Electrochemical determination of chrysophanol based on the enhancement effect of acetylene black nanoparticles

Yuanyuan Zhang \( \text{a}, \) Yanying Wang \( \text{a,} \) Kangbing Wu \( \text{a,} \) Shichao Zhang \( \text{b}, \) Yu Zhang \( \text{b}, \) Chidan Wan \( \text{b,} \)

\( \text{a} \) School of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, Wuhan 430074, China

\( \text{b} \) General Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

\( \text{c} \) College of Chemistry and Materials Science, South-Central University for Nationalities, Wuhan 430074, China

**A R T I C L E   I N F O**

Article history:
Received 6 August 2012
Received in revised form 2 October 2012
Accepted 9 October 2012
Available online xxx

Keywords:
Acetylene black
Nanoparticles
Surface enhancement
Chrysophanol
Electrochemical detection

**A B S T R A C T**

Acetylene black (AB) nanoparticles were readily dispersed into water in the presence of dihexadecyl hydrogen phosphate. After evaporation of water, the surface of glassy carbon electrode (GCE) was coated with AB nanoparticles as confirmed from the scanning electron microscopy measurements. The transmission electron microscopy images indicated that AB nanoparticles possessed porous structure. Electrochemical behavior of chrysophanol was studied, and a sensitive oxidation peak was observed in pH 3.6 acetate buffer solution. Compared with the bare GCE, the AB nanoparticles-modified GCE greatly increased the oxidation peak current of chrysophanol, showing remarkable signal enhancement effect. The influences of pH value, amount of AB, accumulation potential and time on the signal enhancement of chrysophanol were studied. As a result, a novel electrochemical method was developed for the determination of chrysophanol. The linear range was from 1.5 to 200 \( \mu \)g \( \text{L}^{-1} \) and the detection limit was 0.51 \( \mu \)g \( \text{L}^{-1} \) \( (2.01 \times 10^{-8} \text{M}) \) after 2-min accumulation. Finally, this method was used in traditional Chinese medicines, and the results consisted with the values that obtained by high-performance liquid chromatography.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Chrysophanol (1,8-dihydroxy-3-methylantraquinone) is a free anthraquinone compound and a secondary metabolite of medicinal plant rhubarb. Chrysophanol has been reported to have many pharmacological effects such as anti-inflammatory activity [1], anti-microbial activity [2] and anti-cancer action [3,4]. Therefore, the detection of chrysophanol is quite important and interesting. Until now, the widely used method for the detection of chrysophanol is high-performance liquid chromatography (HPLC) [5–8]. Although electrochemical detection possesses high sensitivity, short analysis time, low cost and handling convenience, the study regarding electrochemical determination of chrysophanol is very limited.

Acetylene black (AB), a special kind of carbon black, has attracted much attention and been widely used in the field of electroanalysis because of extraordinary properties such as high accumulation efficiency, excellent electric conductivity and large surface area. For example, the response signal and detection sensitivity of 2-chlorophenol [9], kojic acid [10], erythromycin [11], topotecan [12] and 6-benzylaminopurine [13] were greatly increased by AB nanoparticles.

The main objective of this work is to develop a simple and sensitive electrochemical method for the detection of chrysophanol utilizing the excellent properties of AB nanoparticles. Thus, insoluble AB nanoparticles were firstly dispersed into water and then used to modify the GCE surface, constructing a sensing film for chrysophanol. On the surface of AB nanoparticles-modified GCE (denoted as nano-AB/GCE), the oxidation signal of chrysophanol increased remarkably. Undoubtedly, the sensitivity of chrysophanol detection is greatly enhanced by AB nanoparticles.

2. Experimental

2.1. Reagents

All chemicals were of analytical grade and used as received. Chrysophanol (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China) was dissolved into ethanol to prepare 0.1 g L\(^{-1}\) standard solution, and stored in the fridge at 4 °C. AB nanoparticles (purity >99.99%) was purchased from STREM Chemicals (USA). Dihexadecyl hydrogen phosphate (DHP) was obtained from Sigma.
2.2. Instruments

Electrochemical measurements were performed on a CHI 830C electrochemical workstation (Chenhua Instrument, Shanghai, China). A conventional three-electrode system, consisting of an AB-modified GC working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode, was employed. Scanning electron microscopy (SEM) characterization was conducted with a Quanta 200 microscope (FEI Company, Netherlands). Transmission electron microscopy (TEM) image was measured using a Tecnai G220 microscope (FEI Company, Netherlands).

