Research Article

Semi-Biosynthesis of Magnetite-Gold Composite Nanoparticles Using an Ethanol Extract of *Eucalyptus camaldulensis* and Study of the Surface Chemistry

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Received 18 August 2009; Accepted 9 December 2009

Recommended by Jun Liu

Green synthesis of metal nanoparticles, such as silver or gold nanoparticles, has been attracting increasing attention in recent years. Functionalized magnetite nanoparticles have many uses in various applications, including nanoelectronic devices, molecular recognition, biomedical applications, drug delivery targeting, and optical devices. In this investigation, magnetic cores (Fe_3O_4) were synthesized using a fabrication method involving coprecipitation of Fe^{2+} and Fe^{3+} . In the next step, magnetite-gold composite nanoparticles were synthesized with size ranging from 6–20 nm, using an ethanol extract of *Eucalyptus camaldulensis* as a natural reducing agent. Transmission electron microscopy, energy-dispersive spectroscopy, X-ray diffraction spectroscopy, and visible absorption spectroscopy confirmed the fabrication of magnetite-gold composite nanoparticles. In the UV spectra diagram, a red-shift of the surface plasmon of the Au was evidence that contact between gold and Fe_3O_4 had occurred. The surface chemistry of the as-prepared magnetite-gold nanoparticles was studied using infrared spectroscopy. The presence of organic compounds with a carboxyl moiety was confirmed on the surface of the magnetite-gold nanoparticles fabricated by this combined chemical and biological reducing process, which we have designated as a semi-biosynthesis method.

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1. Introduction

Composite nanoparticles that contain two or more different nanoscale materials are of increasing interest in present day research because of their unique properties. These nanoparticles, have many applications in a number of fields, including use in nanoelectronic devices, molecular recognition, biomedical applications, and optical devices [1]. Recently, different nanocomposite materials, such as SiO_2 -coated Fe nanocapsules, cobalt ferrite core/shell nanoparticles, silver/polystyrene nanospheres, Au- Cu_2O core-shell

nanocubes, and Al_2O_3 -Co nanocapsules have been synthesized by different fabrication methods [2–5]. One of the prevalent nanostructures that has many uses in biomedicine is the magnetic nanoparticle. Magnetic nanoparticles, for instance those composed of magnetite (Fe_3O_4) or maghemite ($\gamma\text{-Fe}_2\text{O}_3$), have unique thermal, chemical, and magnetic properties that make them particularly well suited for medical applications [6].

However, materials that are used in medical diagnoses or as therapeutic agents require specific characteristics, such as colloidal stability [7], biocompatibility [8], resistance against

environmental degradation [2], and nontoxicity [6]. For these applications, nanoparticles must be passivated with an external inert shell in order to protect the magnetic core against oxidation. Moreover, the surfaces of these particles should be functionalized with organic molecules in order to allow them to chemically bind with biomolecules like DNA, proteins, amino acids, and so forth [9].

At present, a number of different methods have been reported for coating magnetic nanoparticles. Protective layers include polymeric molecules such as polyethylene glycol and dextran or inorganic compounds like silica [8]. Although some of these methods have been recently commercialized, most have substantial deficiencies. For example, hydrophobic surfaces arising from polymeric chains can result in improper protein bonding [10]. A nonmetallic coating also potentially decreases the magnetic properties of iron-oxide nanoparticles, thus limiting the applications of these particles [11].

Among the different techniques available for surface modifications, gold-coated magnetic nanoparticles have lately attracted significant attention. Since gold is an inert element from the viewpoint of chemical reactivity, it is very useful as a coating for protecting magnetic nanoparticles. Kinoshita et al. synthesized a gold-iron nanocomposite powder by a reverse micelle method [12]. Similar structures of Fe/Au nanoparticles have been successfully synthesized and the particles used as a magnetic resonance (MR) contrast agent [13]. According to Yu et al. and Au-Fe₃O₄ nanoparticles with dumbbell-like shapes could be prepared via decomposition of iron pentacarbonyl, FeCO₅, onto the surface of gold nanoparticles [14]. In other work, gold and magnetite composite nanoparticles have been prepared by a laser process in water [15]. Gold-coated ferric oxide nanoparticles have been obtained using sodium citrate as the reducing agent, yielding particles with an average size of 25 nm, as reported by Pham et al [6]. Amino acids have also been adsorbed onto the Au/ γ -Fe₂O₃ composite nanoparticles synthesized by gamma-ray irradiation [9].

