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A simple and fast method for the simultaneous determination of Imperatorin, Isoimperatorin and Umbelliferone at β-CD–GO composite film modified GCE

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ABSTRACT

β-CD–GO composite film was immobilized on a glassy carbon electrode (GCE) to develop an electrochemical sensor for simultaneous determination of the isomers of Imperatorin (IMP) and Isoimperatorin (IIMP) and Umbelliferone (UB) for the first time. The electrochemical behaviors of the three components were investigated by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). The peak-to-peak potential differences between IMP and IIMP, IMP and UB, and IIMP and UB were 0.135, 0.540 and 0.405 V, respectively. The calibration curves for IMP, IIMP and UB were obtained in the range of 2.5–90.0 μM, 3.0–90.0 μM and 0.8–20.0 μM with the limits of detection of 0.5, 0.8 and 0.05 μM (S/N = 3), respectively. The modified electrode was successfully applied for the simultaneous determination of IMP, IIMP and UB in traditional Chinese medicine *Angelica dahuricae* Radix and Duliang Diwan with excellent recoveries as confirmed by HPLC method.

1. Introduction

Imperatorin (IMP), isoimperatorin (IIMP) and umbelliferone (UB) are the bioactive components in the traditional Chinese medicine *Angelica dahuricae* Radix or other compound preparation, such as Duliang Diwan, *etc*. And that, IMP and IIMP are isomers. It has been reported that they possessed many physiological activities, such as anti-inflammatory [1], anti-tumor [2], anti-bacteria [3], *etc*. In Chinese Pharmacopoeia, the total content of IMP and IIMP has been an important parameter for evaluating the quality of *Angelica dahuricae* Radix which requires that the sum of their contents is no less than 0.08% [4]. In addition, some Chinese medicine preparations containing *Angelica dahuricae* Radix are widely used in oriental countries for their convenient treatment [5]. Duliang Diwan composed of *Angelica dahuricae* Radix and *Chuanxiong* Rhizoma is one of them for treatment of headache, in which the content sum of IMP and IIMP stipulated in Chinese Pharmacopoeia is no less than 90 μg per pill [4].

Therefore, it is very important and interesting to develope a simple and sensitive method for determination of the isomers of IMP and IIMP or other active substances of interest such as UB. To date, liquid chromatography [6–8] and capillary electrophoresis [9] have been extensively used for determination of IMP and IIMP, and HPLC technology has been utilized to detect UB by P B Shinde, et al. [10]. But to the best of our knowledge, there are still no report on the simultaneous determination of IMP, IIMP and UB by any methods. Although the abovementioned methods have good accuracy and sensitivity, but the needs, for exmple, the cumbersome pretreatment technology, preprocessing processes, *etc*., have increased analysis time and analysis cost. In contrast, the electrochemical analysis method can not only determine some pharmaceutical compounds with redox reaction ability and provide some information on their pharmacological effects [11–13], but also greatly shorten the analysis time and reduce the analysis cost. However, no electrochemical method has been reported for simultaneous detection of the isomers of IMP and IIMP or the three components IMP, IIMP and UB.

The new nanomaterial graphene (Gr) quickly becomes a hotspot in material science because of its excellent physical and chemical properties and wide application prospects [14]. The chemical stability of Gr is high and its surface is inert state. This inert surface structure also makes it weak for interaction with other solvents. At the same time, there is a strong van der Waals force between the pieces of Gr and the chip, so it is easy that the gather phenomenon, caused by its own curl, reunion, and the accumulation of interlayer, may occur and difficult to disperse, which will make it have

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a certain limited in the practical application [15–24]. However, Gr derivatives (GR) are water soluble and have rich oxygen-containing functional groups on their surfaces and easy to be functionalized, which greatly improves the electrical and chemical properties of Gr. Da Chen et al. [25] had given a detailed discussion on the preparation, functionalization and electrochemical applications of graphene oxide (GO). XH Zhou et al. [26] had constructed a sensor immobilized on rGO-aminopyrene (1-AP) composites for detection of hydroquinone and catechol.

Cyclodextrin (CD) is a slightly conical ring compound formed through the combination of D-configuration of ribose glucose by 1, 4-glycosidic bonds, mainly alpha-, beta- and gama-cyclodextrins (α-CD, β-CD and ϒ-CD). β-CD is well known as a host molecule to embed hydrophobic molecules into its cavity, and the hydrophobicity of the cavity had been used to selectively identify molecules. At the same time, because of its good electronic conductivity, β-CD had been used for fabrication of the different separation matrices [27] and sensors [28,29]. It has been reported that CDs can be attached on the surface of GO sheet by the strong hydrogen bonding to make GO more hydrophilic [30]. In this study, the combination of GO and β-CD was chosen for separation and detection of IMP, IIMP and UB simultaneously.

