Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 38 (2005) 528–531
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

The fluorescence characteristic of the yttrium–norfloxacin system and its analytical application

Yunxiao Han, Xia Wu *, Jinghe Yang, Shuna Sun

Key Laboratory of Colloid and Interface Chemistry, Ministry of Education, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, PR China

Accepted 14 January 2005
Available online 7 March 2005

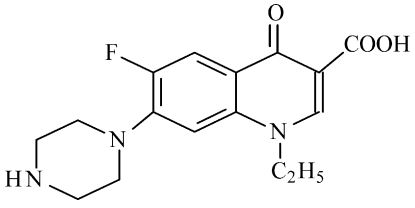
Abstract

In this paper, a simple, rapid and sensitive spectrofluorimetric method based on the formation of yttrium complexes has been developed for the determination of norfloxacin (NFLX). The affecting factors of the enhancement were studied in detail. Under optimum conditions, a linear relationship was obtained between the enhanced fluorescence intensity and the NFLX concentration in the range 1.0×10^{-9} to 1.0×10^{-5} mol/l and the detection limit of NFLX was 3.8×10^{-11} mol/l ($S/N = 3$). The method is applied for the determination of NFLX in actual sample (norfloxcain eye drops), the average recovery is 102.4% ($n = 5$), and the result obtained is satisfactory.

Keywords: Norfloxacin; Yttrium(III); Fluorescence

1. Introduction

Norfloxacin (NFLX) is the third generation quinolone synthetic antibiotic, which has the characteristic of broad bacterium contradicting, small side-effects and cross-resistance with other drugs, so has been widely used for the clinic. It works by entering the bacterial cell and inhibiting a chemical called DNA-gyrase, which is involved in the production of genetic material (DNA). This, therefore, prevents the bacteria from reproducing and their growth is stopped. Norfloxacin reaches high levels in the urine and so is used in the treatment of urinary tract infections. Its structure is shown in the following:



The reported determination methods of NFLX focused on: spectrometry [1], polarography [2], HPLC [3] and spectrofluorimetry [4–5]. Because of the high sensitivity and selectivity, the spectrofluorimetry has been widely used to the analysis of pharmaceutical. Using rare earth Tb^{3+} as fluorescence probe to detect NFLX have been reported [6–9], NFLX can transfer energy to Tb^{3+} and enhance Tb^{3+} fluorescence. The fluorescence of Y^{3+} itself in solution is not observed, but after combine with an organic ligand with a chromophore, Y^{3+} may enhance the fluorescence of the ligand (NFLX). In this paper, the Y–NFLX fluorescence system was studied; the Y^{3+} could form complexes with NFLX, which emitted the strong fluorescence. The proposed method is simple and sensitive.

2. Experimental

2.1. Apparatus

All fluorescence intensities were measured on a F-4500 spectrofluorimeter (Hitachi, Japan). All pH measurements were made with a Delta 320-S pH meter (Mettler Toledo). All

* Corresponding author.
E-mail address: wux@sdu.edu.cn (X. Wu).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.01.033

absorption spectra were recorded with U-5100 spectrophotometer (Hitachi, Japan).

2.2. Reagents and solutions

Stock standard solution of Y^{3+} (1.0×10^{-2} mol/l) was prepared by dissolving the yttrium oxides (Y_2O_3, 99.9%, Yuelong chemical plant, Shanghai, China) in hydrochloric acid (1:1) and heating until nearly dry then diluting with doubly distilled water.

Stock solution of NFLX (Sanxing plant, Shandong, China) (1.0×10^{-3} mol/l) was prepared by dissolving the appropriate amount of NFLX with 0.1 mol/l NaOH, and diluted with doubly distilled water.

A 0.2 mol/l Tris–HCl buffer solution was prepared by dissolving 12.12 g Tris in 500 ml volumetric flask with water and adjusted the pH with HCl.

All the reagents were of analytical grade and doubly distilled water was used throughout.

