Anal Bioanal Chem (2002) 374 : 1187–1190
DOI 10.1007/s00216-002-1553-y

ORIGINAL PAPER

**Li-Hua Nie · Hui-Chun Zhao · Xu Wang · Lin Yi
Yan Lu · Lin-Pei Jin · Hui-Min Ma**

Determination of lomefloxacin by terbium sensitized chemiluminescence method

Received: 28 February 2002 / Revised: 12 August 2002 / Accepted: 13 August 2002 / Published online: 31 October 2002

Abstract A simple, rapid and sensitive chemiluminescence (CL) method was proposed for the determination of lomefloxacin (LFX). This method is based on the fact that the weak CL from the redox reaction of Ce^{4+}-Na_2SO_3 can be greatly enhanced by the complex of Tb^{3+}-LFX. The CL intensity is directly proportional to the concentration of LFX in the range 2.0×10^{-9} to 1.0×10^{-5} mol L^{-1}, and the detection limit (S/N=3) is 1.1×10^{-9} mol L^{-1}. This method has been applied to the detection of LFX in pharmaceutical preparation, urine and serum samples. Recoveries were in the range 95–105%. The CL mechanism of Ce^{4+}-Na_2SO_3-Tb^{3+}-LFX system was proposed to be an intermolecular energy transfer from excited SO_2^* to LFX and an intramolecular energy transfer from LFX to Tb^{3+}.

Keywords Lomefloxacin · Terbium · Chemiluminescence · Energy transfer · Flow injection

Introduction

Lomefloxacin (LFX), 1-ethyl-6,8-difluoro-7-(3-methyl-1-piperazinyl)-4-oxo-1,4-dihydro-3-quinoline carboxylic acid, is one of the third generation synthetic antibacterial fluoroquinolone agents. It has been clinically used widely and successfully, but its toxicity, efficacy and excretion still attract scientists' attention [1, 2, 3], so the detection of LFX in biological fluids is of great importance.

During the last few decades, chemiluminescence (CL) coupled with flow injection analysis has been widely used in chemical analysis and bioanalysis [4, 5] for its sensitivity, rapidity and simplicity. However, the CL intensity of many systems is very weak, so fluorescent substances are often used as sensitizers [6, 7]. In this study, the CL intensity of Ce^{4+}-Na_2SO_3 can be enhanced dramatically by adding Tb^{3+} and LFX simultaneously, and the characteristic fluorescence bands of terbium can be observed in the Tb^{3+}-LFX-Ce^{4+}-Na_2SO_3 system. Based on this phenomenon, a new sensitized CL method for the analysis of LFX was developed. The possible mechanism of this CL system was also discussed. As far as we know, this is the first time the phenomenon of CL enhancement by a Tb^{3+}-LFX complex has been reported.

L.-H. Nie · H.-C. Zhao (✉) · X. Wang · L. Yi · Y. Lu · L.-P. Jin
Department of Chemistry, Beijing Normal University,
Beijing 100875, China
e-mail: huichunzhao@163.net

L.-H. Nie · H.-M. Ma
Center for Molecular Sciences, Institute of Chemistry,
Chinese Academy of Sciences, Beijing 100080, China

Materials and methods

Apparatus. The schematic diagram of the flow injection chemiluminometer (FIA-CL) is shown in Fig. 1. It consists of two basic units, flow injection analyzer and luminescence detector. The FIA-21 flow injection analyzer (East Instrument, Chinese Academy of Sciences, China) was equipped with two peristaltic pumps and a six-way injection valve. All components were connected with PTFE tubes (i.d., 0.8 mm). The CL intensity was measured by the GD-1 luminescence detector (Ruike, Xi'an, China) with a photomultiplier tube, whose sensitivity depends on the value of negative voltage (0–1000 V) used. The kinetic curve was recorded with a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Academia Sinica, China). Fluorescence and CL spectra were recorded with a Hitachi-850 spectrofluorimeter (Japan) equipped with a 150 W xenon lamp.

