Nano-Engineering Of Magnetic Particles For Biocatalysis And Bioseparation

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ABSTRACT

Magnetic nanoparticles encapsulated in a thin coating as magnetic separable *nano-vehicle* for chemical species is a hot but challenging area. The facilitated separation of a small magnetic body carrying biologically active species is of a tremendous interest however; the stability of the magnetic body remains a key issue. We report new syntheses of silica encapsulated magnetic nanosize particles as magnetic separable carriers in large quantities based on simple synthetic techniques. The major advantage of using nano-size magnetic particles as carriers is that they display an excellent mass transfer coefficient (high surface area to volume ratio) comparable to soluble species but can still be easily separated from liquid using magnetic interaction with an external applied inhomogeneous magnetic field (i.e. 50MGOe). It is shown that the external coating surfaces can isolate and protect the magnetic core from destructive reactions with the environment where a wide range of conditions for fine chemical catalysis can be made possible. The functionalized surfaces could also offer anchoring sites for the immobilization of active chemical species of interests (enzymes, DNA oligos and antibodies). Most of these applications require nanoparticles covered with appropriate surface chemical functionalities where a strong magnetic core is essential for the separation of each particles from solution.

***Keywords**:* magnetic nanoparticles, biocatalysis, bioseparation, silica encapsulated iron oxide

1 INTRODUCTION

The synthesis of magnetic nanoparticles has been intensively investigated not only for their electrical, optical and magnetic properties but also for their other technological applications including magnetic assisted bio-separation and bio-catalysis. A number of magnetic nanoparticles have recently been applied in biotechnology areas [1]. In biomedicine, magnetic nanoparticles can be used as labeling or imaging reagents when tagged with biological entities. These approaches are very attractive to industry as the magnetically tagged bio-molecules can be recycled easily using magnetic means from solution (minimize the waste production through regeneration [2].)

In general, the most common synthetic methods for the synthesis of magnetic materials with nanometer-scale dimensions can be classified into three categories. They are physical vapour deposition, mechanical attrition and chemical routes from solution. In both the vapour phase and solution routes, the particles are assembled from individual atoms to form nanoparticles. Alternatively, mechanical attrition involves the fracturing of larger coarse-grained materials to form nanostructures. The chemical routes from solution are widely used for the fabrication of nanoparticles. They often provide the best methods for production of nanoparticles due to their enhanced homogeneity from the molecular level design of the materials and, in many cases, cost effective in bulk quantity production. The solution chemical routes provide mild reaction conditions and require less expensive equipments. They also allow control of particle size and size distribution, morphology, and agglomerate size through the individual manipulation of the parameters that determine nucleation, growth, and coalescence. Surface modification of the particles during synthesis or post-synthesis is easily accomplished, and provides additional functionality to the nanoparticle.

Here, we report a single-step solution based chemical synthesis of silica encapsulated iron oxide nanoparticle using microemulsion technique, which can be adopted in bio-catalysis and bio-separation areas [3]. The silica coated iron oxide nanoparticle is also shown to cover with surface hydroxyl groups which facilitate a strong attachment of protein such as Bovine Serum Albumin (BSA) onto its surface.

2 EXPERIMENTAL

2.1 Synthesis

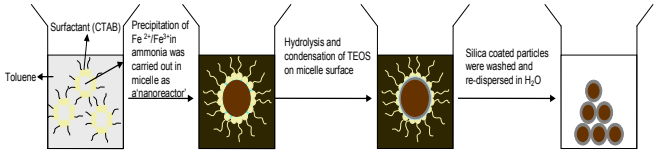
Chemicals: Iron (II) chloride tetrahydrate ($FeCl_2$ $4H_2O$), iron (III) chloride hexahydrate ($FeCl_3$ $6H_2O$), ammonium hydroxide (35 wt%), cetyltrimethylammonium bromide (CTAB) , dried toluene, and tetraethyl orthosilicate (TEOS). All aqueous solutions were prepared with deoxygenated

water. Potassium dihydrogen orthophosphate (99%, Aldrich). Sodium chloride (99%) was received from Fisher. Bovine serum albumin (fraction V, 99%), deionized water was used for all experiments.

Synthesis of Silica Coated Fe_3O_4 Particles in Microemulsion: 7.3 g CTAB was added into 180 mL dried toluene in a 250 mL round bottom flask with stirring for 4 h. In general, the higher Wo value used, the larger of the nanoparticles size we observed. In this case, the mole ratio of water to surfactant (Wo =[water]/[CTAB]) of 20 was fixed. Thus, Fe (II)/Fe (III) salt were dissolved in 7.2 mL deionized water by adding the Fe (II)/Fe (III) solution dropwise into the round bottom flask constantly flushing with nitrogen for 2h. After further four hours, ammonium hydroxide was added dropwise to the same flask with a continuous nitrogen purge. The color changed from light yellow to dark brown without precipitation. 6.951 g TEOS was directly added into the microemulsion and the mixture was allowed to age for 5 days to encourage hydrolysis and condensation of the silica precursor. After aging, the resulting nanoparticles were collected as a precipitate when ethanol was added into the solution. The precipitate was then washed with excess ethanol and re-dispersed in toluene. This step was repeated for five times in order to remove the surfactant from the precipitate (until the FTIR showed no trace of surfactant). At last, the precipitate was washed with acetone and left in air for drying at room temperature overnight. The formation of iron oxide nanoparticle from the chemical reaction of ammonium hydroxide with Fe(II)/Fe(III) species is shown in the equation 1, below and Scheme 1 graphically summaries the preparative procedure.

