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ORIGINAL PAPER

Acetylene black nanoparticle-modified electrode as an electrochemical sensor for rapid determination of rutin

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Abstract Acetylene black nanoparticles were homogeneously dispersed into water in the presence of hydrophobic surfactant and then used to modify the surface of a glassy carbon electrode. An examination of the electrochemistry of rutin showed that this modification of the electrodes resulted in a considerable enhancement of the surface, thus remarkably increasing the signal for rutin. As a result, a sensitive and convenient electrochemical method was developed for the determination of rutin. The linear range is from 20 μg L^{-1} to 5 mg L^{-1}, and the limit of detection is 10 μg L^{-1}. The method was successfully employed to the determination of rutin in traditional Chinese medicines.

Keywords Rutin · Electrochemical sensor · Acetylene black · Modified electrode

Introduction

Rutin (3′,4′,5,7-tetrahydroxyflavone 3β-D-rutinoside), the mainly active component of many traditional Chinese medicines, has been widely used in the clinic because it possesses a broad range of physiological effects such as anti-inflammatory [1], anti-tumor [2], anti-bacteria [3] and significant scavenging abilities on oxygen radicals [4]. Therefore, the detection of rutin is quite important and interesting.

Up-to-now, various electrochemical methods have been applied for the detection of rutin since it contains phenolic hydroxyl group and can be oxidized at the electrode surface. For example, a lead film-modified glassy carbon electrode (GCE) [5], an ionic liquid-modified carbon paste electrode (CPE) [6, 7], a heated graphite electrode [8], a mesoporous silica-modified CPE [9], a poly(vinylpyrrolidone)-modified CPE [10], a carbon nanotube-modified GCE [11–15], a CeO_2 nanoparticle-modified electrode [16] and a carbon-polyurethane composite electrode [17] were reported for the detection of rutin. However, to the best of our knowledge, electrochemical detection of rutin using AB nanoparticle-modified electrode has not been reported.

AB, a special kind of carbon black, has porous structure and possesses many fascinating properties such as excellent electric conductivity, large surface area and strong adsorptive ability. Thus, AB was successfully used for the fabrication of modified electrodes to increase the determining sensitivity of different species such as lead ion [18], 6-benzylaminopurine [19], kojic acid [20], 2-chlorophenol [21] and 1-naphthylacetic acid [22]. Herein, the AB nanoparticles were homogeneously dispersed into water with the aid of dihexadecyl hydrogen phosphate (DHP), a special kind of hydrophobic surfactants with two C-H chains. After that, the resulting AB suspension was added onto the surface of electrode, and the water was evaporated, forming an AB film-modified electrode. The electrochemical behavior of rutin was investigated, revealing that the AB nanoparticles remarkably increase the response signal of rutin. Based on this, a sensitive, rapid and convenient analytical method was developed for rutin.

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Experimental

Reagents

All the reagents were of analytical grade and used as received. Rutin was purchased from Sigma (www.sigma-aldrich.com) and dissolved into ethanol to prepare 1 g L^{-1} standard solution, which was stored at 4 °C. Acetylene black (AB, purity >99.99%) was purchased from STREM Chemicals (USA, www.strem.com). Dihexadecyl hydrogen phosphate (DHP) was obtained from Sigma (www.sigma-aldrich.com).

Instruments

All the electrochemical measurements were carried out using EC 550 Electrochemical Workstation (Gaoss Union Co. Ltd., China, www.gaossunion.com) in a conventional three-electrode system. The working electrode is an AB film-modified GCE, the reference electrode is a saturated calomel electrode (SCE), and the counter electrode is a platinum wire.

Scanning electron microscopy (SEM) was performed with Quanta 200 microscope (FEI Company, Netherlands, www.fei.com).

Preparation of AB film-modified GCE

Ultrasonication agitation for 40 min was used to disperse 10 mg AB and 10 mg DHP into 10 mL redistilled water, giving a stable and homogeneous black suspension (1 mg mL^{-1}). Prior to modification, the GCE was polished with 0.05 μm alumina slurry, rinsed thoroughly with redistilled water, and then sonicated in redistilled water for 2 min. Finally, the GCE was coated with 5 μL AB-DHP suspension and the water was evaporated from the surface under an infrared lamp in the air.

The surface morphology of AB film-modified GCE was characterized using SEM, which was shown in Fig. 1. From the SEM image, it is apparent that the GCE surface was coated with uniform AB nanoparticle, and the average diameter is about 80 nm.

