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Letter

Observations on photochemical fluorescence enhancement of the terbium(III)–sparfloxacin system

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Abstract

Fluorescence of terbium(III) was sensitized when excited in the presence of sparfloxacin (SPFX) in the aqueous solution because a Tb(III)–SPFX complex was formed. The sensitized fluorescence was further enhanced when this system was exposed to 365 nm ultraviolet light. By the spectral properties and contrast experiments, it is proved that irradiation makes this system undergo photochemical reactions and a new terbium complex which is more favorable to the intramolecular energy transfer is formed. The mechanism of photochemical fluorescence enhancement of the Tb(III)–SPFX system is discussed and a new sensitive and selective photochemical fluorimetry for the determination of SPFX is established. Under the optimum conditions, the linear range is $1.0–50 \times 10^{-7}$ M for SPFX, the detection limit is 3.0×10^{-9} M and the R.S.D. for 5.0×10^{-7} M SPFX is 1.3% ($n = 9$). Without any pretreatment the recovery of SPFX in human urine was determined with satisfaction.

Keywords: Terbium ion; Sparfloxacin; Sensitized fluorescence; Photochemical reaction; Body fluids

1. Introduction

Sparfloxacin (5-amino-1-cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid) is a synthetic antibacterial agent and also a fluoroquinolone derivative, which is widely used in the treatment of urinary tract infections because of its excellent activity against various bacteria, low frequency of adverse effects and good absorption on oral administration. So far the high-performance liquid chromatography method (HPLC) [1] and bioassay methods [2,3] have been described for SPFX determination in biological fluids, but all of these methods need tedious pretreatment. In order to establish a simple determination method for SPFX, the sensitized fluorescence of lanthanides with SPFX was investigated, the advantages of which are large stokes shift, narrow emission bands long fluorescence lifetime and hence avoid potential background interferences from the sam-

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ple matrix. We found that SPFX can form a complex with Tb^{3+}, which emits the sensitized fluorescence of Tb^{3+}. Moreover, the sensitized fluorescence is enhanced notably when this system is irradiated by 365 nm ultraviolet light. The mechanism of the fluorescence enhancement and its analytical application were investigated in this paper.

2. Experimental

2.1. Apparatus

Fluorescence spectra were recorded with a Hitachi-850 spectrofluorimeter (Japan). Absorption spectra were recorded on a Shimadzu-UV250 spectrophotometer (Japan). The pH measurements were made with a pHS-2 meter (Shanghai). A ZF-I Ultraviolet analytical meter (Shanghai) was used as the light source for the photochemical reaction. Phosphorescence spectra were measured on a FL-ZTZ spectrofluorimeter equipped with a 193D phosphorimeter (SPEX Co., USA). Fluoride measurement was made with a fluoride selective electrode (model PF-I, Shanghai) as the indicator electrode and a saturated calomel electrode as the reference electrode (model 801, Jiangsu, China).

2.2. Reagents

All reagents were of analytical purity grade. Water was doubly distilled.

Aqueous stock standard solution, 1.0×10^{-3} M of SPFX (Enoxacin and Tosufloxacin, kind gifts from Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijing) was prepared by dissolving SPFX in distilled water with 0.1 M NaOH, stored at 4°C and protected from light. Working standard solutions were prepared by appropriate dilution with distilled water.

Stock standard solution of TbCl3 (5.0×10^{-2} M) was prepared by dissolving Tb_4O_7 (99.99%) in warm 1:1 HCl and evaporating the solution to be almost dry before diluting, stored in a plastic bottle, kept at 4°C, and diluted to the desired concentration when necessary.

Buffer solutions of the required pH were made from sodium acetate and acetic acid, according to Michaelis [4].

2.3. Procedure

2.3.1. Spectral recording

To a 10-ml calibrated tube, solutions were added in the following order: 1.0 ml 5.0×10^{-4} M SPFX, 1.0 ml 5.0×10^{-2} M Tb^{3+} and 5.0 ml NaAc–HAc buffer solution. The mixture was diluted to 10 ml with distilled water, thoroughly mixed by shaking, exposed to 365 nm ultraviolet light for 60 min (the irradiation intensity was 30 mw/cm^2), then the absorption spectrum, the fluorescence spectrum and room temperature phosphorescence spectrum were recorded. The corresponding contrast experiments were made with the unirradiated samples.