Reagents. All chemicals were of analytical grade unless otherwise specified. LFX was purchased from the Institute of Medical

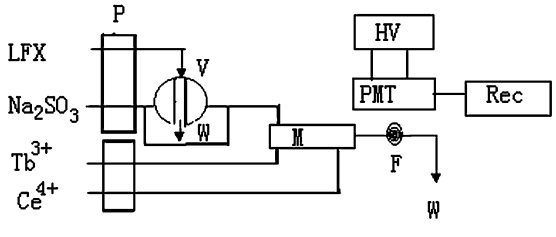

Fig. 1 Schematic diagram of flow injection chemiluminescence (CL) analyzer. *P* Peristaltic pump, *V* sample injection valve, *M* mixing element, *F* flow cell, *W* waste, *HV* high voltage, *PMT* photomultiplier tube, *Rec* recorder

Biotechnology (China). Tb_4O_7 (purity, 99.99%), $(NH_4)_2Ce(NO_3)_6$ and Na_2SO_3 were purchased from Beijing Chemical, China.

Preparation of standard and working solutions. All solutions were prepared in de-ionized, distilled water. A stock standard solution of 1.0×10^{-3} mol L^{-1} LFX was prepared by dissolving 38.8 mg LFX in an appropriate amount of water containing three drops of 0.1 mol L^{-1} NaOH solution, and diluting to 100 ml in a calibrated flask with distilled water. It was kept at 4 °C and protected from light. A stock standard solution of 1.0×10^{-3} mol L^{-1} Tb^{3+} was prepared by dissolving 186.9 mg Tb_4O_7 in hot (75 °C) HCl and evaporating the solution to near-dryness before diluting to 100 ml with water. The working solutions were obtained by serial dilution of the stock standard solutions with water. A working solution of 2.0×10^{-4} mol L^{-1} Ce^{4+} was prepared by dissolving 27.0 mg $(NH_4)_2Ce(NO_3)_6$ in 250 ml of 8.0×10^{-3} mol L^{-1} H_2SO_4 solution, and a working solution of 8.0×10^{-4} mol L^{-1} Na_2SO_3 was freshly prepared by dissolving 10 mg Na_2SO_3 in 100 ml water.

Preparation of pharmaceutical samples. Two tablets (100 mg LFX per tablet) were powdered after weighing, and 162 mg powder was dissolved in water (three drops of 0.1 mol L^{-1} NaOH were added to dissolve the LFX). The solution was filtered and the residue was washed several times with water; all the solutions were collected and appropriate dilution was carried out so that the final concentration of LFX was in the detection range.

Collection of urine samples. The volunteer was administered a tablet, and the urine was collected at different time. These urine samples were stored at 4 °C before analysis.

Pretreatment of serum samples. The serum samples were provided by the hospital of Beijing Normal University. They were deproteinized by using trichloroacetic acid (CCl_3COOH). Briefly, 4 ml of 10% (w/w) trichloroacetic acid was added to 1 ml of serum sample in a plastic centrifuge tube. The mixture was then rotated for 15 min at a rate of 2,000 rev/min, and the upper clear solution was taken for the determination of LFX.

Procedure for CL measurements. A sample solution containing LFX was injected into the flow system through the six-way injection valve and was carried by Na_2SO_3 to the mixing element, where it was mixed with Tb^{3+} and Ce^{4+}. This mixed solution was transferred into the CL cell and the CL signal was observed on the CL recorder immediately. The peak height of the signal was recorded as CL intensity, and the relative CL intensity ΔI (the difference in CL intensity between LFX standard solution and the reagent blank without LFX) was proportional to the concentration of LFX. A calibration graph of relative CL intensity versus LFX concentration was plotted to determine the LFX contents in the samples.

Results and discussion

The optimal conditions for FIA-CL

The optimum injection volume of sample was 120 µl. The coil length from mixing element to flow cell was 22 cm, and pump rate was 4.5 ml/min.

