

## Spectrofluorimetric Determination of Sparfloxacin Using Europium(III) as a Fluorescence Probe in Micellar Medium

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A europium (III)-sensitized, spectrofluorimetric (FL) method is presented for the determination of sparfloxacin (SPAR) using an anionic surfactant, sodium dodecyl benzene sulphonate (SDBS). The method is based on the strong fluorescence (FL) enhancement of SPAR after the addition of  $\text{Eu}^{3+}$  ions as fluorescence probes. The experimental results indicated that the FL intensity of the SPAR- $\text{Eu}^{3+}$  system was enhanced markedly by SDBS. The maximum FL emission signal was obtained at about 615 nm when excited at 372 nm. The experimental conditions that affected the FL intensity of the SPAR- $\text{Eu}^{3+}$ -SDBS system were optimized systematically. The enhanced FL intensity of the system exhibited a good linear relationship with the SPAR concentration over the range of  $1.5 \times 10^{-9}$  -  $1.2 \times 10^{-7}$  mol L<sup>-1</sup> with a correlation coefficient (r) of 0.9987. The limit of detection ( $3\delta$ ) was  $4.15 \times 10^{-10}$  mol L<sup>-1</sup> with a relative standard deviation (RSD) of 1.65%. This method was successfully applied for the determination of SPAR in pharmaceuticals, and human serum and urine samples with higher sensitivity, wide dynamic range and better stability. The possible interaction mechanism of the system is also discussed in detail by ultraviolet absorption spectra and FL spectra.

**Key Words :** Europium(III), Fluorescence, Sparfloxacin, Sodium dodecyl benzene sulphonate

### Introduction

Sparfloxacin (SPAR), [5-amino-1-cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid], is a broad spectrum, third generation fluoroquinolone antibiotic. It is active against both Gram-positive and Gram-negative bacteria and has moderate activity against anaerobes and *Mycobacteria*.<sup>1</sup> It is used in the treatment of various bacterial infections, including urinary tract infections, lung infections such as bronchitis or pneumonia, sinus infections and some other types of infection, because of its excellent activity against various bacteria and good absorption on oral administration.<sup>2</sup> Therefore, it is necessary to develop a simple and sensitive analytical method for the determination of SPAR due to its clinical, pharmaceutical and biological advantages.

Several analytical methods have been reported for determining SPAR including high performance liquid chromatography,<sup>3-6</sup> liquid chromatography,<sup>7</sup> high-performance thin layer chromatography,<sup>8</sup> thin-layer chromatography-fluorimetry,<sup>9,10</sup> spectrofluorimetry,<sup>11-13</sup> capillary electrophoresis-fluorescence,<sup>14</sup> voltammetry,<sup>15</sup> and spectrophotometry<sup>16-18</sup> and Atomic absorption spectroscopy.<sup>19</sup> Some of these methods have suffered from various drawbacks including time consuming sample separation and treatment, expensive instruments and reagents, low sensitivity, inconvenient reagent pretreatment, and inability to assay real samples. Due to the sensitivity, select-

ivity and inexpensive instrumentation, the spectrofluorimetric (FL) method has been widely used in the quantification of pharmaceuticals.

In recent years, the use of lanthanides (especially  $\text{Eu}^{3+}$ ,  $\text{Tb}^{3+}$ ) as fluorescence (FL) probes has become widespread because of their long luminescence life times, large Stokes shifts, and narrow emission bands. Because of these properties, the  $\text{Eu}^{3+}$  complex has been used extensively as the FL probe in the assay of pharmaceuticals and biomolecules.<sup>21-26</sup> SPAR, the carboxylic and keto-oxygen atoms of which are involved in complex with  $\text{Eu}^{3+}$ , shows large Stokes shifts and narrow emission bands. SPAR can easily transfer energy to  $\text{Eu}^{3+}$  and enhance the FL intensity of  $\text{Eu}^{3+}$ . The FL signal of the  $\text{Eu}^{3+}$  ion is very weak in solution due to weak absorption of the metal ion itself, but is enhanced significantly when SPAR combines with  $\text{Eu}^{3+}$  solution.

