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Study on the electrochemical behavior and differential pulse voltammetric determination of rhein using a nanoparticle composite film-modified electrode

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Abstract

A sensitive electrochemical method was developed for the differential pulse voltammetric determination of rhein at a glassy carbon electrode (GCE) modified with a nanoparticle composite film. In the present paper, multi-wall carbon nanotube (MWNT) was dispersed into dihexadecyl phosphate (DHP) to give a homogeneous suspension. After the solvent evaporation, a uniform film of MWNT-DHP composite film was obtained on the GCE surface. The MWNT-DHP composite film-modified GCE exhibited excellent electrocatalytic behavior toward the redox of rhein. Compared with an irreversible reduction of rhein at the bare GCE, a reversible redox behavior of rhein was observed at the MWNT-DHP composite film-modified GCE and the redox current was also enhanced greatly. Based on this, a cathodic differential pulse voltammetry (DPV) was applied for the determination of rhein. The experimental parameters, which influence the current of rhein, were optimized. Under optimal conditions, the cathodic DPV measurements were performed and a linear response of rhein was obtained in the range from 1.0×10^{-8} to 5.0×10^{-6} mol L^{-1} and with a limit of detect (LOD) of 5.0×10^{-9} mol L^{-1}. The proposed procedure was successfully applied to assay rhein in real samples with satisfactory results.

Keywords: Rhein; Electrocatalysis; Differential pulse voltammetry; Nanoparticle composite film-modified electrode

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1. Introduction

Rhein is an active ingredient that extensively exists in plants such as Aloe, Cassitora L, Rhubarb and so on, which has been widely used in traditional medicine for cathartic, febrifugal and antidotal purposes. Pharmacological tests revealed that rhein not only has a strong antibacterial action on *Bacteroids fragilis*, but also may be useful in cancer chemotherapy as a biochemical modulator [1]. It also has been reported that rhein can retard the progression of type-2 diabetic nephropathy [2]. Its therapeutic action and toxicity is still the subject of considerable research and it is necessary to control the dose in biological fluid. So that is very important to establish an efficient method for the determination of rhein. To date, several methods including capillary electrophoresis with two-marker technique [3] or with electrochemical detection [4], high performance liquid chromatography [5], reversed phase high performance liquid chromatography [6] and synchronous fluorescence spectroscopy [7] have been reported for the determination of rhein. However, seldom electrochemical techniques, which are often simple and less expensive, have been used to determine rhein. Here, a direct electrochemical method based on multi-wall carbon nanotube (MWNT)-dihexadecyl phosphate (DHP) composite film-modified glassy carbon electrode (GCE) was developed for the determination of rhein.

There has been much interest in the research of carbon nanotubes since their discovery [8]. Carbon nanotubes are molecular-scale wires with high electrical conductivity, high chemical stability, and extremely high mechanical strength and modulus [9]. Utilization of these properties has lead to application of carbon nanotubes as scanning probes [10],

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electron field emission sources [11], actuators [12], nanoelectronic devices [13], batteries [14], nanotubes-reinforced materials [15] and potential hydrogen storage material [16]. The subtle electronic behaviors of carbon nanotubes reveal that they have the ability to promote electron-transfer reactions when used as an electrode material in electrochemical reactions. However, since carbon nanotubes have a large dimension and hydrophobic surface, the aqueous suspension of intact carbon nanotubes was usually unstable, which limited the applications of carbon nanotubes in electroanalytical chemistry. Our previous work has been developed carbon nanotube-modified GCEs based on the stable dispersion of acid-treated MWNT in the aqueous suspension of DHP and the formation of a stable film of the resulting suspension on the surface of GCE [17–19]. These electrodes have been applied to the sensitive and catalytic detection of some biomolecules [17,18] and heavy metal ions [19]. Compared with other MWNT-modified electrodes, MWNT-DHP composite film-modified GCE has the advantages of easy fabrication, controllable dispersion and high stability as well as good reproducibility.

