

Voltammetric Determination of Vitamin B₆ at Glassy Carbon Electrode Modified with Gold Nanoparticles and Multi-Walled Carbon Nanotubes

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Abstract

In this work, the gold nanoparticles (Au NPs)/multi-walled carbon nanotubes (MWCNTs) composite film modified glassy carbon electrode (GCE) was fabricated, and scanning electron microscopy (SEM) was used to investigate the assemble process of the composite film. In pH 7.0 PBS, an oxidation peak of the vitamin B₆ (VB₆) was only observed at composite film modified electrode. Under the optimized conditions, the current intensity was linear with the concentrations of VB₆ in the range of 1.59 to 102.74 $\mu\text{g}\cdot\text{mL}^{-1}$ with a detection limit of 0.53 $\mu\text{g}\cdot\text{mL}^{-1}$ (S/N = 3). The modified electrode had been applied in pharmaceutical analysis, and obtained good results.

Keywords: Vitamin B₆, Gold Nanoparticles, Multi-Walled Carbon Nanotubes, Differential Pulse Voltammetry

1. Introduction

Vitamin B₆ (VB₆), also known as pyridoxine, is part of the B group vitamins and plays an important role in the synthesis and metabolism of amino acid. The deficiency of VB₆ has been suggested as the cause of many types of illness and disease [1]. Several analytical methods have been described in literature for determination of VB₆, including spectrophotometry [2,3], liquid chromatography [4,5] and electrochemistry method [6,7]. R. Jiménez-Prieto et al determined VB₆ by spectrophotometric techniques in the presence of other vitamins [8]. Fang et al [9] detected VB₆ in pharmaceutical preparations by micellar electrokinetic chromatography with amperometric electrochemical method. Söderhjelm and Lindquist [10] firstly detected VB₆ in pharmaceutical preparations with electrochemical method; however, the interferences from ascorbic acid were existed and had not been repelled. Until now, few papers had been described on the determination of VB₆ using electrochemical method.

Carbon nanotubes (CNTs) have been an important group of nanomaterials with attractive geometrical, electronic and chemical properties. Recently, composite materials based on integration of CNTs and other materials

had gained growing interest, including conducting polymers, redox mediators and metal nanoparticles [11-14]. Gold nanoparticles (Au NPs) were another important nanomaterial, and it had also been used in biosensor of glucose [15,16], DNA [17], pesticides [18], and arsenic (III) [19]. We have used Au NPs/CNTs composite film modified electrode to detect Sal and target DNA [20,21] and obtained good results. From our practical experiment, we find the Au NPs/CNTs composite are good materials, and hope to open more applied window. We want to study medicament analysis based on this ideas. To our knowledge, there was no report of VB₆ detection based on Au NPs and MWCNTs composite film modified electrode.

In this work, Au NPs was electrodeposited on the surface of the MWCNTs modified electrode, and the electrochemical behavior of VB₆ at this modified electrode was investigated, the experiment results showed an oxidation peak of VB₆ was only observed in CVs and the electrochemical response of VB₆ was higher in contract to MWCNTs or Au NPs modified electrode alone. Under optimal experiment condition, the current intensity was linear with the concentrations of VB₆ in the range of 1.59 to 102.74 $\mu\text{g}\cdot\text{mL}^{-1}$ with a detection limit of 0.53 $\mu\text{g}\cdot\text{mL}^{-1}$

(S/N = 3). When the modified electrode was used to detect VB₆ in pharmaceutical preparations, a satisfied result was obtained.

2. Experimental

2.1. Reagents

Multi-walled carbon nanotubes with carboxylic acid groups (MWCNTs, with a diameter of about 30 nm and length of around 30 μm, purity > 95%) were obtained from Chengdu Institute of Organic chemistry, Chinese Academy of Sciences. Vitamin B₆, Vitamin B₁ (VB₁), Vitamin B₂ (VB₂), Ascorbic acid (AA), and HAuCl₄·4H₂O were obtained from Shanghai Chemical Reagent Co., Ltd (China). The phosphate buffer solution (PBS, 0.10 mol·L⁻¹) was prepared by NaH₂PO₄ and Na₂HPO₄, and adjusted the pH with H₃PO₄ and NaOH solutions. All chemicals were of analytical grade and used without further purification. All solutions were prepared with twice-quartz-distilled water.

2.2. Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on a CHI 650C electrochemical workstation (Shanghai Chenhua Instruments Co., China). The three-electrode system consisted of a bare GCE or modified electrode as a working electrode, a platinum wire as a counter electrode, and a saturated calomel electrode (SCE) as a reference electrode. All potentials given in this work were referenced to the SCE reference electrode.