2.3.2. Analytical measurement

Add a proper amount of standard SPFX solution, l.0 ml of 5.0×10^{-2} M Tb^{3+} and 5.0 ml of NaAc–HAc (pH 5.5) buffer solution to a 10-ml calibrated tube with a stopper, then dilute up to the mark with water before shaking. After the sample was irradiated for 60 min under a 365-nm ultraviolet lamp with the irradiation intensity of 30 mw cm^2, the difference of fluorescence intensities ΔI_F between the sample and the blank was determined using excitation wavelength of 310 nm and emission wavelength of 545 nm, with excitation and emission slits of 15 and 20 nm in width, respectively.

2.3.3. Fluoride measurement

Add l.0 ml 5.0×10^{-4} M SPFX solution, 1.0 ml 5.0×10^{-2} MTb^{3+} solution, 5.0 ml NaAC–HAC buffer solution (pH = 5.5) and 3.0 ml water to a 20 ml beaker (the final volume of the sample is 10 ml). Then the sample was irradiated by 365 nm ultraviolet light for 50 min before adding 0.5g NaCl to adjust the total ionic strength. Take a fluoride electrode as the indicator electrode and a saturated calomel electrode as the reference electrode to measure the electromotive force of the cell. The concentration of the fluoride was determined by Gran's plot. The same experiment was made with the unirradiated sample.

3. Results and discussion

3.1. Spetrophotometric studies on the reaction between SPFX and terbium

The absorption spectrum of SPFX is characterized by two absorption bands: 290 and 355 nm (Fig. 1, curve 1). Compared with the absorption bands of SPFX, both absorption peaks have slight red shift (from 290 to 295 nm and from 355 to 360 nm (Fig. 1, curve 2)) and the absorbance of the band at 290 nm increases slightly for the system of SPFX with Tb^{3+}. The red shift shows that the conjugation increases and implies the formation of the Tb(III)–SPFX complex.

3.2. Fluorescence spectra

SPFX fluoresces too weakly to be determined by spectrofluorimetric method, so does the free Tb^{3+} solution under the same conditions, but the intense characteristic fluorescence of Tb^{3+} appears when SPFX is mixed with Tb^{3+}. The result indicates that SPFX and Tb^{3+} form a complex. The energy absorbed by SPFX is transferred effectually to Tb^{3+} by intramolecular energy transfer, Tb^{3+} is then excited and subsequently fluoresces at certain wavelengths. The narrow emission peaks appear at 492, 545, 588 and 623 nm (Fig. 2b(l)), corresponding to the transitions

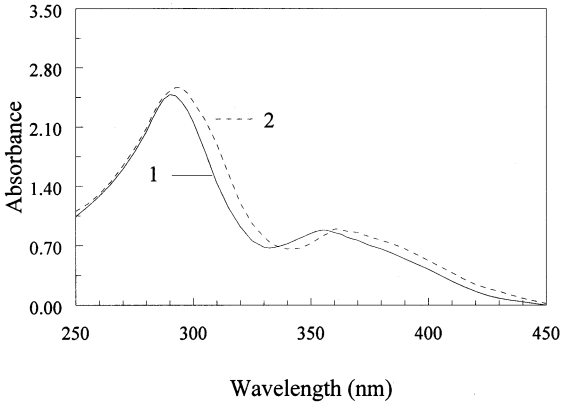


Fig. 1. Absorption spectra of SPFX and the Tb(III)–SPFX complex: (1). [SPFX] = 5.0×10^{-5} M; pH = 5.5; (2) [SPFX] = 5.0×10^{-5} M; [Tb^{3+}] = 5.0×10^{-3} M; pH = 5.5.

