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ORIGINAL PAPER

Modified glassy carbon electrode with Nafion/MWNTs as a sensitive voltammetric sensor for the determination of paeonol in pharmaceutical and biological samples

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Abstract A simple, highly sensitive method was reported for directly voltammetric determination of paeonol in drug samples and human biological samples. Nafion/multi-wall carbon nanotubes' (MWNTs) composite film was coated on the *glassy* carbon electrode. The adsorptive voltammetric behavior of paeonol on the Nafion/MWNTs-modified electrode was investigated using cyclic voltammetry (CV) and differential pulse anodic stripping voltammetry (DPASV). The results indicated that the Nafion/MWNTs-modified electrode could remarkably enhance electrocatalytic activity toward the oxidation of paeonol, and showed an excellent resistance capability toward the electrode passivation. A highly sensitive voltammetric sensor was developed for the detection of paeonol in pharmaceutical and biological samples. Under the optimum conditions, the anodic peak current was proportional to paeonol concentration in the range of 6.0×10^{-7}–6.0×10^{-5} M with a detection limit of 4.0×10^{-7} M. Some kinetic parameters were determined, and multi-step mechanism for oxidation of paeonol was proposed.

Keywords Paeonol · Nafion · Multi-wall carbon nanotubes · Voltammetry

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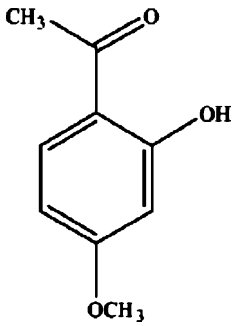
1 Introduction

Paeonol (2-hydroxyl-4-methoxyacetophone, PN) is a major phenolic component of Cortex Moutan. It is known to have anti-aggregatory, anti-oxidant, and anti-inflammatory activities [1]. The molecular structure of paeonol is shown in Scheme 1. Paeonol has been used in the treatment of arthritis and suppress ADP- or collagen-induced human blood platelet aggregation in a dose-dependent manner due to its analgesic, anti-pyretic, and anti-bacterial properties [2]. Many researchers have paid increasing attention to the pharmacokinetic activities of paeonol. Therefore, the technique of quantitative determination of paeonol is crucial to the evaluation and popularization of traditional Chinese medicines as well as drug products containing paeonol [3, 4].

At present, many methods such as gas chromatography–mass spectrometry (GC/MS), liquid chromatography–mass spectrometry (LC/MS), high performance liquid chromatography (HPLC), and micellar electrokinetic capillary chromatography which are all combined with sample pretreatment techniques, have been employed for the determination of paeonol in Cortex Moutan or other products [5–8]. Nevertheless, some of these methods, such as the chromatographic methods are time-consuming, expensive, and need complicated preconcentration or multisolvent extraction as well as trained technicians. Instead, electrochemical methods are characterized by simplicity, high sensitivity, good stability, low-cost instrumentation, and on-site monitoring [9], thus they have been developed for the determination of phenols. For example, Yi et al. prepared Nafion-modified glassy carbon electrode for the determination of phenol [10]. Liu et al. presented a phenol biosensor based on immobilizing tyrosinase to modified core–shell magnetic nanoparticles supported at a carbon paste electrode, with a detection limit of 6.0×10^{-7} M

Scheme 1 Molecular structure of paeonol



[11]. Fernandez et al. declared that phenol could be effectively adsorbed on the hydrotalcite-like clay/anionic surfactants/glassy carbon-modified electrode and its oxidation potential shifted to less positive values [12]. Liu et al. developed a renewable phenol biosensor based on a tyrosinase-colloidal gold modified carbon paste electrode, with a detection limit of 6.1×10^{-9} M [13]. Nevertheless,except Wang's report [14], there is seldom electrochemical literature about paeonol because it is easy to yield compact-insulated polymer arylether on the electrode surface, which can prevent the progression of the electrode reaction on the electrode surface.

Carbon nanotubes (CNTs) are novel nano-materials which have captured worldwide researchers' interests since the discovery in 1991 [15]. CNTs have the ability to hold the potential for wide applications in electrochemistry due to their small dimensions, high surface area, high electrical conductivity, unique structures, significant mechanical strength, and good chemical stability [16–19]. Nafion, a perfluorinated sulfonate polymer is a good cation-exchanger with strong adsorption ability, high surface area, thermal stability, chemical inertness, and mechanical strength. It is widely used to modify electrodes in electrochemical field [20–22].

