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Flow injection chemiluminescence determination of levofloxacin in medicine and biological fluids based on its enhancing effect on luminol–H_2O_2 reaction

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Abstract. Levofloxacin{(–)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid} is a synthetic fluorinated quinolone derivative, having activity against both Gram (+) and Gram (−) bacteria (aerobic and anaerobic) through inhibition of their DNA gyrase. In this paper, a simple flow injection chemiluminescence with luminol–hydrogen peroxide system was described for determining levofloxacin. The chemiluminescence intensity in the presence of levofloxacin was remarkably enhanced compared with that in the absence of it. Under the optimum reaction conditions the chemiluminescence increment produced was proportional to the concentration of levofloxacin in the range of 1.0–700.0 ng ml^{-1} ($R^2 = 0.9992$), with a detection limit of 0.3 ng ml^{-1} (3σ). At the flow rate of 2.0 ml min^{-1}, the whole process including sampling and washing could be completed in 0.5 min offering the sampling efficiency of 120 times h^{-1} accordingly, and the relative standard deviation (RSD) was less than 3.0% ($n = 5$). The recovery for the levofloxacin samples was from 95.9% to 104.5%. It was satisfactory for the application to determine levofloxacin in pharmaceutical preparations, human urine and serum samples.

Keywords: Levofloxacin, chemiluminescence, flow injection, human serum and urine

1. Introduction

Levofloxacin{(–)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid} (Scheme 1) is a synthetic fluorinated quinolone derivative, having activity against both Gram (+) and Gram (−) bacteria (aerobic and anaerobic) through inhibition of their DNA gyrase. Levofloxacin is administered to patients with urinary, respiratory or cutaneous infections, in 500 mg d^{-1} doses. Levofloxacin is mainly excreted in urine (>85%) in unaltered form. No pharmacokinetic differences between oral or intravenous administration have been observed. Final concentrations in serum and urine of treated patients are in the range 2–5 μg ml^{-1} and 200–420 μg ml^{-1}, respectively [1].

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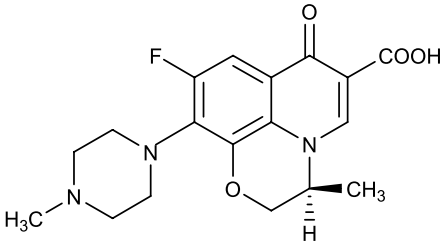

Scheme 1.

From the literature, different methods were employed for the determination of levofloxacin in tablets or biological samples, including high performance liquid chromatography (HPLC) [2–6], spectrophotometry [7,8], fluorimetry [9,10], mass spectrometry (MS) [11], capillary electrophoresis (CE) [12], nuclear magnetic resonance spectroscopy (NMR) [13] and electrochemical methods [14]. However, these methods have relatively complex devices; time-consuming procedure or poor sensitivity. Progress in flow-injection (FI) chemiluminescence (CL) analysis has received much attention in various fields for its high sensitivity, rapidity and simplicity [15–18]. We have recently reported the determination of amoxicillin [19], sudan I [20] and clindamycin [21] with different CL system. Ocana and Barragan using a Ce(IV)–sulphite–fluoroquinolone CL system to detect levofloxacin from 0.5 μg ml^{-1} to 3.5 μg ml^{-1}, with detection limits of 0.10 μg ml^{-1} [22]. In this paper, it was first observed that levofloxacin could remarkably enhance the CL reaction between luminol and hydrogen peroxide, based on which a simple, sensitive as well as rapid procedure was designed to determine levofloxacin. It was found that levofloxacin concentration was proportional to the CL intensity linearly ranging from 1.0 ng ml^{-1} to 700.0 ng ml^{-1} with a relative standard deviation (RSD) less than 3%. At a flow rate of 2.0 ml min^{-1}, a complete determination of levofloxacin, including sampling and washing, could be completed in 0.5 min, offering the sampling efficiency of 120 times h^{-1} accordingly. The proposed procedure was applied successfully to the determination of levofloxacin in tablets, human serum and urine samples without any pretreatment process.

