

Preparation of Stable Luminescent Poly(methyl methacrylate)-Europium Complex Nanospheres and Application in the Detection of Hydrogen Peroxide with the Biocatalytic Growth of Gold Nanoparticles

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ABSTRACT: A simple and effective solvent swelling method was developed for the preparation of poly(methyl methacrylate) (PMMA)/europium (Eu) complex hybrid nanospheres. Transmission electron microscopy and dynamic light scattering results indicate that the as-prepared PMMA–Eu nanoparticles had a spherical morphology, with a narrow particle size distribution ranging from 100–200 nm. The PMMA–Eu nanospheres exhibited strong red emissions with a maximum peak at 612 nm under UV excitation, and the luminescence lifetime of the Eu complex was enhanced after it was swollen into PMMA nanospheres. Furthermore, the luminescence intensity of the PMMA–Eu nanospheres was very stable in various severe media, including aqueous solutions with various pHs, 1 mM Ca^{2+} , 1 mM Fe^{2+} , 1 mM Cu^{2+} , 0.1M phosphate-buffered saline solution, 1 mM citric acid solution, 1 mM lysine, and 1 mM glutamic acid. After the nanospheres were incubated at various temperatures for 1 h, the luminescence properties remained stable when the temperature was less than 40°C yet decreased slightly between 40 and 60°C and decreased rapidly at higher temperatures. These luminescent nanospheres were successfully applied in the luminescence-sensing assay of hydrogen peroxide and exhibited a high sensitivity and broad linear concentration range. Because of their unique luminescence properties, the as-synthesized PMMA–Eu nanospheres are expected to have great potential for use as luminescent labels or probes for long-time imaging and analysis in which severe media situations are present. © 2012 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 000: 000–000, 2012

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INTRODUCTION

In recent several decades, lanthanide-based luminescent complexes [mainly, europium (Eu), terbium, samarium, and dysprosium] have attracted more and more interest because of their unique luminescent properties, including their narrow emission spectra and large Stokes' shift, high emission quantum efficiencies, and long luminescent lifetimes.^{1–4} However, despite their good luminescence features, their poor water solubility, thermal stability, and mechanical properties have limited their practical applications.^{5,6} Furthermore, their luminescence quantum yields are usually reduced substantially by the quenching of water molecules and various cations and anions (hydroxide, phosphate, etc.).^{7–9} To overcome these disadvantages, some researchers have incorporated these complexes into various host matrices, and the incorporated complexes have shown improved photoluminescence properties and thermal stability.^{5,10–14} However, for use in their senior applications in luminescence sensing, labeling, and bioimaging, the complexes need be fabricated into nanoparticles with stable luminescence, so it is necessary to

find a simple method for preparing lanthanide-complex-based nanoparticles.

Among the various methods used to prepare luminescent nanoparticles, the solvent swelling method (also called the soaking method) is a facile, straightforward, and efficient method for loading hydrophobic molecules into polymer microparticles or nanoparticles.¹² In this study, a monodisperse Eu complex incorporated poly(methyl methacrylate) (PMMA) (the complex is abbreviated as PMMA–Eu) nanospheres were prepared by this solvent swelling method. The photoluminescence properties of the PMMA–Eu nanospheres were studied, and we tried to find applications for the as-prepared PMMA–Eu nanospheres in the luminescence sensing of hydrogen peroxide (H_2O_2) with the biocatalytic growth of Au nanoparticles.¹⁵

EXPERIMENTAL

Material and Characterization

Methyl methacrylate, acrylic acid, and divinylbenzene (DVB) were analytical reagents and were redistilled. Poly(vinyl

