Synthesis of the $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{SiO}_2-\text{Tb(PABA)}_3$
Luminomagnetic Microspheres

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In this paper, we describe the synthesis and characterization of a luminomagnetic microspheres with core–shell structures (denoted as $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{SiO}_2-\text{Tb(PABA)}_3$). The luminomagnetic microspheres were characterized by field emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR), vibrating sample magnetometer (VSM), and photoluminescence spectrophotometer (PL). The SEM observation shows that the microsphere consists of the magnetic core with about 400 nm in average diameter and silica shell doped with terbium complex with an average thickness of about 90 nm. It has a saturation magnetization of 15.8 emu/g and a negligible coercivity at room temperature and exhibits strong green emission peak from $^5\text{D}_4 \rightarrow ^7\text{F}_5$ transition of $\text{Tb}^{3+}$ ions. The luminomagnetic microspheres with good magnetic response and fluorescence probe property as well as water-dispersibility would have potential medical applications, such as time-resolved fluoroimmunoassay (TR-FIA), fluorescent imaging, and magnetic resonance imaging (MRI).

Keywords: Fluorescence, Fluorescent Detection, Fluorescent Imaging, MRI, Tb Complex, $\text{Fe}_3\text{O}_4$.

1. INTRODUCTION

Fluorescent nanoparticles and magnetic contrast agents have been widely used in biological detection and imaging.$^1,^2$ For example, magnetic iron oxide nanoparticles are used as contrast agents for magnetic resonance imaging (MRI),$^3$ while lanthanide complex probes are used as fluorescent marker for time-resolved fluoroimmunoassay (TR-FIA)$^3,^4$ and fluorescence imaging.$^5$ Thus, the combination of magnetic and fluorescent properties in one multifunctional composite has recently attracted particular attention due to their broad range of potential applications.$^6,^7$ For example, Wu et al.$^8$ reported preparation of the multifunctional nanoparticles possessing magnetic, long-lived fluorescence and bio-affinity properties. The time-resolved fluorescence imaging results of Hela cells with the nanoparticle-labeled transferrin demonstrated that the background noises caused by cells and matrix can be eliminated. Yu et al.$^9$ synthesized the core–shell structured $\text{Fe}_3\text{O}_4@\text{SiO}_2$ doped with Tb-complex bi-functional nanocomposites with green fluorescence and magnetic behavior. Their morphology, fluorescence and magnetic properties were characterized. Ma et al.$^{10}$ prepared multifunctional magnetic fluorescent nanocomposites composed of anionoxide core and a silica shell doped with terbium ($\text{Tb}^{3+}$) chelate. These multifunctional nanocomposites are thought to have potential use in a variety of biological are as such as bio-imaging, bio-labeling and bioassays. In these processes, (3-aminopropyl)triethoxysilane (APTES) has been demonstrated to play an important role in lanthanide fluorescent complex probes incorporated into the silica shell. However, these products all suffered from relatively weak magnetic response (e.g., 2–8 emu/g of saturation magnetization value) due to the small magnetic nanoparticles with poor crystallinity as magnetic cores and the non-magnetic materials (such as fluorescent labeling and biocompatible shell) coated on the magnetic cores. Most recently, we reported the synthesis of the $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{Eu(DBM)}_3\cdot2\text{H}_2\text{O}/\text{SiO}_2$ luminomagnetic microsphere and improved the magnetic response signal by using the hydrophilic $\text{Fe}_3\text{O}_4$ submicrospheres with high magnetic saturation magnetization and negligible coercivity and remanence as magnetic cores.$^{11}$

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Scheme 1. Synthetic scheme for the Fe\textsubscript{3}O\textsubscript{4}@SiO\textsubscript{2}@SiO\textsubscript{2}–Tb(PABA)\textsubscript{3} luminomagnetic microsphere.

