

Voltammetric Investigation of Vitamin B₆ at a Glassy Carbon Electrode and Its Application in Determination

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The voltammetric behavior of Vitamin B₆ (VB₆) was studied at a glassy carbon electrode in phosphate buffers using cyclic, linear sweep and differential pulse voltammetry. The oxidation process was shown to be irreversible over the entire pH range studied (4.0-10.0) and was adsorption controlled. The adsorption amount of VB₆ on the glassy carbon electrode was examined by chronocoulometry and the value of Δn_0 , product of transfer coefficient and number of electrons transferred in the rate determining step, was determined from Tafel plot. VB₆ was determined by differential pulse voltammetry and the peak current was found linearly with its concentration in the range of 3×10^{-7} - 2×10^{-4} mol L⁻¹. The detection limit was 1×10^{-7} mol L⁻¹. The procedure was successfully applied for the assay of VB₆ in tablets.

Key Words: Vitamin B₆, Glassy carbon electrode, Cyclic voltammetry, Differential pulse voltammetry, Pharmaceutical analysis

Introduction

Vitamin B₆ (VB₆) 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 for the release of stored carbohydrates for energy. It is involved in the production of red blood cells and antibodies and in the maintenance of a healthy skin and healthy digestion. It is also important for normal function of the nervous system and several hormones. VB₆ deficiency may cause weakness, depression, anaemia and skin disorders. Permanent nerve damage may also occur. Therefore, developing a sensitive and simple analysis method for VB₆ is very important in food and pharmaceutical industries.

VB₆ was commonly determined by spectrophotometric method¹⁻⁵ and chromatography.⁶⁻⁸ The separation and determination of VB₆ by chromatography⁹ and electrophoresis¹⁰ with amperometric detection, using a carbon disk electrode as electrochemical detector, have also been published. A few papers have been described in literature concerning the determination of VB₆ using voltammetry. Söderhjelm and Lindquist¹¹ were the first to study the electrochemical behavior of VB₆ by a carbon paste electrode, and then reported a voltammetric method for the determination of VB₆. Gu *et al.* has studied the electrochemical behavior and simultaneous determination of vitamin B₂, B₆, and C at electrochemically pretreated glassy carbon electrode.¹² Its linear concentration range of VB₆ is 2.5×10^{-6} - 7.5×10^{-3} mol L⁻¹ with a detection limit 8.0×10^{-7} mol L⁻¹. After that, two modified electrodes has been used to determine VB₆, one is a carbon paste electrode modified with vanadyl(IV)-salen complex,¹³ the other is a glassy carbon electrode modified with carbon nanotubes.¹⁴ Their detection limit is as low as 3.7×10^{-5} mol L⁻¹ and 2×10^{-7} mol L⁻¹, respectively. However, comparing a bare electrode, a reproducible active

surface of a modified electrode or a pretreated electrode is difficult to control.

The aim of the present study is to examine the oxidative properties and assay of VB₆ at a glassy carbon electrode using a voltammetric method. After optimizing the experimental parameters, differential pulse voltammetry was developed for the direct measurement of VB₆. Compared with other published methods, this proposed method possessed many advantages such as very low detection limit, simplicity and good reproducibility. And it was applied to determine VB₆ in tablets successfully.

Experimental Section

Reagents. VB₆ standard solution (1×10^{-2} mol L⁻¹) was prepared by dissolving an appropriate amount of pyridoxine hydrochloride (Sigma) in redistilled water. The solution was stable for 2 weeks in a refrigerator at about 4 °C. All chemicals were of analytical grade and used without purification, and redistilled water was used throughout. The supporting electrolyte was usually phosphate buffer containing 0.06 mmol L⁻¹ Na₂HPO₄ and NaH₂PO₄. Drug tablets were commercially available and were purchased from market.

