Influence of Magnetic Fe3O4 Nanoparticles on Fluorescence Quenching of Dye Molecules

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Magneto-fluorescent nanocomposites possessing both fluorescent and magnetic properties represent an attractive platform for biomedicine and nanotechnology. One of the great challenges facing magneto-fluorescent nanocomposites is understanding the influence of magnetic component on the fluorescence quenching of dye molecules. However, there was still lack of in-depth and quantitative analysis of fluorescence decay for magneto-fluorescent nanocomposites. Herein, fluorescence intensity decay of acridine orange, a model fluorescent dye, was quantitatively determined with magnetic Fe3O4 nanoparticles in various concentrations ranging from 0 to 6250 ng · ml−1. It was found that when the concentration of Fe3O4 NPs was lower than 0.0625 ng · ml−1, the fluorescence intensity decreased sharply with increasing concentration of Fe3O4 NPs; when the NP concentrations ranged from 0.0625 ng · ml−1 to 1.25 ng · ml−1, the fluorescence intensity of acridine orange decreased linearly with increase of NP concentration; and when the concentration of Fe3O4 NPs exceeded 1.25 ng · ml−1, essentially no fluorescence can be detected. These findings suggested that magneto-fluorescent composites with optimized fluorescence properties could be obtained by carefully controlling Fe3O4 nanoparticle component, and offered a better understanding of interaction between magnetic NPs and luminescence of fluorescent molecules.

Keywords: Magnetic Nanoparticles, Fe3O4, Fluorescent Dye, Acridine Orange, Fluorescence Quenching.

1. INTRODUCTION
Multifunctional nanocomposites have been widely used in biomedical nanotechnology, diagnosis and therapy.1–3 In particular, magneto-fluorescent nanoparticles (NPs) allow for dual read-outs by optical (e.g., flow cytometry, immunofluorescence) and magnetic imaging (e.g., nuclear magnetic resonance, magetoresistive detection).4–6 The magneto-fluorescent NPs can be fabricated using magnetic core, followed by bonding the core with fluorescent dye molecules,2–3,7–9 or incorporating the magnetic and fluorescent components (e.g., organic dyes or quantum dots) into the silica or polymer matrix.10–15

However, with many benefits of magneto-fluorescent nanocomposites, there is still a major difficulty for these multifunctional composites in practical applications. The quenching of the fluorescent signal by the magnetic core makes it difficult to obtain strong fluorescent signal.10 It has been reported that the fluorescent dye molecule can be quenched by magnetic nanoparticle when their distance is less than 10 nm.16–19 Additionally, since the black magnetic nanoparticles intensely absorb visible light, a diminished fluorescence intensity could occur due to the light absorption, even if the distance between two components, where the magnetic core was coated with silica or polymer buffer layers,10 is larger than 10 nm. Despite these reports, very few attempts have been made for an in-depth...
and quantitative analysis of influence of magnetic NPs on fluorescent dye intensity, making it impossible to optimize their fluorescent properties. In the present study, the effect of Fe₃O₄ magnetic nanoparticles on fluorescent properties of acridine orange (AO, a model fluorescent dye) was quantitatively evaluated by using spectral analysis of a mixture of Fe₃O₄ nanoparticles and AO. Moreover, a model was developed to quantitatively evaluate the fluorescent decay of AO. These investigations facilitated the government of fluorescence property of magneto-fluorescent nanocomposites, and pave a way for potential use in multifunctional material synthesis and modification.

2. MATERIALS AND METHODS

2.1. Materials

Fe₃O₄ magnetic nanoparticles (MNPs, 15 nm, coated with oleic acid and dispersed in chloroform) were purchased from Ocean NanoTech, LLC. AO was acquired from Invitrogen. Dopamine (DA), 5-carboxyfluorescein (5-FAM) and 5 mg N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC) were obtained from Sigma-Aldrich. Ethanol and isopropanol were supplied from Fisher Scientific.