In the present work, described here is to investigate the electrochemical responses of rhein at the MWNT-DHP composite film-modified GCE and to develop a convenient and sensitive method for the determination of rhein, based on the unusual properties of carbon nanotube such as strong adsorptive ability, huge specific area, subtle electronic properties as well as excellent electrocatalytic activity. In this report, the MWNT-DHP composite film-modified GCE exhibited strong electrocatalytic activity toward the redox reaction of rhein and a great increase in the magnitude of the redox peak currents. The feature of the electrocatalysis strongly revealed that the electrochemical behaviors of rhein were facilitated and then the determination sensitivity of rhein was significantly improved. Consequently, a differential pulse voltammetric method based on the MWNT-DHP composite film-modified electrode was firstly developed for the determination of rhein. This newly proposed method possesses following advantages such as high sensitivity, rapid response, low cost and simplicity.

2. Experimental

2.1. Reagents and apparatus

Rhein purchased from Chinese National Institute for the Control of Pharmaceutical and Biological Products was used without further purification. Stock solution of rhein (1.0×10^{-4} mol L^{-1}) was prepared by dissolving it into ethanol and lower concentrations of rhein were obtained by simply dilution with water. Dihexadecyl phosphate (DHP) was purchased from Fluka Chemical Reagent Corporation. The 0.1 mol L^{-1} NaAc–HAc buffer (pH 4.5) was using the supporting electrolyte. All other chemicals were of analytical grade and all the solutions were prepared from doubly distilled water. The multi-wall carbon nanotube (obtained from the Chengdu Organic Chemicals Co., Ltd., CAS, China) was synthesized by a catalytic pyrolysis method. Before use, MWNT were purified by refluxing in 2.6 mol L^{-1} HNO_3 for 48 h and treating with 2.0 mol L^{-1} HCl for 12 h before use [20].

All electrochemical measurements were performed with a computer-controlled model 830B electrochemical analyzer (Shanghai Chenhua Co., China). A conventional three-electrode system was employed with a platinum wire as counter electrode; a saturated calomel electrode (SCE) as reference electrode and a MWNT-DHP composite film-modified GCE as working electrode. All potentials were quoted with respect to SCE.

High performance liquid chromatography (HPLC) analysis was carried out with a HPLC system (Shimadzu, Kyoto, Japan) consisted of LC-10AT pump, n Shim-pack ODS column (150 mm × 4.6 mm) and a SPD-IOA UV spectrophotometric detector. The detection wavelength is 258 nm. Mobile phase consisted of 0.2% phosphoric acid solution–acetonitrile–methanol (54:5:41, v/v). The flow rate is 1 mL/min and separation was carried out at 40 °C.

2.2. Preparation of the MWNT-DHP composite film-modified electrode

Glassy carbon electrode of 3 mm in diameter was used. The electrode was sanded in ultrafine sand paper, polished with 0.3 μm and 0.05 μm alumia slurry (CH instrument, Inc., USA) in sequence and sonicated successively in 1:1 HNO_3–H_2O (v/v) and doubly distilled water between each polishing step.

2.5 mg purified MWNTs were added into 5 mL 0.5 mg mL^{-1} DHP suspension. A homogeneous and stable suspension of 0.5 mg mL^{-1} MWNT-DHP was achieved with the aid of ultrasonication agitation for about 30 min. The GCE was coated by casting 5 μL suspension of MWNT-DHP and dried in the air. The freshly prepared MWNT-DHP composite film-modified GCEs were activated in blank supporting electrolyte by using successive cyclic scans from −0.8 V to 0.4 V until the stable voltammograms were obtained.

2.3. Procedure

Voltammetric measurements were performed at room temperature (~ 25 °C). The accumulation step was performed at 0.10 V for 120 s in a stirring solution, and the differential pulse stripping voltammograms were recorded from −0.20 to −0.80 V after 10 s quiescence. The peak currents were measured at −0.50 V for the quantification of rhein. After each measurement, in order to remove the previous deposits completely, the modified electrode surface was refreshed by 15 successive cyclic voltammetric sweeps from −0.80 to 0.20 V at 50 mV/s in a 0.1 mol L^{-1} NaOH solution.