A number of these methods for synthesizing gold-iron oxide nanocomposites may not be suitable for biological application because they use harmful surfactants or organic solvents. In addition, maintaining the magnetic nanoparticles in liquid media and preventing aggregation are difficult in practice. In the current study, Au-Fe₃O₄ composite nanoparticles were prepared with a combined chemical and biological reducing process, designated here as a semi-biosynthesis method. Magnetic cores (Fe₃O₄) were primarily synthesized using a fabrication method consisting of coprecipitation of Fe²⁺ and Fe³⁺. An ethanol extract of *Eucalyptus camaldulensis* [16] was used for the reduction of Au³⁺ on the surface of the Fe₃O₄ magnetic nanoparticles and for the functionalization of the Au-Fe₃O₄ nanocomposite particles.

2. Experimental

Magnetite (Fe₃O₄) nanoparticles were prepared at room temperature by a previously reported method [17]. Briefly, deionized water (200 ml) was deoxygenated by bubbling nitrogen gas for 1.5 hour, then 50 ml of NH₄OH (1 M) was added, and the mixture was stirred with mechanical agitation

at 1000 rpm. Next, 6.76 g FeCl₃·6H₂O (2.5 mmol) and 4.97 g FeCl₂·4H₂O (2.5 mmol) were separately dissolved in 50 ml distilled water to make 0.5 M solutions. Subsequently, 10 ml of the ferrous chloride and 20 ml of the ferric chloride solutions were added to the mentioned ammonium hydroxide solution and the reaction mixture was stirred for 2.5 minutes at 1000 rpm. At this stage, a black precipitate formed and it was washed four times with deionized water. The prepared magnetite (Fe₃O₄) nanoparticles were separated from the solution with a magnet.

The reduction of the Au³⁺ onto the surface of magnetite (Fe₃O₄) nanoparticles was carried out using an ethanol extract prepared from *E.camaldulensis* whole plant [16]. Chloroauric acid was purchased from Merck, Germany. Magnetic nanoparticles (200 mg) were dispersed in 100 mL of aqueous HAuCl₄ (chloroauric acid) solution (1 mM) and mixed with 10 mL of an ethanol extract of *E.camaldulensis* (10 mg/mL). The resulting mixture was allowed to stand for 15 minutes at room temperature, then the prepared magnetite-gold nanocomposites were separated from the colloid by a magnet. Finally, the nanoparticles were washed three times with distilled water for use in further experiments.

The UV-visible spectra of the magnetite nanoparticles and magnetite-gold nanocomposites samples, before and after separation with the magnet, were measured on a Labomed Model UVD-2950 UV-VIS Double Beam PC Scanning spectrophotometer, operated at a resolution of 2 nm. All samples were characterized by transmission electron microscopy (model EM 208 Philips), energy-dispersive spectroscopy (EDS), and X-ray diffraction spectroscopy. The surface chemistry of the separated magnetite-gold nanocomposites was further studied with Fourier transform infrared spectroscopy (Nicolet Magna 550).

3. Results and Discussion

This study reports a new method for preparation of functionalized magnetite-gold nanocomposites that used a combined chemical and biological procedure. In the first stage, Fe₃O₄ magnetite nanoparticles were chemically synthesized and their shape and size were studied by transmission electron microscopy. Figures 1(a) and 1(b) show different representative TEM images recorded from the drop-coated film of the magnetite nanoparticles synthesized by this method [17]. The particle size histogram of the synthesized Fe₃O₄ nanoparticles shows that the particles ranged in size from 6 to 20 nm, with an average size of 10 nm (Figure 1(c)). As can be seen in Figures 1(a) and 1(b), the broad surface area of the nanoparticles and the magnetic forces between them could cause a considerable agglomeration between the fabricated Fe₃O₄ magnetite nanoparticles [18]. The histogram of magnetite nanoparticle size dispersion obtained from TEM images showed diameter ranges between 6–20 nm with an average of 10 nm (Figure 1(c)).

Analysis of the Fe₃O₄ nanoparticles by energy dispersive spectroscopy (EDS) confirmed the presence of Fe and O element signals (Figure 1(d)). The peaks shown in Figure 1(d) were attributed to Fe and O elements, which confirmed the

FIGURE 1: TEM images of magnetic nanoparticles with low (a) and high (b) magnifications, (c) Particle size distribution histogram of Fe_3O_4 nanoparticles, (d) EDS spectrum of magnetite particles.

existence of iron-oxide nanoparticles (copper and carbon peaks existed due to the grid used for TEM imaging). In the second stage, the magnetite-gold nanocomposite was fabricated in the presence of Au^{3+} by adding an ethanol extract of *E. camaldulensis* as a reducing agent. The TEM images of the magnetite-gold nanocomposite illustrated in Figure 2 show gold nanoparticles as dark spots. In contrast, Fe_3O_4 magnetite nanoparticles are observed as a gray color because elemental gold (Au) has a higher electron density than Fe_3O_4 and subsequently more electrons are transmitted in bright field imaging [14].