2.2. Transferring Magnetic Nanoparticles into Isopropanol

The Fe₃O₄ NPs coated with oleic acid (1.5 mg) were washed by hexane/ethanol (1:4, 1 mL), ethanol and isopropanol, successively. Eventually, the processed Fe₃O₄ dispersed in isopropanol well and the suspension could be stable for several weeks. The concentration of Fe₃O₄ NPs was determined gravimetrically by drying NPs isopropanol solution in an aluminum foil cup. The drying experiments were carried out under atmospheric pressure at 70 °C and the experiments were completed when the sample mass was unchanged. The mass of samples were weighed by electrical balance with an accuracy of 0.01 mg. All the experiments were performed several times to ensure reproducibility of results.

The initial AO solution was obtained by dissolving 10 mg AO powder in 1 mL isopropanol. The AO solutions with various concentrations were prepared by diluting the initial AO solution with isopropanol. The mixture of AO and Fe₃O₄ NPs was obtained by adding the Fe₃O₄ NPs solution to pure AO isopropanol solution and consequently sonicated to form a homogeneous solution.

2.3. Estimation of the Maximum Amount of AO Adsorbed by Fe₃O₄ NPs

Assuming that the shape of Fe₃O₄ NP is a cubic to simplify the calculation, the volume and mass of NPs are given as follows:

\[ V_{p,0} = L^3; \quad m_{p,0} = \rho_p L^3; \quad S_{p,0} = L^2 \]

where \( V_{p,0} \) is the volume of one particle, \( m_{p,0} \) the mass, \( \rho_p \) the density of Fe₃O₄ NPs (around \( 5 \times 10^3 \) kg/m³), \( S_{p,0} \) the surface area, \( L \) is the length of one side.

In addition, the diameter of AO molecule is less than 1.5 nm, and its density is around \( 1 \times 10^3 \) kg/m³. Given that the surface of nanoparticle is fully covered by one layer of AO molecules, the diameter of dye-coated NP is increased to 18 nm, and the mass of dye-coated NP is increased by a mass fraction of 14.5%. In other words, the maximum amount of AO adsorbed by 1 ng NPs is 0.145 ng.

2.4. Fluorescence Spectra of 5-FAM-Bonded Fe₃O₄ NPs

In a typical synthesis of DA modified Fe₃O₄ NPs, 50 mg DA was added to 5 mL isopropanol containing 5 mg Fe₃O₄ NPs. After shaking at room temperature for 4 h, DA-modified Fe₃O₄ NPs were obtained by centrifugation and washed with isopropanol for three times.

10 μM 5-FAM and 5 mg EDC were added to 1 mL isopropanol containing 0.5 mg DA-modified Fe₃O₄ NPs. After reaction for 2 h, the 5-FAM-bonded NPs were collected by centrifugation and rinsed with isopropanol for six times. The as-prepared 5-FAM-bonded Fe₃O₄ NPs was stored at 4 °C for further use.

The 5-FAM content (\( C \)) was detected by measuring the absorbance at 490 nm and calculated using Eq. (1).

\[ C = \frac{m_{0} - m_{\text{sup}}}{m_{\text{NPs}}} \times 100\% \]

where \( m_0 \) means the initial mass of 5-FAM added, \( m_{\text{sup}} \) the mass of 5-FAM in the supernatant after reaction, and \( m_{\text{NPs}} \) denotes the mass of Fe₃O₄ NPs added.

2.5. Characterization

Transmission electron microscope (TEM, FEI Tecnai T12) was used to observe the shape and morphology of Fe₃O₄ NPs. The average particle size was analyzed by 90 Plus particle size analyzer (BIC Brookhaven Instruments). UV-Visible and fluorescence emission spectra were collected on Cray 100 Bio spectrophotometer and TECAN GENios Pro fluorescence spectrophotometer, respectively. In emission intensity measurement, the filters of fluorescein (485 nm for excitation and 535 nm for emission) were used to collect fluorescence intensity with a gain value of 36.

