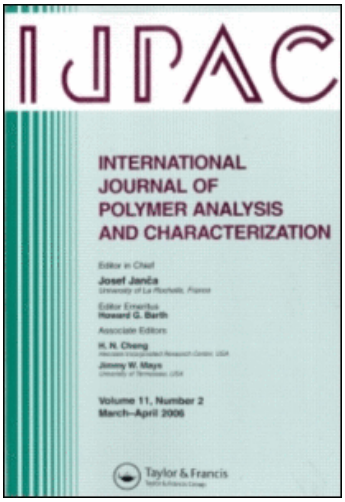

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In Situ Polymerization of Pyrrole in Mesoporous Silica and Application to Electrode Modification

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IN SITU POLYMERIZATION OF PYRROLE IN MESOPOROUS SILICA AND APPLICATION TO ELECTRODE MODIFICATION

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A hybrid system of mesoporous silica (MS) particles coated with polypyrrole (PPy) was constructed through chemical polymerization of pyrrole in the presence of the MS particles. The MS-PPy composite particles with immobilized hemoglobin (Hb) were used to modify a glassy carbon electrode (GCE) for detecting the electrocatalytic response to the reduction of hydrogen peroxide. The structure of composite particles and the performance of biosensors were characterized by SEM, TEM, FT-IR, cyclic voltammetry (CV), and amperometric measurements, respectively. The results show that the PPy shell is uniformly coated over the silica surface without using any agent to modify the MS particles. Under optimal conditions, the sensors had a fast response of hydrogen peroxide (H_2O_2). The catalytic currents are linearly proportional to the concentrations of H_2O_2 in the range from 0.01 to 1.2 mM, and the corresponding detection limits are 0.01 mM (S\N = 3).

Keywords: Biosensors; Conducting polymers; Electrochemistry; Nanocomposites; Polypyrroles

INTRODUCTION

Recently, much attention has been paid to the construction of biosensor systems, which have important medical, environmental, public safety, and defense applications.[1–3] An ideal biosensor would be sensitive, rapid, reliable, robust, and inexpensive.[1] Hemoglobin (Hb) is a kind of polypeptide enzyme consisting of four subunits and has a molecular weight of approximately 64,500 g mol^{-1}. A heme (iron porphyrin) group in each subunit acts as the active center.[2,3] Numerous studies on

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electron transfer characters of Hb have been performed, either homogeneous or heterogeneous.[4,5] However, electron transfer between redox proteins and bare solid electrodes is usually slow and the proteins are easily irreversibly denatured.[6–8] In order to accelerate the electron transfer between the proteins and electrodes and obtain a long-term stable biosensor, the development of novel materials has become the subject of intensive research, for example, the application of conducting polymers[9–11] and silica-based materials[12–15] in electrochemical sensors and biosensors.

Conducting polymers such as polyaniline, polythiophene, and polypyrrole (PPy) have emerged as promising materials for electrochemical sensors because of their electrical properties, exclusion of electroactive and surface-active interferences, capability of loading bio-reagents on tiny electrode surfaces, and other advantages.[16] The most commonly used conducting polymers, which are either doped or modified by biomaterials, exhibit unique catalytic or affinity properties.[17,18] The conductive polymers are often used in designing biosensors. PPy is one of the conductive polymers most used in electrochemical biosensors, since it has much higher chemical stability than other conducting polymers and has no toxicity. It can easily entrap various biological compounds.[19] The study of adsorption of polymers onto PPy showed that this conducting polymer has high surface energy and strong acid-base interactions with macromolecules.[20,21]

Silica particles have been extensively used as stabilizers in the preparation of PPy dispersions or colloids,[22,23] and different hybrid systems consisting of PPy and silica particles have been reported.[24,25] Compared to the other template spheres,[26,27] using silica template particles, especially mesoporous silica (MS), to prepare conducting polymer core-shell particles has some advantages.[28] The synthesis condition and processing of monodispersed MS spherical particles are very simple. To the best of our knowledge, most of the previous research focused on the preparation of silica-PPy composite microspheres; there has been little research exploring the application of this kind of material in Hb immobilization and biosensors fabrication up to now. In this article, MS particle/PPy hybrid systems were constructed through the chemical polymerization of pyrrole in the presence of MS particles. The MS-PPy composite particles were dissolved in a Nafion solution, and the dispersion was dropped onto the surface of a glassy carbon electrode (GCE) to form a composite film modified electrode. The immobilized Hb showed electrocatalytic response to the reduction of hydrogen peroxide.

