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# Fluorescence enhancement of europium(III) perchlorate by benzoic acid on bis(benzylsulfinyl)methane complex and its binding characteristics with the bovine serum albumin (BSA)



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#### HIGHLIGHTS

- Two new rare earth complexes highly stables were synthesized for the first time in this research.
- The Eu<sup>3+</sup> emission intensities of both rare earth complexes were high, with long lifetimes.
- The introduction of the second organic ligand benzoic acid enhanced the fluorescence intensity.
- The binding interactions of Eu (III) complexes and bovine serum albumin (BSA) were studied by fluorescence spectra.

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# G R A P H I C A L A B S T R A C T



#### ABSTRACT

A novel ligand with double sulfinyl groups, bis(benzylsulfinyl)methane L, was synthesized by a new method. Its novel ternary complex, EuL<sub>2.5</sub>·L' (ClO<sub>4</sub>)<sub>2</sub>·5H<sub>2</sub>O, has been synthesized [using L as the first ligand, and benzoic acid L' as the second ligand], and characterized by elemental analysis, molar conductivity, coordination titration analysis, FTIR, TG-DSC, <sup>1</sup>H NMR and UV-vis. In order to study the effect of the second ligand on the fluorescence properties of rare-earth sulfoxide complex, a novel binary complex EuL<sub>2.5</sub>·(ClO<sub>4</sub>)<sub>3</sub>·3H<sub>2</sub>O has been synthesized. Photoluminescent measurement showed that the first ligand L could efficiently transfer the energy to Eu<sup>3+</sup> ions in the complex. Furthermore, the detailed luminescence analyses on the rare earth complexes indicated that the ternary Eu (III) complex manifested stronger fluorescence intensities, longer lifetimes, and higher fluorescence quantum efficiencies than the binary Eu (III) materials. After introducing the second ligand L', the fluorescence emission intensities and fluorescence lifetimes of the ternary complex enhanced more obviously than the binary complex. This illustrated that the presence of both the first ligand L and the second ligand L' could sensitize fluorescence intensities of Eu (III) ions. The fluorescence spectra, fluorescence lifetime and phosphorescence spectra were also discussed. To explore the potential biological value of Eu (III) complexes, the binding interaction among Eu (III) complexes and bovine serum albumin (BSA) was studied by fluorescence spectrum. The result indicated that the reaction between Eu (III) complexes and BSA was a static quenching procedure. The binding site number, n, of 0.60 and 0.78, and binding constant,  $K_a$ , of 0.499 and 4.46 were calculated according to the double logarithm regression equation, respectively for  $EuL_{2.5}$ -L'·( $ClO_4$ )<sub>2</sub>·5H<sub>2</sub>O and EuL<sub>2.5</sub>·(ClO<sub>4</sub>)<sub>3</sub>·3H<sub>2</sub>O systems.

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#### Introduction

Trivalent lanthanide ions  $(Ln^{3+})$  with organic–inorganic ligands have dramatically improved the application of luminescent lanthanide ions in chemosensors [1], polymer-based devices [2,3], phosphors, lasers and optical amplifiers [4–6], and bioimaging probes [7–9]. However, luminescence of  $Ln^{3+}$  originated from electronic transitions between the 4f orbital and these transitions are strongly forbidden by the parity selection rules, which lead to low absorption coefficient [10,11]. Some organic ligands such as sulfoxides are well known to be efficient sensitizers for the luminescence of lanthanide ions, whose rare earth complexes could provide distinct advantages, such as high quantum yields, superior luminescence, high thermodynamic stability and good solubility [12,13].

The lanthanide complexes with carboxylic acids have been studied in considerable detail because they show higher thermal, luminescent stabilities and coordination ability for practical application than other lanthanide complex systems [14,15]. As a result, we choose benzoic acid as the second organic ligand in ternary Eu (III) complex in this paper. On the other side, Eu (III) was chosen as metal ion, because it has excellent luminescence properties making it an ideal probe in spectroscopy-based speciation [16]. The purpose of this study was to search out new fluorescence materials, and to explore the effect of the second ligand (L') on the fluorescence properties of rare-earth complexes. So it was reported [17] that the solid ternary complex of europium perchlorate with L and L'. In order to make a comparison, we report here the synthesis and spectroscopic study of the binary complex. The fluorescence properties of the Eu (III) complexes were discussed and their phosphorescence spectra and fluorescence emission mechanisms were also investigated.

