

Homogenous Thionine-SiO₂ Nanocomposite Spheres: Sonochemical Preparation, Characterization, and Application in H₂O₂ Biosensor

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The electrochemical properties of new homogenous thionine (TH)-doped silica nanoparticles as a mediator were studied. The nanocomposites were synthesized by using sonochemical method. The sonication induced the strong combination between dye molecules and silica in the construction of the composites. By controlling reaction condition, face rougher uniform spheres could be achieved. The as-prepared nanocomposites can be used as the mediator to construct the biosensors with horseradish peroxidase coimmobilized in the gelatine matrix and cross-linked with formaldehyde. The described experimental evidences show the different ratio of ethanol to water can induce the changes in their diameters. As a consequence, the resulting biosensors exhibited amperometric response decreased with the increase of the spherical size. The resulting biosensor mediated by the 100 nm diameter of TH-doped silica nanoparticles exhibited very fast amperometric response, good stability and could be effectively used for the study of the electrocatalytic properties.

Keywords: Sonochemistry, Nanocomposites, Thionine, Mediator, Biosensors.

1. INTRODUCTION

Inorganic/organic nanocomposites have attracted great interest of many researchers, due to the enhanced or unique electronic, optical and magnetic properties derived from the synergistic and hybrid effects of several components. And they have been found wide applications in versatile areas, such as catalysis, optics and chromatography.^{1–3} Silica-based hybrid materials are receiving particular attention especially, because silica has significant properties in biocompatibility and bioconjugation. Various substances such as polymer,⁴ enzymes,⁵ protein,⁶ DNA⁷ and fluorescent dye molecules,^{8–9} have been doped into SiO₂ matrix. Silica nanocomposites can be also used as a superior signaling element in an immunoassay by conjugating them to an antibody. Different types of targets, including proteins, cells, and bacteria have been detected by these nanocomposites.^{10–13} These Hybrids showed significant advantages over the single substances in bioanalysis and biotechnology applications. However, to the best of our knowledge, there are only a few reports about the

hybrid of SiO₂ matrix with the electrochemical active dyes molecular,^{14–16} which may have better performance in electrochemical bioanalysis.

Good stability and high hybrid concentration of the SiO₂-based materials are the keys for their applications and are also the challenge for researchers. The organic dye molecules cannot be easily doped into the silica matrix because the dye molecules and the SiO₂ matrix lack strong enough interaction. And the dye molecules with small size are easy to leak out from the matrix. A variety of techniques are available for the preparation of nanocomposites. So far, the syntheses of dyes-doped silica nanocomposites have two general routes: one is reverse microemulsion, and the other is the Stöber method.¹⁷ In recent years, ultrasonic synthesis has attracted much interest in preparation of nanomaterials. High temperature (~5000 K), high pressure (>1800 atm) and cooling rates in excess of 10¹⁰ K·s⁻¹, can be produced at the centers of bubbles during sonication. This extreme atmosphere provides a unique reaction condition and has been successfully applied to prepare various nanosized materials. Previous reports were only concerning with novel metals¹⁸ or semiconductors¹⁹ silica composite via sonochemical method. In this report, a homogenous

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SiO₂-dye composite material was successfully synthesized by a sonochemical route.

The selected dyes thionine (TH)²⁰ are widely used as electrode surface modifiers. Unfortunately the dyes are easy to leak out of the electrode surface during the electrochemical studies because of the low molecular weight and good dissolvability. Therefore, the as-prepared composites, which have high dye concentrations and are size- and morphology controllable, and combine the efficient electrocatalytic properties of dyes with the physical properties of the SiO₂, can be used as mediator and entrapped efficiently on the electrode surface. Nevertheless, it is interesting that the electrochemical response for the biosensors depended deeply on the size of the spheres.

Herein, the electrochemical properties of new homogeneous thionine (TH)-doped silica nanoparticles as the mediator were studied. The sonication induced the strong combination between dye molecules and silica. The as-prepared nanocomposites can be used as the mediator to construct the biosensors with horseradish peroxidase coimmobilized in the gelatine matrix and cross-linked with formaldehyde. The biosensor exhibited amperometric response decreased with the increase of the spherical size. The biosensor mediated by the 100 nm diameter of TH-doped silica nanoparticles exhibited fast amperometric response, good stability and effectively use in the study of the electrocatalytic properties.

