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Research Article

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Square wave voltammetric determination of pyridoxine in pharmaceutical preparations using cobalthexacyanoferrate modified carbon paste electrode

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ABSTRACT

Square wave voltammetric method was used to study the electrochemical properties of pyridoxine and determine it in pharmaceutical preparations using Cobalthexacyanoferrate modified carbon paste electrode. Various parameters such as pH of the buffer solution, electrode composition, pulse amplitude, step potential and frequency were investigated. The optimized parameter has been made at the electrode composition of 20% CoHCF, 55% graphite and 25% paraffin oil. The peak current depends linearly on the concentration of vitamin B_6 *from* 5.0×10^{-6} *M to* 2.6×10^{-5} *M with a correlation coefficient of* $r = 0.99997$ $(n = 8)$ *and a standard deviation (S) of 0.005. The equation was found to be* I_p $(\mu A) = 2.03277+0.10008C$ (μM) *with the detection limit of* 1.72×10^{-7} *M. The developed voltammetric method was applied for the determination of Pyridoxine in vitamin* B_6 *tablets and it yielded a good mean recovery.*

Keywords: Pyridoxine, cobalthexacyanoferrate, voltammetry, pharmaceutical preparations

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INTRODUCTION

Vitamin B_6 is a very vital cofactor involving in the metabolism of amino acids and lipids, as well as the synthesis of neurotransmitters, histamine and hemoglobin, and other key biologic processes [1]. Its biosynthesis is restricted to bacteria, protozoa, fungi, and plants, but, animals and humans require a constant supply of the vitamin in their diet or drugs [2]. Vitamin B_6 is the derivatives of 3-hydroxy-2-methylpyridine, it includes three chemically, metabolically, and functionally related forms; pyridoxol, pyridoxal and pyridoxamine groups that are located at the fourth position of the pyridine ring [3]. VB_6 deficiency may cause weakness, depression, anaemia and skin disorders. Permanent nerve damage may also occur [4]. Therefore, quantitative analysis of vitamin B_6 is very significant to maintain one's health. Some methods of determining it have been developed, such as ion exchange chromatography [5], liquid chromatography [6], high-performance liquid chromatography [3, 7-9], flow injection-solid phase spectrophotometry [10], fluorescence spectrometry [11-13], gas-chromatography–mass- spectrometry [14]. The separation and determination of VB_6 by chromatography [15] and electrophoresis [16] with amperometric detection, using a carbon disk electrode as electrochemical detector, have also been reported. The amperometric determination using modified electrodes such as vanady(IV)-salen complex [17], carbon nanotube [18], $Ru(bpy)_3^{3+}$[19], Prussian blue [20], poly-methylene blue [21] and carbon electrodes [22-27] also have been reported.

The aim of the present study is to develop suitable square wave voltammetric technique for the electrochemical investigation of pyridoxine and to check the validity of the proposed method by determining the amount of Pyridoxine in different brands of vitamin B_6 e tablets.

EXPERIMENTAL SECTION

2.1. Reagents and Solutions

The reagents and chemicals used in this experiment were pure vitamin B_6 (Univial, China), five different brands of vitamin B_6 tablets (Benadon, B-Long, Pyridoxine, Neurobin and Neurovit), Graphite powder (BDH, England), paraffin oil (Nice), di-sodium hydrogen orthophosphate anhydrous(BDH, England), sodium dihydrogen orthophosphate(Nice), NaOH (Scharlau, Spain), HCl (Nice), Potassium chloride (Nice), cobalt chloride (Nice), potassium hexacyanoferrate III (KiraLight, India). All chemicals are analytical grades. Deionized water was used for the preparation of all solutions. Supporting electrolyte of phosphate buffers in different pH ranging from 2-8 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 drops of 0.1M HCl and/or 0.1M NaOH. Stock solution of Pyridoxine was prepared by dissolving 0.103g of Pyridoxine in 100mL of the supporting electrolyte. The required concentration of pyridoxine solutions were prepared by diluting the stock solution with the supporting electrolyte.

2.2. Apparatus

All voltammetric experiments were conducted using BAS 50W CV voltammetric analyzer which was connected to Dell Pentium personal Computer containing three electrode systems, bare carbon paste electrode (BCPE) and Cobalt hexacyanoferrate (CoHCF) modified carbon paste electrode as a 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 with combination glass electrode. 1mL Syringe (Plastipak, Spain) and Whatman filter paper (Whatman, England) were used for the preparation of the working electrode in the experiment. Origin 6.0 software was used to analyze data.

