

# Determination of norfloxacin by flow injection analysis with chemiluminescence detection

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## Abstract

A new method was described for the determination of norfloxacin (NFLX) by using flow injection chemiluminescence. It is based on the chemiluminescence reaction of the Ce(IV)-sodium hyposulphite-norfloxacin system. The fluorescence and CL spectra of this system were examined and the CL mechanism was developed. Under the optimum conditions, the CL intensity is proportional to the concentration of the drugs in solution with good linearity. The detection limit ( $s/n=3$ ) is  $6 \times 10^{-9}$  g mL<sup>-1</sup>. The relative standard deviation is 1.7% for 11 measurements of  $2.0 \times 10^{-7}$  g mL<sup>-1</sup> NFLX standard solution. The method has been successfully applied to the determination of NFLX in pharmaceutical preparations and biological fluids with the recoveries of 95.0%-102.5%.

**Keywords:** fluorescence, chemiluminescence, norfloxacin, mechanism, flow-injection, pharmaceutical analysis, biological fluids

## Résumé

Nous décrivons une nouvelle méthode pour la détermination de la norfloxacine (NFLX), basée sur la chimiluminescence (CL) en flux continu. Elle repose sur la réaction de chimiluminescence du système Ce(IV)-hyposulfite de sodium-norfloxacine. Nous avons examiné les spectres de fluorescence et de CL de ce système et nous avons élaboré le mécanisme de CL. Sous les

conditions optimales, l'intensité CL est proportionnelle à la concentration des produits en solution avec une bonne linéarité. La limite de détection ( $s/n=3$ ) est de  $6 \times 10^{-9}$  g mL<sup>-1</sup>. L'écart type relatif se situe à 1.7% pour 11 mesures d'une solution standard NFLX de  $2.0 \times 10^{-7}$  g mL<sup>-1</sup>. La méthode a été appliquée avec succès pour la détermination de NFLX dans des préparations pharmaceutiques et des fluides biologiques avec des recouvrements allant de 95.0 à 102.5%.

## Introduction

Norfloxacin [NFLX, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid] is a synthetic, broad-spectrum antibacterial agent which exhibits high antimicrobial activity in vitro against a wide variety of Gram-negative and Gram-positive bacteria, including the gentamicin-resistant *Pseudomonas aeruginosa* and the  $\beta$ -lactamase positive *Neisseria gonorrhoeae* (1). This group of drugs is bactericidal over a wide range of therapeutically achievable concentrations and is effective by selective inhibition of bacterial DNA synthesis. It is related to nalidixic acid, but its potency has been increased by a fluorine atom and by a piperazine at the 7th position. Excellent therapeutic effects have been shown in the treatment of respiratory, biliary and urinary tract infections. Single oral dose of 400 mg gave peak serum level of 1.58 mg mL<sup>-1</sup> and ~30% of the administered dose was recovered in urine as unmetabolized NFLX (2).

Several methods have been reported for the determination of fluoroquinolones. They were either in pure form, in dosage forms or in biological fluids. The

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United States Pharmacopoeia recommends nonaqueous titration methods with potentiometric detection of the end-point for the evaluation of the raw material of NFLX and HPLC methods for determination of their dosage forms (3). The British Pharmacopoeia recommends a non-aqueous titration method for the determination of NFLX in raw material (4). The Chinese Pharmacopoeia recommends a HPLC method for NFLX determination in its tablet form (5). The analytical profile of NFLX and a significant number of references for its determination in pharmaceutical dosage forms and biological fluids have been published (6). Analytical methods were reported for the determination of NFLX in pharmaceutical dosage forms by spectrophotometry (7-9), adsorptive voltammetry (10) and spectrofluorimetry (11-13).

HPLC was used to the determination of fluoroquinolones in biological fluids. A review of the published HPLC assays with UV or fluorescence detection was presented for individual fluoroquinolones in 1998 (14). Separation and determination of synthetic impurities of norfloxacin was described by reversed-phase HPLC (15). A sensitive liquid chromatographic-mass spectrometric assay was used for determination of norfloxacin in poultry tissue (16). HPLC with photoinduced fluorimetric detection and multiemission scanning was developed for the determination of enoxacin, ciprofloxacin, norfloxacin and ofloxacin in urine and serum (17). An HPLC method with fluorescence detection was described for the determination of norfloxacin in chicken tissue with a detection limit of 2.5 ng/mL of homogenate (18). The above methods were complicated, the sensitivity was low, the analytical conditions were harsh, or the instrument was expensive.

