



A sensitive voltammetric sensor for taxifolin based on graphene nanosheets with certain orientation modified glassy carbon electrode



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ABSTRACT

The electrodeposition of reduced graphene oxide (ERGO) film with preferred vertical orientation was fabricated on a glassy carbon electrode by using pulse potential method in a graphene oxide colloidal solution. Using square wave adsorptive stripping voltammetry (SWASV), the ERGO film was applied for the first time, in developing a high-sensitive electrochemical sensor for detection of taxifolin. Compared with bare glassy carbon electrode (GCE), the resulting electrodes (ERGO/GCE) exhibited excellent response toward the redox of taxifolin by significantly enhancing the redox peak currents and decreasing the peak-to-peak separation. Under the selected conditions, the peak currents were linear relationship with taxifolin concentration in the range of 1.0×10^{-8} – 1.0×10^{-6} mol L⁻¹, with detection limit of 2.0×10^{-9} mol L⁻¹. Besides, the ERGO/GCE also exhibited an excellent selectivity, stability, reproducibility and repeatability.

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1. Introduction

Flavonoids have aroused increasing awareness because of their potential health beneficial effect [1]. Taxifolin, 3,3',4',5,7-pentahydroxyflavanon, is the main constituent in extract from the rind of siberian larch *Larix sibirica* leder, and dahurian larch *Larix gmelini* rupr. (Rupr.), syn. *Larix dahurica* turoz. (Pinaceae). It has been widely used in the treatment of cerebral infarction and sequelae, cerebral thrombus, coronary heart disease and angina pectoris [2,3]. Accordingly, accurate analytical method for taxifolin is interesting and necessary. Some detection techniques have been developed, such as high performance liquid chromatography (HPLC) [4–6], UV–vis spectrophotometry [7], thin layer chromatography (TLC) [8] and capillary zone electrophoresis (CZE) [9]. However, some of these methods are time-consuming, expensive or involve a tedious extraction process before detection, which hampers their further application. In contrast, electrochemical method can provide sensitive, fast, facile, and low-cost detection. Recently, some effort has been devoted to electrochemical sensor of taxifolin [10,11], but very limited.

Graphene, a perfect two-dimensional carbon material found in 2004 [12,13], is an ideal material for electrochemistry [14]

because of unusual electronic conductivity, high surface area, high mechanical, thermal and chemical stabilities [15–17]. Hence graphene-based modified electrodes prepared by various methods have been explored as electrochemical sensors platforms [18–22]. Among these, direct electrodeposition of reduced graphene oxide (ERGO) on glassy carbon electrode (GCE) has attracted considerable interest because of its unique several advantages, such as simple, rapid, green and efficient [23–26]. More recently, it has been widely used in both analytical [27–31] and industrial electrochemistry [32,33]. The electrodeposition technique may be cyclic voltammetry (CV) [27–30] or potentiostatic method (PM) [31–33]. As we know, the pulse potentiostatic method (PPM) [34], having advantages, including simplicity, time savings and high purity of the deposits, has little been applied in preparing the ERGO.

In this work, the ERGO film with preferred vertical orientation was fabricated on a glassy carbon electrode by using pulse potential method in a graphene oxide colloidal solution. Using square wave adsorptive stripping voltammetry (SWASV), the ERGO film was applied for the first time, in developing a high-sensitive electrochemical sensor for detection of taxifolin. Compared with bare glassy carbon electrode (GCE), the resulting electrodes (ERGO/GCE) exhibited excellent response toward the redox of taxifolin by significantly enhancing the redox peak currents and decreasing the peak-to-peak separation. Under the selected conditions, the peak currents were linear relationship with taxifolin concentration in the range of 1.0×10^{-8} – 1.0×10^{-6} mol L⁻¹, with detection limit of 2.0×10^{-9} mol L⁻¹. Besides, the ERGO/GCE also exhibited an

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excellent selectivity, stability, reproducibility and repeatability. Based on these results, we believe it is a simple, rapid, green and promising method for determination of taxifolin. And, this work firstly raises an approach to fabricate graphene nanosheets with a certain orientation on the electrode surface.

2. Experimental

2.1. Apparatus and reagents

All the electrochemical measurements were carried out using a CHI 650A electrochemical analyzer (CHI Instrumental, Shanghai, China) and a RST5000 electrochemical workstation (Zhengzhou Shiruisi Instrument Co., Ltd., Zhengzhou, China). A conventional three-electrode system was used, including a GCE ($d = 3$ mm) or a modified GCE as the working electrode, a platinum (Pt) wire as the auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode (KCl saturation). Atomic force microscopy (AFM) images were obtained with a BenYuan CSPM-5500 atomic force microscopy (Guangzhou BenYuan Nanometer Instrument Co., Ltd., Guangzhou, China). Scanning electron microscopy (SEM) images were obtained with a Quonxe-2000 field emission scanning electron microscope (FEI Company, Holland). All the pH measurements were made with a PHS-3C precision pH meter (Leici Devices Factory of Shanghai, China), which was calibrated with a standard buffer solution at 25 ± 0.1 °C every day.