The morphologies of different modified electrodes were obtained using a scanning electron microscopy (SEM) with JEOL JSM-4800F microscopy (Hitachi, Japan).

2.3. Preparation of the Au NPs/MWCNTs/GCE

The bare GCE was carefully polished sequentially with 0.3 and 0.05 μm alumina slurries on microcloth pads followed by rinsing successively in an ultrasonic bath with acetone, absolute alcohol and twice-quartz-distilled water for 3 min, respectively. Afterwards, the electrode was electrochemically treated by cycling the potential between -0.3 and +1.5 V in 0.50 mol·L⁻¹ H₂SO₄ with a scan rate of 100 mV·s⁻¹ until a reproducible cyclic voltammogram was observed, followed it is rinsed with twice-quartz-distilled water thoroughly and dried in a nitrogen stream.

5 μL of 1.0 mg·mL⁻¹ MWCNTs suspensions (10.0 mg of MWCNTs was dispersed in 10.0 mL of ethanol with the help of ultrasonication) were dropped on the surface

of pretreated GCE, and then it was dried at room temperature. The Au NPs/MWCNTs/GCE was obtained by immersing the MWCNTs/GCE into 0.10 mol·L⁻¹ KNO₃ solution containing 1 mmol·L⁻¹ HAuCl₄ and electrodeposited 20 s at -0.2 V (vs. SCE).

2.4. Procedure

The preparation of sample: 1.0 mL of injection (Henan Topfond Pharmaceutical Co., Ltd.) was transferred to a 10.0 mL volumetric flask, diluted to the mark with twice-quartz-distilled water. Next, 2.0 mL of solution above was taken to 10.0 mL volumetric flask, diluted to the mark with twice-quartz-distilled water.

The preparation of tablets: 20 tablets VB₆ (Nanjing Baijingyu Pharmaceutical Co., Ltd.) were taken to grind a fine powder. Next, a suitable amount powder (about one tablet mount) was taken to be dissolved with twice-quartz-distilled water, and it was then transferred to 50 mL volumetric flask, diluted to the mark with twice-quartz-distilled water. Final, it was filtered to remove infusible substance.

Electrochemical measurement: 5.0 mL of 0.10 mol·L⁻¹ PBS containing a suitable amount of VB₆ was added to the 10.0 mL cell. Before experiment, the solution was purged with nitrogen for 10 minutes, and the flow of nitrogen was maintained during the experiment. In DPV measurements, the experiment parameters were following: initial potential: 0.3 V; final potential: 0.9 V; amplitude: 0.05 V; pulse width: 0.05 s; sample width: 0.0167.

3. Results and Discussion

3.1. SEM of the Different Modified Electrodes

Figure 1 displayed SEM morphologies of MWCNTs/GCE (**a**) and Au NPs/MWCNTs/GCE (**b**). It was observed that the MWCNTs distributed uniformly on the surface of GCE and Au NPs had been uniformly decorated onto the surface of MWCNTs (**Figure 1(b)**). The diameter of Au NPs was about 50 nm.

3.2. Electrochemical Behaviors of VB₆ at Bare or Modified Electrode

Electrochemical behaviors of VB₆ at various modified electrode in 0.10 mol·L⁻¹ PBS were investigated by CV technique. **Figure 2** showed the CVs of VB₆ at the bare GCE, the Au NPs/GCE, the MWCNTs/GCE, and the Au NPs/MWCNTs/GCE. For the bare GCE (**Figure 2(a)**) and Au NPs/GCE (**Figure 2(b)**), the responses of VB₆ were poor. When MWCNTs or Au NPs/MWCNTs was

