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A Metal Enhanced Flow-Injection Chemiluminescence Method for the Rapid Determination of Norfloxacin in Pharmaceutical Formulations and Serum Sample

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A simple and highly sensitive chemiluminescence method to determine norfloxacin (NFLX) has been proposed by measuring the chemiluminescence (CL) intensities using a flow injection (FI) system. The CL intensity of the luminol-H_2O_2 system is strongly enhanced by the addition of Cu (II) in alkaline condition. The CL intensity is substantially increased after the injection of NFLX into the luminol-H_2O_2-Cu (II) system. The enhancement effect is attributed to a catalytic effect of Cu (II) due to the interaction with NFLX which forms a complex with the catalyst. Under the optimal conditions, the sensitizing effect of the CL intensity is proportional to the concentration of NFLX in the range of 1.5×10^{-9} - 5.9×10^{-7} mol L^{-1} ($r = 0.9994$) with a detection limit (3σ) of 2.98×10^{-10} mol L^{-1}. The proposed method had good reproducibility with the relative standard deviation (RSD, $n = 5$) of 1.6% for 1×10^{-7} mol L^{-1} of NFLX. The possible reaction mechanism of the CL reaction is also discussed. This method has been successfully applied for the determination of trace amount of NFLX in pharmaceutical preparations and serum samples.

Key Words: Chemiluminescence, Flow injection analysis, Norfloxacin, Copper (II), Luminol

Introduction

Norfloxacin [1-ehtyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline-carboxylic acid] (NFLX), a member of fluoroquinolone derivatives, is a synthetic chemotherapeutic broad spectrum antibiotic that is active against both gram-negative and gram-positive bacteria.[1] NFLX is a third-generation quinolone antibiotic which differs from non-fluorinated quinolones by having a fluorine atom at 6 positions and a piperazine moiety at the 7 position. NFLX shows excellent therapeutic advantages with higher antibacterial activity due to the addition of the two groups.[2] It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV,[3] enzymes necessary to separate bacterial DNA, thereby inhibiting cell division. It is used to treat some bacterial infections such as urinary tract infections, infections of the stomach or intestines, such as traveler's diarrhea. It has some primary side effects such as headache, depression, dizziness, nausea, and vomiting.[4] However, it is the first choice drug for the treatment of diseases caused by *Campylobacter*, *E. coli*, *Salmonella*, *Shigella* and *V. colera*.[5] Thus the determination of NFLX in biological samples and medicine is of great importance for human health, food assurance and quality control because of its increasing usage and influence on human body.

Several methods have been reported for determination of NFLX in pure, dosage form and in biological fluids. Various spectrophotometric methods were described for the determination of norfloxacin.[6,7] It is determined by capillary electrophoresis,[8-10] polarography,[11] voltammetry,[12] high performance liquid chromatography (HPLC),[13] spectrofluorimetry,[14,15] and chemiluminescence.[16,17] Among these techniques CL method combined with flow injection is well known as the powerful analytical technique that provides the advantages of simple equipment, high sensitivity detection and producing low background signals for the emission of CL coming from the chemical reaction. However, a few flow injection CL methods were reported for the determination of NFLX in pharmaceutical and biological samples with different reaction systems such as, Au nanoparticles (NPs)-NFLX-Ce(IV)-Na_2SO_3 system,[18] $[Ag(HIO_6)_2]^{5-}$-H_2SO_4-NFLX system,[4] Ce(IV)-sodium hyposulphite-NFLX system,[1] NFLX-Ce(IV)-$S_2O_4^{2-}$-HNO_3 system,[19] NFLX-Ru(bipy)$_3^{2+}$-Ce(IV)-H_2SO_4 system,[20] luminol-H_2O_2-NFLX-Au NPs system,[21] CL reaction of sulphite with Ce(IV) sensitized by NFLX,[22] micelle-sensitized Ce(IV)-$Na_2S_2O_3$-NFLX system,[23] FQs-soluble manganese (IV)-sulphite system,[24] FQs-Ru(bipy)$_3^{2+}$-Ce(IV) system in sulfuric acid medium.[25]

