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A sensitive electrochemical sensor for cryptotanshinone based on electrochemical reduced graphene oxide and carbon nanotube composite film modified glassy carbon electrode

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The electrodeposition of reduced graphene oxide and carbon nanotube (ErGO/CNTs) composite film modified electrode has been fabricated by electrochemical reduction of graphene oxide (GO) and MWNTs composite film coated on the surface of glassy carbon electrode (GCE). The results show that in 0.2 mol/L NaAc-HAc (pH= 4.5) buffer solution, cryptotanshinone produced a pair of sensitive redox peaks with peak potentials of -0.180 V and -0.207 V (vs SCE). The electrode reaction was two electrons and two protons participate in the completely reversible process of adsorption control. The ErGO/CNTs membrane modified electrode has obvious catalytic and sensitizing effects on the redox of cryptotanshinone. Differential pulse voltammetry (DPV) is used to investigate the relationship between oxidation peak current and cryptotanshinone concentration. It is found that the oxidation current of cryptotanshinone is linear with its concentration in the range of 2.7×10^{-8} ~1.3×10^{-5} mol/L and the determination limit is 1.0×10^{-8} mol/L, which can realize the electrochemical detection of cryptotanshinone in the drug.

Keywords: Graphene, Carbon nanotubes, Cryptotanshinone, Composite film

Cryptotanshinone (CTH) is a monomer extracted from the traditional chinese medicine salvia miltiorrhiza bunge. It has shown promising prospects in cardiovascular disease, antitumor and neuro–degenerative diseases[1-2]. Therefore, it is very important to study the properties of CTH from molecular mechanism and establish a simple, accurate and sensitive new method for the detection of CTH. At present, the determination methods of CTH mainly include room temperature phosphorescence[3], liquid chromatography-mass spectrometer (LC-MS)[4-5], high performance liquid chromatography (HPLC)[6-7], and liquid extraction-capillary electro chromatography[8]. These methods are very sensitive, capable of determining a large number of compounds but they often require tedious pre-treatment procedure, time consumption and/or expensive experimental equipment. In contrast, electrochemical method is simple, rapid, sensitive and inexpensive. Furthermore, the redox properties of drugs can provide insight into their metabolic fate, their redox processes in vivo, and their pharmacological activity. In the case of CTH, electrochemical determination has been reported[9]. However, the traditional electrode has a small specific surface area and weak interaction with organic compounds, resulting in low analytical sensitivity and narrow linear range. Therefore, the sensing layers of electrode are crucial, so more research is still in process[10]. Carbon nanotubes (CNTs) have high conductivity and high surface area, so they have good electrochemical properties and are excellent electrode materials[11]. On the other hand, as a carbon-based nanomaterial, graphene has a surface rich in oxygen-containing functional groups and a large specific surface area, which increases the catalytic active site. Various graphene-based modified electrodes have been developed for electrochemical. The sensor is used for the determination of various substances[12-13]. The traditional method for preparing electrochemical sensing interfaces based on graphene materials is mainly the drip coating method. Because graphene sheets have strong π-π interaction bonds, they are easy to aggregate, and difficult to dissolve in water and common organic solvents, which has caused great difficulties in the research and application of graphene sensors. The graphene modified electrode prepared by electrochemical reduction is considered to be an effective method. This method is simple, green, fast and easy to control the amount of graphene and its micromorphology[14-15].

In this work, we presented a simple and effective method to fabricate graphene/carbon nanotube modified electrode (ErGO/CNTs/GCE) by pulsed potentiostatic method under constant stirring. It was characterized with scanning electron microscopy and cyclic voltammetry. The ErGO/CNTs/GCE was applied in developing a high sensitive electrochemical sensor for determination of CTH. Systematic electrochemical tests demonstrated that the ErGO/CNTs/GCE can effectively increase response due to the large accessible surface area as well as its excellent conductivity. Under the optimum conditions, the electrochemical response showed a linear relationship with CTH concentrations in the range of 2.7×10^{-8}~1.3×10^{-5} mol/L, and a low detection limit of 1.0×10^{-8} mol/L. Based on these results, we believe it will be a potential choice for determination of CTH in medicine.

