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Journal of Pharmaceutical and Biomedical Analysis 36 (2004) 631–635
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Voltammetric determination of pyridoxine (Vitamin B_6) by use of a chemically-modified glassy carbon electrode

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Received 14 March 2004; received in revised form 10 July 2004; accepted 10 July 2004
Available online 13 September 2004

Abstract

A novel carbon nanotube-modified glassy carbon electrode was described for the direct determination of pyridoxine. The electrochemical behavior of pyridoxine was investigated, and a well-defined oxidation peak with high sensitivity was observed at the modified electrode. Owing to the unique structure and extraordinary properties of multi-wall carbon nanotube (MWNT), the MWNT-modified glassy carbon electrode shows obvious electrocatalytic activity to the oxidation of pyridoxine, since it greatly enhances the oxidation peak current of pyridoxine as well as lowers its oxidation overpotential. Based on this, a very sensitive and simple voltammetric method was developed for the measurement of pyridoxine. A sensitive linear voltammetric response for pyridoxine was obtained in the concentration range of 5×10^{-7}–1×10^{-4} mol/L, and the detection limit is 2×10^{-7} mol/L using differential pulse voltammetry. Compared with other voltammetric methods, this proposed method possesses many advantages such as very low detection limit, fast response, low cost and simplicity. The practical application of this new analytical method was demonstrated with pyridoxine drugs.

Keywords: Pyridoxine; Vitamin B_6; Voltammetric determination; Carbon nanotube; Chemically-modified electrode

1. Introduction

Vitamin B_6, also known as pyridoxine, is part of the B group vitamins and is required for both mental and physical health. Pyridoxine is an essential vitamin to aid in the formation of healthy red blood cells and supports more vital bodily functions than any other vitamin. It performs a wide variety of functions in your body and is essential for your good health. For example, vitamin B_6 is needed for more than 100 enzymes involved in protein metabolism. It is also essential for red blood cell metabolism. The nervous and immune systems need vitamin B_6 to function efficiently, and it is also needed for the conversion of tryptophan (an amino acid) to niacin (a vitamin). A vitamin B_6 deficiency can result in a form of anemia that is similar to iron deficiency anemia. Therefore, it is very important and highly valuable to develop a sensitive and simple analysis method for pyridoxine.

To date, various analytical methods have been developed for the determination of pyridoxine. Among these methods, spectrophotpmetric method [1–5] and chromatography [6–8] are commonly used.

A few papers concerning the determination of pyridoxine using voltammetry have been reported. Söderhjelm and Lindquist [9] were the first to study the electrochemical behavior of pyridoxine by a carbon paste electrode, and then reported a voltammetric method for the determination of pyridoxine. After that, the separation and determination of vitamin B_6 by chromatography [10] and electrophoresis [11] with amperometric detection, using a carbon disk electrode as electrochemical detector, have also been published. Recently, a carbon paste electrode modified with vanadyl(IV)-salen complex has been reported for the voltammetric determination of pyridoxine [12]. The detection limit of this

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doi:10.1016/j.jpba.2004.07.016

modified carbon paste electrode is just as low as 3.7×10^{-5} mol/L. To our best knowledge, voltammetric determination of pyridoxine by the use of carbon nanotube-modified electrode has not been reported.

Carbon nanotubes (CNTs) are molecular-scale wires with high electrical conductivity, extremely high mechanical strength and modulus, and can be divided into two categories: single-wall carbon nanotube (SWNT) and multi-wall carbon nanotube (MWNT). Since discovery by Iijima [13], CNTs have attracted considerable attention due to their extraordinary structural, mechanical, electrical, and electrochemical properties as well as their promise in the filed of material science. Now, CNTs have been widely used in electroanalytical chemistry [14–18]. In the current work, a glassy carbon electrode (GCE) modified with a MWNT thin film was firstly described to determine pyridoxine. The electrochemical behavior of pyridoxine suggests that MWNT-modified GCE exhibits obvious electrocatalytic activity to the oxidation of pyridoxine, since it greatly enhances the oxidation peak current of pyridoxine as well as lowers its oxidation overpotential. After optimizing the experimental parameters, a voltammetric method was developed for the direct measurement of pyridoxine. Compared with other published methods, this newly proposed method possesses many advantages such as very low detection limit, fast response, low cost and simplicity.

