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Porous redox-active Cu_2O–SiO_2 nanostructured film: Preparation, characterization and application for a label-free amperometric ferritin immunosensor

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ABSTRACT

A novel Cu_2O–SiO_2 nanostructured particle was synthesized by a solution-phase method and was adopted for construction of a label-free amperometric immunosensor. The porous Cu_2O–SiO_2 nanoparticles had good redox electrochemical activity, large surface-to-volume ratio, film-forming ability and high stability. The physical morphology and structure of Cu_2O–SiO_2 nanoparticles were examined by scanning electron microscope (SEM) and transmission electron microscopy (TEM). The chemical component of Cu_2O–SiO_2 was confirmed by X-ray photoelectron spectroscopy (XPS) and auger electron spectra (AES). The electrode modification process was probed by cyclic voltammetry (CV) and the performance of the immunosensor was studied by differential pulse voltammetry (DPV) measurements. To improve the analytical characteristics of the immunosensor, the experimental conditions were optimized. The immunosensor exhibited a good response to ferritin in ranges from 1.0 to 5.0 and 5.0 to 120.0 ng mL^{-1} with a detection limit of 0.4 ng mL^{-1}. The fabricated immunosensor could make a low-cost, sensitive, quantitative detection of ferritin, and would have a potential application in clinical immunoassays.

1. Introduction

Ferritin, as an iron storage protein, which distributes in body's liver, spleen and marrow, has drawn much attention for its biological properties [1]. It is a tumor marker which plays an important part in iron metabolism in organisms [2]. Normal value of ferritin in a healthy adult serum is less than 200 ng mL^{-1} for men and 90 ng mL^{-1} for women [3].

Nowadays, immunoassay has been applied widely in many fields such as clinical chemistry, food industry and environment analysis. Electrochemical immunosensor is a device based on the specific immunoreaction of antibody and antigen. Along with the progress of biosensors, many kinds of special materials were employed to fabricate biosensors, such as sol–gel materials, polymers and nanomaterials. Sol–gel materials such as organic–inorganic chitosan composite film were often utilized to entrap proteins owing to their excellent film-forming ability, physical rigidity, chemical inertness and thermal stability [4,5]. A molecularly imprinted biocompatible polymer was still used for biosensor modification materials because of its lock–key structure [6]. More importantly, as is known to all, the development of nanomaterials have revolutionized the fields of biosensors. Various nanoparticles were adopted for construction process of biosensors like gold [7], silver [8], and platinum metals [9] for their high surface area, strong adsorption ability, good biocompatibility, and a heterogeneous electron transfer catalyzing ability [10]. Recently, hybrid nanomaterials have drawn much attention by biosensor researchers because they not only show their unexpected combined properties of the original components but also cause changes in optical, chemical or other performances compared with those of the individual components. For example, nanocomposites with carbon nanotubes [11], core–shell nanoparticles [12] were applied to fabricate biosensors. These hybrid nanomaterials show good stability and bioactivity for protein immobilization, but few of them have redox electrochemical activity, which are important and deserve attentions. In present work, the porous Cu_2O–SiO_2 nanoparticles were prepared and they showed the particular performance of good redox electrochemical activity, large surface-to-volume ratio and high stability.

Cu_2O is a brick-red p-type semiconductor with a band gap of about 2.17 eV. It attracts considerable interest as it has latent applications in gas sensors, solar energy conversion, electronics, magnetic storage, catalysis, lithium ion batteries and other fields [13,14]. Different morphology of Cu_2O has been obtained by many methods: electrolysis [13], electrodeposition onto single-crystalline silicon [15], deposition without any template or surfactant [14], reduction of cupric salts [16]. However, it rarely

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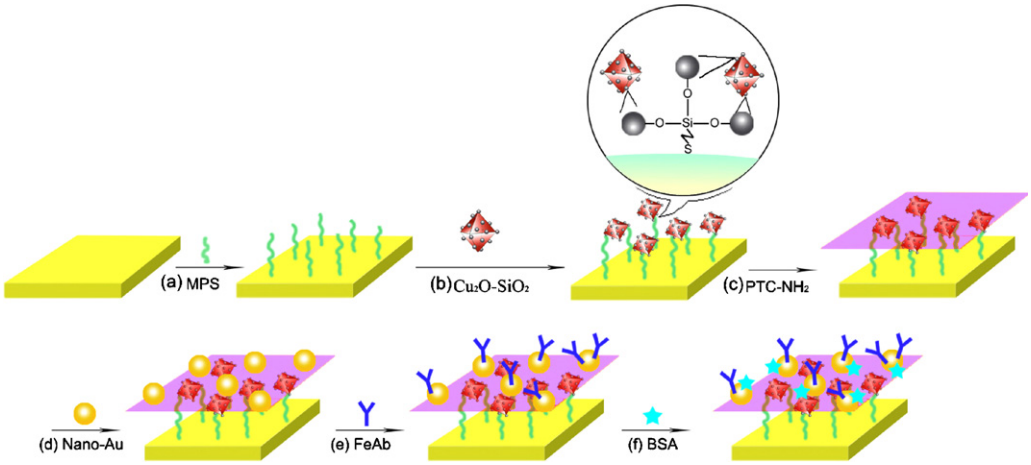

