## 2. Experimental

### Reagents and Materials

Luminol (5-Amino-2, 3-dihydro-1, 4-phthalazinedione) and levofloxacin 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 LVX ($1.0\times10^{-2}$ mol $L^{-1}$) was prepared by dissolving appropriate amount of 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 $1.0\times10^{-2}$ mol $L^{-1}$ luminol stock solution was prepared by dissolving 0.1772 g 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

### Apparatus

A schematic diagram of flow injection analysis (FIA) used in the present study is shown in Fig. 2. Two peristaltic pumps ($P_1$, $P_2$) were used to deliver all solutions. Pump $P_1$ conveyed luminol while pump $P_2$ delivered $CuSO_4$ incorporated with blank/ sample solution in a six-way valve with loop at an equal flow rate for each line. $H_2O_2$ was conveyed by pump $P_2$ at the same flow rate. 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. A pH meter (Model Orion 520A USA) was used for pH adjustment.

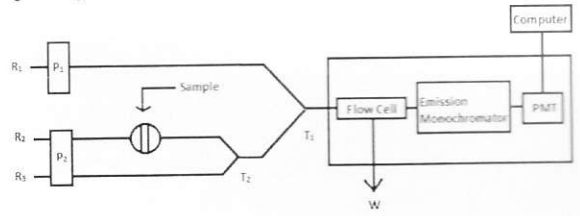


**Fig. 2:** Schematic Diagram of the FIA-CL manifold applied for the determination of Levofloxacin. ($R_1$) luminol; ($R_2$) sample/ blank solution; ($R_3$) $CuSO_4$; ($R_4$) $H_2O_2$; (V) Injection valve; ($P_1$, $P_2$) peristaltic pumps; ($T_1$, $T_2$) Y-pieces; (W), Waste

### General Procedure

The FIA configuration consisted of a three-channel manifold, using two pumps. The schematic diagram of the manifold is shown in Fig. 2. $CuSO_4$ incorporated with LVX sample solution was mixed with a $H_2O_2$ solution stream in a three-way '$T_2$' connector. The resulting stream was combined with the luminol solution in '$T_1$' connector and 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.

### Tablet Sample Preparation

Sample solutions for analysis were prepared as follows. The average tablet weights were calculated from the weight of each of 10 tablets which were selected from the same group randomly. An accurately weighed portion of each homogenized sample containing 250 mg of LVX (Levaquin & Tavanic) were transferred separately into 1000 ml calibrated dark flask containing 500 ml of water and dissolved in ultrasonic bath for 20 min and diluted with water de-ionized to mark. The dissolved sample was filtered through Millipore membrane filter paper and diluted with water to volume to obtain the appropriate concentration for analysis.

## 3. Results and Discussion

### Characteristic of CL Kinetic Curves

The CL kinetic profile of luminol–$H_2O_2$ CL reaction catalyzed by Cu(II) in the absence and presence of LVX antibiotics are shown in Fig. 3. The luminol–$H_2O_2$ CL reaction showed relatively low CL intensity even though CL intensity was not increased markedly with LVX (Fig. 3a, 3b). When $CuSO_4$ was introduced into the luminol-$H_2O_2$ system the CL signal was dramatically increased (Fig. 3c). By the addition of LVX into the luminol-$H_2O_2$-$CuSO_4$ system, the CL intensity was increased more markedly (Fig. 3d) which is proportional to the substances added. By this characteristic, LVX can be determined sensitively with this proposed CL method.

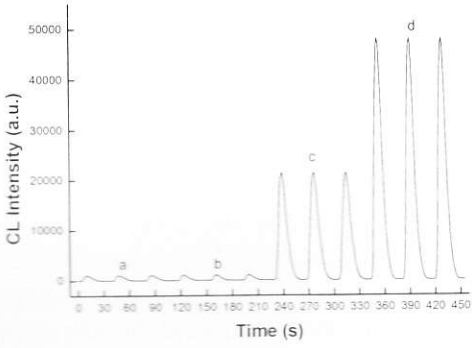


**Fig. 3:** The enhancing effect of LVX on luminol-$H_2O_2$-Cu (II)-LVX CL reaction based on time-scan (a) luminol-$H_2O_2$; (b) luminol- LVX - $H_2O_2$; (c) luminol-$CuSO_4$-$H_2O_2$ (d) luminol-$CuSO_4$- LVX -$H_2O_2$. Conditions: LVX, $5.9\times10^{-7}$ mol $L^{-1}$; $CuSO_4$, $1\times10^{-3}$ mol $L^{-1}$; luminol, $2\times10^{-4}$ mol$L^{1}$; $H_2O_2$, 0.1 mol $L^{-1}$

### Optimization of Experimental Condition for Levofloxacin Detection

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 double distilled 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 has been achieved with the use of $Na_2B_4O_7$ to dilute luminol in this experiment. Therefore, $Na_2B_4O_7$ buffer was chosen to dilute the luminol solution to obtain satisfactory sensitivity of the system.