HPLC detection of chrysophanol was carried out with an Agilent 1100, coupled with a UV-VIS detector. The C18 analytical column (4.6 mm × 150 mm × 5 μm) was used. The mobile phase was methanol–H₂O (85:15, v/v), filtered through a 0.2-μm Millipore filter prior to use. The flow rate was 1 mL min⁻¹, and the sample injection volume was 20 μL. The detection wavelength was 254 nm.

2.3. Preparation of nano-AB/GCE

AB nanoparticles (10.0 mg) and DHP (10.0 mg) were added into doubly distilled water (10.0 mL), and then sonicated in a KQ-50B ultrasonicator for 1 h. Before modification, the GCE with diameter of 3 mm was polished with 0.05 μm alumina slurry, and sonicated in doubly distilled water for 2 min. After that, the GCE surface was coated with 5 μL AB suspension, and the water was evaporated from the surface under an infrared lamp in air. The DHP film-modified GCE was prepared as the same procedure without addition of AB.

2.4. Sample preparation

Four representative rhubarb samples were purchased from a local pharmacy, and then treated as follows. The sample was firstly pulverized, and 50 mg of the powder was accurately weighed and added into 25 mL of ethanol. After 30-min ultrasonication and subsequent 6-min centrifugation at 8000 rpm, the clear liquid phase was collected. The extraction was repeated, and the extract solution was finally diluted to 50.0 mL for further measurement. Spiked samples were prepared by adding a known amount of chrysophanol standard to sample before extraction.

2.5. Analytical procedure

Acetate buffer (0.1 M) at pH of 3.6 was used for the detection of chrysophanol. After 2-min accumulation under open-circuit, the differential pulse voltammograms were recorded from −0.7 to −0.2 V, and the oxidation peak current at −0.48 V was measured for chrysophanol. The pulse amplitude was 50 mV, the pulse width was 40 ms and the scan rate was 40 mV s⁻¹.

3. Results and discussion

3.1. Characterization of AB nanoparticles

The inner structure of AB nanoparticles in suspension was characterized using TEM. As shown in Fig. 1A, the particle size was about 80 nm and porous structure was clearly observed. In addition, the surface morphology of AB film on GCE surface was studied via SEM (Fig. 1B). It was apparent that the GCE surface was coated with homogeneous AB nanoparticles.

![Fig. 1. TEM (A) and SEM (B) images of AB nanoparticles.](image)

3.2. Enhancement effect of AB nanoparticles

The electrochemical responses of low concentration of chrysophanol on different electrodes were compared using differential pulse voltammetry (DPV). Fig. 2 shows the DPV responses of

![Fig. 2. DPV curves of 30 μg L⁻¹ chrysophanol on GCE (a), DHP film-modified GCE (b) and nano-AB/GCE (d). (c) DPV curve of nano-AB/GCE in pH 3.6 acetate buffer without chrysophanol. Accumulation was performed under open-circuit for 2 min.](image)
30 μg L⁻¹ chrysophanol in pH 3.6 acetate buffer. After 2-min accumulation under open-circuit, an oxidation peak at −0.48 V was observed on GCE surface (curve (a)). On the surface of DHP film-modified GCE (curve (b)), the oxidation peak current of chrysophanol decreased slightly. This is because that DHP, a surfactant with poor electric conductivity, forms a perfect film on GCE surface and blocks the electron transfer of chrysophanol. However, the oxidation signal of chrysophanol greatly increased on the surface of nano-AB/GCE (curve (d)). The notable peak current enlargement indicates that AB nanoparticles exhibit strong enhancement effect toward the oxidation of chrysophanol. AB nanoparticles possess strong accumulation ability to chrysophanol, and greatly enhance its surface concentration. As a result, the oxidation peak current of chrysophanol enhances obviously on AB surface. Besides, the response of nano-AB/GCE in the absence of chrysophanol was given in curve (c), and the blank DPV curve was virtually featureless. So the oxidation wave in Fig. 2 was attributed to the oxidation of chrysophanol. In conclusion, the comparison of Fig. 2 clearly demonstrates that the AB film is more sensitive for the detection of chrysophanol.