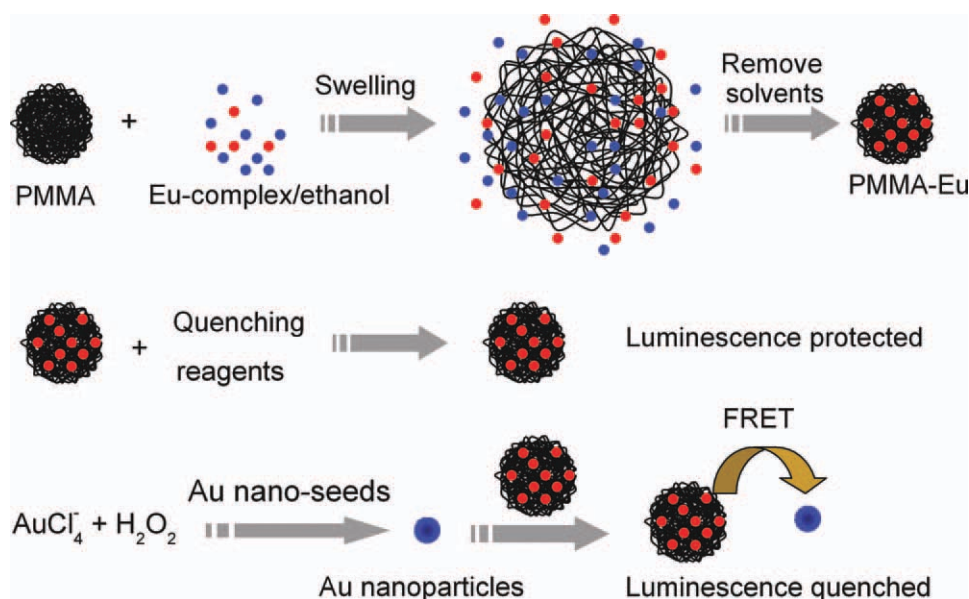


Figure 1. Synthesis scheme of the PMMA–Eu nanospheres. FRET = Fluorescence resonance energy transfer. [Color figure can be viewed in the online issue, which is available at www.wileyonlinelibrary.com.]

pyrrolidone) (K30), potassium persulfate, europium oxide (Eu_2O_3), 2-thenyltrifluoroacetone (TTA), isonicotinic acid (INA), chloroauric acid tetrahydrate ($\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$), sodium borohydride (NaBH_4), sodium hydroxide, sodium citrate, cetyltrimethylammonium (CTAC), and H_2O_2 were all analytical reagents and were used as received. Au nanoparticle seeds were prepared with NaBH_4 as a reductant and were stabilized with sodium citrate according to the literature.¹⁶

Transmission electron microscopy (TEM; H-7000 FA, Hitachi, Japan) were used to characterize the prepared nanospheres. The mean hydrodynamic diameter was obtained by the dynamic light scattering method with a Malvern Zeta-Sizer 3000HS instrument (Worcestershire, United Kingdom). The luminescence spectra and time-decay curves of PMMA–Eu were detected with an F-4600 fluorescence spectrophotometer (Hitachi, Japan).

Preparation of the Eu Complex

The Eu complex was prepared according to a procedure reported earlier with slight modifications.¹⁷ Eu_2O_3 was dissolved in minimum amounts of concentrated nitric acid and was titrated into a solution containing TTA and INA. Then, the Eu complex was collected as a white precipitate and was washed with water three times. The molar ratio of Eu to TTA to INA was controlled to be 1 : 3 : 1 by adjustment of the amounts of the corresponding reactants. The as-synthesized Eu complex is abbreviated as $\text{Eu}(\text{TTA})_3\text{INA}$.

Preparation of the PMMA–Eu Nanospheres

The synthesis of PMMA–Eu mainly consisted of three steps, as follows:

1. PMMA latex was synthesized by a soap-free emulsion polymerization. The polymerization precursor consisted of 4 mL of methyl methacrylate, 0.2 mL of acrylic acid, 0.2 mL of DVB, and 40 mL of a 2% poly(vinyl pyrrolidone) aqueous solution with 100 mg of potassium persulfate as the

initiator. The polymerization was accomplished at 65°C for 5 h under an N_2 atmosphere. After the addition of 10 mL of 0.01 M $\text{Eu}(\text{NO}_3)_3$ to 1 mL of the as-prepared PMMA latex. The PMMA nanospheres were centrifuged and washed with water three times.