2. EXPERIMENTAL DETAILS

2.1. Reagents

Horseradish peroxidase (HRP, BE 1841) was obtained from sigma (USA). Tetraethyl orthosilicate (TEOS) was obtained from Tianjin Reagent Factory (China). Thionine (TH) (not purified before use) was purchased from Third Chemical Factory of Shanghai (Shanghai, China). H₂O₂ (30% w/v solution) was purchased from Shanghai Chemical Reagent Company (China). The concentration of the diluted hydrogen peroxide solutions prepared was determined by titration with cerium (IV) to a ferroin endpoint. All other reagents were of analytical grade. All the solutions were prepared with doubly distilled water.

2.2. Apparatus and Measurements

All electrochemical experiments were carried out in a three-electrode cell controlled by CHI 660 Electrochemical workstation (CH Instruments, USA). An electrode with TH-doped silica nanoparticles and HRP was used as the working electrode. Reference and counter electrodes were a SCE and platinum wire, respectively. Fourier transform infrared (FTIR) spectra by KBr pellets were obtained in the range of 4000~500 cm⁻¹ on a Bruker 22 FT-IR spectrometer at room temperature. UV-visible absorption spectra were obtained with UV-3100 spectrophotometer

(SHIMADZU) at room temperature. The investigation of the morphology and microstructure of the products was carried out by transmission electron microscope (TEM) (JEOL JEM-200CX) and scanning electron microscope (SEM) (JEOL-4000EX).

2.3. Synthesis of SiO₂-Dye Nanocomposites

For the typical synthetic reactions, the 0.01 g of TH was dissolved in a mixed solution with 2.5 mL of TEOS and 14 mL of ethanol in a 100 mL beaker. Then 8 mL of ammonia and 36 mL of distilled water were mixed and added in the tetraethyl orthosilicate (TEOS)/EtOH mixture under stirring. This solution was irradiated with a high-intensity ultrasonic horn (Sonics, Model VCX750, 1.25 cm Ti-horn, 20 kHz, 100 W/cm²) under ambient air for 30 min and a colloid solution was obtained. After cooled to room temperature, the colloid was centrifuged, and washed by ethanol and distilled water in sequence for two times respectively.

2.4. Preparation of the Working Electrodes

Gelatine solution (1%) was prepared by dissolving gelatine flake in hot doubly distilled water. Glassy carbon working electrodes (GCE, 3 mm diameter) were polished successively with 1.0, 0.3, and 0.05 μm alumina powder on chamois leather, 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 the allowed to dry at room temperature.

0.2 mg of HRP and amount of the SiO₂-dye composite were dissolved in 0.5 mL of gelatine solution (1%), and then 10 μL of formaldehyde solution was added into the enzyme solution. The mixture was hand-mixed completely. The GCE was coated with a drop of 10 μL the resulting mixture, and then was left for at least 24 h at 4 °C. Thus, HRP and SiO₂-TH composite were entrapped in the membrane. The enzyme biosensors were stored at 4 °C in a refrigerator when not in use.

3. RESULTS AND DISCUSSION

3.1. Characteristics of the Nanocomposites

The SiO₂-TH nanocomposite spheres were successfully prepared. The selected TEM images in Figure 1 show the morphology of the spheres. The SiO₂-TH composites are uniform spheres with the average sizes of ca. 80 nm respectively (Fig. 1(a)). Compared with the smooth SiO₂ spheres (Fig. 1(b)) prepared in the absence of the dyes, the composite spheres are much rougher. Further observation of a SiO₂-TH composite particle reveals that the composite sphere is consisted of many smaller colloid particles. In the reaction, many dispersed small colloid

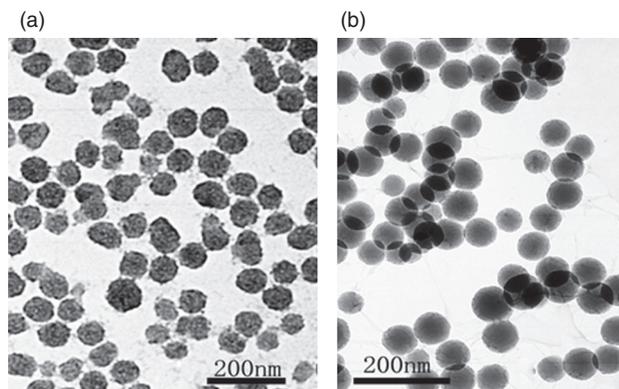


Fig. 1. TEM image of SiO₂/TH (a) and SiO₂ (b) particles.

nanoparticles were also observed and the spheres grew with time until they reached certain size. Hence we considered that the TEOS would first polymerize to form initial small particles and then combined to large spheres gradually. As we mentioned above, with the good solubility in the TEOS/EtOH/water mixture solution, the dyes could be doped into the silica matrix in the polymerization of TEOS. And we think that the extreme atmosphere inside the collapsing bubbles and the strong shock wave generated by the sonication contributed to the formation of the SiO₂-dye composites.^{21–22} In the formation process, TEOS molecules polymerized through Si-O-Si bonds to form SiO₂ small particles. The High temperature (~5000 K) and high pressure (>1800 atm) would promote the polymerization and simultaneously enhance the activity of amid cyanogens on the dye molecules to bond with part of the Si-O- groups. Meanwhile the shock wave also benefited to the growth of the spheres through the enhanced collides of small particles.

