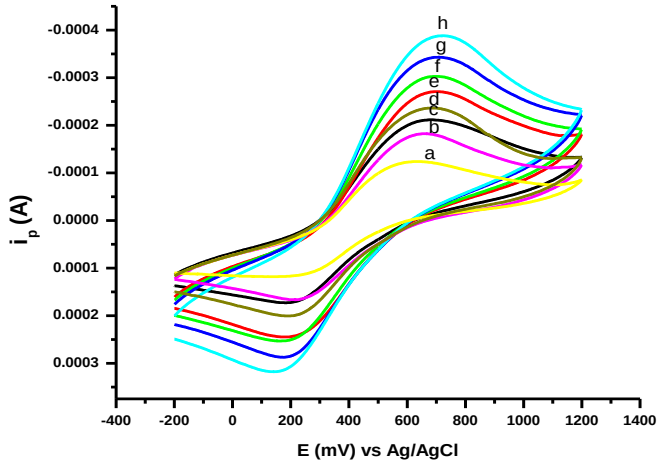

Figure 9: Cyclic voltammograms of different concentrations of pyridoxine at CoHCF in 0.1 M PBS of pH 6.0 with a scan rate 100 mV/s as (a) 5.0 ; (b) 8.0 ; (c) 11.0 ; (d) 14.0 ; (e) 17.0; (f) 20.0 ;(g) 23.0 &(h) 26.0 µM.

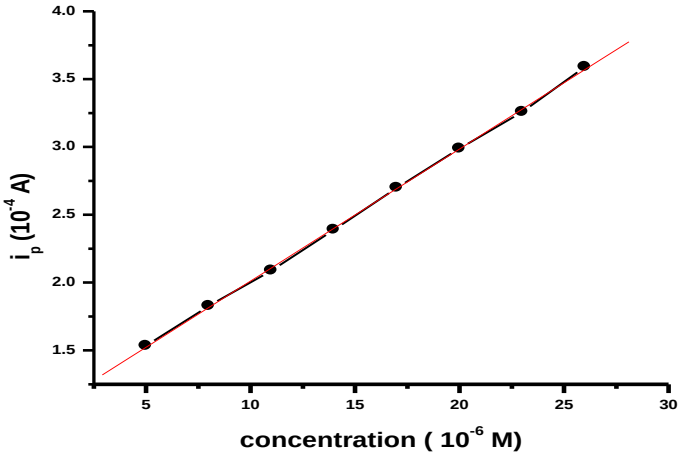

Figure 10: Calibration curve for determination of pyridoxine in 0.1 M PBS of pH 6.0 at Scan rate 100 mV/s at CoHCFE

List of tables

Table 1: Recovery results of pyridoxine from spiked and non-spiked solutions of pharmaceutical formulations of VB_6 under optimized conditions at CoHCFE cyclicvoltametrically.

Sample Brand	Amount of pyridoxine (mg)				Percent Recovery
	Labeled	Spiked	Expected	Recovered[(a)]	
Benadon	40.0	0.0	40.0	38.92±0.32	97.30
		12.0	52.0	50.42± 0.84	96.98
		25.0	65.0	62.79± 0.48	96.60
Neurovit	50.0	0.0	50.0	48.63± 0.56	97.26
		12.0	62.0	60.16±0.67	96.27
		25.0	75.0	72.90± 0.39	97.25
Pyridoxine	50.0	0.0	50.0	48.22± 0.54	97.04
		12.0	62.0	60.37± 0.78	96.58
		25.0	75.0	73.09± 0.27	97.45
B-long	100.0	0.0	100.0	90.67± 0.69	90.67
		12.0	112.0	103.62± 0.48	92.52
		25.0	125.0	113.14± 0.83	90.52
Neurobin	125.0	0.0	125.0	116.85± 0.73	93.50
		12.0	137.0	128.94± 0.52	94.12
		25.0	150.0	142.64± 0.46	95.10

(a)Mean value ± standard deviation, for four replications.

Table 2: Comparison of the electrochemical behavior of CoHCFE in the detection of pyridoxine in real samples with some electrodes, recently reported in literature.