Materials and Methods

Apparatus and reagents

MERLIN series field emission scanning electron microscope (FESEM) (German Zeiss), RST5200 electrochemical analyzer (Zhengzhou Shi Ruisi Instrument Technology Co., Ltd.), standard three-electrode system modified glassy carbon electrode (Φ = 3 mm) as the working electrode, the saturated calomel electrode is the reference electrode and the platinum electrode is the counter electrode.

High-purity graphite powder and multi-walled carbon nanotubes (CNTs, purity ≥95%) were purchased from Nanjing Xianfeng Nano-Materials Technology Co., Ltd., CTH (China National Institute of Pharmaceutical and Biological Products), tanshinone capsules (Hebei Xinglong Xili Medicine Industry Co., Ltd.), all other reagents are of analytical grade, and the test water is quartz sub-boiling double distilled water (DDW).

Preparation of GO-CNTs suspension

Graphene Oxide (GO) with good quality was prepared by a modified Hummers method[16]. After preparation, GO was ultrasonically dispersed in secondary distilled water to form a 3 mg/mL GO suspension. Then carboxylated CNTs was added to the GO suspension, and dispersed by ultrasound for 30 min to obtain a uniform suspension of GO-CNTs.

Preparation of modified electrodes

Prior to modification, the bare glassy carbon electrode (GCE) was polished successively with 1.00, 0.30 and 0.05 µm Al_2O_3 power and rinsed thoroughly with DDW. Then, the GCE was sonicated in ethanol and DDW for 3 min, and dried under N_2 blowing. After that, the electrode was immersed in GO-CNTs suspension, and electrochemical reduction-deposition was carried out by pulsed potentiostatic method. The parameters of electrodeposition were optimized and listed as follows: upper limit potential E_a, 0.1 V; lower limit potential E_c, −1.5 V; cathodic pulse duration t_c, 0.7 s; anodic pulse duration t_a, 0.3 s; and experimental time t_{exp}, 150 s. The obtained electrode was denoted as ErGO/CNTs/GCE. The surface morphology of the modified electrodes was characterized by SEM (Fig. 1). As shown in Fig. 1, the surface of the ErGO/CNTs/GCE showed three- dimensional uniform and wrinkled graphene sheets, which is similar to the characteristic morphology of the literature [17].

Experimental method

A certain volume of stock solution of CTH and 10 mL 0.1 mol/L PBS (pH=6.5) buffer solution were added into an electrochemical cell and then the electrode was immersed into the cell. The cyclic voltammetry (CV), chronocoulometry (CC) or differential pulse voltammetry (DPV) were performed to investigate the electrochemical behaviour of CTH at ErGO/CNTs/GCE.

Real sample assay procedures

The real sample was processed according to the literature[9]. Danshen produced by Hebei Xinglong Xili Pharmaceutical Co., Ltd. Ketone capsules. About appropriate amount of sample (accurately weigh about 300 mg containing CTH) was weighed and extracted with 100 mL anhydrous ethanol for 2 h in an ultrasonic bath. Finally, sample solution was stored in the dark. Just before each measurement, the sample

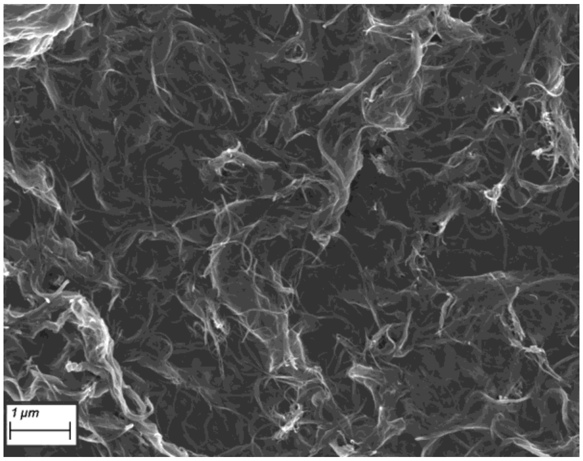


Fig. 1 — SEM images of ErGO/CNTs/GCE

solution was diluted quantitatively using the supporting electrolyte.