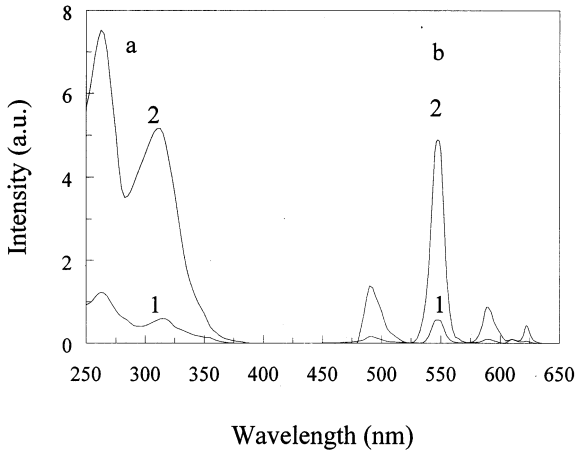


Fig. 2. Fluorescence excitation (a) and emission (b) spectra of the irradiated and unirradiated Tb(III)–SPFX complex systems [SPFX] = 5.0×10^{-5} M; [Tb^{3+}] = 5.0×10^{-3} M: pH = 5.5; (1) $\lambda_{ex} = 315$ nm; $\lambda_{em} = 545$ nm (unirradiated); (2) $\lambda_{ex} = 310$ nm; $\lambda_{em} = 545$ nm (irradiated).

of $^5D_4 \rightarrow {}^7F_6$, $^5D_4 \rightarrow {}^7F_5$, $^5D_4 \rightarrow {}^7F_4$ and $^5D_4 \rightarrow {}^7F_3$, respectively, among which the $^5D_4 \rightarrow {}^7F_5$ emission is the strongest, so 545 nm is selected as the analytical wavelength. In addition, 315 nm is used as excitation wavelength although the intensity of excitation peak at 265 nm is stronger than that at 315 nm (Fig. 2a) in order to avoid the direct excitation of Tb^{3+} [5] and the disturbance of the scattered excitation light (270–280 nm).

It was discovered in the experimental process that irradiation can enhance the sensitized fluorescence of the Tb(III)–SPFX complex system. The relative intensity increases 5–12 times after irradiation using 365 nm ultraviolet light for 50 min, which is dependent upon the concentration of SPFX (Fig. 2, curve 2). This suggests that some chemical changes have taken place in the irradiating process, resulting in the new terbium complex and leading to the more effectual energy transfer.

3.3. The mechanism of the fluorescence enhancement

3.3.1. Spectral analysis

Fig. 3 shows the fluorescence spectra of the irradiated Tb(III)–SPFX complex (a) unirradiated Tb(III)–SPFX complex (b) the phosphores-

cence spectra of the irradiated free SPFX (c) and unirradiated free SPFX (d), which were measured by the phosphorimeter. Because the delayed time for measurement was set to 0.001 ms and the lifetime of the characteristic fluorescence of Tb^{3+} is long (0.1–10 ms) [6], the emission of free SPFX is phosphorescence while that of Tb(III)–SPFX is fluorescence. The phosphorescence intensity of the irradiated SPFX system is higher than that of the unirradiated SPFX system. Moreover, the emission peak has a red shift, which implies that the structure of SPFX has changed and the triplet state energy of the product is lowered.

The phosphorescence of the unirradiated free SPFX decreases when mixed with Tb^{3+} and the characteristic fluorescence of Tb^{3+} appears. The contrast spectra of the two irradiated systems show that the phosphorescence of the ligand after irradiation is too weak to be observed while the sensitized fluorescence of Tb^{3+} after irradiation is enhanced markedly compared with the unirradiated Tb(III)–SPFX complex. This suggests that the intramolecular energy transfer from ligand to Tb^{3+} after irradiation is dominant in the competition with other processes, such as the fluorescence of ligand, the phosphorescence of ligand and non-radiative deactivation [7].