2. Experimental section

2.1. Apparatus

A schematic diagram of the CL flow injection analysis system was shown in Fig. 1. A peristaltic pump was utilized to deliver all flow streams. PTFE tubing (1.0 mm i.d.) was used as connection material in the flow system. A six-way valve with a loop of 100 μl was employed for metering and injection of one of the reagents luminol. The flow cell was made by coiling 30.0 cm of colorless glass tube (2.0 mm i.d.) into a spiral disk shape with a diameter of 2.0 cm and placed close to the photomultiplier tube (PMT) (Hamamatsu, Model IP28). The CL signal produced in the flow cell was detected without wavelength discrimination, and the PMT output was amplified and quantified by a luminosity meter (Xi'an Remax Electronic Science-Tech. Co. Ltd. Model GD-1) connected to a recorder (Shanghai Dahua Instrument and Meter Plant, Model XWT-206).

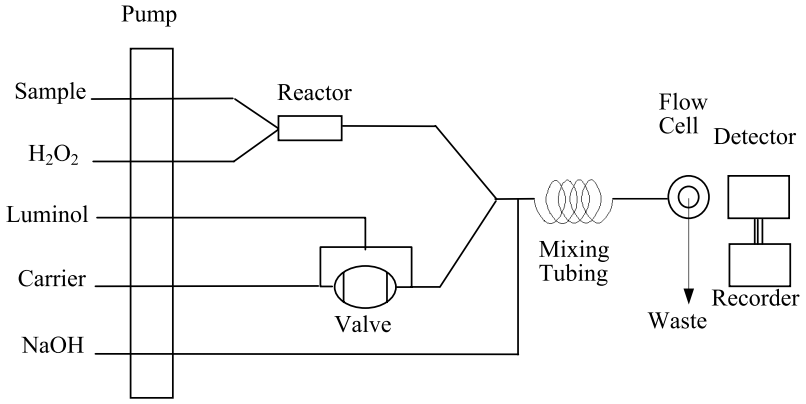

Fig. 1. Schematic diagram of the flow-injection system for levofloxacin determination.

2.2. Reagents

All the reagents were of analytical grade, and the water used was purified in a Milli-Q system (Millipore, Bedford, MA, USA). A stock solution of levofloxacin (Shaanxi Institute for Drug Control) was stored in the refrigerator (4°C). Working standard solutions were prepared daily from the stock solution by appropriate dilution. Luminol (2.5×10^{-2} mol l^{-1}) was prepared by dissolving 4.4000 g luminol (Fluka, Switzerland) in 1.0 liter of 0.1 mol l^{-1} NaOH solution. Hydrogen peroxide (Xi'an Chemical Reagent Plant) was diluted by pure water to give a final concentration of 0.1 mol l^{-1}.

2.3. General procedures

As shown in Fig. 1, flow lines were inserted into the sample, hydrogen peroxide, luminol, water carrier, and sodium hydroxide solutions, respectively. The pump was started at a constant speed of 2.0 ml min^{-1} to wash the whole system until a stable baseline was recorded. Then 100 μl luminol solution was injected into the water carrier stream by injection valve, merged with the mixed solution stream of levofloxacin and hydrogen peroxide. The mixed solution in an alkaline medium was delivered into the CL cell, producing CL emission, detected by the PMT and luminometer. The concentration of the sample was quantified by the increment of CL intensity ($\Delta I = I_s - I_o$), where I_s and I_o were CL signals in the presence and in the absence of levofloxacin, respectively.

2.4. Sample preparation

The proposed procedure for the determination of levofloxacin was applied to commercial pharmaceutical formulation. Also, levofloxacin in spiked human serum and urine was determined.

2.4.1. Determination of levofloxacin in pharmaceutical preparations

Not less than ten tablets of levofloxacin were weighed and ground to a fine powder using a pestle and mortar. The powder was dissolved in water, and the resulting solution was filtered through an ordinary filter paper and diluted to the mark in a 100 ml brown calibrated flask. Suitable aliquots from this solution were taken for the determination of levofloxacin so that the concentration of levofloxacin was in the working range of its determination.