As it is well known, the albumin is the richest protein in blood circulatory system, which can combine with many materials and play an important role in transporting protein [18]. BSA has been studied as a model protein in the group due to its structural homology with human serum albumin (HSA). In addition, the two tryptophan residues in the molecule induce to the intrinsic fluorescence of BSA [19]. So the reaction of the rare earth complexes with BSA was investigated based on fluorescence spectroscopy. The results demonstrated complexes caused the fluorescence quenching of BSA via a static quenching procedure, changing the structures of protein accordingly.

#### Experiment

#### Reagents and apparatus

The purity of lanthanide oxide exceeds 99.99%. The rare earth (III) perchlorates were prepared by dissolving their oxide (99.99%) in HClO<sub>4</sub> (2.0 mol L<sup>-1</sup>). All other chemicals were of analytical reagent grade. The stock solution of BSA (purity 99%) was prepared to be the concentration of  $1.0 \times 10^{-5}$  mol L<sup>-1</sup> by dissolving it in buffer solution at pH 7.4, and kept it in the dark at 0-4 °C. By means of dissolving Eu (III) complexes in a small amount of DMF, then diluting them to be the concentration of  $1.0 \times 10^{-4}$ mol  $L^{-1}$  with DMF, respectively, the working solutions of Eu (III) complexes were obtained. The Trise-HCl buffer solution (pH 7.4) was given via adding dropwise HCl  $(0.1 \text{ mol } L^{-1})$  to Tris solution (0.1 mol L<sup>-1</sup>), which used double distilled water. Elemental analysis was carried out on a HANAU analyzer. Rare earth contents of the complexes were determined by EDTA titration using Xylenolorange as an indicator [20]. Conductivity measurements were made by using a  $10^{-3}$  mol L<sup>-1</sup> solution in DMF on a DDS-11D conductivity meter at room temperature. The thermal behavior was

monitored on SDTQ600 differential scanning calorimeter and thermal gravimetric analyzer. FT-IR spectra were recorded on KBr disk by using NEXUS 670 FT spectrometer in  $4000-400 \text{ cm}^{-1}$  region. The ultraviolet spectra (190-400 nm) of the ligands and the ternary complex were recorded on a Shimadzu UV-265 spectrophotometer and acetone was used as a reference and solvent (concentrations:  $1 \times 10^{-5}$  mol L<sup>-1</sup>). <sup>1</sup>H NMR spectra were measured by Bruker AC-300 instrument in DMSO-d<sub>6</sub>. The fluorescence spectra were determined by FLS920 fluorescence photometer and the excitation and emission slit width of the complexes was 2 nm. The phosphorescence spectra were measured by F-4500 FL spectrophotometer at room temperature, and the excitation and emission slit width was 5.0 and 10.0 nm and excitation wavelength was 300 and 347 nm. Fluorescent decay curves were determined by FLS920 Combined Steady State and Lifetime Spectrometer. Fluorescence measurements of complexes with BSA were recorded on a RF-5301PC spectrofluorophotometer Shimadzu at room temperature.

#### Synthesis of the novel ligand

The synthesis route of the sulfoxide was expressed in Fig. 1.

#### *The synthesis of the sulfide*

The sulfide was synthesized according to the method of Shriner et al. [21].

Sodium hydroxide was dissolved in alcohol. The mechanical stirrer was started and benzyl mercaptan was added in a slow but steady stream. Then dibromomethane was run in drop by drop. The mixture was refluxed, with stirring, on a steam-bath for one and one-half hours. The hot solution was immediately poured on ice powder. A light yellow solid was precipitated, filtered and dried in vacuum. The sulfide was a light yellow crystalline substance which was purified by recrystallization from alcohol. Yield: 60–70%, mp: 46–48 °C. Anal. calcd. For  $C_{15}H_{16}S_2$ : C, 69.23%; H, 6.154%; S, 24.616%; found: C, 69.20%; H, 6.353%; S, 24.32%.

#### Synthesis of the sulfoxide

Bis(benzylthio)methane was dissolved in acetic acid, then 30% hydrogen peroxide was added to it at once. The mixture was stirred continuously at room temperature for 24 h. After the reaction, the mixture was extracted with ether until the pH of mixture was 7.0. Then a white solid was precipitated, filtered and dried in vacuum. Yield: 80%, mp: 212–214 °C. Anal. calcd. For  $C_{15}H_{16}S_2O_2$ : C, 61.64%; H, 5.479%; found: C, 61.33%; H, 5.485%.

#### Synthesis of the ternary Eu (III) complex

4 mmol the novel ligand L and 1 mmol the second ligand L' were dissolved in ethyl ether solution. So did 3 mmol  $Eu(ClO_4)_3$ . During the stirring,  $Eu(ClO_4)_3$  ethyl ether solution was added to ethyl ether solution containing ligands. After a few minutes a white precipitate was formed. The mixture was stirred for 0.5 h



Fig. 1. The synthesis scheme of L.

and precipitate was filtered. The products were washed with ethyl ether for several times, and then dried in drying oven. It was pow-der (yield > 90%).

