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Electrochemical Determination of Phenylephrine Hydrochloride Based on Graphene-TiO_2 Modified Glassy Carbon Electrode

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A novel voltammetric sensor based on TiO_2 nanoparticles and graphene composite (GR/TiO_2) was developed. The nanocomposite modified glassy carbon electrode was characterized using scanning electron microscope (SEM). The electrochemical behavior of phenylephrine hydrochloride (PHE) was investigated on this sensor. The results indicated that the GR/TiO_2 modified electrode exhibited efficient electrocatalytic oxidation for PHE with about 2.87 times higher current response. Using differential pulse voltammetry (DPV), the GR/TiO_2 modified electrode exhibited a linear voltammetric response for PHE in the concentration range of 5.0×10^{-7}-2.0×10^{-5} mol L^{-1}, with the limit of detection (LOD) of 2.0×10^{-7} mol L^{-1} (S/N=3). The sensor was applied to determine PHE in urine and human serum samples were studied.

Keywords: Phenylephrine Hydrochloride; Electrochemistry; Determination.

1. INTRODUCTION

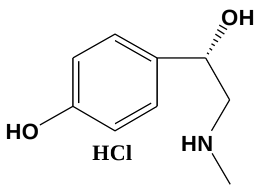


Figure 1. Chemical structure of Phenylephrine hydrochloride

Phenylephrine hydrochloride (PHE, (R)-3-hydroxy-α-[(methylamino) methyl] benzenemethanol hydrochloride, Fig. 1) is a sympathomimetic drug used for nasal congestion, sinusitis and rhinitis. It is also used in ophthalmology as a mydriatic and conjunctival decongestant [1]. It has been used for the following off-label conditions: senile dementia, skull and brain trauma, urinary incontinence after stroke, encephalopathy, mental disorders, and tardive dyskinesia. In the interest of using PHE securely, scientifically and getting better curative effect, it is important to develop a simple, rapid, and sensitive method for the determination of it. Compared with other methods, such as high performance liquid chromatography [2,3], spectroscopy [4,5], and flow injection [6]， the electrochemical technique exhibits its predominance such as rapid response, simple operation, time saving, and low-cost. More inadequate, the pharmacological action of PHE is not observed using other methods. Electrochemical methods also help for identifying the redox of compounds and provide important information about pharmacological actions.

Recently, Zhu *et al* [7] describes a method for the determination of PHE on a glassy carbon electrode (GCE) modified with multi-wall carbon nanotubes (MCNTs). As a novel modification material, the graphene (GR) modified film can greatly enhance the voltammetric response of the analysis due to its unique nanostructure, excellent mechanical properties, and unusual electronic transport properties [8 9]. This tendency is well manifested by several recently published excellent reviews [10, 11]. However, many of the interesting and unique properties of GR can only be realized after it is integrated into more complex assemblies. On the other hand, TiO_2 has become an attractive electrode material for electrochemical sensors and biosensors applications, due to its good biocompatibility, high conductivity and low cost [12]. Although the electrocatalytic activity of GR and TiO_2 films show good results individually, some of the biochemical compounds do not undergo electrocatalysis. The objective of the present work is to develop a simple and sensitive method for PHE determination based on the unusual properties of GR and TiO_2 nanoparticles. The electrochemical oxidation of PHE was facilitated at GR/TiO_2 and the determination sensitivity was significantly improved. The method has been applied to determining PHE in the real sample.

2. EXPERIMENTAL

2.1 Chemicals and reagents

Graphite powder (320 mesh, spectrum pure) was purchased from Sinopharm Chemical Reagent Co., Ltd. Titanium isopropoxide ($Ti(OiPr)_4$) was obtained from Aladdin Chemistry Co., Ltd. Other chemicals used in this study were of analytical grade. All solutions were prepared with double distilled water. The acetate buffer solution (0.1 mol L^{-1}) was made up from HAc+NaAc, and its pH value was adjusted with 0.1 mol L^{-1} HAc and 1.0 mol L^{-1} NaOH. All solutions were made up with ultrapure water (18.2 MΩ, Simplicity Plus, Millipore Corp，US). The human urine and serum samples were obtained from the healthy volunteer and used without further treatment. The direct determination of PHE in human urine and serum samples by employing a high dilution of the sample with the supporting electrolyte.

