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Luminescent Europium Chelates Synthesis and Fluorescence Properties

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The europium(III)-chelating ligand BSPDA containing a thiolated group (BSPDA, an abbreviation of 4,7-bis(sulfhydrylphenyl)-1,10- phenanthroline-2,9-dicarboxylic acid) was synthesized from BCPDA (BCPDA, abbreviated form of 4,7-bis (chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid) by reduction, and then complexed with europium (III, Eu^{3+}) to give 1:1 and 2:1 complexes in molar ratio. The luminescent europium chelates' fluorescence spectra and related lifetimes exceeding several hundreds of microseconds were determined, confirming that the 2:1 complexes have a slightly longer lifetime than the 1:1 complexes because of the more efficient complex interaction and energy transfer from the ligand BSPDA to europium(III) in the former. The results have significant implications to time-resolved-fluorescence-based nanostructuring and detection on biosensor surfaces.

1. Introduction

The challenge and development of micro-electro-mechanical system (MEMS) or microoptical electro-mechanical system (MOEMS) advance modern surface engineering technology, which has been greatly accelerating the emergence of novel nanomaterials and relevant applications in biosensors and microdevices, particularly for the miniaturization of lab on chip (LOC devices) and micro-total analysis systems (μ-TASs). To improve the fabrication and application of biosensor and microdevices, it is necessary to fabricate various single layer or multilayer of tunable and smart films. There is an increasing demand for surface engineering processing techniques such as surface tailoring, surface patterning and surface structuring.[1–3] Among common

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bio-microdevices, DNA chips are typical biosensors whose principle is based on the simultaneous biochemical recognition of arrayed surface molecules.[4] The detection ability of DNA chips can be improved by employing various chemical labeling and physical methods, including direct-signal (such as fluorescence) and indirect-signal (such as chemiluminescence) amplifications from a marker or label of DNA probes for magnifying signaling responses, so that very small negligible differences in hybridization are amplified to be discriminated; the detection sensitivity and specificity of DNA chips could also be greatly improved. These methods include the following various biological and chemical amplification techniques, such as nanolabeling, fluorescence labeling, silver staining, branched-chain DNA assay labeling, and enzyme amplification labeling; and various physical amplification technologies, such as the analysis of optics, photoelectron spectrum, and mass spectrum, electrochemistry and conductometry. Besides these, some biochemical reactions like PCR could repetitively copy and magnify the numbers or amounts of substrates tested, resulting in the amplification of the sensitivity and specificity of DNA chips.[4–8] However, when DNA chips labeled with traditional fluorescence dyes (*e.g.*, Cy3 and Cy5) are being detected, the detection sensitivity and specificity of these conventional dyes are severely compromised owing to background signals coming from light scattering, an endogenous sample's fluorescence, optics and substrate fluorescence and other factors. The lifetimes of traditional dyes are often on the nanosecond scale with a small Stokes shift of approximately several tens of nanometers and relatively broad-banded emission. Such serious background interferences and limitations of the conventional dyes prompted previous investigations and our attention to replace these dyes.[9–13] Some rare-earth ions and related complexes have drawn attention as possible candidates. Generally, rare-earth ions and their complexes have lifetimes on the millisecond scale, a large Stokes shift greater than several hundreds of nanometer and a strong narrow-banded emission in specific transitions, which are typical advantages over conventional dyes. Therefore, the real merits of using rare earth complexes for labeling are the efficient discrimination against unwanted background interferences and convenient time-resolved fluorescence (TRF) detection format. Previous studies have shown the fabrication of a mosaic DNA chip.[14,15]

To apply TRF technology to gene chip detection, a suitable chelator and europium complexes with time-resolved fluorescence characteristics should be synthesized.

In recent years, biolabeling, detection and diagnostics based on the nano-gold nanostructure have been developing rapidly. Common features of these techniques are the utilization sulfhydryl functionalization and the assembly between nano-gold-structure or nanostructured biomolecules and analytes to achieve specific recognition and detection.[16,17] Here, we proposed a time-resolved chelator, BSPDA, containing a thiol functionality (BSPDA, abbreviated form of 4,7- bis(sulfhydrylphenyl)-1,10-phenanthroline-2,9-dicarboxylic acid) synthesis from BCPDA (BCPDA, abbreviated form of 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid) by reduction, and then complexed it with europium(III, Eu^{3+}) to give 1:1 and 2:1 complexes in molar ratio.[18] The europium complexes' fluorescence spectra were determined and related lifetimes exceeding several hundreds of microseconds were determined. We verified that the 2:1 complexes have a slightly longer lifetime than the 1:1 complexes

because of a more efficient complex interaction and energy transfer from the ligand BSPDA to europium(III) in the former. As regards further TRF investigation and possible DNA detection, the discovery is of fundamental interest and has significant implications to time-resolved-fluorescence-based nanostructure construction and detection on biosensor surfaces.[18–21]