Herein, we present a sensitive and simple FL method using  $\text{Eu}^{3+}$  as a fluorescence probe to determine SPAR. SPAR was observed to interact with  $\text{Eu}^{3+}$  and exhibit enhanced, characteristic  $\text{Eu}^{3+}$  FL peak at about 591 and 615 nm, corresponding to the  ${}^5\text{D}_0\text{-}{}^7\text{F}_1$  and  ${}^5\text{D}_0\text{-}{}^7\text{F}_2$  transitions of the  $\text{Eu}^{3+}$  ion, respectively. The FL intensity was enhanced markedly in the presence of sodium dodecyl benzene sulphonate (SDBS), an anionic surfactant. The FL intensity of the  $\text{Eu}^{3+}$ -SPAR-SDBS system was increased with increasing the SPAR concentration. Therefore, an FL method for the determination of SPAR was proposed based on the FL

enhancement by the  $\text{Eu}^{3+}$ -SPAR complex in the presence of SDBS. The interaction mechanism of the  $\text{Eu}^{3+}$ -SPAR complex in the presence of SDBS is discussed in detail. The proposed method affords good precision and accuracy and has been successfully applied to the determination of SPAR in pharmaceutical preparations and biological fluids with satisfactory results.

### Experimental

**Reagents.** SPAR, SDBS,  $\text{Eu}_2\text{O}_3$  and tris (hydroxymethyl) amino methane were purchased from Sigma-Aldrich (St. Louis, USA). All other chemicals were of analytical reagent grade and were used without further purification. Distilled deionized (DI) water (Millipore, MilliQ Water System, USA) was used throughout. A stock solution ( $1.0 \times 10^3 \text{ mol L}^{-1}$ ) of SPAR was prepared in DI water with  $0.05 \text{ mol L}^{-1}$  NaOH. A stock standard solution of  $\text{Eu}^{3+}$  ( $1.0 \times 10^{-3} \text{ mol L}^{-1}$ ) was prepared by dissolving  $\text{Eu}_2\text{O}_3$  (purity, 99.99%) in 1:1 HCl and evaporating the solution to almost dryness before diluting to 100 mL with DI water and finally placing in a refrigerator at  $4^\circ\text{C}$  until use. SDBS ( $1 \times 10^{-3} \text{ mol L}^{-1}$ ) was prepared by dissolving 0.017 g in a 50 mL volumetric flask using DI water and preserved at  $4^\circ\text{C}$ . Tris-HCl buffer was prepared by dissolving an appropriate amount of tris (hydroxy methyl) aminomethane in 100 mL DI water and the pH was adjusted using 0.1 M HCl. Working solutions were prepared daily from the stock solution by appropriate dilution immediately before use.

**Apparatus.** All FL measurements were conducted using a spectrofluorimeter (F-4500, Hitachi, Japan) equipped with a 150 W xenon lamp. Emission lights of a sample solution were measured and transduced to electric signals by a photomultiplier tube (R 928, Hamamatsu, Japan). The high voltage for the photomultiplier tube was set to 950 V. A pH meter (Orion, 520A, USA) was used for pH adjustment. The absorption spectra of the system were measured on a UV-1800 spectrophotometer (Shimadzu, Japan).

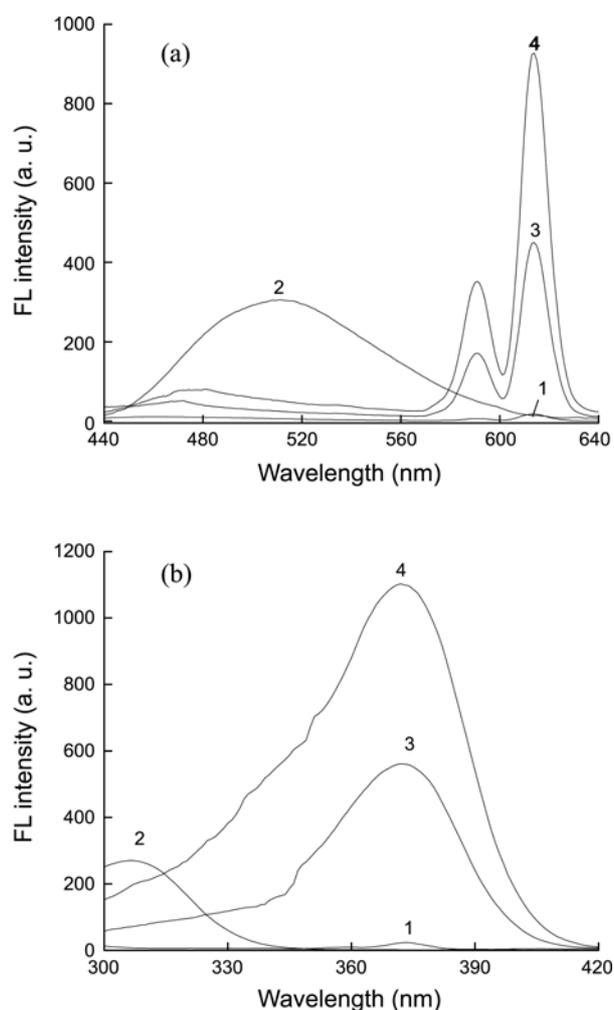
**Preparation of Samples.** Ten commercially available tablets (each containing 200 mg SPAR) were completely crushed to power by a pestle and mortar. An accurately weighed portion of 20 mg was then dissolved in water with  $0.05 \text{ mol L}^{-1}$  NaOH. The resulting solution was then diluted with DI water within the linear range of measurement of SPAR.

