

Surface modification of magnetite nanoparticles for immobilization with lysozyme

M.-J. Kim^a, B. Lim^a, Y.-K. Jeong^b, Y.W. Cho^a and Y.-H. Choa^{a,*}

^aCollege of Engineering Sciences, Hanyang University, Gyeonggi 426-791, Korea

^bNational Core Research Center for Hybride Materials Solution, Pusan University

The surface of magnetite (Fe₃O₄) nanoparticles prepared by a co-precipitation method was modified by the carboxylic acid group of meso-2,3-dimercaptosuccinic acid (DMSA). The egg white lysozyme was then immobilized on the carboxylic acid group-modified magnetite nanoparticles. The magnetite nanoparticles were approximately 10 nm and had a spherical morphology and uniform size distribution. The concentration of lysozyme on the modified magnetite nanoparticles was also investigated by a UV-Vis spectrometer and compared to that of magnetite nanoparticles without surface modification.

Key words: Magnetite nanoparticles, Lysozyme, Protein Immobilization.

Introduction

Fe₃O₄ magnetic nanoparticles are widely studied for their various applications in biology and medicine [1, 2] such as enzyme and protein immobilization [3], magnetic resonance imaging (MRI) [4], RNA/DNA purification [5], drug delivery systems (DDS) [6], and hyperthermia therapy [7]. However, magnetite nanoparticles are difficult to bond directly with biomolecules in aqueous solution. Therefore, the properties of magnetic nanoparticles can be significantly altered by surface modification. A typical approach to tailor the surface properties of magnetic particles is through a coating process or encapsulation [8, 9] but these processes are exceedingly complex and difficult.

In this study, magnetite nanoparticles which were prepared by co-precipitation of Fe²⁺ and Fe³⁺ with NH₄OH were simply modified by the carboxylic acid group of meso-2,3-dimercaptosuccinic acid (DMSA). Then, the egg white lysozyme was immobilized on the carboxylic acid group-modified magnetite nanoparticles.

Experimental Procedure

Magnetite nanoparticles were synthesized by a co-precipitation method. Ferrous chloride tetrahydrate (FeCl₂·4H₂O > 99%) and ferric chloride hexahydrate (FeCl₃·6H₂O > 99%) were used as iron sources, and aqueous ammonia (NH₄OH) was used as the precipitator. Distilled water was used as the solvent. FeCl₂·4H₂O and FeCl₃·6H₂O (Fe³⁺:Fe²⁺ = 1:2) were dissolved in distilled water with vigorous stirring at 80 °C under a

nitrogen atmosphere to prevent oxidation, and then the magnetite nanoparticles were obtained by adding NH₄OH into the solution. After stirring for 30 minutes, the precipitate was repeatedly washed by ethanol.

The 0.3 mol-DMSA (meso-2,3-dimercaptosuccinic acid) was diluted with DMSO (dimethylsulfoxide). One gram of magnetite nanoparticles was added into 10 ml of the DMSA solution with stirring in an air atmosphere for 24 h. This sample was then washed with ethanol five times. 0.4 mol and 0.8 mol of DMSA-functionalized nanoparticles were fabricated by the same process described above.

The adsorption of DMSA on the magnetite nanoparticles was tested by using lysozyme (from chicken egg white, Fluka). To make an activation treatment, 10 mg of functionalized magnetite nanoparticles was added into 0.5 ml of a 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) solution while mixing for 3 h. Then, the DMSA-functionalized magnetite nanoparticles and non-functionalized nanoparticles were dispersed in 0.01 mol of phosphate-buffered saline (PBS: 150 mmol NaCl, 1.9 mmol NaH₂PO₄, 8.1 mmol Na₂HPO₄, pH 7.4). These samples were repeatedly washed with PBS three times. Then, 0.475 mg/ml of the lysozyme solution was added. After stirring for 24 h, lysozyme immobilized magnetite nanoparticles were obtained by a magnetic separation method (Fig. 1).

The crystallographic structures and particle sizes of the samples were characterized by an X-ray diffractometer (XRD; Rigaku, CuK α radiation, λ =1.5405 Å). The XRD measurements were performed with scan speeds of 4°/minute for the crystallographic structures and 0.5°/minute for the particle size calculations. The particle morphology was determined by high resolution transmission electron microscopy (HRTEM; JEM-3000F, JEOL, Japan). The magnetic properties were measured using a vibrating sample magnetometer (VSM-5; TOEI,

*Corresponding author:
Tel : +81-31-407-9067
Fax: +82-31-418-6490
E-mail: choa15@hanyang.ac.kr

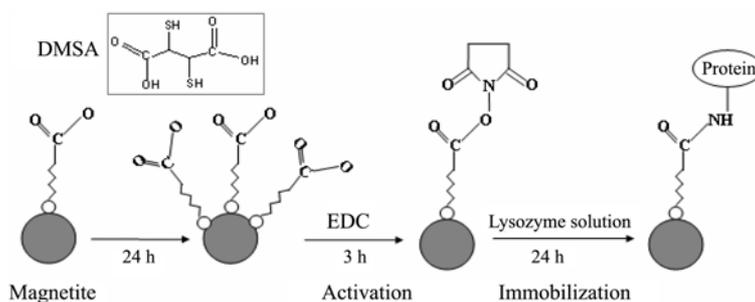


Fig. 1. Schematic diagram of the magnetite nanoparticle modified with DMSA polymerization for lysozyme immobilization.