2. PMMA latex (1 mL) was dispersed into a solution consisting of 2 mL of ethanol, 50 μL of acetone, and 1 mg of $\text{Eu}(\text{TTA})_3\text{INA}$ to accomplish the swelling of the PMMA nanospheres.
3. Ethanol and acetone were removed by centrifugation. The precipitated nanospheres were redispersed into 100 mL of water with the assistance of an ultrasonic cleaner.

Luminescence Sensing of H_2O_2

The luminescence-sensing procedures of H_2O_2 were followed according to previous reports.¹⁷ The Au nanoparticle growth solution consisted of 1.4×10^{-4} M HAuCl_4 and 2.0×10^{-3} M CTAC in 0.01 M phosphate-buffered saline (PBS; pH 7.05). For the detection of H_2O_2 , various concentrations of H_2O_2 were added to 5 mL of the growth solution. Then, 20 μL of 4.5×10^{-4} M Au nanoparticle seeds was added to the growth solution for the catalytic growth of the Au nanoparticles. The growth processes were performed in an ice–water bath for 5 h. Subsequently, 100 μL of 1.1 mg/mL PMMA–Eu was added to the solution. After 20 min at room temperature, the luminescence spectra were recorded with the fluorescence spectrophotometer.

RESULTS AND DISCUSSION

Morphologies of the PMMA–Eu Nanospheres

PMMA nanospheres were synthesized by the classical soap-free emulsion polymerization method,¹⁸ and the simplified procedures are shown in Figure 1. During the experiment, we found that oligomers of acrylic acid could severely quench the luminescence of $\text{Eu}(\text{TTA})_3\text{INA}$. To prevent this quenching effect, $\text{Eu}(\text{III})$ ions were used to presaturate the carboxyl groups of the

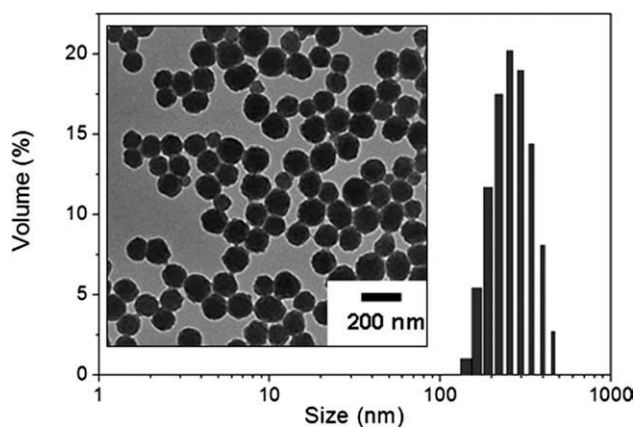


Figure 2. Hydrodynamic diameter distribution and TEM (inset) of the as-synthesized PMMA–Eu nanospheres.

as-prepared PMMA nanospheres. Furthermore, Eu(III) cations could neutralize the negative charges of the PMMA nanospheres and then facilitate the following centrifugation and washing procedures. After centrifugation and several washings, the water-soluble oligomers, residual monomers, and initiators could be removed. Then, the cleaned PMMA nanospheres were dispersed into an organic solution of $\text{Eu}(\text{TTA})_3\text{INA}$. Instead of dissolving into the organic solvent, the macromolecules of PMMA could only swell because of the crosslinking of DVB among different PMMA macromolecules. Therefore, the $\text{Eu}(\text{TTA})_3\text{INA}$ molecules could enter the PMMA nanospheres without destroying their spherical morphologies. After the removal of organic solvent, the $\text{Eu}(\text{TTA})_3\text{INA}$ molecules were isolated from outside species, and a stable luminescence could be retained.

The TEM results indicate that most of the PMMA–Eu nanospheres had a relatively narrow size distribution of about 150 nm; it ranged from about 100 nm to about 200 nm with approximately spherical morphologies (Figure 2). A larger mean hydrodynamic diameter was observed from the dynamic light scattering results; this may have been caused by the aggregation and salvation effect. Most of the nanospheres had a narrow hydrodynamic size distribution, between 100 and 500 nm, and were small enough for their potential applications.