2. Experimental

The reagents used were of analytical grade, which included hydrochloric acid, tin grains, petroleum ether, sodium carbonate, anhydrous magnesium sulfate. They were purified and diluted accordingly prior to use. 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid（BCPDA, 98%）was prepared in the laboratory as reported elsewhere.[9,10]

4,7-Bis(sulfhydrylphenyl)-1,10-phenanthroline-2,9-dicarboxylic acid（BSPDA, 2）was synthesized as follows: A 100-ml, two-necked, round-bottomed flask with a magnetic stirrer was charged with BCPDA (0.068 g, 1.1×10^{-4} mol), hydrochloric acid (50 ml) and tin particles (0.5 g) washed with hydrochloric acid prior to addition. The mixture was heated in a water bath at 40°C for 2 h. After stirring, many tiny bubbles were noted, and the solution gradually changed from yellowish to bright yellow. Stirring was stopped when the tin particles were all dissolved. The reaction mixture was extracted with petroleum ether and rinsed with a saturated aqueous solution of sodium carbonate (20 ml). The organic layer was then dried by adding anhydrous magnesium sulfate, and then placed in a rotary evaporator to evaporate the solvent. A yellow solid (0.045 g) was obtained in about 84% yield and m.p.>300°C. The yellow solid was characterized as follows: nuclear magnetic resonance (NMR), infrared spectrum (IR) and elemental analysis. The characterization results show that the solid is the target product BSPDA (2).

According to the molar ratio of BSPDA to Eu^{3+} of 1:5, a solution containing excessive europium chloride was added to the anhydrous ethanol solution of BSPDA (0.049 g, 0.1 mmol). The mixture was left to stand for one day and the yellow solid was separated. The solid was rinsed with ethanol, filtered, and air-dried to give a BSPDA-Eu^{3+} complex. Similarly, another BSPDA-Eu^{3+} complex in a molar ratio of 1:1 was also prepared.

Given amounts of the above europium complexes (0.01–0.02 g) were taken, heated at 800°C to form europium oxide, which was then dissolved in hydrochloric acid (6M). The resulting solution was evaporated in a water bath to remove excess acid. Europium content in the above complexes was determined by the EDTA (aqueous solution of disodium EDTA, 0.01mol/L) volumetric method using xylenol orange as a indicator and hexamethylene tetramine (40%) as a buffer solution. The samples of BSPDA and BSPDA- Eu^{3+} complexes were laminated with potassium bromide and the corresponding IR spectra were recorded using an AVATAR 360 Fourier transform infrared spectrometer at 4 cm^{-1} resolution. Sixty-four scans were collected per trace. Fluorescence excitation and emission spectra, and fluorescence lifetime were measured using a Spex Fluorolog-3 photon-counting emission spectrometer. The conditions for recording fluorescence excitation and emission spectra were as follows: lamp house, xenon lamp (450 W); Spex 600 grooves/mm double raster; 1200 grooves/mm single raster; excitation wavelength,

338 nm; emission wavelength, 616 nm; slit widths of excitation and emission, both 10 nm; step width, 1 nm; integration time, 0.1 s. The detector used was a Hamamatsu R2658P photomultiplier, with a working voltage of –1500 V. Scattered light was eliminated with a Schott 500 nm (KV 500) filter. The conditions for measuring fluorescence lifetime were as follows: lamp house, xenon lamp (450 W); emitter, Spex 600 grooves/mm double raster; exciter, 1200 grooves/mm single raster; excitation wavelength, 338 nm; emission wavelength, 616 nm; and slit widths of excitation and emission, both 10 nm.

The luminescent decay (the dynamic curve for fluorescence lifetime linear-fitting) at certain emission wavelength was also recorded on a Spex Fluorolog-3 photon-counting spectrometer equipped with Tau-3 Lifetime Modular Fluorescence Measurement System. The excitation wavelength was at 338 nm and 286 nm, an initial delayed time was 0.01ms and an acquisition time interval 0.001ms, excitation frequency 40 ms, and both excitation slit and emission slit were 10 nm. The detector used was a Hamamatsu R2658P photomultiplier, with a working voltage of –1500 V. All of the experiments were done under room temperature.