2.3. Experimental procedure

To a 25 ml test tube, appropriate amount of NFLX, 1.5 ml 1.0×10^{-3} mol/l Y^{3+} and 2.0 ml of 0.2 mol/l Tris buffer solution were added. The mixture was diluted to 10 ml with distilled water, shaken and allowed to stand for 10 min. The fluorescence intensity of the system was measured in a 1 cm quartz cell with excitation and emission wavelengths of 276 and 425 nm, respectively. The excitation and emission slits were both 5 nm.

3. Results and discussion

3.1. Fluorescence spectrum

Excitation and emission spectra of Y^{3+} (1), NFLX (2) and NFLX–Y^{3+} (3) are shown in Fig. 1. It can be seen that NFLX could emits fluorescence. The wavelengths of the excitation and emission peaks were at 276.0 and 425.0 nm, respectively. After adding Y^{3+} solution, the wavelength of the emission peak blue shift and the fluorescence intensity was greatly enhanced. The emission peak shifts to a shorter wavelength from 440 nm (NFLX system) to 425 nm (NFLX–Y^{3+} system), which indicated that complexes were formed. So the emission wavelength of 425 nm was selected for the further experiment.

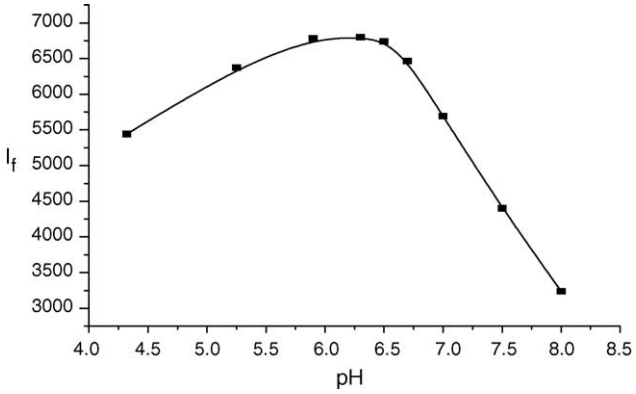


Fig. 2. Effect of pH on the fluorescence intensity. Conditions: 1.5×10^{-4} mol/l Y^{3+}, 1.0×10^{-5} mol/l NFLX, 4.0×10^{-2} mol/l Tris.

3.2. Optimization of the general procedure

3.2.1. Effects of pH and buffers solution

Fig. 2 shows the effect of pH on the fluorescence intensity of the system. The maximum fluorescence intensity obtained in the range of pH 5.8–6.5. Fixing pH 6.2, the effects of different kinds of buffers on the fluorescence intensity of the system are shown in Table 1, which indicates that Tris–HCl is the best of the buffers tested, so the Tris–HCl buffer was chosen for assay and the optimum volume of buffer is 2.0 ml.