To analyze SPAR in serum and urine samples, these samples were spiked with appropriate amounts of SPAR stock solution. Each 1.0 mL serum sample was deproteinized by adding 5 mL of 20% trichloroacetic acid ( $\text{CCl}_3\text{COOH}$ ) in a centrifuge tube, followed by vortexing for 5 min and centrifuging for 10 min at 4000 rpm. A 0.1 mL aliquot of the supernatant of the serum sample was mixed with the standard solutions of SPAR and diluted appropriately within the linear range of determination. No further sample pre-treatment was required for the urine sample except proper dilution in order to shift the SPAR concentrations within the working range.

**Analytical Procedure.** To measure the system's FL, certain amounts of  $\text{Eu}^{3+}$  ion solution, buffer solution, SPAR, and SDBS were added to a 10 mL volumetric flask, diluted with DI water, mixed thoroughly, allowed to stand for about 20 min, and finally placed in a 1 cm quartz cell for measurement of the FL spectra. Both the excitation and emission slits for the FL measurement were set to 10 nm.

### Results and Discussion

**Fluorescence Spectral Characteristics.** The fluorescence emission and excitation spectra of the SPAR- $\text{Eu}^{3+}$ -SDBS system were recorded at room temperature and are shown in Figure 1. The experimental results showed that the  $\text{Eu}^{3+}$  ion solution exhibited a very weak FL emission signal (Fig. 1(a), curve 1) because of weak absorption of the metal ion itself. After the addition of SPAR to the  $\text{Eu}^{3+}$  solution, the characteristic emission signal of  $\text{Eu}^{3+}$  ion appeared at 591 and 615 nm (Fig. 1(a), curve 3) when excited at 372 nm, which correspond to the  $^5\text{D}_0$ - $^7\text{F}_1$  and  $^5\text{D}_0$ - $^7\text{F}_2$  transitions of the  $\text{Eu}^{3+}$



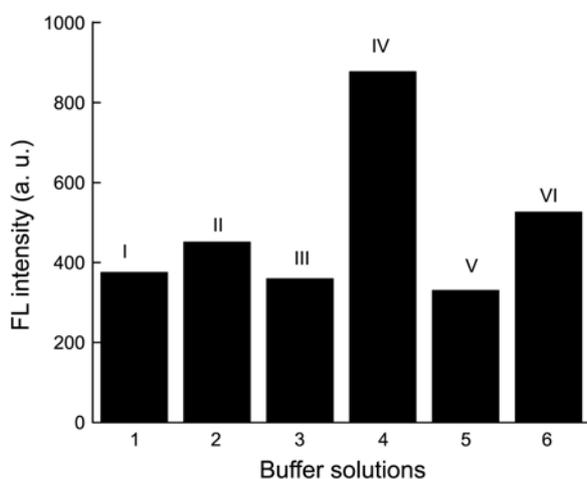
**Figure 1.** Fluorescence emission (a) and excitation (b) spectra of  $\text{Eu}^{3+}$ -SPAR-SDBS system: (1)  $\text{Eu}^{3+}$ , (2) SPAR, (3)  $\text{Eu}^{3+}$ -SPAR, and (4)  $\text{Eu}^{3+}$ -SPAR-SDBS. Conditions: SPAR,  $5.0 \times 10^{-7} \text{ mol L}^{-1}$ ;  $\text{Eu}^{3+}$ ,  $4.7 \times 10^{-5} \text{ mol L}^{-1}$ ; SDBS,  $5.8 \times 10^{-4} \text{ mol L}^{-1}$ ; Tris-HCl,  $0.1 \text{ mol L}^{-1}$ ; pH, 8.8.

