



Fluorescent and Magnetic Monodisperse Fe₃O₄ Nanoparticles

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Fe₃O₄ nanoparticles were prepared by a very promising bottom-up chemical strategy and covered by a biocompatible fluorescent silica coating by an improved microemulsion method. An accurate analysis of some key process parameters, to obtain a controlled nanoparticles covering, has been performed. The co-encapsulation of Fe₃O₄ nanoparticles and large amount of FITC dye molecules together, via silica shell (preventing the fluorescence quenching of the nanoprobe), and the grafting of primary amines (very active functional groups) into the silica surface, have been obtained. Moreover, the as-synthesized nanoprobe can be well-dispersed in aqueous solution.

1. Introduction

Multifunctional nanoprobe combining magnetic nanoparticles with organic dyes have attracted tremendous interest due to their promising applications in biomedical field. Among the nanoprobe, magnetic nanoparticles (Sarno et al., 2014a), such as core/shell Fe₃O₄/silica nanoparticles (Ni et al., 2010) loaded with dye molecules and covered by functionalities are very promising (Ma et al., 2009). It allows the development of biomedical platforms for simultaneous imaging, diagnosis and therapy. However, it is challenging to fabricate fluorescent magnetic nanoprobe with high photostability, high payloads of dyes and desirable outer surface for further modifications with functional or target moieties. Moreover, as far as we are aware, a systematic study of the effect of microemulsion parameters such as the nanoparticles, the SiO₂ precursor, and hydrolysis and condensation promoter amounts, have never been reported.

Here we demonstrate a facile and general strategy for the fabrication of robust fluorescent magnetic nanoprobe with payloads of dye molecules. These nanoprobe not only effectively keep photochemical stability of dyes, but also provide a platform for grafting other functional or targeted moieties into silica surface via primary amines. Moreover, the nanoprobe have uniformly spherical morphology and can be dispersed well in aqueous solution, which are very desirable for biomedical applications.

The nanoparticles were prepared by a very promising bottom-up chemical strategy (Altavilla et al., 2013, Sarno et al., 2014b) offering many advantages, such as experimental easiness and potential low-cost fabrication, through a low temperature thermal decomposition of an iron precursor in organic solvent and in the presence of surfactants. The nanoparticles were further covered by a fluorescent silica coating by adding a fluorescein isothiocyanate–3-aminopropyltriethoxysilane (FITC–APTES) conjugates in a microemulsion (Zhang et al., 2010). An accurate analysis of the key process parameters, to obtain a controlled nanoparticles covering, has been performed. We obtain the co-encapsulation of Fe₃O₄ nanoparticles and large amount of FITC dye molecules together via silica shell, and the grafting of primary amines into the silica surface. This synthetic process enables to prevent the fluorescence quenching of the nanoprobe, because FITC dye is not directly conjugated to Fe₃O₄ nanoparticle; the photochemical stability results protected by the “caging effects” of the silica shell; the primary amines are very active functional groups. Moreover, the as-synthesized nanoprobe can be well-dispersed in aqueous solution.

Transmission Electron Microscopy (TEM) was used for characterization. Particle size distribution of nanoparticles dispersion was determined using a dynamic light scattering (DLS) instrument (HPPS ET-Malvern Instruments).

2. Experimental

The synthesis was carried out using standard airless procedures and commercially available reagents. Absolute ethanol, hexane, and dichloromethane (99%) were used as received. Benzyl ether (99%), 1,2-hexadecanediol (97%), oleic acid (90%), oleylamine (>70%), iron(III) acetylacetonate were purchased from Aldrich Chemical Co (Altavilla et al., 2011).

20 mL of the solvent (benzyl ether), 2 mmol of the precursor ($\text{Fe}(\text{acac})_3$), 10 mmol of 1,2-hexadecanediol used for its high reducing ability, 6 mmol of oleic acid and 6 mmol of oleylamine used as surfactants, were mixed and magnetically stirred under a flow of nitrogen. The mixture was heated to 200°C for 120 min and then, under a blanket of nitrogen, heated to reflux (275°C) for another 60 min. The black-brown mixture was cooled to room temperature by removing the heat source. Under ambient conditions, ethanol was added to the mixture that was precipitated and separated via centrifugation. The black product was then dissolved in hexane.

