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ORIGINAL RESEARCH

A study on the micelle-sensitized Ce(IV)–$Na_2S_2O_3$–norfloxacin chemiluminescence system and its applications

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ABSTRACT: A new chemiluminescence (CL) flow-injection method was developed for the determination of norfloxacin. The method is based on the CL reaction of norfloxacin with sodium thiosulphate and Ce(IV) in sulphuric acid medium sensitized by sodium dodecylsulphate. Under optimum conditions, the CL intensity is proportional to the concentration of the norfloxacin in the range 3.89×10^{-8}–7.18×10^{-6} g/mL. The detection limit (3 s/k) was 2.21×10^{-9} g/mL for norfloxacin. The method has been applied successfully to the determination of norfloxacin in pharmaceutical formulations and human urine. The mechanism for this chemiluminescence system is discussed. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: chemiluminescence; norfloxacin; flow-injection

INTRODUCTION

Norfloxacin [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-1(1-piperazinyl)-3-quinolone carboxylic acid, NFLX] is one of the third-generation members of quinolone antibiotics. Norfloxacin is a broad-spectrum antibiotic for Gram-positive and Gram-negative bacterial infections, with small side-effects (1). Since it appeared on the market in 1980, norfloxacin has been widely applied clinically. At present, the drug is one of the most important synthetic antibacterials used in animal husbandry and agriculture. It can be very harmful to human health, and since norfloxacin can be deposited into the edible animal tissue, the determination of this compound in feed or animal tissue is very important. However, so far it is still a challenge to determine norfloxacin, due to its low concentration in feed or animal tissue.

Some methods have been reported for the determination of norfloxacin, including fluorimetry (2–5), spectrophotometry (6–7), high-performance liquid chromatography (HPLC) (8–11), chemiluminescence (13–15) and electrochemical analysis (12) in dosage or biological samples. Fluorimetry offers the advantage of relatively higher sensitivity for the determination of NFLX; however, it needs special equipment. Chemiluminescence (CL) detection is a sensitive and simple method for some drugs; however, only a few CL methods coupled with flow-injection analysis have been developed (13–15), and these methods have a narrow linear response range and unsatisfactory detection limits (13), low selectivity, and use expensive and poisonous reagents (14) and a complicated Tb agent (15). We have developed a cheaper, steady and simple flow-injection chemiluminescence method for the determination of NFLX with higher sensitivity and selectivity, and based on a CL detection system that can be further developed for use with HPLC.

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EXPERIMENTAL

Apparatus and reagents

The flow-injection analysis system with CL detection is shown in Fig. 1. Polytetrafluoroethylene (PTFE) tubing (0.8 mm i.d.) was used to connect all components in the flow system. Two peristaltic pumps were used to carry

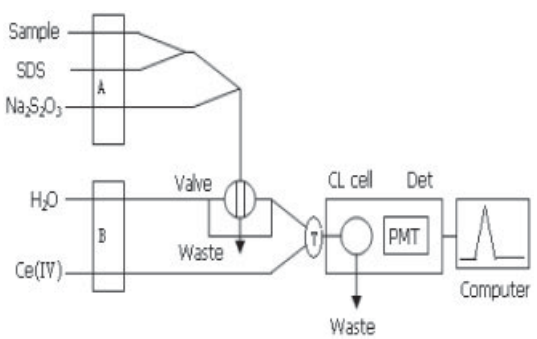

Figure 1. Schematic diagram of the flow-injection system for the determination of NFLX. A,B, peristaltic pumps; valve, injection valve; T, T-piece; PMT, photomultiplier tube; Det, detector.

the reactants at the same flow rate. A six-way valve with a 50 μL sample loop was automatically operated, using a computer in order to inject the sample into the system. Ce(IV) and the other reactants were mixed at the T-piece (see Fig. 1). The flow rate of solutions was 11.2 mL/min. The CL signal was measured with an IFFM-D flow-injection luminescence analyser (RuiMai Electronic Equipments Co., Xian, China). Fluorescence spectra were measured with a 970 CRT spectrofluorimeter (Shanghai, China). Absorption spectra were measured on a UV-1100 spectrophotometer (Beijing, China).