Analytical procedures that apply chemiluminescence (CL) methods combined with flow injection have some advantages such as sensitivity, speed, ease of use and use of simple instrumentation. The flow-injection chemiluminescence methods were described for determination of nescapine, propranolol and ethamsylate in pharmaceutical preparations (19-21). A method for the determination of ciprofloxacin, norfloxacin and ofloxacin was described based on the enhancement by these compounds of the weak CL from peroxytrous acid with a detection limit of ( $s/n = 3$ ) of  $5.9 \times 10^{-8}$  mol L<sup>-1</sup> and linear ranges of  $1.0 \times 10^{-7}$  to  $1.0 \times 10^{-5}$  mol L<sup>-1</sup> for NFLX (22). CL reaction of fluoroquinolones with tris(2,2'-bipyridyl)ruthenium (II) [Ru(bipy)<sub>3</sub><sup>2+</sup>] and Ce(IV) were studied in sulfuric acid medium. This method has been used to determine ofloxacin, norfloxacin and ciprofloxacin hydrochloride in dosage forms and biological fluids with a detection

limit ( $s/n=3$ ) of  $3.1 \times 10^{-8}$  mol L<sup>-1</sup> of NFLX (23). One CL method for the determination of NFLX has been exploited based on the CL reaction of Tb<sup>3+</sup>-NFLX-Ce(IV)-SO<sub>3</sub><sup>2-</sup>, which permitted the determination of  $9.0 \times 10^{-9}$  to  $1.0 \times 10^{-6}$  mol/L of NFLX with a detection limit of  $4.5 \times 10^{-11}$  mol L<sup>-1</sup> (24). Terbium-sensitized luminescence optosensor was developed for the determination of norfloxacin in biological fluids with the detection and quantification limits of 1.5 and 5 ng mL<sup>-1</sup>, respectively (25).

The main purpose of this work is to establish a simple, sensitive and rapid CL method for the determination of NFLX. We found that NFLX could be oxidized by Ce(IV) and low CL signals were produced. However, when sodium hydrosulfite was added to the Ce(IV)-NFLX system, CL signals were enhanced significantly. The CL intensity was strengthened linearly with concentration of NFLX. The Ce(IV)-Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>-NFLX system was used to determine NFLX by flow injection CL method with a good linearity and a detection limit ( $s/n=3$ ) of  $6 \times 10^{-9}$  g mL<sup>-1</sup>. The proposed method has been successfully applied to the determination of NFLX in drug, serum and urine with satisfactory results.

## Experimental

### Chemicals

All chemicals used were of analytical reagent grade. Deionized water was used throughout. NFLX (Institute of Medicinal Biotechnology, Beijing, China) stock standard solution ( $5.0 \times 10^{-4}$  g mL<sup>-1</sup>) was prepared by dissolving 25.0 mg NFLX in 1.5 mL of 0.1 mol L<sup>-1</sup> NaOH and diluting it with deionized water to 50 mL. More diluted solutions were freshly prepared by diluting the stock solution with deionized water. NFLX capsules and injections were provided by China Qianhui Pharmaceuticals Ltd Co. and China Shijiazhuang fourth pharmaceuticals Ltd Co, respectively. A stock solution of Ce(IV) ( $1 \times 10^{-2}$  mol L<sup>-1</sup>) was prepared by dissolving 0.6686 g Ce(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>·4H<sub>2</sub>O in 100 mL of 0.72 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>. A stock solution of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> ( $2 \times 10^{-3}$  mol L<sup>-1</sup>) and a solution of HNO<sub>3</sub> (3 mol L<sup>-1</sup>) were prepared daily.

### Apparatus

The FI system, as shown in Figure 1, is a MPI-B-FI-CL analysis system (Xi'an Remex Electronic science-tech Co. Ltd., Xi'an, China) consisted of two peristaltic pumps working at a constant flow rate (30 rpm) and a six-way injection valve with a sample loop (120 μL), which is automatically operated by a computer equipped operation system of MPI-B flow injection analysis.

PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. The flow cell is a twisted glass tube in order to produce a large surface area exposed to the adjacent photomultiplier tube (PMT), (Hamamatsu, Japan). Since there is no reaction between the Ce(IV) solution and HNO<sub>3</sub> solution, the Ce(IV) solution and HNO<sub>3</sub> solution was mixed. The mixture was used as the carrier stream and was injected into a flow cell through a three-way pipe. Then, a mixture of sample and Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> solution was injected from a sample valve, the CL signals were produced immediately and was recorded by an analyzer. UV spectra were measured with a UV-265 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were recorded with a RF-5301PC spectrofluorometer (Shimadzu, Japan).