In the current study, insoluble-treated multi-wall carbon nanotubes (MWNTs) were homogeneously dispersed into methanol with 0.1% Nafion through ultrasonic. After that, a Nafion/MWNTs film-coated glassy carbon electrode was fabricated through methanol-evaporation. The electrochemical behavior of paeonol was evaluated at the Nafion/MWNTs-modified electrode with great details. The Nafion/MWNTs film-modified electrode not only remarkably enhanced the oxidation peak current with negative shifting of the oxidation peak potential for paeonol (an electrocatalytic event), but also decreased the electrode contamination effectively. Based on the above advantages, a simple, renewable, and sensitive electrochemical sensor was developed for the determination of paeonol. The proposed method was applied for the determination of paeonol in real drug samples and biological samples with satisfactory results.

2 Experimental

2.1 Reagents and solution

Paeonol was purchased from National Institute for the Control of Pharmaceutical and Biological Products (China) and used as received. The stock solution of paeonol (2.0×10^{-3} M) was prepared with doubly distilled water, and diluted with 0.1 M phosphate medium (pH 7.0) before used. Cortex Moutan was purchased from a Chinese medicine store in Zhengzhou (Henan, China). Liuweidihuang Wan was supplied by Henan Wanxi Pharmaceutical Co., Ltd, China. The multi-wall carbon nanotubes (diameter: 10–20 nm, length: 1–2 μm, purity >95%) were obtained from Shenzhen Nanotech Port Co., Ltd, China. Nafion (wt 5%) was purchased from Sigma. Human plasma was supplied by Henan Provincial People's Hospital (Henan, China). The plasma samples were stored at −20 °C. All the other chemicals used were analytical grade without further purification and prepared with double-distilled water.

2.2 Apparatus

RST3000 electrochemical system (Suzhou Risetech Instrument Co., Ltd. Suzhou, China) was employed for all the voltammetric measurement. A conventional three-electrode system was used, including a bare *glassy* carbon electrode (GCE) ($d = 4$ mm) or Nafion/MWNTs film-modified GCE as working electrode, a saturated calomel electrode as reference electrode and a platinum wire electrode as auxiliary electrode. All the pH values were measured with a PHS-3C precision pH meter (Leici Devices Factory of Shanghai, China), which was calibrated with standard buffer solution every day. The scanning electron microscopy (SEM) was performed with a Hitachi X–650 microscope.

2.3 Modification of the Nafion/MWNTs/GCE

The bare GCE was pretreated carefully with 0.05 μm alumina slurry on a polishing cloth, rinsed thoroughly with 1:1 HNO_3–H_2O (v/v), and then washed with pure ethanol and redistilled water, respectively. 10 mg of the untreated MWNTs was added to plentiful concentrated nitric acid (wt 68%), and then sonicated for about 4 h. The mixture was filtrated vacuum and washed with doubly distilled water until the filtrate was litmusless. The treated MWNTs were dried under infrared lamp. Nafion/MWNTs' suspension was accomplished as follows: 5.0 mg of treated MWNTs was sonicated in 10.0 ml 0.1% (w/w) Nafion methanol solution for about 30 min, and then homogeneous suspension would be achieved. The pretreated GCE

was coated with 10.0 μL Nafion/MWNTs' suspension evenly, and allowed to evaporate methanol at room temperature. For contrast, the Nafion/GC electrode was also prepared in this study. The Nafion/MWNTs-modified electrode was stored in phosphate solution (pH 7.0) and can be used for 50 cyclic voltammetric cycles. The microscopic areas of the Nafion/MWNTs/GC electrode and the bare GC electrode were obtained by cyclic voltammetry employing 1.0 mM $K_3Fe(CN)_6$ as a redox probe in the 0.1 M KCl electrolyte at different scan rates [23, 24]. For a reversible process, the anodic peak current i_{pa} is linear to $v^{1/2}$ as follows:

$$i_{pa} = (2.69 \times 10^5) n^{3/2} A C_0 D_R^{1/2} v^{1/2}$$

where i_{pa} refers to the anodic peak current, A the surface area of the electrode, v the scan rate and C_0 the concentration of $K_3Fe(CN)_6$. For 1.0 mM $K_3Fe(CN)_6$, the electron transfer $n = 1$, the diffusion coefficient $D_R = 7.60 \times 10^{-6}$ $cm^2\ s^{-1}$. Thus, from the slope of the i_{pa} versus $v^{1/2}$ relation, the microscopic areas of the Nafion/MWNTs-modified GCE were calculated to be 0.8668 cm^2, which were about seven times greater than the bare GCE (0.1256 cm^2). All studies were performed at room temperature.