Taxifolin was acquired from Shanghai Jinsui Biological Technology Co., Ltd. (Shanghai, China). Stock solution (1.0×10^{-3} mol L⁻¹) of taxifolin was prepared with absolute ethyl alcohol and stored at 4 °C darkly. Graphite was purchased from Nanjing Xfnano Materials Tech Co., Ltd. (Nanjing, China). All other reagents were of analytical grade and were used as received. Double distilled water was used for all preparations.

2.2. Preparation of the modified electrode

Firstly, graphene oxide (GO) was synthesized from graphite by the modified Hummers method [35]. The exfoliated GO was obtained by ultrasound of the GO dispersion, and centrifugation at 4000 rpm for 10 min. The resulting GO deposited on the mica were characterized by AFM. The results revealed that the GO sheets were almost single-layer, see Fig. S1. And the average thickness of single-layer GO sheets was approximately 1 nm.

Prior to modification, the bare GCE was polished successively with 0.3 and 0.05 μm Al₂O₃ power and rinsed thoroughly with doubly distilled water between each polishing step. After that, the GCE was sonicated in ethanol and doubly distilled water each for 2 min, and dried under N₂ blowing. After that, the cleaned GCE was immersed in phosphate buffer solutions (PBS, pH 5.0) containing 1.2 mg mL⁻¹ GO, and electrodeposited the GO by PPM under constant stirring. The optimal parameters of electrodeposition were listed as follows: upper limit potential E_a , 0.1 V; lower limit potential E_c , -1.5 V; anodic pulse duration t_a , 0.6 s; cathodic pulse duration t_c , 0.3 s; experimental time t_{exp} , 60 s. The overall reduction time (t_{re}) can be calculated from the following equation: $t_{\text{re}} = t_{\text{exp}} \times t_c / (t_c + t_a)$. The optimal parameters of electrodeposition (t_a , t_c and t_{re}) were described in Fig. S1. The obtained electrode was denoted as ERGO/GCE.

2.3. Experimental procedures for electrochemical analysis

A certain volume of stock solution of taxifolin and 10 mL 0.1 mol L⁻¹ H₂SO₄ solutions (pH 0.95) were added into an electrochemical cell, and then the electrode was immersed into the cell. The CV, chronocoulometry (CC) or square wave voltammetry

(SWV) were performed to investigate the electrochemical behavior of taxifolin at ERGO/GCE.

2.4. Real sample assay procedures

The real sample was processed according to the literature [11]. The sample powder was obtained by grinding a certain amount of dried *princes-feather fruit*. About 2 g of the powder was weighed and extracted with 50 mL 80% ethanol for 2 h in an ultrasonic bath. Finally, the extractum was extracted with ethyl acetate. Sample solution was stored in the dark. Just before each measurement, the sample solution was diluted quantitatively using the supporting electrolyte.

3. Results and discussion

3.1. Pulse potential electrodeposition of ERGO film on GCE

As applying a positive potential, GO sheets could be adsorbed on the electrode, because GO colloids exhibit negative charges in weak acid [36]. According to the literature [18], the as-adsorbed GO sheets can be electrochemically reduced at $E = -1.1$ V (versus SCE). Here, we used the PPM to achieve the electrodeposition of ERGO films, in which 0.1 V (versus SCE) was used to adsorb GO sheets on GCE, followed by applying -1.5 V to electrochemically reduce the as-adsorbed GO sheets to ERGO sheets.

To illustrate the pulse procedure used for the ERGO film, Fig. 1 shows the evolution in time of the E and i for the process of PPM. After the potential E_c is applied, the i increases sharply and then decreases tending to a steady value as in potentiostatic mode. As potential E_a is imposed, the i drops sharply, reaching values close to zero. This is a so-called “relaxation period” that allows the diffusion of GO sheets to areas where they have been quickly consumed while applying E_a . When a new pulse start, the distribution of GO sheets on the electrode surface is supposed to be more homogenous [37]. Therefore, the PPM can gain more uniform thin films.

