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An electrochemical immunosensor for rapid determination of clenbuterol by using magnetic nanocomposites to modify screen printed carbon electrode based on competitive immunoassay mode

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ABSTRACT

A novel magnetic field controllable and disposable electrochemical immunosensor for rapid determination of clenbuterol (CLB) was fabricated by graphene sheets (GS)-Nafion (Nf) film dropped on the screen-printed carbon electrode (SPCE) and then Fe_3O_4-Au nanoparticles (GoldMag particles, GMP) coated bovine serum albumin-CLB (BSA-CLB) conjugates were absorbed on it with the aid of external magnetic field. X-ray powder diffractometer (XRD), electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM) were employed to characterize the synthesized GS and the construction processes of the modified electrode. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used to study its electrochemical properties. The content of CLB was determined with a competitive immunoassay mode. When different concentration of CLB and 2.0 μg/mL anti-CLB were added to the phosphate buffer solution (PBS) containing 2 mmol/L $K_3[Fe(CN)_6]$, the percentage of anodic peak DPV current increase ratio (CI%) was proportional to the concentration of CLB over the range of 0.5 ng/mL to 200.0 ng/mL after incubation for 15 min at 35 °C. The detection limit was 0.22 ng/mL. The immunosensor was employed to determine CLB in pork samples and the results were consistent with high-performance liquid chromatography (HPLC) method. The proposed electrochemical immunosensor is sensitive, rapid, magnetic field controllable, low sample consumable and disposable, which is suitable for determining trace CLB in real samples.

1. Introduction

Clenbuterol ($C_{12}H_{18}Cl_2N_2O$, CLB) is a member of β-agonists, which is widely used orally in the treatment of asthma. Some studies have been reported that the CLB can promote muscle growth and reduce body fat. Therefore the cost of animal production could be reduced [1]. When animals are treated with CLB, residues could accumulate in their meat and liver, which may have a pharmacological effect on human. Thus, CLB has been banned for feeding animals all over the world. However, driven by economic interests, illegal abuse of CLB was never stopped [2]. So far, the misuse of CLB in animal feeds and the residues of CLB in animal tissues have drawn substantial attention. Accordingly, exploring the sensitive, rapid and simple analytical methods for precise monitoring of CLB is in urgent need [3].

To date, various analytical methods have been reported for the determination of CLB, including gas chromatography–mass spectrometry (GC–MS) [4], liquid chromatography–MS (LC–MS) [5], high-performance LC (HPLC) [6], capillary electrophoresis (CE) [7] and enzyme-linked immunosorbent assay (ELISA) [8]. Although the determination of CLB by these methods is promising because of their high selectivity and sensitivity, these instruments are inherently expensive, pretreatment for samples is involved and the operating processes are complicated and long. From the molecular structure, it is apparent that CLB should be electrochemical active, since it contains phenolic hydroxyl group, which can be oxidized on the electrode surface. Thus, electrochemical methods are also used for the detection of CLB and have attracted growing interests due to their high-sensitivity, portability, low cost and short analytical time. However, direct electrochemical detection of CLB is very limited. For example, the Nafion (Nf)-Au colloids modified glassy carbon electrode (GCE) [9], the Pt nanoparticles modified gold electrode [10], the molecularly imprinted polymer (MIP)-modified solid binding matrix composite electrode (SBMCE) [11], the Nf modified carbon-paste electrode [12], the poly (acid chrome blue K)/graphene oxide-Nf/GCE [13] and

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the acetylene black (AB)-dihexadecyl hydrogen phosphate (DHP) composite film modified GCE [14] were reported for the direct electrochemical detection of CLB in previous papers. Therefore, it is of great importance and interest to develop other novel electrochemical methods for determination of CLB.

Recently, He et al. [15] developed a label-free electrochemical immunosensor based on carbon nanotube for rapid determination of CLB and Zhan et al. [16] introduced amperometric immunosensor for determination of CLB based on enzyme-antibody coimmobilized ZrO_2 nano probes as signal tag. Although all the above methods and materials showed improved signals and performance, new materials, especially novel nanocomposites are still needed to develop highly sensitive and selective CLB sensing platform. Because the nanocomposites have some synergistic effects compared with single nanoparticles [17–19], such as high surface area, rapid electron transport, low detection limit and good signal-to-noise ratio. Graphene sheets (GS)-based material has been developed as a kind of advanced nano-materials for constructing CLB electrochemical immunosensor [20]. The core–shell Fe_3O_4-Au magnetic nanoparticles (GoldMag particles, GMP), with Au coating the magnetic nanoparticles of Fe_3O_4, exhibits suitable intrinsic properties of the magnetic core and Au shell. It is anticipated that incorporation of Au coating on a magnetic core could attain both the advantages of chemical stability, biocompatibility of Au and magnetic separation of Fe_3O_4. The application of GMP in immunosensors has the following advantages: (i) rapid and non-chemical damaging regeneration of the immunosensors and the electrode was easy to renewable by using an external magnetic field; (ii) extremely high surface-to-volume ratio and biocompatibility in the presence of Au, resulting in increased sensitivity; and (iii) prevents cluster aggregation when GMP are directly used as the label [21,22]. Screen-printed carbon electrode (SPCE) is frequently used for the construction of simple portable devices for fast screening purposes and in-field/on-site monitoring, because of their low cost and easy integration into mass-production processes [23,24]. Additionally, Nf is a perfluorinated sulfonated cation exchanger, which consists of a linear backbone of fluorocarbon chains and ethyl ether pendant groups with sulfonic cation exchange sites. It was used as the best performing cation exchange membrane. Some examples of Nf film modified electrodes used to detect CLB have been demonstrated in the literatures [9,12,13]. Those studies demonstrated that GS and GMP based nanocomposites combined with SPCE could well meet the requirements of field detections of CLB.

