

Magnetic nanocomposites of Fe₃O₄/SiO₂-FITC with pH-dependent fluorescence emission

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Abstract

This paper describes the formation of magnetic and fluorescent nanocomposite particles which consist of superparamagnetic Fe₃O₄ core, SiO₂ shell and organic dye (FITC) coating layer on their surface. The obtained nanocomposites possess typical superparamagnetism and exhibit clear green fluorescence image. And their fluorescence emission is pH-dependent, which would be applied to pH sensing.

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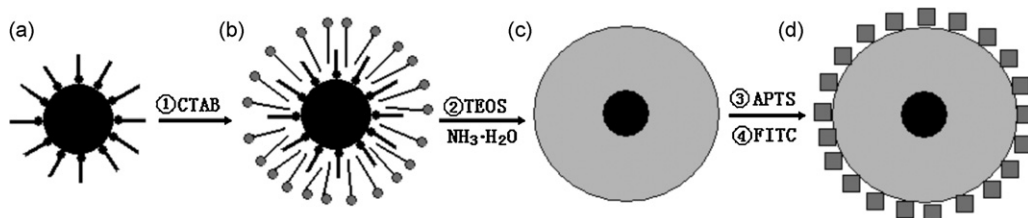
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Magnetic iron oxide nanoparticles are promising candidates for biomedical applications because of their strong magnetic properties and biocompatibility, as well as unique multifunctional properties [1–10]. Indeed, the same magnetic nanoparticles may serve as a carrier for drug delivery [1,2], as a label for bio-separation [3,4], as a sensitive component for biosensing [5,6], as a contrast agent for MRI (magnetic resonance imaging) [7,8], and also as a nanosource of heat for magnetically induced cancer hyperthermia [9,10]. On the other hand, by nanoscale complex technique one also may further increase some additional functions to the magnetic nanoparticles, such as fluorescence [11], bio-targeting [12] and so on. For example, MRI/optical dual-modal probes have been designed, involving attachment of organic dyes to iron oxide nanoparticles [13] or attachment of Ga chelates to quantum dots [14] or to fluorescently tagged polymers [15]. In this paper, magnetic and fluorescent nanocomposite particles, which consist of superparamagnetic Fe₃O₄ core, SiO₂ shell and FITC (fluorescein isothiocyanate) coating layer on their surface, were synthesized and characterized. Besides the magnetic and fluorescent properties, a pH-dependent behavior of fluorescence emission for the nanocomposites was also studied, which would be useful for pH sensing in cells.

Scheme 1 shows a typical synthetic procedure. Monodisperse Fe₃O₄ (magnetite) nanoparticles were used as magnetic cores, which were synthesized by thermal decomposition of iron-oleate, as previously reported [16]. The obtained Fe₃O₄ nanoparticles were capped by oleate with hydrophobic surfaces, as shown in **Scheme 1a**. For the formation of SiO₂ shell on Fe₃O₄ nanoparticles, a similar approach, as reported by Kim et al. [17], was used with a

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Scheme 1. The formation procedure of the magnetic and fluorescent nanocomposite particles. (a) Fe₃O₄ nanoparticles with hydrophobic surface, (b) Fe₃O₄ nanoparticles with hydrophilic surface, (c) Fe₃O₄/SiO₂ core-shell structured nanoparticles and (d) Fe₃O₄/SiO₂-FITC composite nanoparticles.

little modification. Firstly, CTAB (cetyltrimethylammonium bromide) was employed as a secondary surfactant to transfer the hydrophobic Fe₃O₄ nanoparticles from organic phase to aqueous phase, resulting in the formation of hydrophilic CTAB-stabilized Fe₃O₄ nanoparticles as seeds for further forming the SiO₂ shell by subsequent sol-gel reaction. In our experiment, 15 mg of oleate capped Fe₃O₄ nanoparticles dispersed in 1 mL of chloroform were added to a 10 mL of aqueous solution containing 0.2 g of CTAB. After sonication for 10 min, a homogeneous oil-in-water microemulsion was obtained. To eliminate chloroform, heating at 60 °C for 10 min was performed, leading to CTAB-stabilized Fe₃O₄ nanoparticles dispersed in water, as shown in Scheme 1b. Then 0.5 mL of the obtained aqueous solution was diluted with 10 mL of water. To this solution were added 0.3 mL ammonia (28% mass concentration), 0.05 mL TEOS (tetraethylorthosilicate), and 0.5 mL of ethyl acetate. The resulting mixture was stirred for 1 min, and then aged for 3 h. The magnetic separation was used to wash the obtained product (as shown in Scheme 1c) with water and then ethanol for three times, respectively.