2.4.2. Determination of levofloxacin in spiked human urine and serum samples

The urine samples collected from three volunteers and the serum samples supplied by the Hospital of Northwest University were spiked before determination. To prepare the spiked samples, known quantities of standard solution of levofloxacin were spiked into 0.5 ml of urine or serum. After homogenization, dilution with a factor of 5.0×10^4 for urine samples and 5.0×10^5 for serum samples, the samples were determined by the proposed method directly.

3. Results and discussion

3.1. CL intensity–time profile

The kinetic profile for CL intensity of luminol–hydrogen peroxide reaction was tested using 3.6×10^{-6} mol l^{-1} luminol and 2.0×10^{-4} mol l^{-1} hydrogen peroxide in 0.025 mol l^{-1} NaOH. It could be seen from Fig. 2 the CL signal of luminol–hydrogen peroxide reached a maximum at 5 s after initiating the reaction, and tended to be vanishing in the following 16 s. Also, it can be seen that a scintillescent CL signal was detected in the presence of levofloxacin (10.0 ng ml^{-1}), and the CL intensity reached maximum at 3 s, then tended to be vanishing in following 12 s. It was also demonstrated that levofloxacin enhanced the CL reaction and increased the CL intensity greatly.

3.2. Effect of luminol and hydrogen peroxide concentration

Under different concentrations of luminol from 5.0×10^{-8} to 5.0×10^{-4} mol l^{-1} the CL intensity were tested. With an increase concentration of luminol, the CL signal increased steadily until luminol

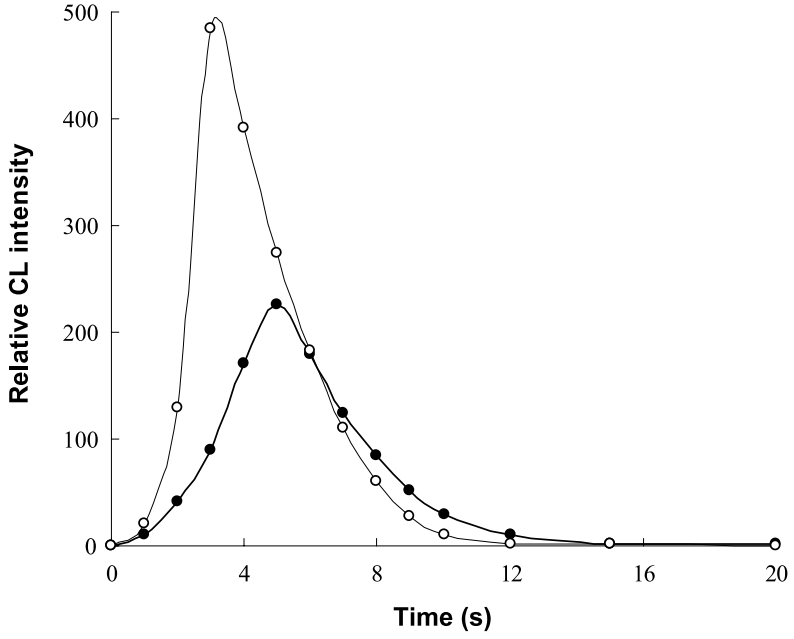

Fig. 2. Kinetic CL intensity–time profile in static system; •: CL intensity in the absence of levofloxacin, ○: CL intensity in the presence of levofloxacin (10.0 ng ml^{-1}).

was 3.6×10^{-6} mol l^{-1}, more than which the CL intensity tended to be stable. Therefore, the luminol solution of 3.6×10^{-6} mol l^{-1} was selected for the present work.

The effect of the hydrogen peroxide concentration was also tested. The CL intensity was recorded for a series of hydrogen peroxide solutions from 5.0×10^{-6} to 5.0×10^{-3} mol l^{-1}. It was found that the CL intensity arrived at the maximum with 2.0×10^{-4} mol l^{-1} hydrogen peroxide and offered approximately constant CL intensity over 2.0×10^{-4} mol l^{-1}. Thus, 2.0×10^{-4} mol l^{-1} hydrogen peroxide was selected.