ion, respectively. The most intense peak was obtained at 615 nm because the  ${}^5D_0-{}^7F_2$  transitions of the  $\text{Eu}^{3+}$  ion is predominantly associated with electric dipole, and their radiative transition probabilities are very sensitive to the nature of the ligand environment. Thus, the luminescence signal at 615 nm was intensified after addition of SPAR (Fig. 1(a), curve 3). SPAR can absorb light energy and transfer it to  $\text{Eu}^{3+}$  through intramolecular energy transfer, and thereby form a binary mixture with  $\text{Eu}^{3+}$  and emit characteristic FL signal of  $\text{Eu}^{3+}$ . After the addition of SDBS to the SPAR- $\text{Eu}^{3+}$  system, SDBS improved the coordination microenvironment of  $\text{Eu}^{3+}$  and SPAR and decreased the combination between the water molecules and the binary complex. Thus, the FL intensity of the SPAR- $\text{Eu}^{3+}$  binary complex was enhanced about 5 times as compared with the SPAR- $\text{Eu}^{3+}$  system in the presence of SDBS (Fig. 1(a), curve 4), which indicated that the existence of  $\text{Eu}^{3+}$  and SDBS synergistically enhanced the FL intensity of SPAR.

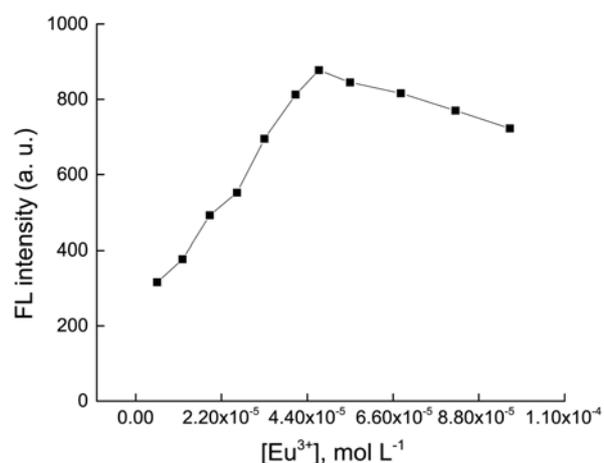
As shown in the excitation spectra (Fig. 1(b)), the SPAR- $\text{Eu}^{3+}$ -SDBS system exhibited the maximum excitation intensity at about 372 nm, while the highest emission intensity was at 615 nm (Fig. 1(a)). Therefore, 372 and 615 nm was selected as the excitation and emission wavelengths for the whole experiment, respectively.

**Effect of pH and Buffers.** pH may have influenced the FL signal of the presented system. Thus, the effect of pH on the FL intensity was investigated in the range of 7-10. The maximum FL intensity was obtained at a pH of 8.8, which was therefore chosen as the optimum pH for this experiment. Buffer solutions may also influence the FL signal. Thus, the effect of the following buffers was examined:  $\text{NH}_4\text{Ac-HAc}$ , borax-HCl,  $\text{NH}_4\text{Cl-NH}_3\cdot\text{H}_2\text{O}$ , tris-HCl,  $\text{KH}_2\text{PO}_4\text{-NaOH}$ , and  $\text{KH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ . The highest FL intensity was obtained using tris-HCl buffer (Fig. 2). Therefore,  $0.1 \text{ mol L}^{-1}$  of tris-HCl buffer was used to maximize the FL intensity.

**Effect of  $\text{Eu}^{3+}$  Ion Concentration.** The effect of the  $\text{Eu}^{3+}$  ion concentration on the FL intensity was examined in the



**Figure 2.** Effect of buffers on the FL intensity of the  $\text{Eu}^{3+}$ -SPAR-SDBS system: (I)  $\text{NH}_4\text{Ac-HAc}$ , (II) borax-HCl, (III)  $\text{NH}_4\text{Cl-NH}_3\cdot\text{H}_2\text{O}$ , (IV) tris-HCl, (V)  $\text{KH}_2\text{PO}_4\text{-NaOH}$  and (VI)  $\text{KH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ .

