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Biomolecule-free, selective detection of clenbuterol based on disposable screen-printed carbon electrode

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ABSTRACT

We report here the development of a selective clenbuterol sensor made of disposable screen-printed carbon electrode (SPCE) without the need of adding any biorecognition element. Good analytical performance was achieved through the proper function of both the oxygen functionalities and edge plane sites on the "preanodized" SPCE (SPCE*). It is the amino group of clenbuterol to effectively form hydrogen bond with the SPCE* to induce the adsorption of clenbuterol. The edge plane sites enhance the electron transfer process and further help the dimer formation of clenbuterol to generate electroactivity for analysis. Square wave voltammetry was applied to increase the detection sensitivity with a linear response in the range of 7–1000 ppb and a detection limit of 0.51 ppb (S/N = 3). In the real sample analysis, results observed were satisfactory with meat, human blood, and human urine. High reproducibility in sensor fabrication further favors the disposable purpose of applications.

1. Introduction

Clenbuterol, a member of β_2 adrenergic agonists, is widely used as a food additive for livestock and a kind of stimulant for athletes. It also acts as a repartitioning agent to improve the performance of food-producing animals. Food safety has been a concern because the residual clenbuterol remaining in organism can cause cardiovascular and central nervous diseases in human beings [1–3]. In 1996, European communities announced Council Directive 96/22/EC that prohibited its use in stimulating the growth and yield of animals [4]. It is thus necessary to develop a fast and cost-effective method to determine clenbuterol level in meat and suspected urine or blood.

Several methods including liquid chromatography tandem mass spectrometry with the help of extraction process, immunosensors with desired antigen–antibody reaction and chemically modified glassy carbon electrodes with proper function of MWCNT–Nafion, graphene oxide, or Nafion–Au nanocomposite have been employed in the detection of clenbuterol [5–14]. We report here a simplified method based on a disposable screen-printed carbon electrode (SPCE) for selective clenbuterol detection without adding biorecognition element. As reported by several groups, either the content of oxygen functional groups or the edge plane site can be tuned to improve the performance of chemical sensors under a suitable preanodization condition [15–22]. We expect that both the oxygen functional groups and edge plane site can effectively help the adsorption and dimer formation of clenbuterol for electroanalysis. Note that the function is similar to those in the Nafion–Au nanocomposite modified glassy carbon electrode [13], where gold colloids acted as an active surface and as an electron-conducting tunnel as well for clenbuterol electron transfer to help the dimer formation and the negatively charged Nafion film can help the accumulation of positively charged clenbuterol. The advantage of disposable sensors to prevent cross-contamination between different samples represents a great attraction for sensing application.

2. Materials and methods

All chemicals and reagents were analytical grade and used as received. Clenbuterol stock solution (100 ppm) was prepared in pH 7.0 PBS and stored at 4 °C. The three-electrode system consisted of an SPCE (Zensor R&D, Taiwan) as working electrode and a platinum wire and Ag/AgCl as auxiliary and reference electrode, respectively. The preanodization process was performed by applying 2.0 V for 300 s at a bare SPCE to form the SPCE*. Radiofrequency oxygen plasma treatment was carried out at 50 Torr, 100 W for 30 s, and the as-form electrode was designated as OPSPCE. The electrochemical experiments were carried out with a CHI 8021 electrochemical workstation (CH Instrument, Austin, TX). Accumulation of clenbuterol on electrode surface was performed by applying a potential of 1.1 V for 10 s and then detected by square wave voltammetry (SWV) in the range of 0.6 V to −0.1 V with potential step = 4 mV, amplitude = 25 mV, and frequency = 25 Hz. Meat samples (20 g in 150 mL water) were heated at 80 °C for 15 min and then filtered with a 0.45 μm paper. After cooling to room temperature, the solution was diluted to 500 mL and stored at 4 °C prior to use.

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Human serum and urine samples were proper diluted with pH 7.0 PBS and then analyzed by standard addition method.

3. Results and discussion

3.1. Voltammetric behavior of clenbuterol at the SPCE*

We first compared the cyclic voltammetric responses at SPCE, OPSPCE, and SPCE* in the presence/absence of 1 ppm clenbuterol in 0.1 M, pH 7.0 PBS. As shown in Fig. 1A, it is interesting to observe that only the SPCE* exhibited a redox peak at ~0.2 V, presumably coming from the peak at ~1.0 V. According to our previous study [16], the OPSPCE contains only oxygen functional groups without much edge plane defects. In contrast, the SPCE* possesses not only oxygen functionalities but also highly electroactive edge plane sites on electrode surface. It is thus confirmed that specific edge plane sites are required to provide electron transfer activity for generating the reversible redox detection signal.