In this paper, a novel lumino magnetic microspheres with core–shell structure (denoted as Fe\textsubscript{3}O\textsubscript{4}@SiO\textsubscript{2}@SiO\textsubscript{2}–Tb(PABA)\textsubscript{3}) (note: \(p\)-aminobenzoic acid, PABA) was fabricated using a modified Stöber method, as illustrated in Scheme 1. The first step involved preparation of Fe\textsubscript{3}O\textsubscript{4} submicrospheres and Fe\textsubscript{3}O\textsubscript{4}@SiO\textsubscript{2} microspheres\textsuperscript{10}. The next step was the synthesis of the \([\text{Tb(PABA)}\textsubscript{3}–\text{Si}]\) conjugated compound through the coupled reaction between –N\textsubscript{C}\textsubscript{S} groups from 3-(triethoxysilyl)propyl isocyanate (TPI) and –NH\textsubscript{2} groups of the PABA from the \([\text{Tb(PABA)}\textsubscript{3}]\) complex. In the last step, the silica doped with Tb(PABA)\textsubscript{3}–Si were coated on the surface of the Fe\textsubscript{3}O\textsubscript{4}@SiO\textsubscript{2} microspheres to form a SiO\textsubscript{2}–Tb(PABA)\textsubscript{3} shell.

2. EXPERIMENTAL DETAILS

2.1. Materials

Terbium oxide (99.99\%) was purchased from Shanghai Yuelong Chemical Factory, China. Tetraethoxysilane (TEOS), 3-(triethoxysilyl)propyl isocyanate (TPI), and \(p\)-aminobenzoic acid (PABA) was obtained from Shanghai Jingchun Chemical Reagent, China. Other reagents were supplied by the Beijing Chemical Factory, China. All chemicals were analytical grade and used as received without further purification. Deionized water was used throughout.

2.2. Synthesis

2.2.1. Preparation of Fe\textsubscript{3}O\textsubscript{4} Particles and Fe\textsubscript{3}O\textsubscript{4}@SiO\textsubscript{2} Microspheres

The magnetic Fe\textsubscript{3}O\textsubscript{4} particles and Fe\textsubscript{3}O\textsubscript{4}@SiO\textsubscript{2} microspheres were prepared according to the methods reported previously\textsuperscript{10}.

2.2.2. Synthesis of [Tb(PABA)\textsubscript{3}]Cl\textsubscript{3} Complex

200 mL of 0.20 mol/L\textsubscript{TbCl\textsubscript{3}} stock solution with pH 4–6 were prepared as follows: 10 mmol of Tb\textsubscript{2}O\textsubscript{3} was adjusted to mash with proper volume of deionized water and 1:1 (v/v) HCl was then added into the system. Tb\textsubscript{2}O\textsubscript{3} mash was dissolved into solution under heating and magnetic stirring. Then, the excess hydrochloric acid and water in the solution were gradually removed by evaporation, finally forming a very ropy solution. After cooled down to ca. 50 °C, 150 mL of absolute ethanol was added into this system under stirring and then it was transferred into a 200 mL of volumetric flask to fix capacity with absolute ethanol.

The \([\text{Tb(PABA)}\textsubscript{3}]\)Cl\textsubscript{3} complex was synthesized as follows: 4 mmol of \(p\)-aminobenzoic acid (PABA) was first dissolved in 60 mL of absolute ethanol to form a solution, which was then adjusted to pH 5–6 with concentrated NH\textsubscript{4}·H\textsubscript{2}O solution and heated to 75 °C. Subsequently, 5 mL of 0.20 mol/L\textsubscript{TbCl\textsubscript{3}} stock solution was added drop wise into the solution under magnetic stirring. Next, the solution was adjusted to pH 7 with concentrated NH\textsubscript{4}·H\textsubscript{2}O solution. After refluxing for 2 h, the reaction was terminated by stopping heating. There was white precipitation formed after placement of more than two hours. The obtained white precipitation was then filtered, washed with absolute ethanol twice, and dried for 2 h at 80 °C.

2.2.3. Synthesis of the [Tb(PABA)\textsubscript{3}–Si Conjugate

0.18 g \([\text{Tb(PABA)}\textsubscript{3}]\)Cl\textsubscript{3} complex was dissolved in 15 mL of N,N-dimethylformamide (DMF) to form a transparent solution under stirring. Then, 0.5 mL of 3-(triethoxysilyl)propyl isocyanate (TPI) was added drop wise into the solution with stirring and refluxed for 8 hours. After cooling, the resulting solution (denoted as Tb(PABA)\textsubscript{3}–Si solution) was used for the following synthesis.