Apparatus. All the electrochemical measurements were carried out with a CHI 830 Electrochemical Workstation (CH Instrument, Austin, USA). A conventional three-electrode system, including a glassy carbon working electrode (3-mm diameter), a saturated calomel reference electrode (SCE) and a Pt wire counter electrode, was employed. To provide a reproducible active surface and improve the sensitivity and resolution of the voltammetric peaks, the working electrode was polished with 0.5 μ m alumina powder on a polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed with methanol and double

distilled water. The electrode cleaning procedures require only 2 min. The active area of the resulted electrode ($3.1 \times 10^{-2} \text{ cm}^2$) was obtained by using cyclic voltammetry in $5 \times 10^3 \text{ mol L}^{-1} \text{ K}_4\text{Fe}(\text{CN})_6$ containing $1 \times 10^{-1} \text{ mol L}^{-1} \text{ KCl}$.

Procedures. After 10 mL of phosphate buffer ($6 \times 10^{-5} \text{ mol L}^{-1}$) was placed in the electrochemical cell, certain volume of standard solution of VB₆ was added into the 10 mL cell containing phosphate buffer. Then the mixture solution was stirred for 2 min and kept unstirred for 20 s at open circuit. The oxidation peak of VB₆ at 0.74 V was recorded in the potential range from 0.2 V to 1.0 V. All measurements were carried out at room temperature.

Results and Discussion

Electrochemical behavior of VB₆ at GCE. The cyclic voltammograms of a GCE in phosphate buffer at pH 7.0 with and without of VB₆ are illustrated in Figure 1. In the potential range from 0.2 to 1.0 V, there is no observable redox peaks for a bare GCE (Fig. 1a). However, upon addition of $1 \times 10^{-5} \text{ mol L}^{-1} \text{ VB}_6$, a well-defined oxidation peak appears at 0.74 V is observed (Fig. 1b). On the reverse potential scan from 1.0 to 0.2 V, there is no corresponding

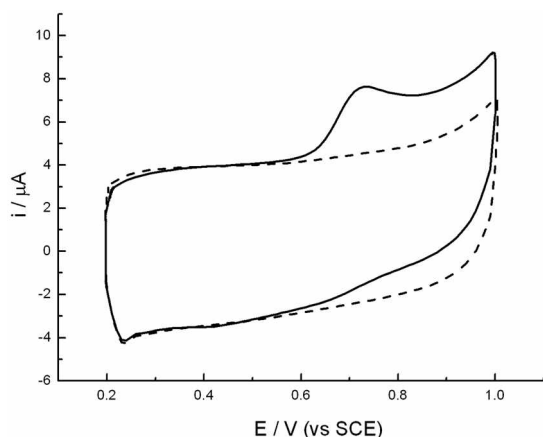


Figure 1. Cyclic voltammograms of bare GC in pH 7.0 phosphate buffers (a): a + $1 \times 10^{-5} \text{ mol L}^{-1} \text{ VB}_6$ (b). Scan rate: 150 mV s^{-1} .

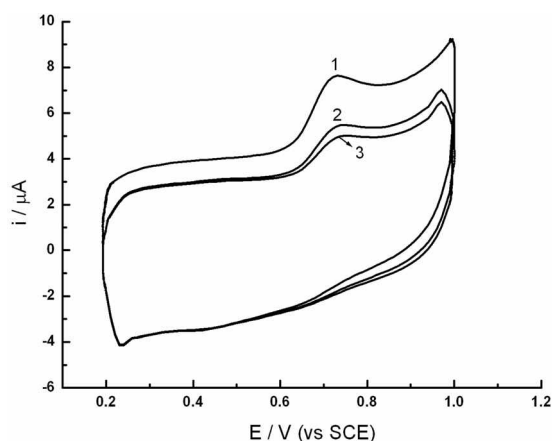


Figure 2. Successive cyclic voltammograms of $1 \times 10^{-5} \text{ mol L}^{-1} \text{ VB}_6$ on bare GC in pH 7.0 phosphate buffers. Scan rate: 150 mV s^{-1} .

reduction peak observed for VB₆. Moreover, the oxidation peak current of VB₆ decreases remarkably during the successive cyclic potential sweeps (Fig. 2). After the second cyclic voltammetric sweep, the peak current decreases slightly and finally almost maintains unchangeable. This phenomenon may be caused by the fact that the adsorption of VB₆ or its oxidative product occurs at the electrode.

