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ORIGINAL PAPER

Sensitive Determination of Norfloxacin by the Fluorescence Probe of Terbium (III)- Sodium Dodecylbenzene Sulfonate and Its Luminescence Mechanism

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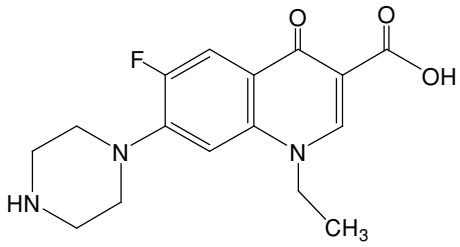
Abstract The fluorescence system of the norfloxacin-Tb^{3+}-sodium dodecylbenzene sulfonate (SDBS) was investigated in this paper. The experiments indicated that the fluorescence intensity of the Tb^{3+}-SDBS was greatly enhanced by the norfloxacin. On the basis of the above findings, a sensitive fluorimetric method for determining the norfloxacin was established. The fluorescence intensity was measured by a 1-cm quartz cell with the excitation wavelength of 290 nm and the emission wavelength of 545 nm. The enhanced fluorescence intensity of the system (Δ F) showed a good linear relationship with the concentration of norfloxacin in the range of 5.0×10^{-9} mol L^{-1}–2.0×10^{-6} mol L^{-1}, its correlation coefficient was 0.9991 and the detection limit ($S/N = 3$) was 1.2×10^{-9} mol L^{-1}. The presented method was used to determine the norfloxacin in real pharmaceutical samples. The luminescence mechanism was also discussed in detail. In the fluorescence system of the norfloxacin-Tb^{3+}-SDBS, the SDBS not only acted as the surfactant, but also acted as the energy donor.

Keywords Terbium(III) · Norfloxacin · Spectrofluorimetry · Enhancement effect · Determination

Introduction

Norfloxacin(1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline- carxylic acid) (Scheme 1) is one of the third generation members of quinolone antibiotics fluorinated in position 6 and bearing a piperazinyl moiety in position 7. Like other fluoroquinolones, norfloxacin (NFLX) is used in the treatment of systemic infections including urinary tract, respiratory, gastro-intestinal and skin infections. It kills bacteria through inhibiting cell DNA-gyrase and prohibiting DNA-replication. Nowadays it is used extensively in the clinical treatment with higher potential and a broad antibacterial spectrum, lower side effects.

Different methods had been reported for determining the fluoroquinolone antibiotics such as spectrophotometry [1, 2], high performance liquid chromatography [3, 4], chemical luminescence [5], nuclear magnetic resonance spectroscopy [6], and so forth. Because of the high sensitivity and selectivity, the spectrofluorimetry has been widely used to estimate the pharmaceuticals [7–9]. Due to rare-earth ions have luminescence characteristics such as narrow spectral width, long luminescence life-time, large stocks shift and strong combination, especially Tb^{3+} and Eu^{3+}, for their resonance energy levels overlap with ultraviolet light. Therefore, the rare earth Tb^{3+} is often used as the fluorescence probes for determining some substances because of the high fluorescence quantum efficiency of the terbium(III) chelates [10, 11]. Although using Tb^{3+} as a fluorescence probe to detect



Scheme 1

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the fluoroquinolone antibiotics had been reported [12–14], its sensitivity was not high, all the detection limits of these methods was in the range of 5.0×10^{-7}–5.0×10^{-8} mol L^{-1}.

However, the experiments indicated that the fluorescence of the Tb^{3+}-SDBS was greatly enhanced by the NFLX. The results showed that its fluorescence intensity could enhance almost 30 times as compared with the NFLX-Tb^{3+}system. The aim of this work was to develop a sensitive spectrofluorimetric method for determining the norfloxacin. The detection limit ($S/N = 3$) of the proposed method could attain 1.2×10^{-9} mol L^{-1}. The method had been successfully applied to determine the norfloxacin in real pharmaceutical samples. In addition, the fluorescence enhancement mechanism was interesting, the SDBS not only acted as the surfactant, but also acted as the energy donor.

