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Electrochemical sensor for toxic ractopamine and clenbuterol based on the enhancement effect of graphene oxide

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ABSTRACT

Graphene oxide (GO) and reduced graphene oxide (RGO) were prepared from graphite powder, and then used to modify the surface of glassy carbon electrode. The electrochemical behavior of ractopamine and clenbuterol, illegally used nutrient repartitioning agents, was investigated on different carbon materials surface. Compared with glassy carbon, graphite and RGO, GO exhibited strong enhancement effect and greatly increased the oxidation signal of ractopamine and clenbuterol. The functional groups were analyzed, and a large number of oxygen-containing groups were introduced on surface of GO, which was the main reason for high electrochemical activities. Furthermore, the application of GO in the simultaneous detection of toxic ractopamine and clenbuterol was studied. Based on the enhancement effect of GO, a sensitive, rapid and simple electrochemical method was developed for the simultaneous detection of ractopamine and clenbuterol. The limits of detection were 17 and 15 $\mu g\,L^{-1}$ for ractopamine and clenbuterol, and the units of sensitivity were 1.22 $\mu A/mg\,L^{-1}$ and 1.39 $\mu A/mg\,L^{-1}$. Finally, it was used to detect the content of ractopamine and clenbuterol in pork samples, and the recovery was over the range from 90.1% to 98.6%.

1. Introduction

Graphene is a single-atom thick, two-dimensional material that has attracted great attention due to its remarkable electronic, mechanical, and thermal properties [1,2]. Graphene mainly consists of GO and RGO [3], depending on the employed chemical reaction. GO is easily prepared by the oxidation of graphite powder with strong oxidants, while RGO can be obtained by the chemical reduction of GO. Compared with the pristine graphite, graphene is water dispersible and possesses many fascinating properties such as large specific surface area, numerous functional groups, strong adsorption ability, and high carrier mobility [4,5]. These features enable graphene to be a novel and promising electrode sensing material. Up to now, graphene was successfully used to modify the electrode surface for greatly improving the response signal of different species such as paracetamol [6,7], dopamine [8,9], ascorbic acid [10,11], caffeine [12], hydrogen peroxide [13,14], α-fetoprotein [15], p-nitrophenol [16], methicillin-resistant *Staphylococcus aureus* DNA [17], NADH [18,19], and hydroquinone/catechol [20,21].

Ractopamine and clenbuterol are β_2-adrenergic agonists, and originally used as drugs for the treatment of pulmonary disease and asthma [22]. However, they are illegally applied as nutrient repartitioning agents in livestock to greatly improve the production of muscle tissues [23]. Because of the potential risk to consumers for adverse cardiovascular and central nervous system effects [24,25], ractopamine and clenbuterol are not licensed for animal production in many countries. Therefore, the detection of ractopamine and clenbuterol residue in animal tissues is quite important. Until now, various methods have been developed for the detection of ractopamine and clenbuterol, including liquid chromatography–mass spectrometry (LC–MS) [26], gas chromatography–mass spectrometry (GC–MS) [27], immunoassay [28], high-performance liquid chromatography [29], and capillary electrophoresis [30].

From their molecular structures, it is apparent that ractopamine and clenbuterol should be electrochemical active since they contain phenolic hydroxyl group, which can be oxidized on electrode surface. So, electrochemical methods were also used for the detection of ractopamine or clenbuterol. For example, a Nafion–Au colloids-modified glassy carbon electrode (GCE) with a detection limit of 1×10^{-6} M [31], a carbon nanotubes–Nafion composite film-modified GCE with a detection limit of 5×10^{-10} M [32], a pyrrole–DNA modified boron-doped diamond electrode with a detection limit of 8.5×10^{-7} M [33], and a Nafion-modified carbon paste electrode with a detection limit of 1.02×10^{-9} M [34], were

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reported for the detection of clenbuterol. However, direct electrochemical detection of ractopamine is very limited. Therefore, it is of great importance and interest to study novel electrochemical method for the simultaneous determination of ractopamine and clenbuterol.

The main objective of this work is to investigate the electrochemical response of ractopamine and clenbuterol on the surface of graphene, and then develop a novel electrochemical sensor for simultaneous detection of ractopamine and clenbuterol utilizing the unique properties of graphene. To address this problem, GO and RGO were prepared and then used to modify the surface of GCE. The electrochemical behavior of ractopamine and clenbuterol showed that GO greatly increased the oxidation signal of ractopamine and clenbuterol, while graphite and RGO did not enhance the response signal. Apparently, GO exhibits high activity to the electrochemical oxidation of ractopamine and clenbuterol, and consequently increases their detection sensitivity remarkably.

2. Experimental

2.1. Reagents

Ractopamine and clenbuterol, as the hydrochloride salt, were obtained from Dr. Ehrenstorfer GmbH (Germany), and individually dissolved into doubly distilled water to prepare 0.1 g L^{-1} standard solution. Graphite powder (spectral pure), H_2SO_4, $K_2S_2O_8$, P_2O_5, H_2O_2, HCl, $KMnO_4$, Na_2HPO_4, $NaBH_4$, and citric acid (analytical grade) were purchased from Sinopharm Chemical Reagent (Shanghai, China). All chemicals were used as received, and the water was doubly distilled.