2.4. Sample preparation

The sample preparation was performed as Ref. [7]. It was briefly described as follows: the samples of Huangdan Yinchen Keli were respectively obtained from Lanzhou Foci Pharmaceutical Company Ltd., Lanzhou, China. 2.5 g of the sample powder was leached with 20 mL ethanol for 24 h. Then the mixtures were filtered through a paper filter and the residues

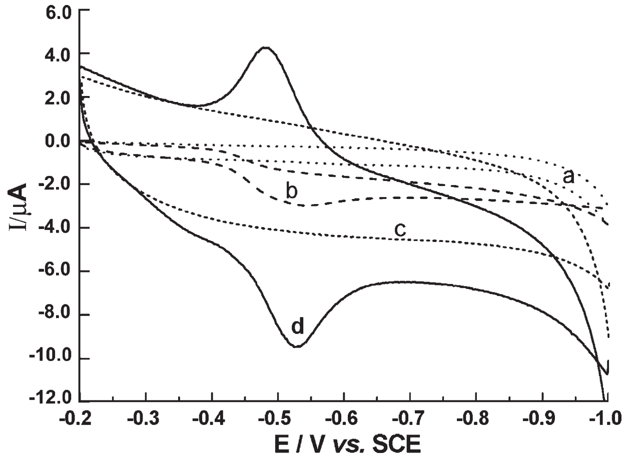

Fig. 1. Cyclic voltammograms. (a) Bare GCE in blank buffer; (b) (a) $+5\times10^{-6}$ mol/L rhein. (c) MWNT-DHP composite film modified GCE in blank buffer; (d) (c)$+5\times10^{-6}$ mol/L rhein. Scan rate: 50 mV s^{-1}.

were washed twice with 10 mL ethanol. The extracts and washings were combined and then diluted to 50 mL in a volumetric flask with ethanol. The extracts were further diluted with ethanol prior to their analysis.

3. Result and discussion

3.1. Electrochemical behavior of rhein at the MWNT-DHP composite film-modified electrode

The electrochemical behaviors of different electrodes in the absence or presence of 5×10^{-6} mol L^{-1} rhein are investigated by cyclic voltammetry (CV) (Fig. 1). When in the absence of rhein, no redox peaks was observed at both bare and modified GCE electrodes during the CV scan within the potential window from −0.20 to −1.00 V (Fig. 1a,c). Compared with the bare GCE (Fig. 1a), a large background current was observed at the MWNT-DHP composite film-modified GCE (Fig. 1c) during the CV scans, which is probable due to high double layer capacity currents [21]. Fig. 1b demonstrates the cyclic voltammogram of a bare GCE in the presence of rhein and the results shows that only a small and broad reduction peak occurs at about −0.54 V and no corresponding oxidation peak of rhein was observed in the reverse scan, which indicates that the electrochemical behavior of rhein at the bare GCE is an irreversible and sluggish process. While at a MWNT-DHP composite film-modified GCE, a pair of redox peaks of rhein with the cathodic peak potential of −0.52 V and the anodic peak potential of −0.48 V was observed (Fig. 2d). The peak currents of anodic and cathodic give similar value and the peak separation is about 40 mV, which clearly indicates that the reversibility of such a redox process was obviously improved at the MWNT-DHP composite film-modified GCE. The obviously increased peak currents and the improvement of the reversibility clearly demonstrate an efficient catalytic reaction between the MWNT-DHP composite film-modified GCE and rhein. This electrocatalytic properties of MWNT-DHP composite film-modified GCE may due to that MWNT can improve the reversibility of the electron transfer processes, hence greatly increases the rate of electron transfer from rhein to the electrode [22]. Additionally, the high aspect ratios of the nanotubes may present a steric effect for more efficient redox reaction of rhein [21].

Fig. 2 demonstrates the effect of scan rates on the electrochemical response of rhein by using cyclic voltammetry. In the range of 0.01–0.3 V s^{-1}, both the anodic and cathodic peak currents had good linear relationships with scan rates, indicating that the electrochemical reaction of rhein at the MWNT-DHP composite film-modified GCE is an adsorption-controlled process.