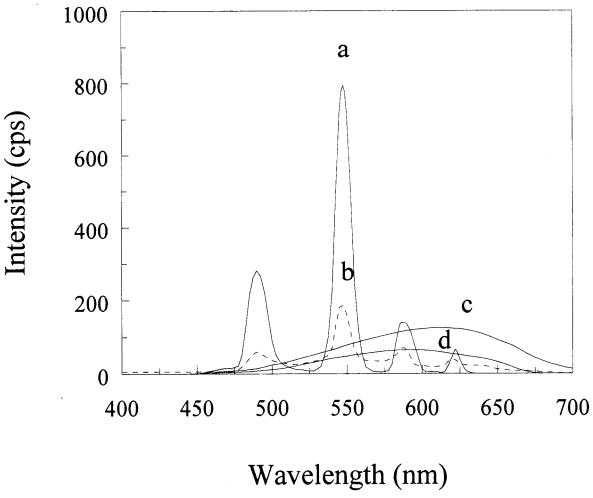


Fig. 3. Emission spectra of the Tb(III)–SPFX complex and free SPFX. (a) the irradiated Tb(III)–SPFX complex; $[SPFX] = 5.0 \times 10^{-5}$ M; $[Tb^{3+}] = 5.0 \times 10^{-3}$ M; pH = 5.5; $\lambda_{ex} = 331$ nm; (b) the unirradiated Tb(III)–SPFX complex; $[SPFX] = 5.0 \times 10^{-5}$ M; $[Tb^{3+}] = 5.0 \times 10^{-3}$ M; pH = 5.5; $\lambda_{ex} = 345$ nm; (c) the irradiated free SPFX; $[SPFX] = 5.0 \times 10^{-5}$ M; pH = 5.5; $\lambda_{ex} = 335$ nm; (d) the unirradiated free SPFX; $[SPFX] = 5.0 \times 10^{-5}$ M; pH = 5.5; $\lambda_{ex} = 334$ nm.

3.3.2. Deduction of the products

It was reported that SPFX degrades when the temperature is higher than 100°C, the product is decarboxylated SPFX [8], but thermodegradation is not relative to our work, because all the experiments were carried out at room temperature (about 20°C). SPFX is sensitive to light and the photodegradation will take place after exposed to sunlight for a long time [9]. But the photoproducts were unknown. Several contrast experiments were made to deduce the products.

(a) The Tb(III)–SPFX complex system ($[SPFX] = 5.0 \times 10^{-5}$ M; $[Tb^{3+}] = 5.0 \times 10^{-3}$ M pH = 5.5) and the free SPFX system ($[SPFX] = 5.0 \times 10^{-5}$ M; pH = 5.5) were irradiated under the same conditions. Tb^{3+} ($[Tb^{3+}] = 5.0 \times 10^{-3}$ M) was added to the irradiated SPFX system, then the fluorescence excitation and emission spectra of the two systems were recorded. It was found that their emission spectra are the same as Fig. 2b(2). and the relative intensities are equal. This indicates that the change of the irradiated Tb(III)–SPFX complex is the structure change of the ligand SPFX.

(b) The fluoride contents in the irradiated and unirradiated Tb(III)–SPFX complex systems were measured. It was found that there is no fluoride in the non-irradiated system while the fluoride ions in the irradiated system are measurable. The same experiments were made with Tb(III)-Enoxacin and Tb(III)–Tosufloxacin complex systems. The structure of these two ligands are similar to SPFX but there are no fluorine atoms on position 8. No fluoride ions were found after irradiation in these two systems.

A conclusion is drawn from Fig. 4. that about 65% fluorine atoms on position 8 (see Scheme 1(A)) were dissociated from the aromatic ring. The result indicates that a photochemical reaction takes place during the irradiation, which is identical with the defluorination of lomefloxacin which has a fluorine atom on position 8 [10,11].

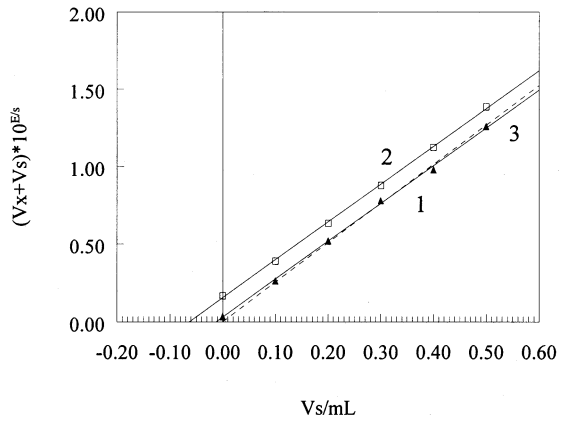


Fig. 4. Graph of fluoride determination [SPFX] = 5.0×10^{-5} M; [Tb^{3+}] = 5.0×10^{-3} M; pH = 5.5; (1) ▲, unirradiated; (2) □, irradiated; (3) —, blank sample.