The effect of potential scan rate on the peak currents of VB₆ was evaluated by linear sweep voltammetry. After background subtraction, the peak currents was found to be proportional to the scan rate over $10\text{-}500 \text{ mV s}^{-1}$ range (Fig. 3), which suggested that an adsorption-controlled process was involved. The peak potential was almost remained unchanged with increasing the scan rate. The value of $n\alpha_0$, product of transfer coefficient (α_0) and number of electrons (n) transferred in the rate determining step, was determined from Tafel treatment ($\log i_p$ vs. E) of the voltammetric curves.¹⁵ The $n\alpha_0$ value was obtained as 0.49. In most systems α_0 turns out to be between 0.3 and 0.7, and it can usually be approximated by 0.5 in the absence of actual measurements.¹⁶ These results, therefore, demonstrate that one electron is involved in the oxidation of VB₆.

The electrooxidation of VB₆ was also studied over pH range of 4.0-10.0 in phosphate buffers by differential pulse

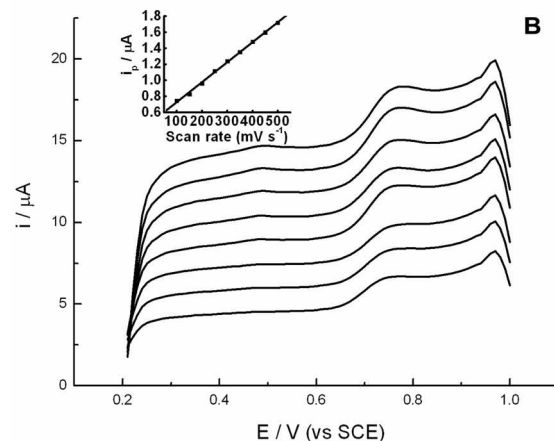
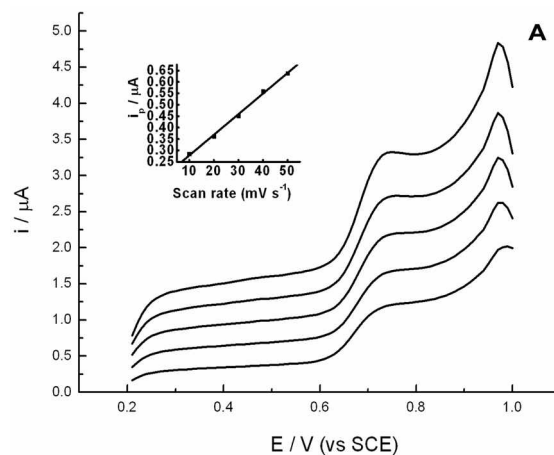


Figure 3. Cyclic voltammograms of $1 \times 10^{-5} \text{ mol L}^{-1} \text{ VB}_6$ on bare GC in pH 7.0 phosphate buffers. Scan rate: 10-50 mV s^{-1} , Inset: i_p vs v . (A), 100-500 mV s^{-1} (B), Inset: i_p vs v .

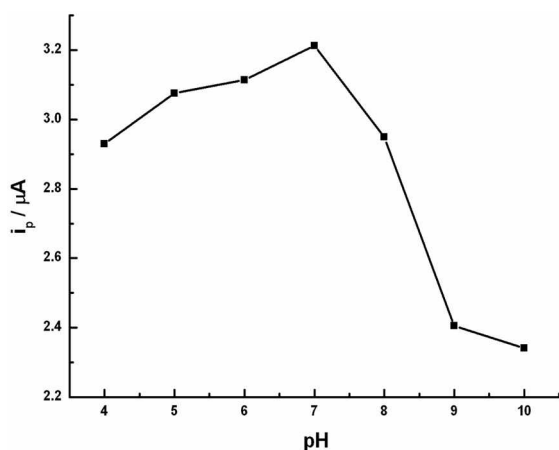


Figure 4. Effect of pH on the peak currents for $1 \times 10^{-5} \text{ mol L}^{-1}$ VB_6 on bare GC in phosphate buffer by differential pulse voltammetry.