All chemicals were of analytical reagent grade. Norfloxacin standards were obtained from Institute of Medical Biotechnology, Beijing, China. Norfloxacin capsule 1 was obtained from Gotian Huamin Antibiotics Co. Ltd, Fujian, China (No. 20030501). Norfloxacin capsule 2 was obtained from Kanmei Pharmaceutical Co. Ltd, Guangdong, China (No. 20030504). Sodium dodecylsulphate (SDS) were purchased from Serva Feinbiochemica Heidelberg/New York (Japan Importation). Cetyltrimethylammonium bromide (CTAB) and $Ce(SO_4)_2{\cdot}4H_2O$ were purchased from Shanghai Chemical Company of the Medicine Group of China (Shanghai, China). TritonX-100 was purchased from the Beijing Chemical Company (Beijing, China). Other chemicals used were of analytical quality or better.

The Ce(IV) solution (3.0×10^{-4} mol/L) was prepared by dissolving the $Ce(SO_4)_2{\cdot}4H_2O$ in 0.07 mol/L sulphuric acid. The sodium thiosulphate solution (1.3×10^{-4} mol/L) was freshly prepared by dissolving sodium thiosulphate in deionized water. The sodium dodecylsulphate stock solution (0.1 mol/L) was prepared by dissolving sodium dodecylsulphate in deionized water. Stock standard solution of norfloxacin (1.62×10^{-4} g/mL) was prepared by dissolving 8.1 mg norfloxacin in 0.005 mol/L sulphuric acid solution and diluting to 50.0 mL with the same acid. Working standard solutions were prepared by appropriately diluting the stock standard solution with 0.005 mol/L sulphuric acid.

Procedures for determination of NFLX

The mixture of sample, SDS and $Na_2S_2O_3$ solution was injected into the flow system using the six-way valve, and then mixed with Ce(IV) solutions at the T-piece; the mixed solution was transferred into the CL cell and the CL signal was then recorded. The relative CL intensity, ΔI (defined as the difference of CL intensity between NFLX standard solution and the blank) was proportional to the concentration of NFLX. A calibration curve was prepared for the determination of NFLX.

Sample preparation

The 100 mg powder containing norfloxacin was accurately weighed and transferred into a 100 mL flask and dissolved with a 0.005 mol/L sulphuric acid solution. The solution was filtered and the residue was washed several times with a 0.005 mol/L sulphuric acid, then diluted appropriately with the same acid so that the final concentration was in the working range.

RESULTS AND DISCUSSION

The kinetic characteristics of the CL reaction

In order to determined the lifetime of the CL, the kinetic characteristics of the CL reaction were examined with a static injection method, using the solution consisted of 3.0×10^{-4} mol/L Ce(IV) in 0.07 mol/L H_2SO_4, 1.3×10^{-4} mol/L $Na_2S_2O_3$ and 0.025 mol/L SDS, and the typical CL kinetic curve was shown in Fig. 2. It can been seen from curve 2,2′ and curve 3,3′ that this CL reaction was rapid and the CL intensity reached a maximum in 0.8 s, and then attenuated quickly to baseline in 50 s. It also showed that the CL intensity was enhanced in the presence of NFLX, and the CL system was steady and repeatable.

Optimization of experimental conditions

Effect of sulphuric acid concentration. Ce(IV) is not readily soluble in water, but becomes stable when it is dissolved in acid solution. Therefore, the effect of acid