EXPERIMENTAL SECTION

Materials

Hb (bovine blood) was purchased from Sigma and used as received. Hexadecylamine was purchased from Fluka. Ethyl silicate, isopropyl alcohol ammonia, ethanol, hydrogen peroxide (30% (w/v) solution), pyrrole, and other reagents were obtained from Shanghai Chemical Co. The concentrations of more diluted hydrogen peroxide solutions were determined by titration with cerium (IV) to a ferroin end point. All chemicals were of analytical grade and used without further purification. Phosphate buffer solutions (PBS, 0.1 M) with various pH values were prepared by

mixing stock standard solutions of K_2HPO_4 and KH_2PO_4 and adjusting the pH with H_3PO_4 or NaOH. All solutions were prepared with doubly distilled water.

Preparation of MS Particles

First, 16 g hexadecylamine was dissolved into the mixed solution consisting of 100 mL isopropyl alcohol and 90 mL water; it was then subjected to ultrasound. A total of 1.4 mL ammonia followed by 5.8 mL ethyl silicate was added to the solution under stirring at room temperature. After 30 min the stirring was stopped, and the mixture was allowed to stand at ambient temperature for one day. The resulting solids were recovered by filtration of the reaction mixture, then extensively washed with the distilled water and ethanol and dried at ambient temperature. The hexadecylamine serving as template was removed by calcination at 600°C for 6 h. The pore size of the obtained microspheres was approximately 3.2 nm.[29]

Pore Size Expanding

A 5.6 g amount of microspheres was added to a 20 mL mixture solution including distilled water, NaCl, LiCl, and KNO_3 at a proportion of 70:20:5:5 (wt) and then heated at 300°C for 2 h. Thus the remodeled MS microspheres with a pore size of approximately 10 nm were produced.[29]

Preparation of MS-PPy Core/Shell Particles

A 0.2 g amount of MS was dispersed in 15 mL of ethanol, and then 0.1 mL of pyrrole monomer was added to the solution. After sonication for 20 min, 15 mL of aqueous solution containing 0.9 g of $FeCl_3$ was added to the suspension. The reaction mixture was stirred for 12 h to yield uniform MS-PPy core/shell particles. Then the resulting composite particles were separated by centrifugation at 4000 rpm for 15 min and washed four times with water. Finally, the composite particles were dried at 50°C for 24 h in a vacuum oven.

Electrode Modification

Glassy carbon electrodes (GCE, 3 mm diameter) were polished successively with 1.0, 0.3, and 0.05 mm alumina powder on silk and rinsed thoroughly with doubly distilled water between each polishing step. Next, the polished electrode was sonicated in 1:1 nitric acid, acetone, and doubly distilled water and allowed to dry under room temperature.

The MS-PPy-modified electrode was prepared by dropping 5 μL of homogeneous black dispersion with 5 μL of 10% Nafion solution onto the electrode, then the electrode was left to dry in air for approximately 5 h. After that, the electrode was rinsed with water and dried under nitrogen atmosphere. Then 5 μL of Hb (20 mg Hb dissolved in 1 mL of 25 mM pH 7.0 PBS) was cast onto the surface of the modified GCE and dried for approximately 6 h at room temperature. Finally, the resultant Hb/MS-PPy modified GCE was immersed in pH 7.0 PBS at 4°C. When not in use, the obtained Hb/MS electrode was stored in 0.1 M pH 7.0 PBS at 4°C.

Apparatus and Measurements

All electrochemical experiments were performed on a CHI 660A electrochemical workstation (CH Instruments Co., Shanghai, China) at 20°C (±2)°C. The working electrode was a modified GCE with diameter of 3 mm. A saturated calomel electrode (SCE) served as reference electrode, and a Pt wire served as counter-electrode. The working solutions were deoxygenated with nitrogen gas for 15 min before measurement, and a nitrogen atmosphere was maintained over the solutions during experiment.