2.2 Instrumentations

All the electrochemical measurements were performed on a CHI 660B electrochemical working station (CH Instrument, Shanghai, China). The three-electrode system consisted of a modified glassy carbon electrode or a bare glassy carbon electrode as a working electrode, a platinum electrode and a saturated calomel electrode (SCE) were used as the auxiliary and reference electrodes, respectively. A scanning electron microscope (SEM, Hitachi H-9000, Japan) was used to characterize the morphology of modifiled electrode. All experiments were carried out at room temperature (25 ± 2 ℃).

2.3 Preparation of GR/TiO$_2$ nanocomposite

Graphene oxide was synthesized from graphite powder by the modified Hummers method [13]. Graphene was prepared by the chemical reduction of a colloidal suspension of exfoliated graphene oxide sheets in water with ascorbic acid [14]. Hydrothermal preparation of GR/TiO$_2$ nanocomposite was carried out by the following procedure. Titanium isopropoxide (0.2 mL) was mixed with graphene (50 mg) in a 25mL Teflon-sealed autoclave, and H_2SO_4 (1 M, 2mL) was then added. The resultant mixture was ultrasonicated for 10 min, and then the autoclave was kept in oven maintained at 170 ^{o}C for 24 h. The final product was isolated by filtration, rinsed thoroughly with deionized water and methanol, and dried in vacuum. The GR/TiO$_2$ nanocomposite was obtained in the form of black powder.

2.4 Preparation of modified electrode

The as-prepared GR/TiO$_2$ nanocomposite (1.5 mg) was dispersed in DMF (1.0 mL) to form a homogenous suspension. The GR/TiO$_2$ modified GCE was prepared by dropping 7 μL of the suspension on the surface of freshly polished GCE, and dried at room temperature. For comparison, 7 μL of the graphene suspension in DMF (1.5 mg/mL) was cast on the bare GCE, and dried at room temperature to get the graphene modified GCE.

3. RESULTS AND DISCUSSION

3.1 Morphology of the multilayer films

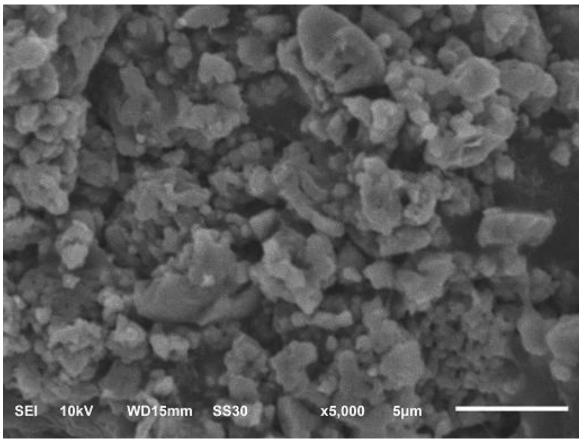


Figure 2. SEM image of GR/TiO$_2$.

Direct morphological observation of the as-prepared GR/TiO_2/GCE was obtained using a SEM. The typical SEM image for the GR/TiO_2/GCE is shown in Fig. 2. It can be seen that that TiO_2 was formed in a highly faceted morphology on the substrates of graphene with ca. 50-100 nm diameter for the clusters. The structure characterization results suggest that hydrothermal reaction proceeded with efficient crystallization of TiO_2 and in situ immobilization on graphene substrate.

