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A molecularly imprinted electrochemiluminescence sensor based on upconversion nanoparticles enhanced by electrodeposited rGO for selective and ultrasensitive detection of clenbuterol

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ABSTRACT

A simple, efficient and sensitive molecularly imprinted electrochemiluminescence sensor (MIECLS) based on reduced graphene oxide (rGO) and upconversion nanoparticles (UCNPs) was developed for determination of clenbuterol (CLB). In this study, rGO generated by electrodeposition of graphene oxide not only acted as carrier for immobilizing UCNPs, but also had a significant impact in boosting electrochemiluminescence (ECL) response of UCNPs thanks to its high conductivity, superior electron transport rate and large specific surface area. UCNPs as an advanced ECL emitter possessed wonderful ECL performance. Furthermore, the introduction of molecularly imprinted polymers (MIP) endowed the ECL sensor a new character of specifically identifying analyte CLB. Under the optimal experimental conditions, the ECL signal was proportional to the logarithm of CLB concentration in the range of 10 nM to 100 μM with a low detection limit of 6.3 nM. The proposed MIECLS combining the advantages of UCNPs-ECL and MIP exhibited good sensitivity, desirable selectivity and favorable stability, indicating enormous potential in the future of food safety detection.

1. Introduction

Electrochemiluminescence (ECL) is an optical radiation from the excited state produced by high energy electron transfer of electrochemically generated reagents at electrode surface (Miao, 2008; Wu et al., 2014b). ECL has been widely applied in various fields, including bioscience, immunoassay and food analysis due to its advantages of rapidity, low background noise, high sensitivity and simplified equipment (Jiang and Wang, 2009; Yang et al., 2015). For ECL emitters, great interest was focused on ruthenium (II) complexes and luminol at the early development of ECL and later much attention was turned to nanomaterials, such as graphene quantum dots (GQDs), carbon nanodots, graphite-like carbon nitride (g-C_3N_4) and noble metal clusters (Fan et al., 2016; Li et al., 2012, 2014a; Lou et al., 2015). It will be an irresistible trend to devote to exploring and studying new and efficient ECL materials.

Rare earth doped upconversion nanoparticles (UCNPs) following anti-Stokes' law can absorb two or more low-energy photons to emit high-energy photons. In recent years, the applications of UCNPs in the fields of bioimaging, analytical testing and clinical medicine have been immensely reported thanks to their good emission stability and fine biocompatibility (Liu et al., 2014; Pokhrel et al., 2014). Nevertheless, the research of UCNPs is rarely reported in ECL field. UCNPs as a new generation of ECL luminescent reagents possess a number of potential advantages such as stable cathode signals, desirable ECL emission and prolonged fluorescence lifetime and thus will expand application scope of ECL (Guo et al., 2016; Zhou et al., 2012). Ren's group first reported the application of UCNPs in ECL investigation in 2012 and they made use of one-step synthesis of graphene-upconversion composites to construct an ECL sensor, in which graphene played an important role in amplifying ECL signal (Yin et al., 2012). Graphene, as a two-dimensional material with single atom structure, shows attractive application prospects in multifarious fields (Kim et al., 2009). The outstanding characteristics of graphene such as large specific surface area, low cost and high electron transfer speed, make it possible to be used for ECL signal amplification (Shan et al., 2009; Pumera, 2011; Zhao et al., 2016a). Reduced graphene oxide (rGO) can be obtained by electrodeposition of graphene oxide (GO) and rGO film yielded by this way not only can be controlled in thickness and size, but also possesses uniformity and continuity (Liu et al., 2011a). However, the method using rGO produced by electrochemical reduction to enhance ECL signal of UCNPs has not been explored and thus it will manifest enormous

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potential in fabricating a novel and sensitive ECL sensor.

Molecular imprinting technique (MIT) is a process to prepare polymers with recognition sites having a specific selectivity for template molecules (Wu et al., 2012). The resulting molecularly imprinted polymers (MIP) have been extensively utilized for sensors due to superb recognition capability and desirable stability. Molecularly imprinted electrochemiluminescence sensor (MIECLS) is the ideal integration of ECL and MIP, and hence MIECLS not only maintains good sensitivity and controllability inherited from ECL, but also shows predominant selectivity of MIP (Yang et al., 2017). However, MIECLS using UCNPs as luminophor remains unexplored.

Clenbuterol (CLB) as adrenaline nervous stimulant plays a positive role in clinical application of treating bronchitis and myasthenia gravis (Lichtenstein and Deckelbaum, 2001). It can also promote animal growth and improve lean percentage. Whereas CLB is easily absorbed by animal body and a large number of trials have shown that CLB residues mainly exist in eyes, liver, kidney and muscle (Zhao et al., 2011). In recent years, CLB is frequently added to animal feed and its abuse has caused lots of incidents of human poisoning (Yan et al., 2014). As a result, many countries including China have banned the use of CLB as animal growth promoter.

Nowadays a number of methods have been developed for CLB detection, such as gas chromatography-mass spectrometry (GC-MS) (Zhao et al., 2010), high-performance liquid chromatography (HPLC) (Chang et al., 2005), liquid chromatography-mass spectrometry (LC-MS) (Lu et al., 2013), capillary electrophoresis (CE) (Wang et al., 2015) and enzyme-linked immunosorbent assay (ELISA) (Ren et al., 2009). GC-MS and LC-MS methods possess high sensitivity, but they need expensive instruments and time-consuming sample pretreatment. Although ELISA method is simple and fast, a primary drawback is high expense of producing antibodies.