3.2. Morphological characterization of the ERGO/GCE

To obtain further information as called ERGO films prepared by PPM and illustrate the difference of electrochemical properties, morphologies of ERGO/GCE were characterized by SEM. As showed in Fig. 2A and B, ERGO film modified GCE showed a cluster of close

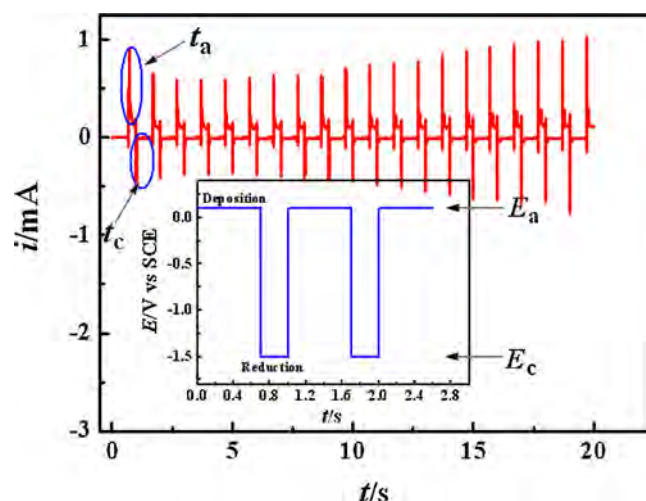


Fig. 1. Characteristic i - t response registered for pulsed electrodeposition; the inset outlines the E - t profile imposed for the method, during the electrodeposition of ERGO films.

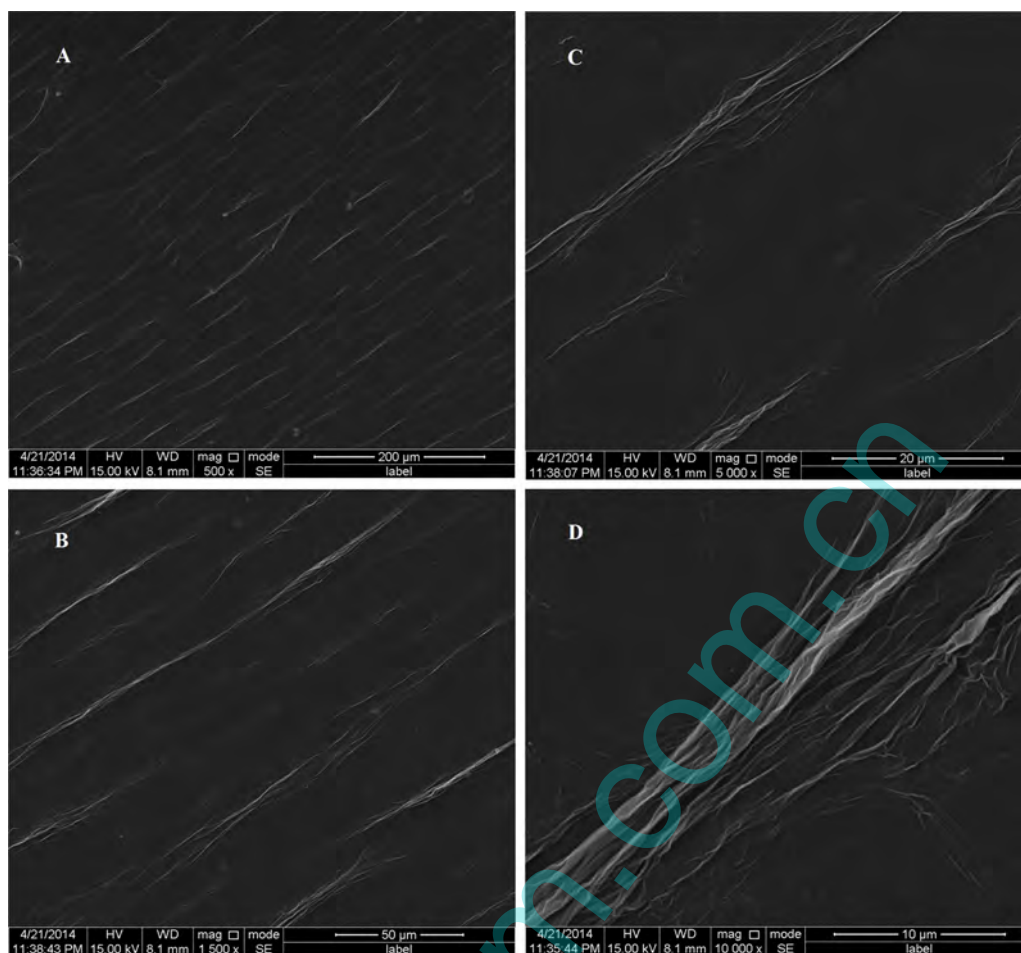


Fig. 2. The different magnification of SEM images obtained from the ERGO/GCE; (A) 500 \times , (B) 1500 \times , (C) 5000 \times , (D) 10,000 \times .

