



Preparation of silica coated cobalt ferrite magnetic nanoparticles for the purification of histidine-tagged proteins

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ABSTRACT

Surface modified cobalt ferrite (CoFe₂O₄) nanoparticles containing Ni-NTA affinity group were synthesized and used for the separation of histidine tag proteins from the complex matrices through the use of imidazole side chains of histidine molecules. Firstly, CoFe₂O₄ nanoparticles with a narrow size distribution were prepared in an aqueous solution using the controlled co-precipitation method. In order to obtain small CoFe₂O₄ agglomerates, oleic acid and sodium chloride were used as dispersants. The CoFe₂O₄ particles were coated with silica and subsequently the surface of these silica coated particles (SiO₂-CoFe₂O₄) was modified by amine (NH₂) groups in order to add further functional groups on the silica shell. Then, carboxyl (-COOH) functional groups were added to the SiO₂-CoFe₂O₄ magnetic nanoparticles through the NH₂ groups. After that N α ,N α -Bis(carboxymethyl)-L-lysine hydrate (NTA) was attached to carboxyl ends of the structure. Finally, the surface modified nanoparticles were labeled with nickel (Ni) (II) ions. Furthermore, the modified SiO₂-CoFe₂O₄ magnetic nanoparticles were utilized as a new system that allows purification of the N-terminal His-tagged recombinant small heat shock protein, Tpv-sHSP 14.3.

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1. Introduction

Magnetic nanoparticles, which exhibit magnetic properties that are different from those of their bulk counterparts, have been used successfully for wide range of applications [1] such as targeted drug delivery [2], enzyme and protein separations [3–5], magnetic resonance imaging contrast agent [6], environmental remediation [7] and biomedical and biological applications (e.g., pull down assays) [8–10]. Small particles tend to form agglomerates due to attractive van der Waals forces, therefore, it is necessary to coat magnetic nanoparticles with a protective layer such as polymer [11], and carbon [7] in order to obtain stable particles. Protective coatings not only stabilize nanoparticles, but can also be used for further functionalization [1]. When the surfaces of magnetic nanoparticles functionalized to create substrate specific surfaces, the

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targeted molecules can be easily captured. Thus, magnetic separation, which does not need pretreatment operations (such as centrifugation and/or filtration), can be used as a convenient and rapid technique for the separation and purification of biomolecules, such as proteins, nucleic acids, and enzymes [12,13].

Non-toxic silica is often used as the coating shell to provide magnetic nanoparticles with water dispersion and biocompatibility in most applications. Silica shell can also prevent the direct contact of the magnetic core with the environment thus preventing undesired interactions. In addition, silica-coated magnetic nanoparticles have hydroxyl (-OH) groups on their surfaces, and these groups can be bonded to various functional groups; such as -NH₂ and -COOH, which cannot be bound directly to magnetic nanoparticles. With the addition of functional groups onto surfaces of the particles, their use can also be extended to bio labeling, drug targeting and drug delivery. Furthermore, the silica coating of magnetic nanoparticles imparts biocompatibility which is important for biomedical applications because silica is a non-toxic material [9].

Magnetic CoFe₂O₄ has been synthesized using various techniques and used widely in many applications due to their unique physicochemical properties [14–17]. It is desirable that CoFe₂O₄

nanoparticles should have a narrow size distribution, high magnetization values, a uniform spherical shape, and superparamagnetic behavior at room temperature for biomedical applications. Many types of synthetic routes have already been employed for the preparation of CoFe₂O₄ nanoparticles, such as hydrothermal, co-precipitation, microemulsion, forced hydrolysis, and reduction–oxidation route. However, the most important issue to be addressed for the as-prepared nanoparticles is that they are severely agglomerated with poor control of size and shape, which greatly restrict their applications [18].

The aim of this study is the preparation of surface modified CoFe₂O₄ nanoparticles as a magnetic separation platform for specific isolation of His-tagged proteins. To this end, first CoFe₂O₄ magnetic nanoparticles were synthesized by using co-precipitation method. Then, the surface of magnetic nanoparticles were coated with silica and modified with Ni–NTA affinity groups. These surface modified CoFe₂O₄ magnetic nanoparticles were used for purification of the N-terminal His-tagged recombinant small heat shock protein Tpv-sHSP 14.3 from cell extract of the *E. coli* pQE-31/775.

2. Experimental

2.1. Materials

Iron(III)chloride (FeCl₃·9H₂O, Riedel-de Haën), Cobalt(II) chloride 6-hydrate (CoCl₂·6H₂O, Surechem), sodium hydroxide pellets (NaOH, Sigma-Aldrich), sodium chloride (NaCl, Fisher Scientific Company), and oleic acid ((9Z)-octadec-9-enoic acid, Fluka) were used for the preparation of CoFe₂O₄ magnetic nanoparticles. In order to prepare silica coating on CoFe₂O₄ nanoparticles, Tetraethyl orthosilicate (TEOS, C₈H₂₀O₄Si, 98%, Aldrich), (3-aminopropyl)trimethoxysilane (APTMS, H₂N(CH₂)₃Si(OCH₃)₃, ≥ 98.0%, Aldrich), and ethanol (EtOH, C₂H₅OH, ≥ 99.9%, Merck) were used. For the functionalization of CoFe₂O₄ nanoparticles with –NH₂ groups, (3-aminopropyl) triethoxysilane (APTES, C₉H₂₃NO₃Si, ≥ 98.0%, Fluka), toluene (C₆H₅CH₃, ≥ 99.0%, Merck), and N,N-dimethylformamide (DMF, C₃H₇NO, ≥ 99.8%, Sigma-Aldrich) were used. The attachment of –COOH group on the nanoparticles were done by using toluene (C₆H₅CH₃, ≥ 99.0%, Merck), N,N-dimethylformamide (DMF, C₃H₇NO, ≥ 99.8%, Sigma-Aldrich), and Succinic (glutaric) anhydride (C₄H₄O₃, ≥ 99%, Sigma-Aldrich). Surface modification of CoFe₂O₄ nanoparticles with NTA was performed with Nα,Nα-Bis(carboxymethyl)-L-lysine hydrate (NTA, C₁₀H₁₈N₂O₆, ≥ 97.0%, Fluka) and 2-(4-morpholino) ethanesulfonic acid Potassium salt (MES K salt, Sigma). For adding Ni (II) ions on the surface modified CoFe₂O₄ nanoparticles, Nickel solution (Nickel (II) ion, 1000 ppm ± 0.5%, Fisher Scientific International Company) was used.