We believe that the dimerization of clenbuterol molecule is responsible for the generated electroactivity at the SPCE*. Cyclic voltammetric

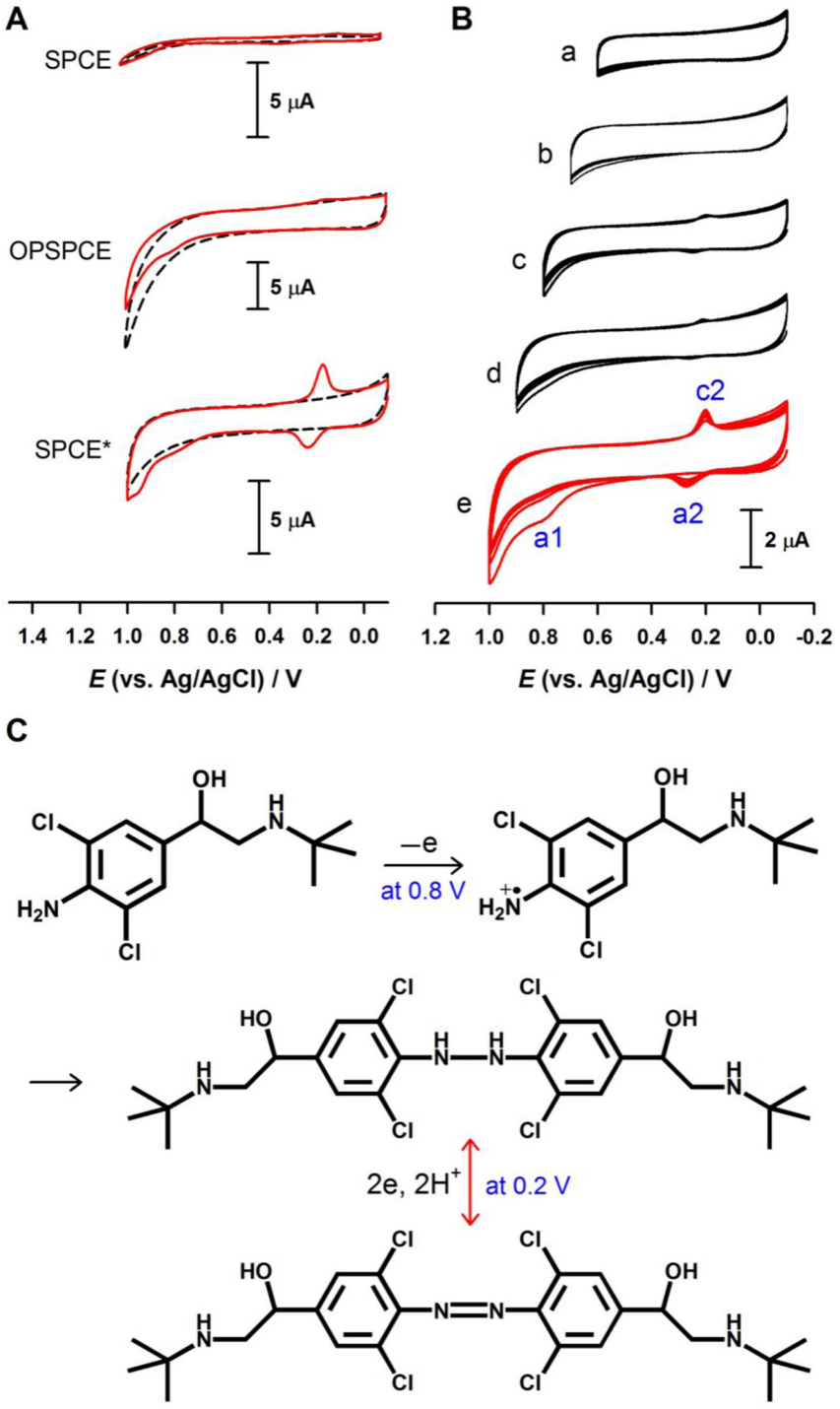

Fig. 1. (A) Cyclic voltammetric responses at SPCE, OPSPCE, and SPCE* in the presence (solid line)/absence (dashed line) of 1 ppm clenbuterol in 0.1 M, pH 7.0 PBS at a scan rate of 50 mV/s. (B) Potential segment experiments of 1 ppm clenbuterol at SPCE* in the range of −0.1 V to (a) 0.6, (b) 0.7, (c) 0.8, (d) 0.9, and (e) 1.0 V in 0.1 M, pH 7.0 PBS. (C) Schematic representation for the formation of clenbuterol dimer through an ECE mechanism.