Before the encapsulation of nanoparticles in silica, a fluorescein isothiocyanate (FITC)-(3-Aminopropyl)triethoxysilane (APTES) conjugate (10 μl of APTES and FITC in molar ratio 2:1 in 2 mL of anhydrous ethanol), through the addition reaction between isothiocyanate groups of FITC and the amine groups of APTES, was prepared. Reactions are carried out in dark for 4 h by slowly shaking. The reaction solution of FITC-APTES conjugate was then stored at 4°C and filtered (0.1 μm) before using.

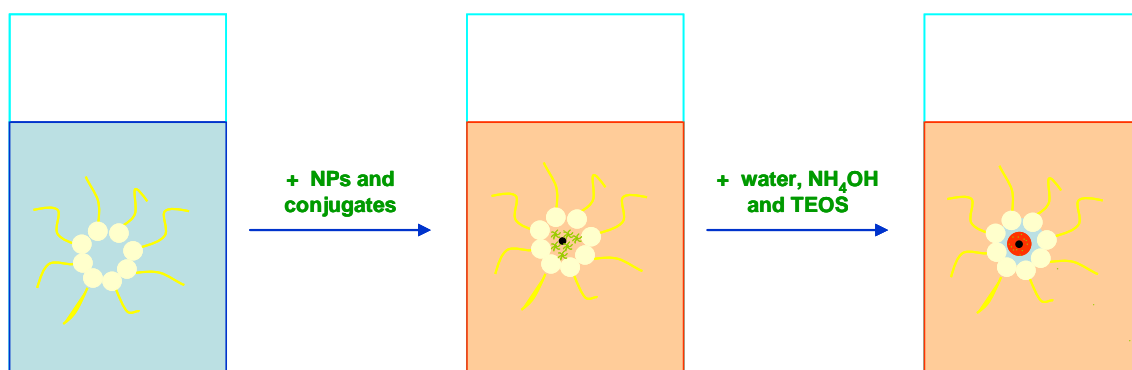


Figure 1: The procedure for encapsulating the as-synthesised Fe_3O_4 nanoparticles

$\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ nanoprobes are finally prepared by an improved microemulsion method. Briefly, Igepal CO-520 (0.46 ml) is dispersed into cyclohexane (9 ml) in a 12 ml glass tubes by stirring for 30 min (see Figure 1). Fe_3O_4 cyclohexane solution and FITC-APTES conjugate (20 μL) were added into the microemulsion and mechanically stirred. Then, TEOS and ammonia solution were added. The mixture was allowed to age for 24 h to hydrolysis and condensation of the silica precursor. To form a surface layer with - NH_2 groups, APES (200 mL) was added and the mixture stirred for 24 h.

Among the number of samples prepared, four of them were selected to give evidence of the effect of some key microemulsion preparation parameters. In particular, the nanoparticles, TEOS and NH_4OH solution amounts were varied, as reported in Table 1.

Table 1: Effect of the nanoparticles, TEOS and NH_4OH solution amounts

	Fe_3O_4 cyclohexane solution		TEOS	NH_4OH solution
	ml	mg/ml	μl	μl
Sample 1	2	1	20	60
Sample 2	0.1	1	20	60
Sample 3	0.1	1	60	80
Sample 4	0.15	1	100	80

The characterization was obtained by the combined use of different techniques. Transmission electron microscopy (TEM) images were acquired using a FEI Tecnai electron microscope operated at 200 KV with a LaB_6 filament as the source of electrons.

3. Results and discussion

Transmission electron microscopy (TEM) images of Fe_3O_4 nanoparticles and $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ nanoproboscopes were shown in Figure 2. Fe_3O_4 nanoparticles, prepared via high-temperature thermal decomposition method, were monodispersed with an average diameter of 5 nm and standard deviation of 0.95 nm (Altavilla et al., 2009). The TEM images are intentionally been made over large areas to give an idea of the high level of organization of the nanoparticles. It has been reported (Sun et al., 2004) that the success of making monodisperse nanoparticles is to heat the mixture to 200 °C first and remain at that temperature for some time before it is heated to reflux at 265 °C in phenyl ether (Fe_3O_4 nanoparticles size distribution centred at ~ 4 nm) or at 300°C in benzyl ether (Fe_3O_4 nanoparticles size distribution centred at ~ 6 nm). To obtain an intermediate particles size, not so large as the 6 nm nanoparticles and to ensure an higher magnetic performance than smaller nanoparticles, we prepare our ~5 nm Fe_3O_4 nanoparticles in benzyl ether solution through a two steps method reaching the highest temperature of 275°C (see the experimental section for details).