Luminescence Properties of the PMMA–Eu Nanospheres

The excitation and emission spectra of the as-synthesized PMMA–Eu nanospheres exhibited a broad excitation band in the UV region, which peaked at 345 nm, and a typical emission peak of Eu^{3+} at 612 nm [$^5\text{D}_0 \rightarrow ^7\text{F}_2$; Figure 3(A)]. Compared with the spectra of $\text{Eu}(\text{TTA})_3\text{INA}$, the spectra of the PMMA–Eu nanospheres were similar; this indicated no obvious chemical interaction between PMMA and $\text{Eu}(\text{TTA})_3\text{INA}$. The Eu complexes have great potential in the field of time-resolved analysis because their lifetimes (on the order of milliseconds) are much longer than those of autofluorescence (on the order of nanoseconds) from organic tissue components. A long lifetime can afford an excellent signal-to-noise ratio and detection limit. Therefore, the influence of PMMA on the lifetime of $\text{Eu}(\text{TTA})_3\text{INA}$ was studied. The lifetime decay patterns of the

red emission at 612 nm of $\text{Eu}(\text{TTA})_3\text{INA}$ in ethanol–water (1 : 1 v/v) and ethanol and the PMMA nanospheres are shown in Figure 3(B). The decay curves fit well as a function of time by a monoexponential equation; this indicated that all of the Eu complex molecules occupied the same local physical chemical environment. Because $\text{Eu}(\text{TTA})_3\text{INA}$ was insoluble in water, a mixed water and ethanol solvent was used to check the influence of water on its luminescence lifetime. The results clearly exhibit that the lifetime of PMMA–Eu was enhanced compared with that of its original counterpart, $\text{Eu}(\text{TTA})_3\text{INA}$ in ethanol. The lifetime decay constant of $\text{Eu}(\text{TTA})_3\text{INA}$ in a 1 : 1 v/v ethanol aqueous solution was 0.384 ms; this increased by 61% to 0.619 ms when anhydrous ethanol was used as the solvent, whereas it increased 85% to 0.710 ms in the case of PMMA–Eu. These results indicate that water acted as an unfavorable factor, hindering the practical applications of the europium complexes in the time-resolved luminescence imaging and immunoassay.^{8,9} Similar results were also observed by other researchers.^{19,20}