3.2 Cyclic voltammograms behaviour of PHE

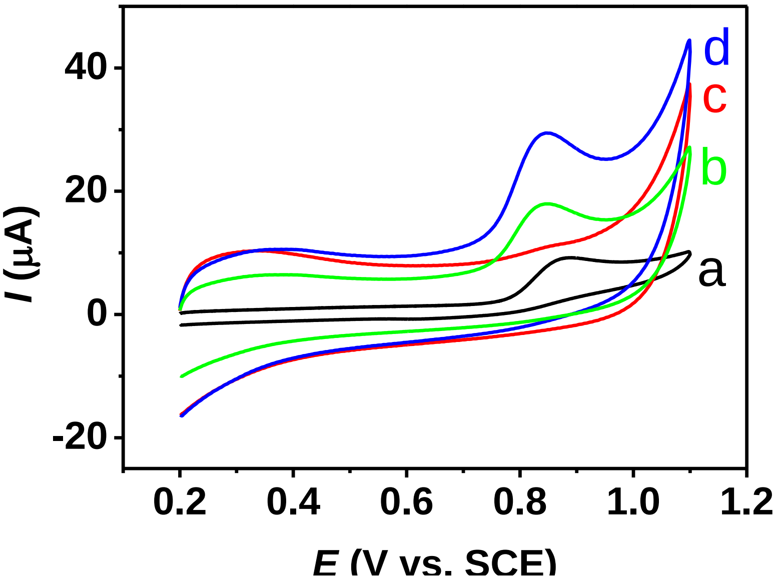


Figure 3. Cyclic voltammograms of 1.0 ×10^{-5} mol L^{-1} PHE at GCE (a), GR/GCE (b) GR/TiO_2/GCE (c), GR/TiO_2/GCE (d) in 0.1 mol L^{-1} HAc-NaAc (pH 4.6). Scan rate: 50 mV s^{-1}.

The electrochemical behavior of PHE at the bare GCE and GR/TiO_2/GCE was investigated in 0.1 mol L^{-1} HAc-NaAc (pH 4.6) using cyclic voltammetry, and the result was shown in Fig. 3. It can be seen an irreversible oxidation peak of PHE appears at the GCE with a potential of 0.873 V and a current of 5.077 μA (curve a). The response on the GR/GCE was a small hump at 0.862 V (curve b), suggesting that the GR film improved the accumulation of PHE. Fig. 3d is a CV curve of GR/TiO_2/GCE in a blank solution, no peaks were observed in blank solution. When 1.0×10^{-5} mol L^{-1} PHE was added into blank solution, a well defined oxidation peak was observed at the modified electrode with the oxidation potential at 0.842 V and a current of 14.552 μA (curve c). No corresponding reduction peak was observed at the reverse scan, indicating that the electrochemical oxidation of PHE was a totally irreversible reaction at the GR/TiO_2/GCE under the above experimental conditions. This phenomenon should be attributed to that more edge plane defects were opened up during the hydrothermal process under the oxidizing medium of deposition and the surface area was significantly increased in the resulting nanocomposite, suggesting the synergistic effect of GR/TiO_2 composite provides an efficient microenvironment for the electrochemical reaction of PHE with enhanced voltammetric response.

3.3 Oxidation mechanism of PHE

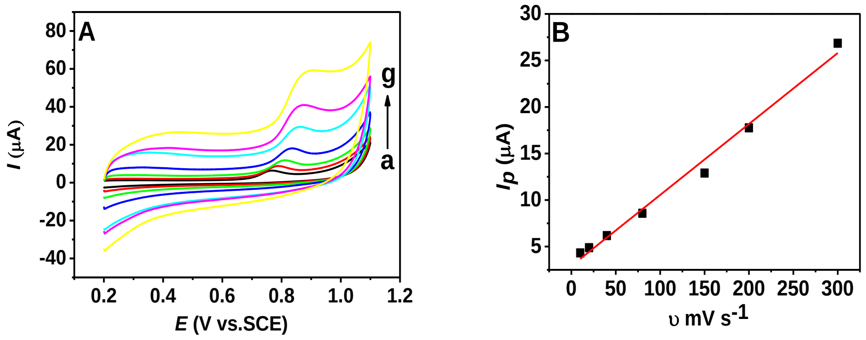


Figure 4. (A) Cyclic voltammograms of PHE at different scan rates. Scan rates (curve a-g): 10, 20, 40, 80, 150, 200, 300 mV s $^{-1}$. (B) A linear relationship between the peak current and the square root of scan rate.

