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Analytical Methods

Sensitive voltammetric sensor based on Isopropanol–Nafion–PSS–GR nanocomposite modified glassy carbon electrode for determination of Clenbuterol in pork

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ABSTRACT

In the present study, poly(sodium 4-styrenesulfonate) (PSS) functionalized graphene (GR) was synthesised via a simple one-step chemical reduction of exfoliated graphite oxides in the presence of PSS. Characterisation of as-made nanocomposite using Fourier transform infrared spectroscopy (FT-IR) and ultraviolet and visible spectroscopy (UV–vis) clearly demonstrate the successful attachment of PSS to graphene sheets. A novel clenbuterol (CLB) electrochemical sensor was fabricated based on isopropanol–Nafion–PSS–GR composite film modified glassy carbon electrode. In the Britton–Robinson buffer (pH 1.2), the sensor exhibited superior electrocatalytic activity towards the oxidation of CLB. Applying linear sweep voltammetry, a good linear relationship of the oxidation peak current with respect to concentrations of CLB cross the range of 7.5×10^{-8}–2.5×10^{-5} mol L^{-1} and a detection limit of 2.2×10^{-8} mol L^{-1} were achieved. The proposed method was successfully applied for the determination of CLB in pork.

1. Introduction

Clenbuterol is a β-agonist drug that originally developed for the treatment asthma of patients. Currently, it has been abused as food additive for livestock because it can enhance the muscle growth and decrease fat deposition (Aharoni, Orlov, Brosh, Granit, & Kanner, 2005), thus reducing the cost of animal production. However, the large accumulation of CLB in animal tissues may pose a severe threat to humans, causing cardiac palpitation, vomiting, nausea, and chills (Brambilla et al., 2000). Therefore, it has been banned from animal husbandry over most of the world (Fan, Miao, Zhao, Chen, & Wu, 2012). To eliminate the abuse of CLB, it is imperative to develop a fast, sensitive method to determine CLB levels in suspected urine or animal products. Many methods including GC–MS (Abukhalaf et al., 2000), HPLC (Wen, Wang, & Feng, 2007), radioimmunoassay (Delahaut et al., 1991), ELISA (Sawaya, Lone, Husain, Dashti, & Saeed, 2000) and electrochemical methods (Bo et al., 2013; Guo, Xu, Wang, & Hu, 2008; Li, Zhen, & Pingli, 2010; Lin, Ni, & Kokot, 2013; Lin, Ni, Li, & Kokot, 2012; Liu, Pan, Du, Xie, & Wang, 2010; Moane, Barreira Rodriguez, Miranda Ordieres, Tuñón Blanco, & Smyth, 1995; Wang et al., 2013; Wu, Sun, Li, & Wu, 2012) have been developed for determination of CLB in meat products or other samples. Among them, electrochemical methods are more attractive due to their high sensitivity, low cost, and simplicity. Although some electrochemical methods have greatly improved the electroanalysis of CLB, there are still some challenges, such as low sensitivity (Bo et al., 2013; Li et al., 2010; Liu, Zhang, Yang, Wang, & Zhu, 2010; Moane et al., 1995; Wu et al., 2012), complexity (Guo et al., 2008; Lin et al., 2013; Wang et al., 2013) and the high cost of platinum or nanoparticulate metal (Bo et al., 2013; Lin et al., 2012; Liu, Pan, et al., 2010; Wang et al., 2013), which adversely affect CLB detection. Therefore, it is essential to identify more sensitive, more selective, and simpler methods for the determination of CLB.

Graphene, as a "rising star" material, has attracted worldwide attention in recent years because of its unique properties. The excellent thermal, mechanical, and electrical (Xu, Bai, Lu, Li, &

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Shi, 2008) properties of graphene afford great promise for many potential applications such as sensors, capacitors, and batteries. However, due to van-der Waals interactions and strong π–π stacking, graphene sheets tend to form irreversible agglomerates, and even restack to form graphite. The propensity to agglomerate may limit its further application, especially for electrochemical sensors, because most of its unique properties are only associated with individual sheets (Liu, Zhang, et al., 2010). Fortunately, this problem has been initially resolved through covalent (Ghosh, Rao, George, & Rao, 2010) or noncovalent (Liu, Tian, et al., 2010; Xu et al., 2008; Yang, Pan, Huang, & Li, 2010) functionalization, of which noncovalent strategies, particularly using polyelectrolytes as functional agents, are more favourable than covalent ones. For PSS, an amphiphilic polymer, the negatively charged poly(sodium 4-styrenesulfonate) units assist the dispersion of graphene in water by electrostatically preventing the restacking of graphene, and thus preserving the intrinsic electronic and structural properties of graphene (Guoxiu et al., 2009; Stankovich et al., 2006). Accordingly, functionalizing graphene with PSS could be an effective method to increase its solubility and provide the opportunity for coating electrodes.

GR and graphene oxidative (GO) have good electrochemical catalytic activity for CLB (Lin et al., 2013; Wu et al., 2012). Relative to GR or GO, PSS–GR may be more favourable to enrich CLB onto the electrode surface because it can bind CLB not only through π–π interaction just as GR (GO) with CLB, but also can bind through electrostatic interaction between the negatively charged PSS and the positively charged CLB under acidic conditions. On the basis of that, it is supposed that PSS–GR will be beneficial to develop highly sensitive electrochemical sensor for the determination for CLB.