In order to better understand the electrochemical properties of MWNT-DHP compote film-modified GCE, the electrochemical active surface areas of bare and modified electrodes were measured by chronoamperometry by using 0.1 mmol L^{-1} potassium ferrocyanide as the redox probe. In chronoamperometric studies, the current, i, for the electrochemical reaction (at a mass-transport-limited rate) of ferrocyanide that diffuses to an electrode surface is described by the Cottrell equation,

$$i = nFAD^{1/2}C^{*}/\pi^{1/2}t^{1/2} \tag{1}$$

where A is the electrochemical active area, D is the diffusion coefficient, C^{*} is the bulk concentration of ferrocyanide and the other parameters have their usual meanings. Under diffusion control, a plot of i vs. $t^{1/2}$ will be linear, and from the slope, the value of A can be obtained, for the precise value of diffusion coefficient of ferrocyanide is well known (6.20×10^{-6} cm^2 s^{-1}). The experiment results showed that the electrochemical active area of bare GCE and MWNT-DHP-modified GCE were 0.11 cm^2 and 0.71 cm^2, respectively. Hence, the enhanced responses of rhein at MWNT-DHP composite film-modified GCE may be arise from the large active area of MWNT-DHP composite film for the adsorption of rhein.

The relationship between i_p and v indicates that the electrode process of rhein is adsorption-controlled. To infer more about the adsorption of rhein on three different electrodes, chronocou-

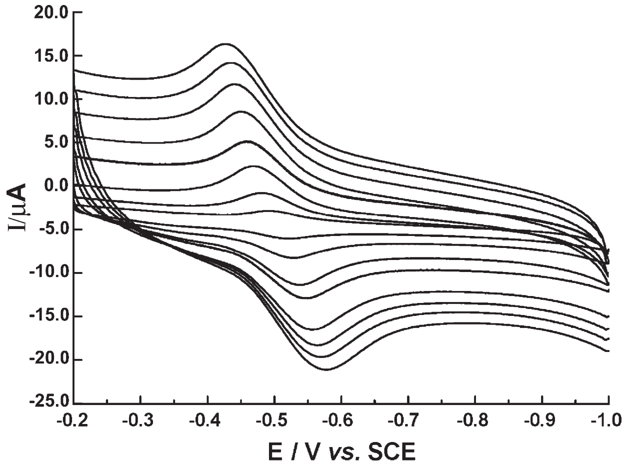

Fig. 2. Cyclic voltammograms of 5×10^{-6} mol/L rhein at differential scan rates at the MWNT-DHP composite film modified GCE. Scan rates from inert to outmost: 10, 25, 50, 100, 150, 200, 250, 300 mV s^{-1}.

lometry was investigated in the presence of 1.0×10^{-6} mol L^{-1} rhein. From the intercepts of the Anson's plots (Q vs. $t^{1/2}$), the excess charge (Q_{ads}) corresponding to the adsorbed component (after background charge correction from the backward step) can be obtained, and the results showed that Q_{ads} of rhein at bare GCE, DHP-modified GCE and MWNT-DHP composite film-modified GCE were 3.5, 1.8 and 21 μC, respectively. Under identical conditions, the value of Q_{ads} of rhein at the DHP-modified GCE is much lower than that at the bare GCE, suggesting that the DHP film blocks the adsorption of rhein. In contrast, the value of Q_{ads} of rhein at the MWNT-DHP composite film-modified GCE increased significantly in comparison with that at the bare GCE, indicating that MWNT offers more effective accumulation of rhein. There is no doubt that the MWNT-DHP composite film-modified electrode gives more sensitive signals to rhein.

3.2. DPV behavior of rhein at the MWNT-DHP composite film-modified electrode

In order to further elucidate the fascinating properties and potential of MWNT-DHP-modified GCE in the determination of rhein, the electrochemical behaviors of 5×10^{-7} mol L^{-1} rhein at three different working electrodes (i.e. bare GCE, DHP-modified GCE and MWNT-DHP composite film-modified GCE) were compared by cathodic differential pulse voltammetry (DPV), and the results are shown in Fig. 3. The response on the bare GCE is a poor reduction peak that occurred at about −0.45 V (curve b). Under the identical conditions, the MWNT-DHP composite film-modified GCE gives significantly increased signals at about −0.50 V (curve c). However, the peak current of rhein decreases obviously at a DHP-modified GCE (curve a). This may be due to that DHP can form a perfect thin film on GCE surface and thus blocks the mass transportation and electron transfer of rhein; therefore, the peak current conversely decreases compared with that at a bare GCE. Based on above discussions, the DPV technique was chosen as the analytic method for the determination of rhein in this paper and the optimized parameters of DPV is as follows: pulse amplitude: 50 mV, scan rate: 20 mV/s, pulse width: 50 ms.

