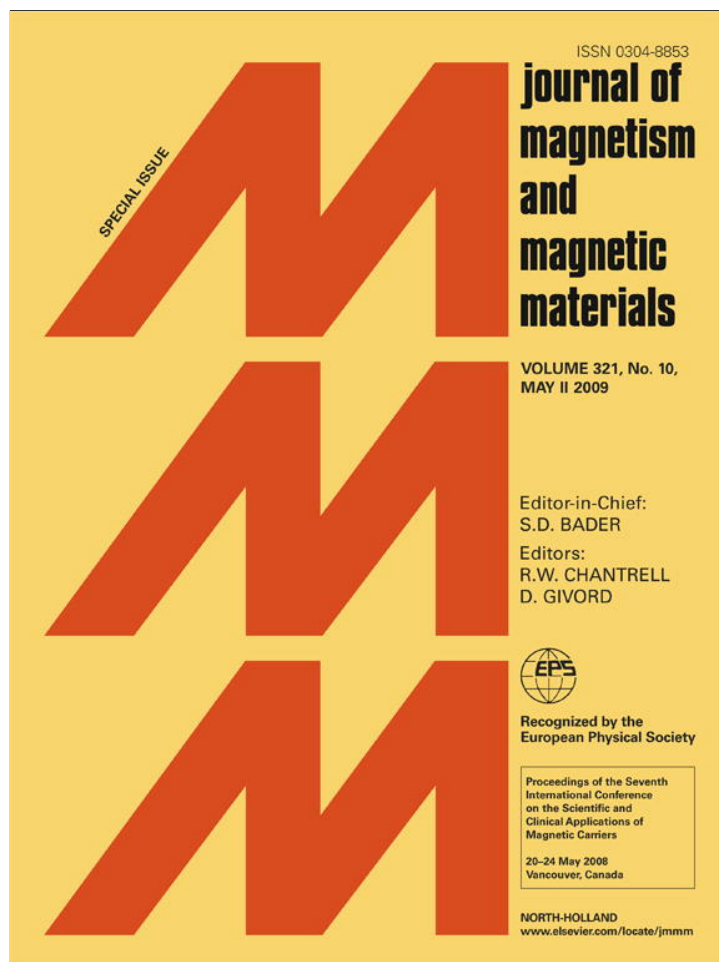


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Synthesis and characterization of multifunctional silica core–shell nanocomposites with magnetic and fluorescent functionalities

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ABSTRACT

Multifunctional core–shell nanocomposites with a magnetic core and a silica shell doped with lanthanide chelate have been prepared by a simple method. First, citric acid-modified magnetite nanoparticles were synthesized by a chemical coprecipitation method. Then the magnetite nanoparticles were coated with silica shells doped with terbium (Tb^{3+}) complex by a modified Stöber method based on hydrolyzing and condensation of tetraethyl orthosilicate (TEOS) and a silane precursor. These multifunctional nanocomposites are potentially useful in a variety of biological areas such as bio-imaging, bio-labeling and bioassays because they can be simultaneously manipulated with an external magnetic field and exhibit unique phosphorescence properties.

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The design and fabrication of multifunctional nanostructures combined with magnetic and fluorescent properties has recently been attracting increased interest for biological and biomedical applications. Magnetic nanoparticles have been widely studied in the field of bioseparation, drug targeting, biosensing, magnetic resonance imaging (MRI), diagnosis, etc. [1–5]. For the fluorescent components, organic fluorophore and quantum dots (QDs) are mostly used [6–8]. Although they have been proven to be quite useful but are still far from perfect because both of them have some inherent limitations. For example, organic fluorophore is prone to photobleaching and has a broad emission and small Stokes shift resulting in cross-talk between excitation and emission signals [9]. QDs are less chemically stable and potentially toxic and may show fluorescence intermittence [10]. Moreover, their inherent short-lived luminescence lifetimes may overlap with the spontaneous background emission sources.

In comparison with organic dyes and QDs, lanthanide ions related compounds, for example, lanthanide chelates, have some unique luminescence properties, such as sharp absorption and emission lines, long lifetimes, superior photostability and effective elimination of short-lived scattering light and background noises [11,12]. Thus they are very favorable for use in bioassays and bio-labeling. They have been used in the time-resolved immunofluorometric assay (TR-FIA), DNA hybridization assay

and fluorescence imaging microscopy. However, the fluorescence intensity of lanthanide chelates is weaker compared with the organic fluorescence dyes since the fluorescence quantum yields and molar extinction coefficients of the chelates are generally smaller than those of the organic fluorescence dyes. Recently, nanoparticles (such as polystyrene and silica) containing thousands of lanthanide chelates have been developed for improving fluorescence intensity and photostability [13,14]. The idea of combining the magnetic nanoparticles with lanthanide ions would lead to a special functionalized magnetic fluorescent nanocomposite that enjoys both the advantages of magnetic nanoparticles and lanthanide ions and offers higher potential applications in biology and biomedicine.