and strip-like graphene nanosheets with the same direction. A more magnified close-up image better exhibited the morphology of the ERGO film, as presented in Fig. 2C and D. The ERGO film displayed closely associated with each other to form thin and crumpled sheets, and the edges of individual sheets were distinguishable with kinked and wrinkled areas. At the same time, a preferred vertical orientation with respect to the substrate was also noted. Such vertical nanosheets may present unique electrochemical properties due to the formation of a large fraction of graphitic edge-plane defects, which can provide a higher surface activity than the graphene nanosheets deposited parallel to the substrate. Therefore, the ERGO film prepared by PPM can be applied as a very promising platform for highly sensitive electrochemistry-based sensors. Moreover, the ERGO films barely showed aggregation, indicating that PPM is an effective way to prepare assembly the ERGO films.

3.3. Electrochemical characterization of the ERGO/GCE

In order to highlight the particular feature of the proposed sensor, its voltammetric response to redox probe $[\text{Fe}(\text{CN})_6]^{3-/4-}$ was compared with at bare GCE. Fig. 3 shows the cyclic voltammograms (CVs) of bare GCE (curve a) and ERGO/GCE (curve b) in $1.0 \times 10^{-3} \text{ mol L}^{-1} \text{ K}_3[\text{Fe}(\text{CN})_6] + 0.1 \text{ mol L}^{-1} \text{ KCl}$ solution. A pair of redox peaks of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ is showed on bare GCE with the peak-to-peak separation (ΔE_p) as 72 mV at the scan rate of 100 mV s^{-1} . While on the ERGO/GCE, both cathodic and anodic peak currents increase obviously with the ΔE_p value decrease to 30 mV. The larger peak currents and the smaller ΔE_p of redox probe $[\text{Fe}(\text{CN})_6]^{3-/4-}$ is observed on the ERGO/GCE. The results suggested that ERGO

films prepared by PPM could efficiently accelerate the electron transfer to amplify the electrochemical signal due to its excellent electric conductivity and large specific surface area. According to Randles–Sevcik formula [38]: $i_{pa} = 2.69 \times 10^5 n^{3/2} A D_0^{1/2} c_0 v^{1/2}$, where i_{pa} refers to the anodic peak current (A); n is the electron transfer number; A is the surface area of the electrode (cm^2); D_0 is the diffusion coefficient ($\text{cm}^2 \text{ s}^{-1}$); c_0 is the concentration of $\text{K}_3[\text{Fe}(\text{CN})_6]$ (mol L^{-1}) and v is the scan rate (V s^{-1}). By exploring the redox peak current with scan rate, the average electroactive

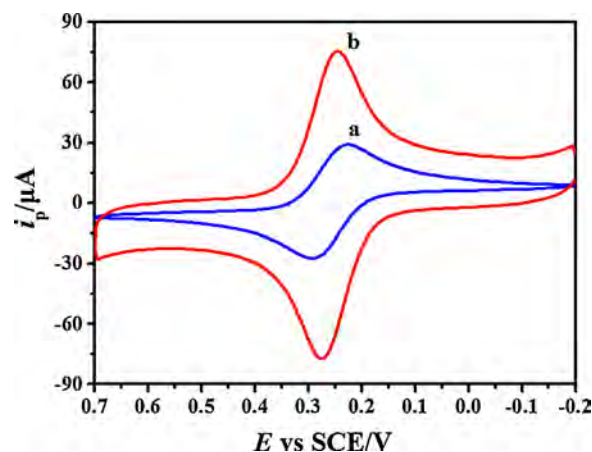


Fig. 3. Cyclic voltammograms of $\text{K}_3[\text{Fe}(\text{CN})_6]$ ($1.0 \times 10^{-3} \text{ mol L}^{-1}$) at bare GCE (a) and ERGO/GCE (b); supporting electrolyte: $0.1 \text{ mol L}^{-1} \text{ KCl}$ solution, $v = 0.05 \text{ V s}^{-1}$.