#### Preparation of the binary rare-earth complex

An ether solution containing 3 mmol rare earth perchlorates was added drop by drop in an ether solution containing 5 mmol ligand L. After a few minutes a white precipitate was formed. The mixture was stirred for 0.5 h and precipitate was filtered. The products were washed with ether for several times, and then dried in drying oven. It was white powder (yield > 90%).

#### BSA binding experiments

To a 5 mL of calorimetric tube, 2 mL BSA stock solution and 10 µm L of Eu (III) complexes working solution (concentration:  $0-25 \times 10^{-7}$  mol L<sup>-1</sup>) was added in turn. The mixture was incubated for 5 min at room temperature. Subsequently, quenching of the emission intensity of BSA at 347 nm was monitored using the Eu (III) complexes as quenchers with the increased concentration. Fluorescence spectra were recorded from 300 to 450 nm at an excitation wavelength of 296 nm.

# **Results and discussion**

### Properties of the complexes

Elemental analysis and molar conductivity values were presented in Table 1, and the composition of the ternary complex was  $EuL_{2.5}$ ·L'·(ClO<sub>4</sub>)<sub>2</sub>·5H<sub>2</sub>O. The binary complex conformed to  $EuL_{2.5}$ (ClO<sub>4</sub>)<sub>3</sub>·3H<sub>2</sub>O. The molar conductivity values of the complexes in DMF were in good accordance with the formula as 1:2

#### Table 1

Composition analysis (%) and molar conductivities (S  $\rm cm^2\,mol^{-1})$  of the rare earth complexes (25  $^\circ C).$ 

Complexes <sup>a</sup>	М	Anal. calcd. (found) (%)		$\lambda_m(\mathbf{a})$	
		С	Н	RE	$(S \text{ cm}^2 \text{ mol}^{-1})$
$EuL_{2.5} \cdot (ClO_4)_3 \cdot 3H_2O$	1231.4	36.24 (36.45)	3.89 (3.73)	11.78 (12.23)	151.3
$EuL_{2.5}{\cdot}L'{\cdot}(ClO_4)_2{\cdot}5H_2O$	1293.1	40.92 (41.33)	4.18 (4.26)	12.32 (11.76)	178.2

<sup>a</sup>  $L = C_6H_5CH_2SOCH_2SOCH_2C_6H_5$ ,  $L' = C_6H_5COO^-$ .



Fig. 2. The TG–DSC curves of  $EuL_{2.5}$ ·L'·(ClO<sub>4</sub>)<sub>2</sub>·5H<sub>2</sub>O.



Fig. 3. Infrared absorption spectrum of L.



Fig. 4. Infrared absorption spectrum of L'.

electrolytes [22]. Those two complexes were white powder, which were stable under atmospheric conditions, and soluble in DMF and DMSO.

#### TG-DSC studies

The TG–DSC analyses were carried out up to 1000 °C in N<sub>2</sub> at a heating rate of 10 °C min<sup>-1</sup>. The TG–DSC curves of EuL<sub>2.5</sub>·L'·(ClO<sub>4</sub>)<sub>2</sub> .5H<sub>2</sub>O was showed in Fig. 2. The TG curve of Eu (III) complex



Fig. 5. Infrared absorption spectrum of the ternary complex. EuL<sub>2.5</sub>·L'·(ClO<sub>4</sub>)<sub>2</sub>·5H<sub>2</sub>O.

**Table 2**Some main IR data of ligand and the rare earth complexes.

were consistent with element analyses.

L	L′	Eu (III) complex
-	-	3415
1622	-	1619
3058	3066	3062
770	709	770
700	681	702
1038	-	1019
2961	-	2966
1404	-	1402
3027	-	3030
1451	-	1454
-	-	1080
-	-	624
-	1416	1402
-	1550	1663
	L - 1622 3058 770 700 1038 2961 1404 3027 1451 - - - - -	L         L'           -         -           1622         -           3058         3066           770         709           700         681           1038         -           2961         -           1404         -           3027         -           1451         -           -         -           -         1416           -         1550

Table 3

Chemical shift data of	<sup>1</sup> H NMR Spectra	(1)	× 10 <sup>-</sup>	-6)
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Ligand and complexes	<sup>1</sup> H NMR		
			-C <sub>6</sub> H <sub>5</sub> -(×2)
L	3.311-4.143	4.246-4.452	7.279–7.379
	(s. 2 H)	(s. 4 H)	(m. 10 H)
$EuL_{2.5} \cdot (ClO_4)_3 \cdot 3H_2O$	3.381–4.153	4.239–4.452	7.288–7.371
	(s. 2 H)	(s. 4 H)	(m. 10 H)
$EuL_{2.5}{\cdot}C_6H_5COO^-{\cdot}(ClO_4)_2{\cdot}5H_2O$	3.839–4.145	4.247–4.454	7.278–7.383
	(s, 2 H)	(s, 4 H)	(m, 10 H)