To infer more about the reaction process of PHE on GR/TiO$_2$/GCE, the effect of the scan rate (*v*) on the peak currents was studied. Fig. 4 A shows CV curves with scan rates of 10，20，40，80，150，200，and 300mV s^{-1}, respectively. The oxidation peak current increased gradually with the increase of scan rate. As shown in Fig. 4 B, the oxidation peak current increased linearly with the scan rate in the range of 10-300 mV s $^{-1}$ and can be expressed as: I_{pa} (μA)=0.0763 *v* (mV s^{-1})+2.918 (R=0.994), which indicated that the oxidation of PHE on GR/TiO$_2$/GCE was a typical absorption-controlled process [15]. Based on the equation [16]: I_p=nFQ*v*/4RT, where n is the number of electrons transferred, F (C mol^{-1}) is the Faraday's constant, Q (C) is the quantity of charge and *v* (V s^{-1}) is the scan rate. The value of n was estimated to be 0.94, which suggested that a total of one electron was involved in the oxidation reaction.

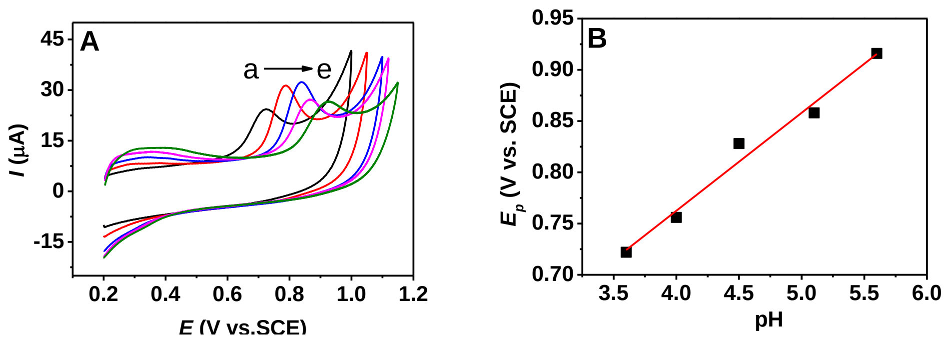
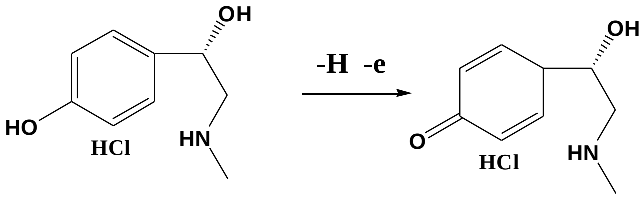


Figure 5. (A) Cyclic voltammograms of PHE at different pH, curve a-e: 3.6, 4.0, 4.6, 5.1, 5.6. (B) A linear relationship between the peak potential and the pH.

The effect of pH on the current response of PHE on the GR/TiO$_2$/GCE was studied in the pH range from 3.6 to 5.6. As shown in Fig. 5A, the oxidation peak current gradually increased with the increase of pH value from 3.6 to 5.6. However, when the pH was further increased to 4.6, the oxidation peak current conversely decreased. Therefore, considering the sensitivity of PHE determination, a pH value of 4.6 was chosen for the subsequent analytical experiments.

The relationship between the oxidation peak potential (E_{pa}) and pH was shown in Fig. 5B. A linear shift of E_{pa} towards negative potential with an increasing pH indicated that protons were directly involved in the oxidation of PHE. It obeyed the following equation: E_{pa}(V)=-0.061 pH+0.398 (R=0.995). The slope is approximately close to the theoretical value of 57.6 mV pH^{-1}, indicating that the electron transfer was accompanied by an equal number of protons in electrode reaction [17]. Thus, it may conclude that the electrode reaction of PHE was accompanied by one proton and one electrons. On the basis of these experimental antecedents and the literature report [7], a possible oxidation mechanism of PHE on GR/TiO$_2$/GCE was proposed as shown in Scheme 1.



