Voltammetric Investigation of Vitamin B_6 at a Glassy Carbon Electrode and Its Application in Determination

Yunhua Wu* and Fajun Song

*Key Lab for Biotechnology of National Commission for Nationalities, College of Life Science, The South Central University for Nationalities, Wuhan 430074, P.R. China. *E-mail: yunhuawu@yahoo.com.cn*
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The voltammetic behavior of Vitamin B_6 (VB_6) 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 VB_6 on the glassy carbon electrode was examined by chronocoulometry and the value of án$_á$, product of transfer coefficient and number of electrons transferred in the rate determining step, was determined from Tafel plot. VB_6 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 VB_6 in tablets.

Key Words : Vitamin B_6, Glassy carbon electrode, Cyclic voltammetry, Differential pulse voltammetry, Pharmaceutical analysis

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 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. VB_6 deficiency may cause weakness, depression, anaemia and skin disorders. Permanent nerve damage may also occur. Therefore, developing a sensitive and simple analysis method for VB_6 is very important in food and pharmaceutical industries.

VB_6 was commonly determined by spectrophotometric method[1-5] and chromatography.[6-8] The separation and determination of VB_6 by chromatography[9] and electrophoresis[10] 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 VB_6 using voltammetry. Söderhjelm and Lindquist[11] were the first to study the electrochemical behavior of VB_6 by a carbon paste electrode, and then reported a voltammetric method for the determination of VB_6. Gu *et al.* has studied the electrochemical behavior and simultaneous determination of vitamin B_2, B_6, and C at electrochemically pretreated glassy carbon electrode.[12] Its linear concentration range of VB_6 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 VB_6, 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 VB_6 at a glassy carbon electrode using a voltammetric method. After optimizing the experimental parameters, differential pulse voltammetry was developed for the direct measurement of VB_6. 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 VB_6 in tablets successfully.

Experimental Section

Reagents. VB_6 standard solution (1×10^{-2} molL^{-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} Na_2HPO_4 and NaH_2PO_4. 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 μm 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 (3.1×10^{-2} cm^2) was obtained by using cyclic voltammetry in 5×10^3 mol L^{-1} $K_4Fe(CN)_6$ containing 1×10^{-1} mol L^{-1} KCl.

Procedures. After 10 mL of phosphate buffer (6×10^{-5} mol L^{-1}) was placed in the electrochemical cell, certain volume of standard solution of VB_6 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 VB_6 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 VB_6 at GCE. The cyclic voltammograms of a GCE in phosphate buffer at pH 7.0 with and without of VB_6 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 1×10^{-5} mol L^{-1} 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 reduction peak observed for VB_6. Moreover, the oxidation peak current of VB_6 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 VB_6 or its oxidative product occurs at the electrode.

The effect of potential scan rate on the peak currents of VB_6 was evaluated by linear sweep voltammetry. After background subtraction, the peak currents was found to be proportional to the scan rate over 10-500 mVs^{-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 $n\alpha_a$, product of transfer coefficient (α_a) and number of electrons (n) transferred in the rate determining step, was determined from Tafel treatment (log i *vs.* E) of the voltammetric curves.[15] The $n\alpha_a$ value was obtained as 0.49. In most systems α_a 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 VB_6.

The electrooxidation of VB_6 was also studied over pH range of 4.0-10.0 in phosphate buffers by differential pulse

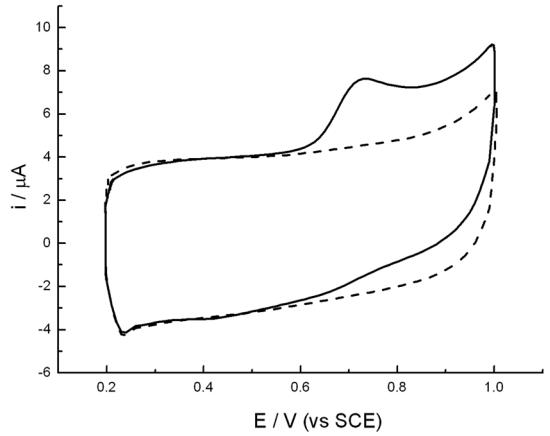

Figure 1. Cyclic voltammograms of bare GC in pH 7.0 phosphate buffers (a); a + 1×10^{-5} molL^{-1} VB_6 (b). Scan rate: 150 mV s^{-1}.