Fig. 1. Schematic illustration of fabrication of the immunosensor.

applies in immunoassays because nano-Cu_2O inclines to aggregate and is hard to be coated on the electrode directly. Nano-SiO_2 is a porous and hydrophilic material which has surface –OH groups [17]. It is often used as a substrate or template for preparation of materials [18]. Some core–shell structured sphere like SiO_2/ZnS and SiO_2/Au had been studied in many literatures [19,20]. In previous work, we have synthesized many types of hybrid nanomaterials such as $CoFe_2O_4/SiO_2$ composite nanoparticles, thionine (TH)-doped magnetic gold nanospheres and so on [21,22]. In this study, a new kind of hybrid nanomaterials, comprised of Cu_2O and SiO_2, was obtained by solution-phase method. The Cu_2O–SiO_2 nanoparticles containing –OH groups were a porous material. Significantly, it exhibited good conductivity and efficient redox activity resulted from the Cu(I) and Cu(II) [23]. Thus, the Cu_2O–SiO_2 nanoparticles with film-forming ability and good biocompatibility would have a potential application in immunoassays.

In present work, a new immunosensor was proposed using anti-ferritin and ferritin as a model system. Firstly, a monolayer of (3-mercaptopropyl) trimethoxysilane (MPS) which had thiol headgroup was formed on the Au electrode by S–Au bond [24]. Subsequently, Cu_2O–SiO_2 can be adsorbed onto the electrode surface to obtain a porous nanostructured film via the covalent bond formed by the methoxysilane groups of MPS pointing outward and the surface –OH groups of SiO_2 [25]. Then one kind of organic compound (PTC-NH_2) was employed to drop on the Cu_2O–SiO_2/MPS/Au electrode, synthesized by ammonolysis of 3,4,9,10-perylenetetracarboxylic dianhydride (PTCDA). PTCDA is a kind of p-stacking organic molecules which has the desirable organic electronic properties and interspaces on the surface [26]. Later, nano-Au was adsorbed on the surface of the PTC-NH_2 film through Au–NH_2 bond. Then the amperometric biosensor for detection of ferritin was constructed by adsorption of anti-ferritin onto the nano-Au monolayer. Lastly, BSA was used to block possible remaining active sites. The Cu_2O–SiO_2 nanoparticles were characterized by scanning electron microscope (SEM), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) and auger electron spectra (AES). The electrode modification process was probed by cyclic voltammetry (CV), the optimization of the experimental conditions and the performance of the ferritin immunosensor were studied by differential pulse voltammetry (DPV).

2. Experimental

2.1. Reagents and materials

Anti-ferritin and ferritin were purchased from ABBOMAX Co. (USA). MPS was obtained from J&K Chemical Co. Ltd. (Beijing, China). PTCDA was purchased from Lian Gang Dyestuff Chemical Industry Co. Ltd. (Liaoning, China). Bovine serum albumin (BSA) (96–99%), gold chloride ($HAuCl_4$) and sodium citrate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Copper sulfate, hydrazine hydrate, ethylenediamine, cetyl trimethylammonium bromide (CTAB), tetraethyl silicate (TEOS) and other chemicals were of analytical grade and purchased from regular company.

Quantities of PTCDA were dissolved in acetone under stirring, and then excessive ethylenediamine was added drop by drop. After the reaction ended, wash the precipitate with acetone and ethanol.

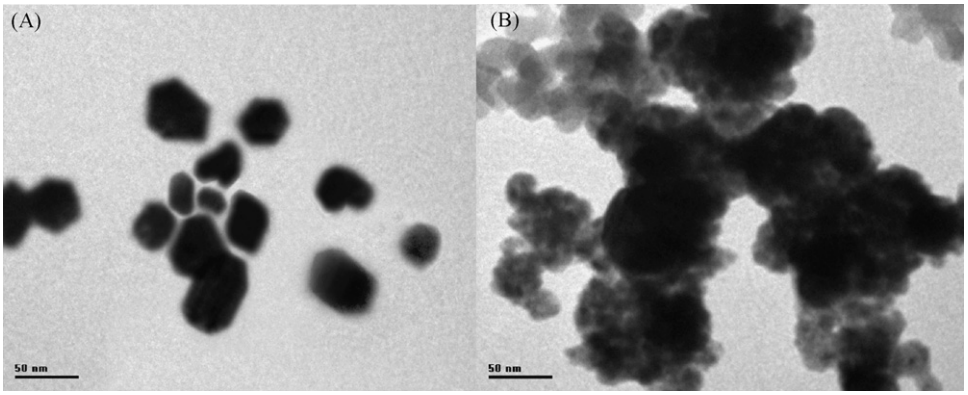

Fig. 2. TEM of pure Cu_2O (A) and Cu_2O–SiO_2 nanoparticles (B).