Scheme 1

3.4 Influence of accumulation time and potential

In consideration of the detection sensitivity and adsorption of PHE on the GR/TiO$_2$/GCE surface, differential pulse voltammetry (DPV) technique, coupled with accumulation procedure, was adopted for researching the analytical method. For a PHE solution (1.0×10^{-5} mol L^{-1}), the peak currents increased with increasing accumulation time, and it reached a maximum value when accumulation time was 200s. After investigation, accumulation potential had little effect on peak currents, so accumulation of PHE was carried out under open circuit for further studies.

3.5 Calibration curve

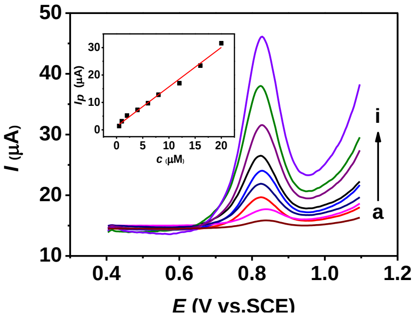


Figure 6. DPV of different PHE concentrations. PHE concentrations (from a to i): 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 16.0, 20.0 × 10^{-6} mol L^{-1}. The insert is linear dependence of the peak current on PHE concentration.

DPV was used to investigate the relationship between peak currents and PHE concentrations under the optimized conditions. The results (Fig. 6) exhibited a linear relationship between peak currents (I_{pa}) and PHE concentrations within the range from 5.0×10^{-7} to 2.0×10^{-5} mol L^{-1}, which can be described by the following linear regression equation: I_p (μA)= 3.514 *c* (10^{-6} mol L^{-1})-5.172, with a correlation coefficient of 0.996. Based on the signal-to-noise ratio of 3, the detection limit was obtained as 2.0×10^{-7} mol L^{-1}.

3.6 Stability and reproducibility and selectivity

The successive stability of the proposed sensor was evaluated. Ten successive scans in the solution containing 1.0×10^{-5} mol L^{-1} PHE were performed. The relative standard deviation value was found to be 2.52 %, indicating excellent stability of the modified electrode. Moreover, after the modified electrode was stored for one week, only a small decrease of the oxidation peak current was observed, demonstrating that the electrode had good stability. Under the optimized experimental conditions described above, the effects of some foreign species on the determination of PHE at the 1.0×10^{-5} mol L^{-1} level were evaluated thoroughly; 200-fold concentration of Al^{3+}, Fe^{3+}, Fe^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}, Ca^{2+}, 100-fold of ascorbic acid, dextrin, glucose and sucrose had almost no influence on the current response of PHE (signal change below 5%). All of these results indicated that the present method had good selectivity for the determination of PHE.

3.7 Sample analysis

In order to evaluate the applicability of the proposed method to the determination of PHE in real samples, the utility of the developed method was tested by determining PHE in urine and human serum samples. The results are summarized in Table1. The accepted recoveries indicated that the presence of ascorbic acid and some other substances, such as glucose and sodium chloride did not interfere with the determination of PHE. It implies a promising application of GR/TiO_2/GCE in direct determination of PHE in real samples.

Table 1. Recovery tests for PHE determination in urine and human serum samples.

Samples	No.	Amounts of added PHE (μM)	Amounts of found PHE (μM)	Recovery (%)
Urine	1	10	9.62	96.2
	2	10	9.88	98.8
	3	10	10.15	101.5
Serum	1	10	9.89	98.9
	2	10	9.96	99.6
	3	10	10.12	101.2

All samples were analyzed using standard addition method (n=5).

4. CONCLUSION

In summary, an electrochemical sensor based on GR/TiO_2 modified electrode was prepared for the detection of trace amounts of PHE. The sensor showed high electrocatalytic activity for the oxidation of PHE at the potential of 0.842 V with about 2.87 times higher current response than the GCE. In addition, The sensor exhibited good sensitivity of PHE with the detection limit of 2.0×10^{-7} mol L^{-1} by DPV under accumulation time of 200 s. The proposed method was further applied for the detection of PHE in real sample with satisfactory results. More importantly, the oxidation reaction of PHE was accompanied by one proton and one electron, which could provide a valuable reference for the pharmacological action of PHE in clinical study.

ACKNOWLEDGEMENTS
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