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Ultrasonic-assisted synthesis and strepavidin conjugation of amino-magnetic nanoparticles

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Abstract MNPs are widely used in biotechnology, magnetic resonance imaging, catalysis and other areas. In this paper, we propose a simple, quick, inexpensive and efficient way to prepare amino-MNPs with sonication-assisted process. The amino-MNPs prepared by hydrolyzing TEOS and APS were characterized by SEM, TEM and FTIR. Conjugation of SA and amino-MNPs was also studied. The biotin bound capacity of prepared SA coated amino-MNPs was 1.824 nmol/mg, which well meet the need of biological application.

Key words Magnetic nanoparticles, Strepavidin, Biotin bound capacity, Sonication-assisted surface treatment

CLC numbers TB383, O482.5

1 Introduction

Nanosized biocompatible magnetic materials have been widely applied in biotechnology, magnetic resonance imaging, catalysis, data storage, and environmental remediation[1,2]. As an example, biocompatible magnetic fluids (MFs), composed of magnetic nanoparticles (MNPs), due to their superparamagnetism, biocompatibility and high reactivity, offer quick and easy separation in biotechnology. Therefore, it is of interest to prepare and modify MNPs to introduce additional functionality[3]. Multifunctional MNPs systems with designed active sites, including ligands, enzymes and other species, seem to be promising for a variety of applications.

MNPs are generally of core/shell structures coated with various organic polymers[4-6] and inorganic components. Co-precipitation is a convenient way to synthesize MNPs. The quality of the MNPs is fully reproducible under fixed synthetic conditions. Silica coating is the most frequently used method to modify surface of MNPs. Silica coated MNPs possess excellent stability, hydrophilicity and easy surface modification characters. They are readily modified with other functional groups.[7-9] Therefore silicon chemistry opens the door of MNPs for advanced applications such as biomolecular attachment, which can be used to deliver specific ligands to target sites *via* antibody-antigen recognition. A fundamental problem often encountered in surface silanization process, however, is formation of hard and large aggregates[10, 11]. Several methods were used to prepare well-dispersed silanized MNPs and power ultrasound has been proven to be an ideal method to solve this problem[12]. Under powerful ultrasonic waves, large particle aggregates can be fragmented into smaller particles resulting from the ultrasonic cavitation effects. Such effects will reduce the particle size and thus increase reactivity in solution.

In this work, a two-step sonication-assisted surface treatment process was developed to synthesize amino-MNPs. Tetraethyl orthosilicate (TEOS) and 3-aminopropyltrimethoxysilane (APTS) were used to introduce silica shell and amino groups onto the MNPs.

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Unlike sonication-free process, no hard aggregation was observed during the coating, and improvement in dispersability of the particles was significant. Further conjugation with strepavidin (SA) experiments offered the excellent conjugation efficiency with MNPs, and SA coated onto MNPs maintained affinity well with biotin conjugation tests.

2 Materials and methods

2.1 Materials and equipment

$FeCl_3 \cdot 6H_2O$, Na_2SO_3, ammonia in concentration of 25wt%, tetraethyl orthosilicate (TEOS), Tween-20 and glutaraldehyde (50wt%) aqueous solution were purchased from Shanghai Chemical Reagent Corp., China. Streptavidin (SA), *d*-biotin *p*-nitrophenyl ester were from Sigma, 3-aminopropyltrimethoxysi-lane (APTS) was from Fluka. All reagents were used as received. The KQ218 (40 kHz, 100W) ultrasonic instrument (Kunshan Ultrasonic Instrument Factory, China) and Eppendorf thermal mixer were used. Magnetic Rack was purchased from Shaanxi Lifegen Co. Ltd.

The samples were characterized with an LEO1530VP SEM, a Hitachi H-600 TEM, and a NICOLET FT-IR 380 in wave numbers of 4000~400cm^{-1}. A Canon A620 digital camera was used to take the photos.

2.2 Preparation of magnetic nanoparticles (MNPs)

MNPs were synthesized with partial reduction method according to Ref. [13]. Briefly, 6.5 g of $FeCl_3 \cdot 6H_2O$ was dissolved in 100 mL of Milli Q water, and freshly prepared sodium sulfite (0.14 mol / L) was added slowly into $FeCl_3$ aqueous solution under N_2 protection while stirring at 30°C for 15 min. After the solution color changed from red to yellow, 40 mL of diluted ammonia (24 mL of concentrated ammonia was diluted with 16 mL of water) was rapidly poured into the solution, the reaction was kept at 60°C for 30min. After being cooled down to RT, the mixture was further maturated for about 2 h at RT. The naked MNPs was collected by magnetic separation and washed for several times with water, then dispersed with 50 mL of water ready for use.