The optimal concentrations of Ce^{4+}, Na_2SO_3 and Tb^{3+}

In this CL system, Ce^{4+} was the oxidant, Na_2SO_3 was the carrier stream and reductant, and Tb^{3+} acted as sensitizer, so their concentrations would affect the CL intensity. The maximum CL intensity was obtained at concentrations of 2.0×10^{-4} mol L^{-1} Ce^{4+}, 8.0×10^{-4} mol L^{-1} Na_2SO_3 and 4.0×10^{-4} mol L^{-1} Tb^{3+}. These concentrations were selected for further investigation. In addition, H_2SO_4 is beneficial to increasing the oxidation efficiency of Ce^{4+}. So, 8.0×10^{-3} mol L^{-1} H_2SO_4 was used, and in this case the system had a pH of 2.

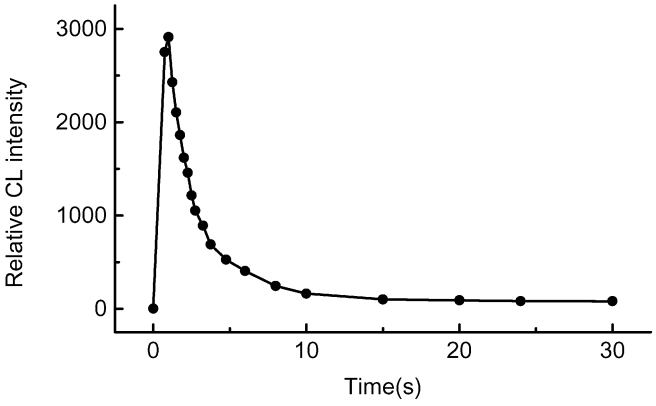

Fig. 2 *CL* kinetic curve of Ce^{4+}-Na_2SO_3-Tb^{3+}-lomefloxacin (LFX) system. LFX, 1.0×10^{-6} mol L^{-1}; Ce^{4+}, 2.0×10^{-4} mol L^{-1}; Tb^{3+}, 4.0×10^{-4} mol L^{-1}; Na_2SO_3, 8.0×10^{-4} mol L^{-1}

Kinetic curve of Tb^{3+}-LFX-Ce^{4+}-Na_2SO_3

The kinetic curve of Tb^{3+}-LFX-Ce^{4+}-Na_2SO_3 was recorded by a BPCL ultra-weak luminescence analyzer (Fig. 2). It can be seen that this CL reaction is a flash-type reaction. The maximum CL intensity was reached at 1 s, and then attenuated to the base line in 20 s.

Calibration curve and detection limit

The calibration curve and the regression equation under different negative voltages are listed in Table 1. The relative standard deviation was found to be 4.8% by ten replicate determinations of 2.0×10^{-8} mol L^{-1} LFX and the detection limit (S/N=3) was 1.1×10^{-9} mol L^{-1}.

Interference studies

In order to assess the applicability of the proposed CL method, the effects of some metal ions and excipients used in pharmaceuticals on the determination of LFX were investigated by analyzing synthetic sample solutions, which contained 1.0×10^{-8} mol L^{-1} of LFX. The recovery results (Table 2) showed that the excipients and most metal ions had no significant effect on the determination of LFX. Reducing agents, such as ascorbic acid and uric acid at a normal concentrations, may produce a little positive but allowable interference as shown in Table 2. This method may therefore be applied to detecting the concentration of LFX in some real samples.

Table 1 Regression equations for lomefloxacin (*LFX*)

LFX mol L^{-1}	Regression equation	Coefficient	Negative voltage V
2.0×10^{-9}–1.0×10^{-8}	$\Delta I = 95.10 \times 10^{8}C - 18$	0.9994	900
1.0×10^{-8}–8.0×10^{-8}	$\Delta I = 124.9 \times 10^{8}C - 66$	0.9998	800
8.0×10^{-8}–1.0×10^{-6}	$\Delta I = 54.97 \times 10^{7}C - 6.3$	0.9992	700
1.0×10^{-6}–1.0×10^{-5}	$\Delta I = 134.1 \times 10^{6}C - 47$	0.9993	550

Table 2 Recovery of 1.0×10^{-8} mol L^{-1} LFX in the presence of foreign substances

