

Facile approach to modification of Fe₃O₄/Au assembled composite nanoparticles

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Fe₃O₄/Au composite nanoparticles have proved to be promising in a wide range of biomedical applications. In order to get monodispersed nanoparticles, Fe₃O₄/Au composite nanoparticles modified with α -thio poly (ethylene glycol) were prepared and the composite nanoparticles could be suspended in PBS buffer several months. The modified magnetic composite nanoparticles not only possessed optical properties based on Surface Plasmon Resonance (SPR), but also exhibited a significant stabilization against aggregation in different buffer. Coupled with biomolecules, the Fe₃O₄/Au composite nanoparticles may accommodate biosensor for simultaneous magnetic and optical detection in the near future.

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1. Introduction

Recently, the incorporation of optically active components onto superparamagnetic iron oxide nanoparticles has attracted much attention. Fe₃O₄/Au nanoparticles with unique structures and functionalities has been extensively studied. One hand, iron oxide or iron nanoparticles with superparamagnetic nature have been used in hyperthermia, drug delivery, MRI, biosensors, and magnetic separation for cell and biomolecules [1-5]. On the other hand, gold nanoparticles have also been used in diagnostic assay, drug and gene delivery, nucleic acid detection and lateral flow immunoassay [6-9] because of their optical property and easy modification of biomolecules. By a marriage of Fe₃O₄ and Au, the Fe₃O₄/Au composite nanoparticle exhibit many performances that are unachievable by separate components, making them ideal candidates for potential applications in biomedical field. These materials can be conveniently manipulated by use of an external magnetic field [11, 12], offer a specific surface plasmon resonance and endows ease, friendly linkage of biomolecules [13-18] as well. For the purposes of *in vivo* applications, synthesis and modified of this composite nanoparticle presents a great challenge. So it is of great importance to devise proper synthetic and modify strategies to obtain these composite nanoparticles with narrow size distribution and without agglomeration in buffer.

The synthesis of Fe₃O₄/Au composite nanoparticles with core/shell structure, with the dumbbell-like, and flower-like structure [10, 13, 17, 19-22] have been reported. The composite particles that the gold nanorod coated with Fe₃O₄ nanoparticles was also studied [23]. In this paper, we report a facile approach to modification of Fe₃O₄/Au assembled composite nanoparticles. The Fe₃O₄/Au composite nanoparticle using iteration hydroxylamine seeding method was prepared and the

optical property, the applications in antibody immobilization and magnetically drug delivery were also studied [14, 24-26]. The free Fe₃O₄ seeds left in the system could be dissolved with acid solution. However, the treatment process for removal of magnetite seeds could cause the aggregation of composite nanoparticles. Thiol-ended PEG (PEG₅₀₀₀-SH), served as a particle stabilizer, have been used for modification on gold surfaces. The structure, colloidal stability and properties of Fe₃O₄/Au composite nanoparticles modified with PEG₅₀₀₀-thiol was also characterized.

2. Experimental procedure

2.1. Chemicals and materials

Chloroauric acid hydrated (HAuCl₄·4H₂O), ammonia, sodium hydroxide, hydrochloric acid, iron (II) chloride tetrahydrate (97%), iron (III) chloride hexahydrate (99%) were purchased from Sigma-Aldrich St. Louis, MO (U.S.A.) without further purification. The chemical, α -thio-poly(ethylene glycol) (SH-PEG, Mw: 5000) was purchased from Rapp Polymer GmbH. Phosphate buffer solution (0.01mol/LPBS, pH 7.4) was used as a supporting electrolyte. The water used was purified through a Millipore system.

2.2. Synthesis of the Fe₃O₄ nanoparticles

The Fe₃O₄ was prepared by the hydrothermal method [37-38]. Sodium oleic acid (0.12 g), ethanol (10 ml), and glycol (5 ml) were dissolved with 5 ml distilled water. 1 ml of ammonia, FeCl₂·4H₂O (0.58 g) and FeCl₃·6H₂O (0.94 g) were added to the system under constant stirring to produce black colloid. Then, the mixed reactants were transferred into a 50 ml autoclave, sealed, and heated at

190 °C for 15 h. The products were washed five times with organic reagent. Finally, the particles were suspended in cyclohexane.