2.3 Preparation of NH_2-MNPs with ultrasonic process

A 0.5 mL of MNP´s suspension was diluted in 30mL methanol (containing 0.5 mL ammonia), 0.2 mL TEOS was added to the solution under ultrasonic, and the reaction went on for 4 h at RT. Then 0.1 mL APS was added and the mixture was ultrasonically dispersed for 4 h. The organic solvent was separated by magnet and the precipitation was washed several times with water to adjust the pH from alkaline to neutral and resuspended in 2 mL of diluted HCl.

Another group was prepared under mechanical stirring, with other conditions being the same as above.

2.4 Immobilization of streptavidin on NH_2-MNPs

As previously reported[14], glutaraldehyde was used to conjugate protein and amino-MNPs. Briefly, 0.2 mL NH_2-Fe_3O_4 was dispersed into 10% glutaraldehyde in phosphate-buffer (0.05 mol / L, pH 7.4) solution with stirring for 3 h at RT. The suspension was washed three times with PBS buffer solution by magnetic separation. It was re-suspended in 2 mL of phosphate-buffer (0.05 mol/L, pH 7.4, and containing 0.05% Tween-20). The solution was added with 0.1 mL of 1g/L SA. The mixture was incubated for 10 h at 4°C with stirring. Excess ethanolamine (0.5 mL, 1mol/L, pH 7.4) was added and stirred for 2 h at RT to terminate the activated residual aldehyde groups. Unreacted SA and other impurities were separated magnetically. The MNPs were washed with water several times and resuspended in 0.5 mL of PBS (50 mmol/L, pH 7.4).

2.5 Colorimetric measurements of SA-MNPs

In order to investigate the biotin binding capacity of SA-Fe_3O_4, a colorimetric measurement was performed by adding excess *d*-biotin *p*-nitrophenyl ester (BNPE). Briefly, the SA-Fe_3O_4 (200 μg) was washed three times with 1 mL of 0.2 mol/L acetate buffer (pH 5.0, containing 0.05% Tween-20). Then 100 μL of the buffer containing 0.5 mg BNPE in 0.1 mL of dimethyl sulfoxide was added. After 30 min incubation under shaking, the particles were rinsed five times with 5 mL of the above buffer, magnetically

separated and transferred to 50 μL of 0.1 mol/L NaOH. The BNPE bound particles hydrolyzed and produced a yellow dye which was analyzed at 400 nm in the supernatant. The visible absorption intensity is proportional to the p-nitryl phenol concentration.

The commercial Streptavidin MagneSphere® Paramagnetic Particles (SA-PMPs) was used as contrast and the whole process was performed as described above.

3 Results and discussions

3.1 SEM of naked MNPs synthesized with co-precipitation method

The MNPs prepared by co-precipitation method were characterized by SEM and TEM (Fig. 1). The photography clearly show that the MNPs were approximately spherical and well-distributed with size of 10~15 nm.

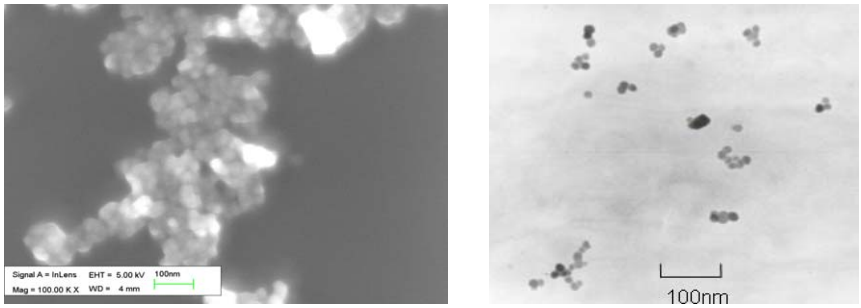

Fig. 1 SEM (left) and TEM (right) images of naked MNPs. The MNPs were well-distributed spherical particles of Φ10~15 nm.

3.2 Preparation and characterization

3.2.1 Sythesis procedures

In absence of surface coating, naked MNPs tend to agglomerate and form large clusters. Silica-coated strategy was reported as an efficient protection method[15-17]. An additional advantage is introduction of more surface silanol groups onto the MNPs surface. The silanol groups can easily react with other silane coupling agents[15], which provide ideal anchorage for covalent bounding of specific ligands.

A two-step method was used to synthesize amino-MNPs[18]. Firstly, the silane coupling agents of TEOS was used to form silane polymers, which offered much more silicic hydroxyl groups than MNPs. Secondly, APTS was used to introduce active amino groups on surface of MNPs by forming a covalent bond with surface hydroxy groups through dehydration.