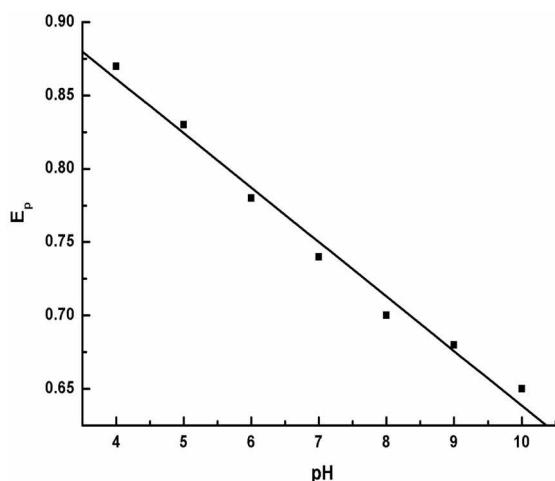
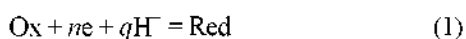


Figure 5. Effect of pH on the peak potentials for $1 \times 10^{-5} \text{ mol L}^{-1}$ VB_6 on bare GC in phosphate buffer by differential pulse voltammetry.

voltammetry. The peak definition was best when using 50 mV pulse amplitude, 30 ms pulse width and 20 mVs^{-1} scan rate. The effect of pH on the oxidation peak current is shown in the Figure 4. The peak current reaches the maximum at pH 7.0, and the pH value is similar to physiological pH, so this pH value was adopted in the following experiment. Figure 5 shows the $E_p \sim \text{pH}$ relationship. The anodic peak potential of VB_6 shifts linearly towards less positive values with increasing the pH by 0.0371 V pH^{-1} . Based on the equation (2), the number of hydrogen ion taking part in the electrode reactions was estimated as 2.

In aqueous solution, for the reaction



The E_p value of the reaction is estimated by the following equation¹⁷:

$$E_{1/2} = E^0 + 2.303(qRT/\alpha_0 nF) \log \text{H}^+ \quad (2)$$

Where $E_{1/2}$ is the half wave potential, E^0 is the formal potential, α_0 is the transfer coefficient, q and n are the

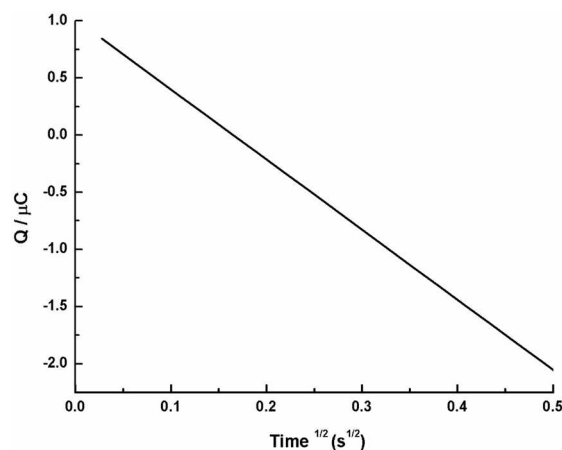


Figure 6. The plot of $Q \sim t^{1/2}$ for a single-potential step chronocoulometric experiment in phosphate buffers (pH 7.0) in presence of $2 \times 10^{-4} \text{ mol L}^{-1}$ VB_6 .

number of the protons and electrons involved in the reaction, respectively. The value of $n\alpha_0$ was determined as 0.49 (see above). Other terms have their usual meanings.