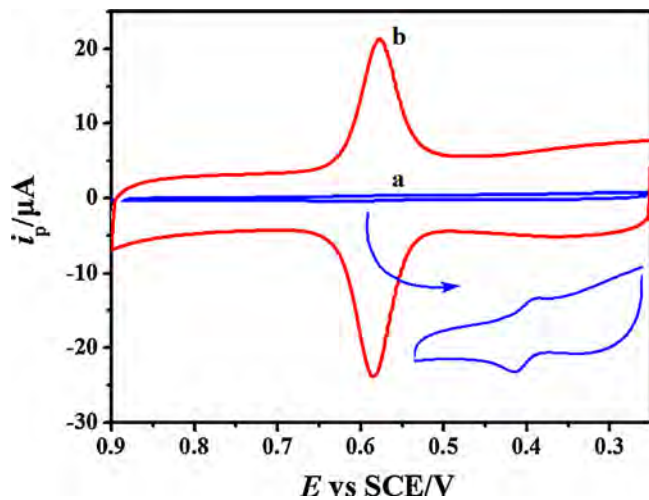


Fig. 4. Cyclic voltammograms of taxifolin ($2.0 \times 10^{-5} \text{ mol L}^{-1}$) at bare GCE (a) and ERGO/GCE (b); supporting electrolyte: $0.1 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ solutions (pH 0.95), $\nu = 0.05 \text{ V s}^{-1}$.

area of bare GCE and ERGO/GCE was calculated as 0.045 cm^2 and 0.093 cm^2 , respectively. The results indicated the ERGO films could availably improve the effective area of the electrode surface.

3.4. Voltammetric behavior of taxifolin at ERGO/GCE

Fig. 4 displays CVs of taxifolin ($2.0 \times 10^{-5} \text{ mol L}^{-1}$) in $0.1 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ solutions (pH 0.95) at bare GCE (curve a) and ERGO/GCE (curve b), respectively. As can be seen, taxifolin showed electrochemical activation on both electrodes. At the bare GCE, a pair of weak redox peaks could be discerned (inset of Fig. 4). In contrast, when the ERGO/GCE was applied, a pair of well-defined redox appeared under the same experimental condition, of which the peak current is about 87-fold higher than that of bare GCE. This results might be ascribed to the unique properties of the ERGO film. Firstly, the ERGO film provides a large specific surface area to increase the loading amount of taxifolin. Secondly, it as a promoter accelerates the electron transfer at the interface of the electrode and solution.

To further elucidate the electrode reaction of taxifolin at ERGO/GCE, the influence of the potential scan rate (ν) on the peak current (i_p) of taxifolin ($2.0 \times 10^{-5} \text{ mol L}^{-1}$) was investigated by CV at various sweep rates. Fig. 5 shows CVs with ν ranging from 0.10 to 1.00 V s^{-1} . The i_p of taxifolin grew with increasing of ν , and there were good linear relationships between i_p and ν , indicating that the redox process of taxifolin at the ERGO/GCE was adsorption-controlled. Based on Laviron's theory of an adsorption-controlled process, the relation of i_p and ν can be described as follows [39]:

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

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 0.10 to 1.00 V s^{-1} , $n=2$ was calculated as an average. Moreover, with the increase of ν , 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 taxifolin was impaired. When $\nu > 0.5 \text{ V s}^{-1}$, the E_{pa} and E_{pc} were linearly dependent on the $\ln \nu$ with the regression equations of $E_{pa} \text{ (V)} = 0.021 \ln \nu + 0.618$ ($R=0.998$) and $E_{pc} \text{ (V)} = -0.019 \ln \nu + 0.555$ ($R=0.995$) (inset of Fig. 5). Based on the Laviron's theory [40] with slopes of the lines $RT/[(1-\alpha)nF]$ and $-RT/\alpha nF$, the value of the electron transfer coefficient (α) was calculated as 0.53.

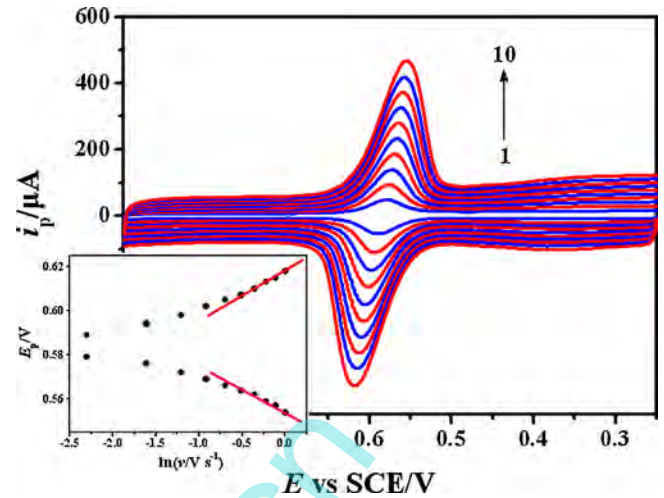


Fig. 5. The superposed voltammograms of taxifolin ($2.0 \times 10^{-5} \text{ mol L}^{-1}$) at ERGO/GCE with different scan rate (from 1 to 10: 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.00 V s^{-1}); insets show the relationship of the peak potential E_p against $\ln \nu$, the other experimental conditions are the same as in Fig. 4.