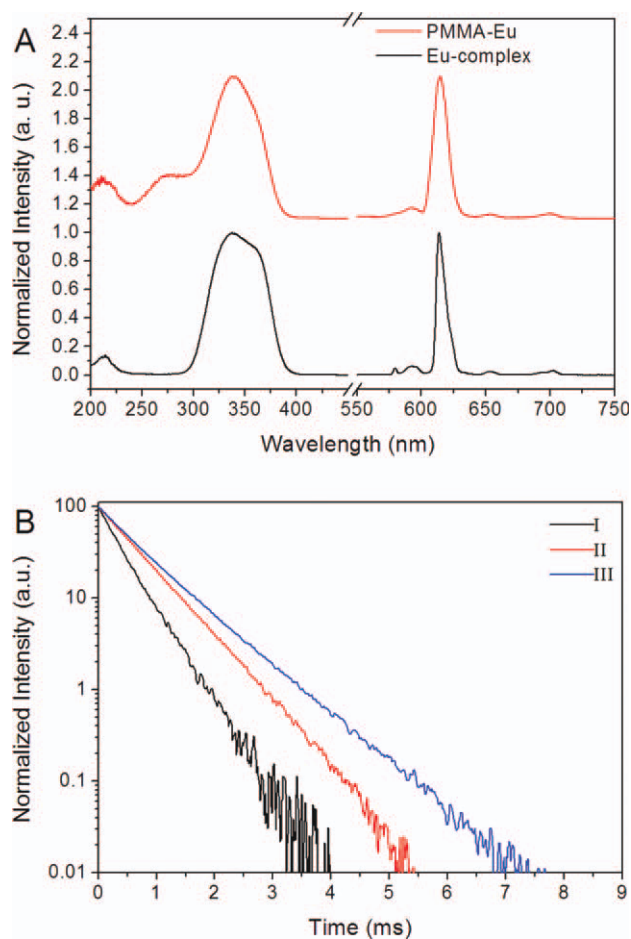


Figure 3. Photoluminescence properties: (A) excitation (emission wavelength = 612 nm) and emission (excitation wavelength = 365 nm) spectra of the PMMA–Eu nanosphere aqueous solution and (B) luminescence lifetimes of $\text{Eu}(\text{TTA})_3\text{INA}$ in different media: (I) ethanol–water hybrid solvent (1 : 1 v/v), (II) anhydrous ethanol, and (III) PMMA–Eu nanosphere aqueous solution. [Color figure can be viewed in the online issue, which is available at www.wileyonlinelibrary.com.]

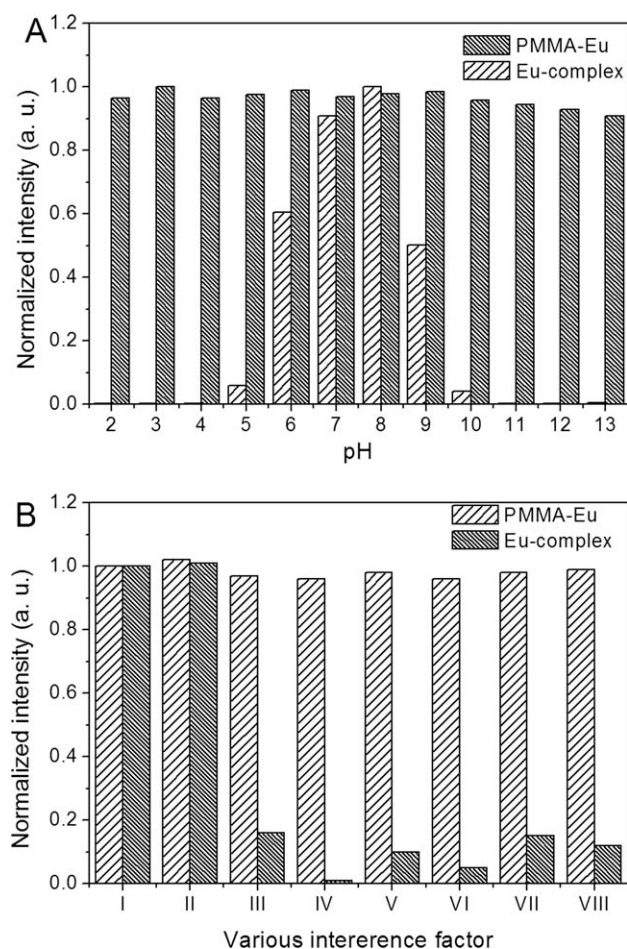


Figure 4. Photoluminescence intensities of the PMMA–Eu nanosphere aqueous solution and Eu complex ethanol–water solution (1 : 1 v/v) with various interference factors: (A) various pHs, (B) (I) water, (II) 1 mM CaCl₂, (III) 1 mM Fe(II), (IV) 1 mM Cu(II), (V) 0.1M PBS solution, (VI) 1 mM citric acid, (VII) 1 mM lysine, and (VIII) 1 mM glutamic acid.

Luminescence of the PMMA–Eu Nanospheres in Various Media

With regard to the applications of the nanospheres, one challenge is the stability of luminescent nanospheres in various media. Although many works have been conducted to overcome this problem, it is still difficult to obtain stable luminescence signals under various conditions. To study the effects of pH, inorganic ions, and organic ligands, the PMMA–Eu nanospheres were incubated in aqueous solutions with various pHs, 1 mM Ca²⁺, 1 mM Fe²⁺, 1 mM Cu²⁺, 0.1M PBS solution (pH 6.88), 1 mM citric acid solution, 1 mM lysine, and 1 mM glutamic acid, and their luminescence spectra were recorded. We found that the luminescence intensity of the as-synthesized PMMA–Eu nanospheres remained stable, whereas the pH varied from 2 to 13 [Figure 4(A)]. However, the Eu(TTA)₃INA in the water/ethanol (1 : 1 v/v) solution suffered severe luminescence quenching when the pH was less than 6 or more than 9. Furthermore, Cu²⁺, Fe²⁺, citric acid, lysine, glutamic acid, and even PBS solution were able to quench the luminescence of Eu(TTA)₃INA, whereas the luminescence of the PMMA–Eu nanospheres