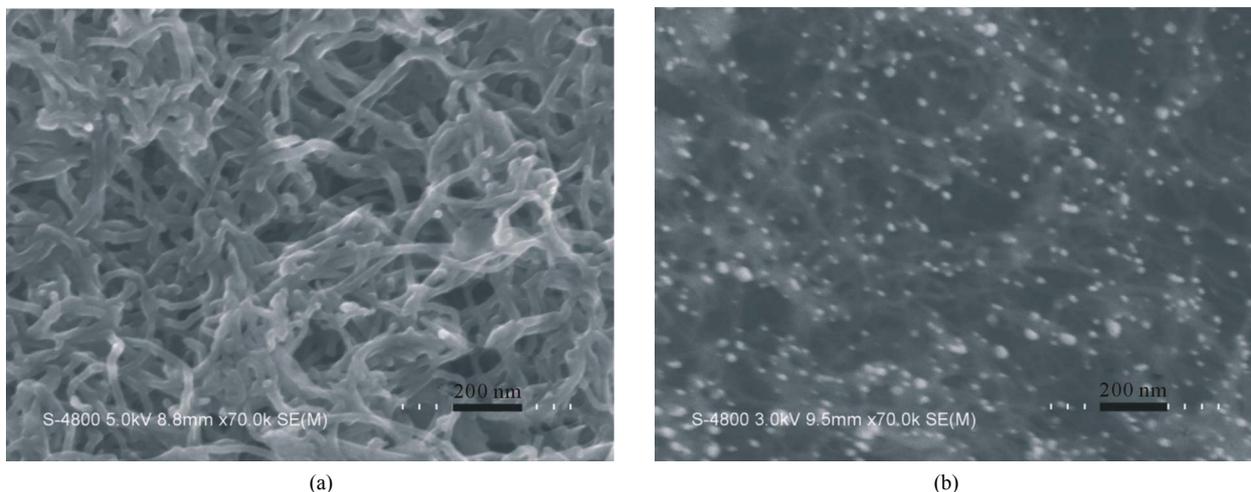


Figure 1. SEM morphologies of MWCNTs/GCE (a) and Au NPs/MWCNTs/GCE (b).

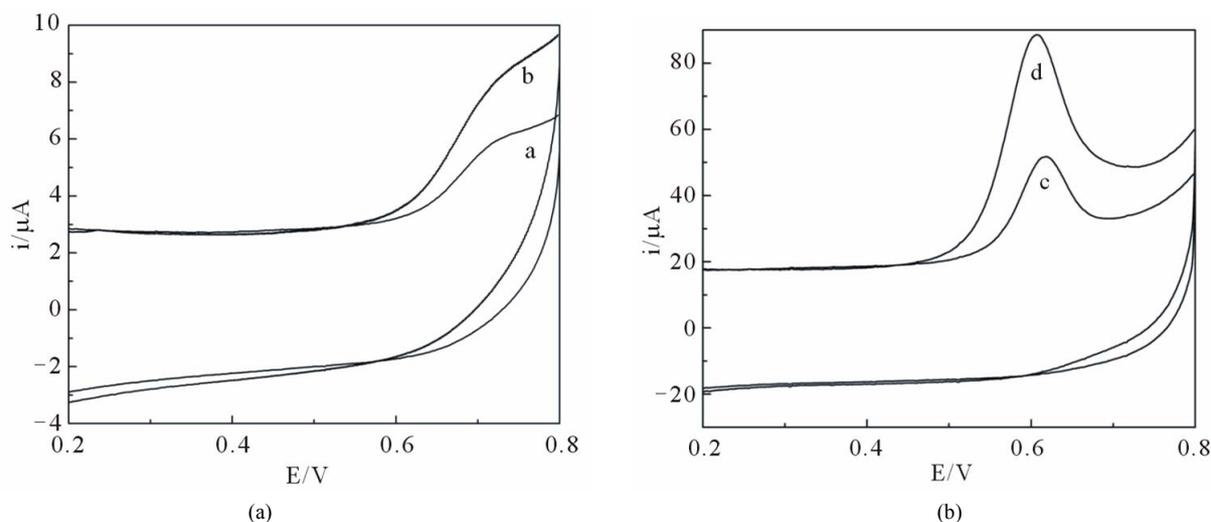


Figure 2. Cyclic voltammograms of VB₆ at GCE (a), Au NPs/GCE (b), MWCNTs/GCE (c) and Au NPs/MWCNTs/GCE (d).

modified on GCE (Figure 2(c)), the response of VB₆ was enhanced; especially, the current intensity of VB₆ was the highest at Au NPs/MWCNTs modified electrode (Figure 2(d)). These facts might attribute to the synergistic effect of Au NPs and MWCNTs. At the same time, there was only an oxidation peak at +0.62 V and no reduction peak could be observed in CVs, indicating that the electrode reaction of VB₆ at the Au NPs/MWCNTs/GCE was irreversible.

The effect of the scan rate on peak current of VB₆ was investigated at the Au NPs/MWCNTs/GCE, and the current intensity was linearly increased with the square of the scan rate, the linear regression equation was: $I(\mu\text{A}) = 1.183 + 109.1v^{1/2} (\text{v/s})$ ($r = 0.9992$), indicating the electrode reaction of VB₆ at modified electrode was a diffusion control process.

3.3. Optimization of the Experimental Conditions

3.3.1. The Effect of Deposition Time

In this work, Au NPs deposition time was optimized by CV technique. The results were shown in Figure 3. As can be seen, the current intensity increased rapidly with the augment of the deposition time increased from 5 to 20 s. The maximum current intensity was observed when the deposition time was 20 s. However, when the deposition times increased continuously, the current intensity decreased gradually. Therefore, 20 s was chosen as deposition time.