3.3. Detection of chrysophanol

Fig. 3 depicts the oxidation responses of chrysophanol on nano-AB/GCE in 0.1 M acetate buffer solutions with different pH values. It was found that the oxidation peak currents of chrysophanol gradually decreased with improving pH value. Moreover, the oxidation peak shifted to more negative potentials with increasing pH value, suggesting that proton was involved in the oxidation process. At high pH value, the shape of oxidation wave became poor, and the oxidation signal decreased. Therefore, 0.1 M acetate buffer at pH of 3.6 was employed for the detection of chrysophanol.

Fig. 4 illustrates the influence of amount of AB suspension on the oxidation peak current of chrysophanol. When gradually improving the volume of AB suspension from 0 to 5 μL, the oxidation peak currents of chrysophanol greatly increased. During this period, the accumulation efficiency of nano-AB/GCE obviously enhances, resulting in remarkable oxidation peak current enhancement of chrysophanol. However, the oxidation peak currents decreased slightly with further improving the amount of AB suspension up to 10 μL, maybe due to the blocking effect of DHP. In order to shorten the time of solvent evaporation and to achieve high sensitivity, 5 μL AB suspension was used to modify the GCE surface.

In order to discuss the effect of accumulation potential, the oxidation peak currents of chrysophanol at different potentials were measured individually. The oxidation peak currents of chrysophanol increased gradually when the accumulation potentials changed from −1 to −0.6 V. In addition, the oxidation peak currents of chrysophanol under open-circuit accumulation were also studied. It was found that the oxidation peak current under open-circuit accumulation was much higher, suggesting that the accumulation of chrysophanol on AB nanoparticles surface is non-electrostatic. In order to achieve high sensitivity, accumulation was performed under open-circuit. Fig. 5 displays the influence of accumulation time on the oxidation peak current of chrysophanol. By extending the accumulation time from 0 to 2 min, the oxidation peak currents remarkably increased, revealing that accumulation is efficient to improve the detection sensitivity. When the accumulation time was longer than 2 min, the oxidation peak currents of chrysophanol enhanced slightly with time. Considering sensitivity and analysis time, 2-min accumulation was employed.

The successive measurements using one nano-AB/GCE were examined, and unfortunately the oxidation peak currents of chrysophanol decreased continuously. The strong surface sorption and fouling were the main reason. Thus, nano-AB/GCE was used for
single measurement, and the reproducibility between multiple electrodes was evaluated by parallel determining the oxidation peak current of 30 μg L⁻¹ chrysophanol. The relative standard deviation (RSD) was 2.2% for ten nano-AB/GCEs, indicative of excellent fabrication reproducibility and detection precision.

The potential interferences on the detection of chrysophanol were investigated. Under the optimized conditions, the oxidation peak currents of chrysophanol were individually measured in the presence of different concentrations of interferents, and then the peak current change was checked. No influence on the determination of 30 μg L⁻¹ chrysophanol was found after the addition of 10,000-fold concentrations of glucose, phenylalanine and valine; 2000-fold concentrations of tryptophan; 1500-fold concentrations of hypoxanthine, glycine, glutamic acid, serine and resorcin; 1000-fold concentrations of xanthine and catechol; 35-fold concentrations of Rhein; and 5-fold concentrations of emodin (peak current change <5%).

The linear range and detection limit were tested using DPV under the optimized conditions. As shown in Fig. 6, the oxidation peak current (iₚ, μA) of chrysophanol was linear with its concentration (C, μg L⁻¹) over the range from 1.5 to 200 μg L⁻¹. The linear regression equation was \( iₚ = 0.0447C \), and the correlation coefficient was 0.997. After 2-min accumulation, the detection limit was evaluated to be 0.51 μg L⁻¹ (2.01 × 10⁻⁹ M) based on three signal-to-noise ratio.

3.4. Reaction mechanism of chrysophanol

The electrochemical behavior of chrysophanol was studied using cyclic voltammetry (CV) to discuss the electrode reaction mechanism. Fig. 7 depicts the CV responses of chrysophanol on nano-AB/GCE that started from different potentials. During the cyclic sweep from −0.7 V to −0.2 V (A), an oxidation peak was observed at −0.445 V, and a reduction peak appeared at −0.475 V on the reverse scan. The separation of peak potentials (ΔEₚ) was 30 mV, and kept constant when the scan rates was lower than 75 mVs⁻¹, suggesting that the oxidation of chrysophanol was reversible and involved two electrons. If we gradually changed the initial potential to more positive directions such as −0.6 and −0.5 V, it was found that the oxidation peak at −0.445 V decreased gradually. This phenomenon indicates that the oxidation peak may be dependent on the reduction product. When the cyclic sweep started from −0.2 V (B), a pair of redox peaks was also observed. From the comparison, we found that the reduction peak in plot B was higher than in plot A.