2. Experimental

2.1. Reagents

Pyridoxine standard solution (1×10^{-3} mol/L) was prepared by dissolving an appropriate amount of pyridoxine hydrochloride (Duchefa) in redistilled water. The solution was stable for 2 weeks in a refrigerator at about 5 °C. All chemicals were of analytical grade and used without purification, and redistilled water was used throughout.

The multi-wall carbon nanotube used was kindly provided by the Institute of Nanometer Materials of Huazhong Normal University in China (purity > 98%).

2.2. 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 MWNT-film-modified GC working electrode, a saturated calomel reference electrode (SCE) and a Pt wire counter electrode, was employed.

2.3. Fabrication of MWNT-modified GCE

According to literature [14], 5 mg MWNT and 5 mg dihexadecyl hydrogen phosphate (DHP) were dispersed into 5 mL of redistilled water, and then sonicated for about 20 min to give a stable and homogeneous MWNT–DHP suspension. Prior to modification, the GCE was mechanically polished with alumina paste of different grades to a mirror finish, rinsed and sonicated (3 min) in redistilled water. Finally, the GCE was coated with 5 μL of the MWNT–DHP suspension and allowed to evaporate water at room temperature in the air. The DHP-film coated GCE was prepared by the same procedure as explained above, but without MWNT.

2.4. Analytical procedure

After 10 mL of pH 6.0 phosphate buffer (0.1 mol/L) was placed in the electrochemical cell, the required volume of pyridoxine standard solution was added by a micropipette. Finally, the differential pulse voltammograms were recorded after 2 min of open-circuit accumulation. The oxidation peak current at 0.76 V was measured for pyridoxine. Prior to and after every measurement, the MWNT-modified GCE underwent cyclic voltammetric sweeps between 0.50 and 1.10 V at 100 mV/s in pH 6.0 phosphate buffer until the voltammograms are stable.

3. Results and discussion

3.1. Electrochemical behaviors of pyridoxine

The cyclic voltammograms of a MWNT-modified GCE in phosphate buffer at pH 6.0 in the absence and presence of pyridoxine were illustrated in Fig. 1. Within the potential window from 0.50 to 1.10 V, there is no observable redox peaks for a MWNT-modified GCE (dotted line). However, upon addition of 5×10^{-5} mol/L pyridoxine, a well-defined and sensitive oxidation peak appears at 0.80 V (solid line). On the reverse potential scan from 1.10 to 0.50 V, there is no corresponding reduction peak observed for pyridoxine. Moreover, the oxidation peak current of pyridoxine decreases

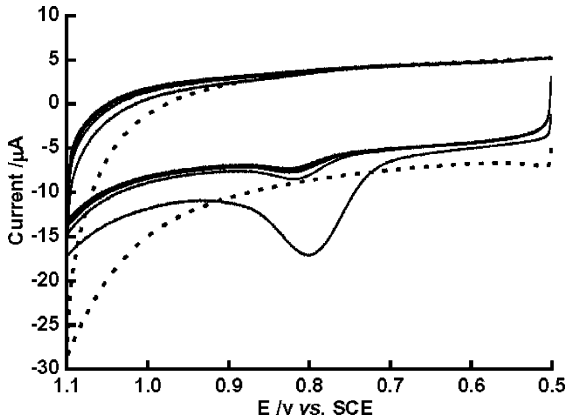

Fig. 1. Cyclic voltammograms of a MWNT-modified GCE: curve (a), in 0.1 mol/L phosphate buffer at pH 6.0; curve (b), (a) + 5×10^{-5} mol/L pyridoxine (scan rate: 100 mV/s).

remarkably during the successive cyclic potential sweeps. 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 pyridoxine or its oxidative product occurs at the electrode.