(c) An air-equilibrated (in the presence of oxygen) Tb(III)–SPFX system and a nitrogen-flushed system (in the absence of oxygen) were irradiated for the same time (their compositions were the same ([SPFX] = 5.0×10^{-5} M, [Tb^{3+}] = 5.0×10^{-3} M, pH = 5.5). It was found that the relative intensity of the former was much higher than that of the latter. The result suggests that the products may be different from each other. The Tb^{3+} ion is bonded to the oxygen atom(s) from the carboxylate group of the ligand SPFX. It is no doubt that no change for the terbium(III) ion takes place during irradiation. It can be deduced that the photochemical reactions for the ligand occurring during irradiation may be as that shown schematically in Scheme 1.

The ligand part in the photoproducts of the irradiated Tb(III)–SPFX complex system may have seven forms: A, B, C, D, E, F and G. In those B, D, E, F, and G were proved experimentally by similar studies of the irradiated lomefloxacin [10,11]. Loss of fluoride leads to cation B, for which mesomeric carbocationic and carbene E forms can be considered. The products C and D are formed when the nucleophiles are chloride and acetate ions. Although the chemical properties of carbenes of the type E are not known, E behaves in an analogous manner to the cyclic carbene, 4-oxocyclohexa-2,5-dienylidene which has been extensively studied [12–14]. Therefore the products should be a phenol F in the presence of nitrogen and a quinoneimine G in the presence of oxygen. The reason of the enhancement of the sensitized fluorescence of Tb^{3+} after irradiation of the Tb(III)–SPFX system is probably that the product (G) forms a new complex with Tb^{3+}. Because the conjugation of G increases, its triplet state energy is relatively lowered and the efficiency of the intramolecular energy transfer is enhanced markedly.