In this study, we developed a facile process to synthesize multifunctional magnetic fluorescent nanocomposites consisting of an iron oxide core and a silica shell doped with terbium (Tb^{3+}) chelate. The synthesis process consisted of preparation of citric acid-modified Fe_3O_4 nanoparticles by a coprecipitation method, coating the Fe_3O_4 nanoparticles with a SiO_2 shell formed by the copolymerization of tetraethyl orthosilicate (TEOS) and a silane precursor containing an organic chromophore (*p*-aminobenzoic acid, PABA) and a chelate (diethylenetriaminepentaacetic acid, DTPA), chelating the Tb^{3+} ions with the resulting nanoparticles to obtain the magnetic fluorescent nanocomposites with core–shell structure. The multifunctional nanocomposites take advantages of strong magnetic responsiveness to external magnetic field, high fluorescence intensity due to thousands of lanthanide chelates encapsulation in one nanoparticle and the high stability without

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any leakage due to the covalently linking of lanthanide chelates to the silica matrix. Moreover, the silica coating endows the nanocomposites biocompatibility, high hydrophilicity, and easiness to modify to conjugate biomolecules. These multifunctional nanoparticles are potentially useful in a variety of biological areas such as bio-imaging, bio-labeling and bioassay because they can be simultaneously manipulated with an external magnetic field and exhibit unique fluorescence properties.

1. Experimental

1.1. Materials

Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), ferrous chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), citric acid (CA), dianhydride of diethylenetriaminepentaacetic acid, 3-aminopropyltriethoxysilane (APTS), tetraethyl orthosilicate, *p*-aminobenzoic acid, ammonium hydroxide (28–30 wt%), terbium chloride (TbCl_3) and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich. All water utilized in the experiments was Milli-Q (Millipore) deionized water.

1.2. Synthesis of citric acid-modified magnetite nanoparticles

Citric acid-modified magnetite (Fe_3O_4) nanoparticles were prepared by a chemical coprecipitation method as described by Prasad et al. with modifications [15]. A complete precipitation of Fe_3O_4 was achieved under alkaline conditions, while maintaining a molar ratio of $\text{Fe}^{2+}:\text{Fe}^{3+} = 1:2$. Typically, to obtain 1 g of Fe_3O_4 nanoparticles, 0.86 g of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and 2.36 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were dissolved under a N_2 atmosphere in 40 mL of deaerated deionized water with vigorous stirring and the solution was heated to 80 °C. 5 mL of ammonium hydroxide was added. After 30 min, 2 g of citric acid in 5 mL H_2O was added dropwise. The mixture was stirred continually for another 90 min. The particles remained dispersed in the fluid even in the presence of an external magnetic field indicating that a stable magnetic fluid had formed.

1.3. Synthesis of PABA–DTPA–APTS (PDA) silane precursor

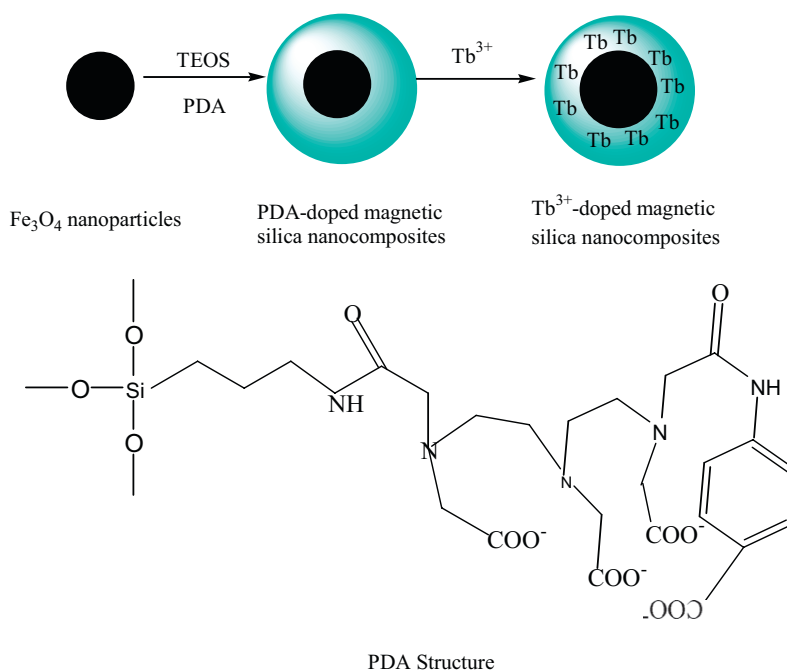
The PABA–DTPA–APTS (PDA) silane precursor was prepared as follows. DTPA 0.5 g was dissolved in 5 mL of DMSO solution containing 0.19 g of PABA and the mixture was stirred for 8 h at room temperature until the solution became clear. APTS 0.33 mL was then added and the resulting mixture was stirred overnight.