3.3.2. The Effect of pH

The effect of the solution pH on the electrochemical response of VB₆ was investigated. The results were shown in Figure 4. As can be seen, the current intensity in-

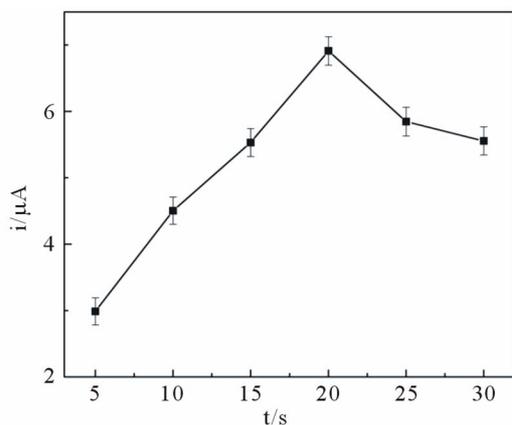


Figure 3. The plots of the peak currents of VB₆ vs electro-deposition time of Au NPs, $c = 30 \mu\text{g}\cdot\text{mL}^{-1}$.

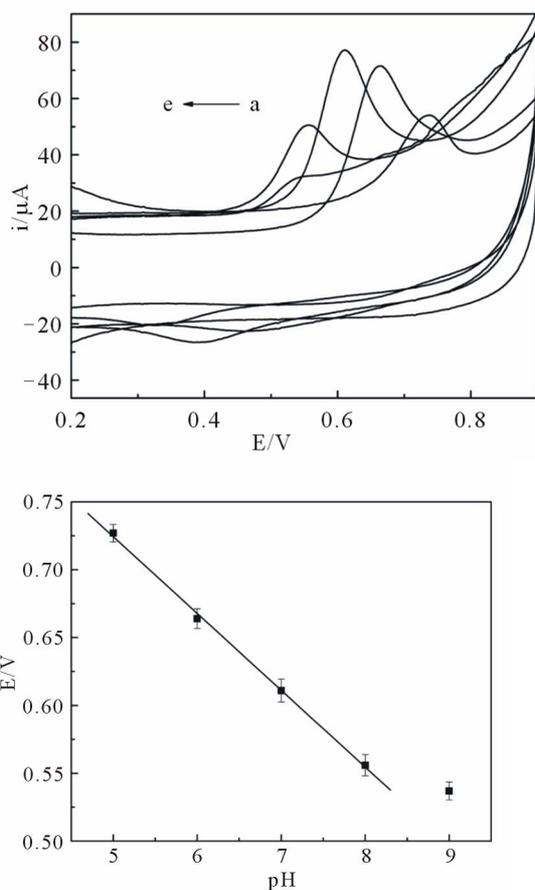


Figure 4. The effect of pH on peak currents (a) and potential of VB₆ (b). The pH value are 5.0, 6.0, 7.0, 8.0, 9.0 (from a to e).

creased with solution pH from 5.0 - 7.0, then it decreased when the pH was over 7.0. The maximum current intensity was observed when pH was 7.0. The peak potential was linearly shifted negatively with the augment of solu-

tion pH, indicating that protons had taken part in the electrode reaction process of VB₆. Therefore, pH 7.0 was selected in all following experiments.

3.3.3. Calibration Curve

The calibration curve of VB₆ detection was performed with DPV technique. Under the optimized experimental conditions, a series of different concentrations of VB₆ were measured. The current intensity at + 0.62 V was used to obtain the calibration curve. The results showed that the current intensity was linear with the concentrations of VB₆ in the range of 1.59 to 102.74 $\mu\text{g}\cdot\text{mL}^{-1}$ as shown in **Figure 5**. The linear regression equation was $I_{\text{pa}} (\mu\text{A}) = 0.2759 c + 0.7007$ ($C - \mu\text{g}\cdot\text{mL}^{-1}$) ($r = 0.9994$) with the detection limit of 0.53 $\mu\text{g}\cdot\text{mL}^{-1}$ ($S/N = 3$).

3.3.4. Stability and Reproducibility

The reproducibility of the modified electrode was investigated by successive measurements of 30 $\mu\text{g}\cdot\text{mL}^{-1}$ VB₆ by DPV ($n = 6$), the obtained peak current value was about the range of 8.79 to 9.24 μA with a relative standard deviation (RSD) of 2.05%. The stability of the modified electrode was investigated after it was storage in air for 2 weeks. The obtained peak currents were almost constant. Therefore, the modified electrode had a good stability and reproducibility.

