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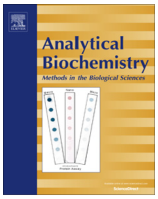

Electrocatalytic activity of core/shell magnetic nanocomposite

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ABSTRACT

Electrically active magnetic nanocomposites (EAMNCs), Au nanoparticles/self-doped polyaniline@Fe_3O_4 (AuNPs/SPAN@Fe_3O_4) with well-defined core/shell structure, were first synthesized by a simple method. The morphology and composition of the as-synthesized AuNPs/SPAN@Fe_3O_4 nanocomposite have been characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier transform infrared (FT–IR), ultraviolet–visible (UV–Vis), X-ray powder diffraction (XRD), and thermogravimetric analysis (TGA). Horseradish peroxidase (HRP)–AuNPs/SPAN@Fe_3O_4 biocomposites were immobilized onto the surface of indium tin oxide (ITO) electrode to construct an amperometric hydrogen peroxide (H_2O_2) biosensor. The effects of HRP dosage, solution pH, and the working potential on the current response toward H_2O_2 reduction were optimized to obtain the maximal sensitivity. Under the optimal conditions, the proposed biosensor exhibited a linear calibration response in the range of 0.05 to 0.35 mM and 0.35 to 1.85 mM, with a detection limit of 0.01 mM (signal-to-noise ratio = 3). The modified electrode could virtually eliminate the interference of ascorbic acid (AA) and uric acid (UA) during the detection of H_2O_2. Furthermore, the biosensor was applied to detect H_2O_2 concentration in real samples, which showed acceptable accuracy with the traditional potassium permanganate titration.

Magnetic nanoparticles have become attractive for exploitation mainly in information storage, ferrofluids, color imaging, drug targeting and delivery, cancer therapy, separations, and catalysis [1–6]. As a representative example, magnetite (Fe_3O_4)[1] has been studied extensively. However, due to the magnetic dipolar–dipolar attraction, Fe_3O_4 nanoparticles are inclined to aggregate into clusters; in addition, the Fe_3O_4 nanoparticles with relatively small sizes may undergo rapid biodegradation on being directly exposed to biological environments [7]. Therefore, surface modification with various useful materials during the synthesis or coating process of superparamagnetic Fe_3O_4 nanoparticles might be helpful to overcome these limitations.

During recent years, core/shell structured magnetic nanocomposites with Fe_3O_4 as the core and inorganic materials or polymers as the shell have been proposed extensively. These specific core/shell materials possess the ability to be modified with different charges, functions, or reactive moieties on the surface with enhanced stability and compatibility, and they provide an avenue for the synthesis of complex nanocomposites. Moreover, these nanocomposites usually combine with the advantageous properties of both core and shell and are promisingly applied in electrocatalysis, drug delivery, and fluorescent biological labeling [8–10]. For example, Jang and coworkers synthesized human natural killer cells (NK-92MI) loaded Cy5.5-SiO_2@Fe_3O_4 core/shell nanocomposites, which could be infiltrated into the target tumor site by an external magnetic field [11]. Govindaiah and coworkers showed that Fe_3O_4/poly(fluorescein *o*-methacrylate) magnetic fluorescent nanocomposites can be used in bioimaging by visualizing the cellular uptake of the nanocomposites into A549 lung cancer cells [12]. Thus, the synthesis of multifarious magnetic core/shell nanocomposites with multiple properties attracts more and more attention.

Among these shell materials, polyaniline (PANI) has been widely investigated. As both an electrical conductor and an organic compound, PANI possesses flexibility, robustness, highly