#### *Sample preparation*

**Capsule treatment.** Not less than 20 capsules were weighed to obtain the mean mass per capsule.

An accurately weighed portion of each homogenized capsule powder containing 100 mg NFLX was dissolved with 3 mL of 0.1 mol L<sup>-1</sup> NaOH in a small beaker. The solution was filtered and the residue was washed with deionized water several times, then it was transferred into a 100 mL volumetric flask and diluted to the mark with deionized water. Working solutions were prepared by appropriate dilution of this sample solution so that the final analyte concentration was within the working range.

**Injected sample treatment.** The injected sample of NFLX was made out of five bottles of NFLX was randomly selected from the same group. The working solutions were directly diluted with deionized water.

**Urine and serum treatment.** The proteins of a 1-mL volume of serum sample was removed by adding 4.0 mL 10% trichloroacetic acid (CCl<sub>3</sub>COOH) in a centrifuge tube, which was then centrifuged for 15 min at 4000 rpm. The supernatant was diluted with deionized water so as to make different concentrations of NFLX in the linear range. No further pre-treatment was required for urine samples. Human serum and urine samples were kindly provided by healthy volunteers.

#### *Procedure*

As shown in Figure 1, 120 µL NFLX solution and Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> solution were injected into a mixed stream of Ce(IV) and HNO<sub>3</sub> solutions. When the mixed solution flowed into the cell, the CL reaction took place. The CL signal produced in the flow cell was recorded by an analyzer. Calibration graphs were constructed by plotting

the intensity (peak height) of the CL signal versus the concentration of NFLX. The intensity of the CL signal of the capsule, injection and spiked urine and serum samples was measured with the procedure as described above. The content was calculated either from a previously plotted calibration graph or by using the regression equation.

## **Results and Discussion**

#### *Effect of sample volume and flow rate*

The role of sample volume and flow rate is critical. For instance, if the sample volume is too small or too large, the maximum CL could not be obtained. The highest emission was when the injected sample volume was 120 µL. The CL intensity increased with increasing flow rate. However, a flow rate of 3.0 mL/min for all solutions is recommended because of greater precision and economy in the use of reagents.

#### *Choice of inorganic acids*

The kind and concentration of the acid used in the reaction have a very significant influence on the CL emission intensity. Therefore, several acids, such as HCl, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, H<sub>3</sub>PO<sub>4</sub> and H<sub>6</sub>P<sub>4</sub>O<sub>13</sub>, were added in the Ce(IV) solution to test the effect of each acid on the CL signal. The highest emission was observed from HNO<sub>3</sub>-treated Ce(IV) solutions, resulting in a stable signal. Hence, HNO<sub>3</sub> was chosen for further work. The concentration of HNO<sub>3</sub> in Ce(IV) solution was subsequently optimized (Figure 2). Hence, 2.0 mol L<sup>-1</sup> HNO<sub>3</sub> was used for further experiments.

#### *Effect of Ce(IV) concentration*

The influence of Ce(IV) concentration in the range of 5.0×10<sup>-5</sup> mol L<sup>-1</sup> to 1.0×10<sup>-3</sup> mol L<sup>-1</sup> on the CL signal was tested (Figure 3). 5.0×10<sup>-4</sup> mol L<sup>-1</sup> Ce(IV) solution was used to provide a maximum CL emission.

#### *Effect of sensitizers*

The Ce(IV)-NFLX system could only produce weak CL emission. Various compounds such as rhodamine B, HCOH, H<sub>2</sub>O<sub>2</sub>, Na<sub>2</sub>SO<sub>3</sub>, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, and Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> were tested as sensitizers for the CL system of Ce(IV)-NFLX. It was found that only Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> could enhance the CL signal for the Ce(IV)-NFLX system. The effect of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> concentration on the CL intensity was studied in the range of 5×10<sup>-5</sup> mol L<sup>-1</sup> to 1×10<sup>-3</sup> mol L<sup>-1</sup>, as shown in Figure 4. The CL intensity was enhanced with increasing Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> concentrations. The maximum CL intensity was

