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A low detection limit penicillin biosensor based on single graphene nanosheets preadsorbed with hematein/ionic liquids/penicillinase

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ABSTRACT

In this study, we reported on a low detection limit penicillin biosensor with layer-by-layer (LbL) film containing single-graphene nanosheets (SGNs) preadsorbed with hematein, ionic liquids (ILs) and penicillinase. The penicillinase catalyzes the hydrolysis of penicillin to penicilloic acid, where H^+ is liberated and monitored amperometrically with hematein as a pH indicator. The SGN-hematein/ILs/penicillinase biosensor exhibited excellent performance for penicillin in PBS with a wide range from 1.25×10^{-13} to 7.5×10^{-3} M, and a low detection limit of 10^{-13} M (0.04 ppt, S/N ≥ 3). Furthermore, the detection of penicillin concentration in real sample (milk) had acceptable accuracy with the assay system.

1. Introduction

Penicillin is a highly effective β-lactam antibiotic based on a β-lactam ring which is responsible for the antibacterial activity, and has variable side chains due to their chemical and pharmacological properties. Therefore, it is widely applied to prevent and treat various bacterial infections in human and livestock [1], and even added to feedstuff to promote animal growth [2,3]. The presence of antibiotic residues in food products, drinking water, and environment constitutes a worldwide concern because of its serious health consequences, including the emergence of antibiotic-resistant bacterial strains, disturbance or disruption of the normal ecological equilibrium, and increasing instances of allergic reactions. Penicillins and other lactam antibiotics are the most frequently used antibiotics and have long been widely used for treating various bacterial infections in human, livestock, poultry, and aquaculture. The presence of penicillin residues represents a potential hazard to public health, since they can cause the formation of antibiotic-resistant bacterial strains and allergic reactions [2]. Approximately 5–10% of the population is hypersensitive to penicillin or other antibiotics, and suffers allergic reactions at concentrations as low as 1 ppb (≈$2.8 \times 10-^9$ M) penicillin [4].

To date, various analytical methods have been reported for the determination of penicillin residues, including capillary electrophoresis (CE) [5], high-performance liquid chromatography (HPLC) [6], liquid chromatograph-mass spectrometer (LC-MS) [7], liquid chromatography–electrospray ionization mass spectrometry (LC-ESI/MS) [2], pressurized liquid extraction and high-performance liquid chromatography with ultraviolet detection (PLE-HPLC-UV) [8] and immunosorbent assays [9–11]. Although the determination of penicillin residues by these methods is reliable and quite sensitive, these instruments are inherently expensive. And also the pretreatment for samples involved as well as these operating processes are complicated and longer. Consequently, it is crucial to develop a simple, rapid and sensitive analysis method for the determination of penicillin residues in food and environmental monitor.

Electroanalysis is an appropriate route for detection of penicillin residues, because penicillinase can catalyze the hydrolysis of penicillin to penicillinic acid, resulting in a decrease of solution pH. Recently, various penicillin sensors have been reported based on the measurement of the change of pH with potentiometric [12,13] and capacitive [14,15] technique, respectively. But they still have a drawback of complex in configurations and relatively high detection limits (0.005–0.5 mM) [12–15]. Another way to simplify the fabrication and improve the detection limit is used by amperometric technique for penicillin biosensors [1,16]. Bi et al. devised an amperometric biosensor for detecting penicillin residues by using hematein as a pH-sensitive redox probe, multi-walled carbon nanotubes (MWCNT) as an electron transfer enhancer, and chitosan as a film-forming polyelectrolyte. The amperometric biosensor has many

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advantages over conventional sensors, such as potentiometric, conductometric, and capacitive sensors, in terms of higher sensitivity, lower detection limit (50 nM), shorter response time, simplicity and lower cost [1].

As known, graphene, an emerging true two-dimensional carbon nanomaterial [17,18], is a monolayer of sp^2-bonded carbon atoms densely packed into a honeycomb crystal structure [19]. Compared to single wall carbon nanotubes (SWCNTs), the single-graphene nanosheets (SGNs) exhibit 60 times more conductivity [19], larger surface area [20], better sensitivity and stability as well as greater sp^2 character [21]. Thus, researches on the graphene-based biosensors are fascinating and increasing for sensing biomolecules like glucose [21], NADH [22], dopamine [23], etc. Besides, ionic liquids (ILs), composed of a bulky organic cation and either an organic or an inorganic anion, have attracted more and more attention in electrochemical biosensors due to its intrinsic properties, such as wide electrochemical window, well biocompatibility, good film stability and high ionic conductivity for the enhanced electrochemical response [24–26]. Maleki et al. developed an ionic liquid modified carbon paste electrode with 1-octylpyridinium hexafluorophosphate (OPFP) as binder and further used it for the detection of some electroactive molecules [27]. It is found that the mixture or combining of graphene and ILs could improve their conductivity, compatibility and stability, inducing more applications of graphene in the electrochemical field [28–30].

In the work, we investigated the performance of modified electrodes with layer-by-layer (LbL) films containing single-graphene nanosheets preadsorbed with hematein (SGN-hematein), ILs and penicillinase for the determination of penicillin residues by employing cyclic voltammetric (CV) and differential pulse voltammetry (DPV). Herein, the SGNs were synthesized by the reducing of graphene oxide with $NaBH_4$ [31]. The surface morphologies and properties of the SGNs samples were researched by UV–VIS, XRD, HRTEM, AFM and electrochemical measurements. In addition, the ILs play an important role in the film-forming, high ionic conductivity and immobilization with negatively charged penicillinase via electrostatic attractions. The resulted SGN–hematein/ILs/penicillinase biosensor is successfully applied to the voltammetric determination of penicillin with high sensitivity. The system was employed to analyze penicillin in milk samples that included penicillin. Finally, the performance features such as reproducibility, stability and specificity were evaluated.