In order to conjugate FITC onto the surface of Fe₃O₄/SiO₂ core-shell nanoparticles, an amine-terminated surface silanizing agent (3-aminopropyltrimethoxysilane, APTS) was used to functionalize the SiO₂ surface. For this, the washed product was dispersed in 2 mL of ethanol, and to this solution was added 0.01 mL of APTS. Subsequently, the mixture was shaken by shaker, and then aged for 24 h. After washing for three times with ethanol by magnetic separation, APTS-functionalized Fe₃O₄/SiO₂ core-shell nanoparticles were redispersed in 2 mL of ethanol, and to this solution was added 0.1 mL FITC solution (1 mg/mL in DMSO). After shaking for 4 h in dark, Fe₃O₄/SiO₂-FITC conjugates (as shown in Scheme 1d) were obtained by washing with ethanol and water and finally dispersed in water.

The morphologies of the samples were characterized by a transmission electron microscope (TEM, JEOL, JEM-2000EX) operated at 100 kV. Fig. 1a shows TEM image of the synthesized Fe₃O₄ nanoparticles capped with oleate. It

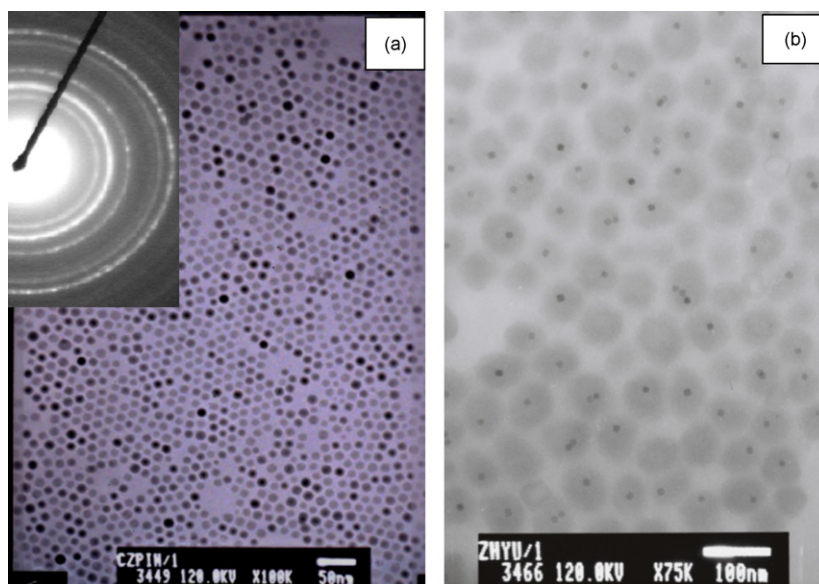


Fig. 1. TEM images of the oleate capped magnetite nanoparticles (inset shows their electron diffraction pattern) (a) and Fe₃O₄/SiO₂ core-shell nanoparticles (b).

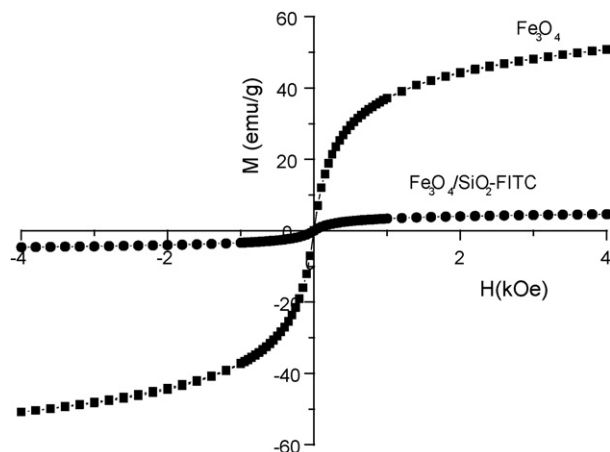


Fig. 2. Magnetic hysteresis curves of the oleate capped magnetite nanoparticles and $\text{Fe}_3\text{O}_4/\text{SiO}_2\text{-FITC}$ nanocomposites at room temperature.