A comparing experiment in the absence of ultrasound was carried out. When the silica spheres was mixed with the dye for two days, the resulting SiO₂-TH nanocomposites were with slight blue color, and the color can be easily washed out. It exhibited that the surface adsorption was not tight. However, such a phenomenon never exhibited on our composites. The colors of the as-prepared nanocomposites were deep blue and were difficult to be washed out, which indicated the sonication induced the strong combination between dye molecules and silica in the construction of the composite.

The combination of the dyes and SiO₂ were further demonstrated by UV-Vis and FTIR measurements. The UV-Vis spectra of the dyes and dye-SiO₂ composites in water were measured. Figure 2 shows the typical UV-Vis absorption spectra of thionine aqueous solution, SiO₂ solution and SiO₂/thionine solution. In spectrum (b), the 599 nm main peak is characteristic of monomeric thionine, while the 560 nm shoulder can be attributed to the H-type dimmer aggregate.²³ As shown in Spectrum (c) the characteristic absorption peaks (about 600 nm) of the pure TH molecules can also be observed in the composite suggests

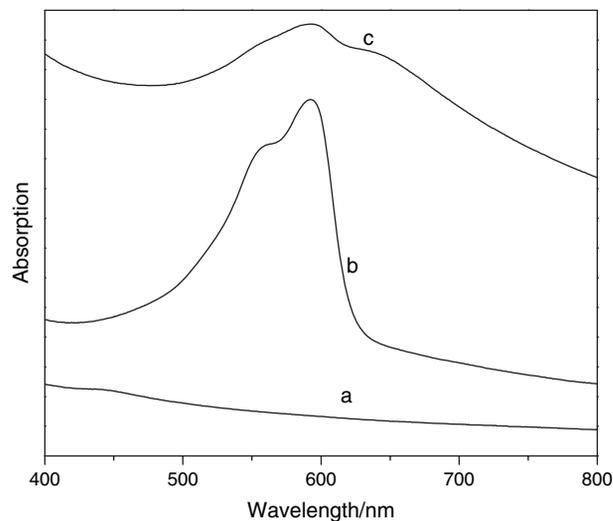


Fig. 2. UV-visible spectra curves: (a) SiO₂, (b) TH, (c) TH-SiO₂.

that thionine is entrapped in SiO₂. However, for the composites there exists a red shift of the peaks (about 630 nm) compared with the pure dyes, which is evidence of interaction between SiO₂ and thionine molecules.²⁴

In the FTIR measurements, for example, the absorption band at 3309 cm⁻¹ and 3166 cm⁻¹ correspond to the vibration of N-H belonging to the characteristic peaks of TH (Fig. 3(a)), which disappeared in SiO₂-TH composite (Fig. 3(c)). The main reason may be attributed to the low concentration of TH in SiO₂ compare with pure TH and the partially band interaction. Furthermore, for the TH-SiO₂ composite, the absorption band at 1103 cm⁻¹ attributes to Si-O-Si (Fig. 3(b)) stretching vibration that shifted slightly to 1097 cm⁻¹. Such results demonstrated that TH was doped in SiO₂ and the TH formed a composite with the SiO₂ by a bonding interaction between the Si-O groups of SiO₂ and the nitrogen of the amidcyanogens of the TH.²⁵

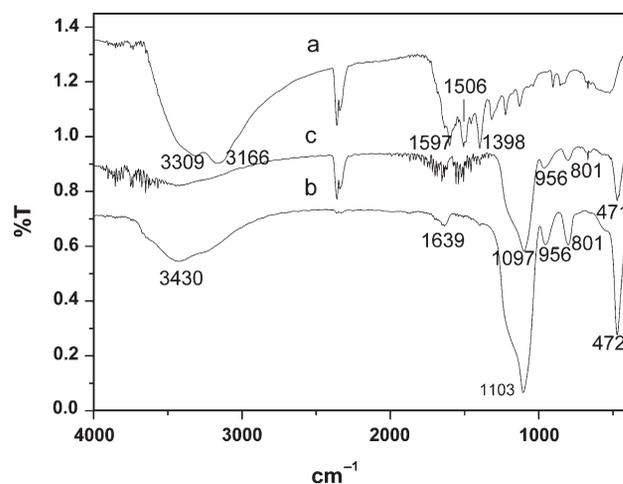


Fig. 3. FTIR spectra of TH-silica composites. (a) TH, (b) SiO₂, (c) TH-SiO₂.