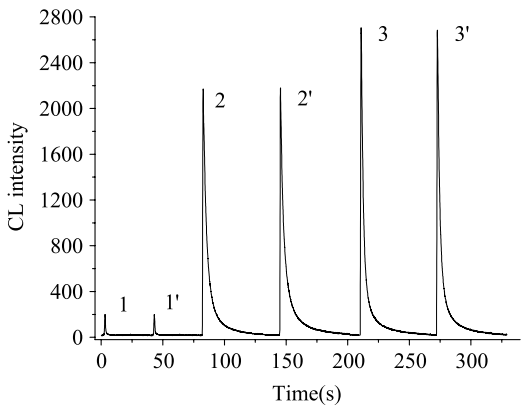

Figure 2. The kinetic curve of the CL reaction. 1, Ce(IV) (in 0.07 mol/L H_2SO_4), 3.0×10^{-4} mol/L; $Na_2S_2O_3$, 1.3×10^{-4} mol/L + SDS, 0.025 mol/L (injection). 1′, Ce(IV) (in 0.07 mol/L H_2SO_4), 3.0×10^{-4} mol/L; $Na_2S_2O_3$, 1.3×10^{-4} mol/L + SDS, 0.025 mol/L (injection). 2, Ce(IV) (in 0.07 mol/L H_2SO_4), 3.0×10^{-4} mol/L; $Na_2S_2O_3$, 1.3×10^{-4} mol/L + norfloxacin, 3.8×10^{-7} g/mL + SDS, 0.025 mol/L (injection). 2′, Ce(IV) (in 0.07 mol/L H_2SO_4), 3.0×10^{-4} mol/L; $Na_2S_2O_3$, 1.3×10^{-4} mol/L + norfloxacin, 3.8×10^{-7} g/mL + SDS, 0.025 mmol/L (injection). 3, Ce(IV) (in 0.07 mol/L H_2SO_4), 3.0×10^{-4} mol/L; $Na_2S_2O_3$, 1.3×10^{-4} mol/L + norfloxacin, 4.8×10^{-7} g/mL + SDS, 0.025 mol/L (injection). 3′, Ce(IV) (in 0.07 mol/L H_2SO_4), 3.0×10^{-4} mol/L; $Na_2S_2O_3$, 1.3×10^{-4} mol/L + norfloxacin, 4.8×10^{-7} g/mL + SDS, 0.025 mol/L (injection).

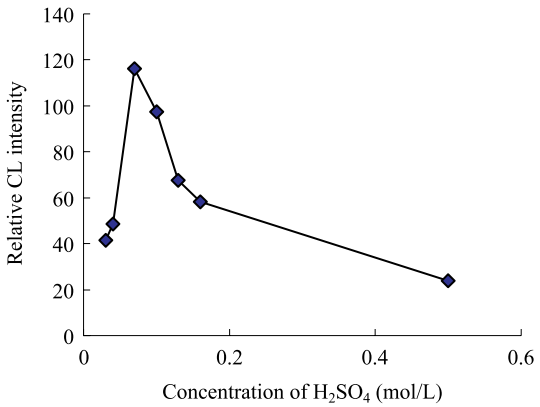

Figure 3. Effect of sulphuric acid concentration on CL intensity. Ce(IV), 3.0×10^{-4} mol/L; $Na_2S_2O_3$, 1.3×10^{-4} mol/L + norfloxacin, 1.56×10^{-6} g/mL; SDS, 0.025 mol/L.

added to the Ce(IV) solution was examined. Solutions of 3.0×10^{-4} mol/L Ce(IV) in 0.07 mol/L hydrochloric acid, phosphate acids, nitric acid and sulphuric acid were prepared and the CL intensity was measured for each of them. The results showed that the highest relative CL intensity (ΔI) could be obtained when 0.07 mol/L sulphuric acid was used. Thus, sulphuric acid was the most suitable medium for the CL measurement of norfloxacin. The effect of sulphuric acid concentration in Ce(IV) solution was examined in the range 0.03–0.5 mol/L, and the result is shown in Fig. 3. The CL emission was highest at 0.07 mol/L sulphuric acid. When the concentration of sulphuric acid was lower than 0.07 mol/L, a remarkable decrease in CL intensity was observed due to the hydrolysis of Ce(IV), forming cerium hydroxide. Hence, 0.07 mol/L sulphuric acid was used throughout the subsequent experiments.

Effect of Ce(IV) concentration. Ce(IV) is one of the important reactants for determination of norfloxacin in this CL system. In order to investigate the effect of the concentration of Ce(IV) on the CL emission intensity, the concentration of Ce(IV) in the range 9.0×10^{-5}–1.5×10^{-3} mol/L in 0.07 mol/L sulphuric acid solution was examined, and the results are shown in Fig. 4. It is known that the maximum emission is observed at 0.3 mmol/L Ce(IV). Therefore, 0.3 mmol/L Ce(IV) was used for subsequent experiments.