3.5. Chronocoulometry investigations

Next, the saturating adsorption capacity of taxifolin at ERGO/GCE was also investigated by employing chronocoulometry (CC). The ERGO/GCE was immersed in a taxifolin solution ($1.0 \times 10^{-4} \text{ mol L}^{-1}$) for several minutes to achieve saturated adsorption. Then, a step potential from 0.2 to 0.9 V was applied and a curve of Q versus t was recorded (Fig. 6, curve b). For control, a curve of Q versus t was recorded in a blank solution too (Fig. 6, curve a). Corresponding plots of Q versus $t^{1/2}$ were also obtained, and were showed as the inset in Fig. 6. According to a formula given by Anson [41]:

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

where c is the substrate concentration, D the diffusion coefficient, n the electron transfer number, Q_{dl} the double-layer charge and Q_{ads} the Faradaic charge from the adsorbed species. Other symbols have their usual meanings. Using Laviron's theory of $Q = nFA\Gamma^*$ (Γ^* is the maximum surface concentration of the surface-attached

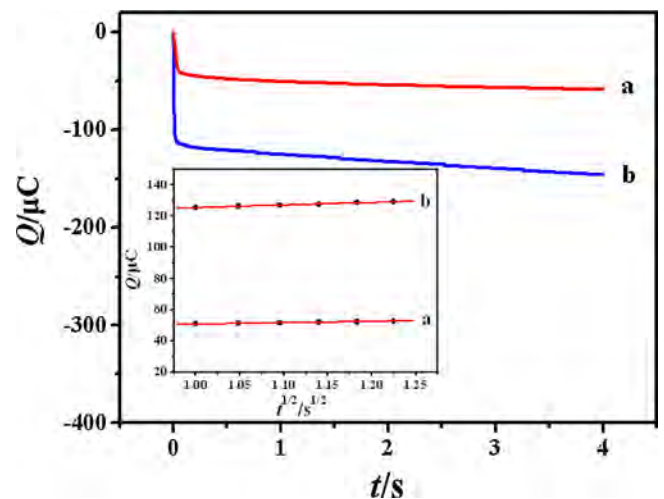
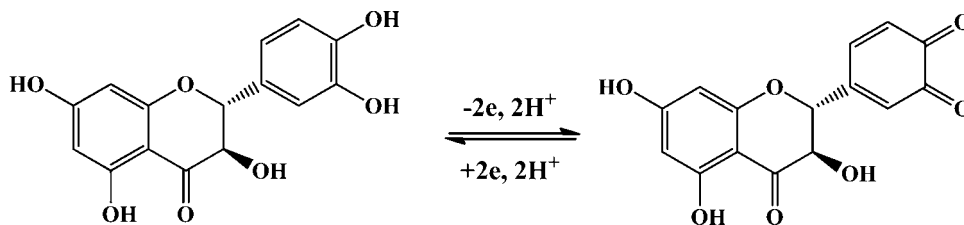


Fig. 6. Chronocoulometric curves of the background (curve a) and taxifolin ($1.0 \times 10^{-4} \text{ mol L}^{-1}$) (curve b) in $0.1 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ at ERGO/GCE; the inset is the corresponding plots of Q versus $t^{1/2}$.



Scheme 1. Redox mechanism of taxifolin at the ERGO/GCE

material, and A is the electrode geometric area) and intercept difference between curves a and b, a Γ^* value of $3.80 \times 10^{-9} \text{ mol cm}^{-2}$ was obtained, which further identify a strong adsorption capacity of the ERGO/GCE.

3.6. Analytical applications and methods validation

3.6.1. Influence of supporting electrolyte and pH

Types of supporting electrolyte played a key role in the voltammetric responses of taxifolin. The current responses of taxifolin ($2.0 \times 10^{-5} \text{ mol L}^{-1}$) were estimated in different supporting electrolytes, such as H_2SO_4 (pH 0.5–3), NaOH (pH 9.0–11.0), phosphate buffer (pH 6.0–8.0), acetate buffer (pH 4.0–7.0), Britton–Robinson (pH 2.0–10.0) and borate buffer (pH 7.5–9.0) solutions. The results showed that a higher peak current and a better peak shape could be obtained in an acid solution. Therefore, $0.1 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ solutions (pH 0.95) was priority adopted for the following experiments.

