Synthesis and luminescence properties of nanoparticles embedded with Europium (III)/coumarin complexes

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A complex of europium (III)/coumarin was synthesized and the complexation reaction was studied by UV-vis, fluorescence, FTIR, ^{1}H NMR spectroscopy, DTA and TG analyses. To improve the stability and solubility, and amplify the optical signal of the complex in aqueous solution, the complex was encapsulated inside into silica nanoparticles through a sol-gel process. The final product could be taken up by cells and showed obviously blue fluorescence by excitation. The study indicated that nanoparticles embedded with europium (III)/coumarin complexes could be applied as luminescent nanoprobes in cell imaging of biological systems.

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Keywords: Synthesis, Europium (III)/coumarin complex, Optical signal, Encapsulation

1. Introduction

Coumarin, known as a natural product, its derivatives have been found to show a wide spectrum of bioactivities including anticoagulant [1, 2], dermal photosensitizing, molluscacidal, spasmolytic [3], anticancer [4] and antifungal [5] activities. In which, 4-methyl-7-hydroxycoumarin is one of the common fluorescent dyes with blue-emitting [6].

Presently, several reports have been emerged on complexation reaction of 4-methyl-7-hydroxycoumarin and metals to get dual properties of both metal ions and coumains or improve the activity of 4-methyl-7-hydroxycoumarin [7, 8]. In view of the above properties, it is worthwhile to synthesize and characterize the metal complexes of coumains.

The complexes of rare earth ions are of much interest because of the importance in bioinorganic and coordination chemistry [9-12]. However, early work suggested the complexes of rare earth ions with 4-methyl-7-hydroxycoumarin were freely soluble in DMSO, soluble in alcohol, but insoluble in water [13-15], which limited their biological application. So, how to improve the water solubility of the complexes remains a priority.

Some reports have shown that the stability and solubility of lipophilic medicines in aqueous solution can be improved by embedding them into silica nanoparticles [16-19], because the silica matrix provided an effective barrier keeping the complex from the surrounding environment [20]. In the present work, we synthesized the complex of 4-methyl-7-hydroxycoumarin and europium (III), and encapsulated the europium (III)/coumarin complexes into silica nanopaticles. The results indicated that the europium (III)/coumarin complex produced a highly amplified optical signal, comparing with a single ligand coumain. Besides, the light stability and water solubility of the europium (III)/coumarin complex was greatly improved; the optical signal was further enlarged after encapsulating into silica nanopaticles. Such nanoparticles can be effectively taken up by THP-1 cells and emitted stronger blue fluorescence than the exposed complexes. Consequently, the blue fluorescent silica nanoparticles could be applied as luminescent nanoprobes for biological detection.

2. Experimental

2.1 Chemicals

4-methyl-7-hydroxycoumarin was supplied from Acros Organics. 3-Aminopropylmethyldiethoxysilane (APMES; 99%) was purchased from Sigma. Anhydrous ethanol and $Eu(NO_3)_3{\cdot}6H_2O$ were both analytical grade.

2.2 Synthesis and characterization of nanoparticles embedded with europium (III)/coumarin complexes

4-methyl-7-hydroxycoumarin sodium salt (Mendiaxon Sodium) as ligand was prepared by the reaction between 4-methyl-7-hydroxycoumarin and sodium hydroxide in deionized water [13]. Europium (III)/coumarin complex was synthesized by mixing ethanol solutions of europium (III) and the ligand. Microporous silica nanoparticles

entrapped with europium (III)/coumarin complexes were prepared by an improved sol-gel method [21].

Transmission electron microscopy (TEM) image was recorded on a FEI-Tevnai G220 S-TWIN electron microscopy with an acceleration voltage of 200 kV. UV-vis absorption spectra were obtained on a Varian Cary5000 spectrophotometer. Fluorescence spectra were performed on a Perkin-Elmer LS-50B spectrofluorimeter with an excitation wavelength of 325 nm. FTIR spectra were taken on a Nicolet Nexus 670 FT-IR infrared spectrometer. The ^{1}H NMR spectra were acquired with an AVANCE 400-MHz spectrometer in DMSO-d6. DTA and TG analyses were measured by a PERKIN-ELMER Diamond TG/DTA, samples were placed in platinum crucibles and the heating rate was 10℃ min^{-1} up to 980℃, with Al_2O_3 as the inert substance.