$$FeCl_2 + 2FeCl_3 + 8NH_3{\cdot}H_2O \rightleftharpoons Fe_3O_4 + 8NH_4Cl + 4H_2O$$

Equation 1: Iron oxide nanoparticle prepared from ammonia precipitation of Fe(II) and Fe(III) according to chemical reaction



Scheme 1 Synthetic procedure for preparing silica encapsulated iron oxide nanoparticles

Directly immobilization of BSA onto silica@iron oxide
5 mL 315µM of BSA solution was added into 63.6 mg of silica@iron oxide in a 10mL vial and the mixture was agitated at room temperature overnight. The solid immobilized BSA was separated from solution by applying an external magnetic field (50MGOe).

2.2 Characterization

Figure 1 shows a typical X-Ray Diffraction (XRD) pattern of iron oxide in silica. There are three strong peaks identified from this spectrum corresponding to the lattice spacing of 2.951, 2.509, and 1.475. These match with either the structures of Fe_3O_4 or γ-Fe_2O_3 as compared with an XRD database. A very broad diffraction peak from 9-23° (2θ) corresponding to the poorly crystalline silica is also observed. The average particle size can be derived using Deby-Scherrer equation from the full width at half maximum (FWHM) of the strongest peak. It is noted that the patterns match well with both crystalline magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) phases. However, assignment to one of this phase or to the mixture of them based entirely on XRD proofs very difficult since their closely related structures (with almost identical patterns) and nano-metric regime (peak broadening)

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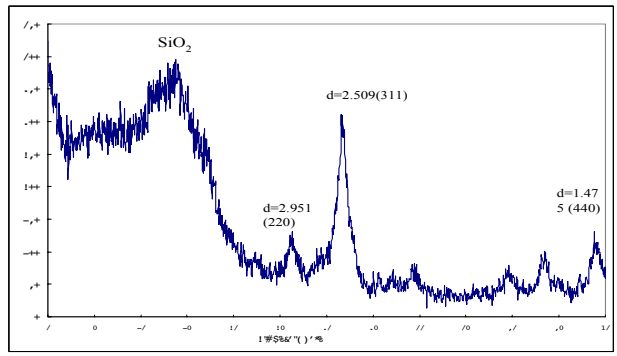

Figure 1: Normalized X-ray diffraction spectrum patterns of silica encapsulated iron oxide in 6.46 nm.

Figure 2 shows a typical high resolution transmission electron microscopic (HRTEM) image of the silica encapsulated iron oxide nanoparticle. It is noted that the image indeed reveals the highly crystalline structure of the iron oxide core (with a lattice spacing of 2.5± 0.1 Å) in an amorphous coating .

Figure 2: HRTEM image of the highly crystalline structure magnetite/ maghemite in silica.

Figure 3a and 3b show general and enlarged vibration saturation magnetization (VSM) responses of the silica encapsulated iron oxide nanoparticle powder upon application of various external magnetic fluxes. The measurements were collected at room temperature. It is evident that the powdered material shows no magnetic hysteresis with both the magnetization and demagnetization curves passing through the origin, which clearly indicates

the superparamagnetic nature of the material. This means that the magnetic material can only be aligned under an applied magnetic field but will not retain any residual magnetism upon removal of the field [3]. Thus, this technique appears to be able to prepare magnetically nanoparticles for magnetic separation purposes.

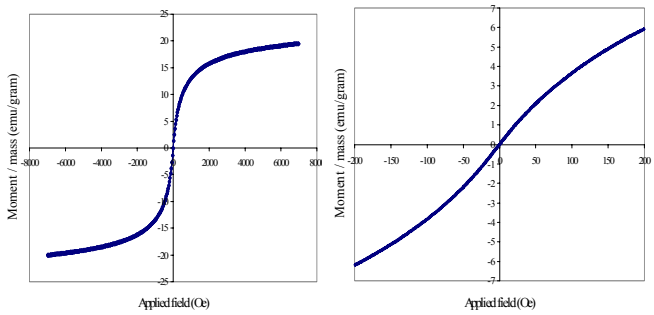

Figure 3: General and enlarged VSM plots of silica encapsulated iron oxide

Figure 4 displays the elemental mapping of the isolated particles of the silica encapsulated iron oxide. After taking the correction of the response factor for each element into account, the atomic ratios of the particle are found to be Fe : O : Si =19.93 : 71.96 : 8.11 with a standard deviation of ± 0.2 %. (excluding carbon analysis because the use of carbon filmed holder which also affects the oxygen analysis).