Experimental

Apparatus

All fluorescence measurements were made with a F-2500 spectrofluorimeter (Hitachi, Japan). All absorption spectra were measured on a UV-2401PC spectrophotometer (Shimadzu, Japan). All pH measurements were made with a pHS-9V acidity meter (Huaguang, China).

Reagents

All chemicals and reagents were of analytical grade, and water was doubly distilled. Stock solutions of NFLX (1.0×10^{-3} mol L^{-1}) was prepared by dissolving the corresponding NFLX (99.9%) in water, working solutions were prepared by appropriate dilution with water. Stock standard solution of Tb^{3+} (0.01 mol L^{-1}) was prepared by dissolving the corresponding oxide (Tb_4O_7, 99.99%) in 1:1(v/v) hydrochloric acid, and the solution was evaporated to near dryness, then the residue was dissolved in water and diluted to the concentration of 0.01 mol L^{-1}. Glycine (5%)-NaOH buffer solution (pH = 7.6) was prepared by dissolving 12.50 g glycine in water and adjusting the pH with 0.1 mol L^{-1} sodium hydroxide solution to give a final total volume of 250 mL. SDBS surfactant solution (0.01 mol L^{-1}) was prepared by dissolving 0.3485 g SDBS in water, then diluting to 100 ml with water. The desired concentrations of SDBS were prepared by appropriate dilution with water.

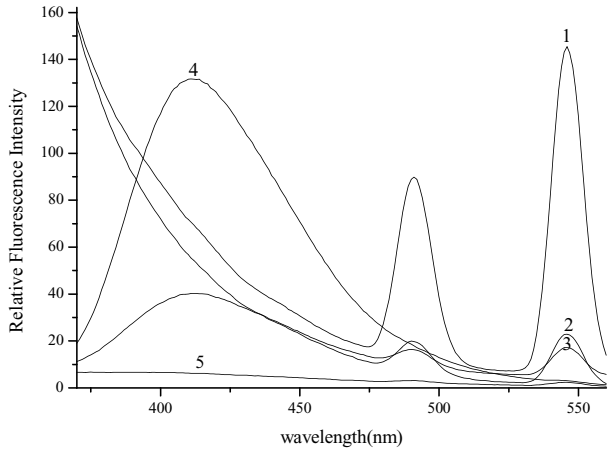
Procedure

To a 10 mL test-tube, solutions were added according to the following order: 1.0 ml of glycine (5%)-NaOH buffer solution (pH = 7.6), 1.0 ml of 5.0×10^{-4} mol L^{-1} Tb^{3+}, 1.0 ml of 5.0×10^{-3} mol L^{-1} SDBS solution, and 1.0 ml of certain concentration NFLX. The mixture was diluted to 10.0 ml with water and allowed to stand for 20–30 min. The entrance and exit slits for all fluorescence measurements were maintained both at 10 nm. The fluorescence intensity was measured by a 1-cm quartz cell with the excitation wavelength of 290 nm and the emission wavelength of 545 nm. The enhanced fluorescence intensity was represented as $\Delta F = F - F_0$. Here, F and F_0 were the fluorescence intensities of the systems with and without NFLX, respectively.

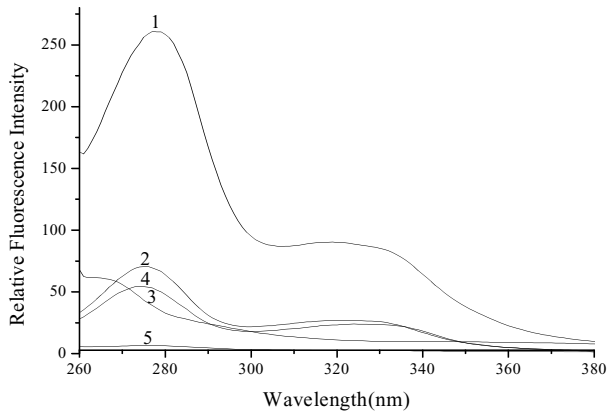
Results and discussion

Fluorescence spectra

The emission and excitation spectra of NFLX-Tb^{3+}-SDBS (1), NFLX-Tb^{3+} (2), Tb^{3+}-SDBS (3), NFLX (4), and Tb^{3+}(5) systems were shown in Fig. 1. From Fig. 1, it could



