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Magnetic glass carbon electrode, modified with magnetic ferriferrous oxide nanoparticles coated with molecularly imprinted polymer films for electrochemical determination of bovine hemoglobin

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ABSTRACT

The modified electrode based on magnetic ferriferrous oxide (Fe_3O_4) nanoparticles covered with molecularly imprinted polymer was fabricated and used for electrochemical recognition of bovine hemoglobin (BHb). First, the magnetic Fe_3O_4 nanoparticles were covered with silicon dioxide. Then dopamine (DA) was self-polymerized on the surface of magnetic nanoparticles in the presence of bovine hemoglobin as the template molecular. The molecularly imprinted polymers were characterized by transmission electron microscope (TEM), Fourier transform infrared spectroscopy (FT-IR) and electrochemical impedance spectroscopy (EIS). Potassium hexacyanoferrate ($K_3[Fe(CN)_6]$) was used as an electroactive probe for measurement signal. Under optimized conditions (70 min for adsorption, pH = 6.0), the DPV peak current regressed linearly with the BHb concentration increase in the range of 5.0×10^{-7} to 1.0×10^{-5} g mL^{-1} ($r = 0.9939$) with a detection limit of 1.184×10^{-8} g mL^{-1} ($S/N = 3$). Several proteins were tested to confirm the imprinting effect of the modified electrode. The results showed the good selectivity of the resulting biosensors. The applicability of the method for complex matrix analysis was also evaluated and the modified electrode showed good stability and reproducibility.

1. Introduction

Molecular imprinting technique is pioneered by Wulff in the early 1970s, and has been widely used to provide molecular assembly with expected structure until now [1,2]. Functional monomer can be polymerized in the presence of template molecules. After that, template molecules are removed to form imprinted holes in polymer matrix. Molecularly imprinted polymers have been widely applied in bioanalysis because of mechanic stability, easy preparation, low cost, long lifetime and so on [3].

Proteins are the material basis of life which performs varies of functions in living organism. However, there are some challenges in the measure of proteins due to their big molecular volumes and complicated unstable structures [4–7]. As a new strategy, molecular imprinting technique has been successfully applied to distinguish and measure proteins. Several achievements have been reported by using spectroscopy in recent years [8–12].

Electrochemical methods with their advantages of higher sensitivity, lower cost and less interferences from non-electroactive substances are preferable to the determination of biological molecular. Many reports about molecular imprinting technique combined with electrochemical methods have been published, such as dopamine [13–15], ascorbic acid [15–17], and quercetin [18]. Not only small molecular, but also macromolecule have been recognized as well. Ramanavicius' group used polypyrrole layer for the determination of DNA [19] and BLV protein gp51 [20]. Wu et al. designed the electrochemical sensor based on molecularly imprinted polymers for the recognition of BHb [21]. Multiwall carbon nanotubes electrode modified with substrate-selective imprinted polymer was assembled by Prasad's group for ultra-trace detection of bovine serum albumin [22]. Li et al. also used the glycoprotein as the template and Fe_3O_4@Au nanofibers as the carrier [23].

Magnetic nanoparticles covered with imprinted polymers have been reported for the recognition and separation of proteins due to their good biocompatible properties, particularly magnetic Fe_3O_4 nanoparticles [24–26]. The cover layer of silica promotes the well-distribution of magnetic Fe_3O_4 nanoparticles and the corrosion resistance of external environment [27]. Magnetic

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nanoparticles could easily be attracted by magnetic electrode, which could reduce the loss of electrode surface modification material in the experimental process and improve the stability of the modified electrode. Dopamine (DA) is often used as the functional monomer to form molecularly imprinted polymer because it can be self-polymerized in weak alkaline solution [28]. Polydopamine is able to adsorb many substances effectively by covalent or non-covalent force [29]. Polydopamine-based molecularly imprinted polymers on silica-modified magnetic nanoparticles have been used for the recognition of BHb with UV–Vis spectrophotometer [8,30]. In this paper, we proposed a new method to prepare magnetic imprinted polymer as the electrochemical sensor for BHb. The biosensor showed high sensitivity, good selectivity and stability for the detection of BHb.

2. Experimental

2.1. Materials

Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ammonium iron(II) sulfate hexahydrate [$(NH_4)_2FeSO_4{\cdot}6H_2O$], potassium chloride (KCl) and aluminum oxide (Al_2O_3) were obtained from the 6th Chemical Reagent Factory (Tianjin, China). Potassium hexacyanoferrate ($K_3[Fe(CN)_6]$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), sodium phosphate monobasic dehydrate ($NaH_2PO_4{\cdot}2H_2O$), ammonium persulfate (APS) and hydrazine hydrate were purchased from Guangfu Technology Development Co., Ltd. (Tianjin, China). Anhydrous ethanol, acetic acid, 2-propanol and ammonia solution were provided by Concord Technology Co., Ltd. (Tianjin, China). Tetraethyl orthosilicate (TEOS) was purchased from J&K Scientific Ltd. (Beijing, China). Dopamine hydrochloride (DA, 99%) was obtained from Alfa Aesar (Shanghai, China). Bovine hemoglobin (BHb, Mw = 64,500 Da), bovine serum albumin (BSA, Mw = 68,000 Da), lysozyme (Lyz, Mw = 14,600 Da), peroxidase from horseradish (HRP, Mw = 40,000 Da), cytochrome C (Cyt C, Mw = 12,384 Da) and sodium dodecyl sulfate (SDS) were supplied by Sigma–Aldrich Co. LLC (St. Louis, MO, USA). All the proteins were ultrapure grade and other reagents were analytical grade. Ultrapure water treated with water purification system (UPH-I-10T, Sichuan, China) was used in all experiments.