To the best of our knowledge there is no report for the determination of NFLX using luminol-H_2O_2 system catalyzed by Cu (II). In this paper we described a simple and sensitive chemiluminescence flow system for the determination of NFLX using luminol-H_2O_2-$CuSO_4$ system. This system is based on the luminescence properties of the luminol-H_2O_2-Cu (II)-NFLX system. Cu (II) exhibited a better catalytic effect, by which the CL intensity of luminol-H_2O_2 catalyzed by Cu (II) was strongly increased in the presence of NFLX which produced satisfactory results with lower limit of detection (LOD) compared to the reported flow-injection analysis (FIA) CL method. The LOD of this proposed method with wide dynamic range proofs the significance of this work. Parameters affecting the reproducibility and CL detection were optimized systematically. Furthermore, the possible mechanism of NFLX which enhanced the luminol-H_2O_2-$CuSO_4$ CL reaction is also discussed and the system was applied for the analysis of pharmaceutical preparations and serum samples.

Experimental

Reagents and Materials. Luminol (5-Amino-2,3-dihydro-1,4-phthalazinedione) and norfloxacin was purchased from Sigma. Hydrogen peroxide was obtained from Junsei Chemical Co. Ltd (Japan). Copper (II) sulfate was from Duksan Pure Chemical Co. Ltd. (South Korea) and Sodium borate (Borax) was from Shinyo Pure Chemical Co. Ltd (Japan). A stock solution of NFLX (1.0×10^{-3} mol L^{-1}) was prepared by dissolving appropriate amount of NFLX solid in 1.5 mL of 0.1 mol L^{-1} NaOH and diluting it with deionized water to 50 mL which was stored at 4 °C. A 1.0×10^{-2} mol L^{-1} luminol stock solution was prepared by dissolving appropriate amount of luminol in 0.1 mol L^{-1} NaOH solutions and diluting it with deionized water to 100 mL and stored in the refrigerator at 4 °C. Hydrogen peroxide solutions were prepared daily before experiment from 30% H_2O_2 (Junsei, Japan). All working solutions were prepared daily from the stock solution by appropriate dilution immediately before used. All experiments were performed with analytical grade reagents used directly without further purification and doubly deionized water was used throughout.

Apparatus. A schematic diagram of FIA used in the present study is shown in Figure 1. Two peristaltic pumps (P_1, P_2) (Model 404, Ismatec, Zurich, Switzerland) were used to deliver all solutions. One pump conveyed copper sulfate, luminol and hydrogen peroxide solution at a flow rate of 2.5 mL min^{-1} while the other delivered the studied drug sample at a flow rate of 1.8 mL min^{-1}. Polytetrafluoroethylene (PTFE) tubing (0.8 mm i.d.) was used to connect all components in the flow system to carry all solutions. 150 μL of sample solution was injected into the reaction stream by a six-way injection valve. An F-4500 spectrofluorimeter (Hitachi, Japan) equipped with a coiled glass flow cell (1.0 mm i.d., 20 mm total diameter) was used for detecting and recording the CL intensity of the reaction product. For the CL measurement, the light source of the spectrofluorimeter was switched off. The high voltage for the photomultiplier tube (Model R 928, Hamamatsu, Japan) was set to 950 V. A pH meter (Model Orion 520A USA) was used for pH adjustment. The UV-1800 (Shimadzu, Japan) spectrophotometer was used to record the absorption spectrum.

Analytical Procedure. The FIA system used in this experiment is shown in Figure 1. Prior to the CL measurement acquisition, luminol, H_2O_2, $CuSO_4$ and blank solution were continuously pumped into the manifold until the steady baseline and good reproducibility of the signal were achieved. The sample solution was incorporated with $CuSO_4$ solution by a six-way injection valve which was mixed with the luminol and H_2O_2 solution stream in a three-way 'T' connector, then reached the flow cell in the fluorimeter, accompanying the remarkable increase of CL intensity. The CL signal produced in the flow cell was recorded. As mentioned above, NFLX was found to enhance the CL signal of the luminol-H_2O_2-$CuSO_4$ system strongly. Determination of NFLX was based on the net CL intensity changes from the with and without NFLX sample solution.