3. Results and discussion

The reaction of the metal-ligand was studied by the UV-vis, fluorescence, FTIR, ^{1}H NMR spectra, DTA and TG. Fig. 1 showed the absorbance spectra of Mendiaxon Sodium and europium (III)/coumarin complex in ethanol. The UV-visible spectrum of Mendiaxon Sodium showed absorption maxima at 321 nm and 375 nm in the wavelength range of 200-500 nm. Upon addition of rare earth ions europium (III), the characteristic absorption at 375 nm disappeared and the intensity of absorption band centered at 321 nm increased. The above results indicated that europium (III) could chelate with coumarin intensely.

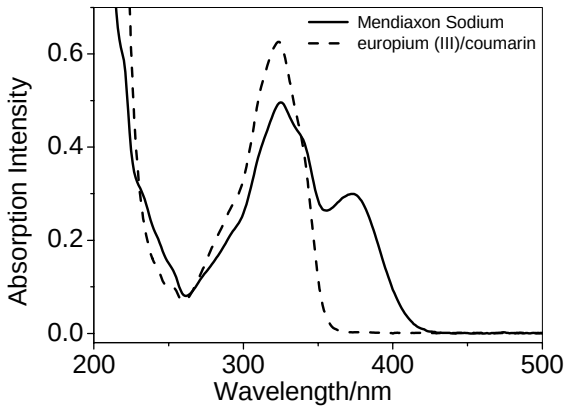

Fig. 1. UV-vis absorption spectra of Mendiaxon Sodium and europium (III)/coumarin complex in ethanol.

In the absence of europium (III), Mendiaxon Sodium emitted blue luminescence and a fluorescence maximum appeared at 449 nm with the excitation band at 325 nm. When the presence of rare earth ions europium (III), a slight blueshift occurred and the fluorescence intensity increased obviously as illustrated in the Fig. 2.

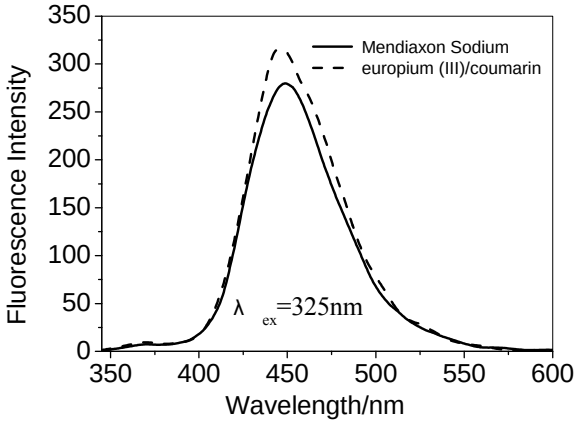

Fig. 2. Fluorescence spectra of Mendiaxon Sodium and europium (III)/coumarin complex in ethanol.

The formation of the complex was determined by molar ratio method. The concentration of Mendiaxon Sodium was fixed at 3.0×10^{-5} M, while that of the europium (III) nitrates was continuously varied in ethanol. The composition of the europium (III)/coumarin complex was detected by measuring the absorbance increase at 321 nm, where the complex exhibited the maximum absorption. As shown in Fig. 3, the extrapolated intersection of the curves occurred at the mole ratio of 0.5:1, corresponding to the ratio of europium (III) to coumarin in the complex. The calculated association constant of the complex was 6.382×10^7 M^{-1}.

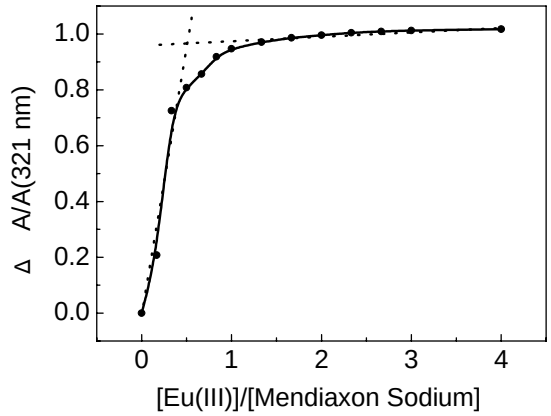

Fig. 3. Mole ratio plot for europium (III)/coumarin complex.

