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Enhanced oxidation and detection of toxic clenbuterol on the surface of acetylene black nanoparticle-modified electrode

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ABSTRACT

Acetylene black (AB) nanoparticles were easily dispersed into water in the presence of a special surfactant: dihexadecyl hydrogen phosphate (DHP). After that, the AB-DHP composite film was coated on the surface of glassy carbon electrode (GCE) after evaporating water. The resulting AB-DHP film exhibited remarkable enhancement to the oxidation of clenbuterol, and greatly increased the oxidation peak current of clenbuterol. The influences of pH value, amount of AB, accumulation potential and time were studied. As a result, a novel electrochemical method was developed for the detection of clenbuterol. The linear range is from 0.02 to 4 mg L^{-1}, and the detection limit is 10 μg L^{-1}. The method was successfully used to detect the concentration of clenbuterol in pork and liver samples. The recovery is in the range from 95.2% to 106.8%.

1. Introduction

Clenbuterol is a β_2-adrenergic agonist, and was originally used as a drug for the treatment of pulmonary disease and asthma [1]. Recently, it has been applied illegally in livestock for diverting nutrients from fat deposition in animals to the production of muscle tissues [2]. The illegal use of clenbuterol in food producing animals for growth promoting purposes had caused adverse health effects on human [3]. Therefore, detection of clenbuterol is quite important.

Various methods have been reported for the detection of clenbuterol, such as gas chromatography–mass spectrometry (GC–MS) [4], immunoassay [5], liquid chromatography–mass spectrometry (LC–MS) [6], capillary electrophoresis with chemiluminescence detection [7], and high-performance liquid chromatography (HPLC) [8,9]. Because of their high sensitivity, short analysis time, low cost and handling convenience, electrochemical methods using different modified electrodes were also used to detect clenbuterol. For example, a Nafion-Au colloids-modified GCE with a detection limit of 1×10^{-6} M [10], a carbon nanotubes-Nafion nanocomposite-modified GCE with a detection limit of 5×10^{-10} M [11], a pyrrole-DNA modified boron-doped diamond electrode with a detection limit of 8.5×10^{-7} M [12], and a Nafion-modified carbon paste electrode with a detection limit of 1.02×10^{-9} M [13] were also reported. However, electrochemical detection of clenbuterol using AB film-modified electrode has not been developed.

Herein, the insoluble AB nanoparticles were easily dispersed into water in the presence of dihexadecyl hydrogen phosphate (DHP). After evaporating solvent, an AB film-modified GCE was successfully prepared. AB nanoparticle is a special kind of carbon black, and exhibits many excellent properties such as large surface area, good electric conductivity, regular porous structure and strong adsorptive ability. The electrochemical behavior of clenbuterol was examined, and two sensitive oxidation peaks were observed. Compared with the unmodified GCE, the AB film-modified GCE greatly enhances the oxidation peak current of clenbuterol. Undoubtedly, the sensitivity of electrochemical determination of clenbuterol was remarkably increased by AB nanoparticles.

2. Experimental section

2.1. Reagents

All the chemicals were of analytical grade and used as received. Clenbuterol, as the hydrochloride salt, was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Stand solution of clenbuterol (1 g L^{-1}) was prepared with re-distilled water, and stored at 4 °C. Acetylene black (AB, purity >99.99%) was purchased from STREM Chemicals (USA). Dihexadecyl hydrogen phosphate (DHP) was obtained from Sigma. Britton–Robinson (B–R) buffer solutions with different pH values were purchased from the Sinopharm Group Chemical Reagent Co. Ltd., China.

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2.2. Instruments

Electrochemical measurements were performed on a CHI 830 C electrochemical workstation (Chenhua Instrument Co. Ltd., Shanghai, China) with 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 a Quanta 200 microscope (FEI Company, Netherlands).

2.3. Preparation of AB film-modified GCE

Ten milligrams of AB and 10 mg of DHP were added into 10 mL redistilled water, and then sonicated for 1 h to form a stable and homogeneous suspension. Before modification, the GCE with diameter of 3 mm was polished with 0.05 μm alumina slurry, and then sonicated in re-distilled water for 2 min. After that, the GCE was coated with 8 μL AB suspension, and the water was evaporated from the surface under an infrared lamp in air. The DHP-modified GCE was prepared by the same procedure but without AB.

2.4. Sample preparation

Different pork and liver samples were purchased from a local supermarket, and treated according to the Standard of Ministry of Agriculture of China (NY/T 486-2006). Briefly, 1.0 g of smashed sample was homogenized for 3 min with 5 mL acetic ether and 0.6 mL 10% $NaCO_3$ solution, and then centrifuged at 3500 rpm for 5 min. The homogenization was repeated twice, and each time the organic phase was collected. Subsequently, 1 mL of 0.1 M HCl was added into the organic phase. After 2-min drastic shaking and 5-min centrifugation, the lower phase was collected. After twice extraction, the extraction liquid was collected and then diluted to 10.0 mL using pH 9.15 B–R buffer solution. Spiked samples were prepared by adding a known amount of clenbuterol standard before the treatment.