#### Infrared spectra

Ligand L and the ternary Eu (III) complex were determined by IR spectrometer and the significant information was presented in Figs. 3–5 and Table 2.

showed that the first mass loss occurred in the range 35.7–188.0 °C (v<sub>S=0</sub>) and the cated that Eu is group. The character the release of the five water molecules (6.97%). The comparatively low temperature for water loss indicated that they were crystal water. At the same time, DSC curve showed small endothermic peaks at 111.2 °C when losing water. In addition, there were two exothermic peaks in DSC curve, which were attributed to the decomposition of the ligands L and L', as well as two obvious weight loss occurred on the TG curve. The final product was found to be EuCl(CO<sub>3</sub>), near 1000 °C. The total weight loss of the complex was closely corresponding to the calculated values. The results

Fig. 5 showed the disappearance of the band at the 1038 cm<sup>-1</sup> ( $v_{S=0}$ ) and the existence of band at 1019 cm<sup>-1</sup> ( $v_{S=0}$ ), which indicated that Eu (III) ions were bonded with oxygen atom in sulfonyl group. The characteristic absorption bands of benzene appeared at approximately 3058 cm<sup>-1</sup> ( $v_{C-H}$ ), 1622 cm<sup>-1</sup> ( $v_{C=C}$ ), 770 cm<sup>-1</sup> ( $\delta_{C-H}$ ) and 700 cm<sup>-1</sup> ( $\delta_{C-H}$ ) in Fig. 3 and they were also found in the spectra of ternary Eu (III) complex.

The  $\nu_{as(COO^-)}$  and  $\nu_{s(COO^-)}$  absorption of L' occurred at 1550 and 1416 cm<sup>-1</sup>, and the  $\Delta\nu$  value was 134 cm<sup>-1</sup> in Fig. 4. The  $\nu_{as(COO^-)}$  and  $\nu_{s(COO^-)}$  absorption bands appeared at 1663 and 1402 cm<sup>-1</sup> in the ternary Eu (III) complex and the  $\Delta\nu$  value was 261 cm<sup>-1</sup> larger than that of L', which could be deduced that carboxyl group was coordinated with Eu (III) ions by monodentate type [23]. When

400



Fig. 6. The UV absorption spectra of (a) L, (b) L', and (c) the Eu (III) complex.



Fig. 7. Fluorescent excitation and emission spectra of the ternary complex  $EuL_{2.5}\cdot L'(CIO_4)_2\cdot 5H_2O.$ 



Fig. 8. Fluorescent excitation and emission spectra of the binary complex  $EuL_{2.5}\text{-}(CIO_4)_3\text{-}3H_2O.$ 

 $ClO_4^-$  was not coordinated, it was Td symmetry and there were two absorption bands. When  $ClO_4^-$  was coordinated, it was  $C_{3v}$  symmetry and there were five absorptions [24,25]. In the spectra of the ternary Eu (III) complex, two absorption bands could be seen clearly at approximately 1080 and 624 cm<sup>-1</sup>. Therefore,  $ClO_4^-$  was Td symmetry. In terms of molar conductivities, it could be suggested that none of  $ClO_4^-$  were bonded with Eu (III) ions.

#### UV absorption spectra

The UV absorption spectra of (a) L, (b) L', and (c) the ternary Eu (III) complex were recorded in Fig. 6 and DMF was used as a reference and solvent. In Fig. 6(a) and (b), the major band of  $\pi$ - $\pi$ <sup>\*</sup> electronic transition in phenyl group was observed at 266 nm and



**Fig. 9.** Fitted curve of (a) binary Eu (III) complex and (b) ternary Eu (III) complex, recorded at room temperature, setting the Eu<sup>3+</sup> emission and excitation wavelength at 612 and 394 nm, respectively.

272 nm, respectively. There was one absorption band at 268 nm in the absorption spectra of (c) the ternary Eu (III) complex. There was no obvious shift in the complex, indicating that the conjugated system of benzoic acid was not influenced obviously after coordinating with the rare earth ions.