Electrodes with reference	Linear range /(M)	Detection limit/ (M)
ssDNA/GCE[10]	$0.1x10^{-3}$-$6.0x10^{-3}$	4.0×10^{-5}
Vanady (IV)-salen complex [18]	$4.5x10^{-4}$ -$3.3x10^{-4}$	3.7×10^{-5}
Prussian blue [19]	$1.0x10^{-6}$ -$8.0x10^{-5}$	8.7×10^{-7}
Activated glassy carbon [20]	$2.5x10^{-6}$ -$7.5x10^{-3}$	8.0×10^{-7}
Carbon disk [21]	$2.5x10^{-6}$ -$1.0x10^{-3}$	1.0×10^{-6}
CoHCFE [This work]	$5.0x10^{-6}$ -$2.6x10^{-5}$	4.8×10^{-7}

Effect of electrode composition

The combination of CoHCF with carbon paste was used to prepare the present sensor and resulted out a strong and significant influence to the observed CV signal as compared to the pure CPE as in Figure 3. Also, it is evident from this figure that the magnitude of peak current increased with increasing amount of CoHCF up to 20% (w/w) whereas for its higher amounts the peak current decreased, significantly.This observation could be authenticated to the fact that the percentage of conducting graphite content in the conductive surface area of the working electrode decreased with further increase of CoHCF content in carbon paste. Thus, the best suitable composition for sensor's preparation was 20% CoHCF, 55% graphite and 25% paraffin oil by weight.

Effect of scan rate

The effect of scan rate on the peak current signals of 1.0 mM pyridoxine using CoHCF modified electrode in phosphate buffer at pH 6.0 was studied by varying the scan rate from 20-100 mV/s. Figure 4 displayed the cyclic voltammograms of current as function of scan rate. This Figure exposed that with increasing scan rate there is a regular rise in the magnitude of both anodic and cathodic peak currents of the redox system. This observation has specified that the current is controlled by linear diffusion with the scan rate. Also, these results could be accredited by a regular variation in magnitude of rates of redox peak currents corresponding to that of rise in scan rate. Another fact illustrated by this figure is a regular increase in the corresponding peak potential separation gap with rise in scan rate in the studied range. The peak-gap is a function of the rate constant of electron transfer process at interface. This suggested that the concentration of redox couple on the surface of sensor increase and favor the electron transfer process at interface. The peak potentials observed change with increasing scan rate which indicated the irreversibility of the electrode process. Figure 4 further revealed that change in value of peak current with regularly increasing scan rate is almost non-uniform and beyond the scan rate 100mV/s, voltamogram get broadened with diminished rise in signal. Thus, a scan rate 100mV/s was chosen for subsequent experiments.

The variation of anodic peak current (I_{pa}) only, vs scan rate (υ) and vs square root of scan rate ($\upsilon^{1/2}$) have been illustrated in Figure 5 and Figure 6, respectively. The plots obtained were nearly straight lines. In the studied range, the variation among the anodic peak current signals found proportional to the scan rate (υ) with negligible deviation from linearity; while with the square root of scan rate ($\upsilon^{1/2}$) found linear, respectively. The observed dependence of anodic peak current on the scan rate and square root of scan rate has been predicting that the nature of electrochemical processes at the interface was governed by both the adsorption and the diffusion controlled mechanisms as evidenced by literature report [12], also.

Effect of pH

The electrochemical behavior of the present sensor was studied over pH range 2 to 8, using 1.0 mM pyridoxine solution in 0.1 M phosphate buffer as supporting electrolyte at a scan rate 100mV/s. A plot of anodic peak current from voltammograms at different pH as a function of pH values using CoHCFE in 0.1 M phosphate buffer solution has been presented in Figure 7. The anodic peak current was increased with increasing pH from 2.0 to 6.0 and then decreased for higher pH values. Moreover, the electrode was not stable and the results were not reproducible at pH values greater than seven. The better sensitivity and shape of the voltammogram was observed at pH 6.0 and engaged as optimal working pH value (Figure 8) for the entire voltammetric experiment. The plot of oxidation peak potentials from voltamograms at studied pH as a function of the pH was reflected in Figure 8. This revealed nearly linear shift of anodic peak potential towards lesser positive side with increasing pH values. The anodic peak potential of pyridoxine was shifted from 697 mV to 592 mV with respect to the pH change from 2.0 to 8.0. This

regular linearity indicated that equal number of protons and electrons were involved in the electrochemical oxidation of pyridoxine at sensor's surface.