remained stable [Figure 4(B)]. Therefore, the luminescence properties of Eu(TTA)₃INA could be improved by the encapsulation of Eu(TTA)₃INA in PMMA nanospheres, and this complex has great potential for use as a luminescent candidate for long-time assays in which intense assay situations are present.

Luminescence of the PMMA–Eu Nanospheres at Various Temperatures

To study the influence of temperature, the as-synthesized PMMA–Eu colloidal solution was incubated at different temperatures for 1 h. It is shown in Figure 5(A) that the luminescence of PMMA–Eu was stable when the temperature was lower than 40°C. However, the luminescence intensities decreased apparently when the temperature was higher than 50°C. After incubation at 80°C for 1 h, the luminescence intensity of the nanospheres decreased nearly 90%. After the PMMA–Eu nanospheres were incubated in water at 70°C for different times, we found, as shown in Figure 5(B), that the luminescence intensities decreased rapidly in 0–20 min but relatively slowly in 20–40 min and remained stable after 40 min. The possible mechanism may have been that the Eu complex molecules in the superficial layers of the PMMA nanospheres were destroyed

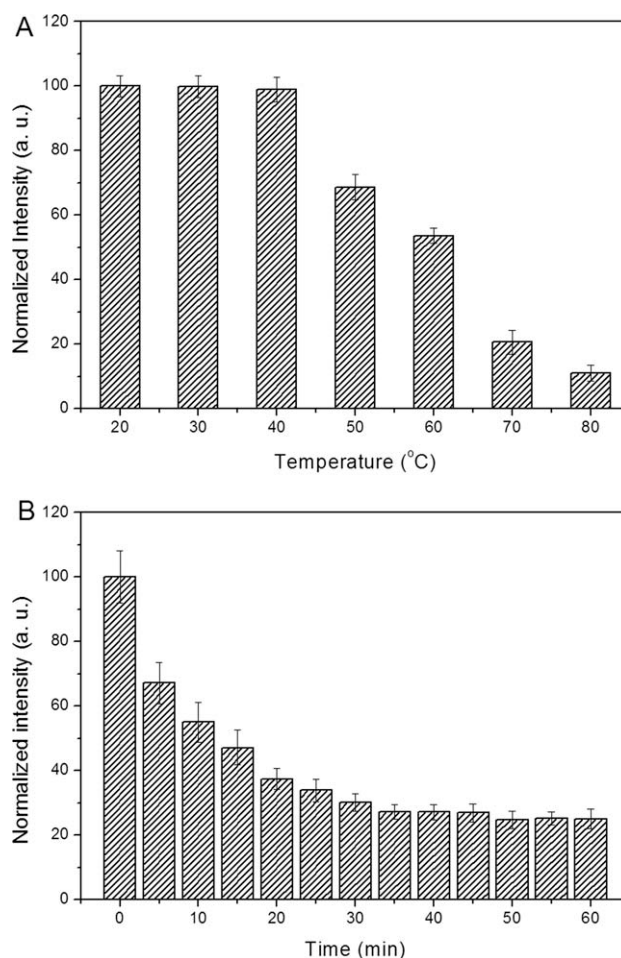


Figure 5. Influence of the temperatures on the luminescence intensities of the PMMA–Eu nanospheres: (A) incubated at different temperatures for 1 h and (B) incubated at 70°C for different times.