TEM investigations was carried out, to provide insights into the morphology, of the resulting $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ nanostructures, are reported in Figure 3. The sample (Sample 1) appears as an aggregate of SiO_2 , dyes and nanoparticles in Figure 3a, but decreasing the nanoparticles amount (Sample 2), results constituted of nanoparticles with a silica layer of about 4.5 nm thick (Figure 3b). In this case, the silica layer is not well-formed and does not adhere well to the nanoparticles. In the sample empty silica nanoparticles, are also visible. Increasing TEOS and NH_4OH solution amounts, a well-defined spherical core-shell nanoproboscopes with a shell of about 6.7 nm around the Fe_3O_4 nanoparticles, can be formed. On the other hand, a number of silica shells do not contain Fe_3O_4 nanoparticles. To overcome this problem we prepare a lot of samples at increasing nanoparticles amount. In particular, in Figure 3d a TEM image of Sample 4, obtained by loading 0.15 mL of nanoparticles solution, is reported.

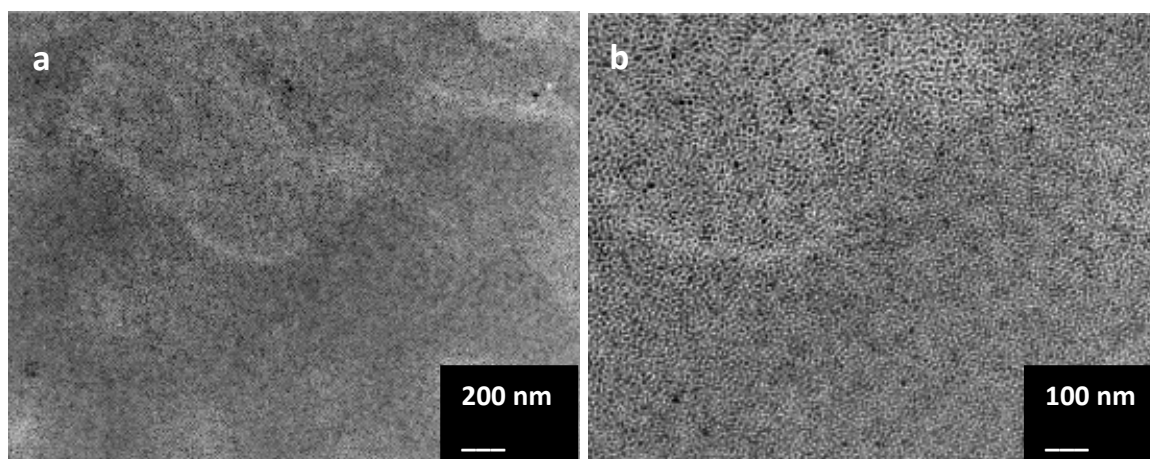


Figure 2: TEM images of Fe_3O_4 nanoparticles at increasing magnification

The thickness of the silica shell can be tuned by varying the volume of TEOS, obviously it increases with increasing the TEOS amount. A thicker layer of silica shell, which was determined to be 20 nm, was formed in situ through hydrolysis and subsequent condensation of silica onto the surface of Fe_3O_4 cores increasing the TEOS amount up to 100 μm . This is preferred where an high payloads of dye molecules to be achieved. It is worth to notice that in any case the number of particles made only by silica can removed operating a magnetic separation of the sample.

The hydrodynamic diameters of the nanoparticles in solution, as determined by DLS, are: in the range 3.8-5.7 nm centered at 5.32, for Fe_3O_4 ; and in the range 37-58 nm centered at 53.4 nm, for $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ nanoparticle (Figure 4).