1.4. Synthesis of multifunctional magnetic silica nanocomposites

The multifunctional magnetic silica nanocomposites were prepared with a modified Stöber method as described before with modifications [16]. 10 mg of Fe_3O_4 nanoparticles were dispersed in a mixture of 20 mL of ethanol, 5 mL of deionized water and 0.5 mL of ammonium hydroxide (28–30 wt%). Finally, 0.2 mL of TEOS was added to the above solution under mechanically stirring. After 2 h, 0.05 mL of PDA silane was added and the reaction proceeded for 12 h. The resulting multifunctional nanocomposites were separated magnetically, washed with ethanol and water several times. The purified nanocomposites were redispersed in 20 mL of 0.02 M TbCl_3 aqueous solution and stirred overnight to obtain Tb^{3+} chelated nanocomposites.

1.5. Characterization

The morphologies and sizes of the as-prepared Fe_3O_4 and magnetic silica nanocomposites were characterized by transmission electron microscopy (TEM, Philips CM-12). Samples were prepared by depositing the particles solution onto a carbon-coated copper grid and allowed to dry at room temperature. The magnetic properties of the magnetic silica nanocomposites were characterized by vibrating sample magnetometer at room temperature. Fluorescence spectra were measured using a Spectramax M2 cuvette/microplate reader (Molecular Devices, Sunnyvale, CA, USA).



Scheme 1. Synthetic scheme for the multifunctional magnetic silica nanocomposites and the chemical structure of PDA silane.

2. Results and discussion

The synthetic procedure for multifunctional magnetic silica nanocomposites and chemical structure of PDA are illustrated in Scheme 1. CA-modified Fe_3O_4 nanoparticles were first synthesized with the widely used coprecipitation method. The CA may be adsorbed on the surface of the magnetite nanoparticles by coordinating via one or two of the carboxylate groups, thus leaving at least one carboxylic acid group exposed to the solvent [15]. Due to the surface charge and hydrophilicity, the CA-modified Fe_3O_4 nanoparticles can form a stable magnetic aqueous fluid.

Fig. 1 shows the TEM image of Fe_3O_4 nanoparticles. The nanoparticles had an average size of 7 nm. Then, magnetic silica nanocomposites were prepared by copolymerization of TEOS and PDA silane precursor in the presence of CA-modified Fe_3O_4 nanoparticles. The method was simple, and the thickness of the silica shell can be readily tuned by control of the amount of TEOS used and the reaction time. The TEM image shown in Fig. 2 indicates that magnetic silica nanocomposites were rather monodisperse and spherical in shape and had an average size of 52 nm. The PDA silane used as a lanthanide complex (shown in Scheme 1) contains three parts: PABA, DAPA and APTS. PABA and APTS were covalently coupled with DAPA by its dianhydride. PABA

acts as an organic chromophore; DAPA chelate serves as a scaffold for binding lanthanide ions in close proximity to the organic chromophore (PABA), which captures the excitation light and sensitizes the lanthanide ion luminescent by fluorescence energy transfer; APTS segment allows the lanthanide complexes to covalently link to the silica matrix via co-hydrolysis and condensation with TEOS, thus the fluorophore leaking problem in bio-labeling and bioassay processes using the luminophore-doped silica nanoparticle can be effectively resolved. Silica coating on the surfaces of Fe_3O_4 nanoparticles could help prevent their aggregation in water and improve their chemical stability. Also silica is nontoxic, biocompatible and the surface can be easily functionalized with various functional groups that can be used for bioconjugation.