3.3.5. Interference Study

In these experiments, we investigated the interference of several species on the determination of VB₆, such as VB₁, VB₂, AA, glucose, starch. The experiment results were shown in **Table 1**, from **Table 1**, there were no interferences existed for the determination of 30 $\mu\text{g}\cdot\text{mL}^{-1}$ VB₆ in the presence of 20-fold VB₁, a 50-fold VB₂, 100-fold AA, glucose and starch.

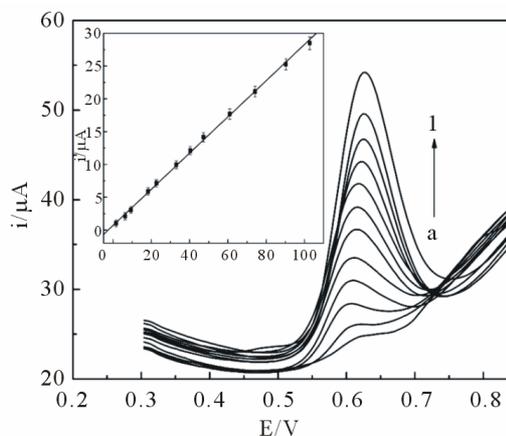


Figure 5. Differential pulse voltammograms of VB₆ at the modified electrode. The concentrations of VB₆ ($\mu\text{g}\cdot\text{mL}^{-1}$) are: 1.59, 6.299, 9.375, 18.32, 22.64, 33.03, 40.31, 47.27, 60.93, 74.20, 90.28, 102.74.

3.3.6. Analytical Applications

In this work, the injection and tablet of VB₆ were analyzed by the standard addition method. For comparison purposes, the obtained results were compared with that of China Pharmacopoeia Method [22] (shown in **Tables 2, 3**). From **Table 2** and **Table 3** we could observe that the obtained values were agreement with the results found by the China Pharmacopoeia Method.

4. Conclusions

The Au NPs/MWCNTs composite film modified electrode showed a strong electrochemical response towards VB₆ in pH 7.0 and AA showed no interference during VB₆ detection. The modified electrode had been applied in medication analysis, and obtained good results.

Table 1. The inference of coexistent substances.

| Coexisting substance | Current ^a μA(n = 3) | Multiple amount added | Current ^b μA(n = 3) | Relative deviation % |
|------------------------|-----------------------------------|-----------------------|-----------------------------------|----------------------|
| Glucose | | 200.0 | 8.738 | -2.39 |
| Starch | | 200.0 | 8.821 | -1.46 |
| Ascorbic acid | 8.952 | 100.0 | 8.708 | -2.73 |
| Vitamin B ₁ | | 20.0 | 9.233 | 3.14 |
| Vitamin B ₂ | | 50.0 | 9.134 | 2.03 |

a. the average peak current of VB₆. b. the average peak current of VB₆ after adding different coexisting substances.

Table 2. Results for the determination of VB₆ in tablets.

| Sample | Labeled mg per tablet | This method | | Pharmacopoeia method | |
|--------|-----------------------------|------------------------|-------------------|------------------------|-------------------|
| | | Found mg per tablet | RSD, n = 5 (%) | Found mg per tablet | RSD, n = 5 (%) |
| 1 | 10.0 | 9.95 | 3.3 | 9.96 | 1.1 |
| 2 | 10.0 | 9.89 | 3.4 | 9.85 | 0.9 |
| 3 | 10.0 | 9.82 | 2.8 | 9.85 | 0.8 |

The tables were obtained from Nanjing Baijinyu Pharmaceutical Co., Ltd.

Table 3. Results for the determination of VB₆ in injections.

| Sample | Labeled mg·mL ⁻¹ | This method | | Pharmacopoeia method | |
|--------|--------------------------------|------------------------------|-------------------|------------------------------|-------------------|
| | | Found mg·mL ⁻¹ | RSD, n = 5 (%) | Found mg·mL ⁻¹ | RSD, n = 5 (%) |
| 1 | 50.0 | 50.4 | 1.7 | 50.1 | 0.9 |
| 2 | 50.0 | 49.5 | 2.4 | 49.3 | 0.6 |
| 3 | 50.0 | 49.3 | 3.2 | 49.2 | 0.5 |
| 4 | 50.0 | 50.2 | 1.9 | 50.1 | 0.9 |
| 5 | 50.0 | 49.7 | 2.7 | 49.9 | 0.8 |

The injections were obtained from Henan Topfond Pharmaceutical Co., Ltd.

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