(a) Emission spectra (λ ex=290 nm)



(b) Excitation spectra (λ em=490 nm)

Fig. 1 Fluorescence spectra. (1) NFLX–Tb^{3+}-SDBS; (2) NFLX-Tb^{3+}; (3) Tb^{3+}-SDBS; (4) NFLX; (5) Tb^{3+}. $[Tb^{3+}] = 5.0 \times 10^{-5}$ mol L^{-1}; $[NFLX] = 1.0 \times 10^{-6}$ mol L^{-1}; $[SDBS] = 5.0 \times 10^{-4}$ mol L^{-1}; [glycine] = 0.5%; pH = 7.6

be seen that almost no characteristic fluorescence of Tb^{3+} was observed in the Tb^{3+} system (5), and weak characteristic fluorescence of Tb^{3+} was observed in the Tb^{3+}-SDBS system (3), which indicated that Tb^{3+} could interact with SDBS and resulted in the energy transfer from the SDBS to Tb^{3+}, then emitted the characteristic fluorescence of Tb^{3+}. There was a strong evidence from the absorption spectra to support the above conclusion (see the luminescence mechanism section). While NFLX was added, the fluorescence intensity of the Tb^{3+}-SDBS was greatly enhanced by the NFLX (see NFLX-Tb^{3+}-SDBS system (1)), which indicated that Tb^{3+} could coordinate with NFLX and also resulted in the energy transfer from the NFLX to Tb^{3+}. This point could be proved that in the NFLX-Tb^{3+}system (2), the native fluorescence of NFLX at 410 nm was decreased obviously in comparison with the NFLX system (4). The emission peaks were at 490 and 545 nm, which corresponded to the transitions from $^5D_4$ level of Tb^{3+} to the $^7F_6$ and $^7F_5$ level, respectively. At the NFLX concentration of 1.0×10^{-6} mol L^{-1}, the results showed that the absolute fluorescence intensity (Δ F) of the NFLX-Tb^{3+}-SDBS system could enhance almost 30 times as compared with the NFLX-Tb^{3+}system which had subtracted the reagent bank.

The maximum excitation wavelength of the NFLX-Tb^{3+}-SDBS system was about 280 nm according to the excitation spectra. Considering the interference of multiple peak (at 560 nm), we selected excitation wavelength at 290 nm for fluorescence intensity measurements. In this paper, $\lambda_{ex} = 290$ nm and $\lambda_{em} = 545$ nm were chosen for further study.

Influence factors on the fluorescence intensity of the system

Effect of pH

Effect of the pH on the fluorescence intensity of the system was studied, the results were shown in Fig. 2. The results indicated that Δ F increased with the increasing of pH when pH was lower than 7.4, and remained maximum when pH was in the range of 7.4–7.7, and then sharply decreased when the pH was above 7.7. The above results could be explained as follows: Tb^{3+} might coordinate with the carbonyl and carboxyl groups of the NFLX in the NFLX-Tb^{3+}complex according to the literatures reported [14]. Obviously, it was disadvantageous of NFLX coordinating with Tb^{3+} ion in the acidic environment when pH was lower than 7.0. Also, Tb^{3+} ion would be deposited in the strong alkaline medium, which blocked the coordination between the NFLX and Tb^{3+} ion. In addition, the changes of pH would influence the compositions and stabilities of the fluorescent complexes and resulted in the changes of the fluorescence characters. So, the suitable pH was very important for the fluorescence characters of the metallic organic complexes, and pH = 7.6 was selected for further research.