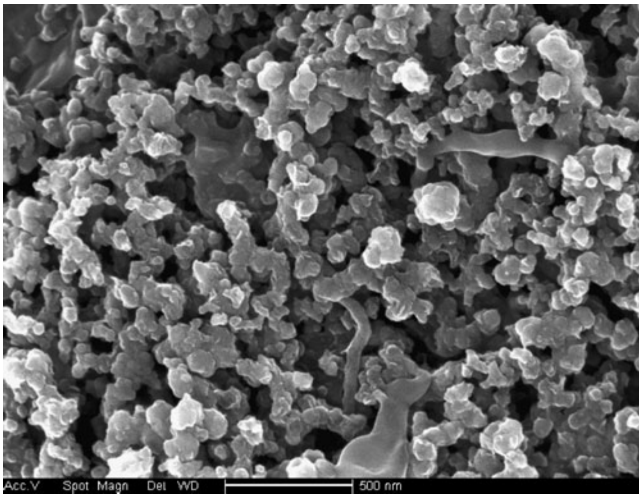

Fig. 1 SEM of AB film

Sample preparation

Traditional Chinese medicines such as Pagodatree Flower Bud and Hawkthorn were purchased from a local Pharmacy, which was dried at 60 °C for 4 h and then pulverized. After that, 2.0 g powder was accurately weighed and added into 10 mL ethanol, followed by 30-min ultrasonication for extracting rutin. The extraction was repeated two times, and the liquid phase was collected in a 50.0-mL volumetric flask every time. Finally, the extract was diluted to volume with ethanol for further analysis. Spiked samples were prepared by adding a known amount of rutin standard to the dry powder sample before extraction.

Analytical procedure

Unless otherwise stated, pH 5 acetate buffer (0.1 M) was used as the supporting electrolyte for rutin. After 3-min accumulation at 0 V (vs. SCE), the differential pulse voltammograms were recorded from 0.0 to 0.6 V, and the peak current at 0.34 V was measured for rutin. The pulse amplitude is 50 mV, the pulse width is 40 ms and the scan rate is 40 mV s^{-1}.

Results and discussion

Electrochemical behavior of rutin

Figure 2 shows the CV response of 5 mg L^{-1} rutin in pH 5 acetate buffer at different electrodes. At the unmodified GCE (curve a), a pair of redox peaks was observed. The oxidation peak potential is 0.364 V, and the reduction peak potential is 0.336 V. So, the peak potential separation is 28 mV at the GCE, suggesting that the oxidation of rutin is reversible and two electrons are transferred. However, the peak potential separation decreases to 4 mV at the AB film-modified GCE (curve b), which is similar to a surface electrochemical process. AB nanoparticles exhibit high accumulation efficiency to rutin, and greatly improve its surface concentration. Therefore, the oxidation of rutin tends to a typical surface process at the AB film. From the comparison, it is also found that the peak current of rutin remarkably increases at the AB film-modified GCE, indicating that AB possesses considerable enhancement effect.

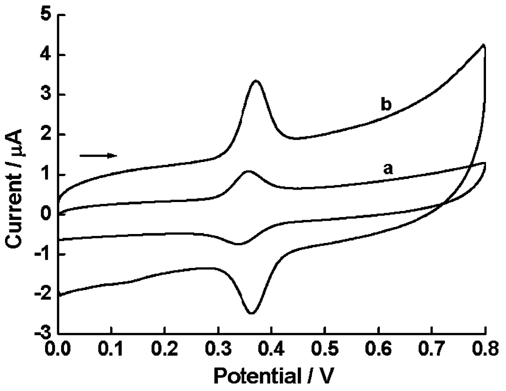

Fig. 2 CV curves of 5 mg L^{-1} rutin at GCE (*curve a*) and AB film-modified GCE (*curve b*) in pH 5 acetate buffer. Scan rate=100 mV s^{-1}