The particle morphologies were examined by scanning electron microscopy (SEM, Philips XL30, operated at 20 kV) and transmission electron microscopy (TEM, JEOL JEM-100CX, operated at 100 kV). Fourier transform-infrared spectra (FT-IR) of the samples were recorded by using KBr on a Bruker VECTOR22 spectrometer.

RESULTS AND DISCUSSION

Morphology of MS Particles

Figure 1 shows the SEM image of MS particles used as the core particles in the synthesis of core/shell particles. It can be seen that MS particles are all spherical in shape and exhibit good monodispersity. The mean diameter is about 1.2 μm. The high-magnification cross section TEM image (inset in Figure 1) indicates that the surface of MS particles is mesoporous.

Morphologies of MS-PPy Core/Shell Particles

Figure 2 presents the SEM image of MS-PPy core/shell particles. The shape of composite particles is still spherical and the particles also have a narrow size

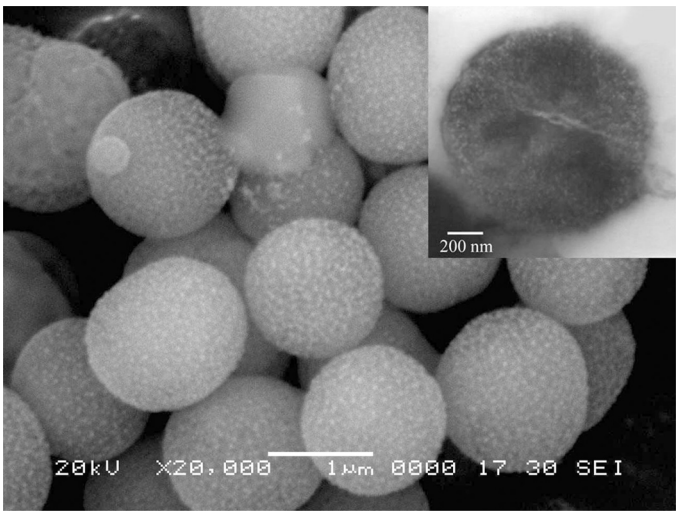

Figure 1. SEM image of MS particles with mean diameter about 1.2 μm. The inset is a high-magnification cross section TEM image indicating that the surface of MS particles is mesoporous.

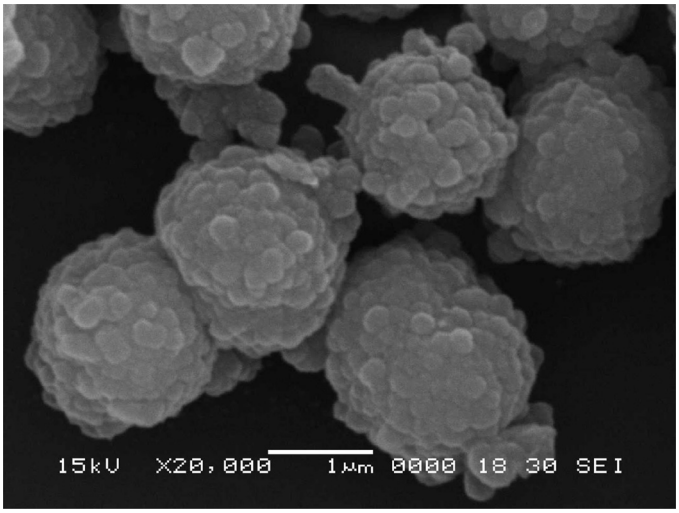

Figure 2. SEM image of MS-PPy core/shell particles. The shape of composite particles is still spherical and the particles also have a narrow size distribution.

distribution. This indicates that PPy has enwrapped the MS particles effectively and formed spherical MS core-polypyrrole shell composite particles. Compared with MS particles, the surface of composite particles is very rough, which is shown in Figure 2. This is due to pyrrole monomer polymerized to form PPy particles and these particles piled on the surface of MS particles.