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[1] *Abbreviations used:* Fe_3O_4, magnetite; PANI, polyaniline; SPAN@Fe_3O_4, self-doped PANI@Fe_3O_4; AN, aniline; SAN, 3-aminobenzenesulfonic acid; AuNP, Au nanoparticle; TGA, thermogravimetric analysis; HRP, horseradish peroxidase; ITO, indium tin oxide; H_2O_2, hydrogen peroxide; APS, ammonium persulfate; PVP, poly(vinylpyrrolidone); PDDA, poly(diallyldimethylammonium chloride); PBS, phosphate-buffered saline; UA, uric acid; AA, ascorbic acid; Nf, Nafion; KCl, potassium chloride; TEM, transmission electron microscopy; FESEM, field emission scanning electron microscopy; FT–IR, Fourier transform infrared; UV–Vis, ultraviolet–visible; SQUID, superconducting quantum interference device; ZFC, zero field-cooled; FC, field-cooled; CV, cyclic voltammetry; EIS, electrochemical impedance spectroscopy; SCE, saturated calomel reference electrode; XRD, X-ray powder diffraction; JCPDS, Joint Committee on Powder Diffraction Standards; EDS, energy-dispersive spectrum; RSD, relative standard deviation.

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controllable properties, simple synthesis, low cost, and environmental stability. Fe_3O_4–PANI core/shell nanocomposites that exhibit the properties of both constituents—magnetic and conducting—have proved to have many applications such as chromatographic solid extraction [13], protein digestion [14], and catalysis [15]. In addition, based on the excellent electrochemical behavior of PANI and on the good biocompatibility of Fe_3O_4, the resultant Fe_3O_4–PANI core/shell nanocomposites might be used to construct a kind of biosensor in which the mediators could be omitted and the fabrication of the biosensors could be greatly simplified.

In this study, electrochemically active self-doped PANI@Fe_3O_4 (SPAN@Fe_3O_4) core/shell nanocomposite was one-pot synthesized from Fe_3O_4, aniline (AN), and 3-aminobenzenesulfonic acid (SAN). It not only was easily manipulated by an external magnetic field but also possessed electroactivity of PANI extending to neutral medium due to the effective doping of sulfonic acid groups. On the other hand, it is well known that Au nanoparticles (AuNPs) are safe in both animals and humans, and they also exhibited good biocompatibility and affinity via amine/thiol terminal groups [16]. Then, AuNPs were modified on the surface of SPAN@Fe_3O_4 through electrostatic adsorption, and the ratio of Au in the AuNPs/SPAN@Fe_3O_4 composites was estimated as 29.7% via thermogravimetric analysis (TGA). The synthetic process of AuNPs/SPAN@Fe_3O_4 nanocomposites was simple, and to our knowledge there is no report of such nanocomposites with multifunction of biocompatibility, electroactivity, conductivity, and magnetism. As an example, horseradish peroxidase (HRP) was successfully immobilized on AuNPs/SPAN@Fe_3O_4 modified indium tin oxide (ITO) electrode. For the first time, an amperometric hydrogen peroxide (H_2O_2) biosensor was fabricated on the basis of HRP–AuNPs/SPAN@Fe_3O_4 modified electrode.

Materials and methods

Materials

Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), sodium acetate (NaAc), polyethylene glycol (PEG) 10000, and ammonium persulfate [$(NH_4)_2S_2O_8$, APS] were purchased from Shanghai Linfeng Chemical Reagent (China). Poly(vinylpyrrolidone) (PVP, 30 kDa) and poly(diallyldimethylammonium chloride) (PDDA, 20 wt%) were purchased from Aldrich. Stock solution of 3% PDDA was prepared in double-distilled water with Tris and NaCl. AN was distilled twice under reduced pressure and stored in the dark at low temperature before use. SAN was purchased from TCI. Chloroauric acid hydrated ($HAuCl_4{\cdot}4H_2O$) was purchased from Nanjing Chemical Reagent (China). H_2O_2 (30% [w/v] solution) was obtained from Sinopharm Chemical Reagent, and its accurate concentration was determined by titration with potassium permanganate. Phosphate-buffered saline (PBS, 0.1 M) was prepared by mixing the stock solutions of KH_2PO_4 and K_2HPO_4 and was adjusted to appropriate pH by the addition of 0.1 M NaOH or H_3PO_4 solution. HRP (250 U mg^{-1}, EC 1.11.1.7), uric acid (UA), and ascorbic acid (AA) were all purchased from Sigma. Nafion (Nf) was obtained from DuPont as 5 wt% solution and was used as 0.5 wt% solution after dilution with water. Potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6]$), and potassium chloride (KCl) were obtained from Shanghai Linfeng Chemical Reagent. Other reagents were of analytical grade and used without further purification. Double-distilled water was used throughout the experiments.