Added substance	Molar ratio of the added substance to LFX	Recovery
Uric acid	1.7	112.5
Ascorbic acid	3	110.4
Glucose	100	103.1
Dextrin	100	108.2
Starch	100	101.0
Fe^{3+}	100	94.3
Ni^{2+}	1000	105.6
Cr^{3+}	1000	98.6
Pb^{2+}	1000	92.7
Na^{+}	1000	98.2
K^{+}	1000	98.5
Ca^{2+}	1000	91.8
Mg^{2+}	1000	90.7

Analytical application

This method was used to detect the content of LFX in pharmaceutical tablets. For a labeling content of 100 mg LFX per tablet, a mean value of 97.2±2.2 mg LFX per tablet (n=3) was obtained with the present method. A recovery test was performed by adding 8×10^{-9}, 1.6×10^{-8} and 2.4×10^{-8} mol L^{-1} of LFX to the solutions made from pharmaceutical tablets, and their recoveries were 103%, 98.8% and 95.0%, respectively.

Using the proposed method, an attempt to detect LFX in urine and serum samples was also made in this study. It was reported that the average concentrations of LFX in urine and serum samples were in the range of 41–332 and 0.20–3.98 mg l^{-1} within 24 h of an oral administration of 400 mg LFX [8], respectively. These values are over the upper limit of the linear range, and therefore a proper dilution is required for analysis. In this work, the urine samples were diluted about 10,000-fold with water before determination. The contents of LFX determined in the urine samples are shown in Table 3. In addition, the recovery test was performed by adding 8.0×10^{-8} mol l^{-1} LFX to the diluted urine samples, and the recoveries, calculated by comparing the found value to the added value, are also displayed in Table 3.

Table 3 Determination and recovery of LFX in urine

Time h	LFX content in urine $\times10^{-4}$ M[a]	Added $\times10^{-8}$ M	Found $\times10^{-8}$ M[a]	Recovery %
2.5	5.68 ± 0.06	8.0	7.97 ± 0.05	99.6
4.5	5.74 ± 0.05	8.0	8.23 ± 0.03	102.9
7.0	2.61 ± 0.06	8.0	7.80 ± 0.05	97.5
10.0	2.01 ± 0.07	8.0	7.86 ± 0.04	98.3

[a]Mean±SD (n=4)

In the serum from the persons not taking LFX, the LFX content was below the detection limit of the present method; whereas in the serum from those persons taking LFX (accurate amount of LFX was unknown), a concentration of $(9.7\pm2.3)\times10^{-9}$ mol L^{-1} LFX (n=3) was found. Further, the recoveries from these two serum samples were in the range 98.8–105%.

Possible CL mechanism

It was reported that Na_2SO_3 can be oxidized by Ce^{4+} to produce excited SO_2*, which emits light at 300–450 nm [9, 10]. However, the CL intensity is very weak for the low luminescence efficiency of SO_2*. By introducing a fluorophore whose absorption falls in the range 300–450 nm, the CL intensity is usually enhanced through energy transfer process from the excited SO_2^* to the fluorophore [9]. Lanthanide ions such as Tb^{3+}, are effective emitters and thus were chosen as sensitizer in this work.

In order to study the CL mechanism of the Ce^{4+}-Na_2SO_3-Tb^{3+}-LFX system, the CL intensities of Ce^{4+}-Na_2SO_3, Ce^{4+}-Na_2SO_3-LFX, Ce^{4+}-Na_2SO_3 -Tb^{3+} and Ce^{4+} -Na_2SO_3-Tb^{3+}-LFX were measured and compared (Fig. 3). It is obvious that Ce^{4+} cannot oxidize LFX to emit light, and LFX can only slightly enhance the CL intensity of Ce^{4+}-Na_2SO_3 under our experimental conditions. In con-