The oxidation behavior of rutin in pH 5 acetate buffer was then examined using differential pulse voltammetry (DPV), which was shown in Fig. 3. After 3-min accumulation at 0 V, a negligible oxidation peak is observed for 0.1 mg L^{-1} rutin at the unmodified GCE (curve a), suggesting that the response activity of rutin is very poor at the GCE surface. However, a very sensitive and well-shaped oxidation peak appears at the AB film-modified GCE (curve b). The peak potential is 0.34 V and the peak current significantly increases. AB nanoparticle, with large surface area, numerous active sites and high sorption ability, facilitates the electron transfer of rutin and considerably increases its oxidation current signal. Therefore, the sensitivity of determination of rutin is greatly improved at the AB film-modified GCE. In addition, the DPV responses of different concentrations of rutin at the AB film-modified GCE were also investigated. When increasing the concentration to 0.25 mg L^{-1} (curve c) and 0.5 mg L^{-1} (curve d), the oxidation peak current of rutin linearly increases. If decreasing the concentration to zero, the oxidation peak at 0.34 V disappears (not shown). These phenomena reveal that the oxidation peak at 0.34 V is attributed to rutin, and the peak current can be used as the analytical signal for rutin.

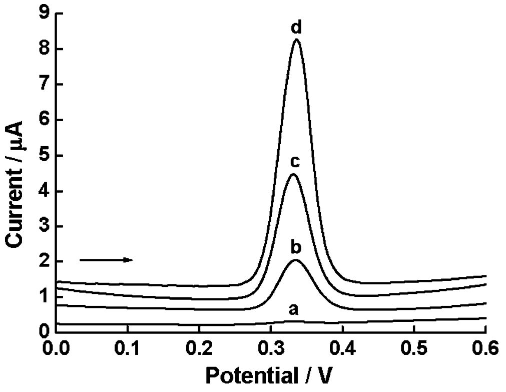

Fig. 3 DPV curves of 0.1 mg L^{-1} rutin at GCE (*curve a*) and AB film-modified GCE (*curve b*) after 3-min accumulation at 0 V. (*c*) and (*d*): 0.25 and 0.5 mg L^{-1} rutin at AB film-modified GCE. Pulse amplitude: 50 mV, pulse width: 40 ms, scan rate: 40 mV s^{-1}

Influence of pH value

The oxidation current of rutin at the AB film-modified GCE was examined using DPV in different supporting electrolytes. The used solutions include 1) 0.1 M acetate buffer solutions with pH of 3, 4, 4.6, 5 and 5.6, 2) 0.1 M phosphate buffer solutions with pH of 6, 6.5, 7, 7.5 and 8. Figure 4 depicts the influence of pH value on the oxidation peak current of 0.1 mg L^{-1} rutin. The oxidation peak current gradually increases with improving pH value from 3 to 5, and then decreases with further increasing pH value from 5 to 8. For the highest sensitivity, the pH 5 acetate buffer was employed.

Effect of amount of AB suspension

The influence of amount of AB suspension on the oxidation peak current of rutin was studied. It is found that the oxidation peak current of rutin remarkably increases with the volume of AB suspension from 0 to 5 μL. During this period, the amount of AB nanoparticles at the GCE surface obviously increases, leading to larger surface area and higher accumulation efficiency. As a result, the oxidation peak current of rutin notably increases. With further increasing the amount of AB suspension from 5 to 15 μL, the oxidation peak current of rutin increases slightly. In order to shorten the time for solvent evaporation and to achieve high sensitivity, 5 μL of AB suspension was used to modify the GCE surface.

Influence of accumulation potential and time

The oxidation peak current of rutin under open circuit and different accumulation potentials such as −0.3 V, −0.2 V, −0.1 V, 0 V, 0.1 V and 0.2 V was individually measured to

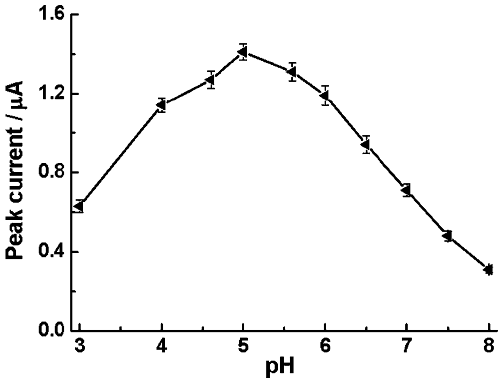

Fig. 4 Influence of pH value on the oxidation peak current of 0.1 mg L^{-1} rutin. Other conditions are the same as in Fig. 3

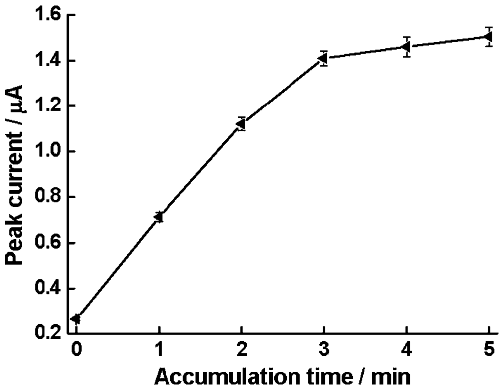

Fig. 5 Effect of accumulation time on the oxidation peak current of 0.1 mg L^{-1} rutin. Other conditions are the same as in Fig. 3

evaluate the influence of accumulation potential. The oxidation peak current of rutin keeps constant, revealing no influence of accumulation potential on the determination of rutin. For the convenience the accumulation step was performed at 0 V.