2.4 Analytical procedures

Except as otherwise stated, 0.1 M NaH_2PO_4–Na_2HPO_4 buffer solution (pH 7.0) was used as the supporting electrolyte for paeonol determination. A stock solution of 2.0×10^{-3} M paeonol was first prepared, and then an aliquot was diluted to the appropriate concentration with 0.1 M phosphate buffer solution (pH 7.0) before commencing the voltammetric scan. Voltammograms were obtained by scanning the potential from 100 to 1,300 mV (vs. SCE). Before each measurement, the three-electrode system was installed in a blank solution, and the cyclic voltammetry scan from 100 to 1,300 mV (vs. SCE) was repeated successively for three times for the modified electrode. The quantitative determination of paeonol was achieved by measuring the oxidation peak current after background subtraction using differential pulse anodic stripping voltammetry (DPASV). The appropriate amount of Liuweidihuang Wan or Cortex Moutan was ground into powder in agate mortar, respectively. 1 g Liuweidihuang Wan or 0.1 g Cortex Moutan of the triturated powder was accurately weighed and dissolved in 25 mL 0.1 M phosphate buffer solution (pH 7.0), respectively. The mixtures were sonicated for an hour, and then filtrated. In order to fit into the linear range of the proposed method, some of the filtrate of Liuweidihuang Wan was diluted by a factor of 1/100 (v/v), Cortex Moutan by a factor of 1/10 (v/v) with phosphate buffer solution for the determination. Plasma and urine samples were diluted by a factor of 1/100 (v/v) with phosphate buffer solution. The dilution process can actually help reduce the matrix effect in real samples.

3 Results and discussion

3.1 Characterization of the Nafion/MWNTs/GCE

Figure 1 displayed the characterization of the Nafion/MWNTs' composite film on the GCE by using SEM method. It was obvious that the Nafion/MWNTs' composite film was uniformly coated on the electrode surface and formed a spaghetti-like porous reticular formation. The special surface morphology offered a much larger real surface area than the apparent geometric area.

3.2 Electrochemical response of paeonol on the Nafion/MWNTs/GCE

As shown in Fig. 2, paeonol exhibited an anodic peak on the Nafion/MWNTs/GCE (Fig. 2a), Nafion/GCE (Fig. 2c), and a bare GCE (Fig. 2b) in 0.1 M phosphate buffer solution (pH 7.0). According to Wang's report [14], paeonol was easy to yield compact-insulated polymer arylether on the bare electrode surface, which could prevent the progression of the electrode reaction on the electrode surface. Nafion, a perfluorinated sulfonate polymer is a good cation-exchanger provided with adsorption ability and high surface area. It maybe effectively prevents the macro-polymer arylether adsorbing on the modified electrode surface and avoid anode fouling by forming a dense film with a low permeability. Thus, when Nafion was present, the anodic peak current (i_{pa})