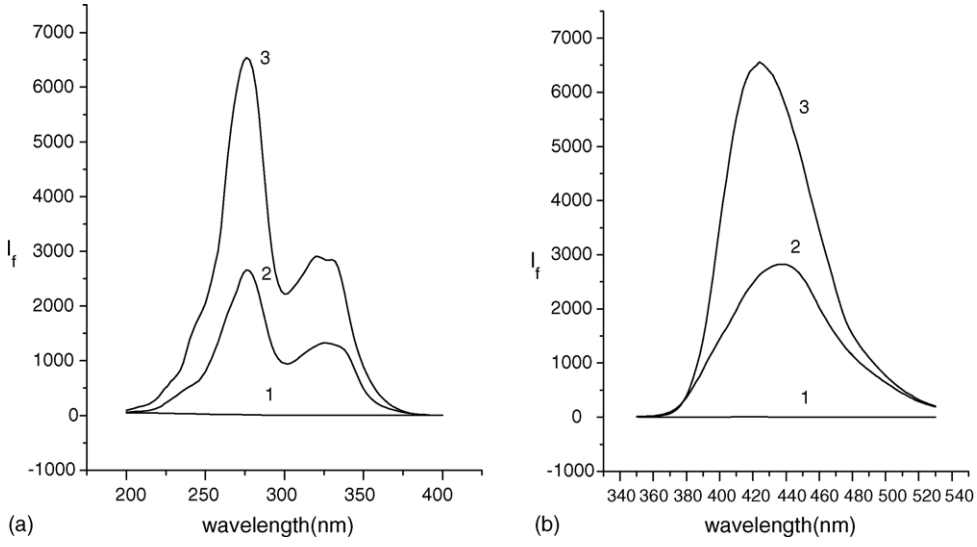


Fig. 1. Fluorescence spectra of Y–NFLX system: (a) excitation spectra (λ_{em} = 425 nm); (b) emission spectra (λ_{ex} = 276 nm). (1) Y^{3+}–Tris, (2) NFLX–Tris, (3) NFLX–Y^{3+} –Tris. Conditions: 1.5×10^{-4} mol/l Y^{3+}, 1.0×10^{-5} mol/l NFLX, 4.0×10^{-2} mol/l Tris, pH 6.2.

Table 1
Effects of buffer solutions

[a]I_f (%)	Buffers
37.4	NH_4Ac–HAc
35.2	Citric acid–citrate
100	Tris–HCl
91.2	HMTA–HCl
36.1	KH_2PO_4–NaOH

[a] The data relative to the Tris–HCl values.

3.2.2. Effect of Y^{3+} concentration

The effect of the Y^{3+} concentration on the fluorescence intensity of the systems was studied with constant concentration of NFLX (1.0×10^{-5} mol/l) (Fig. 3). From Fig. 3, it can be seen that when the concentration of Y^{3+} was lower than 1.2×10^{-4} mol/l, the fluorescence intensity enhanced with the increase of Y^{3+} concentration, then the fluorescence intensity reached a maximum and remained stable. So in further experiments, the concentration of Y^{3+} was fixed at 1.5×10^{-4} mol/l.

3.2.3. Effect of addition order

The effect of addition order on the fluorescence intensity of the system was studied. The results show that the addition order of NFLX–Y^{3+}–Tris is the best.

3.2.4. Stability test

The experiments indicated that at room temperature the fluorescence intensity of NFLX–Y system reached a maximum after 10 min and remained stable at least for 3 days.

3.2.5. Effect of foreign iron

On the optimum experimental conditions, the effects of substances including the familiar metal ions and rare earth irons on the fluorescence intensity of the system were tested, at 1.0×10^{-7} mol/l NFLX and 1.5×10^{-4} mol/l Y^{3+}, the highest permissible molar excesses of the tolerance iron causing a ±5% relative error in the fluorescence intensity of the system were shown in Table 2.

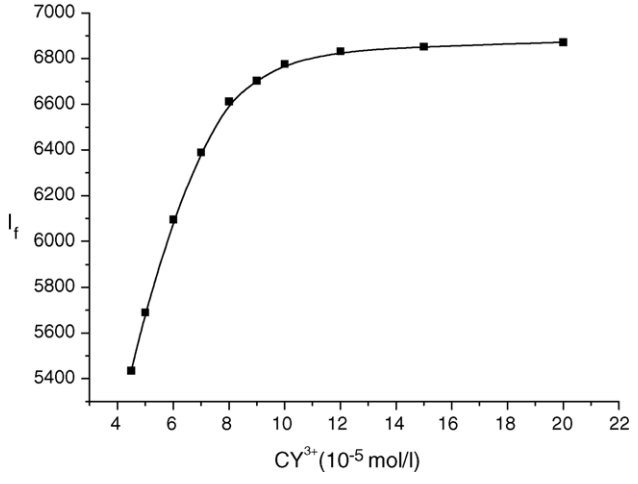


Fig. 3. Effect of Y^{3+} concentration. Conditions: 1.0×10^{-5} mol/l NFLX, 4.0×10^{-2} mol/l Tris, pH 6.2.