Figure 5 illustrates the influence of accumulation time on the oxidation peak current of rutin. When extending the accumulation time from 0 to 3 min, the oxidation peak current of rutin linearly increases, suggesting that accumulation obviously increases the determining sensitivity. However, the oxidation peak current of rutin increases slightly with further increasing the accumulation time from 3 to 5 min, indicating that the amount of rutin tends to a limiting value at the AB film surface. Considering sensitivity and working efficiency, 3-min accumulation at 0 V was employed.

Reproducibility

The AB film-modified GCE was used for single measurement in this work, and the reproducibility between multiple AB film-modifed GCEs was estimated by parallel determining the response of 0.1 mg L^{-1} rutin. The relative standard deviation (RSD) is 4.9% for 10 AB film-modified GCEs, indicative of excellent precision.

Linear range and limit of detection

The linear range and limit of detection (LOD) were studied using DPV under the optimized conditions. The oxidation peak current of rutin (i_{pa}, μA) is proportional to its concentration (C, mg L^{-1}) over the range from 20 μg L^{-1} to 5 mg L^{-1}, obeying the following equation: $i_{pa}=0.1247+12.77\ C$ ($R=0.996$). In addition, the LOD was evaluated to be 10 μg L^{-1} (1.5×10^{-8} mol L^{-1}) based on the 3 signal-noise ratio.

Interference

The potential interference for the determination of rutin was studied. Under the optimized conditions, the oxidation peak current of rutin was individually measured in the presence of different concentrations of interferents and then the change of peak current was checked. It was found that 1000-fold concentrations of vitamin C, glucose, glycine, Cu^{2+}, Fe^{3+}, Al^{3+} and Mn^{2+}; 300-fold concentrations of hesperidin, tangeretin, obiletin, naringin, morin and citromitin; 100-fold concentrations of quercetin and galangin; almost have no influence on the determination of 0.1 mg L^{-1} rutin when the peak current change is below 10%.

Analytical application

In order to assess the performance of this method in real sample analysis, it was used to detect rutin in different traditional Chinese medicines such as Pagodatree Flower Bud and Hawkthorn. Each sample solution undergoes five parallel detections, and the RSD is below 5%, suggesting that the results obtained by AB film-modified GCE are acceptable. The content of rutin was determined by the standard addition method and the results were shown in Table 1. In order to testify the accuracy of AB film-modified GCE, a known amount of rutin standard was spiked in the sample, and the recovery was then tested. The value of recovery is in the range from 95.5% to 98.6%, suggesting that the recovery and accuracy of AB film-modified GCE is satisfactory.

Table 1 Determination and recovery of rutin in traditional Chinese medicines

Sample	Spiked/mgg^{-1}	Expected/mgg^{-1}	Found/mgg^{-1}	Recovery
Pagodatree Flower Bud A	0.00		1.96	/
	2.00	3.96	3.87	95.5%
Pagodatree Flower Bud B	0.00		3.05	/
	5.00	8.05	7.92	97.4%
Hawkthorn A	0.00		3.73	/
	5.00	8.73	8.66	98.6%
Hawkthorn B	0.00		4.57	
	5.00	9.57	9.46	97.8%

Conclusion

An AB nanoparticle-modified electrode was prepared with the aid of a hydrophobic surfactant. Due to the large surface area and numerous active sites, the AB-modified electrode showed high accumulation efficiency to rutin, and greatly increased the oxidation peak current of rutin. The detection conditions were optimized, and an electroanalytical method was developed for rutin. The analytical procedure was simple and rapid, and the accumulation time was just 3 min. The precision and accuracy were excellent, and the values of RSD and recovery were satisfactory. Finally, the method was successfully used to detect rutin in traditional Chinese medicines, and exhibited promising application in real sample analysis.

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