Magnetization curve of magnetic silica nanocomposites was shown in Fig. 3. The nanocomposites exhibit superparamagnetic properties, that is, no remanence once the applied magnetic field is removed. The superparamagnetic properties of the magnetic nanoparticles are critical for their application in biomedicine which prevents aggregation and enables them to redisperse rapidly when the magnetic field is removed. The magnetic saturation (M_s) value of magnetic silica nanocomposites was found to be 4.4 emu g^{-1} . Due to the superparamagnetic property and the screening effect of the silica layer, the magnetic silica nanocomposites could be readily and stably dispersed in water and remained in suspension in the absence of external magnetic

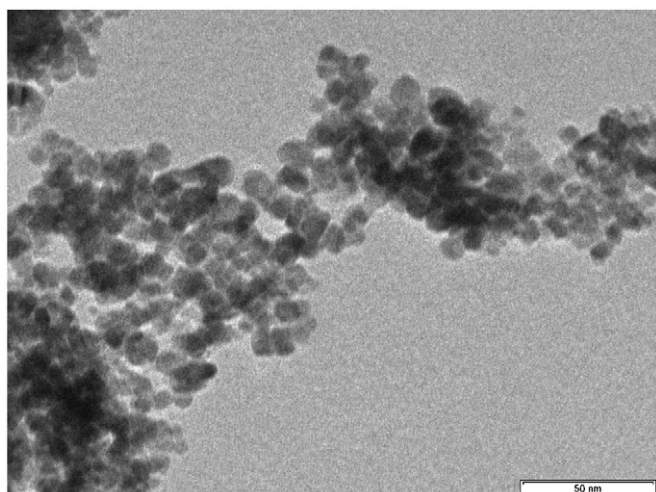


Fig. 1. TEM image of CA-modified Fe_3O_4 nanoparticles.

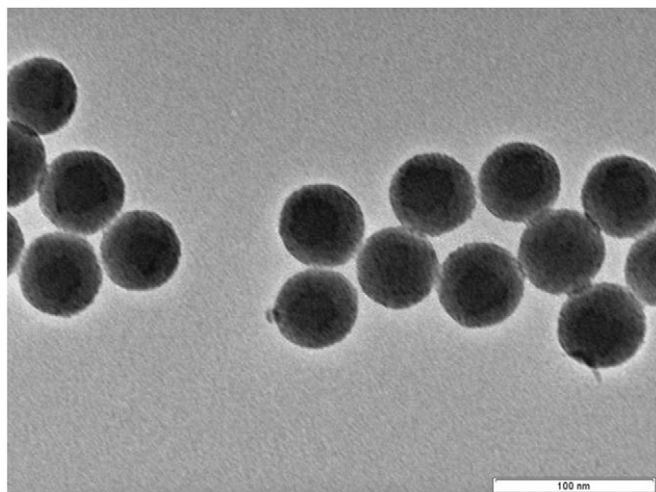


Fig. 2. TEM image of multifunctional magnetic silica nanocomposites.

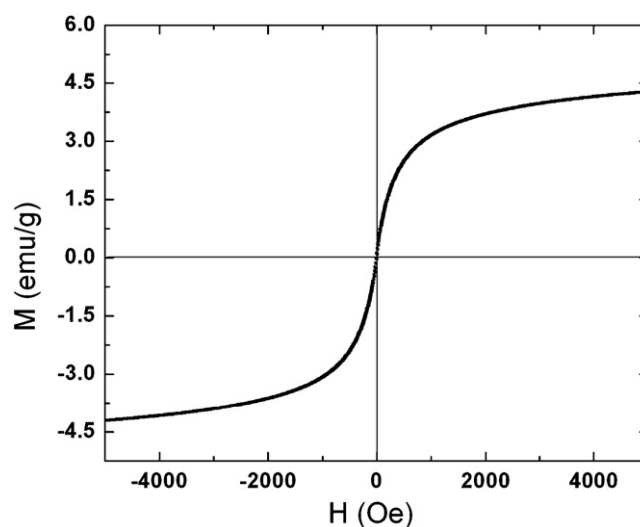


Fig. 3. Magnetization curve of multifunctional magnetic silica nanocomposites at room temperature.

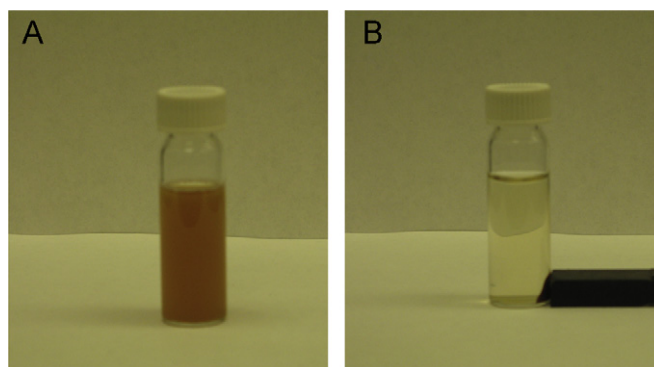


Fig. 4. Pictures of the multifunctional magnetic silica nanocomposites dispersed in aqueous solution (A) without and (B) with an external magnetic field.

