Synthesis of γ -Fe₂O₃/SiO₂/Au magnetic composites for immobilization of bovine serum albumin

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A method for the two-step synthesis of magnetic composites with a γ -Fe₂O₃ core, silica inner layer and numerous gold nanoparticles supported on the surface of the silica (γ -Fe₂O₃/SiO₂/Au) is described. First, thiol-functionalized γ -Fe₂O₃/SiO₂ composites and gold colloids are prepared by modifying γ -Fe₂O₃/SiO₂ composites with mercaptosilane and reduction of Au³⁺ to Au⁰ with citrate, respectively. Gold nanoparticles are then assembled on the surface of the thiol-functionalized γ -Fe₂O₃/SiO₂ composites to form γ -Fe₂O₃/SiO₂/Au composites. The structure of the composite particles is confirmed by transmission electronic microscopy and powder X-ray diffraction. Immobilization studies with bovine serum albumin (BSA) demonstrate that the γ -Fe₂O₃/SiO₂/Au composites can be used to immobilize BSA, making them useful for biomedical and biological applications.

magnetic composite, synthesis, characterization, protein immobilization

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In recent years, magnetic nanoparticles consisting of magnetite (Fe₃O₄) or maghemite (γ -Fe₂O₃) have been studied intensively because of their potential applications in biomedical fields, such as protein separation [1], drug delivery [2,3] and magnetic resonance imaging [4]. However, because of magnetic dipolar attraction, unmodified magnetite nanoparticles tend to aggregate into clusters, which inhibits their activity when they are directly exposed to biological systems [5], limiting their application. One of the main approaches to overcome this limitation is to protect naked magnetic nanoparticles with a coating [6,7]. Among coating materials, noble metals such as Au nanoparticles have received significant attention because of their chemical stability and biocompatibility, as well as their reactivity with thiol compounds. Therefore, the combination of magnetic nanoparticles and Au to form a nanocomposite would produce a unique multifunctional material with broad application prospects [8–11]. However, directly coating magnetic nanopar-

ticles with gold is a difficult task because of the dissimilar nature of the two surfaces [12]. Silica is one of the most common linkers used to minimize contact between the interface of gold and magnetic particles. Ji et al. [13] synthesized hybrid nanoparticles with a γ -Fe₂O₃-silica core and a gold nanoshell using the Stöber process following two-step reduction of HAuCl₄ solution. Salgueiriño-Maceira et al. [14] developed a method to prepare composites of Fe₃O₄/ γ -Fe₂O₃ surrounded by a thick silica shell and further covered these composites with an outer shell of gold. Recently, Xu et al. [15] synthesized nanocomposites composed of a ferrite core coated with mesoporous silica and numerous gold nanoparticles supported on the surface of the mesoporous silica. In this paper, a two-step synthesis of magnetic composites is presented. First, γ -Fe₂O₃/SiO₂ composites were modified with mercaptosilane, and then gold nanoparticles were attached to the surface of the γ -Fe₂O₃/SiO₂ composite *via* Au-S bonds to form γ -Fe₂O₃/SiO₂/Au magnetic composites. The ability of the magnetic composite to immobilize bovine serum albumin (BSA) without a coupling

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was investigated to determine the potential of the composite particles for application in biomedical fields.

1 Experimental

1.1 Synthesis of $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ magnetic composites

Thiol-functionalized $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composites were first synthesized by adding the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite (12.5 mg) to water (10 mL), followed by addition of absolute ethanol (10 mL) [16]. 3-Mercaptopropyltriethoxysilane (14 μL) and ammonia (38 μL) were then added to the solution. The solution was stirred at a speed of 600 r/min for 6 h at 60°C. The products were easily separated from the solution using a NdFeB magnet, and washed 6 times each with absolute ethanol and ultrapure water. The thiol-functionalized composites were dispersed in ultrapure water for characterization.

The gold colloid was prepared according to the Frens method [17]. An aqueous solution containing 0.01% HAuCl_4 (20 mL) was heated to reflux and then a solution of trisodium citrate (1%, 0.2 mL) was quickly added to the heated, stirred solution. After the color of the mixture finished changing, the solution was heated under reflux for an additional 15 min, and then cooled to room temperature.

A $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite was obtained by mixing an aqueous solution of the thiol-functionalized $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite (2.5 mg in 5 mL) with a solution of Au nanoparticles (6 mg in 12 mL) and gently shaking the mixture continuously for 24 h at room temperature. The resulting $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite was separated from the solution with a magnetic separator and washed with ultrapure water six times. The composite was dispersed in ultrapure water for characterization and application.

1.2 Immobilization of BSA

The $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite (1 mg) was added to Tris-HCl buffer solution (300 μL , pH 7.4). A solution of BSA (containing 100 to 700 μg of BSA) was added to the composite solution and shaken at 180 r/min for 20 min at 37°C in an incubator. The composites were separated using a magnet and then washed twice with phosphate-buffered saline containing 0.05% Tween to reduce the nonspecific absorption of BSA. The uncoupled BSA was collected by combining the supernatant fractions.

2 Results and discussion

2.1 Characterization of thiol-functionalized $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composites

TEM images of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ and thiol-functionalized composites are shown in Figure 1. The $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite (Figure 1(a)) appeared quite different to the thiolated

particles (Figure 1(b)). The $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite showed a clear core ($\gamma\text{-Fe}_2\text{O}_3$)-shell (SiO_2) structure with a smooth surface, spherical shape and uniform size, consistent with previous observations [18]. However, after the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite was functionalized with 3-mercaptopropyltriethoxysilane by condensation, the surface of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite became rough, indicating that the surface of the particles has been modified with 3-mercaptopropyl groups.