However, in the coating process with APTS, we found that MNPs was easy to flocculate even under intensive stirring conditions. Therefore an efficient scattering technique was needed to prepare well-dispersed nanoparticles. It is known that ultrasonic was a powerful tool to break the aggregates into splits[19-21].

The ultrasonic-assisted method was used in the silanization process. The small and relax aggregates formed during the modification course was simultaneously broken into well-dispersed fluids again. A stable and well-dispersed black suspension was obtained by sonication, while large aggregates were formed by mechanically stirring method. Fig.2 shows magnetic fluids status after 1h stocking. The irregular amino-MNPs were about 0.5~1 μm by SEM image.

Different types of small particles (nanoparticles of micron-sized particles) are commercially available from several different manufacturers including Bangs Laboratories (Fisher, Indiana); Promega (Madison, WI), Dynal Inc. (Lake Success, New York), etc. Most of the particles are made using conventional techniques, such as grinding and milling, emulsion polymerization, block copoymerization, and microemulsion.

We used a sonication-assisted process, by which the entire preparation procedure could be completed in one day, without complicated time-consuming steps. The irradiation intensity, which is important for dispersing the particles, could be controlled by adjusting the ultrasonic power. Large particles or particle aggregates could be fragmented into smaller particles immediately under powerful ultrasonic waves, which caused impact (jet) milling, attrition milling, knife milling, and direct pressure milling between adjacent

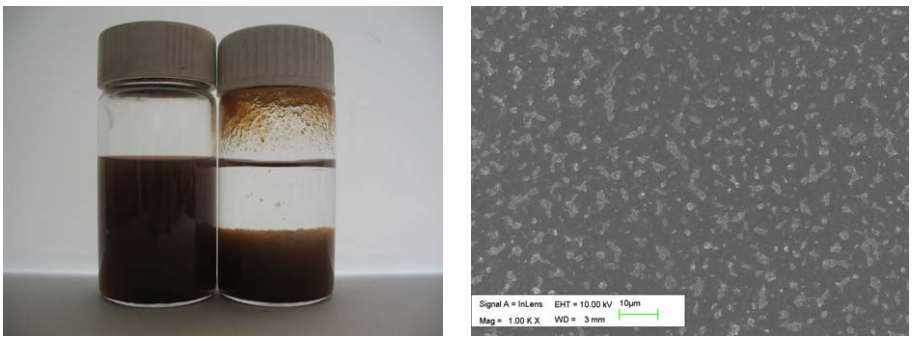

Fig. 2 Magnetic fluids prepared by sonication (left) and stirring method (right), after 1h placing at RT.

nanoparticles. The silanization was slow and continuous process, and the soft, relax and small aggregates formed in the first stages, they will grow into large aggregates without immediately being broken. Obviously, the traditional stirring does not break them while ultrasound does because of the efficient ultrasound cavitation effects[22, 23].

3.2.2 Characterization of amino-MNPs with sonication process

The prepared amino-MNPs were easily separated under magnet and suitable for application in magnetic separation of most biological fields. As shown in Fig.3, the amino-MNPs were precipitated in a few minutes with magnetic separation rack (Promega) and the supernatant was clear. By taking the magnetic rack away, the precipitation was easily redispersed as homogeneous gels. These suggest that the amino-MNPs may completely satisfy the need of magnetic separation in biological and medical fields.

According to FT-IR spectra (Fig.4) of the NH_2-MNPs, both the naked MNPs have the Fe-O absorption bands of Fe_3O_4 at 590 cm^{-1}[8], the absorption

Fig. 3 Amino-MNPs separated by magnet and redispersed. Observations on stability of the amino-MNPs show that placing them at 4°C for over one month, the particles were easily dispersed with gentle shaking.

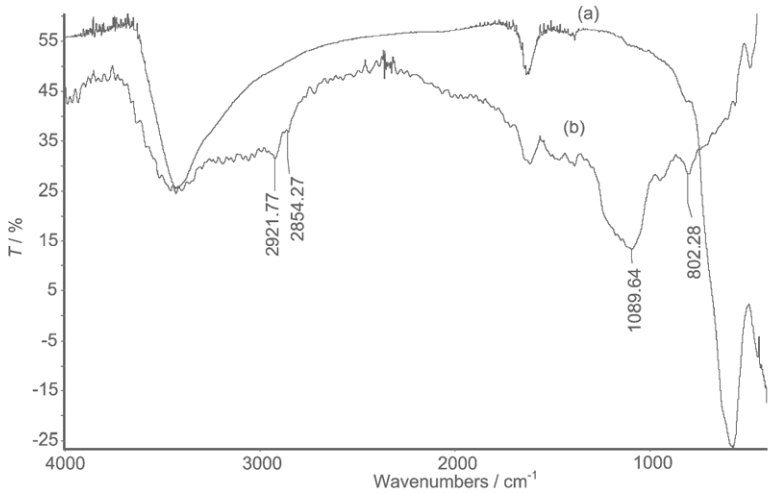

Fig. 4 FT-IR spectra of naked (a) and coated (b) MNPs.