Effect of sodium thiosulphate concentration. The primary investigation showed that addition of a reductant, such as $NaHSO_3$, Na_2SO_3 or $Na_2S_2O_3$, into this CL system enhanced of the CL emission. Therefore, the CL behaviour was examined in the presence of $NaHSO_3$, Na_2SO_3 and $Na_2S_2O_3$, respectively, and the results showed that the highest CL intensity could be obtained when $Na_2S_2O_3$ was used. The effect of sodium thiosulphate concentration on CL intensity was

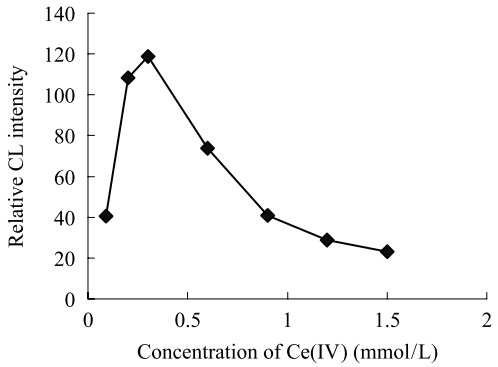

Figure 4. Effect of Ce(IV) concentration on CL intensity. H_2SO_4, 0.07 mol/L; $Na_2S_2O_3$ 1.3×10^{-4} mol/L + norfloxacin, 1.56×10^{-6} g/mL; SDS, 0.025 mol/L.

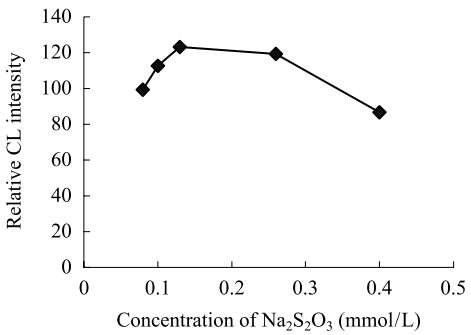

Figure 5. Effect of $Na_2S_2O_3$ concentration on CL intensity. Ce(IV), 3.0×10^{-4} mol/L (in 0.07 mol/L H_2SO_4); norfloxacin, 1.56×10^{-6} g/mL; SDS, 0.025 mol/L.

examined in the range 0.08–0.4 mmol/L, and the results are shown in Fig. 5. The CL signal reached a maximum at 0.13 mmol/L sodium thiosulphate and decreased with increasing sodium thiosulphate concentration after 0.13 mmol/L, so 0.13 mmol/L sodium thiosulphate was employed in further studies.

Effect of sodium dodecylsulphate concentration. Surfactants are often used to enhance the emission intensities of CL reaction. Three type of surfactants, SDS, CTAB and TritonX-100, were used to examine the effect of surfactants on the behaviour of this CL system. It was found that the greatest CL signal could be obtained in SDS micellar medium, while the maxium luminescence intensity achieved in CTAB and TritonX-100 media was only 25% and 60% of that obtained in SDS micellar medium under the optimum conditions, respectively. Therefore, SDS was selected for subsequent experiments. In this sensitized CL system, the key intermediate may be cationic Ce^{3+} or cationic complex $Ce(C_{16}H_{18}FN_3O_3)_2^{3+}$. A large number of Ce^{4+} and Ce^{3+} radical ions were concentrated in the stern layer of the SDS micelles, while the NFLX easily reached the stern layer of the micelles, which made the interaction

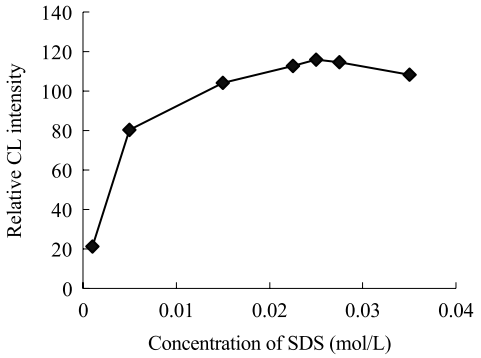

Figure 6. Effect of SDS concentration on CL intensity. Ce(IV), 3.0×10^{-4} mol/L (in 0.07 mol/L H_2SO_4); norfloxacin, 1.56×10^{-6} g/mL; $Na_2S_2O_3$, 1.3×10^{-4} mol/L.