The mode of binding of the coumarin to europium (III) was definite by the FTIR spectra (Fig. 4). The characteristic bands of the free ligand were located at 3662, 3451, 1726, 1603, 1505, 1285, 1142, 1068-981, 830, 613 and 484 cm^{-1}. The bands at 1603 and 1505 cm^{-1} were attributed to the stretching vibrations of the conjugated system. The two bands at 1290 cm^{-1} and 1140 cm^{-1} which can be related to the C-C and C-O stretch were shifted to

1268 cm^{-1} and 1159 cm^{-1} in the complex, respectively. The weak band at 3662 cm^{-1} in the ligand was shifted to 3553 cm^{-1} after coordination. The most change observed was a 40~50 cm^{-1} shift of the C=O band at 1726 cm^{-1} to a lower wavenumber values and the peak intensity decreased obviously on complexation, which indicated that C=O group participated in coordination.

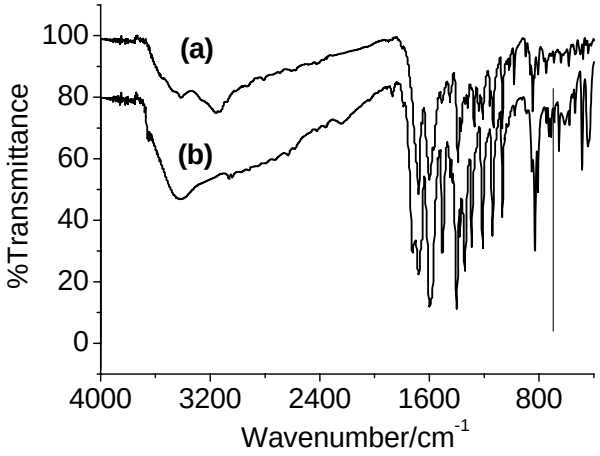

Fig. 4. FTIR spectra of Mendiaxon Sodium (a) and europium (III)/coumarin complex (b).

A clear endothermic peak occurred at 187.6℃ in the DTA-curve of the europium (III)/coumarin complex, which can be attributed to the hygroscopic moisture released (Fig.5). when heated up to 290.4℃ and 355.8℃, there were steady mass losses in the TG-curve of the complex which were due to the elimination of water molecules in the europium (III)/coumarin complex and consequently it testified the existence of water molecules in the complex. On heating the complex, resulting from the decomposition of ligand molecules, the mass loss was in agreement with the composition of the coumarin. When recorded up to 980℃, the dry residue of free ligand remained 20 percent, while about 42 percent for the complex. The above results indicated that the formation of europium (III)/coumarin complex changed thermal stability of the coumarin.

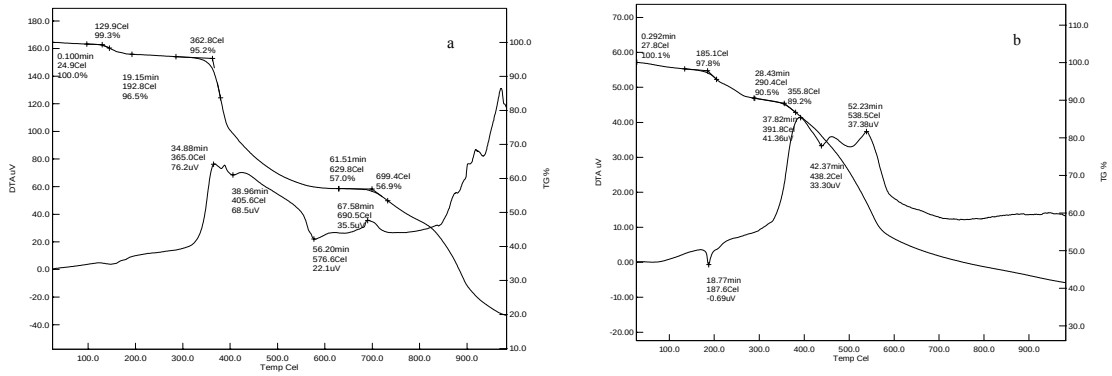

Fig. 5. DTA and TG analyses of Mendiaxon Sodium (a) and europium (III)/coumarin complex (b).