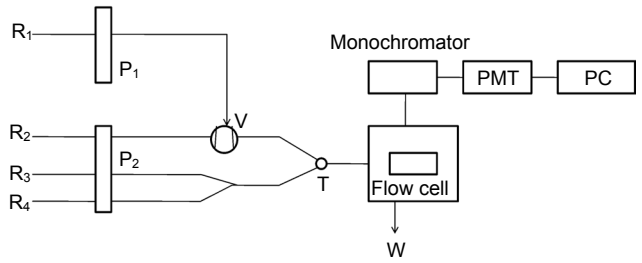

Figure 1. Schematic diagram of the FIA-CL manifold applied for the determination of norfloxacin. (R_1) Sample/ blank solution; (R_2) $CuSO_4$; (R_3) luminol; (R_4) H_2O_2; (V) Injection valve; (P_1, P_2) peristaltic pumps; (T) Y-pieces; (W) Waste.

Sample Preparation. Ten Urekacin tablets (Kukje pharma, South Korea) containing 200 mg NFLX was ground and dissolved in 5 mL 0.1 mol L^{-1} NaOH. The solution was centrifuged for 10 min and the supernatant was transferred into a 25 mL volumetric flask and diluted up to the mark with double distilled water. The working solution was prepared by appropriate dilution of the concentrated sample solution with double distilled water and used for further sample analysis.

A 1.0 mL serum sample was deproteinized by adding 5.0 mL 20% trichloroacetic acid (CCl_3COOH) in a centrifuge tube. This mixture was centrifuged for 15 min at 8000 rpm. The supernatant was dissolved in 1.0 mL deionized water. A known amount of NFLX was added into the protein free serum and then diluted to 50 mL with deionized water in order to obtain a concentration of NFLX in the range of linearity.

Results and Discussions

CL Kinetic Curves of the Systems. The CL kinetic curves of luminol-H_2O_2 CL reaction catalyzed by Cu (II) in the absence and presence of NFLX antibiotics were studied. In order to obtain the life time of the CL reaction, the kinetic characteristics of the CL reaction were examined with a static injection method by using the solution consisted of 2.0×10^{-4} mol L^{-1} luminol, 0.1 mol L^{-1} H_2O_2, 1×10^{-3} mol L^{-1} $CuSO_4$, and the typical CL kinetic curve was shown in Figure 2. It can be observed from Figure 2 that this CL reaction was rapid. The luminol-H_2O_2 CL reaction showed relatively low CL intensity even though CL

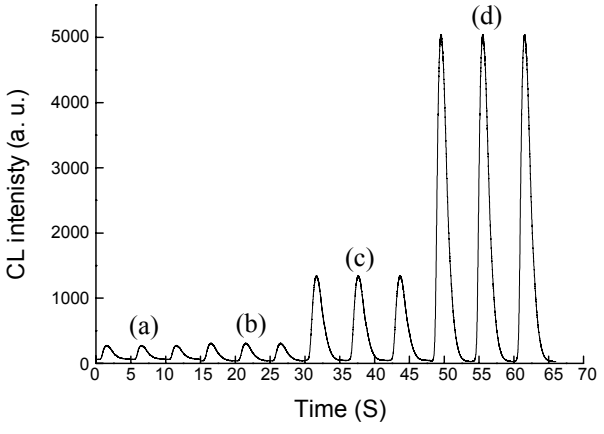

Figure 2. CL Kinetic curve of luminol-H_2O_2-NFLX catalyzed by Cu (II). (a) luminol-H_2O_2; (b) luminol-H_2O_2-NFLX; (c) luminol-H_2O_2-Cu (II); (d) luminol-H_2O_2-Cu (II)-NFLX. Conditions, Luminol: 2×10^{-4} mol L^{-1}; Cu (II): 1×10^{-3} mol L^{-1}; H_2O_2: 0.1 mol L^{-1}; NFLX: 5.9×10^{-7} mol L^{-1}; pH: 10.4.

intensity was not increased markedly with NFLX (Curve a and b, Figure 2). When $CuSO_4$ was introduced into the luminol-H_2O_2 system the CL signal was dramatically increased (Curve c, Figure 2). By the addition of NFLX into the luminol-H_2O_2-$CuSO_4$ system, the CL intensity was increased more markedly (Curve d Figure 2) which is proportional to the substances added. Therefore, it was observed that the CL intensity was highly sensitized in the presence of NFLX, and the CL system was steady and repeatable.

