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Electrochemical Behavior and Determination of Glycyrrhizic Acid at Glassy Carbon Electrode Modified with Graphene

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A simple and rapid electrochemical method is developed for the determination of glycyrrhizic acid, based on the excellent properties of graphene. The graphene-modified glassy carbon electrode constructed and the electrochemical behaviour of glycyrrhizic acid at the electrode is investigated in detail. In 0.2 mol/L pH 7 phosphate buffer solution, the redox peak currents of glycyrrhizic acid increased significantly at graphene modified glassy carbon electrode compared with bare glassy carbon electrode, indicating that graphene possessed electrocatalytic activity towards glycyrrhizic acid. The experimental conditions were optimized and the kinetic parameters were investigated. Under the optimal experimental conditions, the oxidation peak current was proportional to glycyrrhizic acid concentration in the range from 3.12×10^{-8} to 1×10^{-6} mol/L with the correlation coefficient of 0.9915. The detection limit was 2×10^{-8} mol/L. Using the proposed method, glycyrrhizic acid was successfully determined in water samples, suggesting that this method can be applied to determine glycyrrhizic acid in different liquorices.

Keywords: Graphene, Glycyrrhizic acid, Modified electrode, Behavior.

INTRODUCTION

Liquorice (Glycyrrhiza glabra) has long history of use as a medicinal plant throughout the world. Triperpenoids have been widely applied in human medicine since they were developed in the 1982[1-3]. Glycyrrhizic acid (GA) is one kind of the triperpenoids and its structure is shown in Fig. 1. It has a significant effect not only for tracheitis, bronchitis, cough, asthma and other respiratory diseases, but also for gastrointestinal infections, hepatitis B, oral ulcers, gastric ulcer and wonders[4-6]. In addition, glycyrrhizic acid is also broadly applied in production of food additives preparation and cosmetic. As a result, large amounts of glycyrrhizic acid may be useful for our lives. Therefore, the widespread use of glycyrrhizic acid and the need for clinical and pharmacological study require fast and sensitive analytical methods for its determination.

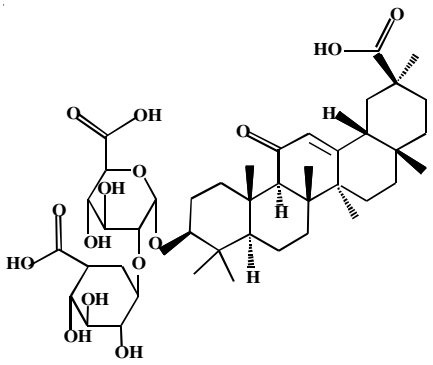

Fig. 1. Structure of Glycyrrhizic acid

Various methods have been proposed for the determination of glycyrrhizic acid, such as spectrophotometry[7,8], high performance liquid chromatography (HPLC)[9,10], high performance liquid chromatography-mass spectrometry (HPLC- MS)[11-13]. However, spectrophotometry is easily interfered by related compounds. Although, HPLC has been widely applied because of its high sensitivity and selectivity and the ability to minimize interferences. It is time-consuming and solvent-usage intensive and expensive. Hence, it is still of great significance to develop sensitive and simple detection methods for natural medicines. During the past decade, considerable interests have been focused on chemically modified electrodes for natural medicines due to the advantages of good reliability, fast response, in expensive instrument, low energy consumption, simple operation, time saving and high sensitivity[14,15]. Huang *et al.*[16] and Chen *et al.*[17] used glassy carbon electrode (GCE) for the determination of rutin and further applied to Chinese medicines samples with satisfactory results. Huang *et al.*[18] and Lida *et al.*[19] used multi-walled carbon nanotubes modified glassy carbon electrode for

the electrochemical behaviour and voltammetric determination of norfloxacin and sulfaguanidine. Ma *et al.*[20] and Tang *et al.*[21] used graphene modified glassy carbon electrode for electrochemical determination of Sudan I in food samples and trace aluminium in biological samples. An *et al.*[22] and Yin *et al.*[23] used graphene-chitosan composite film modified glassy carbon electrode for electrochemical behaviour and voltammetric determination of rutin and 4-aminophenol. Wang *et al.*[24] used mercury electrode for the glycyrrhizic acid electrochemical behaviour.