Adsorption of VB_6 on the glassy carbon electrode measured by single-potential step chronocoulometry. The chronocoulometric method was applied to determine the diffusion coefficient D and Q_{ads} of VB_6 on the glassy carbon electrode, according to the formula given by Anson¹⁸

$$Q = 2nEAC(Dt)^{1/2} / \pi^{1/2} + Q_{dl} + Q_{ads} \quad (3)$$

Q_{dl} is double-layer charge. Q_{ads} the faradaic charge due to the oxidation of adsorbed VB_6 . Q_{dl} is assumed not changed in the presence and absence of VB_6 in our work. The plot of Q vs. $t^{1/2}$ should be linear. From the slope and intercept, the values of D and Q_{ads} can be obtained. In our experiment, the plot of net charge (point by point background subtraction) against $t^{1/2}$ shows straight line (Fig. 6). The values of the slope and Q_{ads} in Figure 6 are 6.587×10^{-6} and $6.134 \times 10^{-6} \text{ C}$, respectively. As the number of electron involved in the oxidation of VB_6 is 1 and $A = 3.1 \times 10^{-2} \text{ cm}^2$, $C = 1.0 \times 10^{-5} \text{ mol L}^{-1}$, it is calculated that $D = 4.38 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. The surface concentration, Γ^s , can be obtained by the equation (4) as $2.09 \times 10^{-9} \text{ mol cm}^{-2}$.

$$\Gamma^s = Q_{ads} / nFA \quad (4)$$

Analysis application

Optimization of experimental parameters: The influence of accumulation potential on the oxidation peak current of VB_6 was examined (Fig. 7). The oxidation peak current of $1 \times 10^{-5} \text{ mol L}^{-1}$ VB_6 was compared under different accumulation potentials after 30s of accumulation. It was found that under positive accumulation potential the oxidation peak current is smaller than that at the accumulation potential of 0 V, which indicated VB_6 is positively charged at pH 7.0. When the accumulation potential is less positive than 0 V, the oxidation peak current gradually becomes higher, and at the accumulation potential of -0.6 V the oxidation peak current reached the maximum. However, when the accumulation potential is less positive than -0.6 V , the oxidation

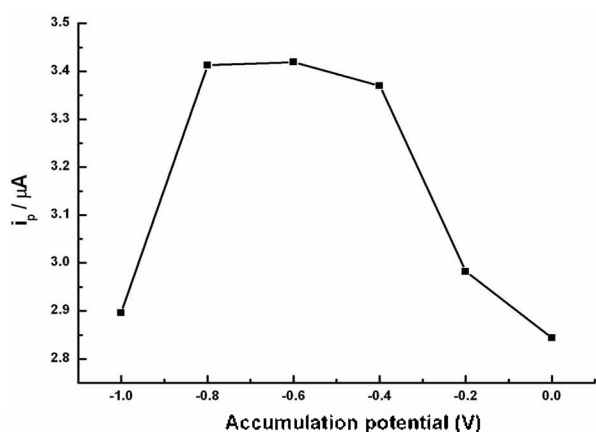


Figure 7. Effect of accumulation potential on the peak currents for $1 \times 10^{-5} \text{ mol L}^{-1}$ VB₆ on bare GC in phosphate buffer by differential pulse voltammetry.

peak current gradually decreases. It may be the polarization of the electrode influence the oxidation of VB₆.

The influence of accumulation time on the oxidation peak current of VB₆ was also investigated. The oxidation peak current increases greatly within 30 s and then remains unchanged, suggesting that the accumulation of VB₆ at the glassy carbon electrode is very rapid to reach saturation.

Calibration graph: Under optimized experimental parameters, the calibration curve was obtained in pH 7.0 phosphate buffers by differential pulse voltammetry. The best parameters on the glassy carbon electrode are accumulation potential = -0.6 V; accumulation time = 30 s; pulse amplitude = 50 mV; scan rate = 20 mV s⁻¹; and pulse width = 50 ms. The linear segment increases from 3×10^{-7} - $2 \times 10^{-4} \text{ mol L}^{-1}$ ($r = 0.995$) with a regression equation of $i_p = 0.19c + 0.11$ ($r = 0.998$, C in $\mu\text{mol L}^{-1}$, i_p in μA). It was found that this method could detect $1 \times 10^{-7} \text{ mol L}^{-1}$ VB₆ after 30 s of accumulation *via* this method. The relative standard deviation (R.S.D.) of 2.1% for $2 \times 10^{-6} \text{ mol L}^{-1}$ VB₆ ($n = 7$) showed good reproducibility. The linearity and LOD for VB₆ by this electrochemical method was compared with other method, such as spectrophotometric method,¹ chromatography¹² and voltammetric methods using a pretreated electrode¹² or modified electrodes.^{13,14} The results were listed in Table 1. It indicated that the presented method possessed the advantages of wide linearity range and very low detection limit for VB₆.