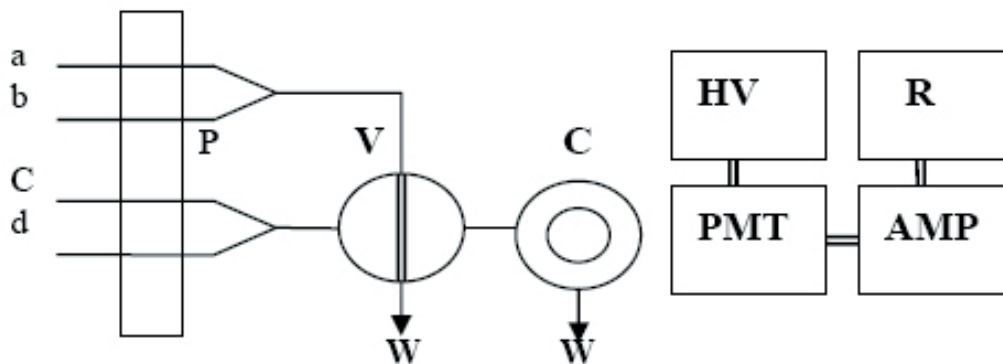


Figure 1. Schematic diagram of flow injection CL analysis system. P—peristaltic pump, V—sampling inlet valve, C—flowing cell, PMT—photomultiplier tube, AMP—amplifier, HV—high voltage, R—recorder, W—waste, a—NFLX solution, b— $\text{Na}_2\text{S}_2\text{O}_4$  solution, c—Ce(IV) solution, d— $\text{HNO}_3$  solution

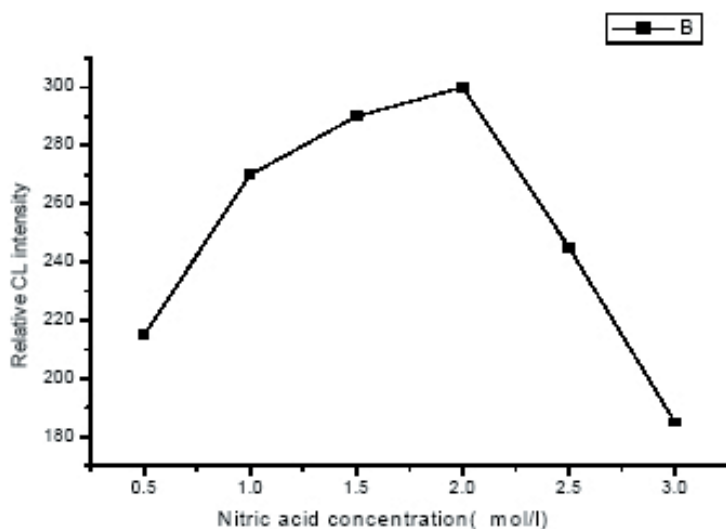


Figure 2. Effect of nitric acid concentration on relative CL intensity NFLX:  $1 \times 10^{-6}$  g  $\text{mL}^{-1}$ , Ce(IV):  $5.0 \times 10^{-4}$  mol  $\text{L}^{-1}$ ,  $\text{Na}_2\text{S}_2\text{O}_4$ :  $1 \times 10^{-4}$  mol  $\text{L}^{-1}$ .

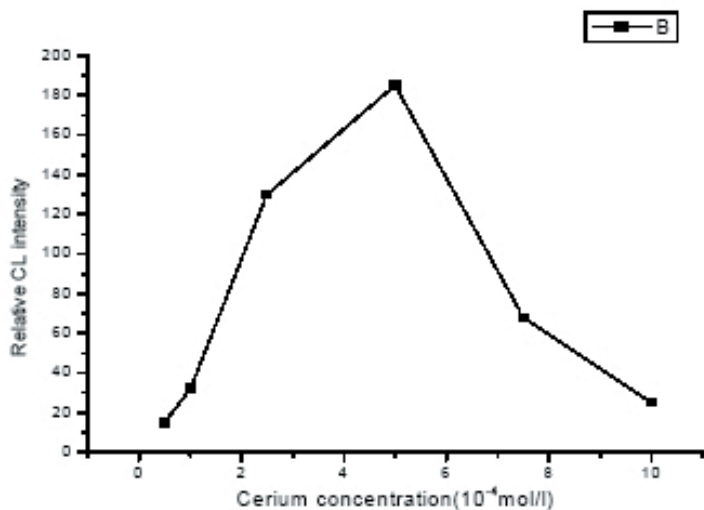


Figure 3. Effect of Ce(IV) concentration on relative CL intensity NFLX:  $1 \times 10^{-6}$  g  $\text{mL}^{-1}$ ,  $\text{Na}_2\text{S}_2\text{O}_4$ :  $1 \times 10^{-4}$  mol  $\text{L}^{-1}$ ,  $\text{HNO}_3$ : 2.0 mol  $\text{L}^{-1}$