In this paper, a simple and fast method for simultaneous determination of IMP, IIMP and UB at β-CD–GO composite film modified GCE using differential pulse voltammetry (DPV) has been presented. The electrochemical behaviors of the three components were investigated by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The performances of the fabricated electrode, such as linear range and detection limit, were evaluated and discussed. The modified electrode was applied for simultaneous determination of IMP, IIMP and UB in *Angelica dahuricae* Radix and Duliang Diwan with satisfactory resolutions and recoveries. Moreover, the accuracy of the obtained data was validated using HPLC technique.

2. Experimental

2.1. Reagents and apparatus

Graphene oxide powder (>99%) was purchased from Nanjing Pioneer Nano Material Science and Technology Co., Ltd. (China). *Angelica dahuricae* Radix was purchased from Kangmei Pharmaceutical Co., Ltd. (China) and Duliang Diwan was purchased from Kowloon Pharmaceutical Co., Ltd. (China). Imperatorin, Isoimperatorin and Umbelliferone were purchased in Chengdu Herbpurify Co., Ltd. (China) and a standard stock solution of 2.0×10^{-2} mol L^{-1} was prepared with anhydrous ethanol and stored in a refrigerator at 4 °Cand diluted to the desired concentration when used. All other chemical reagents were of analytical grade. Double-distilled water was used for all preparations.

2.2. Preparation of β-CD–GO/GCE

2.0 mg of GO was dispersed in 2 ml aqueous solution, and then 20.0 mg β-CD was added and sonicated for 30 min to get a homogeneous black suspension. The pH value of this suspension was adjusted to 9.5 with 5 wt% sodium carbonate solution and stirred for 10 h to obtain β-CD–GO sheets. Five microlitres of β-CD–GO suspension were dropped on the electrode surface and dried under infrared light.

2.3. Analysis of Angelica dahuricae Radix sample

About 0.4 g *Angelica dahuricae* Radix sample was taken, weighed accurately, and placed in 50 ml volumetric flask. 45 ml methanol was added in it and treated by ultrasound for 1 h, cooled and then diluted with methanol to volume. The sample was filtrated and stored in the dark. Just before each quantitative analysis, the sample solution was prepared by dilution with 0.1 M NaOH solution.

2.4. Analysis of Duliang Diwan sample

About 0.5 g Duliang Diwan was taken, weighed accurately, and placed in 100 ml conical flask with cover. 15 ml of methanol aqueous solution (15%) was added in it, mixed well, sonicated for 30 min and then centrifuged. The supernatant was taken and filtered with 0.45 um microporous membrane. 1 mL of filtrate was diluted with 10 ml 0.1 M NaOH solution, and transferred into the voltammetric cell. The differential pulse voltammetry was recorded under optimized conditions.

3. Results and discussion

3.1. Scanning electron microscopic characterization

Scanning electron microscopic (SEM) images were used to characterize the surface morphologies of the GO/GCE (a) and β-CD–GO/GCE (b) modified electrode (Fig. 1). From Fig. 1(a), the wrinkle lamellar structure of graphene oxide nanosheets can be observed clearly. The β-CD–GO composite film (Fig. 1(b)), in addition to the pleat-like structure, can be found out the phenomenon of reunion. As can be seen by T Ogoshi et al. [31], a similar reunion phenomenon can be observed in the modification of graphene oxide with cyclodextrin due to the production of the agglomeration of polymer caused by the cross-linking between them.

3.2. Electrochemical characterization of fabricated electrode

In this paper, the β-CD–GO/GCE electrode was characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The electrochemical behaviors of GCE, GO/GCE and β-CD–GO/GCE electrodes in 0.1 M KCl solution containing 5 mM $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were observed by CV and EIS. As can be seen from Fig. 2 (A), the redox potential difference (ΔEp) on the bare glassy carbon electrode (a) is 100 mV. By contrast, the redox potential differences on the modified electrodes (b and c) have no obvious change. However, the redox peak currents of b and c change significantly: b decreases, but c increases. This may be that the graphene oxide contains negative groups, such as carboxyl groups, *etc*, which impede the transfer of the same negatively charged $[Fe(CN)6]^{3-/4-}$. When the β-CD was modified on GO/GCE, the current was significantly increased compared with GO/GCE and GCE, indicating that the presence of β-CD could promote electron transfer between the electrode and solution. In the meanwhile, this phenomenon also show that β-CD has been modified onto the GO/GCE.

In order to substantiate the result obtained from Fig. 2(A), the EIS analysis was performed on modified sensors as shown in Fig. 2(B). The Nyquist curves clearly indicate that resistance increase dramatically on GO/GCE compared with bare GCE. However, the value of resistance decreases on CD-GO/GCE sensor. It show that the presence of GO has given some resistance to the electrode or solution interface reaction. When β-CD is modified on GO/GCE, it can promote electron transfer and make it have good electron conductivity [29]. The results from CV and EIS all show that the transfer of $[Fe(CN)6]^{3-/4-}$ has been promoted after modification of CD on the GO/GCE, which also indicate that the CD-GO/GCE electrode has a high catalytic activity.