Table 1. Effect of various additives on CL emission intensity

Species added	Mole ratio ($C_{species}/C_{NFLX}$)	Variation of the CL peak height (%)
Fe^{3+}	160	−11.15
Ca^{2+}	750	3.34
Na^{+}	1700	−4.93
NH_4^{+}	1700	−3.39
Al^{3+}	100	−6.96
Zn^{2+}	1200	−9.24
K^{+}	1400	−6.81
Cu^{2+}	50	−9.03
Ni^{2+}	300	−9.74
Pb^{2+}	700	4.53
Co^{2+}	40	−6.75
Starch	800	−0.88
Lactose	1900	−0.95
Glucose	3800	1.19
Dextrin	1900	−1.34

between Ce^{4+} and NFLX, or the energy transfer between the Ce^{3+} radical ions and NFLX adequate more effective. Moreover, the microenvironment created by micelles can protect the excited species from the collisional quenching of light emission, and can increase the excited state lifetimes and decrease the rate of radiationless energy transfer processes. The stability of this CL system was also greatly improved in SDS micelles. The effect of SDS concentration in the range 1.5×10^{-3}–3.5×10^{-2} mol/L on the CL intensity was also examined, and the results are shown in Fig. 6. CL signal reached a maximum value at 0.025 mol/L SDS, hence 0.025 mol/L SDS was chosen for further work.

Linear response range, detection limit and precision

Under the optimum conditions, the calibration curve shows that the CL intensity is linear with the concentration of norfloxacin in the range 3.89×10^{-8}–7.18×10^{-6} g/mL. The regression equation is:

$$I = 48.077 \times 10^6\, C + 7.499 \quad r = 0.9966$$

where I is the emission intensity (mV) and C the concentration of norfloxacin (g/mL). The detection limit of norfloxacin (3 s) is 2.21 ng/mL and relative standard deviation (RSD) is 1.9% for 0.156 μg/mL norfloxacin ($n = 10$).

Interference studies

In order to assess the analytical application possibility of this CL method, under the optimum conditions, the effects of some common excipients in drugs and common metal ions in biological samples on determination of 3×10^{-6} g/mL norfloxacin were investigated, and the results are listed in Table 1. The tolerance limit was taken as the maximum concentration of the foreign substances which caused an approximately ± 10% relative error in the determination. Table 1 shows that no significant interference could be observed for these foreign substances.

Analysis applications

The proposed method was applied to the determination of norfloxacin in two pharmaceutical preparations, and the results obtained are given in Table 2. There were no significant differences between labelled contents and those obtained by the proposed method. Recovery studies were also performed for each of the analysed samples, and recoveries were found to be 91.3% and 101.0%, respectively.

The spiked urine samples were diluted appropriately and the recoveries of urine samples were determined by the standard addition method. The results obtained are listed in Table 3.

Discussion on the CL reaction mechanism

The fluorescence spectra for the solution of Ce(IV), Ce(III), NFLX and the mixture of Ce(IV) and NFLX

Table 2. Results of determination of NFLX in capsules

	Amount (mg)				
Sample	Labelled (mg)	Found (mg)	Added (mg)	Found (mg)	Recovery (%)
Capsule 1	100	100.0	40.0	36.5	91.3
Capsule 2	100	101.3	40.0	40.4	101.0

Table 3. Results of determination of NFLX in urine sample ($n = 5$)

Sample	Added (μg/ml)	Found (μg/ml)	Recovery (%)	R.S.D (%)
Urine 1	0.5	0.493	98.6	2.92
Urine 2	1.0	0.973	97.3	1.95