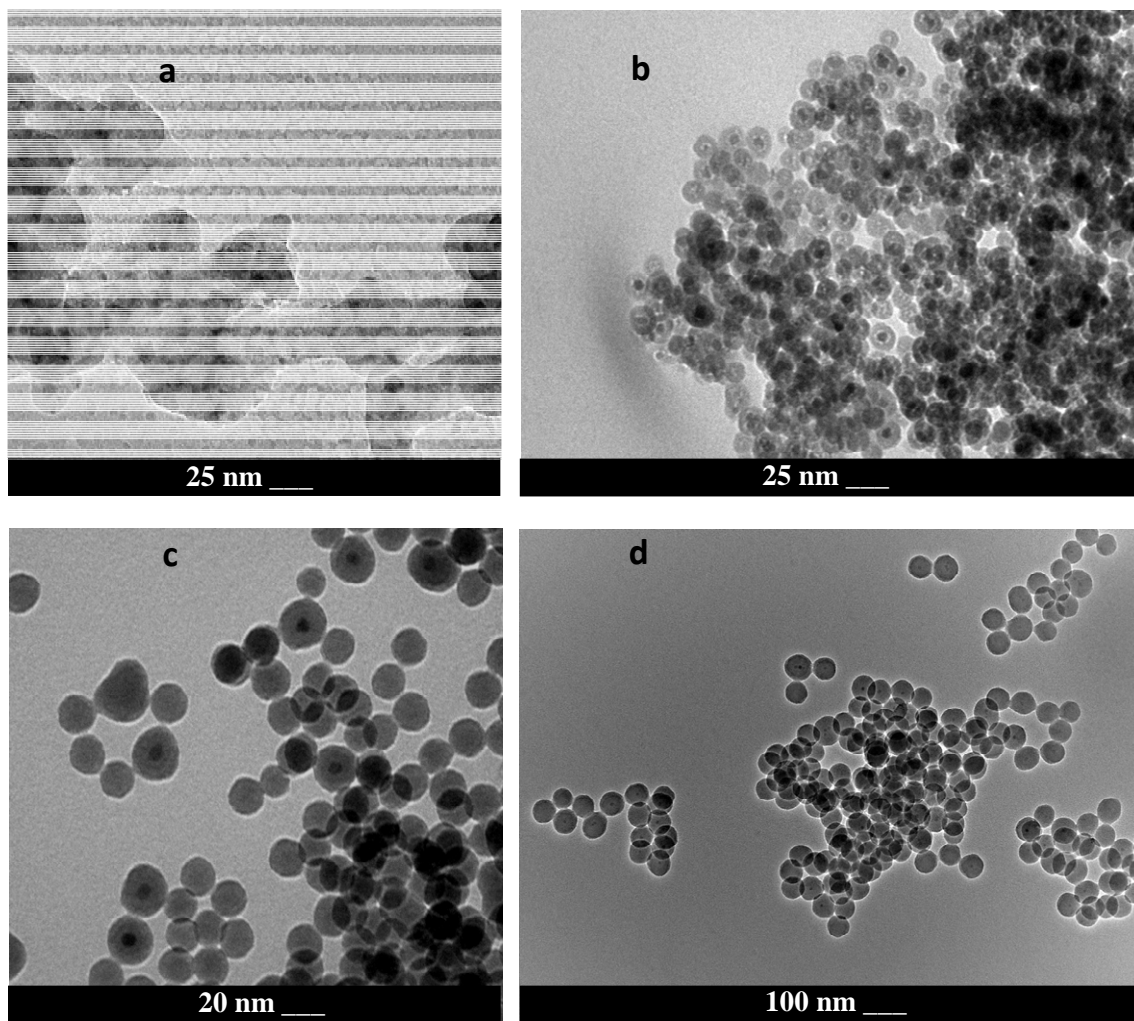


Figure 3: TEM images of $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ Sample 1 (a), Sample 2 (b), Sample 3 (c) and Sample 4 (d)