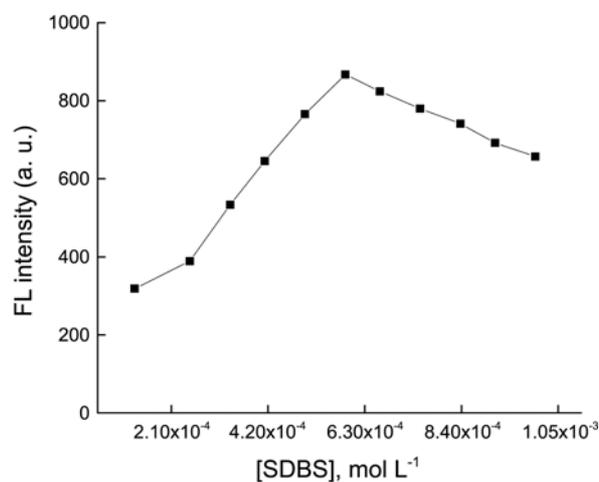


**Figure 3.** Effect of  $\text{Eu}^{3+}$  ion concentration on the FL intensity of the  $\text{Eu}^{3+}$ -SPAR-SDBS system. Conditions: SPAR,  $5.0 \times 10^{-7} \text{ mol L}^{-1}$ ; SDBS,  $5.8 \times 10^{-4} \text{ mol L}^{-1}$ ; Tris-HCl,  $0.1 \text{ mol L}^{-1}$ ; pH, 8.8.

range of  $5.5 \times 10^{-6} - 9.5 \times 10^{-5} \text{ mol L}^{-1}$ . The FL intensity was increased with increasing  $\text{Eu}^{3+}$  ion concentration up to  $4.7 \times 10^{-5} \text{ mol L}^{-1}$  (Fig. 3). Therefore, an  $\text{Eu}^{3+}$  concentration of  $4.7 \times 10^{-5} \text{ mol L}^{-1}$  was selected for further study.

**Effect of SDBS Concentration.** Surfactants are widely used to solubilize hydrophobic compounds and to increase the FL intensity of the weakly fluorescent compounds by improving the microenvironment of the fluorescence. The effect of the surfactants, cetyl trimethyl ammonium bromide (cationic), Triton X-100 (nonionic), Igepal (nonionic), sodium dodecyl sulfate (SDS, anionic), and SDBS (anionic), was investigated and the maximum signal was obtained using SDBS. The effect of SDBS on the FL intensity was determined and the FL intensity was maximized at an SDBS concentration of  $5.8 \times 10^{-4} \text{ mol L}^{-1}$  (Fig. 4), which was thus chosen for the present study.

**Effect of the Addition Order of the Reagents.** The effect of the reagent addition order on the FL intensity was investigated. The results indicated that an addition order of  $\text{Eu}^{3+}$ ,



**Figure 4.** Effect of SDBS concentration on the FL intensity of the  $\text{Eu}^{3+}$ -SPAR-SDBS system. Conditions: SPAR,  $5.0 \times 10^{-7} \text{ mol L}^{-1}$ ;  $\text{Eu}^{3+}$ ,  $4.7 \times 10^{-5} \text{ mol L}^{-1}$ ; Tris-HCl,  $0.1 \text{ mol L}^{-1}$ ; pH 8.8.

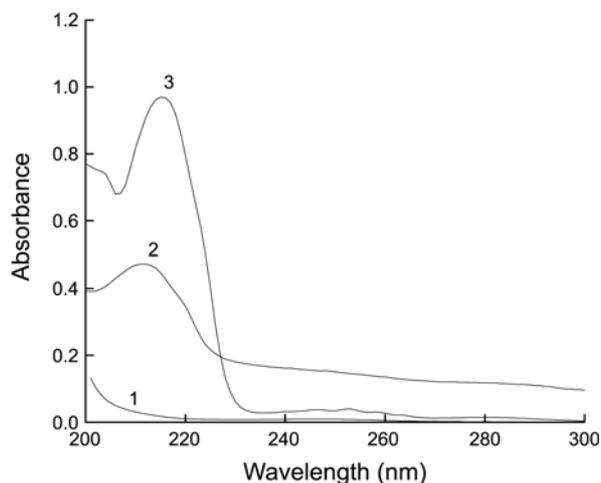
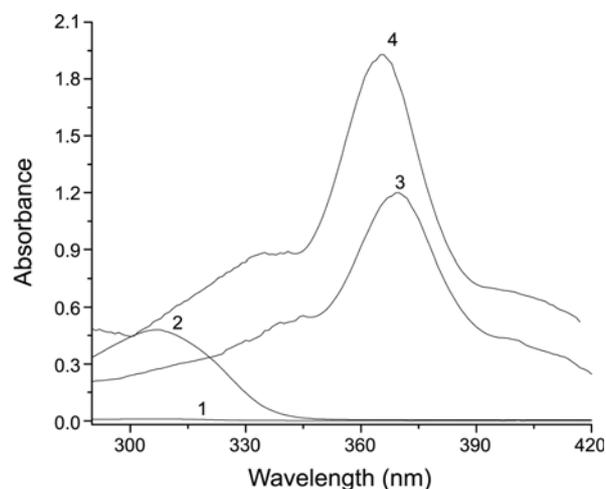
**Table 1.** Tolerance limit of foreign substances in the determination of SPAR