Effect of Flow Rate. In FIA system, flow rate is an important factor which influences not only analytical efficiency but also the sensitivity of the system. Therefore, in order to achieve maximum CL intensity, the effects of flow rate were investigated in the range of 1 - 4.5 mL min^{-1} for $CuSO_4$, luminol and H_2O_2 and 1 - 3 mL min^{-1} for sample respectively. It was observed that the best reproducibility and relatively strong CL intensity was obtained at 2.5 mL min^{-1} flow rate of $CuSO_4$, luminol and H_2O_2 and 1.8 mL min^{-1} of sample, probably because the CL reaction is rapid reaction. Above or below these flow rates, the CL intensity declined, because lower flow rates resulted in lower CL emission while higher flow rates with shorter contact time result insufficient CL reaction and might lead to the irreproducibility and excess consume of the reagents. Therefore flow rates of 2.5 mL min^{-1} for $CuSO_4$, luminol and H_2O_2 and 1.8 for NFLX was chosen for further study.

Effect of the Type and Concentration of Buffer in Luminol. In the preliminary experiments it was found that the luminol-H_2O_2-$CuSO_4$ system had a very low or no CL signal when luminol was diluted with water. In order to obtain high CL signal, the luminol solution was diluted with alkaline buffer. So luminol was diluted in different types of buffer (Na_2HPO_4 NaH_2PO_4, Na_2CO_3-$NaHCO_3$, $Na_2B_4O_7$-NaH_2PO_4, and $Na_2B_4O_7$) and examined the CL intensity. Among the above buffer, it was shown that the highest sensitivity and good reproducibility can be achieved with the use of $Na_2B_4O_7$ at a concentration of 5×10^{-4} mol L^{-1}.

Effect of pH of Luminol Solution. pH is a major factor which influences the luminol CL reaction. The effect of pH of luminol on the CL reaction was investigated in the range of 9.0 - 11.0. We found that the CL intensity increased with increasing pH up to 10.4. As shown in Figure 3a, it was observed that above pH 10.4, the color of the final CL solution changed from yellow to brown followed by the precipitation of some products. This may be because of the fact that with increasing reaction pH, hydroxide ion will increasingly react with the complex to form copper (II) hydroxide, a non-light producing species. Thus, a luminol solution at pH 10.4 was used in all CL experiments for the highest sensitivity and good reproducibility.

Effect of Luminol Concentration. Luminol was used in this system as the CL reagent and its concentration has an influence