3.3. Effect of sodium hydroxide concentration

Owing to the nature of the luminol reaction, which is more favored under alkaline conditions, NaOH was introduced into the luminol solution to improve the sensitivity of the system. A series of NaOH solutions with different concentration (0.005, 0.01, 0.03, 0.05, 0.1 and 0.2 mol l^{-1}, respectively) were tested. The CL intensity versus concentration of NaOH plot reached a peak at about 0.025 mol l^{-1}, and this concentration was employed in subsequent experiments.

3.4. Effect of flow rate and the length of mixing tubing

The effect of the mixing tube length on CL intensity was tested from 5.0 to 25.0 cm. It could be observed that the CL intensity was much stronger using 12.0 cm mixing tube than that of other mixing tube in the presence of 10.0 ng ml^{-1} levofloxacin. Thus, 12.0 cm mixing tube was selected. The influence of flow rate on determination was examined by investigating the signal-to-noise ratio (S/N) under different flow rate. And the flow rate of 2.0 ml min^{-1} offering highest S/N ratio was then chosen as suitable condition considering analytical precision.

3.5. Analytical performance for determination of levofloxacin

Under the optimal conditions, a series of standard solutions of levofloxacin were analyzed by the FI-CL system illustrated in Fig. 1. The increased CL intensity was proportional to the levofloxacin concentration over the range from 1.0 ng ml^{-1} to 700.0 ng ml^{-1} with the detection limit of 0.3 ng ml^{-1} (3σ). And the linear regression equation for levofloxacin was $\Delta I_{\mathrm{CL}} = 6.2199 C_{\mathrm{levofloxacin}} + 14.8000$, $R^2 = 0.9992$.

At the flow rate of 2.0 ml min^{-1}, a typical analysis including sampling and washing, could be completed in 0.5 min giving a throughput of 120 times h^{-1} with a relative standard deviation of less than 3.0% ($n = 5$).

3.6. Interference studies

The interference of foreign species was tested by analyzing a standard solution of levofloxacin (10.0 ng ml^{-1}) into which increasing amounts of interfering analyte was added. The tolerable concentration of foreign species with respect to 10.0 ng ml^{-1} levofloxacin for interference at 5.0% level were over 10,000 for Cl^-, NO_3^-, Ac^-, I^-, SO_4^{2-}, PO_4^{3-}, BrO_3^-, amylum, glucose, borate, malic acid, maltose, ethanol and methionine, and 5000 for NH_4^+, Mg^{2+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, oxalate, methanol, tartrate, sucrose, citrate and salicylic acid, and 1000 for glutin, urea and dextrin, and 500 for cysteine, and 100 for uric acid, and 10 for Cu^{2+}, Zn^{2+}, Ni^{2+}, Cr^{3+}, Fe^{2+}/Fe^{3+}, respectively. Common excipients in tablets and compounds abundant in human urine and serum such as salt, lipid and proteins caused no obvious interference for the determination of levofloxacin.

Table 1
Results of determination for levofloxacin in tablets[a]

Sample No.	Added (ng ml^{-1})	Found (ng ml^{-1})	RSD (%)	Recovery (%)	Content of levofloxacin (mg tab^{-1})	
					By the proposed method	By HPLC
1	0	28.53	2.48	99.7	95.10	98.3
	10.00	38.50	2.30			
2	0	30.43	2.67	98.6	101.43	96.4
	30.00	60.00	1.00			
3	0	31.25	0.71	101.6	104.17	104.2
	50.00	82.03	0.61			
4	0	51.04	2.37	104.5	102.08	98.7
	30.00	82.39	1.08			
5	0	50.40	0.49	96.8	100.80	102.2
	50.00	98.79	0.65			
6	0	47.89	1.75	102.9	95.78	94.8
	70.00	119.94	1.00			
7	0	71.20	2.54	97.5	101.71	97.9
	50.00	119.94	1.34			
8	0	68.00	0.51	97.1	97.14	99.0
	70.00	136.00	0.65			
9	0	68.70	0.95	104.6	98.14	103.7
	100.00	173.28	1.35			

[a]The average of five determinations.
[b]Label claim: 100 mg tab^{-1}.