Table 2
Interfere test

Foreign substance	Coexistance concentration (10^{-6} mol/l))	Changed of ΔI_f (%)
K^+Cl^-	60	−4.7
$Al^{3+}NO_3^-$	2.0	+4.3
$Mg^{2+}SO_4^{2-}$	1.0	+4.6
$Na^+CO_3^{2-}$	30	−4.7
$Ba^{2+}Cl^-$	480	−4.6
$Mn^{2+}SO_4^{2-}$	20	−4.4
$Zn^{2+}SO_4^{2-}$	20	−4.0
$Fe^{3+}Cl^-$	10	−4.7
Na^+Cl^-	300	−4.8
$Fe^{3+}SO_4^{2-}$	0.3	−5.2
$Al^{3+}Cl^-$	80	+4.9
$NH_4^+Cl^-$	200	−3.9
$Na^+SO_4^{2-}$	600	−4.8
$Ca^{2+}Cl^-$	300	−2.2
Tb^{3+}	3.5	−4.7
Gd^{3+}	3.8	+4.4
Dy^{3+}	2.0	−3.9
Sc^{3+}	5.0	−4.6
La^{3+}	4.0	−3.3
Lu^{3+}	2.0	−4.6
Tm^{3+}	1.0	−4.5
Sm^{3+}	3.0	−4.6
Nd^{3+}	3.0	−4.3
Eu^{3+}	4.0	−3.0
Er^{3+}	2.0	−4.3

Condition: 1.5×10^{-4}mol/l Y^{3+}, 1.0×10^{-7} mol/l NFLX, 4.0×10^{-2} mol/l Tris, pH 6.2.

3.3. Analytical application

3.3.1. Calibration curve and detection limit

Under optimum condition, there was a satisfactory linear relationship between enhanced fluorescence intensity and NFLX concentration in the range 1.0×10^{-9} to 1.0×10^{-5} mol/l, the linear equation is $I = 7.34 \times 10^8 C + 145.6$, the correlation coefficient was 0.9937 and the detection limit was 3.8×10^{-11} mol/l ($S/N = 3$). The relative standard derivation calculated from nine determinations at 1.0×10^{-7} and 1.0×10^{-6} g/ml NFLX were 1.18 and 1.36%, respectively.

3.3.2. Recovery test and sample determination

Considering the effects of tolerance ions on the fluorescence intensity of the system, the standard addition method was used for determination of NFLX in actual samples. The results obtained are satisfactory. This method can be applied for the determination of norfloxcain eye drops (Shandong Lukang Cisen Pharmaceutical Co. Ltd.), the recovery of NFLX in actual samples is 102.4 and 111.3% as shown in Table 3. In comparison with the results of ultraviolet spectrophotometry [1], it can be seen that the results of the method are satisfactory.

Table 3
Sample test

Determination method	NFLX sample (10^{-6} mol/l)	NFLX founded (10^{-6} mol/l)	Average recovery (%)	R.S.D. (%)
Proposed	0.94	1.08, 1.01, 1.03, 1.02, 1.09	111.3	2.98
method	1.88	1.94, 1.97, 1.92, 1. 91, 1.89	102.4	1.58
UV	9.4	13.2, 12.3, 11.8, 12.5, 12.6	132.8	4.06

Table 4
Comparison with common fluorescence methods for NFLX

Fluorescence method	Determination wavelength (nm)	LOD (ng/ml)	Reference
Tb^{3+}	545	50[a]	[10]
Sc^{3+}–SDS	430	0.6[a]	[5]
Tb^{3+}–TOPO–cetylpyridinium chloride	546	200[a]	[7]
SDS	435	200	[11]
Sodium bis(2-ethylhexyl)sulfosuccinate (AOT)–water–octane	436	3.2	[12]
Zr, Mo, V or W	423–430	1.214–2.046	[4]
This method	425	0.038	

[a] Units in nM.