2.3. Synthesis of Cobalt (II) Hexacyanoferrate (III)

Cobalt (II) Hexacyanoferrate (III) was prepared by precipitation by mixing a 0.25M potassium hexacyanoferrate (III) solution and 0.5 M cobalt (II) chloride solution with Co: Fe atomic ratio of 2:1. The precipitate obtained was filtered using a whatman filter paper, washed with deionized water several times and dried at room temperature for 4 days [13].

2.4. Preparation of Working Electrode

Unmodified carbon paste was prepared by mixing graphite powder with paraffin oil. The composition of the paste was 75% (w/w) graphite powder and 25% (w/w) paraffin oil. The mixture was homogenized with mortar and pestle for 30 minutes and allowed to rest for 24 hours. The homogenized paste was packed in to the tip of a plastic syringe. A copper wire was inserted from the backside of the syringe to provide electrical contact. Then the surface of the electrode was smoothed against a smooth white paper with a light manual pressure until a shiny surface was emerged.

2.5. Preparation of Modified Carbon Paste Electrode

Modification of carbon paste electrode was achieved by carefully mixing the dispersed graphite powder with CoHCF at varying ratio and subsequently added to 0.250 g of paraffin oil (25% w/w). The mixture was homogenized with mortar and pestle for 30 minutes. The modified carbon paste was packed into an electrode body, consisting of plastic syringe equipped with copper wire serving as an electric contact. Appropriate packing was achieved by pressing the electrode surface against a whatman filter paper.

2.6 Sample Preparation of Vitamin B_6 Tablets

Ten tablets of five brands of Vitamin B_6 were weighed and finely powdered separately portion equivalent to 40mg Benadon, 50mg Pyridoxine, 100mg B-Long, 125mg Neurobin and 50mg Neurovit were transferred into separate 100ml volumetric flask. This volume is completed to the mark using phosphate buffer by shaking thoroughly to dissolve. The square wave voltammograms were recorded in the potential range between -200 and +1200 mV Vs. Ag/AgCl. The amount of Pyridoxine in these tablet samples was determined from the calibration curve.

RESULTS AND DISCUSSION

3.1. Electrochemical properties of CoHCF/MCPE

The modified carbon paste electrode gave large square wave voltammetric peak when compared to unmodified one, as shown in the Fig.1 indicating that the CoHCF modified CPE accelerates the electron transfer reaction at the electrode surface. Hence, CoHCF/CPE was further systematically studied by square wave voltammetry the electrochemical determination of pyridoxine in different brands of pyridoxine tablets.

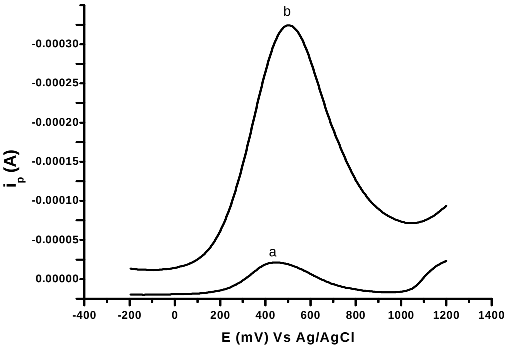

Figure 1:Square wave voltammograms of 1 mM pyridoxine at (a) unmodified carbon paste electrode and (b) CoHCF/CPE in 0.1 M PBS of pH = 6.0 at a frequency of 60 Hz and pulse amplitude of 150 mV

3.2. Effect of Electrode Composition

The amount of CoHCF in the carbon paste shows a significant influence on the voltammetric response of the modified electrode. The peak currents increased with increasing amount of CoHCF up to 20% (w/w). For CoHCF amounts higher than 20% (w/w) the peak currents decreased significantly as indicated in Fig. 2. This occurs due to a decrease in the graphite content in the paste and, consequent reduction of the conductive electrode area. The best carbon paste composition for the electrode was found to be 20% (w/w) CoHCF, 55% (w/w) graphite and 25% (w/w) paraffin oil.