bands near 3400 and 1630 cm^{-1}, originated from vibration of H_2O remaining in the samples. The coated amino-MNPs has absorption bands at 2921.77 cm^{-1} and 2854.27 cm^{-1} due to the -CH_2- groups, at 1089.64 cm^{-1} due to the Si-O bond, and at 885.2 cm^{-1} due to the bending vibration of -NH_2 group. These confirmed that the MNPs had been coated with TEOS and APTS.

3.3 Conjugation of streptavidin with NH_2-MNPs

Magnetic particles are often sensitized with streptavidin (SA) to take advantage of its extremely high affinity interaction with biotin. Because biotin can be easily conjugated to many different diagnostic reagents, a variety of assays for different analytes in several formats can be developed[24].

In this paper, the conjugation of SA with amino-MNPs and its binding capacity with biotin were investigated to evaluate the particle quality. The conjugation of SA and amino-MNPs was performed by glutaraldehyde biofunction molelules, the active groups of -NH_2 to be transformed to aldehyde groups that can easily connect to proteins and enzymes *via* Schiff base linkage.

The biotin binding capacity of SA coated amino-MNPs (Group A) was investigated with a colorimetric measurement by adding excess *d*-biotin *p*-nitrophenyl ester (BNPE)[14], while the commercial SA-Fe_3O_4 (Promega, 1 μm, 1 g/L) was used as a control (Group B).

The yellow dye produced by hydrolyzed BNPE was proportional to the biotin binding capacity. As demonstrated in Fig. 5, the color of Group A was significantly deeper than Group B.

Further quantification was analyzed with UV-Vis spectra, and the absorption intensity of Group A at 400 nm was 0.133 while that of Group B was 0.069. The binding capacity was calculated by

$$C = [AV/(m\varepsilon)]\times 10^6,$$

where C (nmol/mg) refers to the biotin binding capacity of SA-PAMAM-MNP, m (mg) is the dry weight of amino-MNPs used to bind with SA, A is the absorbance at 400 nm, ε is the molar absorption constant of *p*-nitryl phenol (18300 L/mol), and V (mL) is the hydrolyzed volume of supernatant.

The biotin bound capacity of the prepared SA coated amino-MNPs was 1.824 nmol/mg, which was 1.95 times higher than Promega products (0.945 nmol/mg under the same detection conditions).

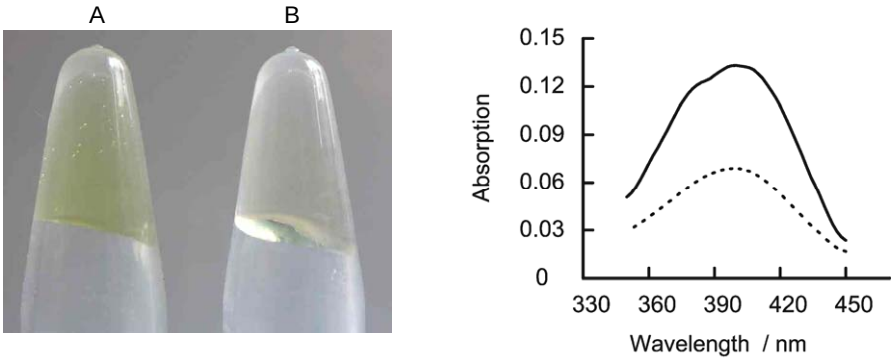

Fig. 5 The biotin binding capacity of SA coated amino-MNPs (solid line: prepared, dash line: commercial).

4 Conclusions

In conclusion, we proposed a simple, quick, inexpensive and efficient way to prepare amino-MNPs with sonication-assisted process. During the silanization process, the power ultrasound effectively broke the nanoparticle aggregates into splits, and well-dispersed amino-MNPs were obtained. The FT-IR, stability, magnetism could satisfy the need of biological separation procedure. Conjugation of SA and amino-MNPs was also studied, and its biotin binding capacity was higher than commercial particles.

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