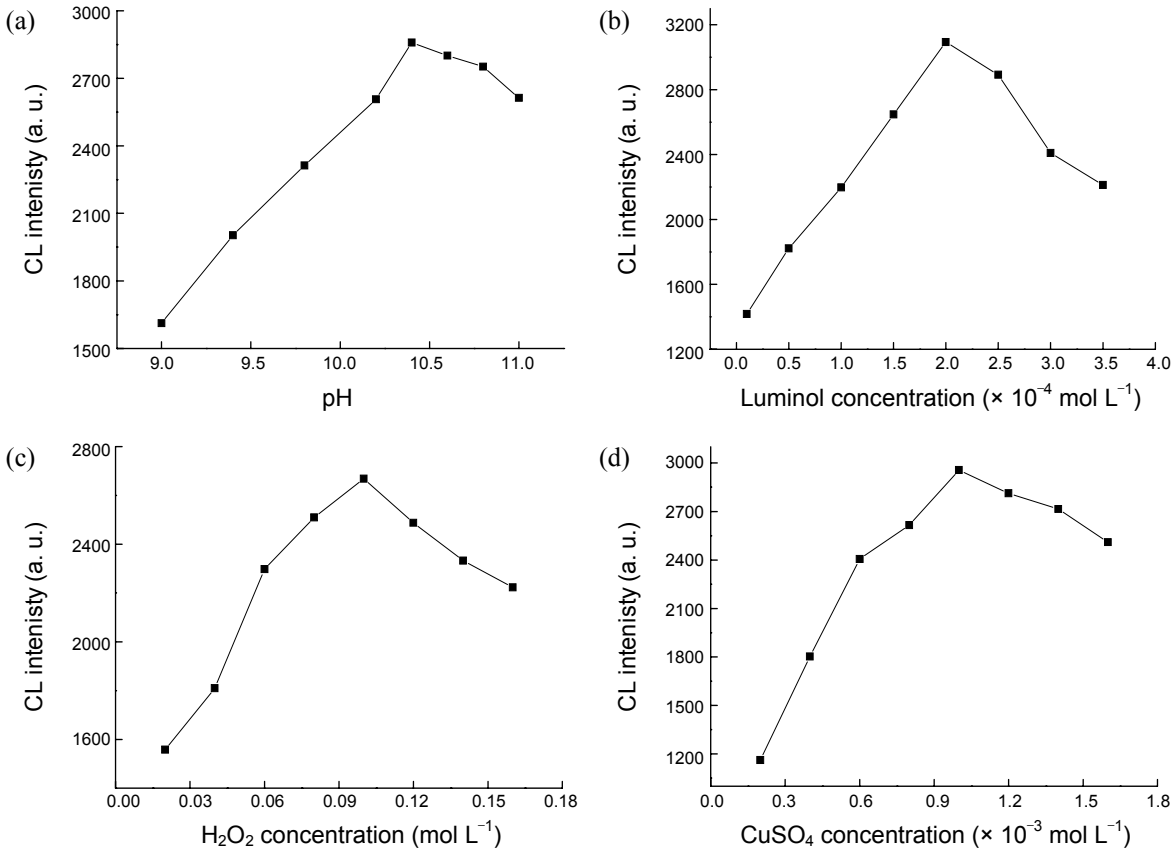

Figure 3. The effect of (a) pH. Conditions, Luminol: 2×10^{-4} mol L^{-1}; Cu (II): 1×10^{-3} mol L^{-1}; H_2O_2: 0.1 mol L^{-1}; NFLX: 5.9×10^{-7} mol L^{-1}; (b) luminol. Conditions, Cu (II): 1×10^{-3} mol L^{-1}; H_2O_2: 0.1 mol L^{-1}; NFLX: 5.9×10^{-7} mol L^{-1}; pH: 10.4; (c) H_2O_2. Conditions, Luminol: 2×10^{-4} mol L^{-1}; Cu (II): 1×10^{-3} mol L^{-1}; NFLX: 5.9×10^{-7} mol L^{-1}; pH: 10.4; (d) Cu (II). Conditions, Luminol: 2×10^{-4} mol L^{-1}; H_2O_2: 0.1 mol L^{-1}; NFLX: 5.9×10^{-7} mol L^{-1}; pH: 10.4.

Table 1. Comparison of several FIA-CL methods with the proposed method for the determination of norfloxacin

CL system	Linear Range (mol L^{-1})	Detection limit (mol L^{-1})	Reference
Ce(IV)-$Na_2S_2O_3$-NFLX	6.0×10^{-5} - 1.0×10^{-8} [a]	1.88×10^{-8} [a]	[1]
$[Ag(HIO_6)_2]^{5-}$-H_2SO_4-NFLX	4.2×10^{-8} - 1.7×10^{-5} [a]	9.71×10^{-9} [a]	[4]
AuNPs-NFLX-Ce(IV)-Na_2SO_3	7.9×10^{-7} - 1.9×10^{-5}	8.2×10^{-8}	[18]
AuNPs-NFLX-luminol-H_2O_2	2.5×10^{-7} - 4.0×10^{-6} [b]	1.0×10^{-8} [b]	[21]
NFLX-Ce(IV)-$S_2O_4^{2-}$-H_2SO_4	1.3×10^{-6} - 1.3×10^{-4} [c]	5.01×10^{-7} [c]	[22]
Ce (IV)-$Na_2S_2O_3$-NFLX-SDS	1.2×10^{-7} - 2.3×10^{-5} [a]	6.92×10^{-9} [a]	[23]
NFLX-Mn (IV)-sulphite system	5.0×10^{-8} - 1.0×10^{-6}	3.0×10^{-8}	[24]
Luminal-H_2O_2-Cu (II)-NFLX-	1.5×10^{-9} - 5.9×10^{-7}	2.98×10^{-10}	Proposed method

[a]g mL^{-1}; [b]ng mL^{-1}; [c]μg mL^{-1}.

Table 2. Application of the proposed method for the determination of NFLX in pharmaceuti-cal preparations

Sample	Amount (mg)		Standard addition method		
	Value labeled	Proposed method ± RSD[a] %	Added (× 10^{-7}mol L^{-1})	Found (× 10^{-7}mol L^{-1}) ± RSD[a] (%)	Recovery (%)
Urekacin tablet (Kukje pharma)	200	199.91 ± 1.08	8.0	7.96 ± 1.12	99.50
			10.0	10.12 ± 0.94	101.20
			12.0	11.89 ± 1.58	99.08

[a]Relative standard deviation of three measurements.