The Fe₃O₄ nanoparticles suspended in cyclohexane was transferred into water with CTAB as surfactant and NaClO as oxidizing agent to oxidize unsaturated bonds of oleic acid. 100 mg of Fe₃O₄ nanoparticles were added to 40 ml of CTAB (10 mmol/L) solution under constant stirring 20 min. Then 3 ml of RuCl₃ (2.2%) and 4.1 ml NaClO were added to the system, the oleic acid on particle surface were oxidized for 2 h. The magnetic nanoparticles were washed three times with ethanol and the hydrophilic Fe₃O₄ nanoparticles were obtained.

2.3. Synthesis of the Fe₃O₄/Au composite nanoparticles

50 ml HAuCl₄ solutions were added into the 80 mg Fe₃O₄ nanoparticles and incubation for 50 min under stirring, 30 ml of NH₂OH·HCl (2 mmol/L) were added into the mixture and stirred for 2 h. A strong color change from black to red indicates that the formation of the Fe₃O₄/Au composite nanoparticles. Then, the Fe₃O₄/Au composite nanoparticles were washed with water for several times. Under shaking (110 rpm) at room temperature, 50 mg of Fe₃O₄/Au composite nanoparticles was treated with 20 ml of HCl solution (2.0 mol/L) for 30 min, and 2 h respectively, the excess Fe₃O₄ can be removed to a varying degree.

2.4. Surface modification of the Fe₃O₄ / Au composite nanoparticles

SH-PEG₅₀₀₀ was used to modify the composite nanoparticle surface. After ultrasonic treatment with a frequency 45 Hz for 30 min, 15 mg of Fe₃O₄/Au nanoparticles were gently mixed with 15 ml CTAB (1 mmol/L) and allowed to stand for 30 min in order to adsorb CTAB on their surface, then 2 mmol/L SH-PEG₅₀₀₀ (10 ml) were added to the Fe₃O₄/Au nanoparticles for standing 6 h with hand shaking during the modification process. The composite nanoparticles was also modified with SH-PEG in the absence of CTAB. After removal of excess SH-PEG and surfactant by washing with water, the PEGylated Fe₃O₄/Au nanoparticles was obtained.

2.5 Characterization of composite nanoparticles

2.5.1. Size and zeta-potential measurement

The Zetasizer Nano (Malvern Instruments) was used to characterize the zeta-potential and average diameter of composite nanoparticles and the nanoparticles modified with SH-PEG.

2.5.2. TEM analysis

The morphology, nanostructure of the SH-PEG modified Fe₃O₄/Au nanoparticles were studied by transmission electron microscopy (JEM-3100II, JEOL) operated at 200 kV. The nanoparticles suspended in water were drop cast onto a carbon-film-coated copper grid, followed by evaporation at room temperature.

2.5.3. Magnetic measurement

Vibrating sample magnetometry (VSM, Lakeshore 7300) was applied to perform magnetization curve of magnetic particles at room temperature.

2.5.4. Optical property measurement

The optical properties of the samples were characterized by a UV-Vis spectrometer (Hitachi U 4100), revealing the surface plasmon resonance characteristic of the Fe₃O₄/Au nanoparticles over range 350-750 nm.

3. Results and discussion

As a modification reagent, SH-PEG has been used to stabilize gold nanorod or gold nanoparticle [27-31]. Herein, Fe₃O₄/Au nanoparticles were synthesized by reducing Au³⁺ to Au in the presence of Fe₃O₄ nanoparticles. After treated with HCl, the composite nanoparticles were further modified by reacting with SH-PEG. The monodispersed PEGylated Fe₃O₄/Au nanoparticles were obtained by washing with water several times.