Interferences: To evaluate the potential effect of foreign species on the determination of VB₆ at $1.0 \times 10^{-6} \text{ mol L}^{-1}$ level, a systematic study was carried out under the above optimized conditions. The peak currents of VB₆ in the absence and presence of foreign species were measured by DPV, respectively, and the error was consequently obtained. The results are listed in Table 2. It is found that $5.0 \times 10^{-4} \text{ mol L}^{-1}$ Ca²⁺, Mg²⁺, Zn²⁺, Al³⁺, Cd²⁺, Pb²⁺, Fe³⁺, Cu²⁺, $6.0 \times 10^{-5} \text{ mol L}^{-1}$ dopamine, ascorbic acid (Vitamin C), uric acid, xanthine, cystine, serine, $2.0 \times 10^{-5} \text{ mol L}^{-1}$ Vitamin B₁, Vitamin B₂, Vitamin B₁₂ and $1.0 \times 10^{-5} \text{ mol L}^{-1}$ glucose almost do not interfere with the oxidation signal of VB₆

Table 1. The linearity and LOD for Vitamin B₆ by various methods

Analyte	Linearity	LOD	Ref.
VB ₆	0.2 - 4 mg L ⁻¹	0.060 mg L ⁻¹	1
VB ₆	2.5×10^{-6} - $1.0 \times 10^{-3} \text{ mol L}^{-1}$	$1.0 \times 10^{-6} \text{ mol L}^{-1}$	9
VB ₆	2.5×10^{-6} - $7.5 \times 10^{-3} \text{ mol L}^{-1}$	$8.0 \times 10^{-7} \text{ mol L}^{-1}$	12
VB ₆	4.5×10^{-4} - $3.3 \times 10^{-3} \text{ mol L}^{-1}$	$3.7 \times 10^{-5} \text{ mol L}^{-1}$	13
VB ₆	5×10^{-7} - $1 \times 10^{-4} \text{ mol L}^{-1}$	$2 \times 10^{-7} \text{ mol L}^{-1}$	14
VB ₆	3×10^{-7} - $2 \times 10^{-4} \text{ mol L}^{-1}$	$1 \times 10^{-7} \text{ mol L}^{-1}$	This method

Table 2. Interferences of foreign species on the oxidation peak current of $1.0 \times 10^{-6} \text{ mol L}^{-1}$ VB₆; accumulation potential = -0.6 V; accumulation time = 30 s; pulse amplitude = 50 mV; scan rate = 20 mV s⁻¹; and pulse width = 50 ms

Foreign species	Tolerance level (mol L^{-1}) ^a
Ca ²⁺ , Mg ²⁺ , Zn ²⁺ , Al ³⁺ , Cd ²⁺ , Pb ²⁺ , Fe ³⁺ , Cu ²⁺	5.0×10^{-4}
dopamine, ascorbic acid, uric acid, xanthine, cystine, serine,	6.0×10^{-5}
vitamin B ₁ , vitamin B ₂ , vitamin B ₁₂	2.0×10^{-5}
glucose	1.0×10^{-5}

^aFor 5% error

(signal change below 5%), suggesting that this proposed voltammetric method has excellent selectivity toward VB₆. This means the selective detection of VB₆ on the bare GCE is possible since the oxidation potential (E_0) of VB₆ is different that those of interferences. In order to determine the oxidation potential (E_0) of water soluble vitamins including Vitamin C, VB₁, VB₂, and VB₁₂ on the electrode response, a study involving these compounds was performed. At the bare GCE in the absence of VB₆ the voltammetric measurements were realized in pH 7.0 phosphate buffers containing $1.0 \times 10^{-6} \text{ mol L}^{-1}$ of Vitamin C, VB₁, VB₂, and VB₁₂, respectively. It is found that the oxidation potential (E_0) of Vitamin C is 0.35 V, and VB₁, VB₂ and VB₁₂ have no any response on this bare GCE in the potential range from 0.2 V to 1.0 V.