For the variation of solution pH from 0.5 to 3.0, it was found that both the anodic peak potential (E_{pa}) and the cathodic potential (E_{pc}) shifted negatively with increasing the solution pH, indicating that the oxidation of taxifolin at ERGO/GCE is losing proton reaction. The relationship between the formal peak potential (E^0) and the pH could be fitted into the regression equation, $E^0 = 0.632 - 0.052 \text{ pH}$ ($R = 0.999$). From the slope value, which is very close to the theoretical value of -59 mV , it indicates that the redox of taxifolin at ERGO/GCE is an equal electron-and proton-process. Therefore, the redox of taxifolin is a two-electron and two-proton electrode reaction process.

Based on results, the electrochemical reaction mechanism was expressed as in Scheme 1.

3.6.2. Square wave voltammetry investigations

To overcome the influence of blank current, we chose the reduction peak as the detection signal and square wave voltammetry (SWV) as analytical technique to establish the analytical method for taxifolin. The optimum instrumental parameters (pulse-amplitude E_{sw} , frequency f) were studied for a $1.0 \times 10^{-6} \text{ mol L}^{-1}$ taxifolin solution. The results indicated that the i_{p} increased with the increasing of E_{sw} from 10 to 50 mV and f from 10 to 40 Hz, but the peak potential shifted to more positive values, and the peak changed unshapely. So 25 mV were chosen as the optimum amplitude and 30 Hz were chosen as the optimum frequency.

3.6.3. Accumulation conditions

For consideration of the adsorption of taxifolin on ERGO/GCE surface, SWV technique coupled with accumulation procedure was used for establishment the calibration curve for taxifolin. With increasing the accumulation time (t_{acc}), the i_{p} increased. When t_{acc} was 210s, i_{pc} achieved a maximum value in a taxifolin solution of $5.0 \times 10^{-7} \text{ mol L}^{-1}$. A plateau appeared for prolonging the t_{acc} afterwards. The accumulation potential had a small effect on the peak current. So the t_{acc} of 210s under open circuit was used for further studies.

3.6.4. Electrode renewal

Electrode renewal is a critical issue in modified electrode applications in that it is impractical to use a brand new electrode for each individual sample. Here we tested in a $0.1 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ or a $0.1 \text{ mol L}^{-1} \text{ NaOH}$ for their potential capability of renewing the modified electrodes. We found when the electrodes were reconditioned in a $0.1 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ in the absence of taxifolin for 5 min under constant stirring, the peaks of taxifolin almost disappeared. And, the electrode was transferred in the same taxifolin solution for cyclic scan and a new voltammogram was obtained and exhibited the same characteristics as that of the first cycle in Fig. 4, curve b. Therefore, electrode renewal was performed in a $0.1 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ in the absence of taxifolin.

3.6.5. Calibration curve, detection limit, repeatability and stability

Fig. 7 displayed the square wave adsorptive stripping voltammograms of different concentrations of taxifolin with the optimum described above. A linear relationship could be established between i_{pc} and the taxifolin concentrations in the range of 1.0×10^{-8} – $1.0 \times 10^{-6} \text{ mol L}^{-1}$ (inset of Fig. 7). The linear regression equation and correlation coefficient are:

$$i_{\text{pc}} = 15.56 + 751.5c \quad (R = 0.999)$$

where i_{pc} was the reduction peak current in μA and c was the concentration of taxifolin in $\mu\text{mol L}^{-1}$. Standard deviations (SD) for the slope and intercept of the calibration curve were 1.667 and 0.842, respectively. Based on the signal-to-noise ratio of 3 (S/N) [42], the detection limit was obtained as $2.0 \times 10^{-9} \text{ mol L}^{-1}$. These values

