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A sensitive electrochemical sensor with sulfonated graphene sheets/oxygen-functionalized multi-walled carbon nanotubes modified electrode for the detection of clenbuterol

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ABSTRACT

A novel electrochemical sensor was designed to determine clenbuterol by using a new hybrid nanomaterials of water-dispersible sulfonated graphene sheets (SGSs) and oxygen-functionalized multi-walled carbon nanotubes (MWCNTs-COOH) modified glass carbon electrode (GCE). The π–π stacking interaction between SGSs and MWCNTs-COOH was verified by UV–vis spectroscopy, fourier transform-infrared spectroscopy and scanning electron microscopy. The anodic peak current (Ipa) of clenbuterol at the GCE modified with SGSs/MWCNTs-COOH was much higher than those at the bare GCE, SGSs/GCE and MWCNTs-COOH/GCE due to the synergistic effect of the modified nanomaterials. Differential pulse voltammetry disclosed a good linear relationship between Ipa and concentrations of clenbuterol (0.01–5.0 μM), with the detection limit of 4.6 nM. Finally, the modified electrode was successfully used to determine clenbuterol in liver samples.

1. Introduction

Clenbuterol, a representative of synthesized β2-adrenergic agonists, was originally developed for the treatment of pulmonary diseases and asthma in humans and animals [1,2]. However, the animals fed clenbuterol usually suffer from side effects such as reduced fat levels and increased muscle protein anabolism. In addition, drug residue of clenbuterol may be prone to accumulation in meat and body tissues for a long time, which causes acute poisoning after eating, giving rise to muscular pain, dizziness, cardiac palpitation and vomiting [3]. Hence, β2-adrenergic agonists including clenbuterol have been banned for animal production in most countries including China to ensure safety [4]. The United Nations Food and Agriculture Organization recommends a daily allowable dose of 0–0.004 μg kg^{-1} for clenbuterol [5], and the European Union has established maximum residue limits of 0.1 μg kg^{-1} in muscles, 0.50 μg kg^{-1} in liver and kidneys, and 0.05 μg kg^{-1} in milk [6]. Therefore, it is necessary to put forward a sensitive method for the determination of clenbuterol.

Nowadays, different analytical methods, including liquid chromatography–mass spectrometry [7,8], gas chromatography–mass spectrometry [9,10], enzyme-linked immunoassay with polyclonal or monoclonal antibodies [11], capillary electrophoresis [12] and electrochemical determination [13], have been developed to detect clenbuterol in animal tissues, feedstuff and body liquids. Although the methods are highly selective and sensitive for determining clenbuterol, the instruments *per se* as well as their running and maintenance are often expensive. Moreover, the enzymes which are intrinsically fragile always require relatively rigorous temperature, pH, humidity and non-toxic chemicals [14,15]. Thus, it is crucial to find out inexpensive and facile methods for analyzing clenbuterol.

Graphene, which comprises two-dimensional (2D) single or few layers of sp^2-hybridized carbon atom sheets, has received huge attention in electrochemical analysis since its discovery in 2004 because of unique nanostructure and extraordinary properties [16]. Modified graphene oxide (GO) sheets such as sulfonated graphene sheets (SGSs) and partially reduced GO sheets are superior to ordinary GO ones. Recently, graphene-based materials have also been utilized as catalysts and biosensors. Graphene-based

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nanocomposites such as polymer/graphene, metal oxides/graphene, partly reduction graphene and SGSs have been successfully designed and used due to the π–π interaction between graphene sheets [17,18].

Carbon nanotubes (CNTs), which were discovered by Iijima in 1991, have been highlighted as electrochemical sensors owing to unique structures and excellent properties [19]. However, CNTs are not easily soluble in a large number of solvents, thus significantly limiting their manipulations and applications, especially in the fabrication of CNTs-modified electrodes. Hence, various functionalized CNTs have been synthesized in order to facilitate the suspension in aqueous solution. For example, Shi et al. investigated the antioxidant behavior of hydroxylated multi-walled carbon nanotubes (MWCNTs) in high density polyethylene [20]. Korani and Salimi prepared an amine-derivative MWCNTs-modified electrode based on the covalent attachment of glucose dehydrogenase and safranin O [21].

In this study, we employed a stable aqueous dispersion of hydrophilic SGSs/MWCNTs-COOH hybrid nanomaterials to prepare a modified electrode for the first time. The electrochemical behavior of clenbuterol was evaluated using the electrode, and the catalytic mechanisms of clenbuterol at the electrode were investigated. The modified electrode provided a large electrochemically active surface area for the catalysis of clenbuterol and effectively accelerated the electron transfer between the electrode and solution, which allowed a more rapid and sensitive current response. Besides, the modified electrode exhibited better sensitivity and selectivity for clenbuterol detection in liver samples with satisfactory recoveries.