Results and Discussion

Electrochemical characterization of different electrode

The electrochemical character of the different electrode was characterized using the electrochemical probe $K_3[Fe(CN)_6]$. Fig. 2 shows the CVs of a bare GCE (curve a), CNTs/GCE (curve b), ErGO/GCE (curve c), and ErGO/CNTs/GCE (curve d) in 1.0×10^{-3} mol/L $K_3[Fe(CN)_6]$ + 0.1 mol/L KCl solution. A pair of redox peaks of $[Fe(CN)_6]^{3-/4-}$ is showed on bare GCE with the peak-to-peak separation (ΔE_p) as 93 mV at the scan rate of 100 mV s^{-1} (curve a). Using the CNTs/GCE and ErGO/GCE, both cathodic and anodic peak currents increase with the ΔE_p value decrease to 75 mV (curve b and c). The results suggested that the CNTs or ErGO film could efficiently accelerate the electron transfer to amplify the electrochemical signal due to its excellent electric conductivity and large specific surface area. When the ErGO/CNTs composite film was modified, the largest peak currents and the smallest ΔE_p of redox probe Fe $(CN)_6^{3-/4-}$ is observed on ErGO/CNTs/GCE (curve d). The results suggested that the method to combine the advantages of both CNTs and ERGO were feasible and effective.

Electrochemical behaviour of CTH at ErGO/CNTs/GCE

Fig. 3 shows the CVs of 5.0×10^{-5} mol/L CTH in 0.2 mol/L NaAc-HAc (pH=4.5) buffer solution at a bare GCE (curve a), CNTs/GCE (curve b), ErGO/GCE (curve c), and ErGO/CNTs/GCE (curve d). As can be seen in Fig. 3, CTH showed electrochemical activation on all electrodes. At bare GCE, electrochemical response of CTH was very weak and only a very small of redox peaks was observed. In contrast, on the CNTs/GCE and/or ErGO/GCE, the redox peak currents (i_p) increased significantly and the ΔE_p value decreased significantly, indicating that the ErGO and CNTs film can accelerated the electron transfer on the electrode surface to amplify the electrochemical signal due to its large surface area and excellent electrical conductivity. When the ErGO/CNTs/GCE was applied, the most well-defined and sensitive redox peaks currents appeared under the same experimental condition and the ΔE_p value was the lowest. This result further testified that the method to combine the advantages of both CNTs and ErGO is successful, which can provide a more efficient interface and microenvironment for the electrochemical response of CTH.

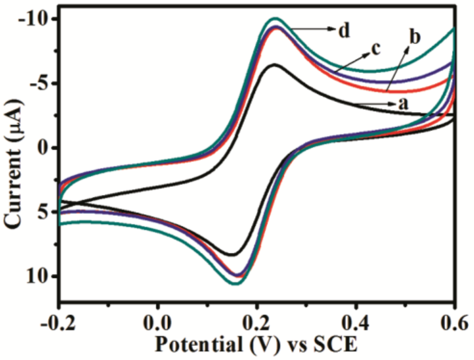


Fig. 2 — CVs of a bare GCE (curve a), CNTs/GCE (curve b), ErGO/GCE (curve c), and ErGO/CNTs/GCE (curve d) in 1.0×10^{-3} mol/L $K_3[Fe(CN)_6]$ + 0.1 mol/L KCl solution, v = 0.1 V/s

Electrochemical redox mechanism of CTH

The kinetics of the electrode reaction was investigated by recording the influence of different scan rates (v) on the redox of CTH. Fig. 4 shows CVs of 5.0×10^{-5} mol /L CTH in 0.2 mol/L NaAc -HAc