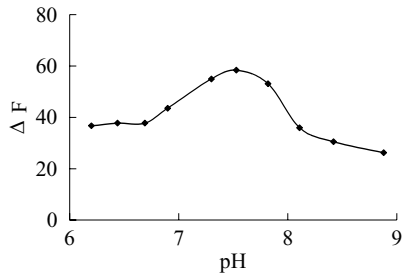


Fig. 2 Effect of pH. $[Tb^{3+}] = 5.0 \times 10^{-5}$ mol L^{-1}; [NFLX] = 1.0×10^{-6} mol L^{-1}; [SDBS] = 5.0×10^{-4} mol L^{-1}; [glycine] = 0.5%

Effect of the buffers

The experiments indicated that the buffers also had a large effect on the fluorescence intensity of the system. Some buffers were tested as follows: KH_2PO_4, Tris-HCl, HMA, NH_4Cl-NH_3, Glycine-NaOH. The results were shown in Table 1. From Table 1, it was found that glycine-NaOH was the most suitable buffer. The proper concentration of glycine was 0.5% in the solution. The glycine-NaOH buffer could obviously enhance the fluorescence of the system, its main effect was to improve the microenvironment of the system, the possible mechanism was as follows: the coordination number of Tb^{3+} in its complex was 6–8, its coordination number cannot be satisfied after coordinating with NFLX. Tb^{3+} would coordinate with H_2O and form NFLX-Tb-$(H_2O)_n$ complex. Tb^{3+} in NFLX-Tb-$(H_2O)_n$ complex after binding with NFLX would easily bind to a amidogen group of glycine and be accompanied with the release of H_2O molecule in NFLX-Tb-$(H_2O)_n$. Thus the non-radiative energy loss through O-H vibration of H_2O molecule in NFLX-Tb^{3+} complex would be decreased and the fluorescence of the system would be enhanced [12].

Table 1 Effect of the buffers (pH = 7.60 ± 0.05)

Buffers	Water	HMA	KH_2PO_4	NH_4Cl-NH_3	Tris-HCl	Glycine-NaOH
Δ F(%)	17.2	60.0	NO	24.3	37.8	100

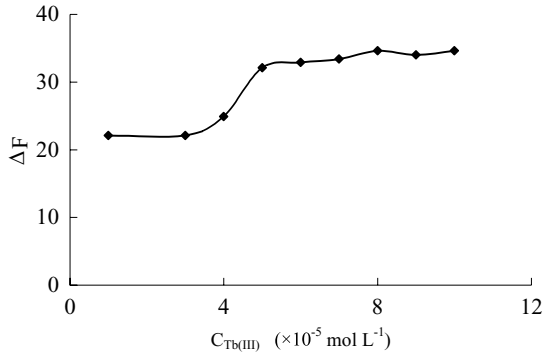


Fig. 3 Effect of terbium(III) ion concentration. [NFLX] = 1.0×10^{-6} mol L^{-1}; [SDBS] = 5.0×10^{-4} mol L^{-1}; [glycine] = 0.5%; pH = 7.6

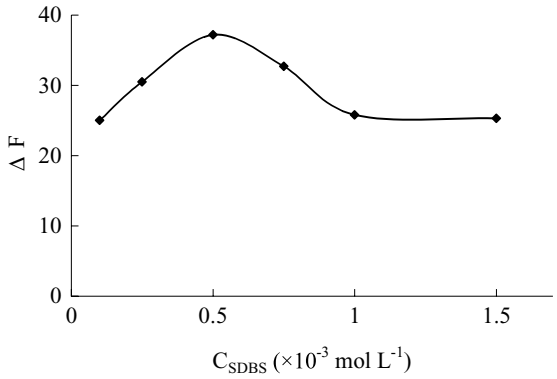


Fig. 4 Effect of SDBS concentration. [Tb^{3+}] = 5.0×10^{-5} mol L^{-1}; [NFLX] = 1.0×10^{-6} mol L^{-1}; [glycine] = 0.5%; pH = 7.6