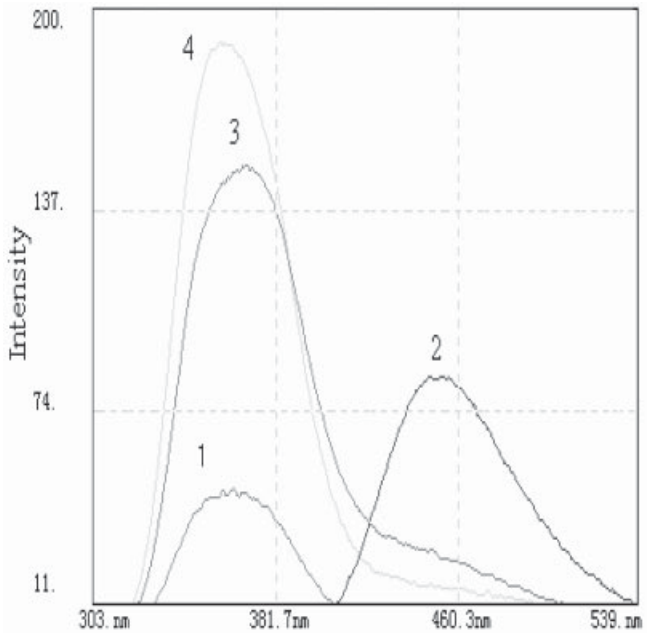

Figure 7. Fluorescence spectra. 1, Ce(III) $\lambda_{em} = 360$ nm, $\lambda_{ex} = 254$ nm; 2, norfloxacin, 6.3×10^{-6} mol/L, $\lambda_{em} = 454$ nm, $\lambda_{ex} = 254$ nm; 3, $2 + Ce(SO_4)_2$ (in 0.07 mol/L H_2SO_4) 1.5×10^{-4} mol/L $\lambda_{em} = 362$ nm, $\lambda_{ex} = 254$ nm; 4, $3 + SDS$ 6.1×10^{-3} mol/L $\lambda_{em} = 356$ nm, $\lambda_{ex} = 254$ nm.

were measured under the same excited wavelength and shown in Fig. 7. The fluorescence spectrum for the mixture solution of 1.5×10^{-4} mol/L $Ce(SO_4)_2$ in 0.07 mol/L H_2SO_4 solution and 6.3×10^{-6} mol/L norfloxacin solution (see Fig. 7, curve 3) is very similar to that of Ce(III) (see Fig. 7, curve 1), and does not show significant fluorescence emission in the wavelength range 420–540 nm, while the norfloxacin solution gives obvious fluorescence emission in this range (see Fig. 7, curve 2). Moreover, Ce(IV) is non-fluorescent (16). Consequently, it appears that the luminophor is attributed to the Ce(III) ions or the complex formed between Ce(III) and norfloxacin, not to the norfloxacin alone. It was known that in a sensitized CL system the key intermediate frequently involves a high energetic species; $Ce(C_{16}H_{18}FN_3O_3)_2^{3+*}$ is most probably such a high energetic intermediate complex in this CL system (17). Curve 4 in Fig. 7 is the fluorescence spectrum of the mixture solution of 1.5×10^{-4} mol/L $Ce(SO_4)_2$ in 0.07 mol/L H_2SO_4, 6.3×10^{-6} mol/L norfloxacin and 6.1×10^{-3} mol/L SDS solution. Based on the discussion above, the mechanism for this CL system can be proposed as follows:

$$S_2O_3^{2-} + 8Ce^{4+} + 5H_2O \rightarrow 8Ce^{3+*} + 2SO_4^{2-} + 10H^+$$

$$Ce^{4+} + NFLX^{Red} \xrightarrow{SDS} Ce^{3+*} + NFLX^{Ox}$$

$$Ce^{3+*} \rightarrow Ce^{3} + h\nu$$

and/or

$$Ce^{3+*} + NFLX \xrightarrow{SDS} Ce(C_{16}H_{18}FN_3O_3)_2^{3+*}$$

$$Ce(C_{16}H_{18}FN_3O_3)_2^{3+*} \rightarrow Ce^{3+} + NFLX^{Ox} + h\nu$$

where NFLX = norfloxacin, SDS = sodium dodecylsulphate, Red = reduced form and Ox = oxidized form.

CONCLUSION

A new flow-injection analysis method based on the Ce(IV)–$Na_2S_2O_3$–NFLX–SDS CL system has been developed. Compared to the present CL method for determination of quinolones, the proposed method has advantages in sensitivity, selectivity and accuracy. This method has been successfully used in the determination of NFLX in pharmaceutical preparations and spiked human urine.

Acknowledgements

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