The Preparation of Gold Magnetic Nanoparticles Through Layer-by-Layer Assembly Technique

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Abstract. The gold magnetic composite particle has been widely applied to the clinical and biochemical analysis. In this study, a simple synthetic method to prepare gold magnetic composite particles were established. In this paper, gold magnetic composite particles were prepared through layer-by-layer assembly technique. Fe₃O₄ magnetic nanoparticles (MNPs) were prepared by coprecipitation method, then the magnetic nanoparticles were coated by silica and carboxyl modified by APTES-COOH. Following by coating the bovine serum albumin on the surface and adsorbing gold nanoparticles then MNP@SiO₂@BSA@Au nanocomposites were prepared.

1. Introduction
In the design and fabrication of immune detector, the development of a simple and effective strategy for the construction of nanoparticles which can sensitively connect antibody and then capture antigen is a crucial step. For the special paramagnetism and bulk effect, the study of magnetic nanoparticles and their applications in biomedicine has drawn many attention [1]. Magnetic nanoparticles can be used to realize the enrichment, rapid separation and detection of the target and remove the distractor just in a magnetic field after magnetic nanoparticles combined with the target molecules. Due to these advantages, magnetic nanomaterials can be applied in many fields, especially in biomedical fields [2]. Fe₃O₄ is one of the commonly used magnetic nanoparticle, nevertheless, it has poor stability and has no functional groups on its surface which can attach biomolecules. The surface functionalization is crucial for their application such as immunological recognition and molecule targeting.

Gold nanoparticles have special physical and chemical properties with high electron density and dielectric property. Gold nanoparticles can combine firmly with antibody or antigen without affecting the biological activity of biomolecules [3]. The molecular recognition between the gold nanoparticles-antibodies and antigens has been used in immunoassay and achieve great improvement in rapid detection of biomarkers. In recent years, gold nanoparticle has been widely used in the immunity [4], calibration, tracer, biosensor, disease diagnosis, gene extraction, etc.

Combining the properties of gold and magnetic particle, the gold magnetic nanoparticle have the characteristics of dissociability in magnetic field and the rapid immobilization of colloidal gold [5]. Thus, the synthesis and application of gold magnetic nanoparticles have become a hot topic. Hence, there is a need to develop methodologies for the synthesis of the composite particles which did not require complicated sample preparation or technical expertise.
2. Materials and experiment

2.1 Materials
FeCl₃•6H₂O, FeSO₄•7H₂O, citric acid monohydrate, tetraethyl orthosilicate (TEOS), maleic anhydride, HAuCl₄•4H₂O, 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride (EDC), N-hydroxy-succinimide (NHS) and hydroxylammonium chloride and 2-morph-o-loinethanesulfonic acid (MES) were purchased from Sinopharm Chemical Reagents Company. Aminopropyl triethoxysilane (APTES) and bovine serum albumin (BSA) were obtained from Sigma Aldrich. Deionized water (18.2 MU cm) used for all experiments was obtained from Millipore (Millipore, Bedford, MA).

2.2 Characterization
Hydrodynamic diameter (Dh) measurements were conducted by dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS90 (Malvern, UK) instrument using a He–Ne laser at a wavelength of 632.8 nm. Transmission electron microscopy (TEM) images were taken on a JEM-2100F transmission electron microscope at an accelerating voltage of 200 kV. UV-vis spectra were recorded by using a SHIMADZU UV-1800 UV-vis spectrophotometer (Japan).