2.2.4. Synthesis of Fe\textsubscript{3}O\textsubscript{4}@SiO\textsubscript{2}@SiO\textsubscript{2}–Tb(PABA)\textsubscript{3} Luminomagnetic Microspheres

2.0 ml of the Tb(PABA)\textsubscript{3}–Si solution were dissolved in a mixing solvent containing absolute ethanol (100 ml) and deionized water (50 mL). 0.030 g of the Fe\textsubscript{3}O\textsubscript{4}@SiO\textsubscript{2} particles was then added into the solution. After sonication treatment for 20 min, the system was dispersed to form a uniform brown-black suspension. Subsequently, 2.0 ml of concentrated NH\textsubscript{4}·H\textsubscript{2}O solution was added dropwise into the solution, which was then under magnetic stirring for two hours. Next, another 3.0 ml of concentrated NH\textsubscript{4}·H\textsubscript{2}O solution was added into the solution again and stirred for 3 h. The resulting microspheres were magnetically separated using an NdFeB permanent magnet, washed with ethanol or water three times, and then dried at 80 °C in air.

2.3. Characterization

Morphological images of samples were obtained by a HITACHI S4800 field emissions cannon electron microscope.
magnetic property of the sample was measured by a Lakeshore 7300 vibrating sample magnetometer. The excitation and emission spectra were recorded on a HITACHI F-7000 FL spectrophotometer equipped with a 150 W xenon lamp as the excitation source. The conditions of scan speed (1200 nm/min), slits (excitation slit, 5 nm; emission slit, 5 nm) and filters (excitation filter, void; emission filter, 430 nm) were used for the measurements. All the measurements were performed at the room temperature (RT).

3. RESULTS AND DISCUSSION

3.1. Morphologies of the \( \text{Fe}_3\text{O}_4@\text{SiO}_2@\text{SiO}_2-\text{Tb(PABA)}_3 \) Microsphere

Figure 1 shows SEM images of \( \text{Fe}_3\text{O}_4 \) magnetic particles, \( \text{Fe}_3\text{O}_4@\text{SiO}_2 \) composite microspheres and \( \text{Fe}_3\text{O}_4@\text{SiO}_2@\text{SiO}_2-\text{Tb(PABA)}_3 \) luminomagnetic microspheres. The \( \text{Fe}_3\text{O}_4 \) particulates in Figures 1(a and b) are uniform spherical particles with sizes in the range of 370–450 nm. The \( \text{Fe}_3\text{O}_4 \) submicrometer particle is composed of many reunited nanoparticles and thus results in its rough surface. Nevertheless, the \( \text{Fe}_3\text{O}_4 \) microsphere with the relative coarse surface is in favor of silica coating on its surface. Most importantly, it possesses magnetism with negligible coercivity and remanence because...
sizes of the magnetic nanoparticle consisting of the Fe₃O₄ microsphere are close to the critical size of super paramagnetic Fe₃O₄ nanocrystal. The Fe₃O₄@SiO₂ composite particles in Figures 1(c and d) are still uniform and spherical with an average diameter of ca. 480 nm and they have smoother surface than that of the bare Fe₃O₄ cores. Obviously, the Fe₃O₄ microspheres have been coated by silica and thus form core–shell structural microspheres. The smooth Fe₃O₄@SiO₂ composite microspheres have a magnetic core with the average diameter of about 400 nm and silica shell with thickness of ca. 80 nm on average. We can easily adjust the thickness of the silica shell by simply varying the initial amount of TEOS. Compared with the precursor of the Fe₃O₄@SiO₂, the Fe₃O₄@SiO₂@SiO₂–Tb(PABA₃) microspheres with an average size ca. 490 nm in Figures 1(e and f) have a coarse surface, indicating that the Tb(PABA)₃–Si together with SiO₂ has been deposited on the surface of the Fe₃O₄@SiO₂ microspheres to form a core–shell structure. The TEM images of the Fe₃O₄@SiO₂@SiO₂–Tb(PABA₃) luminomagnetic microspheres in Figure 2 show clear core–shell structures as well. For Fe₃O₄@SiO₂ microspheres in Figures 2(a, b, and c), they are composed of the ca. 430 nm of the black magnetic cores and ca. 90 nm of gray silica shell. In cases of Fe₃O₄@SiO₂@SiO₂–Tb(PABA₃), microspheres shown in Figures 2(d, e, and f), the light gray silica shell doped with the Tb(PABA)₃–Si exhibits an increased surface area. The poor compatibility of hydrophobic organic ligands in the Tb(PABA)₃–Si fluorescent complex with hydrophilic silica surfaces could be responsible for the formation of coarse surfaces.