To our best of knowledge, electrochemical determination of glycyrrhizic acid using graphene modified glassy carbon electrode (GCE) has not been reported yet. In this work, the graphene modified glassy carbon electrode was fabricated and characterized. The electrochemical behaviour of glycyrrhizic acid was investigated at the modified electrode. It was found that graphene showed significantly electrocatalytic activity towards glycyrrhizic acid.

EXPERIMENTAL

Graphite powder was obtained from Qindao Graphite Corporation, glycyrrhizic acid was purchased from U.S. Sigma company and 1×10^{-4} mol/L glycyrrhizic acid stock solution was prepared by dissolving it in absolute ethanol and distilled water. All other chemical reagents (Analytical-reagent grade) were obtained from Tianjin Chemical Reagent Company (Tianjin, China). Phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of 0.2 mol /L Na_2HPO_4 and 0.2 mol/L NaH_2PO_4. All aqueous solutions were prepared in distilled water.

Electrochemical measurements were performed with a CHI660E electrochemical workstation (CH Instrumental, Shanghai, China) with a conventional three-electrode cell. The conventional three-electrode cell was used with a saturated calomel electrode (SCE) as reference electrode, a Pt wire as counter electrode and a bare or graphene modified glassy carbon electrode was used as working electrode, respectively. All the used electrodes were procured from CHI Co. All the measurements were carried out at room temperature (25 ± 0.5 °C).

Preparation of graphene/glassy carbon electrode: 1.5 mg graphene was added into distilled water and then sonicated for 2 h to give a homogeneous solution. The glassy carbon electrode was polished with 0.05 mm alumina powders and rinsed thoroughly with distilled water. After the glassy carbon electrode was dried, 5 µL of graphene dispersion was dropped on glassy carbon electrode surface. The modified electrode was dried at room temperature and rinsed with distilled water before use. The obtained electrode was noted as graphene/glassy carbon electrode.

Analytical procedure: The graphene coated glassy carbon electrode was first activated in phosphate buffer (0.2 mol/L, pH 7) by cyclic voltammetric sweeps between -0.2 and 0.8V at a scan rate of 100 mV/s until stable cyclic voltammograms were obtained and then transferred into another 10 mL of phosphate buffer (0.2 mol/L, pH 7) containing a certain concentration of glycyrrhizic acid. The oxidation peak current at 0.34 V was measured and all electrochemical experiments were carried out at room temperature. Upon completion of each scan, the modified electrode was placed in the pure base solution and cyclic scan was continued until no peak comes out, then the electrode was washed with water and dried with filter paper for reuse.

RESULTS AND DISCUSSION

Electrochemical behaviour of glycyrrhizic acid: The electrochemical behaviour of glycyrrhizic acid was investigated by cyclic voltammetry. Graphene/glassy carbon electrode in phosphate buffer (pH 7) were shown in Fig. 2. It can be seen that the glycyrrhizic acid oxidation peak was sharper and the peak current was increased significantly at graphene/glassy carbon electrode than the bare glassy carbon electrode, indicating that the graphene film can significantly catalyze the oxidation process of glycyrrhizic acid and the electron transfer rate of glycyrrhizic acid in the graphene film is much faster. This may be attributed to the special chemical and nano-mesh structure of graphene, which has a large specific surface area and a large number of defects. All these unique physical and chemical properties make the glycyrrhizic acid reactivity at the modified electrode significantly improved and the response signal greatly increased. From the cyclic voltammetries of glycyrrhizic acid at the modified electrode, the redox peak potential located at 340 mV (E_{pa}) and -730 mV (E_{pc}). $I_{pa}/I_{pc} <$ 1, which indicates that the reaction process of glycyrrhizic acid at the modified electrode is a reversible redox process. These results further demonstrated the advantages of graphene as described above.