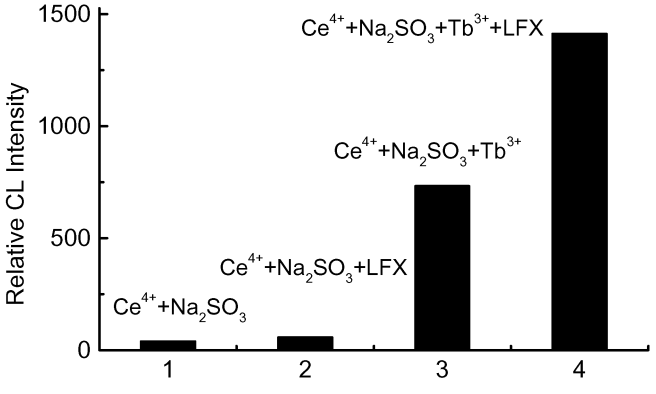

Fig. 3 The relative CL intensity of different systems. *LFX*, 1.0×10^{-7} mol L^{-1}; Ce^{4+}, 2.0×10^{-4} mol L^{-1}; Tb^{3+}, 4.0×10^{-4} mol L^{-1}; Na_2SO_3, 8.0×10^{-4} mol L^{-1}

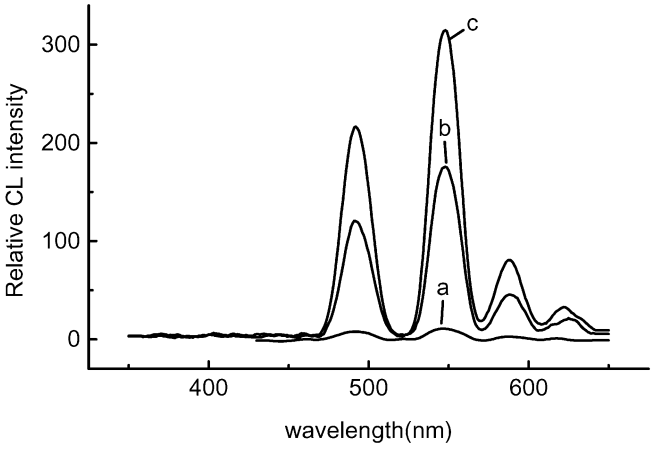

Fig. 4 CL spectra. *a* Ce^{4+}-Na_2SO_3 -Tb^{3+}, *b* Ce^{4+}-Na_2SO_3 -Tb^{3+}-LFX, *c* Ce^{4+}-Na_2SO_3 -Tb^{3+}-FLX. LFX, 1.0×10^{-5} mol L^{-1}; FLX, 2.0×10^{-5} mol L^{-1}; Ce^{4+}, 2.0×10^{-4} mol L^{-1}; Tb^{3+}, 4.0×10^{-4} mol L^{-1}; Na_2SO_3, 8.0×10^{-4} mol L^{-1}

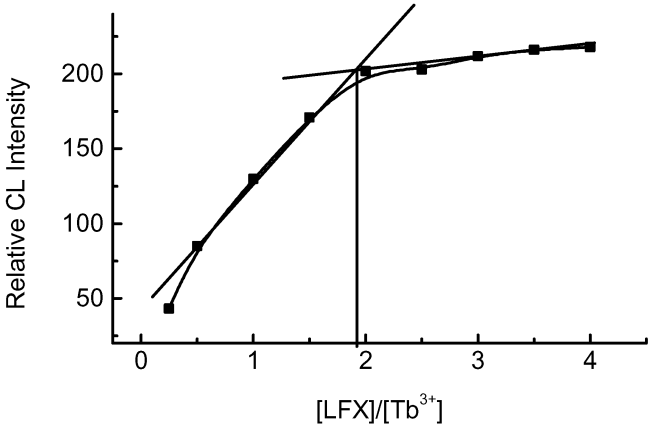

Fig. 5 Molar ratio of Tb^{3+}-LFX complex

trast, the introduction of Tb^{3+} and Tb^{3+}-LFX can substantially enhance the CL intensity of Ce^{4+}-Na_2SO_3 system. To further understand the CL mechanism, the CL spectra of Ce^{4+}-Na_2SO_3-Tb^{3+} and Ce^{4+}-Na_2SO_3-Tb^{3+}-LFX systems were recorded (Fig. 4).