The shift of plasmon resonance for Fe₃O₄/Au composite nanoparticles during the modification process could be determined by UV/Vis absorbance spectra. The result (Fig. 1) show the changes of the monodispersion property of pre- and post-modified Fe₃O₄/Au composite nanoparticles. Curve a denotes the characteristic spectrum of composite particles with a characteristic peak at 535 nm, it is red shift with a peak at 560 nm and spectral broadening occurs after treated with HCl for 30 min (curve b). The peak of composite nanoparticles modified with SH-PEG₅₀₀₀ became narrow (curve c) and the maximum peak shifted to 532 nm. The results demonstrate that particle size of PEGylated composite particles are homogeneity. The Fe₃O₄/Au composite nanoparticle surface treated with HCl has negative surface charge (zeta-potential: -20 mV), the positive charge of surfactant CTAB could interact and adsorb a surfactant layer before SH-PEG modified on their surface with Au-S. however, the SH-PEG modified particles aggregated without the addition of CTAB (curve d). This result was confirmed by the change of particle surface charge, the zeta potential values of Fe₃O₄/Au composite nanoparticles treated with HCl was 18 ± 2.0 mV with negative charge, and it shifted to 26.5 ± 2 mV with positive charge due to addition of CTAB. Obviously, CTAB molecules adsorbed electrostatically on the surface of the Fe₃O₄/Au composite nanoparticle could prevent aggregation of nanoparticles in the next step for SH-PEG modification.

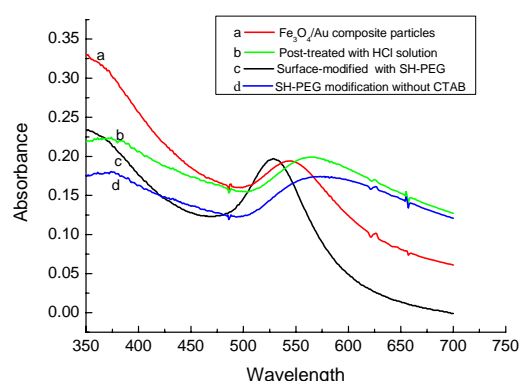


Fig. 1. UV-Visible spectra of pre- and post-modified Fe₃O₄/Au composite nanoparticles with SH-PEG

The Fig. 2 shows the TEM images of Fe₃O₄/Au composite nanoparticles of pre- and post-modified with SH-PEG₅₀₀₀. The Fe₃O₄/Au composite nanoparticles tend to aggregate (Fig. 2a). After the surface modification of nanoparticles with SH-PEG₅₀₀₀, the particle is monodisperse and has a kind of sunflower like structure. The average diameter of the Fe₃O₄/Au composite nanoparticles is about 30 nm, the hydrodynamic diameter (Fig.3) of the modified composite nanoparticles determined by Malven Nanosizer is consistent with TEM results and the size distribution of the composite particle was narrow. Gold is much more electron dense than iron or iron oxides, and the TEM image formation theory indicates that it is unlikely for an Fe₃O₄ core to appear darker than a surrounding gold shell [34]. From TEM images analysis of Fe₃O₄/Au composite nanoparticles, a layer of Fe₃O₄ nanoparticles with 5 nm diameter assembled on the core surface, however, the electron dense of the particle core is not homogeneous, indicate that the core is not a purely gold component, but is a composite core as illustrated in Fig. 2b.

The particle structure was also validated by the following experiment: the Fe₃O₄/Au composite nanoparticles were etched with HCl at a concentration of 2.0 M HCl for 2 h. Fig. 4a show the TEM pictures of a 2 h etching. However, the color of nanoparticle turning into black and still has responsiveness to an external magnetic field (Fig. 4b) and the saturation magnetization of particles about 3.15emu/g. This results indicate that the nanoparticles have a core/shell composite structure

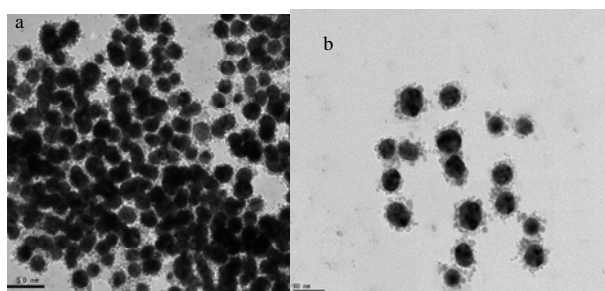


Fig. 2 The TEM images of sunflower-like Au/Fe₃O₄ Nanoparticles (a) and surface-modified with PEG-SH (b)