Otherwise, the electrochemical behaviors of pyridoxine at different scan rates from 10 to 200 mV/s were investigated by using cyclic voltammetry (CV), only an oxidation peak was obtained even at 10 mV/s. This suggests that the electrode reaction of pyridoxine at the MWNT-modified electrode is totally irreversible.

In order to illustrate the electrocatalytic effect of MWNT toward pyridoxine, the electrochemical properties of pyridoxine at three different kinds of working electrodes were examined using cyclic voltammetry, and the results shown in Fig. 2. At bare GCE, 5×10^{-5} mol/L pyridoxine yields a very low oxidation peak at 0.85 V in phosphate buffer at pH 6.0 (curve a). Under the identical conditions, the oxidation peak height of pyridoxine at the DHP-modified GCE decreases by two times in contrast to that at bare GCE (curve b). Dihexadecyl hydrogen phosphate forms a perfect thin film on GCE surface, and thus, inhibits the electron transfer between pyridoxine and GCE. The oxidation peak current, therefore, decreases compared with that at bare GCE. However, the oxidation peak current of pyridoxine at the MWNT-modified GCE increases significantly and the peak potential shifts towards more negative potential (curve c), in comparison with that at bare GCE. The remarkable peak current enhancement and the fall of oxidation overpotential undoubtedly testify the electrocatalysis of the MWNT-modified GCE to the oxidation of pyridoxine. In conclusion, MWNT-modified GCE greatly improves the determining sensitivity of pyridoxine on account of the unusual structure and properties of MWNT (such as very large specific area, strong adsorptive ability, subtle electronic properties).

3.2. Optimizing of supporting electrolyte

The electrochemical oxidation behaviors of 2×10^{-6} mol/L pyridoxine in various medium such as pH 5.0–8.0 phosphate buffer, pH 1.0–5.0 sodium citrate–HCl buffer, pH 2.0–8.0 Macllvaine buffer, pH 2.0–10.0 Britton–Robinson buffer (each 0.1 mol/L), were compared by cyclic voltammetry. The excellent oxidation response was obtained in pH 6.0 phosphate buffer because the peak current is highest and the peak shape is well-defined. Thus, 0.1 mol/L phosphate buffer at pH 6.0 was chosen as the determining medium for pyridoxine.

3.3. Effect of the amount of MWNT–DHP suspension

The amount of MWNT–DHP suspension on the GCE surface directly determines the thickness of the MWNT–DHP film. The relationship between the oxidation peak current of pyridoxine and the amount of MWNT–DHP suspension is illustrated in Fig. 3. It is found that the oxidation peak current gradually increases, while gradually improving the volume of the MWNT–DHP suspension from 0 to 5 μL. Further improving the amount of MWNT–DHP suspension, the peak current almost remains stable. However, when the amount of MWNT–DHP suspension exceeds 15 μL, the peak current conversely decreases. MWNT is an ideal electrode material with excellent electrical conductivity. In principle, the oxidation peak current is almost independent to the thickness of MWNT film. However, DHP is an insulator and blocks electron transfer. Due to lowering the electronic conductivity of the cast film on the GCE surface, the peak current conversely decreases when the MWNT–DHP film is too thick.

3.4. Optimization of accumulation conditions

The influence of accumulation potential on the oxidation peak current of pyridoxine was examined. The oxidation peak current of 2×10^{-6} mol/L pyridoxine was compared after