2.5. Analytical procedure

Unless otherwise stated, pH 9.15 B–R buffer solution was used as the supporting electrolyte for the detection of clenbuterol. After 3-min accumulation at −0.2 V, the differential pulse voltammograms were recorded from −0.2 to 1.2 V, and the oxidation peak current at 0.61 V was measured. The pulse amplitude is 50 mV, the pulse width is 40 ms, and the scan rate is 40 mV s^{-1}.

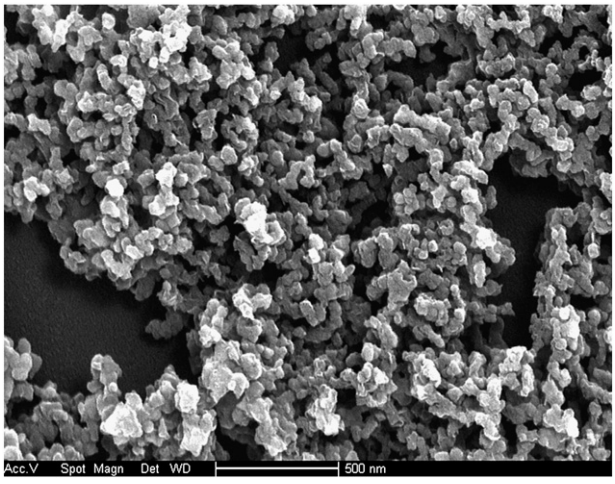

Fig. 1. SEM image of AB film.

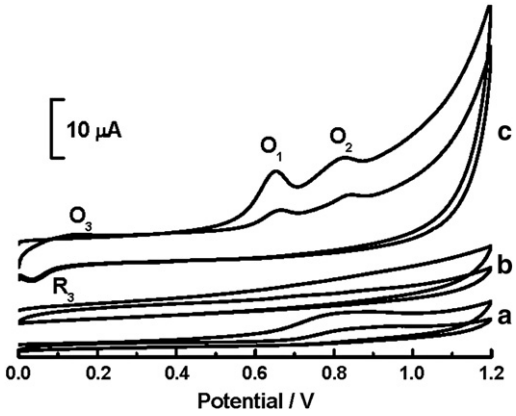

Fig. 2. CVs of 20 mg L^{-1} clenbuterol in pH 9.15 B–R buffer solution on GCE (a), DHP film-modified GCE (b) and AB film-modified GCE (c). Scan rate: 100 mV s^{-1}.

3. Results and discussion

3.1. SEM image of AB film

The morphology of AB nanoparticles on the GCE surface was characterized using SEM. As shown in Fig. 1, it was found that the GCE surface was successfully modified with AB nanoparticles, and the particle size is about 50 nm.

3.2. Surface enhancement of AB to clenbuterol

The electrochemical behavior of clenbuterol was studied using cyclic voltammetry (CV), and the results are displayed in Fig. 2. In pH 9.15 B–R buffer solution, an oxidation peak is observed on the GCE surface after addition of 20 mg L^{-1} clenbuterol (curve a). The oxidation peak is broad, and the peak potential is 0.89 V. However, the oxidation peak vanishes on the DHP film-modified GCE (curve b), and the curve becomes featureless. This suggests that DHP film blocks the electron transfer of clenbuterol. When using AB film-modified GCE (curve c), two oxidation peaks are observed at 0.65 V (O_1) and 0.83 V (O_2) during the anodic sweep from 0 to 1.2 V, and then a reduction peak appears at 0.04 V (R_3) during the reverse scan. In the second anodic sweep, another oxidation peak (O_3) is observed at 0.13 V, and the peaks of O_1 and O_2 obviously decrease. From the comparison, it is apparent that the oxidation behavior of clenbuterol shows great difference on the AB film surface, which is attributed to the unique properties of AB nanoparticles. In addition, the CV

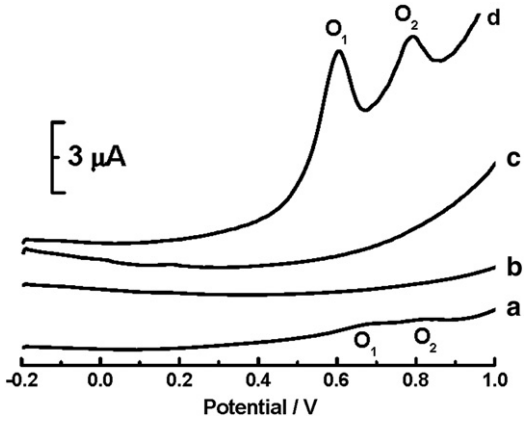

Fig. 3. DPV curves of 2 mg L^{-1} clenbuterol in pH 9.15 B–R buffer solution on GCE (a), DHP film-modified GCE (b) and AB film-modified GCE (d). (c) DPV curve of AB film-modified GCE without clenbuterol. Accumulation potential: −0.2 V; accumulation time: 3 min.