2.3 Preparation of magnetic nanoparticles
The coprecipitation method were applied to prepare Fe₃O₄ NPs as follows: FeCl₃•6H₂O (1.892 g, 7 mmol), FeSO₄•7H₂O (0.973 g, 3.5 mmol) were dissolved into 100 ml ultrapure water by ultrasonic, then the mixture was stirred at 45°C for 20 min. NH₃•H₂O (25 ml) were added to the solution drop by drop. After that, Citric acid monohydrate (0.1%, w/v) were added to the mixture at 80°C, then the mixture were stirred for 1 h. During the whole process, the solution was under a N₂ atmosphere. Finally, the prepared Fe₃O₄ NPs was separated by magnetic decantation and washed with ultrapure water. The product was then dispersed in water. 10 mL of the above solution containing Fe₃O₄ NPs was mixed with 90 mL ethanol, followed by addition of 150 µL of tetraethyl orthosilicate (TEOS). After the suspension was stirred for 10 min, 1mL NH₃•H₂O was added dropwise and further reacted for 2 h. Then 50 mg of APTES–COOH was added into the solution and continued to react for 7 h. Finally the mixture was washed with ethanol and water by magnetic separation, and the product was dispersed in water.

2.4 Preparation of gold nanoparticles
Gold NPs (AuNPs) were prepared using the method reported by Frens [6] with some modification: 50 mL HAuCl₄ (0.01%, w/v) was added to a flask, then 1 mL of 1% sodium citrate solution was added into boiling HAuCl₄ solution. After 15 min, the solution was cooled to room temperature and stored at 4°C before use.

2.5 Preparation of gold magnetic nanoparticles
The synthesis procedures of MNP@SiO₂@BSA@Au NPs are schematically illustrated in Figure 1. The surface of Fe₃O₄ MNPs was first coated with BSA, as follows: 30 mL (1 mg/mL) Fe₃O₄ NPs solution was washed with phosphate buffer (PB 10 mM, pH 7.4) 3 times by magnetic separation, and then re-suspended in 15 mL PB, adding 25 mL containing 0.1M EDC, 0.05 M NHS. The solution
was incubated for 1 h at room temperature and then add 50 mg BSA. After 2 h incubation, the precipitate was magnetically separated and then resuspended in 10 mL PB. Finally the separation and re-suspension process was repeated 3 times to remove the excess BSA. After that, a 50 mL AuNP colloid was mixed with the MNP@SiO$_2$@BSA suspension, followed by 2 h incubation. After the incubation, the AuNPs were attached to the surface of MNP@SiO$_2$@BSA NPs, due to the electrostatic interaction between AuNPs and BSA. Finally the NPs were washed 3 times with PB by magnetic separation.

3. Result and discussion
The nanocomposites were characterized by DLS, TEM and UV. The hydrodynamic diameter of the MNP@SiO$_2$ measured by DSL was 230.2 nm, and the hydrodynamic diameter of MNP@SiO$_2$@BSA@Au was 316.1 nm as shown in figure 2, the increase of the hydrodynamic diameter has indicated that the AuNPs were successfully deposited on the surface. The gold magnetic composite nanoparticles were examined in a transmission-electron microscope (TEM), figure 3 shows its TEM images. Figure 4 presents the UV-vis absorption spectra of the MNP@SiO$_2$@BSA@Au suspension, AuNPs and MNP@SiO$_2$. No obvious UV-vis absorption peak was observed for MNP@SiO$_2$. The AuNPs’ typical surface plasmon resonance (SPR), absorption peak at around 520 nm was observed. MNP@SiO$_2$@BSA@Au showed an absorption peak at around 535 nm contributed by the deposited AuNPs, which could further confirm that the AuNPs were successfully coated on the surface of MNP@SiO$_2$@BSA.

![Figure 2](image1.png)  
**Figure 2** The DLS results of MNP@SiO$_2$ NPs (a) and MNP@SiO$_2$@BSA@Au NPs (b)

![Figure 3](image2.png)  
**Figure 3** The TEM images of MNP@SiO$_2$@BSA@Au NPs
4. Conclusion
Both gold nanoparticles and magnetic nanoparticles have been widely used in immunoassay. In this study, we focused on the preparation of the combination of these two nanoparticles, the particle has the properties of the two kinds of nanoparticles. This paper reported a simple and convenient method to prepare highly dispersible MNP@SiO$_2$@BSA@Au NPs in aqueous solutions.

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