There are four peaks, at 490, 545, 585 and 620 nm, in both spectra. They are the characteristic fluorescence spectra of terbium, indicating clearly that the excited Tb^{3+} is the emitter, and there must exist an intermolecular energy transfer process from SO_2* to Tb^{3+} in Ce^{4+}-Na_2SO_3-Tb^{3+} system. When LFX was added into this system, it would combine with Tb^{3+} to form a complex, whose molar ratio (LFX to Tb^{3+}) was found to be 2:1 (Fig. 5).

Elbanowski et al. [11] have reported that there usually exists an energy transfer from ligand to Ln^{n+} in CL systems containing lanthanide ions. So, CL enhancement of Ce^{4+}-Na_2SO_3-Tb^{3+}-LFX may be attributed to an intermolecular energy transfer from SO_2* to LFX and then an intramolecular energy transfer from the ligand LFX to Tb^{3+}. Moreover, the combination of LFX and Tb^{3+} can remove the coordinated water molecules and reduce the fluorescence quenching of Tb^{3+} by water.

Therefore, the CL process of Tb^{3+}-LFX-Ce^{4+}-Na_2SO_3 system may be described as:

$$Ce^{4+} + HSO_3^- \rightarrow HSO_3^{\bullet} + Ce^{3+} \quad (1)$$

$$2HSO_3^{\bullet} \rightarrow S_2O_6^{2-} + 2H^+ \quad (2)$$

$$S_2O_6^{2-} \rightarrow SO_4^{2-} + SO_2^* \quad (3)$$

$$SO_2^* + \left[Tb\,(LFX)_2\right]^{3+} \rightarrow SO_2 + \left[Tb\,(LDX)_2^*\right]^{3+} \quad (4)$$

$$\left[Tb\,(LFX)_2^*\right]^{3+} \rightarrow \left[Tb^*\,(LFX)_2\right]^{3+} \quad (5)$$

$$\left[Tb^*\,(LFX)_2\right]^{3+} \rightarrow \left[Tb\,(LFX)_2\right]^{3+} + h\upsilon \quad (6)$$

On the other hand, fleroxacin having similar properties to LFX (including absorption spectra, matchable energy transfer to Tb^{3+}) could also give such CL (Fig. 4), implying that the CL mechanism proposed above may be reasonable.

In this paper, a new and simple CL method for the determination of LFX has been established and preliminarily subjected to analysis of pharmaceutical preparation, urine and serum samples. The mechanism of CL enhancement was studied, and an inter- and intra-molecular energy transfer process is proposed for the present system. Such an explanation would be useful for understanding some CL processes.

Acknowledgments The authors express thanks to Mr. Zhonglun Zhang and Mrs. Yanzheng Zheng for providing a BPCL ultra-weak luminescence analyzer. NNSFC (No. 29971005), the Natural Science Foundation of Beijing (2022007) and State Key Project of Fundamental Research (G1998061308) financially support this work.

References

1. Forster C, Rucker M, Shakibaei M, Baumann W, Vormann J, Stahlmann R (1998) Arch Toxicol 72:411–419
2. Sudoh T, Fujimura A, Harada K, Sunaga K, Ohmori M, Sakamoto K (1996) Eur J Clin Pharmacol 51:95–98
3. Jauch A, Fsadni M, Gamba G (1999) Graefe's Arch Clin Exp Ophthalmol 237:705–713
4. Roda A, Pasini P, Guardigli M, Baraldini M, Musiani M, Mirasoli M (2000) Fresenius J Anal Chem 366:752–759
5. Palilis LP, Calokerinos AC (2000) Anal Chim Acta 413:175–186
6. Koukli I, Calokerinos AC (1990) Analyst 115:1553–1557
7. Rao Y, Tong Y, Zhang XR, Luo G, Baeyens WRG (2000) Anal Chim Acta 416:227–230
8. Shi YG, Cao YJ, Wang L, Zhang QJ, Zhang YY (1994) Chin J Antibiot 19:248
9. Huang YM, Zhang C, Zhang XR, Zhang ZJ (1999) Anal Chim Acta 391:95–100
10. Pearse RWB, Gaydon AG (1976) The identification of molecular spectra (4th edn) Chapman and Hall, London
11. Elbanowski M, Makowska B, Staninski K, Kaczmarek M (2000) J Photochem Photobiol A Chem 130:75–81