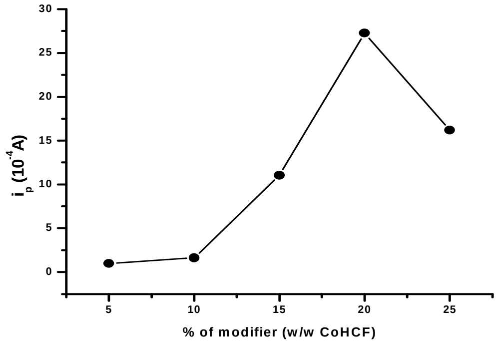

Figure 2: Effect of electrode composition on anodic peak current in 1 mM pyridoxine at 0.1 M PBS of pH = 6.0 ranging from 5 to 25% CoHCF modifier at a frequency of 60 Hz and pulse amplitude of 150 mV

3.3. Effect of pH

The electrochemical behavior of the carbon paste electrode modified with CoHCF was studied over a pH range of 2 up to 8 in solution containing 1.0 mM of pyridoxine in 0.1 M phosphate buffer solution as supporting electrolyte at a scan rate of 100mV/s. The graph of the anodic peak current as a function of different pH values at CoHCF in 0.1 M phosphate buffer solution is shown in Fig.3. The anodic peak current increased with increasing pH from 2.0 to 6.0 and then decreased for higher pH values. At higher pH values, the electrode was not stable and the results were not reproducible, especially above pH = 8.0. The better sensitivity and shape of the voltammogram was observed at pH = 6.0 as indicated in Fig. 3. Therefore, pH = 6.0 was chosen for the entire voltammetric experiment.

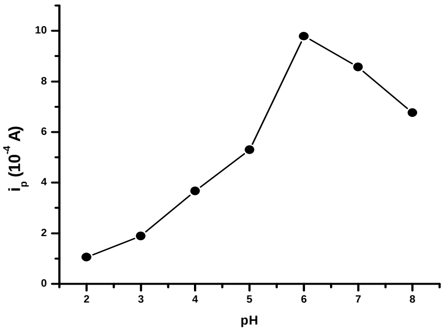

Figure 3: Effect of variation of pH on the anodic peak current of 1.0 mM pyridoxine in 0.1 M phosphate buffer solution at CoHCF at a frequency of 60 Hz and pulse amplitude of 150 mV

3.4. Effect of Frequency

The effect of frequency on the oxidation peak current of 1.0 mM of pyridoxine in 0.1 M phosphate buffer solution at pH = 6.0 using CoHCF was studied by varying the frequency from 15 to 90 Hz as shown in Fig. 4. The peak current increased with increasing of frequency up to 60 Hz. A frequency of 60 Hz was chosen for subsequent experiments because above 60 Hz of frequency the peak current became leveled off or decreased.

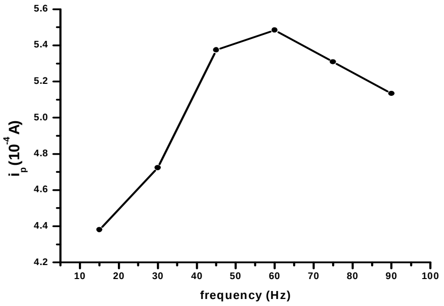

Figure 4: Effect of the frequency on the square wave voltammetric peak current of 1.0 mM of pyridoxine in 0.1 M PBS of pH = 6.0 at pulse amplitude of 150 mV

3.5. Effect of Pulse Amplitude

The effect of square wave pulse amplitude on the oxidation peak current of 1.0 mM of pyridoxine in 0.1 M PBS of pH = 6.0 at CoHCF was studied by varying the square wave pulse amplitudes from 25 mV to 150 mV at a frequency of 60 Hz (Fig. 5). Upon increasing the square wave pulse amplitude, a linear increase in the peak current was observed as shown in Fig. 6 and hence, 150 mV was chosen as the pulse amplitude for the subsequent experiment.