#### <sup>1</sup>H NMR spectra

The <sup>1</sup>H NMR spectra data of L and its complexes in DMSO-d<sub>6</sub> could be seen from Table 3. The <sup>1</sup>H NMR of the ligand showed the proton resonance of two phenyl group peaks was at  $\delta7.279 \sim 7.379$  ppm, and the integral intensities showed that it had ten protons. The proton resonance of two methylene group peaks was at  $\delta4.246 \sim 4.452$  ppm, the integral intensities of peaks showed that it had four protons. There was also one methylene group in the ligand and the proton resonance of its peaks was at  $\delta3.311 \sim 4.143$  ppm, and the integral intensities showed that it had two protons. And the ratio of resonance peaks area was 10:2:4.

In the <sup>1</sup>H NMR spectrum of the ternary Eu (III) complex, the proton resonance peaks of phenyl and methylene group were very clear, which had shifted at different degree. The reason may be concerned with coordinate effect. A broad proton resonance peak was found between  $\delta$ 7.278 and  $\delta$ 7.383 ppm, which belonged to the phenyl group absorption peak of L overlapped with that of L'.

Table 4

Comparison of fluorescent emission spectra data of Eu (III) binary and ternary complex.

Complex	$\lambda_{ex}$ (nm)	$\lambda_{em}$ (nm)	I (a.u.)	Energy state transitions
$EuL_{2.5}$ ·(ClO <sub>4</sub> ) <sub>3</sub> ·3H <sub>2</sub> O	394	612	8153	${}^{5}D_{0} \rightarrow {}^{7}F_{2}$
$EuL_{2.5} \cdot C_6H_5COO^- \cdot (ClO_4)_2 \cdot 5H_2O$	394	612	34,510	${}^{5}D_{0} \rightarrow {}^{7}F_{2}$



**Fig. 10.** The fluorescence decay curves of (a) binary Eu (III) complex and (b) ternary Eu (III) complex, recorded at room temperature, setting the  $Eu^{3+}$  emission and excitation wavelength at 612 and 394 nm, respectively.

Table 5Photoluminescent data of the compounds.

	$EuL_{2.5} \cdot (ClO_4)_3 \cdot 3H_2O$	$EuL_{2.5} \cdot C_6H_5COO^- \cdot (ClO_4)_2 \cdot 5H_2O$
v <sub>00</sub>	17,271	17,271
U01	16,892	16,863
v <sub>02</sub>	16,340	16,340
v <sub>03</sub>	15,385	15,385
v <sub>04</sub>	14,327	14,327
I <sub>01</sub>	2298	7920
I <sub>02</sub>	8153	34,510
$I_{02}/I_{01}$	3.548	4.357
A <sub>00</sub>	12.470	4.518
A <sub>01</sub>	50	50
A <sub>02</sub>	183.388	224.841
A <sub>03</sub>	8.624	16.448
A <sub>04</sub>	69.984	231.395
A <sub>r</sub>	324.467	527.203
τ	0.434	0.641
$1/\tau$	2304	1560
η%	14.08	33.80

#### Fluorescence spectra

The excitation and emission slit width of the complexes was 2 nm. The fluorescence excitation and emission spectra of europium perchlorate ternary and binary complexes were measured at room temperature. According to the fluorescence spectra of the complexes reported in Figs. 7 and 8 and Table 4, we could see that the ternary complex had excellent fluorescence properties.

The excitation spectra of the two Eu (III) complexes were obtained by monitoring their emissions at 612 nm; the maximum peaks were at 394 nm. The excitation spectra of the two complexes were similar (shown in Figs. 7 and 8). There were five emission peaks in the fluorescence emission spectra of the binary and ter-



Fig. 11. The phosphorescence spectrum of L.

nary complexes, which were attributed to the characteristic  ${}^{5}D_{0}-{}^{7}F_{J}$  (J = 0, 1, 2, 3, 4) transitions of Eu (III) at about 579, 592, 612, 650, 698 nm, and the  ${}^{5}D_{0}-{}^{7}F_{2}$  transition of Eu (III) presented the higher emission intensity at about 612 nm.

Under the same conditions, the intensity of the strongest fluorescence emission peak of Eu (III) ion was 8153 a.u. in the Eu (III)-sulfoxide binary system. It is worth mentioning that the fluorescence emission intensity peak of the Eu (III) ion was 34,510 a.u. in the Eu (III)-sulfoxide-benzoic acid ternary system. It was 4.23 times as great as that of the binary system. In the ternary complex, L and L' could play a mutually synergistic part in the energy transfer process of the ligand to Eu (III) ion. The luminescence of the Eu (III) ion in the ternary system was more enhanced than that of the binary systems.