Foreign Substances	Maximum tolerable concentration ratio	Change in fluorescence intensity (%)
Na <sup>+</sup> , K <sup>+</sup> , Ba <sup>2+</sup> , Ca <sup>2+</sup> , Pb <sup>2+</sup>	2700	-2.12
Al <sup>3+</sup> , Cu <sup>2+</sup> , Mg <sup>2+</sup> , Fe <sup>3+</sup> , Co <sup>2+</sup>	2200	-3.19
Sm <sup>3+</sup> , La <sup>3+</sup>	1700	+1.71
Starch, glucose, fructose, lactose	1100	+1.11
Sucrose, dextrin, galactose	950	+1.23
Citric acid, ascorbic acid, uric acid	350	-4.3
Amoxicillin, ibuprofen,	150	-2.9
Furosemide, sulfamethoxazole	50	+1.45

tris-HCl, SPAR, and SDBS maximized the FL intensity and so it was chosen as the optimum reagent addition order. Furthermore, the FL intensity of the system peaked at 20 min after all the reagents had been added and then remained stable for 2 h.

**Effect of Interfering Substances.** The effect of potential interferents that may have influenced the analytical results by suppressing or enhancing the FL signal was systematically studied. Therefore, the effect of potential foreign substances was investigated by preparing a set of solutions, each containing  $3.5 \times 10^7$  mol L<sup>-1</sup> of SPAR and different concentrations of the chemical species to be tested. A foreign species is considered to interfere if it produces an error greater than 5% in the determination of SPAR. The results, summarized in Table 1, indicated that the foreign substances did not significantly affect the FL intensity of the system for the determination of SPAR.

**Possible Interaction Mechanism.** In order to investigate the interaction between Eu<sup>3+</sup>, SPAR and SDBS, several absorption spectra of the system were recorded and are shown in Figures 5 and 6. SPAR is a less polar and hydrophobic compound and its solubility were improved by SDBS. Thus, when the SPAR-Eu<sup>3+</sup> complex was dispersed and gathered

**Figure 5.** Absorption spectra of SDBS in the Eu<sup>3+</sup> system: (1) Eu<sup>3+</sup>, (2) SDBS, and (3) Eu<sup>3+</sup>-SDBS.**Figure 6.** Absorption spectra of SPAR in various systems: (1) Eu<sup>3+</sup>, (2) SPAR, (3) Eu<sup>3+</sup>-SPAR, and (4) Eu<sup>3+</sup>-SPAR-SDBS.

around the micelles, the microenvironment of the complex was changed significantly, which may have decreased the non-radiative energy loss through the molecule collisions and improved the quantum efficiency of FL.<sup>27</sup> Moreover, the coordination number of Eu<sup>3+</sup> in its solution is usually 6-10, but this coordination number could not have been satisfied after SPAR-Eu<sup>3+</sup> complex formation. Thus, Eu<sup>3+</sup> in the SPAR-Eu<sup>3+</sup> complex could interact with SDBS by ionic interaction, which facilitated energy transfer not only from SPAR to Eu<sup>3+</sup>, but also from SDBS to Eu<sup>3+</sup>, and thereby enhanced the FL of the system markedly. This explanation was proved by Figures 5 and 6. The absorption of SDBS was enhanced when the Eu<sup>3+</sup> solution was mixed with SDBS (Fig. 5, curves 2 and 3), indicating that SDBS may have interacted with Eu<sup>3+</sup>. As shown in Figure 6, the absorption peak of SPAR (305 nm) was increased and the wavelength was red shifted from 305 to 369 nm (Fig. 6, curves 2 and 3), thus revealing the formation of SPAR-Eu<sup>3+</sup> complex. However, the absorption of SPAR was enhanced significantly with the addition of both Eu<sup>3+</sup> and SDBS, which was in accordance with the FL increment of the FL excitation spectrum of the SPAR-Eu<sup>3+</sup>-SDBS system (Fig. 1(b)), and the maximum absorption wavelength showed a slight blue shift from about 369 to 365 nm (Fig. 6, curves 3 and 4). Moreover, a multiple ionic associate that was formed in the SPAR-Eu<sup>3+</sup>-SDBS system increased the effective absorption cross-section of the complex and thereby increased the molar absorbance index. Thus, the FL intensity of the system was significantly increased by the SDBS addition. In addition, the optimal SDBS concentration in the present study was approximated to the critical micelle concentration (0.63 mmol L<sup>-1</sup>) of SDBS,<sup>27</sup> which indicated that the micelle formation strongly enhanced FL intensity of the system.