VB₆ assay in tablets: Ten tablets were weighted accurately and crushed to a fine powder. 675.6 mg of these powders were transferred to a 50 mL flask and were dissolved in 50 mL pH 7.0 phosphate buffers. After sonication it was filtered. An aliquot of the filtrate was placed in a 100 mL calibrated flask and diluted with pH 7.0 phosphate buffers. The prepared solution from drug tablets was detected on the glassy carbon electrode by differential pulse voltammetry. After 1 mL of the prepared solution from drug tablets was placed in the electrochemical cell, then 9 mL of pH 7.0 phosphate buffers was added. After accumulation for 30 s at the accumulation potential of -0.6 V, the differential pulse voltammograms were recorded (Fig. 8).

The amount of VB₆ present in tablet is calculated from the calibration equation and the results are listed in Table 3. It exhibited the relative standard deviation of 0.30-1.0%, indicating the applicability of the proposed method. Further, in order to establish the suitability of the proposed method,

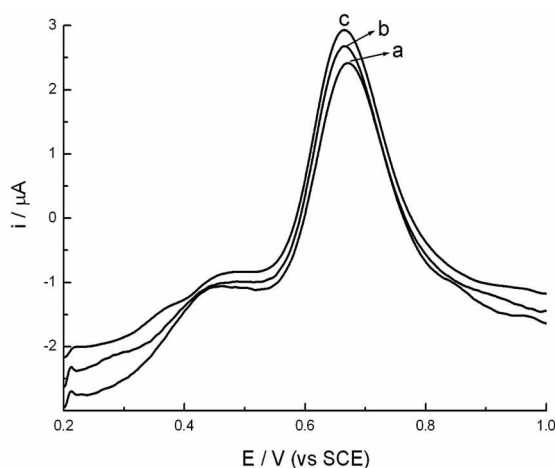


Figure 8. Differential pulse voltammetry of VB₆ tablets in pH 7.0 phosphate buffers (a); a + 1.0×10^{-5} mol L⁻¹ VB₆ (b); a + 5.0×10^{-5} mol L⁻¹ VB₆. IncrE(V) = 0.001, Amplitude (V) = 0.05, Pulse width (s) = 0.05, Pulse period (s) = 0.2.

Table 3. Determination of Vitamin B₆ in drug tablets by the proposed voltammetric method^a

Sample No.	Lable claim (mg)	Found (mg) ^a	RSD (%)	Recovery (%)
1	10.0	9.92	0.80	102.1
2	10.0	10.10	1.0	98.5
3	10.0	9.93	0.70	98.9
4	10.0	10.03	0.30	101.4
5	10.0	10.06	0.60	100.5

^aAverage of seven determinations

known amounts of the standard VB₆ were added into the analytical solution of the VB₆ tablets and the same procedure was applied. The recoveries indicate that the accuracy and repeatability of the proposed voltammetric method are very good. The effect of excipients on the voltammetric response of VB₆ was investigated. It was found that microcrystalline cellulose, hydroxypropylmethylcellulose, and lactose did not cause interferences.

Conclusion

In this paper, the electrochemical behavior of VB₆ on

glassy carbon electrode has been investigated by cyclic, linear sweep, differential pulse voltammetry and chrono-coulometry. The glassy carbon electrode provided a good platform to detect VB₆, and it was applied to detect VB₆ in tablets with good results. The proposed method was a good alternative for the analytical determination of VB₆ because it was simple, low cost and low detection limit, and it had sufficient precision, accuracy and sensitivity.

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