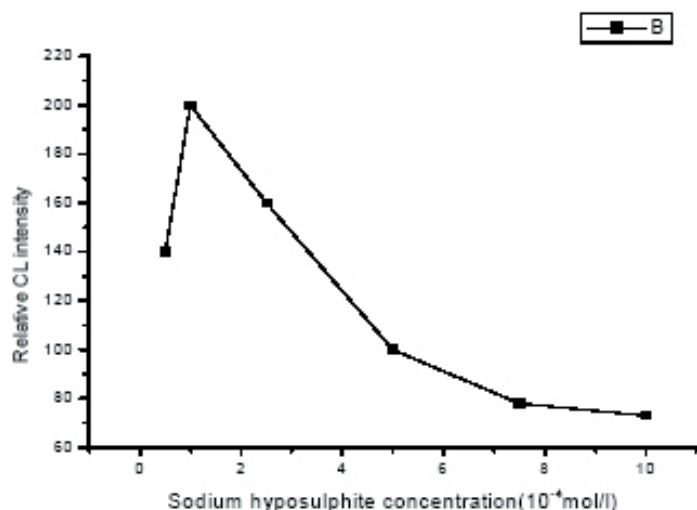


Figure 4. Effect of sodium hyposulphite concentration on relative CL intensity NFLX:  $1 \times 10^{-6}$  g mL $^{-1}$ , Ce(IV):  $5.0 \times 10^{-4}$  mol L $^{-1}$ , HNO $_3$ : 2.0 mol L $^{-1}$ .

obtained when the Na $_2$ S $_2$ O $_4$  concentration was  $1 \times 10^{-4}$  mol L $^{-1}$ . Hence, the Na $_2$ S $_2$ O $_4$  working concentration was fixed at this concentration.

#### Interference studies

The influence of some common excipients used in drugs, metal ions in human body and several organic compounds on the CL intensity was investigated. The tolerance content, defined as the amount of foreign species that produced an error not exceeding  $\pm 5\%$  in the determination of  $1 \times 10^{-6}$  g mL $^{-1}$  NFLX, was presented as follows: 100-fold magnesium stearate, lactose, sodium citric acid, starch, sucrose, glucose; 50-fold fructose, dextrin, galactose, polyglycol; 25-fold EDTA; 10-fold sodium benzoate, ascorbic acid; 100-fold Zn $^{2+}$ , Co $^{2+}$ , Ca $^{2+}$ , Mg $^{2+}$ , Ba $^{2+}$ , Ni $^{2+}$ , Cl $^{-1}$ , SO $_4^{2-}$ , NO $_3^{-}$ ; 50-fold Cu $^{2+}$ , 2-fold Fe $^{3+}$ , Fe $^{2+}$ . There was feeble interference from equal amounts of hemoglobin, myoglobin, vitamin B1, I $^{-}$  and Br $^{-}$ .

#### The kinetic characteristics of CL reaction

CL kinetic characteristics for the reactions of NFLX, Na $_2$ S $_2$ O $_4$ , and acidic Ce(IV) in solution were studied in detail. It was found that the reaction rate in solution was very fast, only 3s was needed from reagent mixing to peak maximum, and it took 9s for the signal to return to zero again.

#### Performance of NFLX measurement system

Under optimum conditions described above, the linearity for the determination of NFLX was investigated in the range of  $1.0 \times 10^{-8}$  to  $1.0 \times 10^{-5}$  g mL $^{-1}$ . The calibration graph of NFLX consisted of four parts in order to

improve the applicability. The linear regression equations are given in Table 1.

The detection limit (s/n=3) for NFLX is  $6 \times 10^{-9}$  g mL $^{-1}$ . The relative standard deviation (R.S.D.) is 1.7% for 11 determinations of  $2.0 \times 10^{-7}$  g mL $^{-1}$  NFLX.

#### Analysis of pharmaceutical preparations

The proposed method and the UV-method (8) were applied to the determination of NFLX in capsules and injections. The repeatability of the proposed method was calculated in terms of the variation coefficient from the CL intensity of seven independent replicate analyses. The results are summarized in Table 2, along with labeled contents.

Statistical analysis of the results using Student t-test and the variance ratio F-test showed no significant difference (p=0.95) between the performance of the two methods in terms of accuracy and precision.

#### Analysis of spiked urine and plasma samples

NFLX has been found in body tissues, blood, serum and urine in a few hours after oral administration. Single oral dose of 400 mg gave peak serum level of 1.58 mg mL $^{-1}$  and  $\sim 30\%$  of the administered dose was recovered in urine as unmetabolized NFLX (2). Once the proteins from the serum sample was removed and centrifuged, the supernatant was used to investigate the % recovery. In order to make the sample concentration of the drug within the linear range of determination, the serum sample was diluted appropriately. The standard addition method was used to avoid matrix effects. The urine samples were diluted properly with deionized water and analyzed by the standard addition method. The results

Table 1. Regressive equations and linear ranges of the calibration curves.