## Luminescence decay times $(\tau)$ and emission quantum efficiency $(\eta)$

The fluorescence decay curves of the binary complex and the ternary complex were measured. Fig. 9 showed the fitted curve of (a) binary Eu (III) complex and (b) ternary Eu (III) complex. Fig. 10 showed the decay curves of (a) binary Eu (III) complex and (b) ternary Eu (III) complex. The fluorescence lifetime values of the Eu (III) complexes were calculated by the double exponential mode  $[\tau = (A_1\tau_1^2 + A_2\tau_2^2)/(A_1\tau_1 + A_2\tau_2)]$ . From the results, the fluorescence lifetime of the ternary complex was 641.07 µs, and the fluorescence lifetime of the binary complex was 434.14 µs.

According to the emission spectrum and the lifetime of the Eu<sup>3+</sup> first excited level ( $\tau$ , <sup>5</sup>D<sub>0</sub>), the emission quantum efficiency ( $\eta$ ) of the <sup>5</sup>D<sub>0</sub> Eu<sup>3+</sup> excited state can be calculated. There is a hypothesis that only nonradiative and radiative processes are substantially involved in the depopulation of the <sup>5</sup>D<sub>0</sub> statea,  $\eta$  can be confirmed as follows [26]:

$$\eta = \frac{A_r}{A_r + A_{nr}} \tag{1}$$

where  $A_r$  and  $A_{nr}$  are radiative and nonradiative transition rates, respectively.  $A_r$  can also be obtained by summarizing the radiative rates  $A_{0l}$  for each  ${}^5D_0 \rightarrow {}^7F_l$  (J = 0-4) transitions of Eu<sup>3+</sup>.

$$A_r = \sum A_{0J} = A_{00} + A_{01} + A_{02} + A_{03} + A_{04}$$
<sup>(2)</sup>

The branching ratio for the  ${}^{5}D_{0} \rightarrow {}^{7}F_{5,6}$  transitions can be neglected as they are not measured experimentally, whose influence can be elided in the depopulation of the  ${}^{5}D_{0}$  excited state. Since  ${}^{5}D_{0} \rightarrow {}^{7}F_{1}$  belongs to the isolated magnetic dipole transition, it is practically independent of the chemical environments around the Eu<sup>3+</sup> ion, and thus can be considered as an internal reference for the whole spectrum, the experimental coefficients of spontaneous emission,  $A_{0l}$  can be calculated according to the equation [27–30].



Fig. 12. The phosphorescence spectrum of L'.



Fig. 13. Triplet state of ligands and excited state of the Eu (III) ion.

$$A_{0J} = A_{01}(I_{0J}/I_{01})(v_{01}/v_{0J})$$
(3)

Here,  $A_{0J}$  is the experimental coefficient of spontaneous emission.  $A_{01}$  is the Einstein's coefficient of spontaneous emission between the  ${}^{5}D_{0}$  and  ${}^{7}F_{1}$  energy levels. In vacuum,  $A_{01}$  as a value of 14.65 s<sup>-1</sup>, when an average index of refraction *n* equal to 1.506 was considered, the value of  $A_{01}$  can be determined to be 50 s<sup>-1</sup> approximately ( $A_{01} = n^{3}A_{01(vac)}$ ) [31].  $I_{01}$  and  $I_{0J}$  are the integrated



$$I_{i-i} = h \varpi_{i-i} A_{i-i} N_i \approx S_{i-i} \tag{4}$$

where *i* and *j* are the initial (<sup>5</sup>D<sub>0</sub>) and final levels (<sup>7</sup>F<sub>0-4</sub>), respectively,  $\omega_{i-j}$  is the transition energy,  $A_{i-j}$  is the Einstein's coefficient of spontaneous emission, and  $N_i$  is the population of the <sup>5</sup>D<sub>0</sub> emitting level. On the basis of reference [32–36], the value of  $A_{01} \approx 50 \text{ s}^{-1}$  and the lifetime ( $\tau$ ), radiative ( $A_r$ ), and nonradiative ( $A_{nr}$ ) transition rates are related through the following equation:

$$A_{tot} = 1/\tau = A_r + A_{nr} \tag{5}$$

On the basis of the above discussion, the quantum efficiencies of the two kinds of europium complexes can be determined, as shown in Table 5. From the equation of  $\eta$ , it can be seen the value  $\eta$  mainly depends on the values of two quantum: one is lifetimes and the other is  $I_{02}/I_{01}$ . As can be clearly seen from Table 5, the quantum efficiencies of EuL<sub>2.5</sub>·L·(ClO<sub>4</sub>)<sub>2</sub>·5H<sub>2</sub>O ( $\eta$  = 33.80%) is higher than that of EuL<sub>2.5</sub>·(ClO<sub>4</sub>)<sub>3</sub>·3H<sub>2</sub>O·(14.08%).