In this work, a novel and ultrasensitive MIECLS was exploited for the detection of CLB. GO was electrochemically reduced on electrode surface to generate rGO, and then the resulting rGO as signal amplifying agent and UCNPs as luminophor were used for fabricating an ECL sensor. Driven by the electrostatic interaction, positively charged UCNPs and negatively charged rGO can be assembled together making ECL signal more stable. And rGO by virtue of its super properties of wonderful conductivity and excellent electron transport can tremendously boost ECL intensity of UCNPs and improve ECL performance of UCNPs. Besides, the ECL response of UCNPs/rGO modified electrode gradually reduced with increasing CLB concentration and the possible ECL mechanism and quenching mechanism were explored in detail. MIP was further introduced to the ECL sensor based on rGO and UCNPs to specifically recognize template CLB and endowed the ECL sensor with good selectivity realizing quantitative determination for CLB. Therefore, the proposed MIECLS with stable and strong cathodic ECL signal would provide a new way for CLB detection in food safety analysis.

2. Experiments

2.1. Materials

Carboxyl graphene oxide dispersion (GO, 2 mg mL^{-1}) and SiO_2-NH_2 modified upconversion nanoparticles ($NaYF_4$:Yb,Er) were supplied by XFNANO (Nanjing, China). *O*-phenylenediamine (*o*-PD, 99.5%) was supplied by J&K Scientific Ltd. (Beijing, China). Isoprenaline (ISO, 99.2%), salbutamol (SAL, 99.7%) and terbutaline (TER, 99.8%) were purchased from Chinese Food and Drug Inspection Institute. CLB (95%) and potassium peroxodisulfate ($K_2S_2O_8$) were achieved from Sigma-Aldrich. Ractopamine (RAC, 97.0%) was purchased from Dr. Ehrenstorfer GmbH (Germany). Millipore system purified water was used in all of the experiments.

2.2. Apparatus

All ECL experiments were tested with a LK5100 ECL analyzer (Tianjin Lanlike Chemical and Electronic High Technology Co., Ltd., China). Electrochemical impedance spectroscopy (EIS) was taken by a PARSTAT 2273 electrochemical workstation. All of the above measurements employed a typical three-electrode system, which used a glassy carbon electrode (GCE, 4 mm), a saturated calomel electrode (SCE) and a platinum wire electrode as working, reference and auxiliary electrodes, respectively. Ultraviolet-visible (UV–vis) spectra were measured using a UV–vis spectrophotometer (Evolution 300, Thermo, America). Fluorescence spectra were obtained on an F-2500 fluorescence spectrophotometer (Hitachi, Japan) with an external 980 nm exciter. Scanning electron microscopy (SEM), which was used to track the surface morphologies of the modified electrodes, was performed on a LEO-1530VP scanning electron microscopy (LEO, Germany). Transmission electron microscopy (TEM, JEOL, Japan) was carried out on a JEM-2010FEF transmission electron microscopy to observe the structure morphology of the nanomaterials.

2.3. Fabrication of UCNPs/rGO/GCE

GO (2 mg mL^{-1}) was diluted into 0.5 mg mL^{-1} with deionized water under sonication for 10 min. UCNPs (2 mg mL^{-1}) were also dispersed in aqueous solution to the final concentration of 0.5 mg mL^{-1}. The bare GCE was successively polished with 1.0, 0.3 and 0.05 μm alumina slurry to a smooth plane followed by rinsing entirely with deionized water and then measured with cyclic voltammetry in 1 mmol L^{-1} $K_3[Fe(CN)_6]$ containing 0.2 mol L^{-1} KNO_3 until the peak potential difference was below 80 mV. The resulting electrode was immersed in the above GO dispersion for electrodeposition from 0.6 to −1.4 V with a scan rate of 50 mV s^{-1} for 5 cycles to obtain a rGO modified electrode (rGO/GCE). Subsequently, 10 μL UCNPs solution (0.5 mg mL^{-1}) was dropped on rGO/GCE surface and dried at room temperature to obtain a UCNPs/rGO modified electrode (UCNPs/rGO/GCE).

2.4. Fabrication of MIP/UCNPs/rGO/GCE

The CLB-imprinted UCNPs/rGO/GCE was prepared in the deoxygenated acetate buffer (0.1 mol L^{-1}, pH 5.2) consisting of 6 mmol L^{-1} *o*-PD and 1.2 mmol L^{-1} CLB under darkness using cyclic voltammetry from 0 to 0.8 V with a scan rate of 50 mV s^{-1} for 7 cycles. The obtained electrode was placed in 25 mL methanol/acetic (2:1, *v/v*) solution with continuous magnetic stirring for 20 min to remove the embedded template and subsequently rinsed with deionized water and allowed to dry naturally. The NIP/UCNPs/rGO/GCE (non-imprinted polymers, NIP) as a control experiment was also prepared with the same electropolymerization process as the MIP/UCNPs/rGO/GCE but no template CLB. The fabrication procedure of MIP/UCNPs/rGO/GCE was illustrated in Scheme 1 (A).

2.5. ECL measurement

The MIECLS was immersed in various concentrations of CLB to adsorb template molecules for 6 min and then detected in 5 mL 0.1 mol L^{-1} $K_2S_2O_8$ solution containing 0.1 mol L^{-1} PBS (pH 7.4). In the process of ECL emission, cyclic voltammetry and electrochemiluminescence were simultaneously adopted. And the voltage of photomultiplier tube was placed at 800 V, meanwhile cathodic potential ranged from 0 to −2.5 V with a scan rate of 100 mV s^{-1}. EIS performed from 100 mHz to 100 kHz with AC amplitude of 10 mV was used for characterization of different modified electrodes.