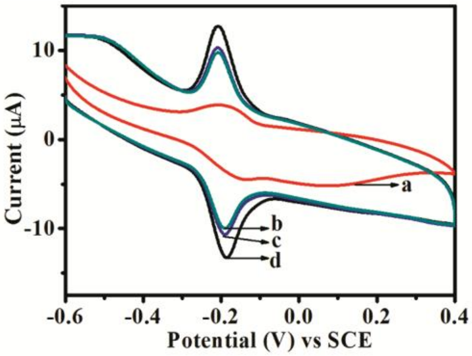


Fig. 3 — CVs of 5.0×10^{-5} mol /L CTH at a bare GCE (curve a), CNTs/GCE (curve b), ErGO/GCE (curve c), and ErGO/CNTs/GCE (curve d) in 0.2 mol/L NaAc-HAc buffer solution (pH=4.5), v = 0.1 V/ s

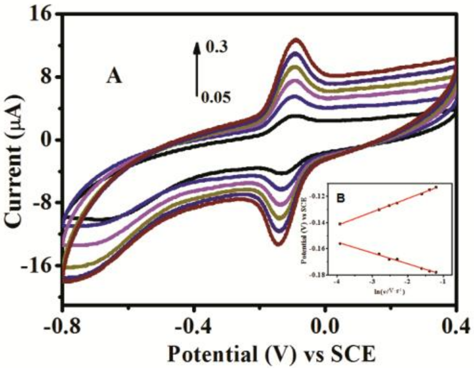


Fig. 4 — (A) CVs acquired on ErGO/CNTs/GCE with 5.0×10^{-5} mol/LCTH in supporting electrolyte of 0.2 mol/L NaAc –Hac (pH=4.5) at different scan rates from 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 V/s ; (B) The relationship between E_p and ln v

buffer solution (pH=4.5) at the ERGO/GCE with the v ranging from 0.05∼0.30 V/s . The redox peak currents were found to be linearly proportional to the scan rate. The linear relationships were established with two linear regression equations as i_{pa} (μA)= 60.718 V + 0.289 (R = 0.998) and i_{pc} (μA) = -56.802 V - 0.189 (r = 0.997) (Fig. 4b). This result demonstrates that the redox reaction of CTH on ErGO/CNTs/GCE is an adsorption-controlled process.

According to the Laviron's theory [18], the i_p–v relation can be described as follows:

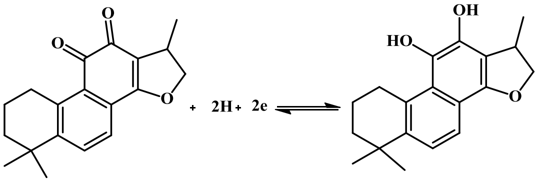
$$i_p = \frac{n^2F^2A\Gamma^*v}{4RT} = \frac{nFQv}{4RT}$$

This means that the electron-transfer number, n, can be calculated as long as the CV peak area, Q, is obtained under a certain scan rate. As the scan rates varied from 0.05 to 0.3 V/ s, n = 2 was calculated as an average. Moreover, with the increase of v; the oxidation peak potential (E_{pa}) was positively shifted and the reduction peak potential (E_{pc}) was negatively shifted, indicating that the redox reversibility of CTH was impaired. The E_{pa} and E_{pc} were linearly dependent on the ln v with the regression equations of E_{pa} = 0.01029 ln$v$ - 0.1010 (R = 0.9994)and E_{pc} = -0.0083 ln$v$ - 0.1881(R = 0.9957) (Fig. 4b). Based on the Laviron's theory[19] with slopes of the lines RT/ [(1-α)nF] = 0.01029 and -RT/αnF , the value of the electron transfer coeffcient (α) were calculated as 0.65. Furthermore, the rate constant (k_s) of the heterogeneous electron transference can be calculated using the following equation[20]:

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log \alpha - \log \frac{RT}{nFV} - \alpha(1-\alpha)\frac{nF\Delta E_p}{2.3RT}$$

The k_s for ErGO/CNTs/GCE is estimated to be 1.17 s^{-1}.