The EDS pattern of the separated Fe_3O_4 magnetite-gold nanocomposite indicated the presence of gold, iron, and oxygen, as illustrated in Figure 3. The EDS experiment was repeated for different nanoparticles and the obtained EDS spectra confirmed that the formed nanoparticles were comprised of both elemental gold and Fe_3O_4 magnetite ingredients. The UV-Visible spectra were examined before and after separation of the magnetic nanoparticles from

the gold nanoparticles (Figure 4). Plasmon resonance peaks detected after removing the magnetic nanoparticles from the reaction mixture established the formation of magnetite-gold nanocomposites. It should be noted that pure iron-oxide did not show a significant plasmon resonance peak in the absence of gold nanoparticles. As seen in Figure 4, the highest absorbance was observed for gold nanoparticles located at 530 nm (solid line curve), whereas Au- Fe_3O_4 composite nanoparticles showed a maximum peak at 608 nm (dotted line) which agreed with other previous reports [2, 9, 14, 19]. This wavelength shift in the gold surface plasmon, known as red-shift, is a probable occurrence and has been reported previously [9, 12, 14, 19].

The XRD method was further used to determine the chemical composition of the nanocomposites (Figure 5). The Fe_3O_4 and Au peaks were observed and again verified the connection of magnetite nanoparticles with elemental gold. The pattern of gold nanoparticles was face-centered cubic, and the dominant crystal plane for these particles

FIGURE 2: TEM images of magnetic-gold composite nanoparticles with low (a) and high (b) magnifications. Dark particles represent gold, and gray particles are magnetite.

FIGURE 3: EDS pattern of magnetite-gold composite nanoparticles.

was (111). The surface chemistry of the prepared magnetite-gold nanocomposites was studied using infrared (IR) spectroscopy and the IR peaks centered at 3426 cm^{-1} and 1675 cm^{-1} confirmed the presence of a carboxyl group on the surface of the semi-biosynthesized magnetite-gold nanocomposites (spectrum not shown).

4. Conclusion

In this study, the green synthesis and functionalization of a $\text{Au-Fe}_3\text{O}_4$ nanocomposite (from Fe_3O_4 magnetic nanoparticles) were performed using an ethanol extract of *E. camaldulensis*. As one advantage, a carboxyl group was detected on the surface of the prepared $\text{Au-Fe}_3\text{O}_4$ nanocomposite, which would make these particles suitable for rapid separation and purification of biomolecules by magnetic field and drug delivery. It should be noted that the importance of the external inert shell in protecting the magnetic core against oxidation is well established but however, in this study we prepared the $\text{Au-Fe}_3\text{O}_4$ nanocomposites that may have contained some portions of $\text{Au-Fe}_3\text{O}_4$ core-shell nanoparticles. Further treatments such as acid washing can be used for separation of the $\text{Au-Fe}_3\text{O}_4$ core-shell fraction and merit further investigation. Moreover, since surfactant has not been used for functionalization of these nanocomposites, this makes these nanoparticles also potentially useful for biomedical applications.

FIGURE 4: Absorbance spectra of gold nanoparticles (solid line) and magnetite-gold composite nanoparticles (dotted line).

FIGURE 5: X-ray diffraction pattern of $\text{Au-Fe}_3\text{O}_4$ composite nanoparticles.

Acknowledgment

This work has been supported by Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