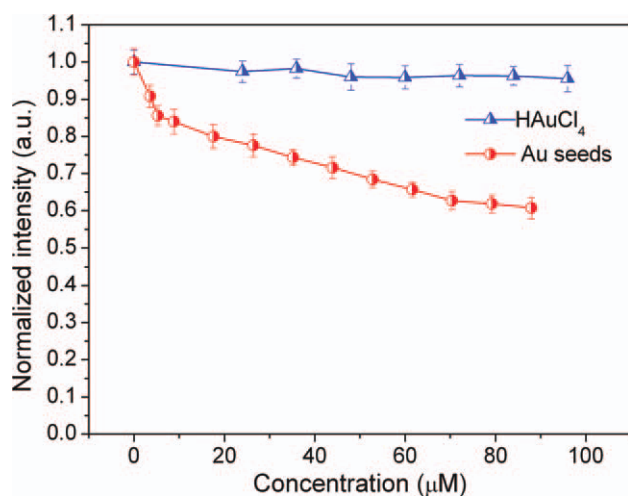


Figure 6. Effects of HAuCl₄ and Au nanoparticles on the luminescence intensities of the PMMA–Eu nanospheres. [Color figure can be viewed in the online issue, which is available at www.wileyonlinelibrary.com.]

rapidly at high temperature, whereas the complexes in the inner core were able to remain stable. However, most of the Eu(TTA)₃INA molecules were at the superficial layers, as the sizes of the PMMA–Eu nanospheres were very small. Therefore, although the hydrophobic shell of PMMA could improve the luminescence properties of the Eu complexes, their luminescence intensities decreased greatly at high temperatures. Herein, what should be mentioned is that the temperature in most biomedical applications is usually below 50°C, and the luminescence intensity of the as-prepared PMMA–Eu was able to remain at about 70% in this situation; this would be enough for most potential practical applications.

Luminescence Sensing of H₂O₂

H₂O₂ is a well-known reactive species associated with cell damage and an important regulator of eukaryotic signal transduction. Therefore, the sensing of H₂O₂ is of prominent significance, and many methods have been developed for this purpose. Among these sensing strategies, the optical method is a straightforward approach based on the biocatalytic growth of Au nanoparticles due to the surface plasmon resonance of the catalytic generated Au nanoparticles at a wavelength of about 540 nm.²¹ Because of the high extinction coefficient and broad absorption spectra, Au nanoparticles are commonly used as a luminescence quencher and energy acceptor. Accordingly, an inhibition assay of H₂O₂ was developed on the basis of fluorescence resonance energy transfer between the PMMA–Eu nanospheres and Au nanoparticles. Herein, the as-synthesized PMMA–Eu nanospheres were used to develop a luminescence-sensing strategy for H₂O₂ based on the biocatalytic growth of the Au nanoparticles. H₂O₂ was used to reduce the Au(III) salts to Au nanoparticles under the catalysis of Au nanoparticle seeds. The changes in the luminescence intensity of the PMMA–Eu nanospheres resulted from the changes in the concentrations of HAuCl₄ and Au nanoparticles during the growth process. Interestingly, in contrast to CdTe quantum dots, which can be

severely quenched by both HAuCl₄ and Au¹⁶ HAuCl₄ had very weak quenching effects on the luminescence of the PMMA–Eu nanospheres, whereas the Au nanoparticles were able to severely quench the luminescence of PMMA–Eu (Figure 6). The possible reason may have been that quantum dots have nude surfaces and are very sensitive to many chemicals, whereas the luminescent Eu complexes were encapsulated and, thus, protected by PMMA nanospheres in the case of the as-prepared PMMA–Eu nanospheres.

The addition of H₂O₂ to a PBS, including HAuCl₄, Au nanoparticle seeds, and CTAC, resulted in a decrease in the luminescence intensity of the PMMA–Eu nanospheres. The Au nanoparticle seeds acted as catalysts for the reduction of AuCl₄[−] by H₂O₂ and resulted in the enlargement of the Au nanoparticle seeds and enhanced absorbance features.¹⁵ Figure 7(A) shows the evolution of the luminescence spectra in the system in the presence of variable concentrations of H₂O₂. Only a decrease in the luminescence intensity could be observed, whereas the luminescence emission maxima appeared at the same 612 nm. This