3.2. Surface Properties of the Fe₃O₄@SiO₂@SiO₂–Tb(PABA₃) Microsphere

Figure 3 gives FTIR spectra of the Fe₃O₄@SiO₂@SiO₂–Tb(PABA₃) microspheres. The absorption band at 3406 cm⁻¹ originates from stretching vibration of the N–H bonds. The absorption bands at 2980, 2927 and 2852 cm⁻¹ can be assigned to stretching vibrations of C–H bonds from –CH₂ and –CH₃ groups. The absorption bands at 1632 and 1558 cm⁻¹ belong to the stretching vibrations of the C=O bonds and C=C bonds from the PABA, respectively. The absorption bands at 1096 and 800 cm⁻¹ can be attributed to stretching vibration and bend vibration of the Si–O–Si, respectively. The absorption bands at 949 cm⁻¹ is due to stretching vibration of Si–OH. The absorption bands at 639 and 580 cm⁻¹, and 469 cm⁻¹ originate from the Fe–O bonds. These results indicate that the silica shell doped with Tb(PABA)₃–Si has been coated on the surface of the magnetite core. Such Fe₃O₄@SiO₂@SiO₂–Tb(PABA₃) microspheres can be well dispersed in an aqueous medium and further conjugated with bioactive molecules (e.g., drug, markers) by reactions of amino groups on the microsphere surfaces.

3.3. Magnetic Properties of the Fe₃O₄@SiO₂@SiO₂–Tb(PABA₃) Microsphere

Figure 4 presented the magnetic hysteresis loops of the Fe₃O₄, Fe₃O₄@SiO₂, and Fe₃O₄@SiO₂@SiO₂–Tb(PABA₃) in an applied field of 10 kOe at 300 K. It is clearly seen from these magnetic hysteresis loops that they have stronger magnetism with a negligible coercivity at room temperature. With increase of thickness of
the shell, their saturation magnetizations (i.e., 67.2, 21.5 and 15.8 emu/g) decrease in turn, since the nonmagnetic shell resulted in an increased proportion of low magnetic content. The smaller difference of the saturation magnetization between Fe₃O₄@SiO₂ and Fe₃O₄@SiO₂–Tb(PABA)₃ shows that the outer layer shell (SiO₂–Tb(PABA)₃) is thinner than the inner silica layer shell. This is in agreement with the observation of the SEM and TEM. Though the saturation magnetization of the Fe₃O₄@SiO₂–Tb(PABA)₃ is less than that of the bare magnetic core, it still possesses enough magnetic response signal for biomedical applications such as MRI and effectively magnetic separation.

3.4. Fluorescent Properties of the Fe₃O₄@SiO₂@SiO₂–Tb(PABA)₃ Microsphere

The excitation spectrum of the Fe₃O₄@SiO₂@SiO₂–Tb(PABA)₃ microspheres shown in Figure 5(a) was obtained using 545 nm green light as the monitored wavelength. It can be seen from the excitation spectrum that there are two broad excitation peaks at 272 nm which are due to light absorption by the organic ligands. The emission spectrum in Figure 5(b) was measured with 272 nm as the excitation wavelength. There are four narrow emission peaks located at 490, 545, 585, and 622 nm, which originate from transitions of the 5D₄ excited state level to the 7F₃ ground state levels, respectively. The green light at 545 nm is the strongest one, which is from the transition of 5D₄–7F₅. These results indicate that fluorescent Tb(PABA)₃ complex has been incorporated into the silica shell of the Fe₃O₄@SiO₂@SiO₂–Tb(PABA)₃ microspheres and there is potential application in bioimages.

4. CONCLUSIONS

We synthesized and characterized the luminomagnetic Fe₃O₄@SiO₂@SiO₂–Tb(PABA)₃ microspheres with core–shell structures using a simple method. The luminomagnetic Fe₃O₄@SiO₂@SiO₂–Tb(PABA)₃ microsphere improved the poor magnetic response and reduced
magnetic conglomeration through the monodispersive submicrometer Fe₃O₄ particulates with negligible coercivity and a larger saturation magnetization as magnetic cores. Meanwhile, it eliminates fluorescence quenching through the SiO₂ isolation layer between Fe₃O₄ particulates and the terbium fluorescent complex and increases photostability through protection of the silica shell. The luminomagnetic microspheres with good magnetic response and fluorescence probe property as well as water-dispersibility would have potential biomedical applications.

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References and Notes


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