Table 3. Determination of NFLX in serum sample

Sample	Amount found (× 10^{-8} mol L^{-1}) ± RSD[a] (%)	Standard addition method		
		Proposed method	Added (× 10^{-6} mol L^{-1})	Found (× 10^{-6} mol L^{-1}) ± RSD[a] (%)
Serum	5.3 ± 1.57	2.00	1.98±1.22	99
		4.00	4.08±1.52	102
		6.00	6.03±1.02	100.5

[a]Relative standard deviation of three measurements.

on the CL intensity. The effect of luminol concentration was investigated from 1.0×10^{-5} - 3.5×10^{-4} mol L^{-1}. It was shown that the relative CL intensity was increased with the increase of concentration of luminol from 1.0×10^{-5} - 2.0×10^{-4} mol L^{-1} (Figure 3b). When the concentration of luminol was too low (1.0×10^{-5} mol L^{-1}) or too high (3.5×10^{-4} mol L^{-1}), the CL intensity and the sensitivity of signal decreased and reproducibility was poor. Since luminol can act as a bidentate chelate, a high concentration of luminol should lead to a decrease in CL because luminol will tie up with copper and decrease the concentration of 3-aminophalate ions.[26] Thus, considering the sensitivity and stability of the CL intensity, luminol of 2.0×10^{-4} mol L^{-1} was selected as optimum for the determination of NFLX.

Effect of H_2O_2 Concentration. The concentration of H_2O_2 played an important role in the CL reaction. The CL intensity increased markedly in the range of 0.01 - 0.25 mol L^{-1} of H_2O_2 concentration and the CL emission was gradually increased with the increase in H_2O_2 concentration up to 0.1 mol L^{-1} (Figure 3c). Above the concentration 0.1 mol L^{-1} the CL intensity decreased and the background signal and noise increased. On the other hand, too high H_2O_2 concentrations caused the formation of gaseous bubbles in the flow line. So the concentration of 0.1 mol L^{-1} H_2O_2 was chosen for this experiment.

Effect of $CuSO_4$ Concentration. $CuSO_4$ played an important role in this CL reaction. When luminol was mixed with H_2O_2, the CL emission was relatively low. However, CL emission could be greatly enhanced by the addition of $CuSO_4$ as catalyst to the reaction solution. In this study, we examined five transition metal ions, Cu^{2+}, Co^{2+}, Ni^{2+}, Cr^{2+} and Fe^{2+}. Among the five metal ions, Cu^{2+} exhibited strongest CL intensity. So the effect of Cu^{2+} concentration on CL intensity was studied by the flow injection system in the range of 2×10^{-4} - 1.6×10^{-3} mol L^{-1}. The results are shown in Figure 3d. From the results, it was shown that the CL intensity was increased with the increase of Cu^{2+} concentration up to 1×10^{-3} mol L^{-1}. Over 1×10^{-3} mol L^{-1} concentration of Cu^{2+}, the CL intensity was gradually decreased and a brown precipitate appeared in the flow line. Considering these factors and higher selectivity and reproducibility, a 1×10^{-3} mol L^{-1} $CuSO_4$ solution was used for all CL measurements.

Interference Study. The presence of interfering substances in the real sample may suppress or enhance the CL signal, although they have no significant effect on the intensity. The tolerance level was defined as the amount of foreign species that produce an error not exceeding 5% in the determination of the analytes. Thus, the effect of potential interfering sub-

stances and metal ions was investigated by preparing a set of solutions, each one with 1×10^{-6} mol L^{-1} NFLX plus a different concentration of a chemical species to be tested. The results implied that the foreign species did not interfere the determination of NFLX with 500 fold for K^+, Ca^{2+}, Zn^{2+}; 200 fold for Cl^-, SO_4^{2-}, PO_4^{3-}; 100 fold for Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}; 50 fold for amylum, dextrin, glucose, lactose, fructose; 10 fold for sodium benzoate, EDTA; and 5 fold for oxalic acid, urea. The results indicate that the proposed method holds good selectivity which can be selectively applied for the determination of NFLX in pharmaceutical preparations and serum sample.