In this work, PSS–GR was successfully synthesised via a simple one-step chemical reduction. Then, electrochemical behaviours of CLB were studied on an isopropanol–Nafion–PSS–GR modified glassy carbon electrode, which gave favourable electrocatalytic behaviour towards the electro-oxidation of CLB. And moreover, the method was applied to the determination of CLB in real samples.

2. Materials and methods

2.1. Reagents

Natural graphite flakes with the average diameter of 200 mesh was purchased from Sigma–Aldrich. PSS (MW = 70 000) was purchased from Sigma–Aldrich. Nafion (wt. 5%) was purchased from the Shanghai HeSen Electric Co., Ltd (Shanghai, China). Clenbuterol was purchased from the food and drug verification research institute (Beijing, China). Britton–Robinson buffer (pH = 1.2) was prepared using 11.5 mL acetic acid (99%), 13.5 mL phosphoric acid (85%), 12.44 g boric acid and 650 mL H_2O. A 0.05% solution of Nafion was obtained by a 1:1 water:isopropanol dilution of the 5% stock solution. All the other chemicals used were of analytical grade without further purification. Redistilled water was used throughout.

2.2. Apparatus

A Bruker IFS 66v/s infrared spectrometer was used for recording the IR spectra. UV–vis spectra were obtained on a T6 UV–Vis Spectrophotometers (Purkinje General, Beijing, China). Electrochemical measurements were conducted on a CHI 660E Electrochemical Workstation (Chen-hua, Shanghai, China). A conventional three-electrode system was used, including a bare GCE (diam. = 3 mm) or an isopropanol–Nafion–PSS–GR modified GCE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire electrode as the auxiliary electrode. All of the pH values were measured by a PHS-3C precision pH meter (Leici Devices Factory of Shanghai, China), which was calibrated with standard buffer solution every day.

2.3. Preparation of PSS–GR nanocomposite

Graphene oxide (GO) was synthesised using the modified Hummer method (Bose et al, 2010). PSS–GR nanocomposite was prepared as follows: 0.2 g GO was added into 200 mL PSS solution (0.01 mg mL^{-1}). After being sonicated vigorously for 30 min, 10 mL hydrazine monohydrate was added and heated at 95 °C for 3 h. Finally, the mixture was centrifuged to remove excess hydrazine and PSS. The product was dried under vacuum at 50 °C overnight and named as PSS intercalated graphene (PSS–GR).

2.4. Preparation of isopropanol–Nafion/PSS–GR modified GCE

Before modification, the bare GCE was polished to a mirrorlike surface with a 0.03 μm alumina slurry and washed cleanly. Next, 4.0 mg of the PSS–GR composite was added into 8 mL of isopropanol–Nafion solution and sonicated for 1 h to obtain a homogenous mixture of isopropanol–Nafion–PSS–GR. Then, 10 μL of the isopropanol–Nafion–PSS–GR composite was deposited on the polished GCE surface and dried under an infrared lamp. The isopropanol–Nafion modified electrode was fabricated with a similar procedure.

2.5. Electrochemical measurements

CV and linear sweep voltammetry (LSV) measurements were carried out in an unstirred Britton–Robinson buffer (pH = 1.2) at room temperature. In a typical process, 5 mL of Britton–Robinson solution was transferred into a clean electrochemical cell, then the required volume of CLB or sample solution was added by micropipette. After accumulation for 180 s at −0.2 V, CV was performed from 0 to 1.2 V with the scan rate of 50 mV s^{-1}. LSV was carried out from 0.5 to 1.2 V with the following parameters: scan rate, 50 mV s^{-1}; sample interval, 1 mV; quiet time, 2 s. Due to the strong absorption of clenbuterol onto the electrode surface, after each determination, the used electrode was cleaned by immersing it into 0.01 mol L^{-1} NaOH for 3 min (Guo et al., 2008).

2.6. Preparation of pork sample

The analysed pork sample was purchased at a local supermarket. A 20.0-mL aliquot of 0.1 mol L^{-1} HCl and a 1.0 mL aliquot of trichloroacetic acid were added to a 2.0 g ground pork sample. After shaking for 20 min, the mixture was centrifuged at 11 000 rpm for 10 min. The obtained supernatant was adjusted pH to 12.0 with sodium hydroxide and extracted with 20.0 mL of ethyl acetate. The organic phase was evaporated to dryness with nitrogen. The residue was dissolved in 2.0 mL of 0.1 mol L^{-1} HCl.

3. Results and discussion

3.1. Characteristics of PSS–GR composite

As shown in Fig. 1A, the different colour of PSS–GR from that of GO indicates that the reduction of the GO had been achieved. Furthermore, the as-prepared PSS–GR dispersion with a concentration of 1.0 mg mL^{-1} was very stable and hardly any precipitate appeared after six months of standing. From the UV–vis spectra given in Fig. 1B, the GO dispersion displays a maximum absorption at 228 nm (attributed to the $\pi-\pi^*$ transitions of C=C bonds) and a