PPy deposition was first performed on the surface of MS particles. Up to now, most of the work about fabrication of MS-conducting polymers introduced modified agents such as silane coupling agent and kinds of surfactants to improve the surface character of MS particles.[28] However, it is a surprising finding that PPy can be successfully coated on MS particles without using any agents to modify the MS particles. We assumed that MS particles, with pore size of 2–10 nm, possess uniform pore size and large surface area as well as mechanical and chemical resistance. They can adsorb pyrrole monomer and then polymerize to form PPy shell. Besides the effective deposition of PPy due to electrostatic interaction, successful anchoring of a layer of PPy on MS particles can be attributed to the hydrogen bonding interaction between MS and pyrrole.

Infrared Spectra of Samples

Figure 3 shows the infrared spectra of MS particles and MS-PPy composite particles. Compared to the IR spectrum of MS particles, the important peaks near 1178 and 924 cm^{-1} imply the doping state of polypyrrole.[30,31] We also find that the absorption peaks at 1100 cm^{-1} of MS-PPy composite particles are lower. This is due to the effect of MS templates in composite particles.

Electrochemical Studies

Figure 4 shows the cyclic voltammograms of different modified electrodes in 25 mM pH 7.0 PBS at a scan rate of 100 mVs^{-1}. No redox peaks were observed at

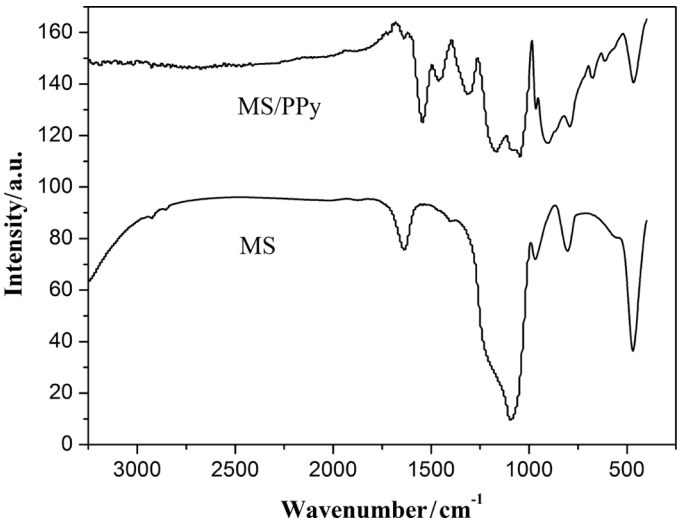

Figure 3. FT-IR spectra of MS particles and MS-PPy composite particles.

the bare GCE (Figure 4, curve a) or MS-PPy/GCE (Figure 4, curve b), however, Hb/GCE (Figure 4, curve c) and Hb/MS-PPy/GCE (Figure 4, curve d) gave a couple of redox peaks at −300 and −410 mV, which was the characteristic of heme Fe(III)/Fe(II) redox couple of the protein.[32] Obviously, the response of Hb/MS-PPy/GCE is attributed to the redox of the electroactive centers of the immobilized Hb. The results showed that direct electron transfer between this heme protein

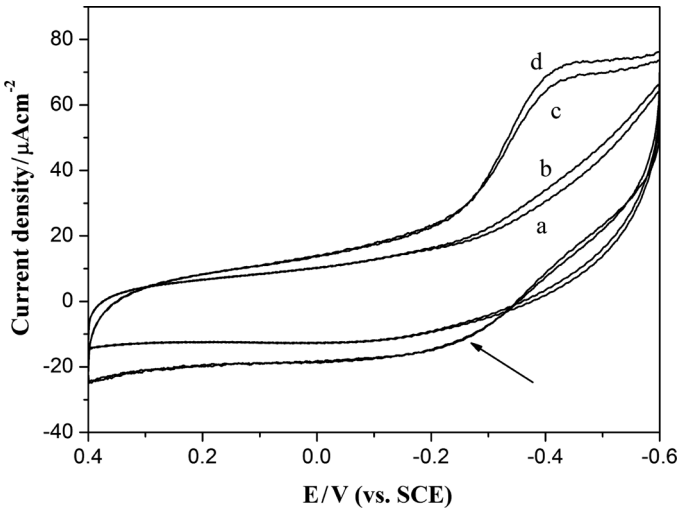

Figure 4. CVs obtained at GCE (curve a), MS-PPy-modified GCE (curve b), Hb-modified GCE (curve c), and Hb/MS-PPy-modified GCE (curve d) in 0.10 M N_2-saturated phosphate buffer. Scan rate, 100 mV s^{-1}.