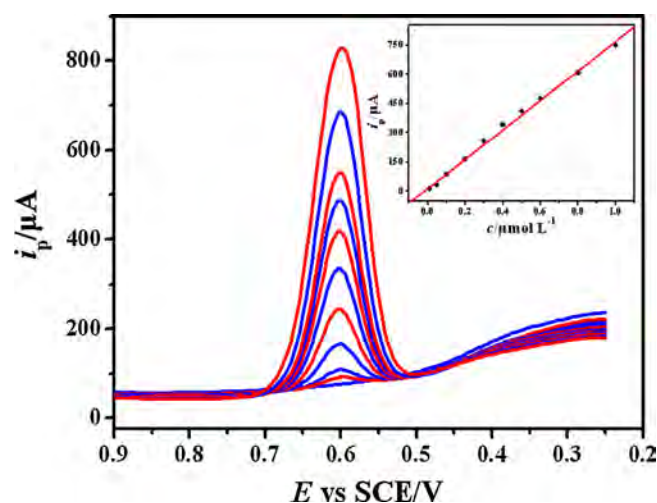


Fig. 7. Square wave anodic stripping voltammograms and their associated calibration plot (inset) for increasing concentrations of taxifolin at ERGO/GCE under optimum conditions; taxifolin concentration: (1) 0.0 mol L^{-1} , (2) $1.0 \times 10^{-8} \text{ mol L}^{-1}$, (3) $5.0 \times 10^{-8} \text{ mol L}^{-1}$, (4) $1.0 \times 10^{-7} \text{ mol L}^{-1}$, (5) $2.0 \times 10^{-7} \text{ mol L}^{-1}$, (6) $3.0 \times 10^{-7} \text{ mol L}^{-1}$, (7) $4.0 \times 10^{-7} \text{ mol L}^{-1}$, (8) $5.0 \times 10^{-7} \text{ mol L}^{-1}$, (9) $6.0 \times 10^{-7} \text{ mol L}^{-1}$, (10) $8.0 \times 10^{-7} \text{ mol L}^{-1}$, and (11) $1.0 \times 10^{-6} \text{ mol L}^{-1}$.

Table 1
Determination results of taxifolin in the *princes-feather fruit* sample by SWASV and UV–vis.

| SWASV ($n=3$) | | | | | UV–vis | |
|-------------------------------------|---------|------------------------------------|------------------------------------|--------------|-------------------------------------|---------|
| Amount found (mg g^{-1}) | RSD (%) | Stand added (mg g^{-1}) | Total found (mg g^{-1}) | Recovery (%) | Amount found (mg g^{-1}) | RSD (%) |
| 4.784 | 2.7 | 2.104 | 6.814 | 96.5 | 4.764 | 1.3 |

confirmed the sensitivity of the proposed method for determination of taxifolin.

The stability, reproducibility and repeatability of ERGO/GCE are also estimated. It is seen that the modified electrode remains 95.6% of its initial current response to taxifolin after 24 days' storage, indicating the good stability of the taxifolin sensor. The reproducibility was examined from the response to $1.0 \times 10^{-6} \text{ mol L}^{-1}$ taxifolin at five modified electrodes prepared in the same conditions and a relative standard deviation (RSD) of 5.7% was obtained. The RSD of the response to $1.0 \times 10^{-6} \text{ mol L}^{-1}$ taxifolin was 4.7% for 5 successive measurements, indicating the good repeatability of the taxifolin sensor. All these observations indicate that ERGO/GCE exhibit good stability, reproducibility and repeatability for detection of taxifolin.

3.6.6. Interference studies

For the possible analytical application of the proposed method, various possible interfering species were evaluated, with a fixed taxifolin concentration of $1.0 \times 10^{-6} \text{ mol L}^{-1}$. The tolerance limit for a foreign species was taken as the largest amount yielding a relative error $< \pm 5\%$ for the current response of taxifolin. The experiment results showed that no interference was aware for following organic compounds: glucose (50), citric acid (50), glutamic acid (50), oxalic acid (50), ascorbic acid (50), uric acid (5), quercetin (3), norepinephrine (5) and 5-hydroxytryptamine (10), where the data in brackets denote the molar ratio of interfering compound to taxifolin. At the same time, the inorganic species, such as Ca^{2+} , Zn^{2+} , Cu^{2+} , Mg^{2+} , NH_4^+ , Cl^- , PO_4^{3-} and Ac^- did not interfere. The results indicate the present method was adequate for the determination of taxifolin in real samples.

3.7. Determination of taxifolin in the real sample

In order to evaluate the validity of the proposed method, it was employed for determination of the content of taxifolin in the *princes-feather fruit*. The pretreatment of samples were presented in Section 2.4. Five parallel samples were analyzed with RSD of 2.7% (Table 1). After determining the content of taxifolin, some standard taxifolin was added in the five samples respectively and the total content of taxifolin were determined again to calculate the recovery (Table 1). For testing the accuracy of the proposed method, the same samples were analyzed using UV–vis method and the results were listed in Table 1 too. The contents obtained from the proposed method and UV–vis method were compared using *t*-test under 95% confidence levels. The results showed that no significant difference between them.

4. Conclusions

In conclusion, it is demonstrated that the ERGO film prepared by PPM was applied for the first time, in developing a high-sensitive electrochemical sensor for detection of taxifolin. The proposed modified electrode showed enhanced electron transfer properties and high resolution capacity to the taxifolin. Wide linear concentration ranges, low detection limits, and excellent selectivity, stability, reproducibility and repeatability were achieved on the modified electrode. We believe it is a simple, rapid, green and promising method for determination of taxifolin. And, this work firstly raises

an approach to fabricate graphene nanosheets with a certain orientation on the electrode surface.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.snb.2014.11.008>.

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