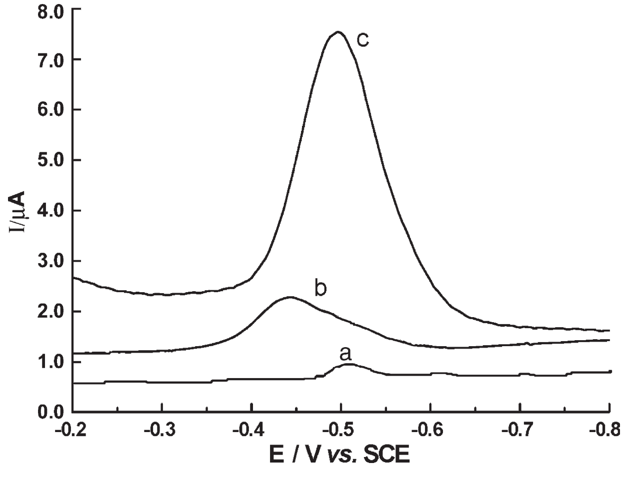

Fig. 3. Differential pulse voltammograms of 5×10^{-7} mol/L rhein at three different electrodes after 120 s accumulation at 0.1 V. Pulse amplitude: 50 mV, scan rate: 20 mV/s, pulse width: 50 ms. Curve (a): DHP film modified GCE; curve (b): bare GCE; curve (c): MWNT-DHP composite film-modified GCE.

3.3. Effect of the amount of MWNT-DHP suspension

Since DHP film can prohibit the electron exchange between rhein and the electrode and block the adsorption of rhein, the concentration of DHP in the MWNT-DHP suspension solution should be kept as low as possible. However, the lower concentration of DHP leads to a poor dispersion characterization and hence affects the casting of MWNT-DHP composite film. In present case, the DHP concentration of 0.5 mg mL^{-1} is suitable. The relationship between the amount of MWNT-DHP suspension solution on the GCE and the DPV peak current of rhein has been examined. The results illustrated that the peak current increases gradually with the increasing of the amount of the MWNT-DHP over the electrode surface at firstly. When the amount of MWNT-DHP suspension solution exceeds 5.0 μL, no obvious change in the peak current is observed, which suggests that the MWNT-DHP composite film has genuine catalytic function towards the electrochemical behavior of rhein and that the diffusion of electron transfer processes within the film and at the film–solution interface are sufficiently fast and not the rate-controlled steps. Thus, the thickness of film does not affect the catalytic redox of rhein. However, the charging current increased with the amount of the MWNT-DHP on the electrode surface, thus preventing from determining rhein at low concentration level. Then the amount of MWNT-DHP on the GCE surface is chosen for 5.0 μL.

3.4. Choice of supporting electrolyte

For optimization of the rhein determination, the effects of pH and buffer systems, such as phosphate buffer, B-R buffer, acetate buffer and Macllvaine buffer, were examined. The results showed that the highest DPV peak current of rhein was obtained in acetate buffer and, moreover, the cathodic peak was also well defined. The effect of pH of the supporting electrolyte on the peak current of rhein at the MWNT-DHP composite film-modified GCE is investigated over the range of pH 3.0–8.0. When the pH of the solution is lower than 4.0, the DPV peak current of rhein increases with the pH increase and little change in the peak current is observed over the pH range of 4.0–5.5. At the pH higher than 5.5, the peak current of rhein decreases with the pH increase. When at the higher pH, rhein become deprotonated and the solubility of rhein become larger. Hence, the adsorption of rhein on the electrode at higher pH is inefficient. Thus, after each measurement, the MWNT-DHP composite film-modified GCE was multi-cyclic potential scanning in a 0.1 mol L^{-1} NaOH solution to regenerate the surface of MWNT-DHP composite film-modified GCE.