The elemental analysis was performed on a Perkin-Elmer 2000 analyzer and the ^{1}H-NMR spectroscopy was recorded on a BRUKER DSX-300 NMR spectrometer (300 MHz, the solvent was $(CD_3)_2SO$).

3. Results and Discussion

3.1 *BSPDA-Eu^{3+} complexes' complexing property*

The IR spectra of BSPDA, BSPDA-Eu^{3+} and BCPDA showed similar characteristics. However, BSPDA-Eu^{3+} had one sulphydryl absorption maximum at 2539 cm^{-1}. Meanwhile, the positions of the carbonyl absorption maximum of BSPDA-Eu^{3+} complexes shifted to 1652cm^{-1}, 1622 cm^{-1} while that of BSPDA, BCPDA to 1731 cm^{-1}, 1724 cm^{-1}. The red shift of the carbonyl absorption maximum might be due to the complexation between Eu^{3+} and the carbonyl bond, which results in carbonyl bond weakening and bond force constant reduction.[12,13] Furthermore, the weakening of the carbonyl double bond in the product obtained was lower when the BSPDA-to-Eu^{3+} molar ratio was 1:5 than when the ratio was 1:1. Actually, the larger the number of carbonyl residues in the complex, the less likely the shift in the extent of maximum carbonyl absorption. From the above analysis, we can conclude that stable complexation exists between BSPDA and Eu^{3+}. Results of an element analysis supported the speculation. The element analysis showed that the molar ratio of Eu^{3+} to molecular N was 2.02–2.06:1 in the 1:5 product, whereas the molar ratio of Eu^{3+} to molecular N was 0.95–1.12:1 in 1:1 product. The above data indicate that BSPDA and Eu^{3+} could form stable complexes either in a molar ratio of 1:1 or 2:1, which was very similar to those of the complexes between Eu^{3+} and BCPDA or PDA.[9,10,12,13]

3.2 *Fluorescence of solid BSPDA- Eu3+ complexes*

The fluorescence spectra of the complexes BSPDA-Eu^{3+} (2:1) and BSPDA-Eu^{3+} (1:1) are shown in Fig.1. It shows that the BSPDA-Eu^{3+} complexes show strong fluorescence

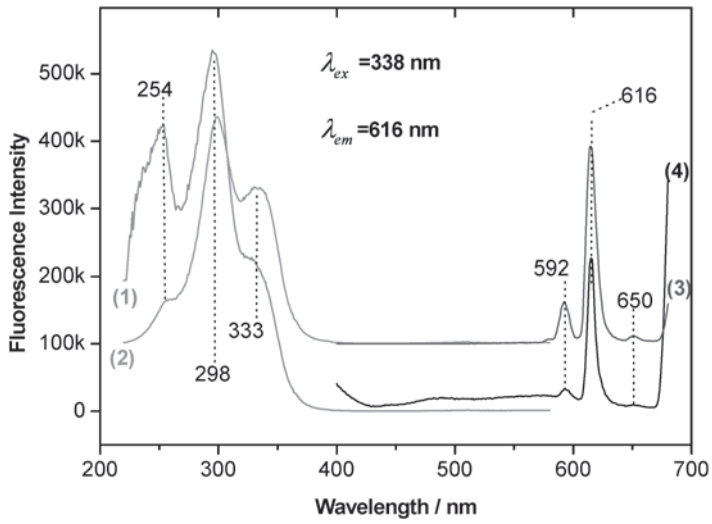


Fig. 1. Fluorescence excitation and emission spectra of different BSPDA-Eu^{3+} samples. (1) BSPDA:Eu=1:1 excitation; (2) BSPDA:Eu=2:1 excitation; (3) BSPDA:Eu=2:1 emission; (4) BSPDA:Eu=1:1 emission.