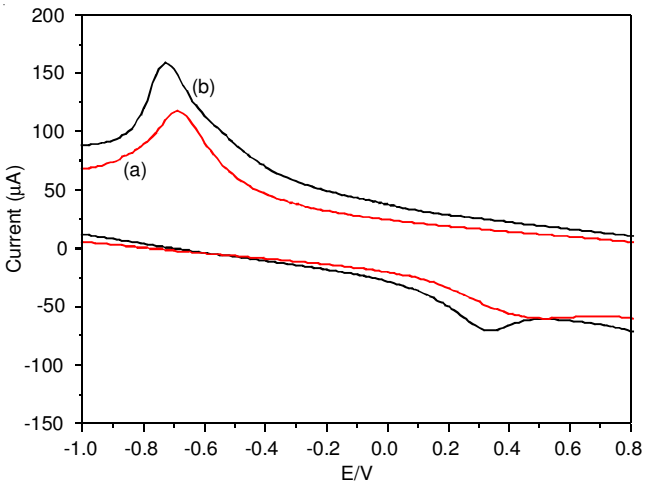

Fig. 2. (a) Typical cyclic voltammograms of 1×10^{-5} mol/L glycyrrhizic acid at a bare glassy carbon electrode (red line), (b) Graphene modified glassy carbon electrode (black line) in phosphate buffer (0.2 mol/L, pH 7). Scan rate: 100 mV/s

Choice of supporting electrolyte: The electrochemical responses of glycyrrhizic acid show difference in different supporting electrolytes , choice of suitable supporting electrolyte is of great importance. In this work, the electrochemical oxidation responses of glycyrrhizic acid in a variety of supporting electrolyte, such as Na_2HPO_4-NaH_2PO_4 buffer, K_2HPO_4-KH_2PO_4 buffer and Na_2HPO_4- citric acid buffer, respectively, were investigated. It was found that better sensitivity and peaks were observed in Na_2HPO_4-NaH_2PO_4 buffer. Thus, PBS was chosen as the supporting electrolyte. Fig. 3

shows the influence of solution pH on glycyrrhizic acid oxidation peak current. Using PBS of various pH, results show that, in a pH range from 6 to 8, oxidation peak current firstly increases with increasing pH and reaches maximum at pH 7.

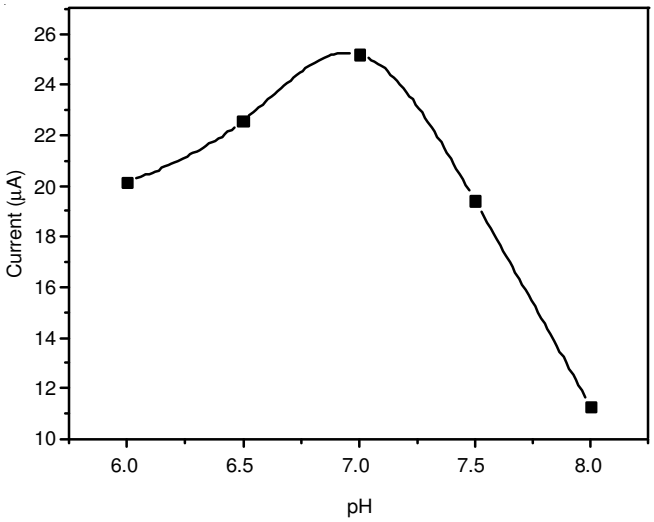

Fig. 3. Effect of pH on the redox peak current of glycyrrhizic acid

Effect of scan rate: Useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. The effect of scan rate on the redox reaction of glycyrrhizic acid at the graphene/glassy carbon electrode was investigated at a concentration of 1×10^{-6} mol/L glycyrrhizic acid in pH 7 PBS (Fig. 4). It was found that, with the increase of scan rate, the oxidation potential shifted in the positive direction. The oxidation peak currents linearly increased with the scan rates ranging from 50 to 120 mV/s with the linear regression equations expressed as Ipa(-A) = $105.65 \times 10^{-6} + 1.2266 \times 10^{-6}$ v(mV/s), r = 0.959, suggesting that the electrochemical behaviours of glycyrrhizic acid at the graphene/glassy carbon electrode was an adsorption process. Scan rate of 100 mV/s gave the best redox peaks of glycyrrhizic acid, therefore, 100 mV/s was chosen as the best scan rate.