To further investigate the mechanism of the electrochemical redox of CTH, the effect of the solution pH on the response of CTH was also studied by CV (Fig. 5). The results shows that both the anodic peak potential (E_{pa}) and the cathodic potential (E_{pc}) shifted negatively with increasing the solution pH (3.0-6.0), indicating that the electrocatalytic reaction of CTH at ErGO/CNTs/GCE is a proton-involved reaction. The linear regression equation is E_{pa} = -0.05955pH + 0.1380 (R = 0.9983), demonstrating that the electrode process is equal to proton–electron transfer (Fig. 5b). Based on these results, the electrochemical reaction mechanism was expressed as follows:



Chronocoulometry investigations

In view of the electrode process being controlled by adsorption, the saturated adsorption capacity (Γ^*) of CTH on the ErGO/CNTs/GCE surface was calculated. Fig. 6 shows chronocoulometric curves of ErGO/CNTs/GCE in the background (curve a) and 5.0 × 10^{-5} mol/L CTH (curve b). The corresponding Q~ $t^{1/2}$ plots were also performed and shown as inset in Fig. 6. According to the formula given by Anson[21]:

$$Q = \frac{2nFAc(Dt)}{\pi^{1/2}} + Q_{dl} + Q_{asd}$$

where Q_{dl} is the double-layer charge, and Q_{ads} is the Faradic charge caused by the oxidation of adsorbed

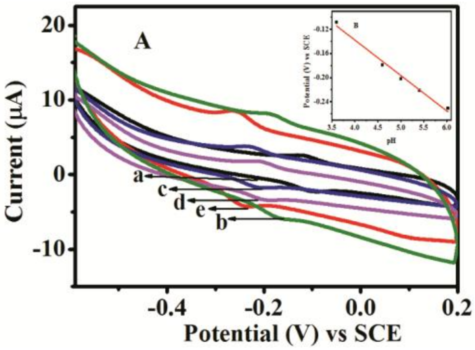


Fig. 5 — (A) CVs of CTH (5.0 × 10^{-5} mol /L) in 0.2 mol/LNaAc –Hac (pH=4.5) with different pH values of 3.5, 4.5, 5.0, 5.4 and 6.0 (a- e), scan rate: 0.1V/ s; (B) The relationship between formal potential ($E^{0'}$) and the pH value

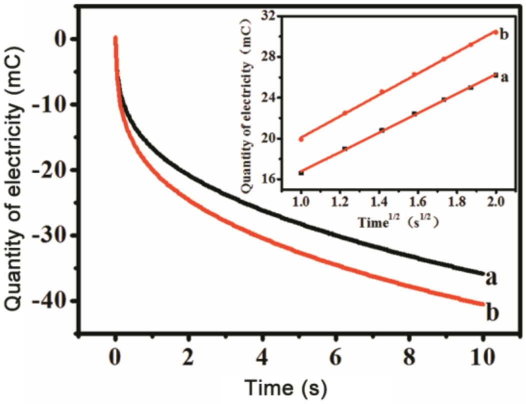


Fig. 6 — CTH curves of the background (curve a) and 5.0 × 10^{-5} mol/L cryptotanshinone (curve b) in 0.2 mol/L solutions (pH = 4.5) at the ErGO/CNTs/GCE; the inset shows the corresponding relationship of Q versus $t^{1/2}$

CTH. In this experiment, Q_{dl} was considered the same in the presence and absence of CTH. Using Laviron's theory[19] of $Q = nFA\Gamma^*$, Γ^* is the maximum surface concentration of the surface-attached material (mol/cm^2), and A is the electrode geometric area (cm^2) and intercept difference between curves a and b, the Γ^* is obtained to be 2.31 × 10^{-10} mol/cm^2.

Accumulation conditions

For consideration of the adsorption of CTH on the ErGO/CNTs/GCE surface, differential pulse voltammetry (DPV) technique coupled with accumulation procedure was used for study. With an increase in accumulation time (t_{acc}), the i_{pa} increased greatly within 200 s and then enhanced slowly. It is well-known that a longer t_{acc} will result in a better detection limit, but will also lead to a narrower linear range. For comprehensive consideration, 200 s was chosen as accumulation time. Moreover, the accumulation potential (E_{acc}) had a little effect on the effect on the i_{pa}. So the accumulation was carried out under an open circuit.