**Linear Range and Detection Limit.** Under the optimal conditions described above, a calibration curve for the determination of SPAR was constructed by plotting the SPAR concentration versus FL intensity. The experimental results indicated that the enhanced FL intensities of the system

**Table 2.** Figure of merits of comparable methods to determine SPAR

Methods	Analytical ranges	LODs	Ref.	Comments
TLC-Fluorimetry	$1.0 \times 10^{-5}$ - $4.2 \times 10^{-4}$ mol L <sup>-1</sup>	$1.6 \times 10^{-6}$ mol L <sup>-1</sup>	[10]	Need sample separation, pretreatment & expensive instrument
Spectrofluorimetry	$8.0 \times 10^7$ - $1.4 \times 10^5$ mol L <sup>-1</sup>	$9.01 \times 10^8$ mol L <sup>-1</sup>	[11]	Less sensitive & low detection limit than presented method
Spectrophotometry	$4.41 \times 10^{-12}$ - $4.07 \times 10^{-11}$ mol L <sup>-1a</sup>	$5.38 \times 10^{-14}$ mol L <sup>-1a</sup>	[17]	Expensive reagents, inconvenient reagent pretreatment and time consuming
AAS conductometry and colorimetry	$2.5 \times 10^{-11}$ - $3.5 \times 10^{-10}$ mol L <sup>-1a</sup>	$5.86 \times 10^{-12}$ mol L <sup>-1a</sup>	[19]	Expensive apparatus, reagents and unable to assay real sample
Spectrofluorimetry	$1.5 \times 10^{-9}$ - $1.2 \times 10^{-7}$ mol L <sup>-1</sup>	$4.15 \times 10^{-10}$ mol L <sup>-1</sup>	Proposed method	Simple, good sensitivity, inexpensive instruments & reagents and able to assay real sample

<sup>a</sup>μg mL<sup>-1</sup>**Table 3.** Determination of SPAR in pharmaceutical preparations and recovery results

Sample	Amount (mg)		Added ( $\times 10^{-7}$ mol L <sup>-1</sup> )	Found ( $\times 10^{-7}$ mol L <sup>-1</sup> ) $\pm$ RSD <sup>a</sup>	Recovery (%)
	Labeled (mg)	Found by the proposed method $\pm$ RSD <sup>a</sup>			
Tablet	200 mg of SPAR	197.85 $\pm$ 1.45	1.00	0.95 $\pm$ 1.33	95
			2.00	2.04 $\pm$ 1.17	102
			3.00	3.03 $\pm$ 1.25	101
			4.00	4.11 $\pm$ 1.05	102.75
			5.00	4.99 $\pm$ 1.19	99.8

<sup>a</sup>Relative standard deviation for five replicate measurements

showed an excellent linear relationship with the SPAR concentration in the range of  $1.5 \times 10^{-9}$  -  $1.2 \times 10^{-7}$  mol L<sup>-1</sup>. The linear regression equation was  $Y = 5.19 \times 10^9 C_{\text{SPAR}} + 328$ , where  $C_{\text{SPAR}}$  is the SPAR concentration and Y the FL intensity in arbitrary unit (a. u.), with a correlation coefficient of 0.9987. The limit of detection was  $4.15 \times 10^{-10}$  mol L<sup>-1</sup> for SPAR with a relative standard deviation (RSD) of 1.65% for 5 replicate determinations of  $5 \times 10^{-7}$  mol L<sup>-1</sup> SPAR. Thus, the presented method offers higher sensitivity (shown in Table 2) to determine SPAR with simple and cheap instrumentation.