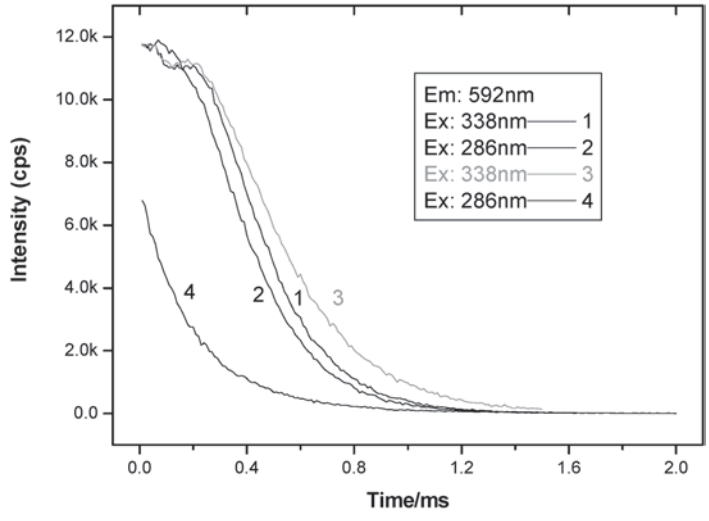
excitation in the ultraviolet areas. The maximum excitation positions of the BSPDA-Eu^{3+} (1:1) complex were at 254 nm, 298 nm, 333 nm, of which that at 298 nm was the highest peak (curve 1 in Fig. 1), whereas the maximum excitation positions of the BSPDA-Eu^{3+} (2:1) complex were similar to these of the BSPDA-Eu^{3+} (1:1) complex (curve 2 in Fig. 1). The maximum intensity of the BSPDA-Eu^{3+} (2:1) complex at 254 nm decreased, whereas that at 333 nm increased compared with that of the BSPDA-Eu^{3+} (1:1) complex. This is attributed to the stronger complexation interaction in the BSPDA-Eu^{3+} (2:1) complex, and the more efficient transfer of excitation energy from the ligand BSPDA to the central europium(III), so that less energy is needed for excitation. Therefore, the fluorescence excitation wavelength of the 2:1complex demonstrated a red shift compared with that of the 1:1 complex, and the property was consistent with and was cross-verified by the red shift law for the complexes' IR spectra. At the same time, the fluorescence lifetime of the BSPDA-Eu^{3+} (2:1) complex was longer than that of the BSPDA-Eu^{3+} (1:1) complex. This will be cross-verified in the measurement of fluorescence lifetime in § 3.3.

When the europium ion (Eu^{3+}) was excited by applying an energy (ν>17267 cm^{-1}) such as through ultraviolet light exposure, it demonstrated europium emissions characteristic of the $^5D_0 \rightarrow {}^7F_J$ (J=0, 1, 2, 3, 4) transition.[19,20] Typical emission lines of Eu^{3+} were observed at approximately 580 nm ($^5D_0 \rightarrow {}^7F_0$), 590 nm ($^5D_0 \rightarrow {}^7F_1$, strong), 616 nm ($^5D_0 \rightarrow {}^7F_2$, very strong), 650 nm ($^5D_0 \rightarrow {}^7F_3$) and 695 nm ($^5D_0 \rightarrow {}^7F_4$). It is shown in Fig. 1 that the complexes' emission lines at 592 nm and 616 nm were strong, particularly in the $^5D_0 \rightarrow {}^7F_2$ emission. Furthermore, the emission intensity of the BSPDA-Eu^{3+} (2:1) complex was stronger than that of the BSPDA-Eu^{3+} (1:1) complex.

3.3 *Fluorescence lifetimes of solid BSPDA-Eu^{3+} samples*

The law of fluorescence decay follows $I_t = I_0\, e^{-t/\tau}$, where I_t is fluorescence intensity, t is the delayed time, and τ is the fluorescence lifetime. When $I_t = I_0$, then $t = \tau$, so that $\ln I_t = \ln I_0 - (1/\tau) \times t$. τ can be obtained from the slope B (B=$-1/\tau$) of linear relation: $\ln I_t = A + B*t$. By using this method, the fluorescence decay curves for the BSPDA-Eu^{3+} complex (592 nm ($^5D_0 \rightarrow {}^7F_1$), 616 nm ($^5D_0 \rightarrow {}^7F_2$), 650 nm ($^5D_0 \rightarrow {}^7F_3$)) were linear-fitted and fluorescence lifetimes were obtained (Figs. 2, 3, 4). The lifetimes in specific emissions are summarized in Table 1. We could see from Table 1 that the BSPDA-

(a)



(b)