3. RESULTS AND DISCUSSION

The commercially available Fe₃O₄ magnetic NPs were synthesized by high temperature decomposition method, and their surface was coated with oleic acid, resulting in well dispersion in hydrophobic solvent. AO, a nucleic acid selective fluorescent cationic dye useful for cell cycle determination, was utilized as a model fluorescent dye in this work. AO is difficult to dissolve in hydrophobic solvent (e.g., chloroform and hexane), but soluble in...
polar solvent (e.g., DMF and alcohol). Hence, in order to obtain the alcohol-dispersible magnetic NPs, the abundant oleic acid was removed by successively rinsing in hexane/ethanol (1:4, 1 mL), ethanol and isopropanol. The as-prepared magnetic NPs can be well dispersed in isopropanol for several weeks. As shown in Figure 1, raw NPs exhibited good dispersion in chloroform owing to an oleic acid layer on the surface (Fig. 1(a)). The average particle size of NPs was determined to be 14.1 nm ± 0.6 nm. After removal of most oleic acid, the NPs can be transferred into isopropanol. Although the particle size of NPs was almost the same, the NPs tend to slightly aggregate with each other (Fig. 1(b)).

3.1. Absorption spectra of Fe$_3$O$_4$ NPs
The Fe$_3$O$_4$ NP solution exhibits dark brown or black color due to the full absorption of visible light. As shown in Figure 2(a), the NPs can absorb the visible light in the range of 300–800 nm with a decreased absorbance. The spectra indicated that long wavelength light was better transmitted than the short wavelength light. The decrease in absorption with increasing wavelength resulted in a slightly yellow-brown appearance of the NP suspension. In addition, the absorbance was proportional to the concentration of magnetic NPs based on Lambert–Beer law, indicating the well-dispersion of NPs in isopropanol (inset in Fig. 2(a)). Furthermore, when an external magnetic field was applied to collect the suspended NPs in isopropanol solution, the suspension spectra was nearly identical with that of pure isopropanol (Fig. 2(b)). Additionally, it’s worth to note that no fluorescence of NPs was detected by use of a fluorescent spectrophotometer.

3.2. Absorption and Fluorescence Emission Spectra of AO
AO was utilized to evaluate the interaction between dye molecule and magnetic Fe$_3$O$_4$ NPs. As shown in Figure 3, the maximum absorption of AO located at 488 nm, and its emission maximum was around 525 nm. In addition, both absorption and emission intensity were linearly proportional to the concentration of AO.

3.3. Absorption Spectra of the Mixture of AO and NPs
Figure 4 shows the absorption spectra of the mixture of AO and NPs. The solid line shows the absorption spectra of their mixture, which is composed of combined absorption spectra of AO and NPs. The maximum absorption peak of AO still appeared, and the baseline was elevated due to the absorption of NPs. Nevertheless, when NPs were removed from the suspension by an external magnetic field, the
spectra became similar to that of pure AO solution. These findings indicated that in the mixture of AO and NPs, most of AO molecules remained in the solution rather than absorbed onto the surface of Fe₃O₄ NPs, since the removal of NPs from the bulk solution did not diminish the absorbance of AO.

3.4. Fluorescence Emission Intensity of the Mixture of AO and NPs

The effect of Fe₃O₄ NP concentration on the fluorescence intensity of AO was investigated. It was found that the fluorescence intensity of AO changed significantly after the addition of Fe₃O₄ NPs. As shown in Figure 5, a sharp decrease in fluorescence intensity occurred over concentrations ranging from 0 to 0.0625 ng · ml⁻¹, a linear decline occurred at concentrations ranging from 0.0625 to 1.25 ng · ml⁻¹, and a plateau followed at concentrations ranging from 1.25 to 6250 ng · ml⁻¹.

On the other hand, the influence of AO on the fluorescence intensity of the mixture of AO and NPs was studied. Figure 6 and Table I illustrate the fluorescence intensity plots as a function of AO concentration, and their corresponding linear fitting curves. It can be seen that the fluorescence intensity of the mixture increased linearly as a function of AO concentration with NP concentration of 0, 0.625, 62.5 and 312.5 pg · ml⁻¹. Moreover, the fluorescence intensity of the mixture decreased with higher NP concentrations, which is consistent with the observation shown in Figure 5.