Regressive equation*	Correlation coefficient	Concentration of NFLX (g mL <sup>-1</sup> )
I = 1.3C + 15.1	0.9975	1.0 × 10 <sup>-8</sup> – 1.0 × 10 <sup>-7</sup>
I = 10.5C + 15.8	0.9979	1.0 × 10 <sup>-7</sup> – 1.0 × 10 <sup>-6</sup>
I = 37.2C + 88.5	0.9991	1.0 × 10 <sup>-6</sup> – 1.0 × 10 <sup>-5</sup>
I = 96.7C + 379.5	0.9983	1.0 × 10 <sup>-5</sup> – 6.0 × 10 <sup>-5</sup>

\*I: CL intensity, C: NFLX concentration

Table 2. Determination results of NFLX in capsule and injection sample.

Sample	Batch number	Nominal	Proposed method*	UV method*
capsule (mg/capsule)	050406	100	99.7 ± 1.80	100.3 ± 1.71
injection (mg/100 mL)	03112046	200	198.2 ± 3.37	196.9 ± 3.54

\* Mean ± S.D. (n = 7)

Table 3. Determination results of recovery of serum and urine.

Sample	Added (× 10 <sup>-6</sup> g mL <sup>-1</sup> )	Found (× 10 <sup>-6</sup> g mL <sup>-1</sup> )	Recovery (%)	RSD % (n = 5)
serum	0.4	0.39	97.5	2.0
	4.0	4.10	102.5	1.9
urine	0.2	0.19	95.0	2.1
	2.0	1.97	98.5	2.0

are given in Table 3.

#### CL mechanism

The fluorescence and CL spectra were examined in order to obtain more information about the enhanced CL mechanism. The fluorescence emission spectra of NFLX and Ce(IV)-NFLX system were observed in the range 300–700 nm. As shown in Figure 5, they showed different emission spectra.

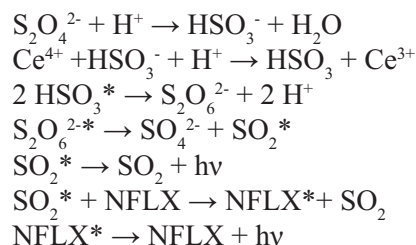
The native fluorescence emission of NFLX shows a broad peak at 436 nm. When mixed with Ce(IV), the fluorescence emission shows a broad peak at 369 nm, while the fluorescence emission for Ce(IV)-Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> shows a broad peak at 371 nm. For the NFLX-Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> system with Ce(IV), the fluorescence emission shows a broad peak at 361 nm.

The reaction of Ce(IV) with SO<sub>3</sub><sup>2-</sup> or S<sub>2</sub>O<sub>4</sub><sup>2-</sup> in acidic medium was studied comparatively. Ce(IV)-SO<sub>3</sub><sup>2-</sup> CL reaction has a lower quantum efficiency and thus exhibited a weaker luminescence. Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> in acidic medium can produce HSO<sub>3</sub><sup>-</sup>. Ce(IV) can oxidize HSO<sub>3</sub><sup>-</sup> to be a hydrogen sulfite radical HSO<sub>3</sub><sup>\*</sup>, which can then form S<sub>2</sub>O<sub>6</sub><sup>2-</sup>. S<sub>2</sub>O<sub>6</sub><sup>2-</sup> will result in the excited intermediate product SO<sub>2</sub><sup>\*</sup>. When SO<sub>2</sub><sup>\*</sup> relaxes to its ground state, a photon is emitted (26). The excited state SO<sub>2</sub><sup>\*</sup> emits in

the spectral region 300-450 nm (27).

In order to prove that the products generate CL at 369 nm and 436 nm, the CL spectrum was examined further by using a series of interference filters. The CL spectra for the Ce(IV)-Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>-HNO<sub>3</sub> system in the absence and presence of NFLX were recorded in the range of 330–695 nm. When NFLX was absent, no obvious CL spectrum was observed.