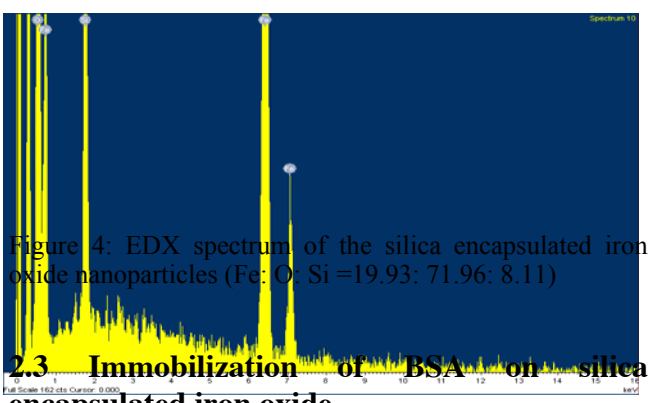

Figure 4: EDX spectrum of the silica encapsulated iron oxide nanoparticles (Fe: O: Si =19.93: 71.96: 8.11)

2.3 Immobilization of BSA on silica encapsulated iron oxide

The ability of the silica encapsulated iron oxide for binding protein is clearly illustrated in the experiment of BSA attachment to the particle. Figure 5 shows the FTIR spectral characteristics of silica encapsulated iron oxide with and without the BSA attachment. Both spectra show an absorption peak near 960 cm^{-1} suggesting the presence of hydroxyl groups. It is noted that the fresh silica encapsulated iron oxide sample appears to contain more hydroxyl groups from its spectrum. It is evident that the appearance of new absorption peaks at 1648 and 1540 cm^{-1} which are the characteristic features of the BSA, is seen from the sample upon the BSA immobilization. A similar FTIR spectrum was obtained in a previous work who used naked iron oxide for the BSA immobilization [5,6]. It is particularly noted that the high peak near 1648 cm^{-1} corresponds to the Amide I of the BSA [7]. This peak has been regarded as a good indicator whether the immobilized BSA still remains functional as compared to the native free form. If the immobilized BSA is somehow denatured, its secondary structure will be altered leading to a significant shift in this Amide I region [8]. Therefore, the FTIR analysis clearly suggests that the BSA is successfully immobilized onto the silica encapsulated nanoparticle probably via the hydroxyl linkage and the attached protein likely remains functional (no peak shift).

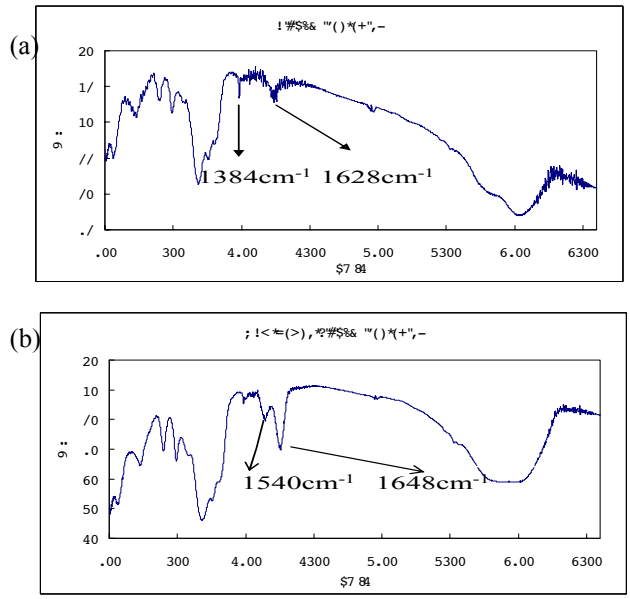

Figure 5 FT-IR of (a) silica encapsulated iron oxide and (b) BSA-bound silica encapsulated iron oxide

3 CONCLUSION

To summarize, we describe a one-step chemical preparative procedure for the synthesis of silica encapsulated iron oxide nanoparticles in solution using microemulsion method. Characterization suggests the material matches with the magnetite or maghemite phases in silica and the sample shows superparamagnetic properties. The material is also shown to cover with hydroxyl groups which assist for the immobilization of BSA thereupon. It is known that BSA is a protein of 66,000 Dalton molecular weight and with approximately 4 × 14 nm diameter. Each albumin molecule has at least 6 distinct binding sites for drugs and endogenous compounds. As a result, this new magnetic immobilized protein may find applications in magnetic drug delivery/administration. Nevertheless, the important point is that the reported technique may be employed for the tagging of a wide variety of biological entities for biomedical or biochemical applications useful for magnetic switch, recording storage and magnetic bio-separations applications.

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