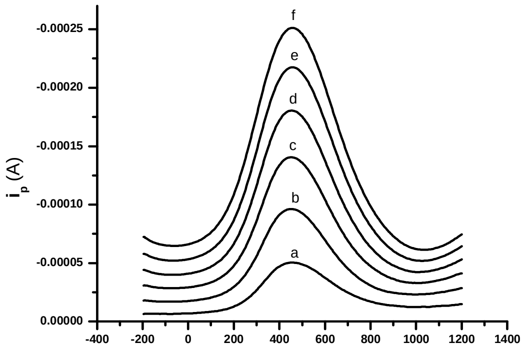

Figure 5: Square wave voltammograms of 1.0 mM of pyridoxine in 0.1 M PBS of pH = 6.0 at a frequency of 60Hz and square wave pulse amplitudes of (a) 25; (b) 50; (c) 75; (d) 100; (e) 125; (f) 150 mV

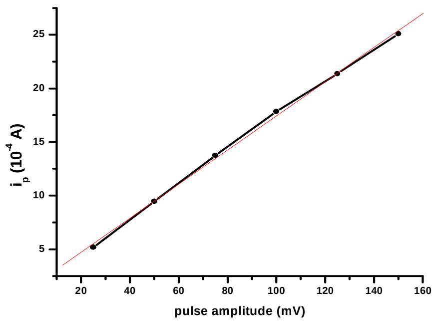

Figure 6: Square wave voltammetric peak currents of 1.0 mM of pyridoxine in 0.1 M PBS of pH = 6.0 at CoHCF at a frequency of 60Hz and different pulse amplitudes

3.6. Effect of Square Wave Step Potential

The effect of square wave step potential on the peak current of CoHCF was studied by varying the step potential from 2 mV to 10 mV at frequency of 60 Hz and amplitude of 150 mV. The peak current increases as the square wave step potential increases up to 6 mV and then the peak current declined slightly as shown in Fig. 7. Hence square wave step potential of 6 mV was chosen as optimal condition for the subsequent voltammetric experiment.

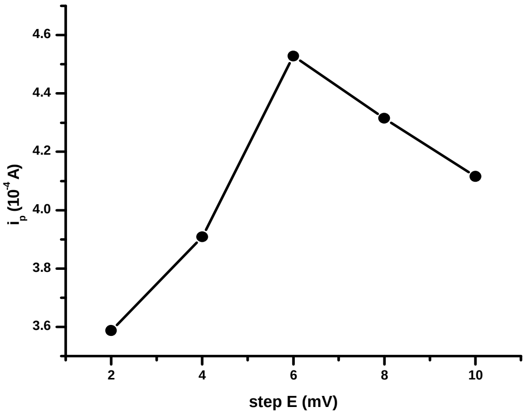

Figure 7: Square wave voltammetric peak currents of 1.0 mM of pyridoxine in 0.1 M PBS of pH = 6.0 at CoHCF at different step potentials frequency of 60Hz and pulse amplitudes of 150 mV

3.7. Optimum Experimental Conditions

The optimum experimental conditions from experimental parameters for square wave voltammetric investigation and determination of pyridoxine at CoHCF has been summarized as follows in Table 1.

Table 1: Optimum experimental conditions for the voltammetric study and determination of pyridoxine by square wave voltammetry at CoHCF/MCP

Parameters	Optimum value
pH of Buffer solution	6.0
Frequency	60Hz
Step E	6
Pulse amplitude	150mV
Modifier Composition (%)	20

3.8. Linear range and detection limit

The square wave voltammetric peak current of CoHCF/MCPE versus peak potential as a function of concentrations of pyridoxine ranging from 5 x 10^{-6} to 2.6x10^{-5}M were recorded based on the optimum experimental conditions as shown in Fig. 8. The plot of square wave voltammetric peak current versus the respective concentrations of pyridoxine was found to be linear in the range of 5.0×10^{-6}M to 2.6×10^{-5}M with a correlation coefficient of r = 0.99997 (n = 8) and a standard deviation (S) of 0.005 (Fig. 9).

The equation was found to be: I_p (µA) = 2.03277+0.10008C (µM) with the detection limit of 1.72×10^{-7}M, where I_p is peak current and C is concentration.

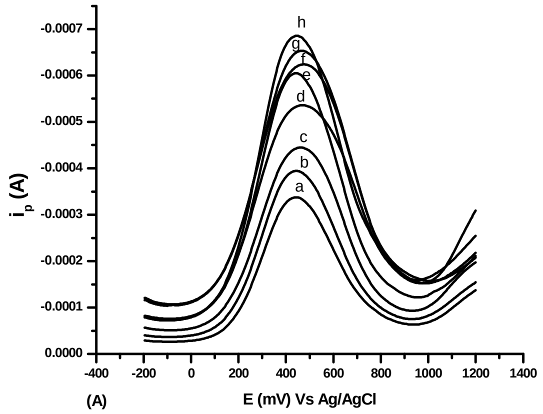

Figure 8: Square wave voltammograms of different pyridoxine concentrations of (a) 5.0; (b) 8.0; (c) 11.0; (d) 14.0; (e) 17.0; and (f) 20.0; (g) 23.0; (h) 26.0 µM in 0.1 M PBS of pH = 6.0 at a frequency of 60 Hz and pulse amplitude of 150 mV at CoHCF