As shown in Figure 6, the CL spectra for the Ce(IV)-NFLX-Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>-HNO<sub>3</sub> systems show two bands at 366 and 436 nm. Fluorescence emission spectrum at 371 nm is produced by the product SO<sub>2</sub><sup>\*</sup>. The energy of the partially excited SO<sub>2</sub><sup>\*</sup> is transferred to NFLX, which then fluoresces at 436 nm. The CL spectra for Ce(IV)-NFLX-Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>-HNO<sub>3</sub> system show the two broad peaks. The possible CL mechanism for this system can be described as follows:



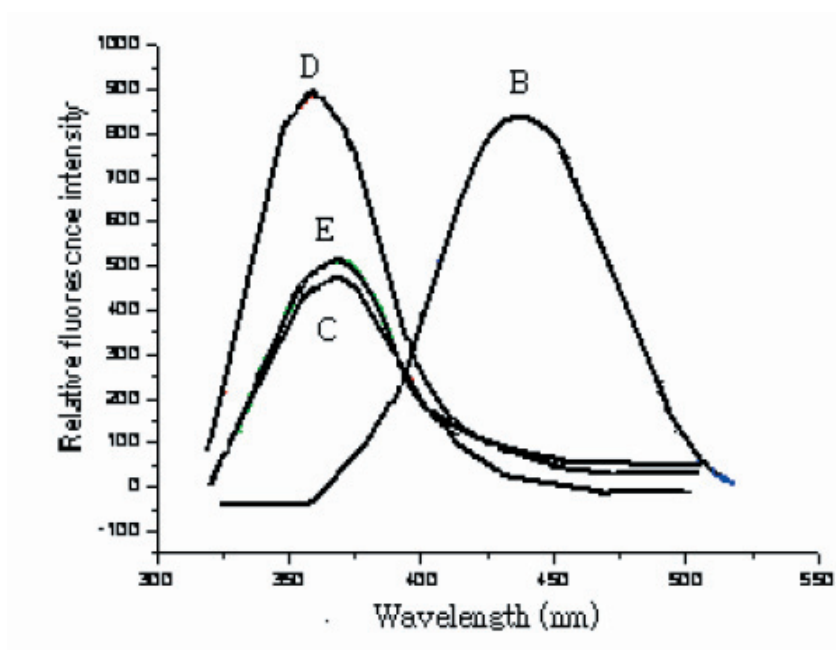


Figure 5. Fluorescence emission spectra of NFLX(B), Ce(IV)-NFLX(C), Ce(IV)-NFLXNa<sub>2</sub>S<sub>2</sub>O<sub>4</sub>(D) and Ce(IV)-Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>(E)  $\lambda_{\text{ex}}=275$  nm; NFLX:  $2 \times 10^{-7}$  g mL<sup>-1</sup>; Ce(IV):  $7.5 \times 10^{-4}$  mol L<sup>-1</sup>, Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>:  $2.5 \times 10^{-4}$  mol L<sup>-1</sup>.

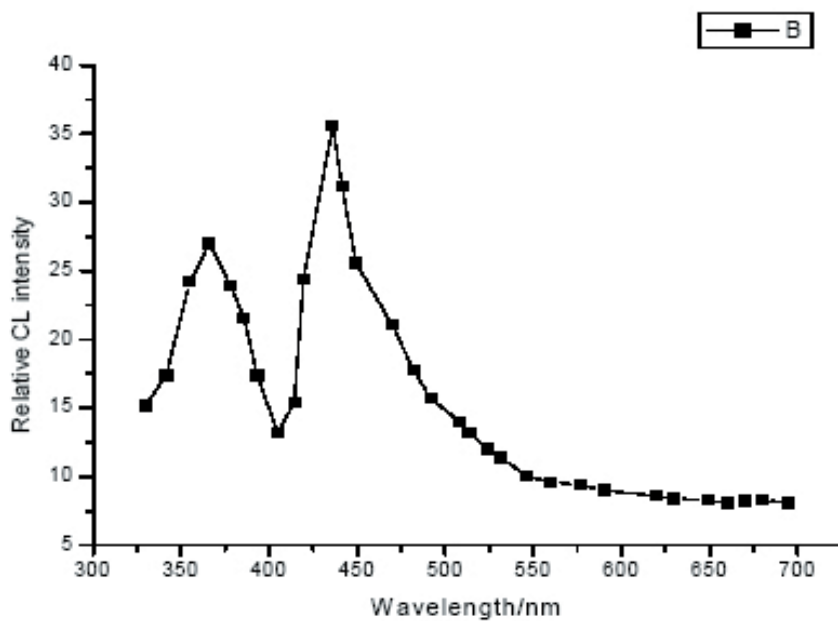


Figure 6. CL spectra of Ce(IV)-NFLX-Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>-HNO<sub>3</sub> HNO<sub>3</sub>: 2 mol L<sup>-1</sup>; Ce(IV):  $5 \times 10^{-4}$  mol L<sup>-1</sup>; Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>:  $1 \times 10^{-4}$  mol L<sup>-1</sup>; NFLX:  $2 \times 10^{-5}$  g mL<sup>-1</sup>

## Conclusion

The proposed flow-injection enhanced CL method has a simple, rapid, inexpensive and high sensitivity for the determination of NFLX based on the Ce(IV)-NFLX- $\text{Na}_2\text{S}_2\text{O}_4$ - $\text{HNO}_3$  system. The proposed CL method can process up to 60 samples per hour, and can be used to routinely determine NFLX in pharmaceutical preparations and biological fluids.