The signal intensity of luminol CL system is particularly dependent on reaction pH. So the effect of luminol solution pH on the CL reaction was investigated over the range of 8-12. At pH higher than 10.0, the CL intensity decreased. The maximum CL emission was obtained at pH 10.0. Therefore, a pH of 10.0 was chosen for this system (Fig. 4a).

The effect of luminol concentration was investigated from the concentration level of $1.0\times10^{-5}$ to $3.5\times10^{-4}$ mol $L^{-1}$. The relative CL intensity increased with the increase of concentration of luminol from $1.0\times10^{-5}$-$2.0\times10^{-4}$ mol $L^{-1}$. Above $2.0\times10^{-4}$ mol $L^{-1}$ the CL signal was decreased dramatically. So the concentration of $2.0\times10^{-4}$ mol $L^{-1}$ luminol was selected for the determination of LVX (Fig. 4b).

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 $H_2O_2$ and the maximum CL intensity was obtained at 0.1 mol $L^{-1}$. So the concentration of 0.1 mol $L^{-1}$ $H_2O_2$ was chosen for this experiment (Fig. 4c).

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. The effect of $Cu^{2+}$ concentration was investigated from $2\times10^{-4}$-$1.6\times10^{-3}$ mol $L^{-1}$. From the results, it was shown that the CL intensity was increased with the increase of $Cu^{2+}$ concentration up to $1\times10^{-3}$ mol $L^{-1}$. Over $1\times10^{-3}$ 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 $1\times10^{-3}$ mol $L^{-1}$ $CuSO_4$ solution was used for all CL measurements (Fig. 4d).

## Analytical Characteristics

A calibration curve of CL intensity versus LVX concentration was obtained at the optimized conditions given above. The linearity for the determination of LVX was investigated and it can be clearly seen that the CL intensity is increased linearly with the concentrations of LVX in the range of $5.6\times10^{-10}$-$2.2\times10^{-7}$ mol $L^{-1}$ with a regression equation of $I=7.01\times10^{9}C+ 1609$ ($r = 0.9996$) where I is the CL intensity and C is the concentration of LVX (mol $L^{-1}$). The limit of detection (LOD) was found to be $5\times10^{-10}$ mol $L^{-1}$ and the relative standard deviation (RSD) is 1.29% for 5 determinations of $1.0\times10^{-7}$ mol $L^{-1}$ LVX.