2.2. Apparatus

The LK98BII Electrochemical Workstation (Lanlike, Tianjin, China) was used for the cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments. The LK2010 Electrochemical Workstation (Lanlike, Tianjin, China) was applied for the electrochemical impedance spectroscopy (EIS). A conventional three-electrode system was used with a magnetic glass carbon electrode (Gaossunion, Wuhan, China) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the counter electrode, respectively. A UV-2450 UV–Vis spectrophotometer (Shimadzu, Japan), FT-IR spectrometer (Nicolet 6700, USA) and JEM100CXII transmission electron microscope (JEOL, Japan) were used for the characterization of the nanoparticles and imprinted polymers.

2.3. Synthesis of BHb-imprinted magnetic nanoparticles

Fe_3O_4 magnetic nanoparticles were prepared in the following steps by co-precipitation method. 5.0 g of $FeCl_3{\cdot}6H_2O$, 2.1 g of $(NH_4)_2Fe(SO_4)_2$ and 1.0 mL of hydrazine hydrate were dissolved in 50 mL water, then 10.0 mL of ammonia solution (27% *w*/*v*) was added into the mixed solution instantly after all the reactants were dissolved completely. After stirring for 30 min at room temperature, the mixed solution was heated to 80 °C and kept for 1 h. The magnetic nanoparticles were separated from the turbid solution with a magnetic plate and washed by water until the solution was neutral. Finally, the resultant particles were washed by anhydrous ethanol and dried under vacuum at 70 °C for 24 h.

In order to prepare silica-covered Fe_3O_4 magnetic nanoparticles, 0.3 g of Fe_3O_4 nanoparticles was dispersed in the mixed solvent (60 mL 2-propanol and 4.5 mL H_2O) by ultrasonic vibration for 15 min. 1.0 mL of ammonia solution (27% *w*/*v*) and 4.0 mL of TEOS were added into the mixed solution and kept stirring at room temperature for 12 h. The SiO_2 covered nanoparticles were isolated by magnetic plate and washed with water till the washing solution was neutral. Then the product was dried at 70 °C under vacuum for 24 h.

40 mg of dopamine with 8 mg BHb as template molecular was dissolved in 10.0 mL phosphate buffer solution (PBS, 0.1 M, pH = 7.5) and stirred for 2 h at 15 °C. Then 140 mg of silica-covered Fe_3O_4 magnetic nanoparticles was added and kept stirring for 1 h. 10 mg of APS as the initiator of the polymerization reaction was added into the solution, followed by stirring for 12 h. The imprinted magnetic nanoparticles (MIP-NPs) were isolated by magnetic plate combined with centrifugation and washed with a mixed solution of SDS (0.1% *w*/*v*) and acetic acid (3% *v*/*v*) overnight. The removal of the template was detected by the UV–Vis spectrophotometer until no template protein was observed in the eluent. The MIP-NPs was dried at 70 °C under vacuum.

The non-imprinted magnetic nanoparticles (NIP-NPs) were also prepared as the procedure mentioned above except for the addition of BHb. Both MIP-NPs and NIP-NPs were dark brown solids. The basic steps of synthesizing BHb-imprinted magnetic nanoparticles were shown in Fig. 1.

2.4. Preparation of MIP-NPs and NIP-NPs modified sensors

Magnetic glass carbon electrode (GCE *d* = 4 mm) was polished to a mirror-like surface with 0.3 and 0.05 μm of alumina aqueous slurry. Then it was washed ultrasonically in ethanol and ultrapure water respectively and dried in air. The prepared magnetic nanoparticles (MIP-NPs or NIP-NPs) were dispersed uniformly into absolute ethyl alcohol under ultrasonic. 15.0 μL of dispersed solution (1 mg/mL) of MIP-NPs or NIP-NPs was dropped smoothly on the surface of GCE (5.0 μL at a time, 3 times), and dried in air. Then, CV experiments with a scan rate of 100 mV/s were carried out on the MIP-NPs or NIP-NPs in the solution containing 10 mmol/L $K_3[Fe(CN)_6]$ and 0.5 mol/L KCl until the steady voltammograms were obtained.

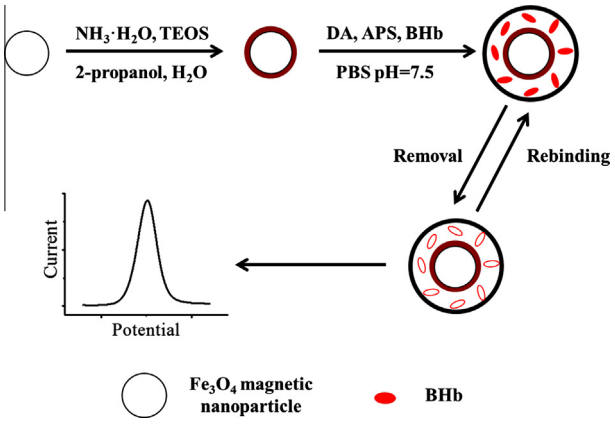

Fig. 1. Schematic procedure for synthetizing of BHb-imprinted magnetic nanoparticles.