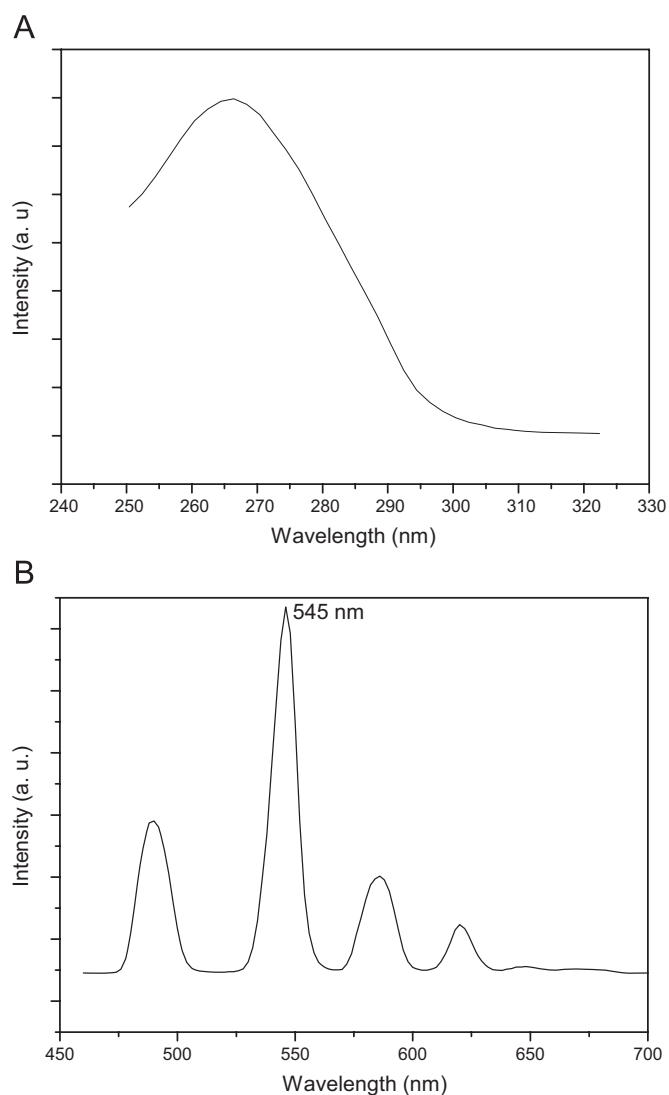


Fig. 5. Excitation (emission at 544 nm) (A) and emission (excitation at 265 nm) (B) spectra of Tb^{3+} -doped multifunctional magnetic silica nanocomposites.

field (Fig. 4A). The magnetic silica nanocomposites could also be completely separated from the solution within minutes when subjected to a strong magnetic field and redisperse very well with a slight agitation when the magnetic field is removed as shown in Fig. 4B. This suggests that these magnetic silica nanocomposites have high dispersibility and high sensitivity to the magnetic field, which are two important factors for bio-applications.

Fig. 5A shows the excitation spectra of magnetic silica nanocomposites obtained by monitoring the emission at 544 nm. A maximum absorption peak appears at 265 nm, the characteristic absorption of PABA arising from the efficient energy

transition based on the conjugated double bonds of the aromatic ligand. The excitation spectrum of magnetic silica nanocomposites shows clearly that Tb^{3+} can be efficiently sensitized by the aromatic ligand, which is called the “antenna effect”, a light-conversion process via an absorption energy transfer–emission sequence involving distinct absorption by the ligands (here aminobenzoic acid segment) and emission by Tb^{3+} . When the ligands (aminobenzoic acid segment) were excited from ground state to excited state at 265 nm, the energy transfer from excited state of the ligands to 5D_4 level of Tb^{3+} will occur, the spiked emission peaks at 489, 544, 588 and 620 nm were due to the energy transfer from the 5D_4 level to the 7F_J ($J = 6, 5, 4, 3$) levels, of which the 540 nm (5D_4 to 7F_5) emission is the most prominent one [14] (Fig. 5B).

3. Conclusion

Multifunctional silica nanocomposites with both magnetic and unique lanthanide fluorescent properties have been successfully prepared with a modified Stöber method. The magnetic fluorescent nanocomposites can be easily separated using an external magnetic field with an easily functionalized silica surface and unique fluorescent properties of Tb^{3+} chelate. They have great potential applications in bio-imaging, bioassays and bio-labeling.

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