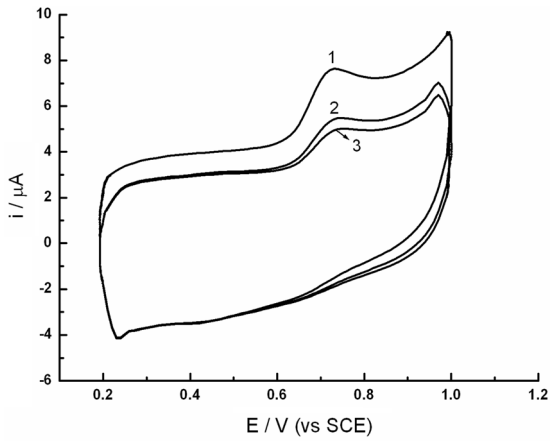

Figure 2. Successive cyclic voltammograms of 1×10^{-5} molL^{-1} VB_6 on bare GC in pH 7.0 phosphate buffers. Scan rate: 150 mV s^{-1}.

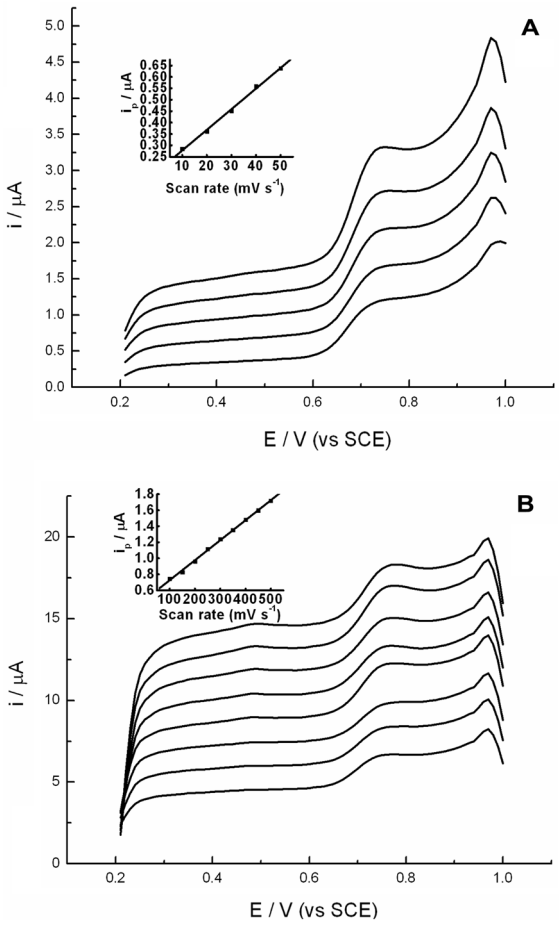

Figure 3. Cyclic voltammograms of 1×10^{-5} molL^{-1} VB_6 on bare GC in pH 7.0 phosphate buffers. Scan rate: 10-50 mV s^{-1}, Inset: i_p *vs v.* (A); 100-500 mV s^{-1} (B), Inset: i_p *vs v.*

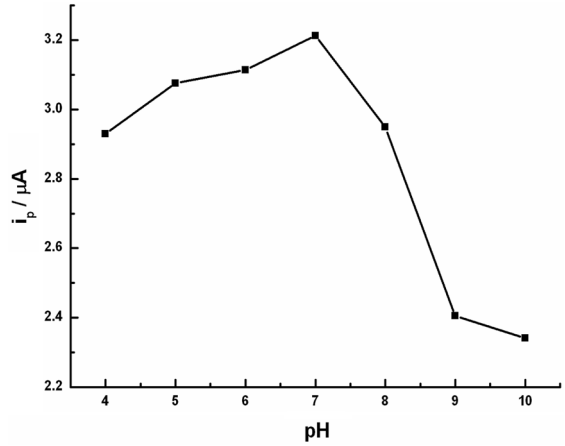

Figure 4. Effect of pH on the peak currents for 1×10^{-5} mol L^{-1} VB_6 on bare GC in phosphate buffer by differential pulse voltammetry.

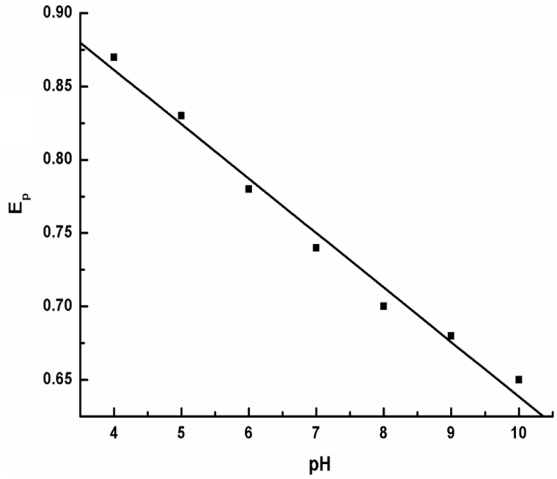

Figure 5. Effect of pH on the peak potentials for 1×10^{-5} mol L^{-1} VB_6 on bare GC in phosphate buffer by differential pulse voltammetry.