Effect of the terbium(III) ion concentration

When the concentration of NFLX was fixed at 1.0×10^{-6} mol L^{-1}, effect of the Tb^{3+} concentration on the fluorescence intensity of the system was studied. The results was shown in Fig. 3. It could be seen that Δ F almost remained constant and maximum when the concentration of Tb^{3+} was up to 5.0×10^{-5} mol L^{-1}. Although Δ F had a slightly increase when the concentration of Tb^{3+} was more than 5.0×10^{-5} mol L^{-1}, the slight increase only resulted from the characteristic fluorescence of the Tb^{3+} itself, in other words, the fluorescence increase of the Tb^{3+} itself was neglected and not be subtracted, just because the increase of F_0 was very limited with the increase of Tb^{3+} concentrations at low concentrations, F_0 was always regarded as a constant. So, the Tb^{3+} concentration of 5.0×10^{-5} mol L^{-1} was selected for further research.

Effect of the surfactants

Surfactants were frequently employed to solutize hydrophobic compounds as well as to increase the fluorescence intensities of the weakly fluorescent compounds, because micellar solutions could improve the microenvironment of luminescence. The Experiments indicated that the surfactants had a large effect on the fluorescence intensity of the system. Under the same condition the following surfactants were tested: SDBS, SDS, Tween-20, TX-100, β-CD, CTAB. The results were shown in Table 2. From Table 2, it was found that different kinds of surfactants could increase the fluorescence intensity of the system to different extents, and the most effective surfactant was SDBS. The effect of the concentration of SDBS on the fluorescence intensity of the system had also studied, the results were shown in Fig. 4. The results indicated that the Δ F increased with the increasing of the SDBS at low concentration, it attained maximum when the SDBS concentration was 5.0×10^{-4} mol L^{-1}, and then decreased when the concentration of SDBS was above 5.0×10^{-4} mol L^{-1}. The above results could be explained as follows: the Δ F increased with the increasing of the SDBS when its concentration was close to the critical micelle concentration (CMC) of SDBS, just for a multiple ionic associate was formed in the micellar solution, which was beneficial to increase the effective absorption cross section of the complex and resulted in the increase of molar absorbancy index. When the concentration of SDBS was more than CMC, maybe the surplus SDBS would extinguish the fluorescence of the system. So, the SDBS concentration of 5.0×10^{-4} mol L^{-1} was chosen for further research.

Stability test

The experiments indicated that the fluorescence intensity of the system reached a maximum in 20 min after all the reagents had been added and remained stable for at least 1 h.

Interferences of the foreign substances

Interferences of the common metal ions

Under the optimum conditions, interferences of the foreign metal ions on the fluorescence intensity of the system were studied. At the NFLX concentration of 1.0×10^{-6} mol L^{-1}, the highest permissible molar excesses of the foreign metal

Table 2 Effect of the surfactants

Surfactants	NO	SDBS	SDS	Tween-20	TX100	β-CD	CTAB
ΔF	2.4	26.0	2.7	24.6	2.1	NO	2.2

Table 3 Recovery test in pharmaceutical samples

Species (1.0×10^{-6} mol L^{-1})	Added NFLX ($\times 10^{-6}$ mol L^{-1})	Found NFLX ($\times 10^{-6}$ mol L^{-1})	x ± s	Recovery (%)
Cefalexin	0.20	0.187,0.214,0.204,0.191,0.217	0.203 ± 0.013	101.3
	1.00	0.91, 1.06,0.89,1.04,0.96	0.972 ± 0.076	97.2
Cefradine	0.20	0.183,0.213,0.190,0.198,0.215	0.200 ± 0.014	100.0
	1.00	0.96,1.04,1.14,1.10,1.08	1.064 ± 0.068	106.4
Acetaminophen	0.20	0.205,0.202,0.199,0.194,0.233	0.207 ± 0.015	103.5
	1.00	1.07,1.11,1.09,1.09,0.95	1.062 ± 0.064	106.2
Cimetidine	0.20	0.188,0.190,0.197,0.192,0.199	0.193 ± 0.005	96.5
	1.00	0.99,1.08,1.04,1.03,1.02	1.032 ± 0.033	103.2

ions causing a ±10% relative error in the fluorescence intensity were as follows: 50-fold molar excess of Mg^{2+}, Zn^{2+}, Cu^{2+} and Ca^{2+}, and 5-fold molar excess of Fe^{3+} and Al^{3+}. Therefore, these common metal ions did not interfere with the determination when its concentration was not high enough.