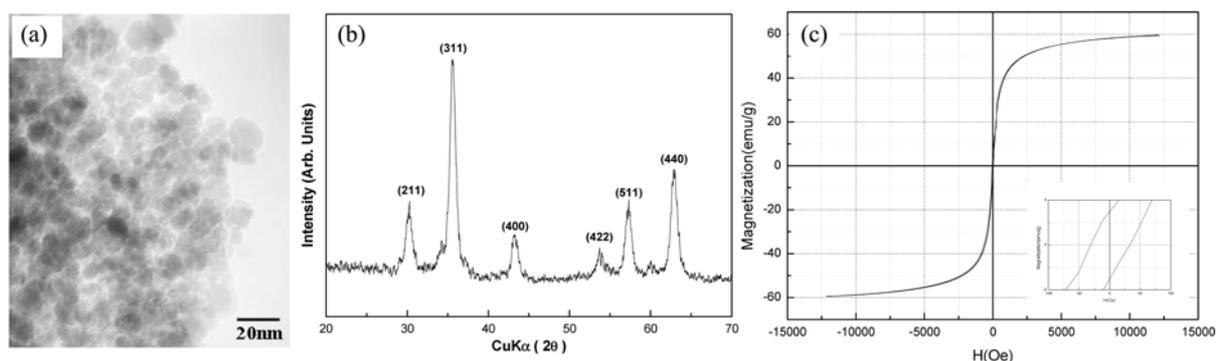


Fig. 2. (a) TEM image, (b) XRD pattern, and (c) M-H hysteresis loop of magnetite nanoparticles prepared by the co-precipitation method.

Japan). The M-H hysteresis loop measurements were carried out at 300 K with an applied magnetic field of up to 12,000 Oe. Fourier transform infrared spectroscopy (FT-IR; FTS6000, BIO-RAD, USA) was used to characterize the surface composites of the magnetite nanoparticles modified by DMSA. The concentration of the lysozyme adsorption on the surface of functionalized magnetite nanoparticles was determined by UV-vis spectroscopy (UV-vis; Optizen 2120UV, Mecasys Co., Korea).

Results and Discussion

Figure 2 shows various characterizations of the magnetite nanoparticles. The mean particle diameter was also calculated from the XRD patterns utilizing the line width of the (311) plane diffraction peak in the Scherrer equation;

$$D = \frac{0.9\lambda}{B\cos\theta} \quad (1)$$

Equation (1) uses the reference peak width at an angle θ , where λ is the X-ray wavelength (1.5405 Å), and B is the width of the XRD peak at half height. The resulting mean particle diameter was approximately 10 nm for the magnetite nanoparticles. This was in agreement with the result obtained from the TEM image of Fig. 2(a). In Fig. 2(c), the magnetite nanoparticles had a coercive force (H_c) of 31 Oe and a magnetization force (M_s) of 59.3 emu/g.

FT-IR spectra of the magnetite nanoparticles modi-

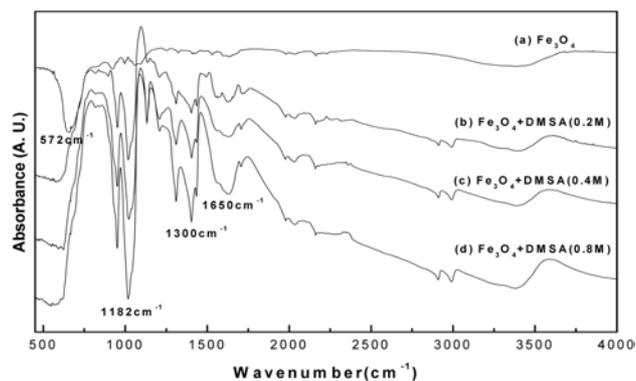


Fig. 3. FT-IR spectra of magnetite nanoparticles modified by DMSA.

fied by DMSA are shown in Fig. 3. All the samples have a strong absorption band of Fe-O bond of magnetite nanoparticles at 572 cm^{-1} , and another broad band appeared in the 3200-3600 cm^{-1} region, corresponding to the O-H stretching vibration. The vibration of asymmetry of the carboxyl group (-COOH) for samples (b), (c), and (d) was located at 1650 cm^{-1} . Other vibrational features at 1300 cm^{-1} and 1182 cm^{-1} are assigned to the C-O stretching vibration. Accordingly, we believe that these characteristics indicate the adsorption of DMSA on the Fe_3O_4 surface. The results also confirmed that the intensity of DMSA peaks (-COOH group and C-O stretching) increased by increasing the DMSA concentration from 0.2 mol to 0.8 mol.