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## References

1. B. Swanson, V. Boppana, P. Vlasses, H. Rotmen, R. Ferguson, *Antimicrob. Agents Chemother.*, **23**, 284 (1983).
2. M. Córdoba-Borrego, M. Córdoba-Díaz, D. Córdoba-Díaz, *J. Pharm. Biomed. Anal.*, **18**, 919 (1999).
3. The United States Pharmacopoeia, 24 Revision, The National Formulary 19, USP Convention, INC. Rockville, MD, 2000.
4. The British Pharmacopoeia, Her Majesty's Stationery Office, London, 1998.
5. Pharmacopoeia of People's Republic of China (Part II), Beijing, Chemical Industry Press, 2000: 753.
6. C. Mazuel, in: K. Florey (Ed.), *Analytical Profiles of Drug Substances*, vol.20, Academic Press, New York, 1991, pp. 557–559.
7. Z. Sonia, Khateeb El, A. Sawsan, Rizek Abdel, M.M. *Analysis*, **17**, 829 (1998).
8. Hong Chen, Dianhui Zheng, *Journal of Taizhou University*, **26**, 64 (2004).
9. Nafisur Rahman, Yasmin Ahmad, Azmi Hejaz, Najmul Syed, *European Journal of Pharmaceutics and Biopharmaceutics*, **57**, 359 (2004).
10. M.M. Ghoneim, A. Radi, A. M. Beltagi, *J. Pharm. Biomed. Anal.*, **25**, 205 (2001).
11. J. L. Vilchez, O. Ballesteros, J. Taoufiki, G. Sánchez-Palencia, A. Navalón, *Anal. Chim. Acta*, **444**, 279 (2001).
12. M. Córdoba-Borrego, M. Córdoba-Díaz, I. Bernabé, D. Córdoba-Díaz, *J. Pharm. Biomed. Anal.*, **14**, 977 (1996).
13. Li Ming Du, Ai Ping Lin, Ya Qin Yang, *Anal. Lett.*, **37**, 2175 (2004).
14. G. Carlucci, *J. Chromatogr. A*, **812**, 343 (1998).
15. R. Nageswara Rao, V. Nagaraju, *J. Pharm. Biomed. Anal.*, **34**, 1049 (2004).
16. Jonghwan Lim, Byungkwon Park, Hyoin Yun, *J. Chromatogr. B, Analytical Technologies in the Biomedical and Life Sciences*, **772**, 185 (2002).
17. A. Espinosa-Mansilla, A. Muñoz de la Peña, D. González Gómez, F. Salinas, *J. Chromatogr. B, Analytical Technologies in the Biomedical and Life Sciences*, **822**, 185 (2005).
18. C. Kowalski, Z. Roliński, T. Sławik, B. K. Głód, *J. Liq. Chromatogr. Relat. Technol.*, **28**, 121 (2005).
19. Yafeng Zhuang, Xilan Cai, Junsheng Yu, Huangxian Ju, *J. Photochem. Photobiology A: Chemistry*, **162**, 457 (2004).
20. A. Townshend, J.A. Murillo Pulgarín, M.T. Alañón Pardo, *Anal. Chim. Acta*, **488**, 81 (2003).
21. Fengzhen Yang, Chao Zhang, Willy R.G. Baeyens, Xinrong Zhang, *J. Pharm. Biomed. Anal.*, **30**, 473 (2002).
22. Yaodong Liang, Junfeng Song, Xiaofeng Yang, *Anal. Chim. Acta*, **510**, 21 (2004).
23. Fatma A. Aly, Salma A. Al-Tamimi, Abdulrahman A. Alwarthan, *Talanta*, **53**, 885 (2001).
24. Ninglian, Chunyansun, Huichun Zhao, *Chinese Journal of Analytical Science*, **18**, 111 (2002).
25. E.J. Llorent Martínez, J.F. García Reyes, P. Ortega Barrales, A. Molina Díaz, *Anal. Chim. Acta*, **532**, 159 (2005).
26. D.A. Paulls, A. Townshend, *Analyst*, **121**, 831 (1996).
27. R.W.B. Pearse, A. G. Gaydon. *The Identification of Molecular Spectra*, fourth ed., Chapman and Hall, London, 1976, pp. 297–298.