2. Experiment

2.1. Reagents and apparatus

Hydrazine (98 wt% in water) and graphite powder (99%, 40 nm) used for synthesizing GO and SGSs were obtained from Aladdin Inc. (Shanghai, China). MWCNTs (95%, 10–20 nm in diameter and 10–30 μm in length) used for synthesizing oxygen-functionalized MWCNTs (MWCNTs-COOH) were purchased from DKnano Co., Ltd. (Beijing, China). Clenbuterol was purchased from National Institutes for Food and Drug Control (Beijing, China). Phosphate buffer solutions (PBS) with pH ranging from 5.0 to 10.0 were prepared by mixing stock solutions of 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4, and pH was adjusted with H_3PO_4 (1.0 M) and NaOH (1.0 M) solutions. Unless otherwise stated, all the other chemicals were of analytical grade and purchased from Guangzhou Chemical Reagent Co., Ltd. (Guangzhou, China). Deionized water was used to prepare the aqueous solutions.

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on a CHI 850 C electrochemical workstation (CH Instrument, Shanghai, China). A conventional three-electrode system was used for all electrochemical experiments. Bare or modified glass carbon electrode (GCE, Φ = 3 mm) acted as the working electrode, and an Ag/AgCl-saturated KCl electrode and a platinum electrode were used as the reference electrode and the auxiliary electrode (Gaoss Union, Wuhan, China), respectively. UV–vis absorption spectroscopy was conducted with a Shimadzu 2550 probe spectrophotometer (Kyoto, Japan). Fourier-transform infrared (FT-IR) spectrum was obtained on a Mattson Cygnas 100 FT-IR spectrometer (Shelton, USA). The surface of the modified electrode was examined by a scanning electron microscope (SEM), and images were obtained from a Quanta 400F thermal field emission environmental FEI-SEM (Philips, Amsterdam).

2.2. Preparation of MWCNTs-COOH and SGSs modified materials

MWCNTs-COOH were synthesized according to a previously reported method [22]. In a typical experiment, 75.0 mL of H_2SO_4 (98%) and 25.0 mL of HNO_3 (65%) were mixed and added to 1.0 g of MWCNTs in a round-bottomed flask and refluxed under constant circumfluence at 50 °C for 8.0 h. After the experiment, MWCNTs-COOH were obtained, and then washed to neutral by deionized water. The residue was then filtered and vacuum-dried.

Water-dispersible SGSs were synthesized from graphite powders. Three steps were involved in this process. Initially, GO was synthesized according to the modified Hummer's method reported by Kovtyukhova et al. [23]. The obtained GO was dispersed in deionized water, yielding a dark brown suspension. Secondly, sodium borohydride was used to reduce GO. Thereafter, as reported previously by Li et al. [15], a black suspension was prepared by 4 h of reaction with aryl diazonium salt of sulfanilic acid in an ice bath that kept the temperature below 5 °C. Thirdly, 98 wt% hydrazine was added into the dispersion. The mixture was kept at 100 °C for 24 h under stirring to complete the reduction. The powders were obtained by being centrifuged, washed with deionized water several times and dried at 60 °C for 24 h.

The hybrid nanomaterial (SGSs/MWCNTs-COOH) was prepared according to a previous literature with slight modifications [24]. Particularly, 5.0 mg SGSs were mixed with 5.0 mg formal MWCNTs-COOH in 10 mL deionized water and sonicated for 2 h. The resulting suspension was then centrifuged so that the as-prepared SGSs/MWCNTs-COOH hybrid nanomaterial could be well dispersed in water and stored as a 0.5 mg mL^{-1} aqueous solution.

2.3. Preparation of the SGSs/MWCNTs-COOH modified electrode

Prior to modification, the prepared GCE (Φ = 3 mm) was mechanically polished twice with 0.05 μm α-Al_2O_3 to a mirror finish. Thereafter, the polished electrode was rinsed with deionized water, sonicated in HNO_3 (1:1), dehydrated alcohol and deionized water successively, and dried in air at room temperature. Then 10 μL of the hybrid SGSs/MWCNTs-COOH dispersion was cast on the electrode surface with a micro-syringe and the solvent was evaporated at room temperature in air to obtain the SGSs/MWCNTs-COOH/GCE. Before each measurement, the modified electrode was rinsed with PBS (0.1 M) and then scanned from −0.2 V to 1.0 V, and the analyte adsorbed on the modified electrode surface after measurement was readily removed by soaked in the KOH (0.5 M) solution for half a minutes.

2.4. Preparation of samples

Pork liver samples were purchased from a local supermarket, and were pretreated according to previous literatures [25,26]. Briefly, 1.0 g smashed sample was homogenized using 2 mL of 0.1 M $HClO_4$, ultrasonicated for 20 min, and then heated at 80 °C for 30 min. After cooling and 15 min of centrifugation at 3000 rpm, the clear liquid phase was collected. Then pH of the collected liquid was adjusted to 9–10 by using 1.0 M Na_2CO_3, into which 1.6 g NaCl was then added. Subsequently, clenbuterol was extracted twice by using 20 mL of ethyl ether. Finally, clenbuterol was reversely extracted by 2 mL of 0.1 M HCl solution. The reverse extraction was repeated, and the sample solution was diluted to 10 mL by using pH 7.0 PBS.

3. Results and discussion

3.1. Characteristics of SGSs/MWCNTs-COOH/GCE

The improvement in the physicochemical properties of the SGSs/MWCNTs-COOH composite depends on the distribution of SGSs in the MWCNTs-COOH bundles as well as the interfacial bonding between the SGSs and MWCNTs-COOH bundles. The surface