Interferences of the other pharmaceuticals

Under the optimum conditions, interferences of these pharmaceuticals which usually used as the compatibility of medicines on the fluorescence intensity of the system were studied. At the NFLX concentration of 1.0×10^{-6} mol L^{-1}, the highest permissible molar excesses of other pharmaceuticals causing a ±10% relative error in the fluorescence intensity were as follows: more than 200-fold molar excess of amoxicillin, 200-fold molar excess of ibuprofen, 50-fold molar excess of cimetidine, 5-fold molar excess of furosemide and sulfamethoxazole (SMZ), and 1-fold molar excess of ranitidine hydrochloride.

Analytical application

Calibration curve and detection limit

Under the optimal conditions, the calibration graphs for the determination of norfloxacin were constructed. The enhanced fluorescence intensity of the system (Δ F) showed a good linear relationship with the concentration of norfloxacin in the range of 5.0×10^{-9} mol L^{-1}–2.0×10^{-6} mol L^{-1}, its correlation coefficient was 0.9991. The detection limit ($S/N = 3$) was 1.2×10^{-9} mol L^{-1}.

Recovery tests

The recovery tests in cefalexin, cefradine, acetaminophen, and cimetidine solutions were examined with the standard addition method. The results were shown in Table 3. From Table 3, it could be seen that these ordinary pharmaceuticals did not interfere with the determination, all recoveries were in the range of 96.5–106.2%.

Sample determination

The norfloxacin capsule (Zhejiang DE EN Pharmaceutical Co. Ltd., China) and norfloxacin eye drops (Nanjing LI YE Pharmaceutical Co. Ltd., China) were directly determined by the proposed method. The results were shown in Table 4. From Table 4, it showed that the concentrations of the samples determined by the presented method were in accordance with the mark concentrations of the samples determined by other method.

Luminescence mechanism

The mechanisms of the fluorescence enhancement caused by the SDBS were explained as follows. Firstly, NFLX was lower polar and hydrophobic compound, and its solubility in the micellar solution could be improved obviously. When the complexes of the NFLX-Tb^{3+} were dispersed and gathered together with the micelles, its microenvironment was greatly changed, which could decrease the nonradiative energy loss through molecule collisions and improve the quantum efficiency of fluorescence [15]. Secondly, the coordination number of Tb^{3+} in its complex was 6–8, furthermore, the concentration of Tb^{3+} was overplus in

Table 4 Sample determination

Sample species	Units	Found	Average	RSD (%)	Mark conc
Norfloxacin capsule	(%)	52.6, 54.1, 52.8, 50.1, 55.5	53.02	3.77	56.0
Norfloxacin eye drops	(g/L)	2.66, 3.02, 2.97, 2.74, 2.72	2.82	5.67	3.0