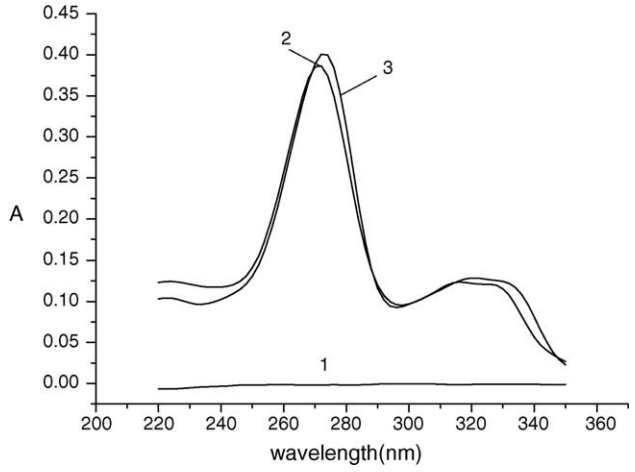


Fig. 4. Absorption spectra. (1) Y^{3+}–Tris, (2) NFLX–Tris, (3) NFLX–Y^{3+}–Tris. Conditions: 1.5×10^{-4} mol/l Y^{3+}, 1.0×10^{-5} mol/l NFLX, 4.0×10^{-2} mol/l Tris, pH 6.2.

3.4. Luminescence mechanism

The absorption spectra (Fig. 4) showed that the absorption peak of the NFLX–Y system shifts to a longer wavelength than that of NFLX system. From Fig. 1, it can be seen that after adding Y^{3+} to the system of NFLX, the fluorescence spectrum of the system changed in wavelength shift and the fluorescence intensity was enhanced. This indicated that the fluorescence of this system belong to Y^{3+} perturbed fluorescence of ligand or L^{*}–L luminescence.

4. Conclusion

It is found that NFLX and Y^{3+} can form complexes, which emit strong fluorescence. Based on the formation of yttrium complexes, a new method for determination NFLX is proposed. The detection limits is 3.8×10^{-11} mol/l ($S/N = 3$). This method is used for the determination of NFLX in actual sample, and the result obtained is satisfactory. This method is sensitive comparison with other methods (Table 4).

Acknowledgements

This work was supported by Natural Science Foundation of Shandong Province (No. Y2003B02), and by Visiting Scholar Foundation of Key Lab in the University. Thank for Professor Lizeng Wang offered NFLX.

References

[1] J. Tuma, W.H. Connors, D.H. Stitelman, C. Richert, J. Am. Chem. Soc. 124 (2002) 4236–4246.
[2] D.B. Luo, S.G. Tang, Yaowu Fenxi Zazhi 11 (1994) 43–45.
[3] P.G. Gigosos, P.R. Revesado, O. Cadahía, C.A. Fente, B.I. Vazquez, C.M. Franco, A. Cepeda, J. Chromatogr. A 871 (2000) 31–36.
[4] M.E. El-Kommos, G.A. Saleh, S.M. El-Gizawi, M.A. Abou-Elwafa, Talanta 60 (2003) 1033–1050.
[5] A.I. Drakopoulos, P.C. Ioannou, Anal. Chim. Acta 354 (1997) 197–204.
[6] Y. Wu, T.L. Zhang, Anal. Commun. 36 (1999) 231–233.
[7] C.J. Veiopoulou, P.C. Ioannou, E.S. Lianidou, J. Pharm. Biomed. Anal. 15 (1997) 1839–1844.
[8] L. Wang, B.Z. Han, Chin. J. Pharm. 20 (2000) 75–77.
[9] Y. Wu, T.L. Zhang, H.C. Zhao, L.P. Jin, Anal. Lett. 33 (2000) 3303–3314.
[10] H.C. Zhao, H.L. Zhang, Y.A. Zhang, Fenxi Shiyanshi 17 (1998) 27–29.
[11] L.Y. Xu, Y. Cai, Lihua Jianyan, Huaxue Fence 38 (2002) 487–488.
[12] Z.H. Liu, Z.Y. Huang, R.X. Cai, Analyst (Camb) 125 (2000) 1477–1481.