and GCE was enhanced on the MS-PPy composite film. But it should be noted that the enhancement was not so important. The reason may be that although the MS-PPy composite film provided a favorable microenvironment for Hb immobilization, which would accelerate the direct electron transfer between Hb and the conductor surface,[33] it still represents the obstructions to electron transfer of the redox probe.

The reduction peak current of Hb/MS-PPy/GCE shows strong dependence on the pH of external solution. When solution pH changes from 5.0 to 9.0, Figure 5 shows the maximum response current value at pH 7.0. Thus pH 7.0 of 0.1 M phosphate buffer was used to support the electrolyte for H_2O_2 detection in most cases. Therefore, the electrochemical reaction can be expressed as follows[34]:

$$\mathrm{HbFe(III)} + \mathrm{H^+} + \mathrm{e^-} \rightarrow \mathrm{HbHFe(II)} \quad (1)$$

Electrochemical Catalysis

It is well known that heme proteins, such as horseradish peroxidase and Hb, are able to electrocatalyze the reduction of H_2O_2, O_2, and so on.[12,35–37] To test the potential application of Hb/MS-PPy/GCE, the electrocatalytic reduction of H_2O_2 on the electrode was studied. As shown in Figure 6, upon the addition of H_2O_2 to pH 7.0 PBS, the CV of Hb/MS-PPy/GCE changes dramatically. The cathodic current increases, but for MS-PPy/GCE the change of CVs is indiscernible. This indicates that the immobilized Hb has electrocatalytic effect on the reduction of H_2O_2. The electrocatalytic process can be described as follows:[38]

$$\mathrm{H_2O_2} + 2\mathrm{HbHFe(II)} \rightarrow 2\mathrm{HbFe(III)} + 2\mathrm{H_2O} \quad (2)$$

Figure 7 shows a typical current-time plot of Hb/MS-PPy/GCE at the applied potential of −410 mV upon the additions of successive aliquots of H_2O_2 to the pH

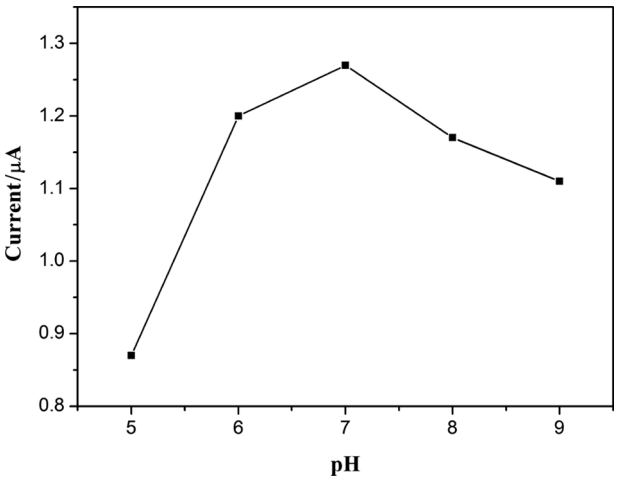

Figure 5. Effect of pH on the electrocatalytic currents of 0.1 M PBS containing 1 mM H_2O_2 at −410 mV.