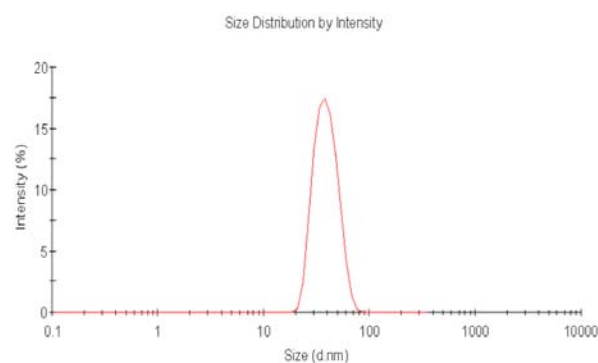


Fig. 3. The particle size distribution of Fe₃O₄/Au composite nanoparticles surface-modified with PEG-SH.

As-synthesized composite nanoparticle appears red-brown (Fig. 5A). Optical adsorption measurement shows that optical adsorption peak located at the wavelength of 532 nm. The color of Fe₃O₄/Au composite nanoparticles turned into blue after treated with HCl for 30 min (Fig.5B). After modified with SH-PEG, the color of composite particle suspended in water turned into red color again (Fig.5C). The saturation magnetization of surface-modified composite particles about 14.5emu/g (Fig.6) and also had efficient and fast-acting to an external magnetic field when they were suspended in PBS solution (Fig. 5D).

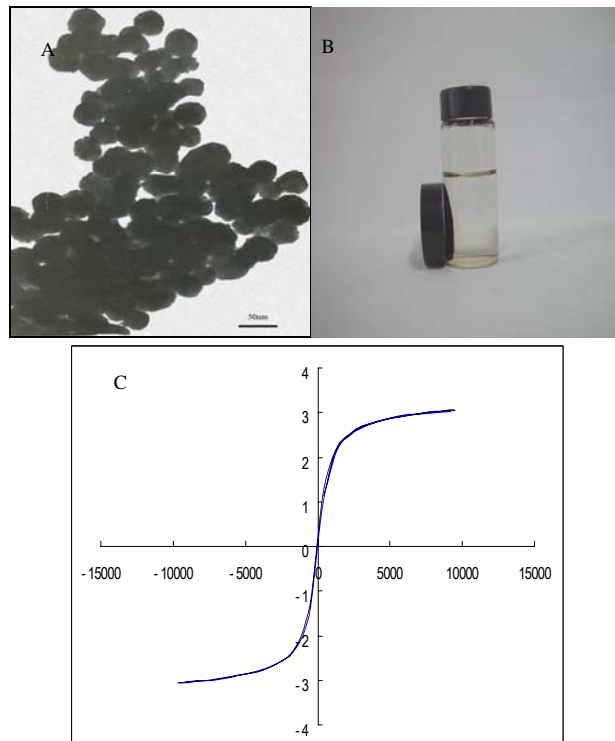


Fig.4. the TEM image of Fe₃O₄/Au composite nanoparticles treated with HCl solution for 2 h (A) The magnetic responsiveness of particle core in an external magnetic field (B) and hysteresis loops of the Fe₃O₄/Au composite nanoparticles (C).

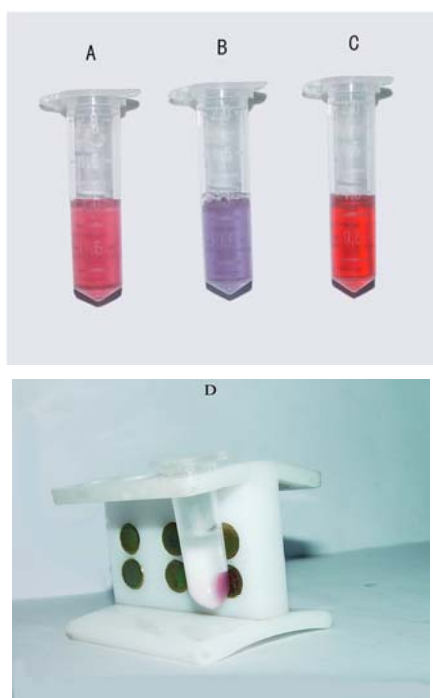


Fig. 5 The colors of $\text{Fe}_3\text{O}_4/\text{Au}$ composite nanoparticles pre-treated (A) and (B) post-treated with HCl solution the surface-modified nanoparticles with SH-PEG (C) The magnetic responsiveness of surface-modified $\text{Fe}_3\text{O}_4/\text{Au}$ composite nanoparticles with SH-PEG in external magnetic field (D)