**Determination of SPAR in Tablets.** In order to demonstrate the applicability of the presented system, it was applied to the analysis of tablets containing SPAR. The results obtained by the proposed method, listed in Table 3, indicated that the amount of SPAR obtained by the proposed method was in close agreement with the labeled contents. Recovery was performed by applying standard addition method. It was evaluated by determining the agreement

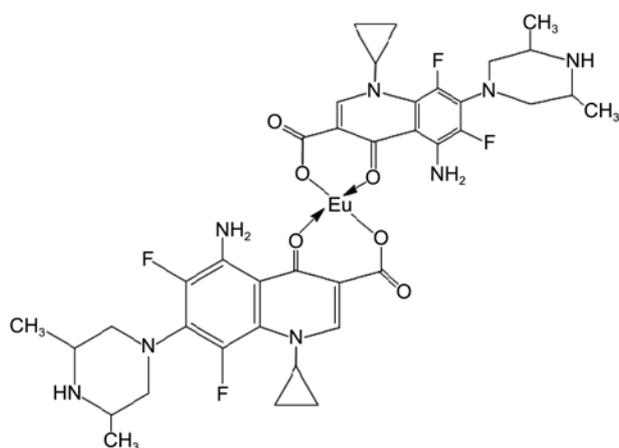
between the measured concentration of the standard and the added known concentration of the sample. The test was performed by spiking the pre-analyzed powdered tablet sample at different concentrations with pure SPAR. Each test was repeated five times and the recoveries were in the range of 95-102.75% for SPAR.

**Determination of SPAR in Spiked Serum and Urine Samples.** The proposed method was used to determine the SPAR contents in the serum and urine samples. In order to adjust the sample SPAR concentration within the linear range of determination, after deproteinization and centrifugation of serum and urine samples, the supernatant was used to investigate the recovery by standard addition method where known amounts of SPAR were added. The results of the spiked serum and urine samples are shown in Table 4. Recoveries of SPAR contents in serum and urine samples were 97.83-101.57% and 98.5-104.5%, respectively. Therefore, the proposed method can be applied to determine SPAR in serum and urine samples with good accuracy.

**Table 4.** Recovery of SPAR in serum and urine samples

Samples	Standard addition method					
	Serum			Urine		
	Added ( $\times 10^{-7}$ mol L <sup>-1</sup> )	Observed ( $\times 10^{-7}$ mol L <sup>-1</sup> ) $\pm$ RSD <sup>a</sup> (%)	Recovery (%)	Added ( $\times 10^{-7}$ mol L <sup>-1</sup> )	Observed ( $\times 10^{-7}$ mol L <sup>-1</sup> ) $\pm$ RSD <sup>a</sup> (%)	Recovery (%)
SPAR	2.0	1.98 $\pm$ 1.19	99	2.0	2.09 $\pm$ 0.92	104.5
	4.0	4.07 $\pm$ 1.21	101.75	4.0	4.02 $\pm$ 1.41	100.5
	6.0	5.87 $\pm$ 1.05	97.83	6.0	5.91 $\pm$ 1.15	98.5

<sup>a</sup>Relative standard deviation for five replicate measurements



**Figure 7.** Proposed structure of the  $\text{Eu}^{3+}$ -SPAR complex.

**Determination of Coordination Ratio.** For better understanding of the complexation of SPAR- $\text{Eu}^{3+}$ , the coordination ratio of  $\text{Eu}^{3+}$ : SPAR was determined to be 1:2 by Job's method of continuous variation of equimolar solution. The SPAR molecule contains two types of coordinating atoms: oxygen and nitrogen. The oxygen atom has a tendency to form a stable complex with a lanthanide element, due to its strong ability of coordination with the lanthanide compounds. However, the stability of the complex formed by a nitrogen atom and a lanthanide element is relatively poor. Usually, oxygen atoms in an organic ligand coordinate with the lanthanide compounds in one of two ways: the negatively charged oxygen atom forms a stable ionic bond with the lanthanide ion or the electro-neutral oxygen atom forms a coordinate bond with the lanthanide ion.<sup>28</sup> Based on the above discussion, a structure was proposed for the binary complex of  $\text{Eu}^{3+}$  and SPAR and is shown in Figure 7.

### Conclusion

A sensitive and cost effective FL method was proposed for determining SPAR based on the interaction of  $\text{Eu}^{3+}$  and SPAR in the presence of SDBS. The proposed method offers higher sensitivity, selectivity and accuracy with a lower limit of detection ( $4.15 \times 10^{-10}$  mol  $\text{L}^{-1}$ ) and wide linear range ( $1.5 \times 10^{-9}$  -  $1.2 \times 10^{-7}$  mol  $\text{L}^{-1}$ ). The presented method was successfully applied to the determination of SPAR in formulated pharmaceutical tablets, and serum and urine samples.

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