To the best of our knowledge, there is no report based on GS and GMP nanocomposites for determination of CLB. In this paper, we report on a novel electrochemical immunosensor for the rapid determination of CLB by using GS-Nf film and GMP coated bovine serum albumin (BSA)-CLB conjugates modified SPCE (SPCE|GS-Nf/GMP-BSA-CLB) based on competitive immunoassay mode. The resulting immunosensor combines the advantages of GS, Nf, GMP and SPCE. In addition, the fabrication of the modified electrode is simple, fast and reproducible. Finally, the modified electrode has been successfully applied to analyze CLB in pork samples with good recovery and accuracy, demonstrating its potential use for real sample analysis.

2. Experimental

2.1. Reagents

CLB, rabbit anti-CLB antibody (anti-CLB) and BSA-CLB conjugates were purchased from Taizhou Jinxin biotechnology limited company (Wuhan, China). BSA (Sigma–Aldrich). Graphite powder was obtained from green battery material limited company (Changsha, China). GMP were purchased from Xi'an Goldmag Nanobiotech Co., Ltd. (particle size: 30 nm, 5 mg/mL, Xi'an, China). Nf (5% ethanol solution), 30% H_2O_2 solution and other chemicals were of analytical-reagent grade and used without further purification were obtained from Sinopharm Medicine Holding Co., Ltd. (Shanghai, China). The 0.1 mol/L phosphate buffer solution (PBS) at various pH values were prepared by mixing the stock solutions of 0.1 mol/L NaH_2PO_4 and 0.1 mol/L Na_2HPO_4 with different proportion. Doubly distilled water (18.2 MΩ resistance) was used throughout. Pork samples (Jiahui Supermarket, Huaihua, July, 2013). All experiments were carried out under N_2 protection.

2.2. Apparatus

Electrochemical measurements were performed on a CHI 660D electrochemical workstation (CHI Instruments, Shanghai, China). SPCE was purchased from DropSens corporation (Spain, the working electrode was modified, the auxiliary and reference electrode was screen-printed carbon and Ag/AgCl electrode, respectively). The morphology of different electrodes was characterized by scanning electron microscopy (SEM, Hitachi S-4800N). The X-ray powder diffractometer (XRD, Rigaku Ultima IV, Cu Kα radiation) was used to determine the phase purity and crystallization degree of GS. The HPLC (Shimadzu, LC-10AT) was also used for the measurements of the concentration of CLB in pork samples.

2.3. Preparation of GS solution

Firstly, the graphite oxide was synthesized from natural graphite powder according to the literature with a modified Hummers method [25]. Then, exfoliation of graphite oxide to graphene oxide (GO) was achieved by ultrasonication of the dispersion for 30 min. Finally, a bright yellow homogeneous aqueous dispersion was obtained. As a large number of carboxyl, hydroxyl hydrophilic groups have been introduced between the layers of carbon atoms, GO is soluble in water. The resulting homogeneous dispersion of GO (0.1 g) was mixed with 50 μL hydrazine solution. After being vigorously shaken or stirred for a few minutes, the solution was stirred for 24 h at the temperature of 80 °C to form GS. GS tend to form irreversible agglomerates or even restack to graphite through strong π–π conjugation and van der Waals interaction. Thus, it is difficult for GS to be directly dispersed into solvent to form a uniform dispersion. This brings about the difficulty for the construction of electrochemical sensing platforms. Herein, Nf was selected as a stabilizer to disperse GS into an aqueous solution. Due to its excellent capability for film formation, nontoxicity, biocompatibility, mechanical strength and good water permeability, Nf is commonly used to disperse nanomaterials and immobilize enzymes for constructing immunosensors [26].

2.4. Preparation of the GMP-BSA-CLB conjugates

GMP was first pretreated with Tris–HCl as coupling agent. About 100 μL of GMP solution was suspended in 100 μL of 0.02 mol/L Tris–HCl solution (pH = 7.5). The mixture was then allowed to react under gentle stirring, followed by collection under an external magnetic field. Subsequently, 100 μL of BSA-CLB solution (1 mg/mL) and 50 μL GMP solution (1 mg/mL) were added to 5 mL Tris–HCl solution. The mixture was shaken at 35 °C for 20 min at 180 rpm. In order to keep the formed monolayer insulating and pin-free, and to ensure a high sensitivity, BSA was used to block uncovered spaces on the GMP surface. Thus, the resulting conjugates (labeled as GMP-BSA-CLB) were treated with 1 mL BSA solution (w = 0.25%)