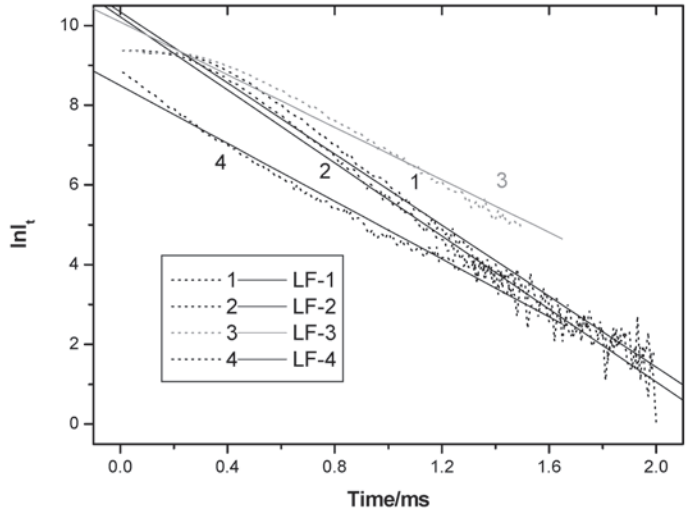


Fig. 2. Fluorescence decay curves for samples* at 592 nm emission (a) and linear-fit curves for natural logarithm of fluorescence intensity vs time (b).

*Curve 1 for BSPDA-Eu^{3+} complex 1:1; Curve 2 for BSPDA-Eu^{3+} complex 2:1 Curve 3 for BSPDA-Eu^{3+} complex 1:1; Curve 4 for BSPDA-Eu^{3+} complex 2:1.

(a)

(b)

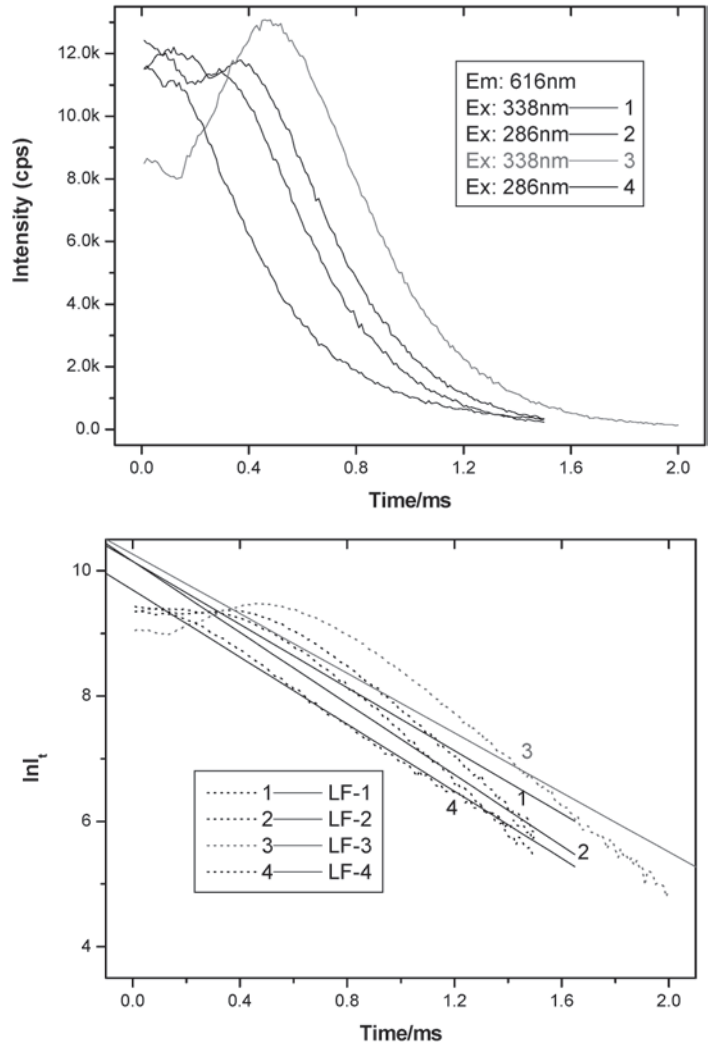


Fig. 3. Fluorescence decay curves for samples* at 616nm emission (a) and linear-fit curves for natural logarithm of fluorescence intensity vs time (b).

*Curve 1 for BSPDA-Eu^{3+} complex 1:1; Curve 2 for BSPDA-Eu^{3+} complex 2:1 Curve 3 for BSPDA-Eu^{3+} complex 1:1; Curve 4 for BSPDA-Eu^{3+} complex 2:1.