The fluorescence intensity decay should not only be due to the fluorescence energy resonance transfer of dye fluorescence by NPs, but also due to the strong optical absorption capability of NPs.¹² Fluorescence energy resonance transfer is the nonradiative transfer of energy from an excited donor fluorophore to an acceptor through nonradiative dipole–dipole coupling.²³ Since the efficiency of the energy transfer is inversely proportional to the sixth power of the distance between donor and acceptor, fluorescence energy resonance transfer only occurs when the distance of molecules is less than 10 nm. Based on the calculation (see Section 2.3), it was found that 1 ng of Fe₃O₄ NPs can only adsorb 0.145 ng of AO, which was consistent with the result of Figure 2. It’s notable that more than 1 µg AO was utilized in the experiments (Figs. 2, 5 and 6), in other words, the proportion of AO adsorbed by NPs was neglectable. Hence, it is safe to conclude that the probability of occurrence of fluorescence energy resonance transfer was very low. On the other hand, the Fe₃O₄ NPs were able to absorb the full spectrum of visible light (Fig. 2), which covered both the excitation (488 nm) and emission (535 nm) region of AO (Fig. 3). Therefore, it can be concluded that the fluorescence intensity decay of AO in presence of Fe₃O₄ NPs should be mainly attributed to the absorption of Fe₃O₄ rather than the fluorescence resonance energy transfer between AO and NPs.

The change in fluorescence intensity ∆Eᵣᵣ is linearly proportional to the change in absorption intensity ∆I (∆Eᵣᵣ = kᵣ · ∆I), where kᵣ represents the quantum efficiency of AO dye. According to Lambert-Beer law, the amount of absorbed light is proportional to the concentration of NPs Cₙᵣ (ΔI = kᵣ · Cₙᵣ) (Fig. 2(a)). On the other hand, the change in emitted fluorescence ∆Eₑₑ is proportional to the concentration of NPs as well (∆Eₑₑ = kₑₑ · Cₙᵣ). Hence, the overall fluorescence intensity change ∆Eᵣᵣ is deduced by the equation,

\[
\Delta Eᵣᵣ = \Delta Eₑₑ + \Delta Eᵡᵡ = kᵣ · kₑₑ · Cₙᵣ + kₑₑ · Cₙᵣ = k · Cₙᵣ
\]

(2)

Based on Eq. (2), the intensity decay was proportional to the concentration of NPs in a certain concentration range (i.e., from 0.0625 to 1.25 ng · ml⁻¹). While, when the concentration below 0.0625 ng · ml⁻¹, the sharp decrease in intensity absorption may be due to the assembling of NPs in solution. As shown in Figure 7, the NPs were inclined to aggregate loosely at high concentrations. When the concentration of NPs was lower than
In this study, the effect of magnetic Fe₃O₄ nanoparticles on fluorescence quenching of dye molecules was quantitatively studied. It was found that the absorption intensity of Fe₃O₄ NPs, dispersed well in isopropanol solution, was proportional to their concentration. We further investigated their effect on fluorescence intensity of AO. It has been demonstrated that the fluorescence intensity of AO decreased significantly with increasing NP concentration. Specifically, when the concentrations was in the range of 0.0625 to 1.25 ng·ml⁻¹, a linear decrease in fluorescence intensity was found; when the concentration of NPs was lower than 0.0625 ng·ml⁻¹, the fluorescence intensity decreased rapidly; and when the concentration of NPs exceeded 1.25 ng·ml⁻¹, most of fluorescence was absorbed by NPs, resulting in a plateau with weak fluorescence. All these results and calculation offered clear evidence that the fluorescence decay of dye molecules was mainly contributed by the absorption of Fe₃O₄ NPs. Our investigation into the interaction between magnetic nanoparticles with dye molecules might help to rationally tailor the fluorescent properties of magnetofluorescent nanocomposites by modulating the content of NPs for structure design, reaction optimization and quality assessment.

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References and Notes

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Zhang et al.


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