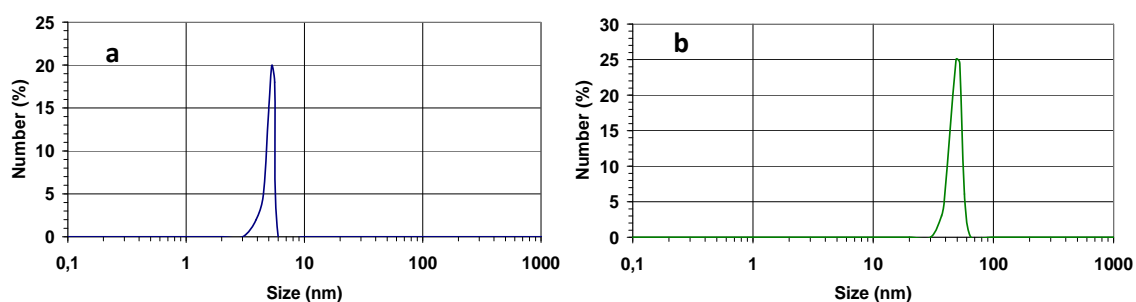


Figure 4: PSD of Fe_3O_4 (a) and $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ (Sample 4) (b) nanoparticles, as determined by DLS technique

In Figure 5 the excitation and emission spectra of $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ nanoprobe, are reported. In particular, the excitation and emission spectra $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ nanoprobe and pure FITC dye (not shown here) are similar, indicating that the silica shell did not alter the spectra characteristics of FITC dye. The nanoparticles results well-dispersed in aqueous solution even after 12 months (Figure 6a), thanks to the final surface layer incorporating primary amines, which was formed in situ through hydrolysis of APTES. The fluorescence photograph of $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ nanoprobe was shown in Figure 6d. The encapsulation

of FITC dye in $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ nanoprobe endowed the nanoprobe with excellent fluorescent property. We observed that the nanoprobe emitted green fluorescence under a UV lamp ($\lambda = 365 \text{ nm}$).

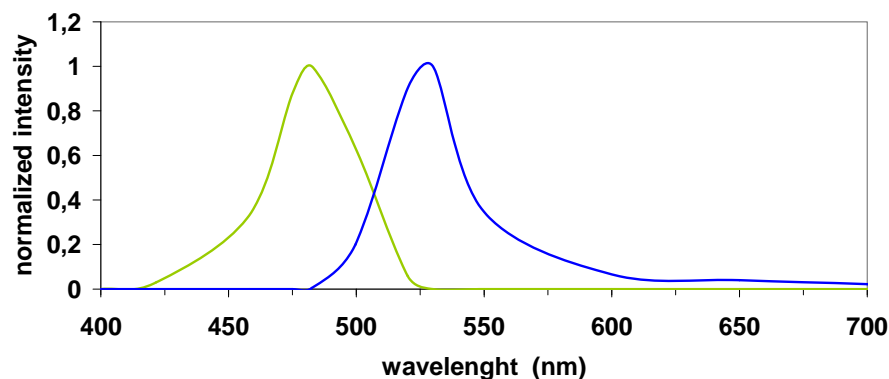


Figure 5: Excitation green line and emission blue line spectra of $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ nanoprobe (Sample 4)



Figure 6: Photograph showing the high stability in water of $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ (a). Magnetism of Fe_3O_4 nanoparticles (b) and of $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ (c) in response to a Gd magnet. Fluorescence photographs of $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ in water under a UV lamp ($\lambda=365 \text{ nm}$) (c). *(the Eppendorf in pink contains a water solution of $\text{Fe}_3\text{O}_4(\text{RBITC})@\text{SiO}_2\text{-NH}_2$, the fluorescent molecules are rhodamine-B-Isothiocyanate, RBITC)

4. Conclusions

Magnetic $\text{Fe}_3\text{O}_4(\text{FITC})@\text{SiO}_2\text{-NH}_2$ nanoparticles were prepared by a very promising bottom-up chemical strategy and covered by a biocompatible fluorescent silica coating by an improved microemulsion method. Some key process parameters has been tuned to obtain a controlled nanoparticles covering, well-defined spherical core-shell nanoprobe with a fluorescent shell were formed. The dyes in the nanoprobe because isolated from external environment via silica shell can keep their photochemical stability, while the primary amines of silica surface provide a platform to graft other functional and targeted moieties, the nanoprobe are highly water-soluble and can act as labelling materials due to their excellent magnetic and fluorescent properties.

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