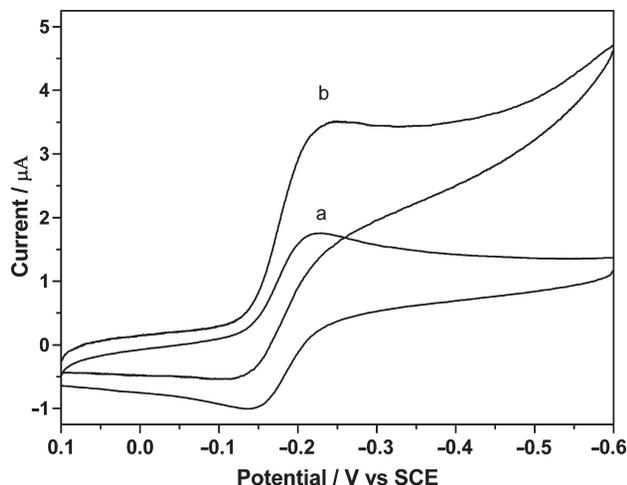


Fig. 4. Cyclic voltammograms of SiO₂-TH-gelatin-GC in the absence of H₂O₂ (a), and in the presence of 1.1 mM H₂O₂ (b) in pH 7.0 PBS at scan rate of 100 mV/s.

3.2. Electrochemical Studies of the Nanocomposites

The as-prepared SiO₂-dye nanocomposites exhibited good electrochemical activity and stability when serving as electron transfer mediator. Due to the homogeneous state, high concentration and strong combination between SiO₂ and the dye, the electron can easily transfer through the nanoparticles directly. At the same time, the leakage of the low molecular weight dye from the electrode surface could be avoided. Furthermore, the rough surface was also more effective in the adsorption of the protein molecular. Herein, the spheres were used as the mediator to construct the H₂O₂ biosensor with horseradish peroxidase coimmobilized in the gelatine matrix and cross-linked with formaldehyde.

Figure 4 shows the typical cyclic voltammetric curve of the enzyme electrode by using 80 nm TH-SiO₂ particles as the mediator. In blank phosphate buffer solution, the enzyme electrode only gave the electrochemical behavior of thionine. There was a pair of quasi-reversible anodic and cathodic waves. When 1.1 mM H₂O₂ was added into the solution, cathodic peak current increased significantly. A little peak-potential shift toward negative direction was observed with the increase of H₂O₂ concentration. These phenomena demonstrated that TH-SiO₂ incorporated in this matrix could effectively shuttle electrons between the base GCE and the bioactive center of HRP in the membrane.²⁶

3.3. Size and Morphology Effect on the Electrochemical Properties of TH-SiO₂ Nanocomposite

The effect of the ratio of EtOH/water on the morphology and size was investigated. Regular compact spheres with enough dye concentration can be obtained just in a certain range of ratio of EtOH/water. By carefully adjusting

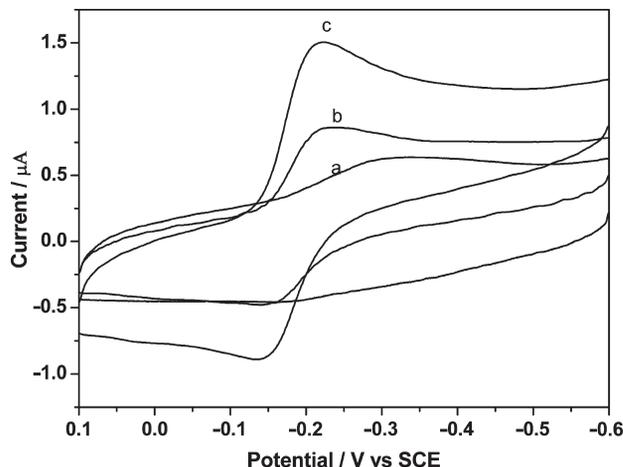


Fig. 5. The cyclic voltammetric behavior of prepared SiO₂-TH spheres with different diameters in the presence of 1.1 mM H₂O₂ in pH 7.0 PBS at scan rate of 100 mV/s (a) irregular spherical particles with the diameter from 150 nm to 200 nm (b) regular particles with the diameter of 100–120 nm (c) Regular particles with the diameter of about 80 nm.

the ratio from 13:37 to 15:35 in the appropriate range, the average diameter of the spherical SiO₂-TH composites increased from 50 nm to 120 nm. This indicates that the diameters increased with the ratio in a certain range. However, if the ratio of EtOH/water was further increased, the dye concentration of the products was very low, and silica spheres became smooth. The reason is that the polymerization rate increased at the elevated ratio and the TH molecules could not be doped into silica matrix easily.