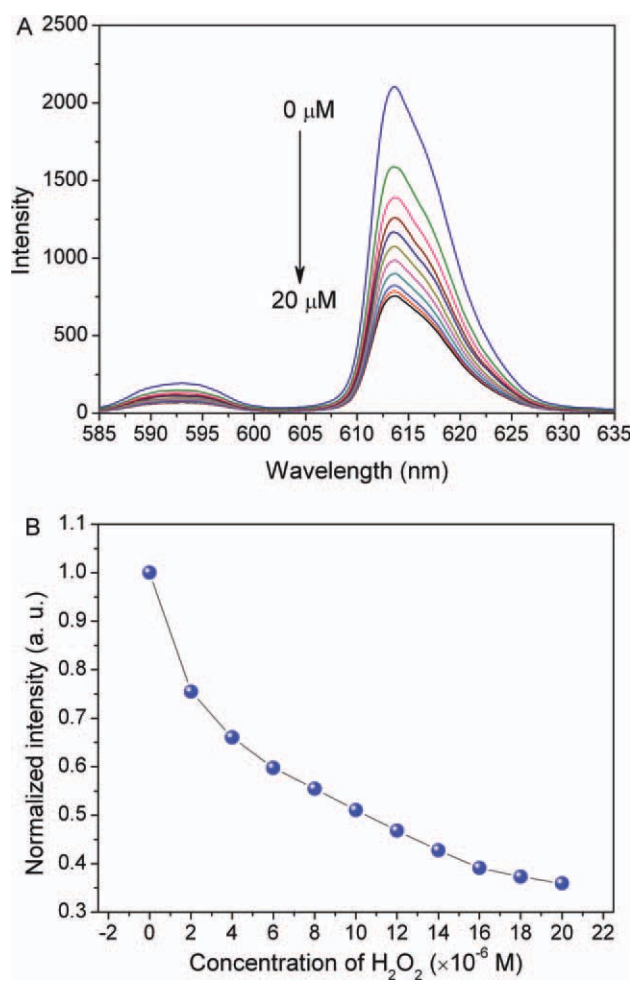


Figure 7. (A) Variation in the luminescence spectra of the PMMA–Eu nanospheres mixed with 5 mL of Au nanoparticle growth solution reacted with different concentrations of H₂O₂. (B) Normalized luminescence intensity at 612 nm versus H₂O₂ concentration. [Color figure can be viewed in the online issue, which is available at www.wileyonlinelibrary.com.]

is one of the unique properties of lanthanide complexes, and it is useful for the improvement of analytical accuracy. Figure 7(B) shows the derived calibration curve corresponding to the luminescence sensing of H_2O_2 by the catalytically enlarged Au nanoparticles. The limit of detection for H_2O_2 is 2.0×10^{-6} M. The linear concentration range for H_2O_2 is from 4.0×10^{-6} to 1.6×10^{-5} M. These results were comparable with those of previous reports based on near-infrared CdTe quantum dots.¹⁶ What should be mentioned is that CdTe quantum dots have been criticized for their use of heavy metals and their potential cytotoxicity, whereas lanthanide complexes are considered to be environmentally friendly.

CONCLUSIONS

A simple and effective solvent swelling method was used to prepare PMMA–Eu nanospheres, which exhibited spherical morphologies, water-dispersability, narrow size distribution, and a strong red emission with a maximum peak at 612 nm under UV excitation. The luminescence lifetime of the as-prepared PMMA–Eu nanospheres was extended toward $\text{Eu}(\text{TTA})_3\text{INA}$, and their luminescence properties were stable in various severe media. The size of the luminescent nanospheres could be easily controlled by changes in the diameter of the PMMA nanospheres. These luminescent nanospheres were successfully applied in a luminescence-sensing assay of H_2O_2 and exhibited a high sensitivity and broad linear concentration range. Because of their unique luminescence properties, the as-synthesized PMMA–Eu nanospheres are expected to have great potential for use in luminescent labels or probes for long-time imaging and analysis in which severe media situations are present. Furthermore, this solvent swelling method is easy to scale up and does not need any expensive devices or complicated procedures. Therefore, we believe that this method has great potential for the synthesis of other kinds of luminescent nanospheres for use in various fields.

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