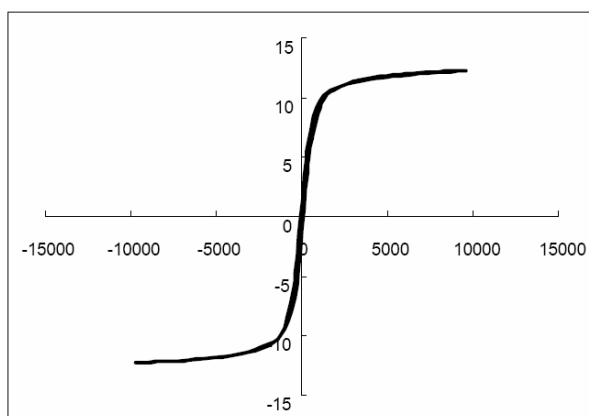


Fig.6 Hysteresis loops of $\text{Fe}_3\text{O}_4/\text{Au}$ composite nanoparticle surface-modified with SH-PEG

The $\text{Fe}_3\text{O}_4/\text{Au}$ composite nanoparticle have similar optical properties as colloid gold particles. The shift of characteristic peak for the particle suspension in the range of 350–750 nm was used to evaluate the monodispersion and stability. The UV–Vis spectra of SH-PEG₅₀₀₀ modified $\text{Fe}_3\text{O}_4/\text{Au}$ nanoparticles in different buffers (Fig. 7) indicate that the composite magnetic nanoparticles have narrow spectra, with peak at 532. The composite nanoparticles could stably suspended in 0.1 mol/L PBS, 1mol/L Tris-HCl or 0.2 mol/L Borate buffer for several weeks. In contrast, the non-PEGylated $\text{Fe}_3\text{O}_4/\text{Au}$ nanoparticles tend to aggregate when suspended in these

buffers. Therefore, the surface modification and functionalization of these nanoparticles would greatly expand the range of applications of these materials.

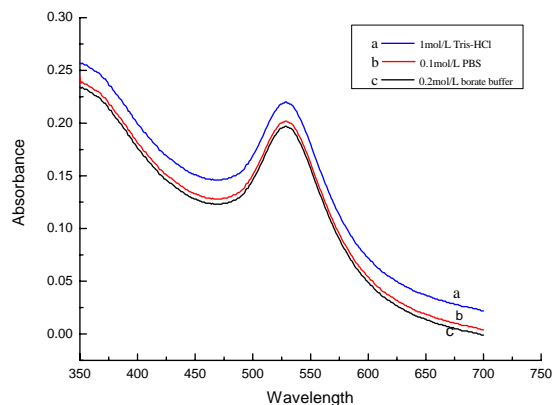


Fig. 7 UV-Visible spectra of $\text{Fe}_3\text{O}_4/\text{Au}$ composite nanoparticle surface-modified with SH-PEG in different buffer

4. Summary

In this work, we prepared sunflower-like $\text{Fe}_3\text{O}_4/\text{Au}$ composite nanoparticles, Fe_3O_4 nanoparticle loosely attach on the surface with composite particle. We prepared the composite nanoparticles by using iteration hydroxylamine seeding method. The free Fe_3O_4 seeds were dissolved with an acid solution. Finally, $\text{Fe}_3\text{O}_4/\text{Au}$ nanoparticles were surface-modified through the reaction with SH-PEG₅₀₀₀. The nanoparticles exhibited the similar characteristic absorption peak as colloid gold particles and had significant stabilization against aggregation in different solvents, and had good magnetism. This nanoparticle could be used in antibody immobilization, as a carrier for magnetically targeted drug delivery. The advantages of these composite particles make them very promising for applications in biomedical area. The $\text{Fe}_3\text{O}_4/\text{Au}$ nanoparticles have optical and electronic properties of nanogold, so the particle offers an enormous potential in nanoscale science and engineering applications.

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