Calibration Curve and Detection Limit. A calibration curve of CL intensity versus NFLX concentration was obtained at the optimized conditions given above. The linearity for the determination of NFLX was investigated and it can be clearly seen that the CL intensity is increased linearly with the concentrations of NFLX in the range of 1.5×10^{-9} - 5.9×10^{-7} mol L^{-1} with a regression equation of $I = 2.63 \times 10^9 C + 1612$ ($r = 0.9994$) where I is the CL intensity and C is the concentration of NFLX (mol L^{-1}). The limit of detection (LOD) as defined by IUPAC ($C_{LOD} = 3 \times Sb/m$, where Sb is the standard deviation of the blank signals and m is the slope of the calibration graph) was found to be 2.98×10^{-10} mol L^{-1} and the relative standard deviation (RSD) is 1.29% for 5 determinations of $1.0 \times 10 - 7$ mol L^{-1} NFLX. A comparison between the sensitivity of the proposed method and other FIA-CL method is shown in Table 1. The summarized results (Table 1) indicate that the sensitivity of the proposed method is higher than the other FIA-CL methods.

Analytical Application of the Proposed Method. In order to evaluate the validity of the proposed method, commercially available NFLX pharmaceutical preparations such as Urekacin tablets (Kukje pharma, South Korea) were studied. The results are given in Table 2. As shown in Table 2, the NFLX found through the proposed method was in close agreement with the labeled quantities. Recovery studies were also performed for each of the analyzed sample. For the recoveries study, standard addition method was applied and it can be seen that the recoveries of Urekacin tablets were found to be 99.08 - 101.20%.

The proposed CL method was also applied for the determination of NFLX in serum sample. The results are shown in Table 3. For the assay of NFLX in serum sample the freshly prepared sample was diluted appropriately within the linear range of determination. In order to compensate the effect of the biological matrix in the measurement, standard addition method was applied to the quantification of NFLX in the serum sample. From the Table 3, it can be seen that under optimum conditions and with the proposed method, the recoveries of NFLX from serum sample were 99 - 102%. Therefore, the proposed method can be easily performed and affords good precision and accuracy when applied to serum sample.

Overall results showed that the method is easy, sensitive and reliable and can be applied for the determination of NFLX in pharmaceutical preparation and biological samples.

Possible CL Reaction Mechanism. It is well known that 3-aminopthalate ion is the luminophore of chemiluminescence reaction between luminol and H_2O_2 and produces light at wavelength of 425 nm. In our presented study it was observed that

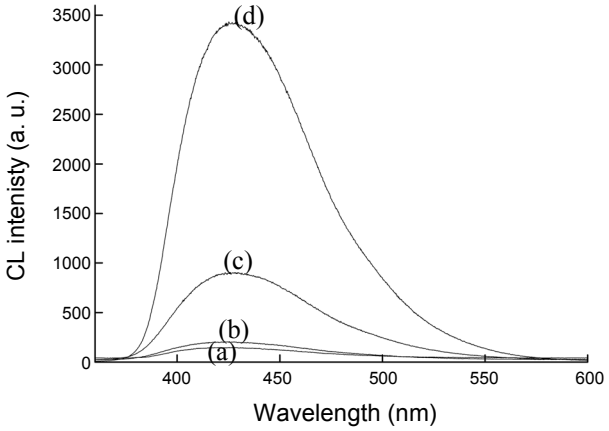

Figure 4. CL emission spectra. (a) luminol-H_2O_2; (b) luminol-H_2O_2-NFLX; (c) luminol-H_2O_2-Cu (II); (d) luminol-H_2O_2-Cu (II)-NFLX. Conditions, NFLX: 5.9×10^{-7} mol L^{-1}; $CuSO_4$, 1×10^{-3} mol L^{-1}; luminol, 2×10^{-4} mol L^{-1}; H_2O_2, 0.1 mol L^{-1}; pH: 10.4.

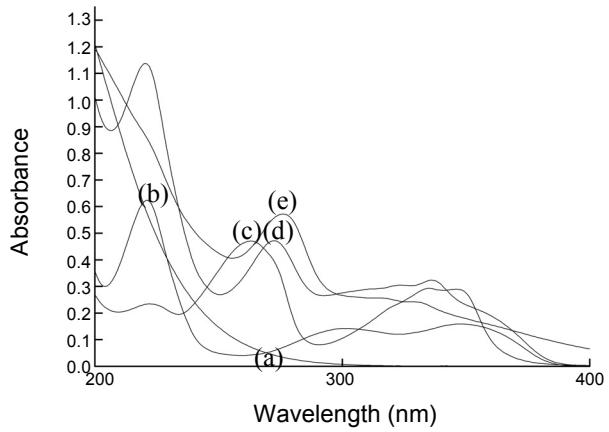

Figure 5. UV-vis absorption spectrum. (a) H_2O_2; (b) luminol; (c) NFLX; (d) luminol-H_2O_2 NFLX; (e) luminol-H_2O_2.Cu (II)-NFLX.