Eu^{3+} complexes could emit a europium line characteristic of the $^5D_0 \rightarrow {}^7F_J$ transition when excited with ultraviolet light, and exhibited a fluorescence lifetime exceeding several hundreds of microseconds. The lifetime of the 2:1 complexes was longer than that of the 1:1 complexes at correlative emission peaks. This shows that the fluorescence decay of the 2:1 complexes was slower than that of the 1:1 complexes because of the more efficient complex interaction and energy transfer from the ligand BSPDA to europium(III) in the former. The result was cross-verified to be similar to the red shift of carbonyl adsorption in the IR spectrum and the photoluminescence excitation shift and changes.

(a)

(b)

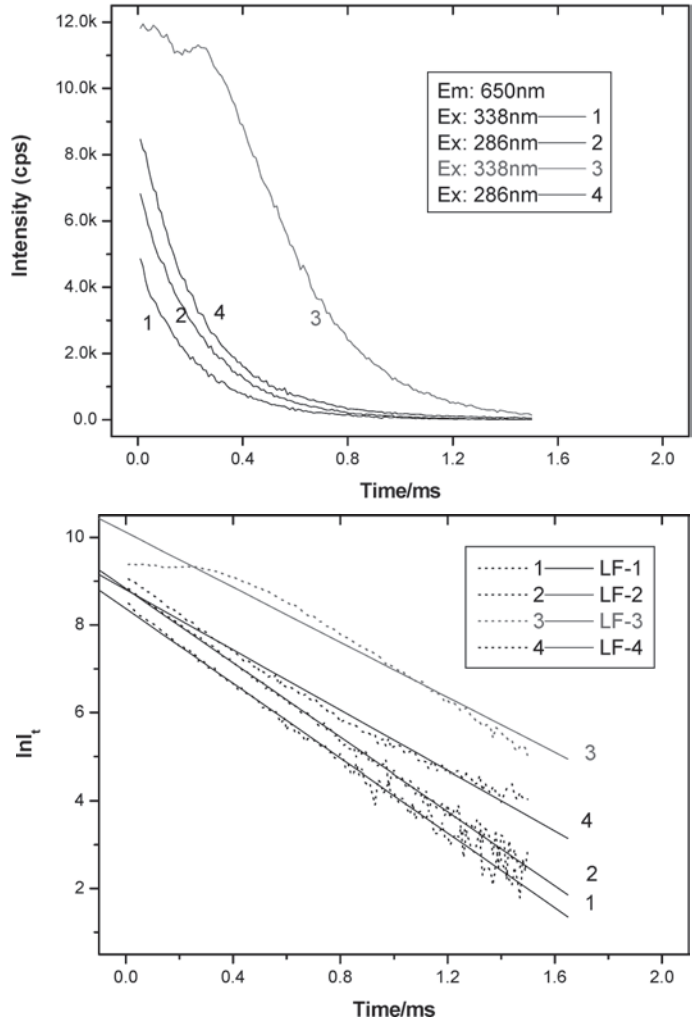


Fig. 4 Fluorescence decay curves for samples* at 650nm emission (a) and linear-fit curves for natural logarithm of fluorescence intensity vs time (b)

*Curve 1 for BSPDA-Eu^{3+} complex 1:1; Curve 2 for BSPDA-Eu^{3+} complex 2:1 Curve 3 for BSPDA-Eu^{3+} complex 1:1; Curve 4 for BSPDA-Eu^{3+} complex 2:1.

4. Conclusions

When excited by ultraviolet light, BSPDA-Eu^{3+} complex chelates could emit characteristic europium emissions and show fluorescence lifetimes exceeding several hundreds of microseconds. The lifetime of the 2:1 complex was longer than that of the 1:1 complex at specific emission peaks because of the more efficient complex interaction and energy transfer from the ligand BSPDA to europium(III) in the former. These europium complexes might be applied to the fabrication of time-resolved fluorescence sensitive films and corresponding biosensors (such as DNA chips).

Table 1
Fluorescence lifetimes of BSPDA- Eu^{3+} complexes.

Sample (BSPDA: Eu^{3+})	Excitation wavelength/nm	Fluorescence lifetime τ (ms) $^5D_0 \rightarrow {}^7F_1$ 592 nm	$^5D_0 \rightarrow {}^7F_2$ 616 nm	$^5D_0 \rightarrow {}^7F_3$ 650 nm
	338	0.227	0.389	0.254
1:1	286	0.213	0.339	0.241
	338	0.303	0.420	0.319
2:1	286	0.276	0.373	0.291

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