2.2. Instruments

Electrochemical measurements were performed on a CHI 830C electrochemical workstation (Chenhua Instrument, Shanghai, China) with a conventional three-electrode system. The working electrode is a graphene-modified GCE, the reference electrode is a saturated calomel electrode (SCE), and the counter electrode is a platinum wire. Scanning electron microscopy (SEM) was conducted with a Quanta 200 microscope (FEI Company, Netherlands). Fourier Transform-Infrared (FT-IR) spectra were obtained with a VERTEX 70 spectrometer (Bruker, Germany) with a KBr plate. Particle size analysis was performed with a LB-550 Dynamic Light Scattering Particle Size Analyzer (HORIBA, Japan). X-ray photoelectron spectroscopy (XPS) was measured using a VG Multilab 2000 spectrometer (USA) with Al Kα radiation (1486.6 eV) as the X-ray source for excitation.

2.3. Preparation of GO and RGO

GO was prepared from the natural graphite powder according to modified Hummer's method [35]. In brief, the graphite powder was firstly oxidized by H_2SO_4, $K_2S_2O_8$ and P_2O_5 at 80 °C for 5 h. After that, the resulting product was then oxidized using concentrated H_2SO_4 and $KMnO_4$ in ice bath for 2 h. Finally, the mixture was filtered and washed with 10% HCl solution to remove metal ions.

RGO used in this work was prepared by the chemical reduction of GO with $NaBH_4$ in a steam bath [36].

2.4. Fabrication of sensors

The resulting GO solid (10 mg) was dispersed into doubly distilled water (10 mL) through 60-min ultrasonication, giving a stable and brown GO suspension. Subsequently, the GCE 3 mm in diameter was polished with 0.05 μm alumina powder, and then sonicated in re-distilled water for 2 min. After drying, the GCE surface was coated with 3 μL GO suspension, and the water was evaporated from the surface under an infrared lamp in the air. For the comparison, RGO and graphite powder were also used to modify the GCE surface according to above procedure.

2.5. Sample preparation

Different pork samples were purchased from a local supermarket, and treated according to the No. 985 Bulletin of Ministry of Agriculture of China in 2007. Briefly, smashed sample (5.0 g) was homogenized using 10 mL 0.1 M $HClO_4$, then ultrasonicated for 20 min, and heated at 80 °C for 30 min. After cooling and 5-min centrifugation at 10,000 rpm, the clear liquid phase was collected. After that, the pH value of collected liquid was adjusted to 10 using 10% Na_2CO_3, and 4 g NaCl was added. Subsequently, ractopamine and clenbuterol were extracted twice using 10 mL ethyl acetate. Finally, ractopamine and clenbuterol were reversely extracted to 2 mL, 0.1 M HCl solution. The reverse extraction was repeated, and the sample solution was diluted to 10 mL using pH 3 disodium hydrogen phosphate-citric acid buffer. Spiked samples were prepared by adding a known amount of ractopamine and clenbuterol standard before the treatment.

2.6. Analytical procedure

Disodium hydrogen phosphate–citric acid buffer solution with pH of 3 was used as supporting electrolyte for the detection of ractopamine and clenbuterol. After 3-min accumulation under open-circuit, the differential pulse voltammetry (DPV) curves were recorded from 0.3 to 1.2 V, and the oxidation peak current at 0.81 and 1.03 V was individually measured for ractopamine and clenbuterol. The pulse amplitude is 50 mV, the pulse width is 40 ms and the scan rate is 40 mV s^{-1}.

3. Results and discussion

3.1. Properties of GO and RGO

The surface morphology of unmodified, graphite-modified, GO-modified and RGO-modified GCEs was individually characterized using SEM. As displayed in Fig. 1A, the unmodified GCE surface is smooth. After modification with graphite, large and irregular particles were observed in Fig. 1B, revealing poor water dispersion of graphite powder. On the surface of GO-modified GCE (Fig. 1C) and RGO-modified GCE (Fig. 1D), homogeneous, flexible and wrinkled sheets were observed, suggesting excellent water dispersion ability. In addition, the particle size of graphite, GO and RGO was compared. Fig. 2 shows the size distribution of GO suspension (A) and RGO suspension (B). The size distribution of graphite is not given because its size is larger than 6 μm, beyond the measurement range. Undoubtedly, the pristine graphite is very difficult to be dispersed into water. However, the average size of GO and RGO decreased to 368 and 246 nm. The remarkable size decline clearly indicates that GO and RGO are water dispersible. In conclusion, GO and RGO exhibit excellent water dispersion compared with graphite, resulting in great difference on SEM properties.

3.2. Electrocatalytic activity to the oxidation of ractopamine and clenbuterol

The electrochemical behavior of ractopamine and clenbuterol was studied using DPV on the unmodified, graphite-modified, GO-modified and RGO-modified GCEs. Fig. 3 displays the oxidation