3.5. Accumulation conditions

Accumulation, either open-circuit or close-circuit accumulation, is a common and effective tool to improve determining sensitivity. The influence of accumulation potential on the DPV peak current of rhein was investigated at different potentials from −0.2 to 0.4 V and open-circuit. When the accumulation potential shifted from −0.2 to 0.1 V, the DPV peak current of rhein increased. As the accumulation potential becoming more positive, the adsorption of rhein at the electrode surface became more efficient and, consequently, the DPV peak currents increased. When the accumulation potential is more positive than 0.1 V, the DPV current decreased slightly and, at the same time, the background current increased. The experiment also showed that the accumulation of rhein at open-circuit was less efficient than that at 0.1 V. Hence, the best accumulation potential is 0.1 V.

The effect of accumulation time on the DPV peak current of 5×10^{-7} mol L^{-1} rhein is shown in Fig. 4. The DPV peak current increases rapidly with the increase of accumulation time. Rapid adsorption of rhein on the surface of the MWNT-DHP composite film-modified GCE is responsible for this phenomenon. The peak current increases linearly with the accumulation time over the range 15 s to 120 s. However, for a longer accumulation time, the plot levels off, indicating, presumably, that a limiting amount of rhein on the electrode surface has been adsorbed. Further increase of the accumulation time leads to only slight increase the amount of rhein adsorbed on the electrode owing to surface saturation. The sensitivity for lower rhein concentrations could be increased by increasing the accumulation time, but the linear concentration range was diminished.

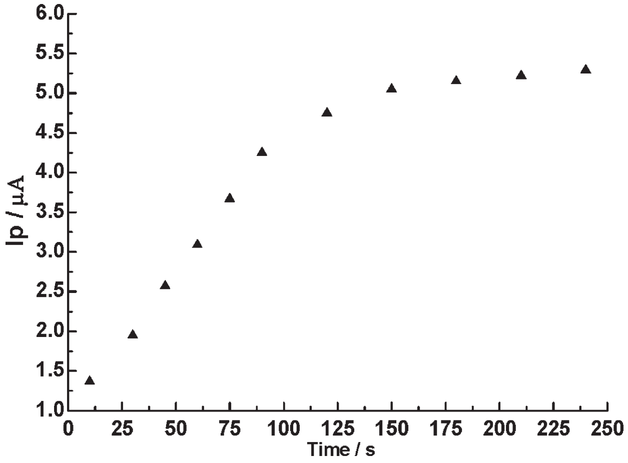

Fig. 4. Variation of the DPV peak current of 5×10^{-7} mol/L rhein versus the accumulation time. Other conditions are the same as in Fig. 3.

3.6. Electrode reaction mechanism of rhein

The pair of symmetrical peaks of rhein (Fig. 1d) at the MWNT-DHP composite film-modified GCE indicates that it belongs to a typical reversible adsorptive redox reaction. The cyclic voltammogram of rhein demonstrates that the width at a half-height of either the cathodic or the anodic peak is about to 47 mV. The electron number (n) can be calculated by following equation [23]:

$$\Delta E_{\mathrm{p},1/2} = 3.53RT/nF = 90.6/n\ (\mathrm{mV},\ 25\ ^{\circ}\mathrm{C}) \qquad (2)$$

Thus, the n of rhein involved in the electrode reaction is 2. The relationship between pH value and the redox peak potentials of rhein has also been studied and the results showed that the pH value strongly affects the peak potentials of rhein. It shows that both the peak potentials shift toward more negative potentials as pH increasing from 3.0 to 8.0, and a good linear relationship was observed between pH value and peak potentials with a slope of −53 mV/pH. The slope of −53 mV/pH indicates that the number of protons and electrons involved in the electrode reaction of rhein is equal. From the structure of rhein, it could be proposed that the electroactive group is quinonyl. Thus, the possible electrode reaction of rhein at the MWNT-DHP composite film-modified GCE is described as in Fig. 5.