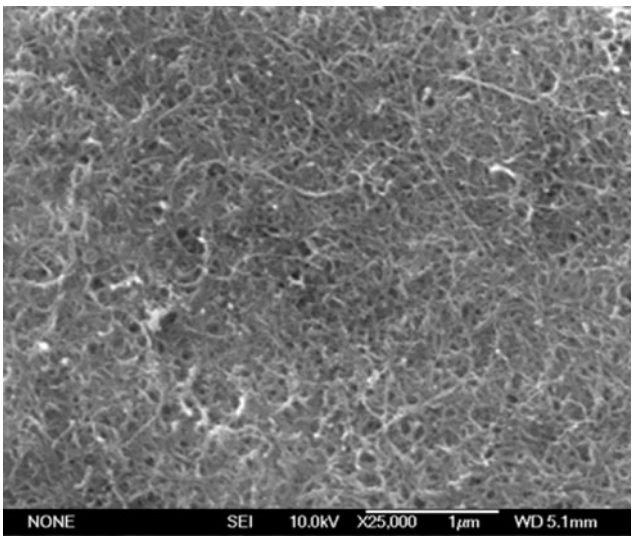


Fig. 1 SEM image of Nafion/MWNTs' composite film on the glassy carbon electrode

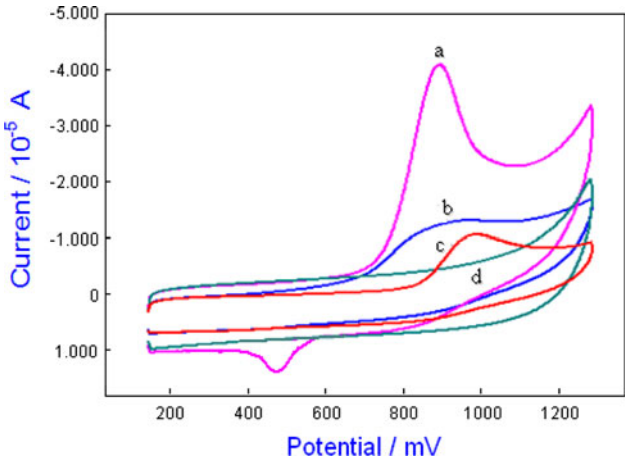


Fig. 2 Cyclic voltammograms on the Nafion/MWNTs-modified GCE (**a**), the bare GCE (**b**), Nafion-modified GCE (**c**) containing 8.0×10^{-5} M paeonol and Nafion/MWNTs modified GCE (**d**) without paeonol in 0.1 M NaH_2PO_4–Na_2HPO_4 (pH 7.0) medium; scan rate 100 mV s^{-1}

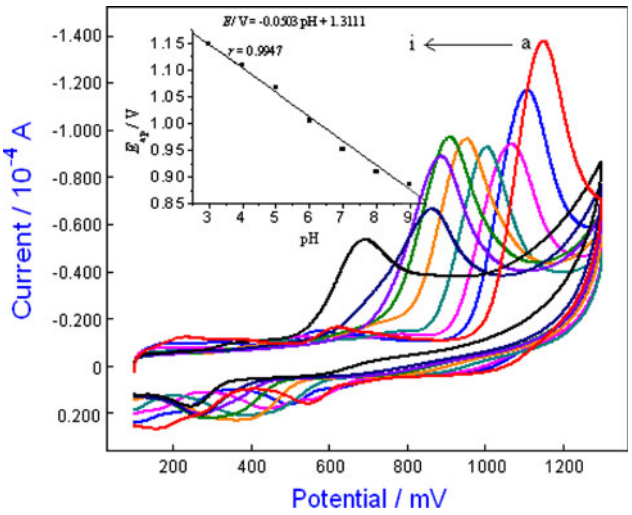


Fig. 3 Cyclic voltammograms of the Nafion/MWNTs/GCE in the presence of 4.0×10^{-4} mol L^{-1} paeonol with different pH of 0.1 M phosphate buffer ($a \rightarrow i$): 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0; scan rate 100 mV s^{-1}

was effectively separated from the background current (curve c). When Nafion and MWNTs were combined together, the peak potential moved in the negative direction slightly at about 836 mV (vs. SCE) with the obvious enhancement of the peak current (curve a). This phenomenon maybe related to the influence of MWNTs on the electron transfer rate, the effective electrode area, as well as the accumulation amount of paeonol. The resulting composite materials-coated electrode exhibited more sensitive response to paeonol, meaning that composite materials could cooperate with each other to enhance the voltammetric response of paeonol. In addition, it was observed that paeonol also produced a slight cathodic peak at about 440 mV (vs. SCE) on the Nafion/MWNTs/GCE, which indicated that Nafion/MWNTs/GCE was much more sensitive than Nafion/GCE in improving paeonol response under the same conditions.

3.3 Influence of pH

The voltammetric behavior of paeonol was examined in 0.1 M phosphate buffer with different pH values (see Fig. 3). It was found that the peak currents of paeonol decreased from pH 3.0 to pH 5.0 first, and then increased from pH 5.0 to pH 7.0. Finally, the peak currents of paeonol decreased from pH 8.0 to pH 11.0. Meanwhile, the anodic peak potential and cathodic peak shifted toward negative potential with the increasing pH values. The phenomenon indicated that the electrochemical reaction of paeonol was easier with the increasing pH values and combined with the proton-coupled electron transfer, which was in agreement with Wang's report [14]. pH 7.0 phosphate buffer was taken as the supporting electrolyte, which was close to the biological environment.

The value of proton in the electrode reaction was estimated by the following equation [25]:

$$E_p = E^{o\prime} - 0.059\, p/n \text{ pH}$$

p is the value of proton in the electrode reaction, and n, the number of electron involved in the reaction. Based on the above equation, the plot of E_{pa} versus pH was linear with a slope of 0.0503 (see Fig. 3 inset), which suggested that the number of electron transfer was equal with that of hydrogen ions taking part in the electrode reaction.