A comparison of the ^1H NMR spectra of the europium (III)/coumarin complex and of the ligand were observed in Table 1, a remarkable shift for H_8 and slight shifts for H_3, H_5 and H_6 occurred. Chemical shifts of these protons in the ^1H NMR spectra indicated the coordination of coumarin to europium (III). We considered that these shifts were valuable for confirming the coordination.

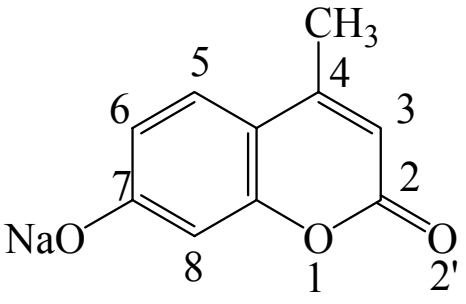

Fig. 6. Chemical structure of 4-methyl-7-hydroxycoumarin sodium salt.

Table 1. ^1H NMR spectra of 4-methyl-7-hydroxycoumarin sodium salt and its europium (III)/coumarin complex.

C_n-H	δ (ppm)	
	Ligand ($C_{10}H_7O_3Na$)	europium (III)/coumarin complex
C_8-H	5.82	6.72
C_6-H	6.10	6.86
C_5-H	7.12	7.58
C_3-H	5.40	6.18
CH_3	2.19	2.49

Fig. 7 showed a TEM image of silica nanoparticles embedded with europium (III)/coumarin complexes. It was suggested that the nanoparticles were spherical, with an average size of 45 nm, having a good dispersibility in aqueous solution. The study indicated that water solubility of the complex was improved a lot by encapsulation.

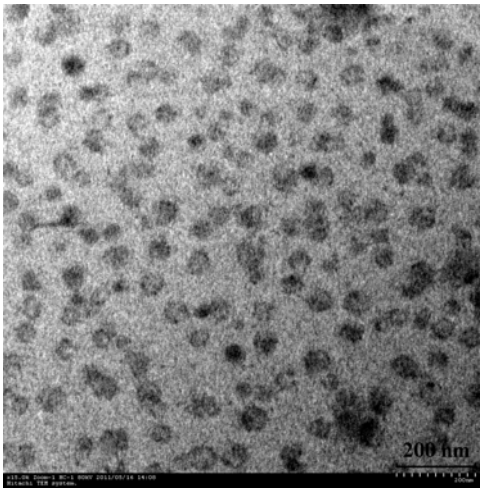

Fig. 7. TEM image of silica nanoparticles embedded with europium (III)/coumarin complexes (Bar=200 nm).

The UV-vis absorption spectra of europium (III)/coumarin complex in ethanol solution before and after encapsulation were shown in Fig. 8. The characteristic absorption peak of the complex which was located at 321 nm had a 38 nm redshift and the intensity slightly increased. While it was observed that there was a new small peak at about 393 nm and a marked redshift from 446 nm to 451 nm occurred in the fluorescence spectra after encapsulation. In addition, the optical signal was further amplified in the luminescent silica nanoparticles (Fig. 9).

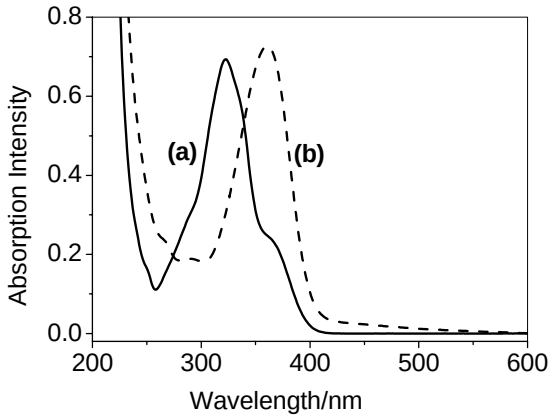

Fig. 8. UV-vis absorption spectra of europium (III)/coumarin complex in ethanol before (a) and after encapsulation (b).