The presence of thiol groups attached to the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite was confirmed by Fourier transform infrared (FTIR) spectroscopy, as shown in Figure 2. The spectrum of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite (Figure 2(a)) exhibited bands at 3428 and 1658 cm^{-1} that correspond to the O–H vibrations of residual H_2O , bands at 1071, 798 and 467 cm^{-1} corresponding to the Si–O–Si stretching vibration, a band at 953 cm^{-1} corresponding to Si–OH bending vibrations [19], and bands at 1071 and 614 cm^{-1} corresponding to C–O and Fe–O stretching vibrations, respectively [13]. The spectrum of the thiol-functionalized $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite (Figure

Figure 1 TEM images of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite (a), and thiol-functionalized $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite (b).

Figure 2 FTIR spectra of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite (a), and thiol-functionalized $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite (b).

2(b)) showed some extra bands in addition to the bands from $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$. Additional bands were observed at 2927, 2890 and 1447 cm^{-1} corresponding to CH_3 and CH_2 vibrations, 2570 cm^{-1} corresponding to SH stretching vibrations, and 791 cm^{-1} corresponding to Si-C stretching vibrations [20]. The presence of these additional bands in the FTIR spectrum of the thiol-functionalized $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite indicates that thiol functionalization was successful.

2.2 Characterization of Au nanoparticles

The prepared Au nanoparticles were investigated by TEM to determine their size and dispersion (Figure 3(a)). The prepared Au nanoparticles are uniform in size with a diameter of 10 nm and are well-dispersed in aqueous solution. The absorption spectrum of a solution of the Au nanoparticles is shown in Figure 3(b). A broad absorption peak from 400 to 650 nm with a maximum at 516 nm was caused by local surface plasmon resonance of the Au nanoparticles.

Figure 3 (a) TEM image and (b) UV-Vis spectra of Au nanoparticles. (1) Before assembly, and (2) after assembly with the thiol-functionalized $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite.

2.3 Assembly process of the Au nanoparticles

To investigate the assembly process of the Au nanoparticles onto the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite, the absorbance of the Au nanoparticles at 516 nm was monitored. The optical density of the solution of Au nanoparticles was 0.799 before mixing with the thiol-functionalized $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite. After assembly, the optical density of the solution decreased to 0.041 (Figure 3(b), (2)), indicating that the Au nanoparticles have attached onto the surface of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite to form a $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite. A TEM image of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite (Figure 4) clearly shows that the surface of the larger particles are covered with nanoparticles with a diameter of 10 nm and low electron penetrability. This confirms that the decrease in the absorbance of the solution of Au nanoparticles can be ascribed to assembly of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite.

2.4 Structure of $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite

The structural features of the Au nanoparticles and $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite were further examined by X-ray diffraction (XRD). The XRD patterns obtained for the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ and $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composites are shown in Figure 5(a) and (b), respectively. In the case of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite, six major peaks were observed from 30° to 63° that were consistent with diffraction from the (200), (311), (400), (422), (511), and (440) planes of $\gamma\text{-Fe}_2\text{O}_3$ (JCPDS card No.79-0418). The amorphous SiO_2 layer in the composite results in a broad diffraction peak at 25° . For the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite, besides the peaks from $\gamma\text{-Fe}_2\text{O}_3$, four additional peaks were observed at 38.2 , 44.4 , 64.6 , and 77.6° that could be assigned to diffraction from the (111), (200), (220) and (311) planes of Au, respectively (JCPDS card No. 04-0784). These results confirmed that Au nanoparticles were present in the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite.

Figure 4 TEM image of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite.

Figure 5 XRD patterns of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ composite (a) and $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite (b).

2.5 Application of BSA immobilization

To assess the possibility of using the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite in biological fields, the immobilization of BSA on the composite was investigated. The capacity and efficiency of immobilization of BSA was determined by measuring the absorbance at 280 nm of the uncoupled BSA solution [21]. Absorption spectra of the solution containing 300 μg of BSA before and after immobilization are shown in Figure 6(a). The optical density at 280 nm decreased from 0.902 to 0.206, giving a capacity for BSA of 264 μg for the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite.

Figure 6 (a) UV-Vis spectra of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite (1) before, and (2) after immobilization of 300 μg of BSA. (b) The amount of BSA immobilized by the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite as the amount of BSA in solution changes.

SiO_2/Au composite. The relationship between the capacity and amount of BSA added is shown in Figure 6(b). As the amount of BSA added increased, the amount of BSA immobilized on the composite particles increased. When 700 μg of BSA was added, the amount of BSA immobilized on the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite was 427 μg , giving an efficiency of 61%. The efficient immobilization of BSA on the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite particles without any coupling reagent is mainly ascribed to the formation of Au-S covalent bonds, because BSA contains a large number of thiol-based substituents.

3 Conclusions

In summary, a $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite was successfully synthesized. Characterization of the composite by TEM and XRD confirmed its structure and composition. Because of the presence of Au nanoparticles on the surface of the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2$ particles, the $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{Au}$ composite has a large specific area and good biochemical activity without requiring coupling reagents. The immobilization of BSA on the composites revealed that they may find use in immunoassays, bioseparation, nucleic acid purification, targeted drug delivery, immunology testing and many other biological fields.

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