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Analytical standard curve and detection limit

The effect of varying concentration of pyridoxine was studied at CoHCFE in 0.1 M phosphate buffer solution of pH 6.0 at a scan rate 100 mV/s. Figure 9 has shown enhancement in the magnitude of redox peak current density with successive intensification of pyridoxine concentration from 5.0 μM to 2.6 μM. At each concentration of pyridoxine within studied range, there was a continuous enhancement in I_{pa} and I_{pc} magnitudes along with increasing peak potential difference ($\Delta E = E_{pa} - E_{pc}$) by shifting E_{pa} towards more positive and E_{pc} towards more negative side with analyte concentration. By using the data of Figure 9, the plot of peak current vs the concentrations of pyridoxine portrayed in Figure 10. Its behavior was found linear in the range 5.0 μM to 26.0μM with a correlation coefficient, R=0.9998 (n =10) and a standard deviation 'S' = 0.01535. The linear regression equation obtained was given as:

I_p (μA) = 0.09668C (μM) + 1.04561 ... I

The observed enhancement in peak current signals with increasing VB_6 concentration could be authenticated by the increase in number of:

i) electroactive species near electrode, and

ii) effective collisions in electrode active region.

These factors could enhance the flow of electrons across the interface and facilitate the electrochemical process there.

The sensitivity of the method was 0.09668μA/μM. The quantification limit 'QL' and detection limit 'DL' values were determined by considering the standard deviation (S) from the mean of 10 voltammograms of the blank and the slope (m) of the analytical curve, according to IUPAC allusions [17], by using the formula as DL=3S/m and QL=10S/m. These were found 4.8×10^{-7} M and 1.56×10^{-6}M respectively in present investigation.

Determination of pyridoxine in VB_6 Tablets

The proposed method was applied for determination of pyridoxine in five different products of pharmaceutical formulations. Table 1 offered a graceful look on the results of the present work. The labeled values, the spiked amount, the expected extent and the recovered quantity of pyridoxine using the proposed CV method in different studied brands of VB_6 tablet samples have been presented there. The recovery results were determined by the application of analytical standard curve. The percentage recovery of analyte from analyzed samples suggested the legitimacy of the proposed technique for both qualitative and quantitative analysis of pyridoxine in pharmaceutical formulations and other kinds of samples.

Comparison of the electrochemical behavior of sensor

Recent works of some researchers' [10, 18-21] as reported in literature regarding detection and estimation of pyridoxine in real samples along with their studied linear range and detection limit by using the CV technique at the interface of the analyte and different electrochemical sensors. A comparative chart of the recent works and the present study has been tabulated in Table 2.

Effect of Interferences

To evaluate the potential effect of foreign species on the determination of vitamin B6, at $1.0 \times 10^{\square 3}$mol/Llevel, a systematic study was carried out under the aboveoptimized and experimental conditions. The species are fructose, maltose, glucose, urea, vitamin C, vitamin B1 and vitamin B12, respectively. The tolerable limit of interferer'scould be their concentration in samplethat gave ± 5.0% variation in the determination of vitamin B6. It has been observed that 1.0 mM vitamin C, 0.1mM vitamin B1 and 0.01mM vitamin B12 have some inference signals; while 100 mM of fructose, maltose, glucose and urea have no inference signals indetermination of 1.0mM vitamin B6. This suggests an excellent selectivity of the proposed voltammetric method toward VB6. The selectivity of the method is thus acceptable and it may be applied to determine the content of vitamin B6 in medicinal samples.

Conclusion

This work demonstrates the ability of CoHCFE for the electrochemical investigation of pyridoxine and its determination in real samples. The results obtained illustrates that the electro-catalytic oxidation of pyridoxine at the surface of the modified electrode occurs at a potential about 347mV less positive than that of bare carbon paste electrode. The anodic peak current density of pyridoxine varied directly proportional to the concentration in the range 5.0×10^{-6} to 2.6×10^{-5} M using CV, with a detection limit 4.8×10^{-7}M and correlation coefficient of 0.9998. Moreover, simple preparation, sensitivity, good stability, excellent reproducibility, cost effective, easy handling and rapid analysis make the developed method suitable for its utilization as routine tool to determine pyridoxine in fruit/food-drinks and pharmaceutical related industries.

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