![Fig. 6](image1.png)

**Fig. 6.** Calibration curve for chrysophanol. Accumulation was performed under open-circuit for 2 min. Error bar represents the standard deviation of triple measurements.

![Fig. 7](image2.png)

**Fig. 7.** CV curves of 2 mg L⁻¹ chrysophanol in pH 3.6 acetate buffer that started from −0.7 V (A) and from −0.2 V (B). The arrow indicated the sweeping direction. Scan rate: 50 mVs⁻¹.

![Fig. 8](image3.png)

**Fig. 8.** Electrode reaction mechanism of chrysophanol on nano-AB/GCE.
Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Detection</th>
<th>Recovery test</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPLC/mg g⁻¹</td>
<td>Nano-AB/GCE/mg g⁻¹</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.61</td>
<td>5.20</td>
<td>−7.31%</td>
</tr>
<tr>
<td>2</td>
<td>4.72</td>
<td>4.60</td>
<td>−2.54%</td>
</tr>
<tr>
<td>3</td>
<td>3.95</td>
<td>3.60</td>
<td>−8.68%</td>
</tr>
<tr>
<td>4</td>
<td>6.18</td>
<td>6.53</td>
<td>5.67%</td>
</tr>
</tbody>
</table>

In order to test the accuracy, the content of chrysophanol was also detected by HPLC. It was found that the results obtained by HPLC and nano-AB/GCE were in good agreement, suggesting that this new method is accurate and feasible. In addition, the recovery of chrysophanol standard was also performed, and the value of recovery was over the range from 87.9% to 102.5%, indicating that this method has promising application.

4. Conclusion

The oxidation of chrysophanol was greatly enhanced on the surface of AB nanoparticles. AB nanoparticles exhibited high accumulation efficiency to chrysophanol and significantly increased the oxidation peak current of chrysophanol. Based on the remarkable enhancement effect of AB nanoparticles, a novel electrochemical method was developed for chrysophanol detection. This new method possessed high sensitivity, rapid response, good reproducibility and excellent accuracy. It was successfully used in rhubarb samples, and showed promising application in real sample analysis.

Acknowledgements

This work was supported by the National Basic Research Program of China (973 Program, No. 2009CB320300), the National Natural Science Foundation of China (Nos. 61071052 and 30972798), and the Program for New Century Excellent Talents in University (NCET-11-0187). The Center of Analysis and Testing of Huazhong University of Science and Technology was also thanked for its help in the SEM and TEM observation.

References


and shifted slightly to more negative potential, while the oxidation peak kept unchanged. Based on the above results, we concluded that quinonyl of chrysophanol was firstly reduced at −0.7 V, and then the reduction product was oxidized during the anodic sweep from −0.7 to −0.2 V. The proposed electrode reaction is consistent with the published results [14] and can be described using Fig. 8.

3.5. Analytical application

In order to evaluate the practical application of this new method, it was used to detect chrysophanol in rhubarb samples. After adding 50 μL sample solution into 10.0 mL pH 3.6 acetate buffer, the DPV curve from −0.7 to −0.2 V was recorded after 2-min accumulation under open-circuit. As seen in Fig. 9a, two oxidation peaks at −0.48 V (O₁) and −0.39 V (O₂) were observed on nano-AB/GCE. In order to confirm which peak was attributed to chrysophanol and also to obtain its concentration, a known amount of chrysophanol standard solution was added into, and the DPV curve was then recorded in Fig. 9b. It was found that the oxidation peak current at −0.48 V (O₁) increased correspondingly after addition of chrysophanol standard, while the oxidation peak of O₂ at −0.39 V kept unchanged. Thus, the oxidation peak at −0.48 V (O₁) is due to chrysophanol, and the content can be achieved according to the oxidation peak current ratio. Each sample was determined by three parallel detections, and the RSD was below 5%, revealing excellent precision. The content of chrysophanol was obtained by the standard addition method, and the results were listed in Table 1.

Fig. 9. DPV curves of rhubarb sample (a) and spiked rhubarb sample (b) on nano-AB/GCE. Accumulation was performed under open-circuit for 2 min.