It was found that the electrochemical response for the biosensor depended on the size of the spheres. Figure 5 showed the cyclic voltammetric behavior of the biosensor using three different diameters SiO₂-TH spheres as mediator. In this figure, it was observed that the redox current decreased with the increase of the sphere size and the current was the most sensitive by using the nanocomposites with 80 nm diameter. Thus behavior might be attributed to the long electron transfer distance, the decreased content of TH and the increased resistance of the composites. On the other hand, though the decrease of size for the composite spheres was benefit to increasing electrochemical response, the stability of the biosensor for their leakage from the gelatine matrix could be affected. In order to acquire high sensitivity, fast response and good stability, the regular and relative compact spheres with the diameter around 80 nm were used in the experiments.

3.4. Electrochemical Studies

Various factors influenced on the performance of the sensor. In order to obtain a high sensitivity and fast response, applied potential, buffer pH and temperature were investigated. For reaching the maximum current response, the operating potential, pH, and the temperature were selected as -0.3 V, 7.0, and 50 °C, respectively.

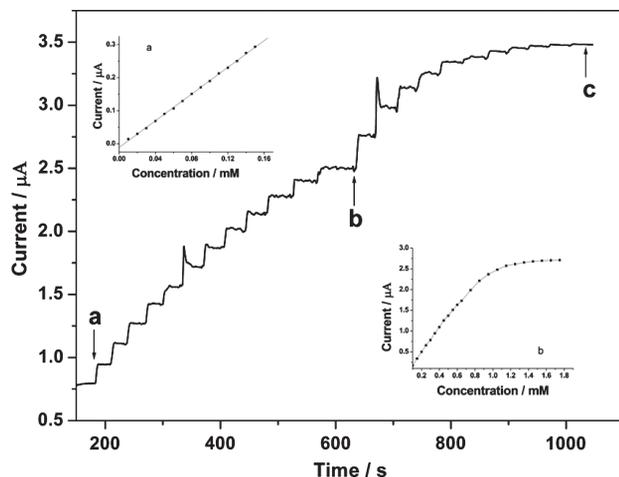


Fig. 6. Dynamic response of the H₂O₂ biosensor to successive addition of (a–b) 0.05 mM (b–c) 0.1 mM H₂O₂ steps in the solution at the working potential of -0.3 V. Inserted figure (a) shows the calibration plot between the current and the lower concentration of H₂O₂; inserted figure (b) shows the calibration plot between the current and the concentration of H₂O₂.

Figure 6 displayed the dynamic response of the electrode under the optimal experimental conditions with successive injection of H₂O₂ to the phosphate buffer solution under stirring. The trace clearly demonstrated the fast response and high sensitivity of the electrode to H₂O₂. The time required to reach 95% of the maximum steady-state current was less than 20 s. The response to H₂O₂ was linear in the range from 0.01 to 1.0 mM. The detection limit was 5.3 μ M when the signal to noise ratio is 3.

The stability of the biosensor was also investigated by the amperometric measurements in the presence of 0.05 mM H₂O₂ periodically. It was found that the biosensor also retained its original response after one month of testing. At the same condition, when the directly modified dyes served as the mediator, the comparing biosensor lost the response only after two days. The good stability can be attributed to the great stability of the mediator, the good biocompatibility, and the stabilizing microenvironment around the enzyme provided by the organically modified gelatine composite matrix.

4. CONCLUSIONS

An ultrasonic-assisted method to synthesize a series of novel different diameter and morphology SiO₂-TH nanocomposites was developed. Ultrasound can lead to the strong interaction of TH molecules and SiO₂ matrix in the formation of the uniform homogeneous SiO₂-TH nanocomposites. Adjusting the ratio of Ethanol/water can get controllable the size and morphology of the nanocomposites. The nanocomposites can be successfully entrapped

into gelatine cross-linked matrix to provide a three-dimensional interface to adsorb the enzyme and exhibited perfect behavior as mediators. The resulting biosensor exhibited high sensitivity and excellent stability. The stable structure of such kind of composite would have wide applications in electrochemistry and optics. Herein, the nanocomposites were just used as mediators, and other applications are being further developed in our laboratory.

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