CL emission spectrum generating from the luminol-H_2O_2 system and luminol-H_2O_2-$CuSO_4$-NFLX system was found at about 425 nm (Figure 4). It implied that 3-aminopthalate ion which is the oxidation product of luminol was still luminophore of the investigated system.

Many studies reported that fluoroquinolone could enhance the CL intensity.[27-29] In this work, we found that only NFLX could not enhance the CL intensity of luminol-H_2O_2 system remarkably as shown in Figure 4. Results showed that CL intensity of luminol-H_2O_2 system in alkaline medium was stepped up by about four times when $CuSO_4$ was added in that system (Figure 4). Catalytic activity was reported to be responsible for the enhancement of CL intensity. It has been reported that Cu (II) ion act as a good catalyst in the decomposition of H_2O_2 to produce superoxide radical ion. Then superoxide radical ion reduces Cu^{2+} to Cu^+ through electron transfer mechanism and superoxide radical is produced which can interact with the ground state of 3-aminophthalate ions, and form more excited 3-aminophthalate ions. During relaxation process the excited 3-aminophthalate ions return to the ground state with enhanced CL intensity.[30] In this study, relative emission intensity was found to

be dramatically increased when NFLX was injected into the luminol-H_2O_2-$CuSO_4$ system.

It has been reported that Cu (II) form complex with fluoroquinolones (FQs).[31] In this study, change of absorption peak with the addition of NFLX and $CuSO_4$ in to luminol-H_2O_2 system was observed (Figure 5) which indicates that there was an interaction between Cu (II) and NFLX. Individual absorption spectra of H_2O_2 (Curve a, Figure 5), luminol (Curve b, Figure 5), NFLX (Curve c, Figure 5) and their mixtures were investigated. The results showed that the absorption peak of NFLX at 265 nm was found to be disappeared in the absorption spectrum of luminol-H_2O_2-NFLX (Curve d, Figure 5). When $CuSO_4$ was added to the luminol-H_2O_2-NFLX, the absorption peak of luminol-H_2O_2-NFLX was further changed and absorption peak of luminol-H_2O_2-NFLX was also disappeared. Therefore, it has been proposed that Cu (II) ions could interact with NFLX to form a complex which might serve as a center for production of superoxide radical from decomposition of H_2O_2 by its strong catalytic activity because homogeneous transition metal complexes contribute to rapid decomposition of H_2O_2.[32]

The decomposition of hydrogen peroxide (H_2O_2) catalyzed by transition metal ions or their complexes in homogeneous and heterogeneous systems have been extensively studied and different mechanisms have been suggested for these reactions.[33-37] From above description, possible mechanism can be suggested as

$$CuSO_4 + NFLX \rightarrow [NFLX\text{-}Cu]^+$$

$$[NFLX\text{-}Cu]^+ + H_2O_2 \rightarrow \text{Superoxide Radical}$$

$$\text{3-aminopthalate} + \text{Superoxide Radical} \rightarrow [\text{3-aminopthalate}]^*$$

$$[\text{3-aminopthalate}]^* \rightarrow \text{3-aminopthalate} + \text{Light (425 nm)}$$

Conclusion

A rapid, simple and sensitive FIA-CL method is described for the determination of NFLX, based on the sensitizing effect of NFLX on the CL of luminol-H_2O_2 catalyzed by Cu (II). When NFLX was mixed with Cu (II) in the system, the CL intensity was increased dramatically. Under the optimum condition, the CL intensity was proportional to the concentration of NFLX. The present method possesses higher sensitivity and selectivity than the reported FIA-CL method for the determination of NFLX. However, the proposed FIA-CL method exhibits satisfactory results and sensitivity for the determination of trace amount of NFLX in pharmaceutical preparations and serum sample which indicates a system is of great analytical potentials.

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