References

- [1] L. Wang, F. Wang, and D. Chen, "Fabrication and characterization of silver/polystyrene nanospheres with more complete coverage of silver nano-shell," *Materials Letters*, vol. 62, no. 14, pp. 2157–2160, 2008.
- [2] X. F. Zhang, X. L. Dong, H. Huang, et al., "Synthesis, structure and magnetic properties of SiO₂-coated Fe nanocapsules," *Materials Science and Engineering A*, vol. 454–455, pp. 211–215, 2007.
- [3] M. Pita, J. M. Abad, C. Vaz-Dominguez, et al., "Synthesis of cobalt ferrite core/metallic shell nanoparticles for the development of a specific PNA/DNA biosensor," *Journal of Colloid and Interface Science*, vol. 321, no. 2, pp. 484–492, 2008.
- [4] Y. Q. Wang, K. Nikitin, and D. W. McComb, "Fabrication of Au-Cu₂O core-shell nanocube heterostructures," *Chemical Physics Letters*, vol. 456, no. 4–6, pp. 202–205, 2008.
- [5] X. G. Liu, D. Y. Geng, J. M. Liang, and Z. D. Zhang, "Magnetic stability of Al₂O₃-coated FCC-Co nanocapsules," *Journal of Alloys and Compounds*, vol. 465, no. 1–2, pp. 8–14, 2008.
- [6] T. T. Pham, C. Cao, and S. J. Sim, "Application of citrate-stabilized gold-coated ferric oxide composite nanoparticles for biological separations," *Journal of Magnetism and Magnetic Materials*, vol. 320, no. 15, pp. 2049–2055, 2008.
- [7] P. Tartaj, M. del Puerto Morales, S. Veintemillas-Verdaguer, T. González-Carreño, and C. J. Serna, "The preparation of magnetic nanoparticles for applications in biomedicine," *Journal of Physics D*, vol. 36, no. 13, pp. R182–R197, 2003.
- [8] J. Jeong, T. H. Ha, and B. H. Chung, "Enhanced reusability of hexa-arginine-tagged esterase immobilized on gold-coated magnetic nanoparticles," *Analytica Chimica Acta*, vol. 569, no. 1–2, pp. 203–209, 2006.
- [9] T. Kinoshita, S. Seino, Y. Mizukoshi, et al., "Magnetic separation of amino acids by gold/iron-oxide composite nanoparticles synthesized by gamma-ray irradiation," *Journal of Magnetism and Magnetic Materials*, vol. 293, no. 1, pp. 106–110, 2005.
- [10] Z. Wang, H. Guo, Y. Yu, and N. He, "Synthesis and characterization of a novel magnetic carrier with its composition of Fe₃O₄/carbon using hydrothermal reaction," *Journal of Magnetism and Magnetic Materials*, vol. 302, no. 2, pp. 397–404, 2006.
- [11] J. Lin, W. Zhou, A. Kumbhar, et al., "Gold-coated iron (Fe@Au) nanoparticles: synthesis, characterization, and magnetic field-induced self-assembly," *Journal of Solid State Chemistry*, vol. 159, no. 1, pp. 26–31, 2001.
- [12] T. Kinoshita, S. Seino, K. Okitsu, T. Nakayama, T. Nakagawa, and T. A. Yamamoto, "Magnetic evaluation of nanostructure of gold-iron composite particles synthesized by a reverse micelle method," *Journal of Alloys and Compounds*, vol. 359, no. 1–2, pp. 46–50, 2003.
- [13] S.-J. Cho, B. R. Jarrett, A. Y. Louie, and S. M. Kauzlarich, "Gold-coated iron nanoparticles: a novel magnetic resonance agent for T1 and T2 weighted imaging," *Nanotechnology*, vol. 17, no. 3, pp. 640–644, 2006.
- [14] H. Yu, M. Chen, P. M. Rice, S. X. Wang, R. L. White, and S. Sun, "Dumbbell-like bifunctional Au-Fe₃O₄ nanoparticles," *Nano Letters*, vol. 5, no. 2, pp. 379–382, 2005.
- [15] K. Kawaguchi, J. Jaworski, Y. Ishikawa, T. Sasaki, and N. Koshizaki, "Preparation of gold/iron-oxide composite nanoparticles by a unique laser process in water," *Journal of Magnetism and Magnetic Materials*, vol. 310, no. 2, pp. 2369–2371, 2007.
- [16] N. Ramezani, Z. Ehsanfar, F. Shamsa, et al., "Screening of medicinal plant methanol extracts for the synthesis of gold nanoparticles by their reducing potential," *Zeitschrift für Naturforschung B*, vol. 63, no. 7, pp. 903–908, 2008.
- [17] I. Martínez-Mera, M. E. Espinosa-Pesqueira, R. Pérez-Hernández, and J. Arenas-Alatorre, "Synthesis of magnetite (Fe₃O₄) nanoparticles without surfactants at room temperature," *Materials Letters*, vol. 61, no. 23–24, pp. 4447–4451, 2007.
- [18] Q. H. Lu, K. L. Yao, D. Xi, Z. L. Liu, X. P. Luo, and Q. Ning, "Synthesis and characterization of composite nanoparticles comprised of gold shell and magnetic core/cores," *Journal of Magnetism and Magnetic Materials*, vol. 301, no. 1, pp. 44–49, 2006.
- [19] J. Lim, R. D. Tilton, A. Eggeman, and S. A. Majetich, "Design and synthesis of plasmonic magnetic nanoparticles," *Journal of Magnetism and Magnetic Materials*, vol. 311, no. 1, pp. 78–83, 2007.