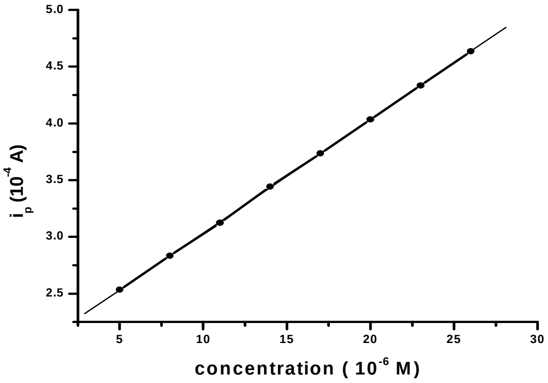

Figure 9: Calibration curve for determination of pyridoxine in 0.1 M PBS of pH = 6.0 at CoHCF at a frequency of 60Hz and pulse amplitude of 150 mV with different pyridoxine concentrations: (a) 5.0; (b) 8.0; (c) 11.0; (d) 14.0; (e) 17.0; and (f) 20.0; (g) 23.0; (h) 26 µM

3.9 Comparison of the Electrochemical Behavior of CoHCF/MCPE with other modified electrodes

Comparison of the electrochemical behavior of CoHCF in the detection of pyridoxine with some previously reported electrodes has been summarized Table 2

Table 2: Comparison of the electrochemical behavior of CoHCF in the detection of pyridoxinewith some previously reported electrode

Electrode	Linear range (M)	Detection limit(M)	Method	Reference
ssDNA/ GCE	$0.1x10^{-3}$-$6.0x\ 10^{-3}$	$4\ x\ 10^{-5}$	CV	30
CuHCF/ CPE	$1.2x10^{-6}$ -$6.9x10^{-4}$	$4.1\ x10^{-7}$	CV	31
CrHCF/ GCE	$1.33\ x10^{-6}$ – $1.32x10^{-5}$	$3.46\ x\ 10^{-7}$	CV	32
CoHCF/ CPE	$5\ x\ 10^{-6}$ – $2.6\ x10^{-5}$	$1.72\ x\ 10^{-7}$	SWV	This work

3.10. Quantitative determination of pyridoxine

The validity of the proposed modified electrode for the determination of pyridoxine using square wave voltammetry was proved by examining five different brands of pyridoxine. The concentration of pyridoxine (VB_6) present in the tablets was determined from the calibration curve as shown in Table 3. It exhibited that the relative standard deviation of 0.34-1.3%, indicating the applicability of the proposed method. In order to establish the suitability of the proposed method, known amounts of the standard VB_6 were added into the analytical solution of the VB_6 tablets and the same procedure was applied. The recoveries indicate that the accuracy and repeatability of the proposed voltammetric method are in a good agreement.

Table 3: Amount of pyridoxine detected from each solutions of brands of pyridoxine

Brands	Labeled pyridoxine**(mg)**	**Pyridoxine recovered (mg)**	**% Recovery**
Benadon	40	38.73 ± 0.83	96.83
Pyridoxine	50	48.12 ± 1.3	96.24
B-Long	100	90.94 ± 0.76	90.94
Neurobin	125	116.95 ± 0.34	93.46
Neurovit	50	49.23 ± 0.65	98.46

CONCLUSION

The voltammetric determination of pyridoxine was successfully conducted by SWV using CoHCF modified carbon paste electrode. The linear working range was much lower and the detection limit was greatly improved to allow a sensitive detection of pyridoxine. The very low detection limit and its high sensitivity suggest that the modified carbon paste electrode can act as a useful electrochemical sensor for the determination of pyridoxine in real samples. The optimum experimental conditions for the oxidation of pyridoxine were determined. The proposed method was a good alternative for the analytical determination of VB_6 because it is simple, low cost and low detection limit, and it has sufficient precision, accuracy and sensitivity.

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