3.4. Analytical application

3.4.1. The optimum experimental conditions

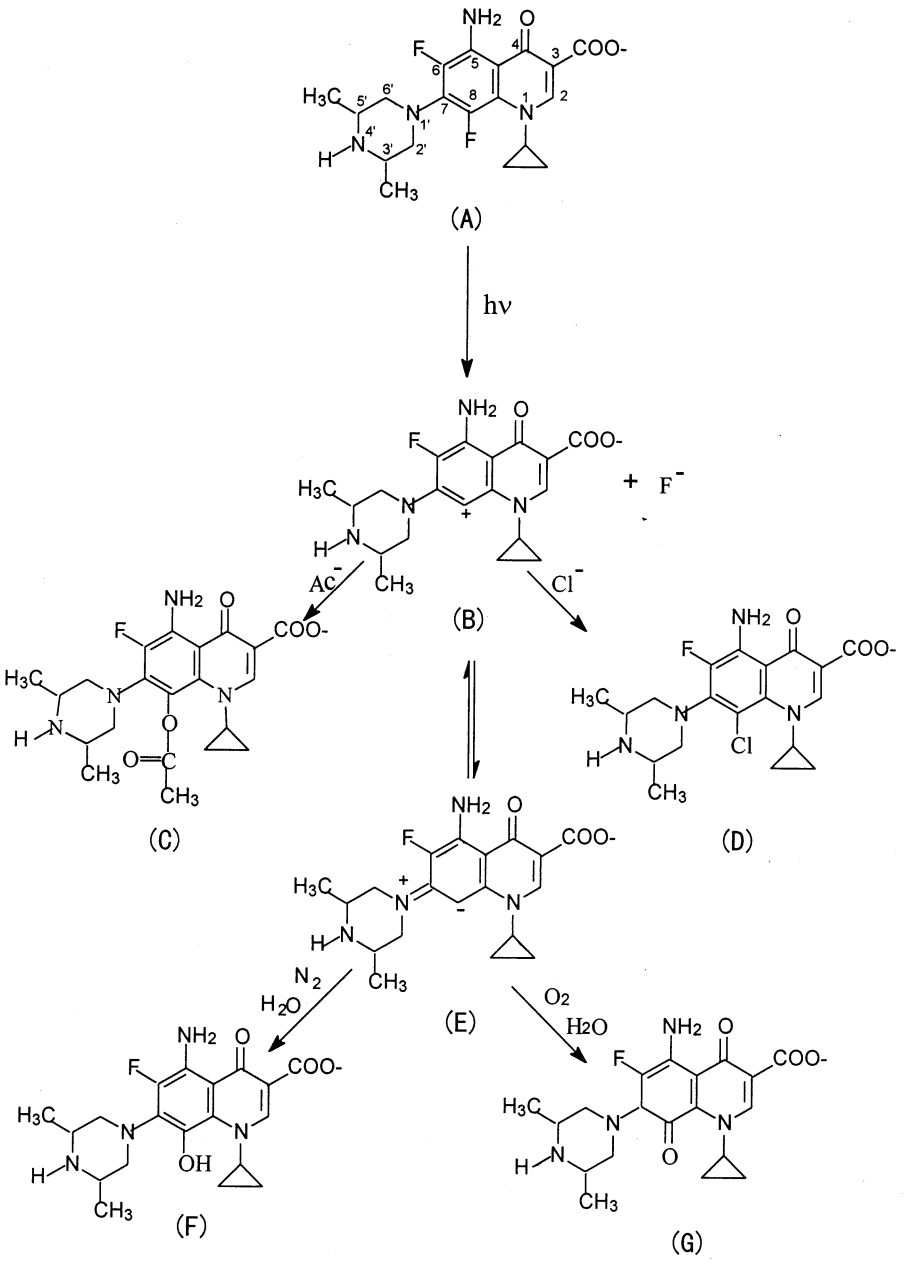
It was found that the sensitized fluorescence intensity of the Tb^{3+} for 2.0×10^{-6} M SPFX was the highest and stable under the following conditions: the irradiation time is more than 40 min, the pH is in the range of 4.5–6.5 and the concentration of Tb^{3+} is over 2.0×10^{-3} M. So, the irradiation time of 60 min, pH5.5 (Hac–NaAc) buffer solution and 5.0×10^{-3} M Tb^{3+} were chosen in the calibration graph drawing.

3.4.2. Calibration graph and detection limit

Under the optimum conditions described above, the SPFX calibration graph is linear in the range $1.0–50 \times 10^{-7}$ M and the regression formula is $\Delta I_F = 0.0566 + 1.114 \times 10^6$ C_{SPFX}(M), with correlation coefficient $r = 0.9992$. The relative standard deviation (R.S.D.) for 5.0×10^{-7} M SPFX is 1.3% ($n = 9$). The detection limit, defined as three times the standard deviation for the reagent blank signal is 3.0×10^{-9} M SPFX ($n = 10$).

3.4.3. Sample determination

In order to evaluate the validity of this method for SPFX determination in human urine, the recovery studies were carried out on urine samples where known amounts of SPFX were added by standard addition method. The results obtained are shown in Table 1.



Scheme 1. Photochemical changes of the ligand SPFX in the irradiated Tb(III)–SPFX complex.

4. Conclusion

Irradiation of the Tb(III)–SPFX complex causes photochemical reactions taking place in the ligand part. The product is more favorable to the intramolecular energy transfer, resulting in the enhanced sensitized fluorescence of Tb^{3+}. On this basis a new photochemical fluorimetric method

Table 1
Recovery of sparfloxacin added to urine ($n = 5$)

Sample	SPFX added (M, $\times 10^{-6}$)	SPFX found (M, $\times 10^{-6}$)	Recovery (%)	R.S.D. (%)
Urine	0.40	0.43	107.5	4.6
	1.00	1.04	104.0	2.3

for the determination of SPFX has been established. Because of its high sensitivity, selectivity, accuracy and good repeatability, it was successfully used in the determination of urine samples without any prehandling but only by appropriate dilution of the samples. It is a simple and rapid method for the determination of SPFX in body fluids.

Acknowledgements

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