#### Phosphorescence properties of ligands and luminescence mechanism

The phosphorescence spectra of ligands L and L' were recorded using a F-4500 FL spectrophotometer in solid state and are shown in Figs. 11 and 12. The excitation and emission slit width of the complexes was 5.0 nm and 10.0 nm, and excitation wavelength was 300 nm and 347 nm, respectively.

According to the energy transfer and intra-molecular energy transfer mechanism [37,38], intra-molecular energy transfer efficiency chiefly lied on two energy transfer processes: one was the transitions from the triplet state energy level of ligand to the excited states of the Eu (III) ion by Dexter's resonant exchange interaction [39], the other was just an inverse energy transfer process by the thermal deactivation mechanism [40]. Fig. 11 shows that two bands could be seen clearly at 519 and 471 nm which corresponded to the triplet state energy level of ligand L T<sub>1</sub> (19,268 cm<sup>-1</sup>) and T<sub>2</sub> (21,231 cm<sup>-1</sup>), respectively. The triplet state energy level T<sub>1</sub> was appropriately higher than <sup>5</sup>D<sub>0</sub> of Eu (III) ion (17,241 cm<sup>-1</sup>). Fig. 12 showed that the triplet state energy level of the second ligand L' was at 500 nm (T<sub>1</sub>: 20,000 cm<sup>-1</sup>) and 470 (T<sub>2</sub>: 21,276 cm<sup>-1</sup>), which was also higher than <sup>5</sup>D<sub>0</sub> of Eu (III) ion. These results are shown in Fig. 13, which also shows that the triplet state energy level T<sub>1</sub> of the ligands L and L' was higher than the



**Fig. 14.** Effect of (a) EuL<sub>2.5</sub>·L'(ClO<sub>4</sub>)<sub>2</sub>·5H<sub>2</sub>O, (b) EuL<sub>2.5</sub>·(ClO<sub>4</sub>)<sub>3</sub>·3H<sub>2</sub>O on fluorescence spectrum of BSA (Room temperature, pH = 7.4,  $\lambda_{ex}$  = 296 nm). a–f, c(BSA) = 1.0 × 10<sup>-5</sup> mol L<sup>-1</sup>, c(a and b) (×10<sup>-7</sup> mol L<sup>-1</sup>): 0, 5.0, 10.0, 15.0, 20.0 and 25.0, respectively.



Fig. 15. The Stern–Volmer curves of BSA quenched by (a) EuL<sub>2.5</sub>·L'·(ClO<sub>4</sub>)<sub>2</sub>·5H<sub>2</sub>O and (b) EuL<sub>2.5</sub>·(ClO<sub>4</sub>)<sub>3</sub>·3H<sub>2</sub>O.

# Table 6

# Fluorescence quenching constants of complexes-BSA system.

Complexes	pН	$\begin{array}{l} K_{SV} \\ (\times 10^4  \mathrm{L}  \mathrm{mol}^{-1}) \end{array}$	$K_q$ (×10 <sup>12</sup> L mol <sup>-1</sup> s <sup>-1</sup> )	<i>R</i> <sup>2</sup>
$\begin{array}{l} EuL_{2.5} \cdot L' \cdot (ClO_4)_2 \cdot 5H_2O \\ EuL_{2.5} \cdot (ClO_4)_3 \cdot 3H_2O \end{array}$	7.4	6.97 7.32	6.97 7.32	0.99854 0.99834

excited state energy level  ${}^{5}D_{0}$  of Eu (III). It was deduced that two ligands could absorb energy effectively and transfer energy to the Eu (III) ion, and enhanced the fluorescence emission intensity. Therefore, the type of luminescence of Eu (III) ion was  $L \rightarrow M$ . Because both of the triplet state energy level  $T_{1}$  of L and  $T_{1}$  of the L' was higher than the excited state energy level  ${}^{5}D_{0}$  of Eu (III), they could be matched better with the  ${}^{5}D_{0}$  energy level of the Eu (III) ions. In the Eu (III)-sulfoxide–benzoic acid ternary system, L and L' were able to transfer energy to the Eu (III) ion together. Therefore, in the ternary complex, the two ligands could play a mutually synergistic part in the energy transfer process of the ligands to the Eu (III) ion, making the fluorescence emission of the Eu (III) ion increase exponentially in the ternary complex. All these suppositions have been confirmed by experiment.