Calibration curve, detection limit, reproducibility and stability

Under the optimum conditions, the calibration curve for determination of CTH was established. Fig. 7 displays the response of different concentration of CTH by DPV. A linear relationship could be established between i_{pa} and the concentration of CTH in the range of 2.7 × 10^{-8} ~ 1.3 × 10^{-5} mol/L (the inset of Fig. 7). The linear regression equation and correlation coefficient are:

$$i_{\mathrm{pa}}(\mu\mathrm{A}) = 1.45821c + 1.12557,\ \mathrm{R} = 0.991$$

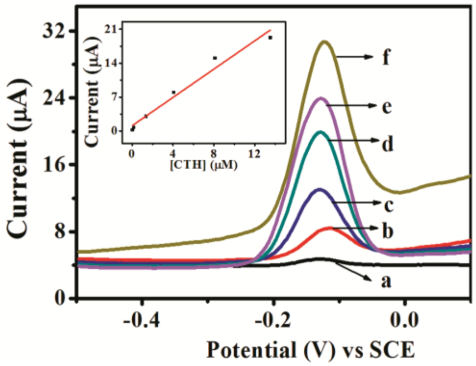


Fig. 7 — Differential pulse voltammograms and their associated calibration plot (insert) for increasing concentrations of CTH at the ErGO/CNTs/GCE under optimum conditions, following accumulation: tacc = 200s and open circuit; cryptotanshinone concentration: (a) 2.7 × 10^{-8} mol/L, (b) 1.3 × 10^{-7} mol/L, (c) 1.3 × 10^{-6} mol/L, (d) 4.0 × 10^{-6} mol/L, (e) 8.1 × 10^{-6} mol/L and (f) 1.3 × 10^{-5} mol/L

where i_{pa} is the oxidation peak current in µA and c is the concentration of caffeine in µmol/L. The limit of detection (LOD) for CTH is was found to be about 1.0 × 10^{-8} mol/L based on the signal-to-noise ratio of 3 (S/N) [22]. To estimate the reproducibility, the R.S.D. of five times successful measurement the peak current of 5.0 × 10^{-6} mol/L CTH was calculated to be 3.0%, which demonstrated the good reproducibility of the proposed electrode. The ErGO/CNTs/GCE can be stored about 2 weeks and the decrease of the response was got as 4.2%, which indicated that ErGO/CNTs/GCE had good stability.

The proposed ErGO/CNTs/GCE for CTH determination was compared with the literature[9] reported. The ErGO/CNTs/GCE can achieve wider linear concentration ranges and low detection limit. The result suggested that the modified electrode as voltammetric sensor might be a very promising direction in trace analysis of electrochemistry.

Interference studies

Under optimum experimental condition, the influences of some coexisting substances on the determination of CTH (5.0 × 10^{-6} mol/L) were studied. It is found that 500-fold oxalic acid, tartaric acid, citric acid, L-Glutamate, β-cyclodextrin, glucose, lactose and saturated starch water, 100-fold L-cystine do not influence the current response of CTH (signal change below 5%), suggesting that this proposed electrochemical sensor has excellent selectivity toward CTH .

Determination of CTH in the real sample

In order to evaluate the feasibility of the developed method for the analysis of real samples, the sample was diluted with the supporting electrolyte to operate in the linear range of the method. After the content of CTH was evaluated, a standard CTH solution was added into the sample and the total content of matrine was determined to calculate the recovery (Table 1). As listed in Table 1, the recovery and RSD values indicate that the newly-developed method is accurate and has promising application.

Table 1 — Determination results of cryptotanshinone in real samples by DPV

Sample	Cryptotanshinon concentration (10^{-6} mol/L)			Recovery (%)
	Amount found	Stand added	Total found	
1	1.90	2.50	4.25	96.6
2	2.02	2.50	4.28	94.7
3	2.04	2.50	4.76	104.8

Conclusions

In this work, we have demonstrated PPM to prepare ErGO-CNTs film for the sensitive detection of CTH. The proposed modified electrode showed exhibits larger accessible surface area as well as higher conductivity by incorporation of CNTs into the ErGO network. It showed wider linear range and low detection limit for determination of CTH. We believe it is a simple, rapid, green and promising method for determination of CTH content in real samples.

Acknowledgement

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