3.4 Effect of the amount of Nafion/MWNTs

The volume of Nafion/MWNTs' suspension coated on the electrode can change the properties and functions of the electrode surface. Figure 4 illustrated the relationship between anodic peak current of paeonol and the amount of Nafion/MWNTs' suspension coated on GCE. It was found that the oxidation peak current gradually increased with the improvement of the volume of Nafion/MWNTs' suspension from 4.0 to 10.0 μL. When the amount exceeded 10.0 μL, the peak current conversely decreased. In the beginning, on increasing the amount of modifier, the sites of electron exchange increased, and the adsorption of paeonol on the Nafion/MWNTs-modified electrode was enhanced. Hence, the peak current increased. However, when the amount of Nafion/MWNTs' composite solution exceeded a certain volume (10.0 μL), the film became thicker and retarded the electron transfer and mass transportation between paeonol and electrode, which resulted in the higher electric

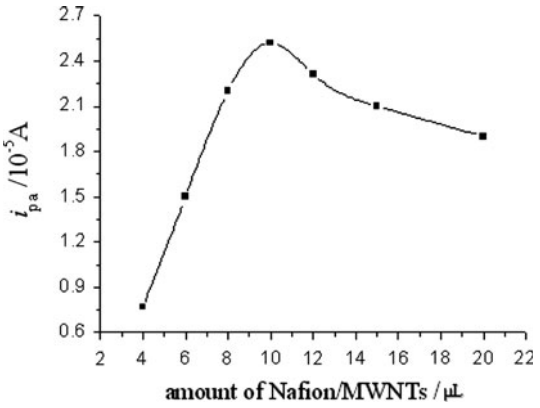


Fig. 4 Effect of the amount of Nafion/MWNTs suspension on the oxidation peak current of 6.0×10^{-5} M paeonol. Other conditions as in Fig. 2

resistance. All those factors could cause a decrease of the anodic peak current. For above-mentioned reasons, the volume of Nafion/MWNTs' suspension on the GCE surface was employed as 10.0 μL in the present investigation.

3.5 Effect of the scan rate on the peak current and peak potential

The effect of the scan rate (v) on the peak current and peak potential at the modified GCE was investigated. As shown in Fig. 5, the peak current (i_{pa}) versus v gave a straight line until 400 mV s^{-1}. The linear regression equation was:

$$i_{pa} = 0.0245v + 3.5539 \quad (i_{pa} \text{ in } 10^{-5}\text{A},\ v \text{ in mV s}^{-1},\ r = 0.9979).$$

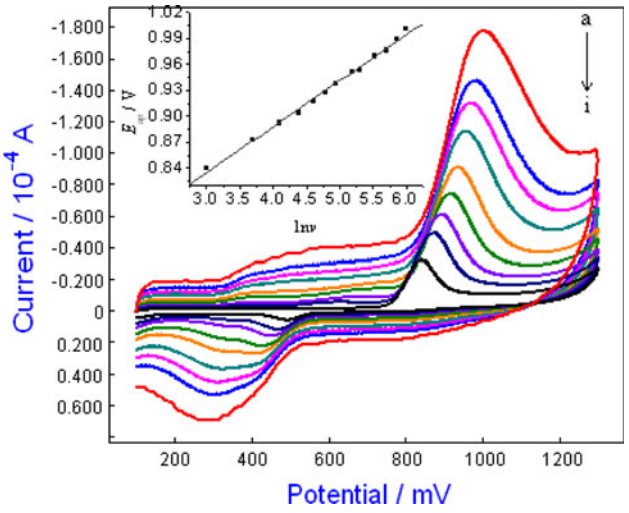


Fig. 5 Dependence of peak current of the Nafion/MWNTs/GCE on different scan rates in the presence of 2.0×10^{-4} M paeonol ($a \rightarrow i$): 400, 300, 250, 200, 140, 100, 60, 40, 20 mV s^{-1}; inset is the E_{pa} versus ln v plot; other conditions as in Fig. 2

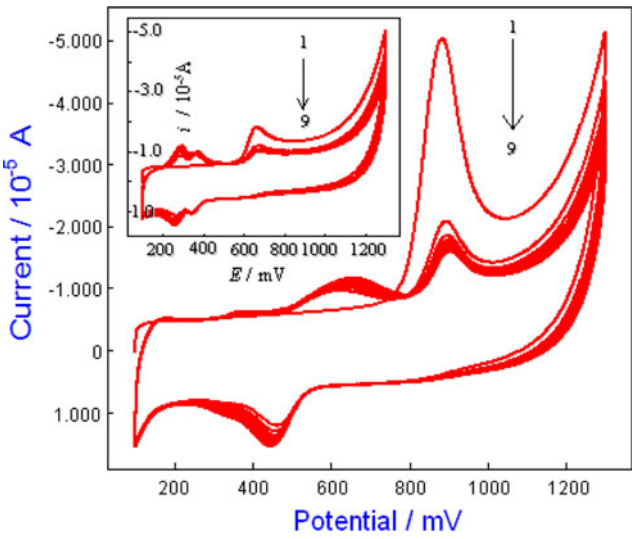


Fig. 6 Nine times of repetitious cyclic voltammograms of Nafion/MWNTs/GCE in the presence of 8.0×10^{-5} M paeonol; inset: cyclic voltammograms of 8.0×10^{-5} M phenol; other conditions as in Fig. 2

This indicated an adsorption-driven oxidation process occurring at the modified GCE.