# BSA binding studies

# Quenching mechanism of fluorescence of BSA by Eu (III) complexes

At the excitation wavelength of 296 nm, the fluorescence spectra of BSA with varying concentrations of Eu (III) complexes are shown in Fig. 14. The naturally fluorescence of BSA at around 347 nm was gradually quenched along with the increasing concentration of Eu (III) complexes. Results above indicate that there are strong interactions and radiationless energy transfer between Eu (III) complexes and protein [41–43]. In given conditions (such as pH 7.4 and room temperature), fluorescence quenching may result from ground complex formation, energy transfer and dynamic quenching processes [44]. Dynamic quenching is a process that the fluorophore and the quencher come into contact during the lifetime of the excited state, whereas static quenching refers to fluorophore–quencher complex formation [43]. Fluorescence quenching data can be analyzed by the Stern–Volmer equation [45]:

$$F_0/F = 1 + K_{SV}[Q] = 1 + \tau_0 \kappa_q[Q]$$
(1)

where  $F_0$  and F are the fluorescence intensities in the absence and presence of quencher, respectively.  $K_{SV}$  is the Stern–Volmer quenching constant. [Q] is the concentration of quencher.  $\tau_0$  is the average fluorescence lifetime of biomolecule at about  $10^{-8}$  s [46].  $\kappa_q$  is the biomolecule quenching rate constant and the value of the maximum



Fig. 16. Double log plots of (a)  $EuL_{2.5}$ ·L'-(ClO<sub>4</sub>)<sub>2</sub>·5H<sub>2</sub>O and (b)  $EuL_{2.5}$ ·(ClO<sub>4</sub>)<sub>3</sub>·3H<sub>2</sub>O quenching effect on BSA fluorescence.

scattering collision quenching constant is  $2.0 \times 10^{10} \text{ mol}^{-1} \text{ s}^{-1}$  [47]. Fig. 15 shows the Stern–Volmer plots of  $F_0/F$  versus [Q], the values of  $K_{SV}$  and correlation coefficient  $R^2$  are outlined in Table 6. The results show the values of  $\kappa_q$  are much larger than that of the maximum scattering collision quenching constant, which indicate that the main quenching mechanism of BSA by Eu (III) complexes should be a static quenching procedure [43].

# Binding constants and binding sites

As we have known from results above, the fluorescence quenching coming from Eu (III) complexes formation between protein and quencher is static quenching process, hence the equilibrium between free and bound molecule can be expressed by the following double logarithm regression equation [48]:

#### Table 7

Binding constants *K*<sub>a</sub> and number of binding sites n for complexes-BSA system.

Complexes	$K_a (\times 10^3 \mathrm{L}\mathrm{mol}^{-1})$	n	$R^2$
$\begin{array}{l} EuL_{2.5} \cdot L' \cdot (ClO_4)_2 \cdot 5H_2O \\ EuL_{2.5} \cdot (ClO_4)_3 \cdot 3H_2O \end{array}$	0.499	0.60	0.98070
	4.46	0.78	0.98933

$$\lg\left(\frac{F_0 - F}{F}\right) = \lg K_a + n \lg[Q]$$
(2)

where  $K_a$  and n are the binding constant and the number of binding site, respectively.

From Eq. (2), the binding parameters can be obtained by a plot of  $\lg[(F_0 - F)/F]$  versus  $\lg[Q]$ . As it is expressed in Fig. 16 and Table 7, the value of  $K_a$  is large, indicating the combination of BSA with Eu (III) complexes is relatively strong. Both  $EuL_{2.5}\cdot L' \cdot (ClO_4)_2 \cdot 5H_2O$  and EuL<sub>2.5</sub> (ClO<sub>4</sub>)<sub>3</sub>·3H<sub>2</sub>O presented similar values of n, which were calculated as 0.60 and 0.78, respectively.

#### Conclusion

A novel ligand and its corresponding ternary and binary Eu (III) organic luminescence complexes were synthesized. These complexes had strong luminescence properties. Fluorescence spectroscopy showed that the strongest characteristic fluorescence emission intensity of the ternary complex was 4.23 times as great as the binary complex. The experimental results showed that fluorescence emission intensity of Eu (III) in the complex could increase with the increase of fluorescence lifetime and fluorescence quantum efficiency.

The introduction of the second organic ligand influenced the fluorescence intensity of the rare-earth organic complexes. When triplet state energy level of the second ligand was higher than the excited state energy level <sup>5</sup>D<sub>0</sub> of Eu (III) ion, the second ligand transferred energy to the Eu (III) ion. This ligand could match well with the Eu (III) ion's energy level and sensitized the Eu (III) ion luminescence. The luminescent material which we synthesized had good solubility in DMF and DMSO.

The binding study of Eu (III) complexes and BSA was carried out by fluorescence spectroscopy. The fluorescence quenching results indicated the fluorescence quenching mechanism of BSA with Eu (III) complexes was a static quenching process. The combination of BSA with Eu (III) complexes is relatively strong. The binding constants and the number of binding sites were also given in this paper.

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