Effect of accumulation time: It was important to fix the accumulation time when adsorption studies were undertaken. Measurements were made with various accumulation time at a glycyrrhizic acid concentration of 1×10^{-6} mol/L. The peak current increased rapidly with increasing accumulation time, which induced rapid adsorption of glycyrrhizic acid on the surface of the modified electrode. The peak current reached the maximum after 50 s and then decrease. This is because excess potential caused by concentration difference of glycyrrhizic acid on electrode surface becomes smaller with better mixing, resulting increased response current. Hence, a 50s accumulation period was used for the determination of glycyrrhizic acid.

Linearity range and limit of detection: The relationship between the oxidation peak current and the concentration of glycyrrhizic acid was examined by cyclic voltammetry and the results are shown in Fig. 5. It was found that, in pH 7 PBS, the oxidation peak current of glycyrrhizic acid at the graphene/glassy carbon electrode is linearly proportional to its concentration

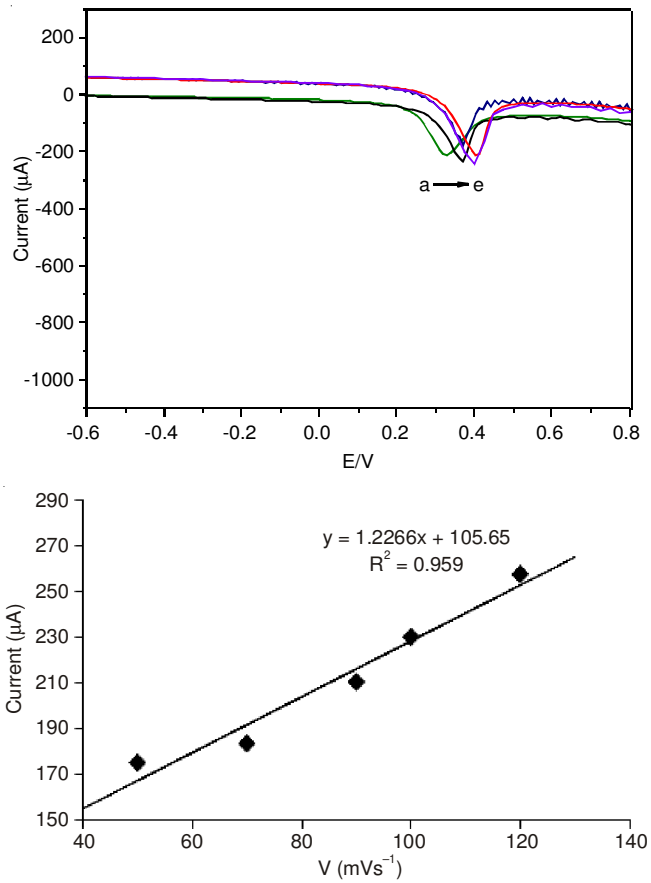

Fig. 4. Cyclic voltammograms of 1×10^{-6} mol/L glycyrrhizic acid in pH 7 PBS at different scan rates. Each of the letters from a to e corresponds to scan rates of 50, 70, 90, 100, 120, respectively, (in mV/s). Inset is the plot of oxidation glycyrrhizic acid peak currents *versus* scan rates

over the range from 3.12×10^{-8} to 1×10^{-6} mol/L, with a correlation coefficient of 0.9915. The linear regression equation can be expressed as Ipa(-A) = $53.823 \times 10^{-6} + 45.565 \times 10^{-6}$ (mol /L). The limit of detection was estimated by gradually decreasing the concentration levels of glycyrrhizic acid. When the concentration of glycyrrhizic acid was decreased to 2×10^{-8} mol /L, the redox peaks can still be observed, but the redox peaks almost disappear when the concentration was further decreased. Therefore, the limit of detection was evaluated to be 2×10^{-8} mol/L.