In order to clarify the electrode reaction nature of paeonol, the repetitious cyclic voltammograms of paeonol were recorded. Figure 6 demonstrated that the anodic peak current in the second cyclic scan decreased remarkably compared with that of the first cyclic scan. After the second cyclic scan, the peak current decreased slightly. This phenomenon may be another evidence for the adsorption-driven oxidation process of paeonol occurring at the modified GCE. Wang et al. reported that after the second consecutive cyclic voltammetric scan of paeonol, no electrochemical signal was recorded on Pt electrode because of the rapid electrode passivation [14]. Figure 6 illustrated that after the ninth cyclic voltammetric scan of paeonol, higher peak current could be observed, which declared that the Nafion/MWNTs/GCE had the excellent resistance capability toward electrode passivation of the electrochemical reaction of paeonol. However, after the first potential scan, another smaller anodic peak appeared at about 640 mV.

The anodic peak potential shifted in the positive direction with the increasing scan rate. The variation of peak potential with scan rate from 20 to 400 mV s^{-1} followed the regression equation:

$$E_{pa} = 0.6760 + 0.0530 \ln v \quad (E_{pa} \text{ in V},\ v \text{ in mV s}^{-1},\ r = 0.9972).$$

Based on the theory of Laviron [26], for an irreversible anodic reaction, the linear relationship of $E_p - v$ should obey the following equation:

$$E_p = E^o - \frac{RT}{\alpha nF}\ln\frac{RTKs}{\alpha nF} + \frac{RT}{\alpha nF}\ln v$$

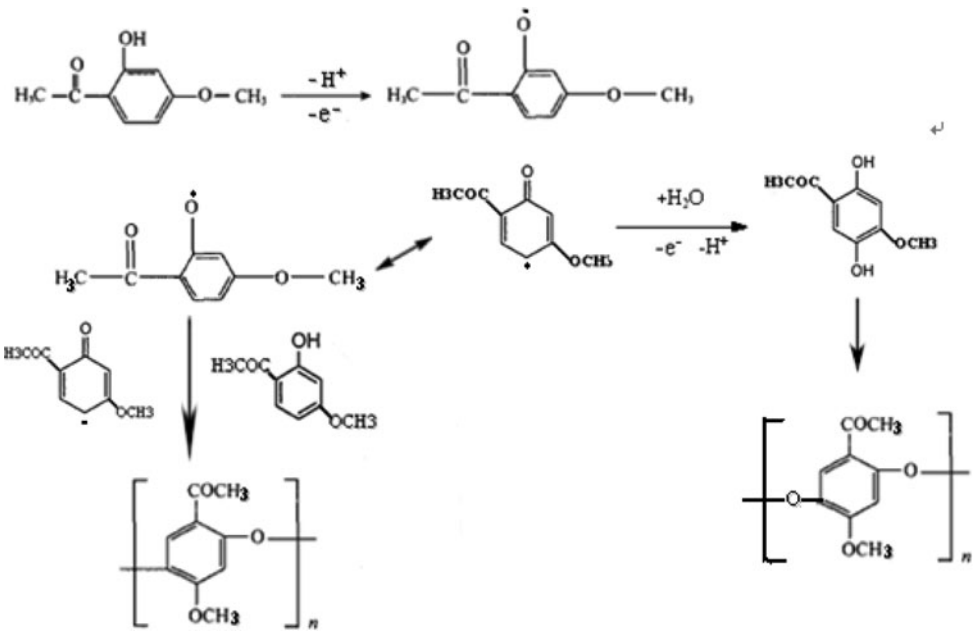
where E^0 is formal standard potential; α is the charge transfer coefficient; n is the number of the electron transferred; F is the Faraday constant (96,500 C mol^{-1}) and k_s is the standard heterogeneous reaction rate constant. R and T have their usual meaning. From the slope of E_{pa} versus ln v, $\alpha n = 0.46$ could be obtained and the electron participated in the electrode reaction process could be calculated to be 1, when assuming a was 0.5. Assuming $n_a = n$, the value of α was 0.46. The value of E^o of 0.870 V was got from the intercept of linear relationship E_p against v on the ordinate by extrapolating the line to $v = 0$, and from the intercept of the straight line of E_{pa} versus ln$v$, $k_s = 733\ s^{-1}$ was calculated. The results calculated were in agreement with the literature reported [14]. However, the scan rate of 100 mV s^{-1} which had higher current signal, lower peak potential and smaller background current was selected as the optimum value for the study of paeonol.