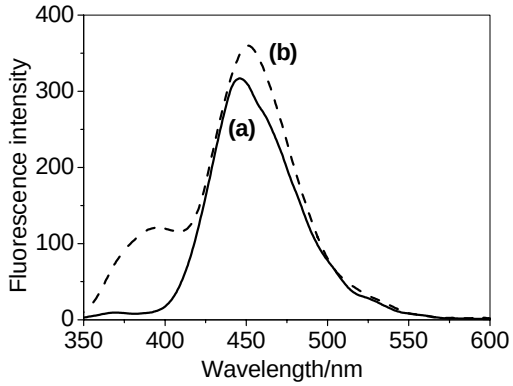

Fig. 9. Fluorescence spectra of europium (III)/coumarin complex in ethanol before (a) and after encapsulation (b).

Fig.10 showed the comparisons of the fluorescence stability of europium (III)/coumarin complexes and silica nanoparticles embedded with the complexes. The results showed that the fluorescence intensity of europium (III)/coumarin complexes at 446 nm was decreased 64.73%, but only 28.49% for encapsulated complexes after 2.5 min irradiation. Consequently, encapsulating the europium (III)/coumarin complexes into silica nanoparticles could significantly enhance the light stability.

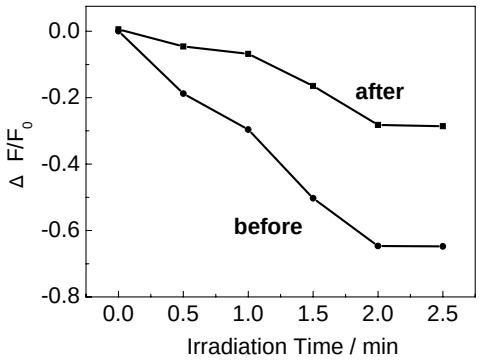

Fig. 10. The relative curves between $\Delta F/F_0$ and irradiation time at 446 nm in ethanol before and after encapsulation, λ_{ex}=325 nm.

Europium (III)/coumarin complexes and silica nanoparticles embedded with the complexes could both be taken up by THP-1 cells and showed blue fluorescence signal after excitation (Fig. 11). In vitro imaging of THP-1 cells indicated active uptake and accumulation of the luminescent complexes and silica nanoparticles. Moreover, it could be observed that the optical signal amplified markedly after encapsulation. So, the result product could be applied as fluorescent nanoprobes in biological imaging and improved the detection sensitivity.

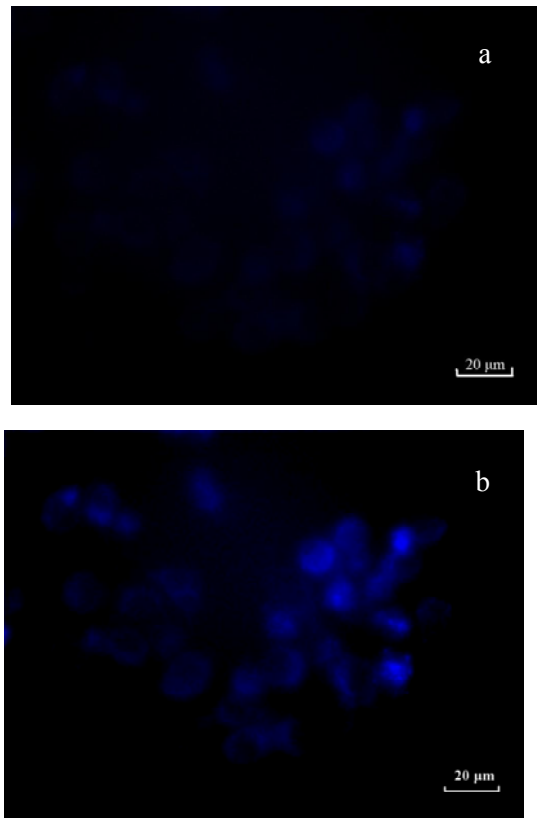

Fig. 11. Fluorescence image of THP-1 cells treated with europium (III)/coumarin complex (a) and complex-loaded silica nanoparticles (b) (Bar=20 μm).

4. Conclusions

The synthesis and characterization of europium (III)/coumarin complex were studied, and then embedded it into silica nanoparticles by an improved sol-gel method. The nanoparticles embedded with europium (III)/coumarin complexes had a spherical morphology, with high photo-stability, good water-solubility and amplified optical signal. Such nanoparticles can be effectively taken up and accumulated by THP-1 cells and emitted stronger blue fluorescence than the exposed complexes in vitro studies. From the above results, we concluded that the final products can be used as a promising fluorescent nanoprobe.

Acknowledgements

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