can be seen that the Fe_3O_4 nanoparticles are monodisperse with an average diameter of 12 nm. The inset gives the electron diffraction pattern which is characteristic of cubic spinel structure of Fe_3O_4 . Magnetic measurement using VSM (vibrating sample magnetometer, Lakeshore 7407) demonstrated their superparamagnetic behavior at room temperature, with saturation magnetization of ca. 50 emu/g (Fig. 2). Fig. 1b shows TEM image of the obtained $\text{Fe}_3\text{O}_4/\text{SiO}_2$ nanoparticles, indicating spherical core-shell structure with an average overall size of 60 nm. Note that aggregation between Fe_3O_4 nanoparticles prior to or during the coating process sometimes led to the trapping of multiple nuclei in a single SiO_2 shell. As shown in Fig. 1b, few linear dimers or trimers can be seen in SiO_2 shell. For increasing the ratio of monomers, it may be favored to decrease Fe_3O_4 seed concentration and to ensure that seed particles are well dispersed by sonication before coating begins. To verify their magnetic properties, a 0.3 T external magnetic field was applied to inducing $\text{Fe}_3\text{O}_4/\text{SiO}_2$ nanoparticles to generate aggregation. As expected, linear aggregates were observed by optical microscope (ZEISS Axiovert 200), due to magnetic interaction between particles [18]. Fig. 2 also shows the result of VSM measurements for the obtained $\text{Fe}_3\text{O}_4/\text{SiO}_2\text{-FITC}$ nanocomposites. A reduced saturation magnetization of ca. 4.5 emu/g can be seen, attributed to the presence of nonmagnetic contents of silica and surface capping molecules [16].

The fluorescence properties were demonstrated by a fluorescence inverted microscope (ZEISS Axiovert 200). As shown in Fig. 3a, the obtained $\text{Fe}_3\text{O}_4/\text{SiO}_2\text{-FITC}$ nanocomposites exhibit clear green fluorescent image, which is sufficient for biological fluorescent label. Some large fluorescent aggregates can be observed due to the aggregation of nanocomposites induced by solvent evaporation in the process of sample preparation. Since the fluorescein dye is capped on SiO_2 shell surface away from the iron oxide core, its fluorescence is not quenched significantly by the iron oxide. In general, FITC is a pH-sensitive organic fluorescein dye. Like FITC, the $\text{Fe}_3\text{O}_4/\text{SiO}_2\text{-FITC}$ nanocomposites also exhibited pH-dependent fluorescence emission at 515 nm, measured by luminescence spectrometer (LS 55,

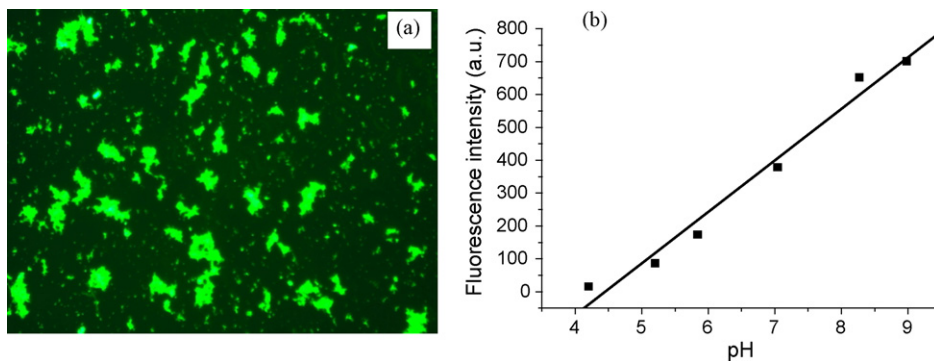


Fig. 3. Fluorescent microscopy image (200 \times), (a) and fluorescence intensity as a function of pH value for $\text{Fe}_3\text{O}_4/\text{SiO}_2\text{-FITC}$ nanocomposites (b).

PerkinElmer) with 490 nm exciting light at different pH values through adjusting their aqueous solutions with 1 mol/L of HCl and NaOH solutions. The fluorescence intensity is enhanced with increasing pH value of solution. Fig. 3b shows the linear relationship between the fluorescence intensity and pH value in a pH range of 5–9. It is significant to detect the pH variation in physiological range.

In conclusion, the multifunctional Fe₃O₄/SiO₂-FITC nanocomposites with magnetic, fluorescent and pH-sensitive properties have been synthesized and characterized. The nanocomposites may be applied to MRI/optical dual-modal label and detection, as well as pH sensing in cell, etc.

Acknowledgments

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