The overall oxidation reaction mechanism with multi-electron–proton transfer step and complicated polymerization could be speculated based on the report [27]. Paeonol anion gives rise to phenoxy radical during the first step of oxidation. On the one hand, the phenoxy radical can be oxidized very quickly again to produce a new product which can couple to produce the polymer. On the other hand, a phenoxy radical can react irreversibly with other radicals or with unreacted paeonol anions through C–C or C–O coupling to form the different polymer. Summing up all the above experimental results, a possible oxidation mechanism of paeonol could be expressed in Scheme 2. According to the literature [27], the cathodic peak response can be attributed to the reduction of quinone compounds, i.e., of a side product in the proposed mechanism leading to the polymer, and the smaller anodic peak appeared, maybe, is ascribed to the oxidation of the resultant products of the reductive quinone compounds. A comparison with cyclic voltammograms of an authentic phenol sample was recorded under the same conditions. It was found that the voltammetric curves of phenol were very similar to that of paeonol (see Fig. 6 inset).

3.6 Effect of various differential pulse anodic stripping voltammetric parameters

Differential pulse anodic stripping voltammetry (DPASV) was used due to its high sensitivity and excellent separation from background current. Since the anodic peak current was dependent on the various DPASV parameters, such as accumulation time, accumulation potential, pulse amplitude, pulse increment, pulse width and pulse period, and the preliminary experiments were employed to choose the best parameters. The results indicated that the anodic peak current was increased with the increasing of accumulation time from 0 to 20 s or accumulation potential in the range of 500–700 mV. When the accumulation time was more than 20 s, the anodic peak current was almost constant. As the accumulation potential was over 700 mV, the anodic peak current decreased. Therefore, 20 s was chosen as the accumulation time and 700 mV was selected as the optimum accumulation potential. The pulse increment was tested in the range of 1–8 mV. Experiments revealed that the anodic peak height increased with the increasing of pulse increment. When the pulse increment was greater

Scheme 2 Oxidation mechanism of paeonol with electron–proton transfer step



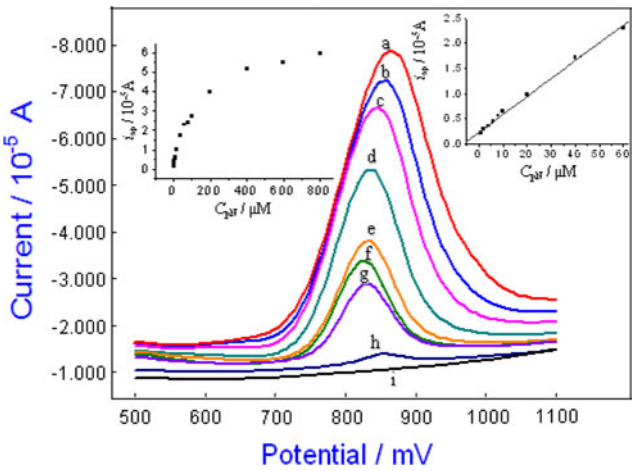


Fig. 7 Differential pulse anodic stripping voltammograms of Nafion/MWNTs/GCE in different concentrations of paeonol in 0.1 M phosphate buffer solution ($a \rightarrow i$): 800, 600, 400, 200, 100, 60, 40, 4.0, 0 μM; insets are the i_{pa} versus C_{PN} plot

than 6 mV, the anodic peak current did not change. Thus, 6 mV was selected as the optimal pulse increment. Any increase of the pulse amplitude, pulse width, or the pulse period resulted in the increasing of the anodic peak current. The response of the anodic peak current was increasing until the pulse amplitude, the pulse width, or the pulse period were up to 50 mV, 40 ms, and 150 ms, respectively. It was also discovered that the changing of these parameters resulted in slight effect on the anodic peak potential.

3.7 Calibration curve

In order to test the feasibility of the exploited method for the quantitative analysis of paeonol, the relationship between the anodic peak current and the concentration of paeonol was studied using DPASV. Under the optimum instrumental conditions (accumulation time: 20 s, accumulation potential: 700 mV, pulse amplitude: 50 mV, pulse increment: 6 mV, pulse width: 40 ms and pulse period: 100 ms), when the concentration of paeonol changed from 6.0×10^{-7} to 8.0×10^{-4} M, the anodic peak current and paeonol concentration declared linear relationship in the range of 6.0×10^{-7}–6.0×10^{-5} M (Fig. 7 inset). The regression equation was: $i_{pa} = 0.22598 + 0.0356\ C_{PN}$ (i_{pa} in 10^{-5}A, C_{PN} in μM, $r = 0.9977$). Based on the signal to noise ratio of 3, the detection limit of 4.0×10^{-7} M was obtained.