Determination of glycyrrhizic acid in real samples: The application of the method has been demonstrated by the analysis of glycyrrhizic acid in licorice from different regions. The results shown in Table-1 indicated the proposed method was accurate and it can be recommended for routine analysis in the majority of drug quality control laboratories. After the determination, three equal samples from different regions were spiked separately with 2×10^{-5} mol/L glycyrrhizic acid standard solution and then similarly analyzed.

TABLE-1
RESULTS GLYCYRRHIZIC ACID
IN LICORICE FROM DIFFERENT REGIONS

Regions	Jiuquan [Ref. 25]	Minqin [Ref. 26]	Hongsibao [Ref. 27]
Proposed method (%)	2.07	3.82	2.96
Certified value (%)	2.04	3.41	2.8-3.3

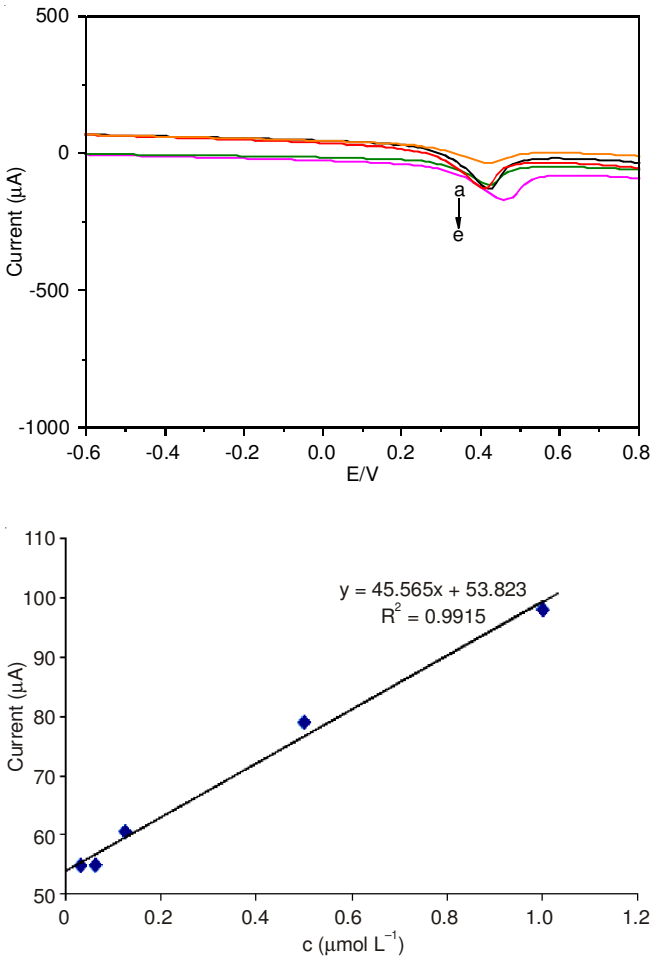

Fig. 5. Cyclic voltammograms of different concentrations of glycyrrhizic acid at graphene/glassy carbon electrode in pH 7 PBS. Each of the letters from a to e corresponds to concentrations of 0.031, 0.062, 0.125, 0.5, 1, respectively, (in μmol/L)

Conclusion

The results of the present work revealed that the graphene modified glassy carbon electrode exhibited excellent electrocatalytic activity towards the electrochemical oxidation of glycyrrhizic acid could significantly enhance the sensitivity of the determination. Therefore, the electrochemical responses of glycyrrhizic acid were greatly increased at the graphene/glassy carbon electrode. The peak currents obtained by cyclic voltammetry were linearly proportional to glycyrrhizic acid concentrations in a range from 1×10^{-6} to 3.12×10^{-8} mol/L with a detection limit of 2×10^{-8} mol/L. Due to its convenience, fastness, high sensitivity and selectivity, the method provides a practicable solution for determining glycyrrhizic acid in medicines.

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