4. Applications

4.1. Determination of levofloxacin in pharmaceutical preparations

Following the procedure detailed in the sample preparation section, the proposed method was applied to the determination of levofloxacin in tablets (Beijing Jingfeng Pharmaceutical Co. Ltd.) from the local market, and the results were summarized in Table 1, with recovery from 96.8% to 104.6%. To verify the results obtained by the proposed method, HPLC (Model Agilent-1100, Agilent Technologies) was applied to determine the samples. The results obtained by the proposed method agreed well with the results obtained by HPLC method.

4.2. Determination of levofloxacin in spiked human urine

The proposed CL method was applied successfully to the determination of levofloxacin in spiked human urine. In order to evaluate the validity of the proposed method, recovery studies were carried out on samples to which known amounts of levofloxacin were added. The results were given in Table 2, with recovery from 95.9% to 103.7%. The concentrations of levofloxacin determined by the proposed CL method were compared with the concentration spiked, and good agreement was obtained.

4.3. Determination of levofloxacin in spiked human serum

The proposed CL method was also applied successfully to the determination of levofloxacin in spiked human serum. The results were given in Table 3, with recovery from 96.0% to 104.5%. The concentra-

Table 2
Results of determination for levofloxacin in spiked human urine[a]

Sample No.	Added (ng ml^{-1})	Found (ng ml^{-1})	RSD (%)	Recovery (%)	Content of levofloxacin (mg ml^{-1})	
					By the proposed method	Spiked
1	0	30.70	1.76	102.3	1.54	1.50
	10.00	40.93	0.82			
2	0	29.64	1.12	102.4	1.48	1.50
	30.00	60.36	1.64			
3	0	29.47	1.22	98.7	1.47	1.50
	50.00	78.81	0.89			
4	0	50.80	0.92	101.1	2.54	2.50
	30.00	81.14	1.86			
5	0	50.98	2.53	98.0	2.55	2.50
	50.00	100.00	1.89			
6	0	49.76	2.56	97.6	2.49	2.50
	70.00	118.07	1.57			
7	0	69.57	1.84	103.7	3.48	3.50
	50.00	121.43	1.22			
8	0	69.05	2.01	95.9	3.45	3.50
	70.00	136.22	1.95			
9	0	71.47	0.40	96.9	3.57	3.50
	100.00	168.36	1.09			

[a]The average of five determinations.

tions of levofloxacin determined by the proposed method were compared with the concentration spiked, and good agreement was obtained.

5. Conclusions

Based on the enhanced effect of levofloxacin on the luminol–hydrogen peroxide CL system, a simple FI-CL system for the determination of levofloxacin was proposed, which improved general sensitivity for the levofloxacin assay. Compared with other methods for the assay of levofloxacin, CL method offers advantages of simplicity of apparatus, less reagent consumption, higher sensitivities and higher sample throughput. The satisfactory performance in an assay of levofloxacin in pharmaceutical preparations and biological fluids demonstrated that the method was practical and suitable not only for quality control analysis but also for complex biological samples, confirming the promise for pharmacological and clinical research.

Acknowledgements

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Table 3
Results of determination for levofloxacin in spiked human serum[a]

Sample No.	Added (ng ml^{-1})	Found (ng ml^{-1})	RSD (%)	Recovery (%)	Content of levofloxacin (mg ml^{-1})	
					By the proposed method	Spiked
1	0	29.00	2.62	96.0	14.50	15.00
	10.00	38.60	1.80			
2	0	30.19	1.07	104.5	15.10	15.00
	30.00	61.56	1.69			
3	0	29.82	2.04	100.9	14.91	15.00
	50.00	80.28	1.42			
4	0	51.23	0.89	99.2	25.62	25.00
	30.00	81.00	1.72			
5	0	51.39	0.56	102.8	25.70	25.00
	50.00	102.78	0.81			
6	0	49.41	1.24	98.5	24.70	25.00
	70.00	118.38	0.75			
7	0	71.05	0.42	97.4	35.52	35.00
	50.00	119.74	0.79			
8	0	69.10	1.40	101.3	34.55	35.00
	70.00	140.00	1.43			
9	0	69.17	2.00	99.2	34.58	35.00
	100.00	168.33	1.32			

[a]The average of five determinations.

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