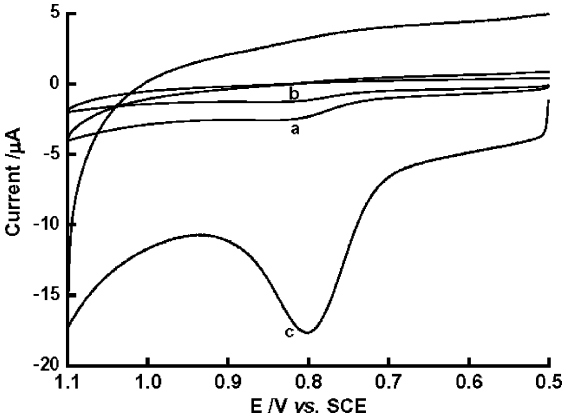

Fig. 2. Cyclic voltammograms of 5×10^{-5} mol/L pyridoxine in pH 6.0 phosphate buffer at three different electrodes: curve (a), bare GCE; curve (b), DHP-film-modified GCE; curve (c), MWNT–DHP film-modified GCE (scan rate: 100 mV/s).

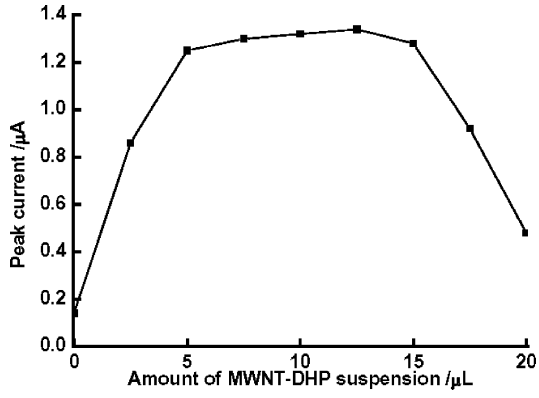

Fig. 3. Effects of the amount of MWNT–DHP suspension on the oxidation peak current of 2×10^{-6} mol/L pyridoxine at a MWNT-modified GC: accumulation time = 2 min; pulse amplitude = 50 mV; scan rate = 20 mV/s, and; pulse width = 50 ms.

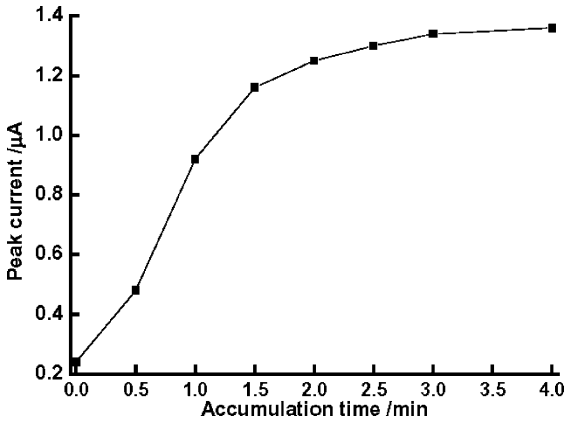

Fig. 4. Oxidation peak current of 2×10^{-6} mol/L pyridoxine responses for the accumulation time (other conditions are the same as in Fig.3).

2 min of accumulation under different potentials. The peak current almost remained unchangeable as the accumulation potential shifted from −0.20 to 0.50 V, indicating that the accumulation potential had no effects on the oxidation peak current of pyridoxine at the MWNT-modified GCE. Thus, an open-circuit accumulation was employed.

Fig. 4 shows the influence of accumulation time on the oxidation peak current of 5×10^{-6} mol/L pyridoxine. The oxidation peak current increases greatly within the first 2 min and then levels off, suggesting that the accumulation of pyridoxine at the MWNT-modified GCE is very rapid to reach saturation.

3.5. Calibration graph

The calibration curve for pyridoxine in pH 6.0 phosphate buffer was measured by differential pulse voltammetry (DPV). The best parameters on the MWNT-film coated GCE are accumulation time = 2 min; pulse amplitude = 50 mV; scan rate = 20 mV/s; and pulse width = 50 ms. For the MWNT-modified GCE, the linear segment increases from 5×10^{-7} to 1×10^{-4} mol/L (r = 0.997) with a regression equation of $i_p = 0.05 + 0.61 \times 10^6$ C (r = 0.997, C in mol/L, i_p in μA). It is found that this method can detect 2×10^{-7} mol/L pyridoxine after 2 min of accumulation via experiment. The relative standard deviation (R.S.D.) of 4.9% for 2×10^{-6} mol/L pyridoxine (n = 8) showed good reproducibility. However, the linear range is from 7.5×10^{-6} to 1×10^{-4} mol/L (r = 0.995), and the detection limit is just 3×10^{-6} mol/L for pyridoxine at a bare GCE.