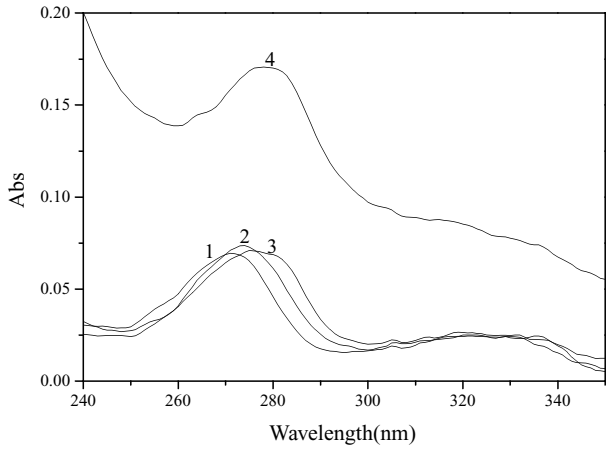


Fig. 5 Absorption spectra of the NFLX-Tb^{3+}-SDBS system. 1. NFLX; 2. NFLX-Tb^{3+}; 3. NFLX-SDBS; 4. NFLX-Tb^{3+}-SDBS. [NFLX] = 2.5×10^{-6} mol L^{-1}; [Tb^{3+}] = 5.0×10^{-5} mol L^{-1}; [SDBS] = 5.0×10^{-4} mol L^{-1}; [glycine] = 0.5%

the NFLX-Tb^{3+}-SDBS system. Therefore, the coordination number of Tb^{3+} could not be satisfied after the formation of the NFLX-Tb^{3+} complex, and Tb^{3+} in the NFLX-Tb^{3+} complex could interact with the SDBS by an ionic attraction. Therefore, it was possible that the energy was transferred not only from NFLX to Tb^{3+}, but also from SDBS to Tb^{3+}, and caused the great fluorescence enhancement of the system [16]. The above conclusion could be explained from Fig. 5 and 6. From Fig. 6, it could be seen that the absorption of SDBS (had subtracted the background absorption from all the reagents by using the reagents as a reference solution except SDBS which being evaluated) increased obviously after the Tb^{3+} was added, which indicated that SDBS might interact with the Tb^{3+}, and could result in the energy transfer from the SDBS to Tb^{3+}. This was the reason why the SDBS could enhance the characteristic fluorescence of Tb^{3+} in the Tb^{3+} solution(see Fig. 1(a)). From Fig. 5, it could be seen

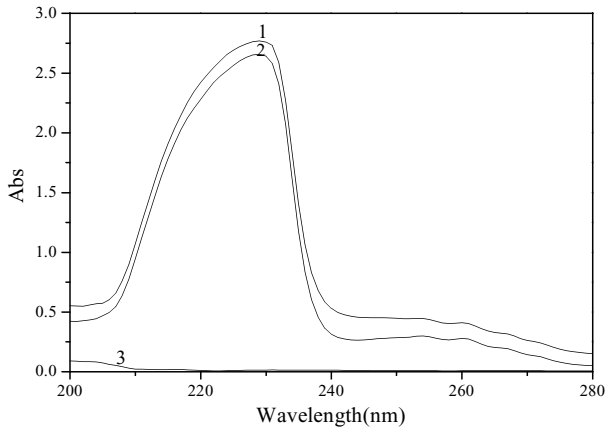


Fig. 6 Absorption spectra of the Tb^{3+}-SDBS system. 1. Tb^{3+}-SDBS; 2. SDBS; 3. Tb^{3+}. [Tb^{3+}] = 5.0×10^{-5} mol L^{-1}; [SDBS] = 5.0×10^{-4} mol L^{-1}; [glycine] = 0.5%

that the absorption of NFLX (had subtracted the background absorption from all the reagents by using the reagents as a reference solution except NFLX which being evaluated) increased appreciably and its maximum absorption wavelength underwent a red shift after both the Tb^{3+} and SDBS solution were added. However, the adsorption of NFLX increased greatly when the Tb^{3+} together with SDBS were added, which was in accordance with the fluorescence enhancement of the excitation spectra in the NFLX-Tb^{3+}-SDBS system (see Fig. 1(b)), and its maximum absorption wavelength underwent a red shift from 271 nm to 278 nm. Thirdly, a multiple ionic associate was formed in the NFLX-Tb^{3+}-SDBS system, which was beneficial to increase the effective absorption cross section of the complex and resulted in the increase of molar absorbancy index. Therefore, the fluorescence intensity of the system was greatly enhanced by the SDBS. In addition, the optimal SDBS concentration approximated to the critical micelle concentration (CMC) of SDBS (CMC = 0.63 mmol L^{-1}) [17], which indicated that the formation of micelles had a great effect on the increase in the fluorescence intensity of the system.

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