2. Experimental

2.1. Materials

Graphite powder (99.95%, 325 mesh) was obtained from Alfa Aesar (Tianjin, China). Penicillin G sodium salt was purchased from Aladdin Chemistry Co. Ltd (Shanghai, China). Penicillinase (1 ml will inactivate 120,000 units of Penicillin stored at 4 °C) and hematein were provided by J&K Scientific Ltd. (Shanghai, China). The ionic liquids, trihexyltetradecylphosphonium bis (trifluoromethylsulfonyl) imide ($[P(C_6)_3C_{14}][Tf_2N]$, purity: 98%), were supplied by Sigma-Aldrich and used as received. The phosphate buffer solution (PBS) was prepared using K_2HPO_4 and KH_2PO_4. The penicillin G solutions and penicillinase solutions were prepared with 1 mM pH 7.0 PBS (containing 0.1 M NaCl) in all experiments. All other chemicals used were of analytical reagent grade. Ultra-pure water was obtained with a Milli-Q plus water purification system (Millipore Co. Ltd., USA) (18 MΩ). Milk samples, containing protein ≥2.9% (w/w) and fat ≥3.3% (w/w), were purchased from a local supermarket. The spiked milk samples were filtrated by a hollow fiber membrane module to remove protein and fat, and then the supernatants were collected as real sample in PBS.

2.2. Apparatus

UV–VIS measurements were conducted at a UV-1800 SHIMADZU UV–VIS spectrophotometer (SHIMADZU, Japan). High-resolution transmission electron microscope (HRTEM) image was obtained using Tecnai G2 F20 S-TWIN, 200 kV (FEI Company, USA). The samples were prepared by diluting a few milliliters of the SGN suspension with ethanol, followed by pipetting a few microliters onto a copper grid, and then dried under ambient temperature. Atomic force microscopy (AFM) images were recorded using a Nanoscope IIIa multimode atomic force microscope (Veeco Instruments, USA) in tapping mode to simultaneously collect height and morphology data. A droplet of SGN dispersion (about 0.01 mg mL^{-1}) was casted onto a freshly cleaved mica surface, then dried in N_2. X-ray diffraction (XRD) measurements of the samples were recorded on a Rigaku-miniflex X-ray powder diffractometer using Cu Kα radiation ($\lambda = 1.5306$ A) with scattering angles (2θ) of 5–80°. Zeta-potential value measurement of the samples was performed on Zeta Sizer3000 Laser Particle Size and Zeta Potential Tester (Malvern Corporation, UK) in aqueous solution.

The electrochemical properties of as-prepared electrodes were characterized by cyclic voltammetric (CV), electrochemical impedance spectroscopy (EIS) and differential pulse voltammetric (DPV). The electrochemical measurements were performed with a three-electrode configuration on a CHI 660D electrochemical workstation (CH Instrument Company, Shanghai, China). The working electrode was a modified glassy carbon electrode (i.e., the SGN-hematein/ILs/penicillinase electrode), and counter electrode and reference electrodes were a platinum wire and a KCl saturated Ag/AgCl electrode, respectively. In biosensing measurements, the CV and DPV measurements of the penicillinase electrode were proceeded in a corresponding PBS solution by applying voltage in the range between −0.4 and 0.8 V versus Ag/AgCl. The resulted current changes were correlated to the final concentrations of penicillin G in the tested solution. The EIS measurements of the penicillinase electrode were recorded at a formal potential of 237 mV (vs. Ag/AgCl) within the frequency range of 1Hz–100 kHz. 5 mV amplitude of sine voltage signal was applied to the three-electrode system under open circuit potential and the number of data points per frequency decade was 12. During the analysis of real sample, the amperometric response of the penicillinase electrode was monitored at 0.2 V in a 20 mL-static electrochemical cell containing 10 mL of 1 mM pH 7.0 PBS-0.1 M NaCl as the supporting electrolyte solution. Aliquots of 1–10 μL of penicillin G spiked in PBS and milk sample solutions were successively injected into the electrochemical cell under magnetic stirring. The resulted current changes were correlated to the final concentrations of penicillin G in the tested solution. All measurements were performed at room temperature.

2.3. Synthesis of graphene

Graphene oxide (GO) was prepared by a modified Hummer's method, graphite powder as raw materials [32]. Then, the as-purified GO suspensions were dispersed in water to yield a yellow–brown dispersion (0.05 wt.%) by ultrasonication for 1 h, following that the dispersion was centrifuged at 8000 rpm for 15 min to remove unexfoliated GO (usually presented in a very small amount). Subsequently, a $NaBH_4$ solution (1 M, 15 mL) was added to GO (75 mL) suspensions under stirring. A 10 wt.% sodium carbonate (Na_2CO_3) solution was used as an acidity regulator to adjust the pH to around 10. After being vigorously shaken or stirred for a few minutes, the vial was put in an oil bath (90 °C) for 1 h. The stable black dispersion was then obtained. The mixture was then allowed to cool at room temperature and filtered with a nylon membrane (0.22 μm) to obtain SGNs. Finally, the resulted-SGNs redispersed readily in water by ultrasonication.

2.4. Preparation of the amperometric penicillin biosensor

Prior to preparation of biosensor, the glassy carbon electrode (GCE) with a diameter of 3 mm was polished on a polished cloth with 0.3 and 0.05 μm alumina powder, respectively. Then the GCE was rinsed with deionized water, ultrasonicated in deionized water and ethanol bath,