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 ~ pH relationship. The anodic peak potential of VB_6 shifts linearly towards less positive values with increasing the pH by 0.0371V 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

$$Ox + ne + qH^+ = Red \quad (1)$$

The E_p value of the reaction is estimated by the following equation[17]:

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

Where $E_{1/2}$ is the half wave potential, $E^{0'}$ is the formal potential, α_a is the transfer coefficient, q and n are the number of the protons and electrons involved in the reaction, respectively. The value of $n\alpha_a$ was determined as 0.49 (see above). Other terms have their usual meanings.

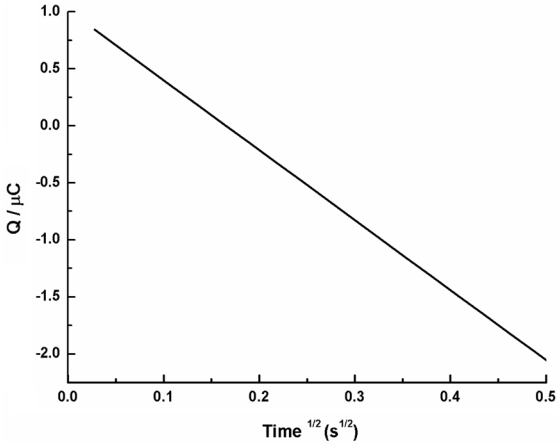

Figure 6. The plot of Q ~ $t^{1/2}$ for a single-potential step chronocoulometric experiment in phosphate buffers (pH 7.0) in presence of 2×10^{-4} molL^{-1} VB_6.

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 Anson[18]

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

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 6.134×10^{-6} C, respectively. As the number of electron involved in the oxidation of VB_6 is 1 and $A = 3.1 \times 10^{-2}$ cm^2, $C = 1.0 \times 10^{-5}$ mol L^{-1}, it is calculated that $D = 4.38 \times 10^{-6}$ cm^2 s^{-1}. The surface concentration, Γ^s, can be obtained by the equation (4) as 2.09×10^{-9} 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 1×10^{-5} 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

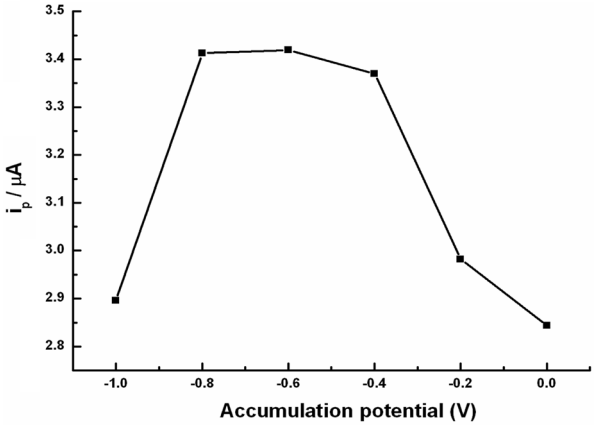

Figure 7. Effect of accumulation potential on the peak currents for 1×10^{-5} mol L^{-1} VB_6 on bare GC in phosphate buffer by differential pulse voltammetry.

peak current gradually decreases. It may be the polarization of the electrode influence the oxidation of VB_6.

The influence of accumulation time on the oxidation peak current of VB_6 was also investigated. The oxidation peak current increases greatly within 30 s and then remains unchanged, suggesting that the accumulation of VB_6 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} - 2×10^{-4} molL^{-1}, (r = 0.995) with a regression equation of $i_p = 0.19\,c + 0.11$ (r = 0.998, C in μmol L^{-1}, i_p in μA). It was found that this method could detect 1×10^{-7} mol L^{-1} VB_6 after 30 s of accumulation *via* this method. The relative standard deviation (R.S.D.) of 2.1% for 2×10^{-6} mol L^{-1} VB_6 (n = 7) showed good reproducibility. The linearity and LOD for VB_6 by this electrochemical method was compared with other method, such as spectrophotpmetric method,[1] chromatography[9] and voltammetric methods using a pretreated electrode[12] 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 VB_6.