Regeneration and reproducibility are two important characteristics for the modified electrode, which should be investigated. The same modified GCE was used for seven times successive measurements of 8.0×10^{-5} M paeonol. After each measurement, the surface of the Nafion/MWNTs/GCE was regenerated by successively cycling between 100 and 1,300 mV in 0.1 M phosphate buffer solution for three cycles. The electrode exhibited good regeneration and reproducibility, and the relative standard deviation (RSD) of the anodic peak current was 2.3% for seven times successive measurements.

3.8 Interference studies

In order to evaluate the selectivity of the Nafion/MWNTs/GCE, the influence of some coexistent interference substances was examined in phosphate buffer solution (pH 7.0) containing 6.0×10^{-5} M paeonol. The results showed that when the concentration of K^+, Na^+, Cl^-, SO_4^{2-}, PO_4^{3-}, and acetate were 500 times more than that of paeonol, ascorbic acid, and carbamide 50 times, as well as amino acid 20 times, no observable interference were observed in the determination of paeonol according to the relative error $<\pm 10\%$.

3.9 Analysis of real samples

In order to fit into the linear range of the method, Liuweidihuang Wan and Cortex Moutan employed for

Table 1 Determination of the content of paeonol in pharmaceutical and biological samples on the Nafion/MWNTs/GCE

Sample	Original (μM)	Spike (μM)	Found (μM)	Average recovery (%)	RSD of recovery (%)
LiuweidihuangWan[a]	7.71 ± 0.19	40	47.52 ± 0.52	98.7	2.3
		8	15.83 ± 0.24	101.5	2.0
		2	9.63 ± 0.17	96.0	1.5
Cortex Moutan[b]	48.26 ± 0.24	40	88.10 ± 0.21	99.6	2.0
		8	57.92 ± 0.23	108.2	3.2
		2	50.30 ± 0.14	102.0	2.3
Urine[a]	–	80	79.80 ± 0.31	99.8	2.6
		20	20.25 ± 0.10	101.3	1.7
		2	1.99 ± 0.22	99.5	1.9
Plasma[a]	–	80	81.20 ± 0.42	101.5	3.0
		20	21.00 ± 0.19	105.0	3.7
		2	1.96 ± 0.20	98.0	2.2

Number of samples assayed: 5

[a] Dilution factor: 1/100

[b] Dilution factor: 1/10

operation were accurately diluted with the supporting electrolyte. Liuweidihuang Wan was diluted by a factor of 1/100 (v/v), Cortex Moutan by a factor of 1/10 (v/v) for determination. Plasma and urine samples were diluted by a factor of 1/100 (v/v) with phosphate buffer solution. The dilution process can actually help reduce the matrix effect of real samples. The Nafion/MWNT/GCE was applied to the determination of paeonol in Liuweidihuang Wan and Cortex Moutan and the results were listed in Table 1. Total value of paeonol in Liuweidihuang was 3.20 ± 0.75 mg/g, which was close to the revealed value of 3.22 mg/g [28]. Total value of paeonol in cortex moutan was 20.05 ± 0.50 mg/g, which was consistent with the reported value of 20.61 ± 0.986 mg/g [29]. The accuracy of the method was evaluated by its recovery during spiked experiments. Confirming those quantitative and reproducible results of this method, the direct determination of paeonol in spiked Liuweidihuang Wan, Cortex Moutan, urine, and plasma samples were carried out, and the results were also displayed in Table 1. The recoveries of paeonol from the drug matrices and biological samples, such as urine and plasma demonstrated that this proposed method could be applied to the detection of paeonol in pharmaceutical and biological samples with excellent sensitivity and selectivity.

4 Conclusions

The electrochemical response of paeonol was studied in phosphate buffer solution (pH 7.0) on the Nafion/MWNTs' composite film-modified glassy carbon electrode. The Nafion/MWNTs/GCE not only remarkably enhanced the oxidation peak current of paeonol, but also decreased the oxidation potential. Moreover, the Nafion/MWNTs/GCE had an excellent resistance capability toward the electrode passivation of the electrochemical reaction of paeonol. The kinetic parameters and electrochemical oxidation mechanism of paeonol were evaluated. The peak current of DPASV was linear to the concentration of paeonol in the range of 6.0×10^{-7}–6.0×10^{-5} M with a lower detection limit of 4.0×10^{-7} M. The proposed method was successfully applied to the quantification of paeonol in drug and biological samples.

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