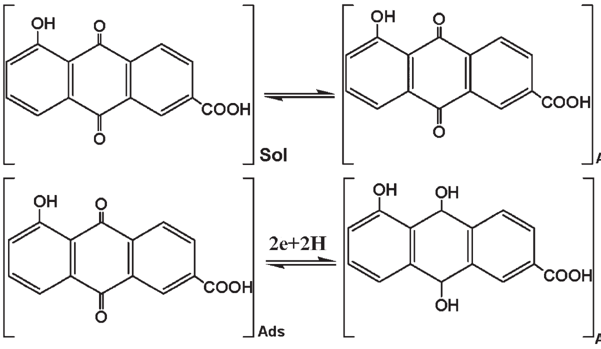

Fig. 5. Scheme of the redox reaction of rhein at the MWNT-DHP composite film modified GCE.

3.7. Calibration plot

The calibration plot for the rhein determination was constructed based on results of cathodic differential pulse voltammetry. Its linear segment increases from 1.0×10^{-8} to 5.0×10^{-6} mol L^{-1} (R=0.9976). For the concentration of rhein higher than 5.0×10^{-6} mol L^{-1}, the peak currents increased only slightly and approaching a constant value due to saturation of the MWNT-DHP composite film-modified electrode surface. The detection limit was 5.0×10^{-9} mol L^{-1} at a 3:1 signal-to-noise ratio for accumulation time of 120 s. The relative standard deviation of 4.7% for determination of 5.0×10^{-7} mol L^{-1} rhein (n=10) indicates excellent reproducibility.

Table 1
Results of rhein analysis in real samples (n=6)

Samples	Detected (mg/mL)	Added (mg/mL)	Recoveries (%)	Measured with HPLC (mg/mL)
1	0.32	0.20	102.5	0.34
1	0.34	0.40	98.1	0.33
2	0.28	0.20	103.6	0.29
2	0.30	0.40	102.2	0.31
3	0.34	0.20	98.7	0.34
3	0.35	0.40	103.8	0.34

The stability tests were carried out in room temperature. The current response decreased only by around 3.8% of its initial response after 2 weeks exposing the electrode in air, which shows the MWNT-DHP composite film-modified GCE has good long-time stability for determination of rhein.

4. Interferences

The effect of coexistent substances on the determination of rhein was investigated. Under optimal conditions, the proposed method was used for the determination of 1.0×10^{-7} mol L^{-1} rhein. The experimental results showed that at least a 200-fold excess of Ca^{2+}, Mg^{2+}, Ba^{2+}, L-histidine, DL-alanine, DL-phenyl alanine, proline, thymine, a 100-fold excess of adenine, thiourea, urea, epinephrine, guanine, uric acid, and a 10-fold excess of aloe-emodin and emodin do not interfere with the determination of 1.0×10^{-7} mol L^{-1} rhein within an error of 5%.

5. Sample analysis

In order to confirm the sensitivity and generality of the proposed method, we have used this method for the determination of rhein in sample solutions of Huanddan Yinchen Keli by using standard addition method. The results are listed in Table 1. The recoveries obtained were found to be 98.1–103.8%, which indicate that this method is accuracy enough for practical application. The reliability for the detection of rhein at MWNT-DHP composite film-modified electrode was also proved by comparative experiment using HPLC, and Table 1 also shows that results obtained with MWNT-DHP composite film-modified electrode are in good agreement with reference values obtained with HPLC.

6. Conclusion

An easy prepared MWNT-DHP composite film-modified GCE was firstly performed to investigate the electrochemical behavior of rhein in detail. The reported modified electrode improved the reversibility of redox reaction of rhein and increased the electrochemical response of rhein significantly, which clearly demonstrates the excellent electrocatalytic activity of the MWNT-DHP composite film toward the redox reaction of rhein. The electrode reaction mechanism of rhein at the MWNT-DHP composite film was also investigated. Based on this, a sensitive cathodic differential pulse voltammetric method was proposed for the determination of rhein in real samples. Its sound results showed that the method is quite valuable and seems to be of great utility for further sensor development.

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