Interferences: To evaluate the potential effect of foreign species on the determination of VB_6 at 1.0×10^{-6} mol L^{-1} level, a systematic study was carried out under the above optimized conditions. The peak currents of VB_6 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 5.0×10^{-4} mol L^{-1} Ca^{2+}, Mg^{2+}, Zn^{2+}, Al^{3+}, Cd^{2+}, Pb^{2+}, Fe^{3+}, Cu^{2+}, 6.0×10^5 mol L^{-1} dopamine, ascorbic acid (Vitamin C) , uric acid, xanthine, cystine, serine, 2.0×10^{-5} mol L^{-1} Vitamin B_1, Vitamin B_2, Vitamin B_{12} and 1.0×10^{-5} mol L^{-1} glucose almost do not interfere with the oxidation signal of VB_6 (signal change below 5%), suggesting that this proposed voltammetric method has excellent selectivity toward VB_6. This means the selective detection of VB_6 on the bare GCE is possible since the oxidation potential (E_0) of VB_6 is different that those of interferences. In order to determine the oxidation potential (E_0) of water soluble vitamins including Vitamin C, VB_1, VB_2, and VB_{12} on the electrode response, a study involving these compounds was performed. At the bare GCE in the absence of VB_6 the voltammetric measurements were realized in pH 7.0 phosphate buffers containing 1.0×10^{-6} mol L^{-1} of Vitamin C, VB_1, VB_2, and VB_{12}, respectively. It is found that the oxidation potential (E_0) of Vitamin C is 0.35 V, and VB_1, VB_2 and VB_{12} have no any response on this bare GCE in the potential range from 0.2 V to 1.0 V.

Table 1. The linearity and LOD for Vitamin B_6 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} - 1.0×10^{-3} mol L^{-1}	1.0×10^{-6} mol L^{-1}	9
VB_6	2.5×10^{-6} - 7.5×10^{-3} mol L^{-1}	8.0×10^{-7} mol L^{-1}	12
VB_6	4.5×10^{-4} - 3.3×10^{-3} mol L^{-1}	3.7×10^{-5} mol L^{-1}	13
VB_6	5×10^{-7} - 1×10^{-4} mol L^{-1}	2×10^{-7} mol L^{-1}	14
VB_6	3×10^{-7} - 2×10^{-4} mol L^{-1}	1×10^{-7} mol L^{-1}	This method

Table 2. Interferences of foreign species on the oxidation peak current of 1.0×10^{-6} mol L^{-1} VB_6. 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})*
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}

*For 5% error

VB_6 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 VB_6 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,

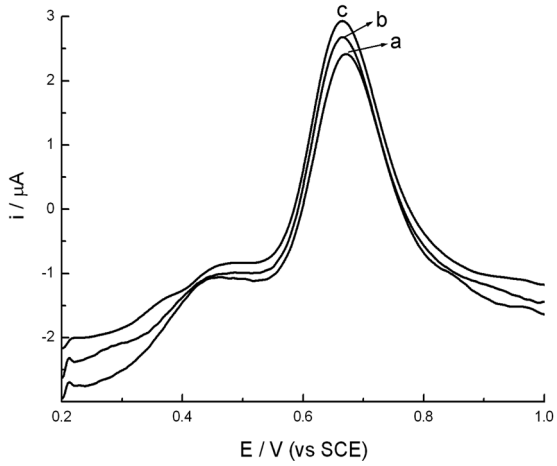

Figure 8. Differential pulse voltammetry of VB_6 tablets in pH 7.0 phosphate buffers (a); a + 1.0×10^{-5} mol L^{-1} VB_6 (b); a + 5.0×10^{-5} mol L^{-1} VB_6. IncrE(V) = 0.001, Amplitude (V) = 0.05, Pulse width (s) = 0.05, Pulse period (s) = 0.2.

Table 3. Determination of Vitamin B_6 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

[a]Average of seven determinations

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 very good. The effect of excipients on the voltammetric response of VB_6 was investigated. It was found that microcrystalline cellulose, hydroxypropylmethylcellulose, and lactose did not cause interferences.

Conclusion

In this paper, the electrochemical behavior of VB_6 on glassy carbon electrode has been investigated by cyclic, linear sweep, differential pulse voltammetry and chronocoulometry. The glassy carbon electrode provided a good platform to detect VB_6, and it was applied to detect VB_6 in tablets with good results. The proposed method was a good alternative for the analytical determination of VB_6 because it was simple, low cost and low detection limit, and it had sufficient precision, accuracy and sensitivity.

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