The long-term stability of the MWNT-modified GCE was evaluated by measuring the current responses at a fixed pyridoxine concentration of 2×10^{-6} mol/L over a period of 3 weeks. The MWNT-modified GCE was used daily and stored in the air. The experimental results indicated that the current responses deviated only 5.2%, revealing that the MWNT-modified GCE fabricated by this way possesses long-term stability.

Table 1
Interferences of foreign species on the oxidation peak current of 2×10^{-6} mol/L pyridoxine

Foreign species	Tolerance level (mol/L)*
Vitamin B_1, Vitamin B_2, Vitamin B_{12}, Vitamin C	2×10^{-5}
Uric acid (UA), dopamine (DA), Vitamin A	6×10^{-5}
Vitamin E, progesterone, caffeine	1×10^{-4}

* For 5% error.

3.6. Interferences

To evaluate the interferences of foreign species (especially other vitamins) on the determination of pyridoxine at the level of 2×10^{-6} mol/L, a systematic study was carried out. The results are given in Table 1. It is found that the MWNT-modified GCE can tolerate interferences from other organic compounds. For example, 10-fold concentration of vitamin B_1, vitamin B_2, vitamin B_{12}, vitamin C, 30-fold concentration of uric acid (UA), dopamine (DA), vitamin A, and 50-fold concentration of vitamin E, progesterone and caffeine almost do not influence the current response of 2×10^{-6} mol/L pyridoxine (signal change below 5%), suggesting that this proposed voltammetric method has excellent selectivity toward pyridoxine.

3.7. Analysis of pyridoxine in tablets

The average mass of ten tablets was determined and finely powdered. Then the required amount of sample to prepare a solution of ca. 10^{-3} mol/L was transferred into a 100 mL standard flask containing 80 mL of phosphate buffer (pH 6.0). The contents of flask were stirred magnetically for 15 min and then diluted to volume with the same supporting electrolyte. The solution was filtered and the first 20 mL of the filtrate was removed. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrate and diluting them with supporting electrolyte mentioned above.

Voltammograms were recorded as in standard pyridoxine. The content of pyridoxine was calculated from the regression equation, and the results shown in Table 2. The results obtained by the MWNT-modified GCE are in good agreement with the declared pyridoxine content. Further, in order to establish the suitability of the proposed method, known amounts of the standard pyridoxine were added into the analytical solution of the pyridoxine tablets and the same

Table 2
Determination of pyridoxine in tablet samples by the proposed voltammetric method

Samples	Declared pyridoxine content (mg per tablet)	Declared found content (mg per tablet)	Recovery (%)
1	10.0	10.08	102.4
2	10.0	9.94	101.6
3	10.0	9.90	99.7
4	10.0	10.04	99.4
5	10.0	9.96	100.8

procedure was applied. The recoveries indicate that the accuracy and repeatability of the proposed voltammetric method are very good. From above experimental results, it is very clear that this novel MWNT-modified electrode has great potential for practical sample analysis.

4. Conclusion

It is very important and useful to develop a sensitive and convenient method for the measurement of pyridoxine. In this work, a novel chemically-modified electrode, MWNT-modified GCE, was easily fabricated for the determination of pyridoxine. Owing to the unique properties of MWNT, such as high specific surface area, subtle electronic properties and strong adsorptive ability, the MWNT-modified GCE shows remarkable electrocatalytic ability to the oxidation of pyridoxine. As a result of all this, the MWNT-modified GCE greatly enhances the determining sensitivity of pyridoxine.

Acknowledgement

The authors are grateful to financial supports from the National Science Foundation of China (No. 30370397 and 60171023).

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