2.2. Instruments

Field Emission Scanning Electron Microscopy (FE-SEM) with energy-dispersive X-ray analyzer (EDX) (FE-SEM, Quanta 400F, FEI) and Transmission Electron Microscopy (TEM, JEOL JEM-2010F (FEG, 80–200 kV)) were used for morphological and chemical characterization of CoFe₂O₄ and SiO₂–CoFe₂O₄ nanoparticles. For the FE-SEM and TEM measurements, the magnetic nanoparticle suspensions were dropped on carbon-coated copper grids and then they were dried at room temperature overnight. Number-length (arithmetic) mean size ($D[1,0]$) and volume weighted mean size ($D[4,3]$) of CoFe₂O₄ and SiO₂–CoFe₂O₄ nanoparticles were determined from FE-SEM images using the following equations:

$$D[1, 0] = \frac{\sum d_i N_i}{\sum N_i} \quad (1)$$

$$D[4, 3] = \frac{\sum d_i^4 N_i}{\sum d_i^3 N_i} \quad (2)$$

where d_i is the diameter of primary particles and N_i is the number of primary particles. Saturation magnetization of the CoFe₂O₄ magnetic nanoparticles was measured using a Vibrating Sample Magnetometer (ADE Magnetics Model EV9) at room temperature. Fourier Transform Infrared Spectroscopy (FTIR) (Alpha, Bruker) was used for characterization of nanoparticles in the range of 300–4500 cm^{–1}. Determination of Ni, Cu and Ag ions attached on the surface modified CoFe₂O₄ nanoparticles were performed using an inductively coupled plasma spectrometer (ICP–OES, Direct Reading Echelle, Leeman Labs INC.). Particle size and distribution of the CoFe₂O₄ and SiO₂–CoFe₂O₄ agglomerates were determined using the dynamic light scattering method (Malvern Nano ZS90).

2.3. Methods

2.3.1. Preparation of cobalt ferrite nanoparticles

CoFe₂O₄ nanoparticles were synthesized by the co-precipitation method [14]. According to this procedure, after mixing 0.54 g FeCl₃·6H₂O, 0.238 g CoCl₂·6H₂O and 10 mL deionized water in a test tube, an orange colored solution was obtained and it was put in a 50 mL beaker. The solution was then transferred into a beaker and 10 mL 3 M NaOH was added drop wise with Pasteur pipette by continuous stirring by using a magnetic stirrer to obtain a black colloidal suspension. 200 μL oleic acid was added to the black colloidal suspension and the stirring was continued for 1 h at 80 °C. Then the suspension was cooled to room temperature, the black precipitates (CoFe₂O₄ nanoparticles) were collected with a magnet and the supernatant was discarded. The CoFe₂O₄ nanoparticles were washed three times with deionized water–ethanol solution. The black precipitate was dried overnight at 100 °C, and then heated to 600 °C for 10 h to remove residual water. The CoFe₂O₄ nanoparticles were redispersed in deionized water and stored in 15 mL plastic tubes. Alternatively, sodium chloride (NaCl) was used as a dispersant. In this case, 5 mL 1.5 M NaCl solution was added to the orange solution together with 10 mL 3 M NaOH. Following the same protocol, the stirring was carried out using an ultrasonic bath instead of the magnetic stirrer to see the effect of ultrasound application on the size of CoFe₂O₄ nanoparticles and their agglomerates.

2.3.2. Silica coating of cobalt ferrite magnetic nanoparticles

The CoFe₂O₄ magnetic nanoparticles were coated with a silica shell using a sol–gel method [19]. First, 80 mL ethanol, 169 μL TEOS and 14.4 μL APTMS were mixed in a beaker and subsequently 20 mL CoFe₂O₄ colloidal suspension was added to the mixture and stirred for 3 h at room temperature. Subsequently, the silica coated cobalt ferrite (SiO₂–CoFe₂O₄) particles were collected with a permanent magnet and washed three times with deionized water.

2.3.3. Functionalization of silica coated cobalt ferrite nanoparticles with amine groups

After coating the CoFe₂O₄ nanoparticles with a silica shell, the surfaces of SiO₂–CoFe₂O₄ nanoparticles were modified with amine groups using APTES [9]. For the coating process, 12 mL DMF and 8 mL toluene were vortexed in a 50 mL tube and this solution was put in a 50 mL beaker, and subsequently 200 μL APTES was added drop by drop into this mixture under magnetic stirring for 24 h. Then, the functionalized particles were collected with the magnet

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