Characterization

The structures and morphologies of the Fe_3O_4, SPAN@Fe_3O_4, and AuNPs/SPAN@Fe_3O_4 were characterized by a Philip X'Pert X-ray diffractometer taken with a Cu KR X-ray source, transmission electron microscopy (TEM, Jeol JEM-200CX), and field emission scanning electron microscopy (FESEM, Hitachi S4800). Fourier transform infrared (FT–IR) spectra of KBr powder-pressed pellets were recorded on a Bruker model VECTOR22 Fourier transform spectrometer. Ultraviolet–visible (UV–Vis) spectra were recorded on an Agilent UV–Vis spectrophotometer. TGA was performed on a NETZSCH STA 449C. Zeta potential analysis was performed with a PALS Zeta Potential Analyzer (version 3.43, Brookhaven Instruments). The static water contact angles were measured at 25 °C by a contact angle meter (Rame–Hart 100) using drops of pure deionized water. The readings were stabilized and taken within 120 s after the addition. The magnetic properties of the samples were measured using a 7-T Quantum Design superconducting quantum interference device (SQUID) magnetometer. The zero field-cooled (ZFC) and field-cooled (FC) measurements were performed by cooling the sample to 5 K at zero field or in the presence of an external field of 100 Oe, respectively. All of the magnetic measurements during the warming runs were carried out in a field of 100 Oe.

Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and amperometric measurements were performed using a CHI660D electrochemical workstation (Shanghai CH Instruments, China). A three-compartment electrochemical cell contained a saturated calomel reference electrode (SCE) against which all potentials were measured, a platinum wire auxiliary electrode, and the modified ITO magnetism electrode ($\Phi = 3$ mm) working electrode. The CV and EIS characterizations were performed in 0.1 M KCl solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1). The determination of H_2O_2 was conducted in 0.1 M PBS. The sensor responses were measured as the difference between total and residual currents.

Preparation of AuNPs/SPAN@Fe_3O_4 magnetic nanocomposite

The magnetic Fe_3O_4 microspheres were prepared through a solvothermal reaction (see online supplementary material) [17]. Core/shell structured SPAN@Fe_3O_4 magnetic nanocomposite was prepared as follows. First, 0.3 g of PVP and 0.06 g of Fe_3O_4 nanoparticles were dispersed into 66 ml of H_2O under ultrasonication at room temperature for 30 min. Then, 28.2 μl of AN and 0.05 g of SAN with 9 ml of 0.2 M HCl were added into the mixture, followed by adding 60 ml of H_2O. After the solution was shaken for 30 min, the oxidant APS with a concentration of 0.06 M was added drop by drop to start the copolymerization reaction under vigorous stirring, and the reaction was allowed to proceed for 8 h at 4 °C. After the reaction, the precipitate was magnetic separated and washed successively with H_2O and ethanol three times and then was dried under vacuum at 60 °C for 6 h for further use.

An electrostatic attraction method was employed for the immobilization of AuNPs onto the SPAN@Fe_3O_4 core/shell nanocomposite to form the AuNPs/SPAN@Fe_3O_4 magnetic nanocomposite. To induce positive charges at the surface of the SPAN@Fe_3O_4 nanocomposite, 10 mg of SPAN@Fe_3O_4 was dispersed in 4 ml of H_2O with 1 ml of 3% PDDA aqueous solution, and the mixture was sonicated for 3 min. The residual PDDA was removed by magnetic separation, and the nanocomposite was rinsed with water at least three times. Subsequently, the positively charged SPAN@Fe_3O_4 nanocomposite was added to 30 ml of Au colloid solution and stirred for 8 h. The AuNPs were electrostatically attracted on the surface of the SPAN@Fe_3O_4 nanocomposite. Finally, the resultant dark purple AuNPs/SPAN@Fe_3O_4 product was removed from the solution by applying an external magnetic field, and the supernatant liquor was colorless. After washing with water and ethanol three times, the AuNPs/SPAN@Fe_3O_4 nanocomposite was dried under vacuum at 50 °C for 6 h.