A comparison between the amount of lysozyme immobilized on the non-functionalized magnetite parti-

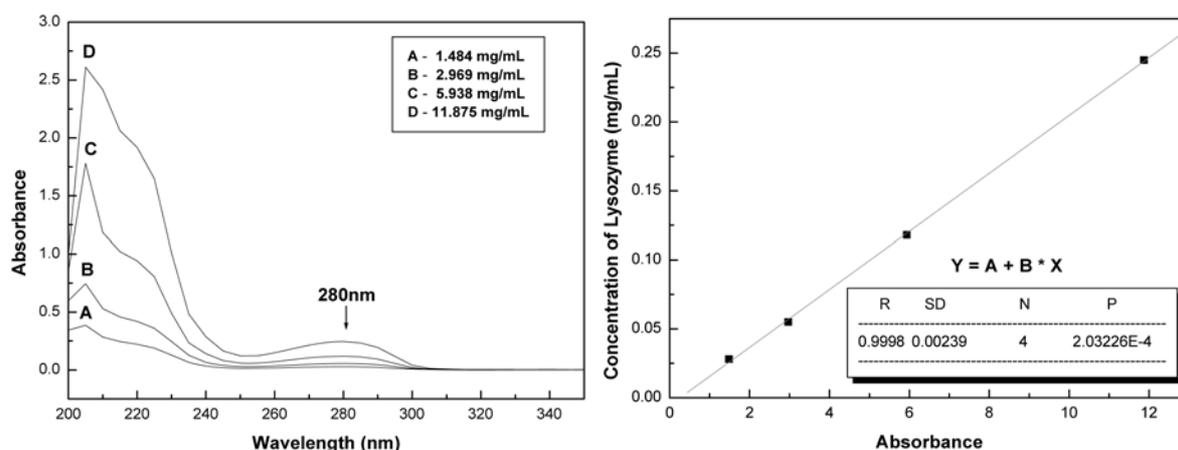


Fig. 4. UV-vis spectra of (a) lysozyme solution and the supernatants of samples after the magnetic separation and (b) calibration standard line of lysozyme.

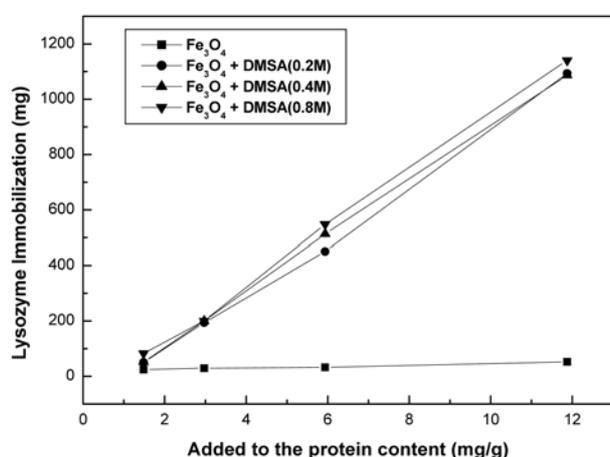


Fig. 5. The amount of lysozyme immobilized on non-functionalized magnetite particles and DMSA-functionalized magnetite particles.

cles and that of DMSA-functionalized magnetite particles detected by UV-vis absorption spectra at a wavelength of 280 nm is shown in Fig. 4. The amount of immobilized protein on 10 mg of magnetite nanoparticles was calculated from the calibration standard line of lysozyme in Fig. 4(b). These values of lysozyme immobilization for DMSA-functionalized samples, in general, are higher than those for the non-functionalized sample. In addition, the protein content increased linearly with an increase in the concentration of lysozyme as shown in Fig. 5.

Conclusions

Magnetite nanoparticles were prepared by a co-precipitation method. The sample particles had a uniform size distribution and small size (10 nm). After functionalization of the surfaces of the magnetite nanoparticles with DMSA, lysozyme was immobilized on the magnetite surfaces. The presence of DMSA on magnetite surfaces was confirmed by FT-IR spectra. The amount

of lysozyme immobilized on DMSA-functionalized samples was much higher than that of the non-functionalized sample. In addition, the protein content increased linearly with an increase of the concentration of lysozyme. In this study, a simple modification method was developed to functionalize magnetite nanoparticles with an active carboxyl group (-COOH) by attaching it with DMSA. Therefore, magnetite nanoparticles functionalized by a carboxyl group have a great potential for specific applications such as in cells, protein, DNA sorting or separation, diagnosis, DDS, and hyperthermia therapy.

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