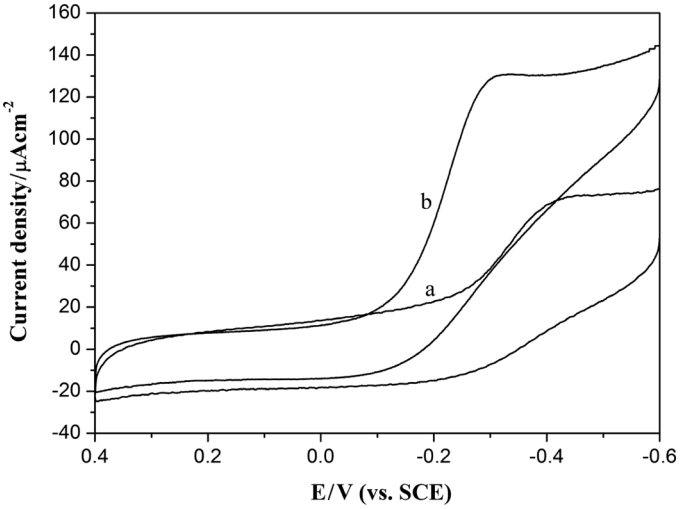

Figure 6. CVs obtained at Hb/MS-PPy/GC electrodes in the absence (curve a) and presence (curve b) of 0.10 mM H_2O_2 N_2-saturated phosphate buffer. Scan rate, 100 mV s^{-1}.

7.0 PBS. The biosensor exhibited a rapid and sensitive response to the changes of H_2O_2 concentration and the reduction current increased steeply to reach a stable value. So the electrocatalytic response could be used efficiently for H_2O_2 detection. The amperometric response showed a linear relation with H_2O_2 concentration from 0.01 to 1.2 mM with a correlation coefficient of 0.998 (inset in Figure 7). The detection limit was estimated to be 0.005 mM with a signal-to-noise ratio of 3. This value is better than 50 μM to 1.2 mM for Hb entrapped in gelatin (gel) films.[39]

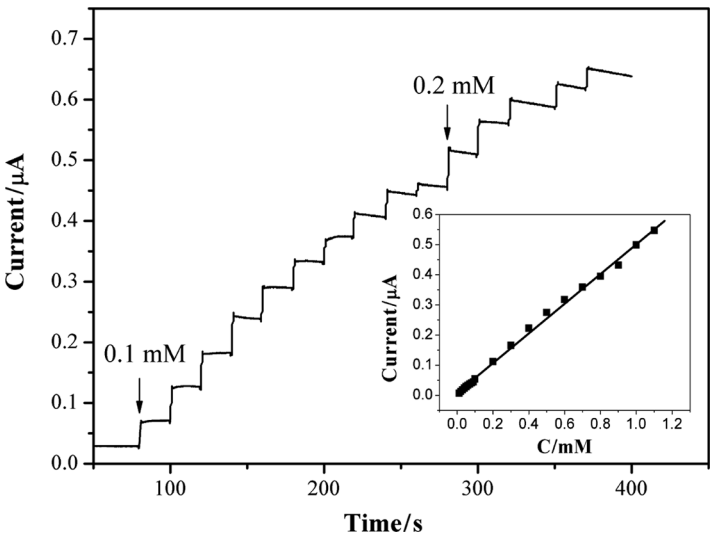

Figure 7. Amperometric responses of Hb/MS-PPy-modified GC electrodes in stirred solutions to the successive additions of H_2O_2. Applied potential, −410 mV vs. SCE.

The operational stability of the Hb/MS-PPy electrode was investigated by consecutive measurements of its response to 0.5 mM H_2O_2. About 86% initial activity remained after 100 measurements. When we stored the electrode under 4°C at dry conditions and measured it daily for 30 days, the response to 0.5 mM H_2O_2 maintained over 80% of the first-day response. A conclusion can be drawn from the above results that it was efficient for retaining the electrocatalytic activity of Hb and preventing it from leaking off the electrode.

CONCLUSION

In this study, we prepared the MS-PPy composite microspheres without introducing modified agents and immobilized Hb onto the MS-PPy-modified GCE. The immobilized Hb retains its biological activity well and shows high catalytic activity to the reduction of H_2O_2. Under the optimized experimental conditions, the catalytic currents are linearly proportional to the concentration of H_2O_2 in the range from 0.02 to 30 mM and the corresponding detection limits are 0.01 mM ($S/N = 3$). The sensor shows good reproducibility and stability. The fabrication method of the biosensor opens a new opportunity for the development of other enzymes that are simple and reliable.

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