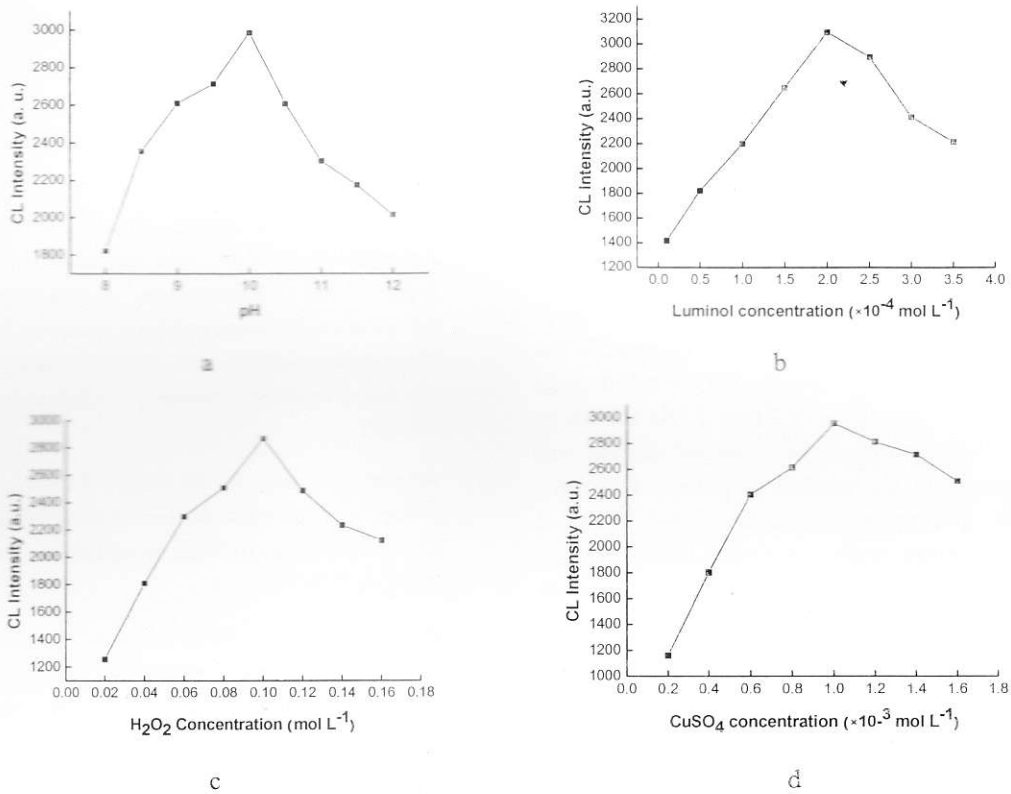


**Fig. 4:** The effect of (a) pH. Conditions: Luminol: $2 \times 10^{-4}$ mol $L^{-1}$; Cu(II), $1 \times 10^{-3}$ mol $L^{-1}$; $H_2O_2$, 0.1 mol $L^{-1}$; LVX, $5.9 \times 10^{-7}$ mol $L^{-1}$; (b) luminol. Conditions: Cu(II), $1 \times 10^{-3}$ mol $L^{-1}$; $H_2O_2$, 0.1 mol L-1; LVX, $5.9 \times 10^{-7}$ mol L–1; pH, 10.0; (c) $H_2O_2$. Conditions: Luminol, $2 \times 10^{-4}$ mol $L^{-1}$; Cu (II), $1 \times 10^{-3}$ mol L-1; LVX, $5.9 \times 10^{-7}$ mol $L^{-1}$; pH, 10.0; (d) Cu(II). Conditions: Luminol, $2 \times 10^{-4}$ mol $L^{-1}$; $H_2O_2$, 0.1 mol $L^{-1}$; LVX, $5.9 \times 10^{-7}$ mol $L^{-1}$; pH, 10.0.

**Table 1:** Application of the proposed method for the determination of levofloxacin in pharmaceutical preparations.

| Sample | Active ingredient labeled (mg) | Found±RSD[b] | Added ($\times10^{-6}$mol $L^{-1}$) | Found ($\times10^{-6}$mol $L^{-1}$, ±RSD[b] %) | Recovery (%) |
|---|---|---|---|---|---|
| Levaquin | 200 | 199.85±1.05 | 8.0 | 7.96±1.22 | 99.0 |
| | | | 10.0 | 10.01±0.91 | 100.2 |
| | | | 12.0 | 12.10±1.08 | 101.5 |
| Tavanic | 100 | 101.08±2.05 | 4.0 | 4.08±1.02 | 101.3 |
| | | | 5.0 | 4.89±0.89 | 97.2 |
| | | | 6.0 | 5.92±1.06 | 99.1 |

[b] Relative standard deviation of three measurements

### Interference Study

In order to assess the possible analytical applications of this CL method, the effect of potential interfering substances and metal ions ($K^+$ , $Ca^{2+}$, $Zn^{2+}$, $Cl^-$, $SO_4^{2-}$, $PO_4^{3-}$, dextrin, glucose, oxalic acid, urea) was investigated by preparing a set of solutions, each one with $1\times10^{-6}$ $molL^{-1}$ LVX plus a different concentration of a chemical species to be tested. The emission signal was measured for these solutions using a FIA-CL system based on the emission of luminol-$H_2O_2$-$CuSO_4$ system. No significant interference was observed by adding these common excipients to the solution which produced an error not exceeding ±5% for the determination of LVX. So the present method could be selectively applied to the determination of LVX in pharmaceutical preparations.

### Determination of Levofloxacin in Pharmaceutical Preparations

In order to evaluate the validity of the proposed method, commercially available Levofloxacin pharmaceutical preparations such as Levaquin tablets (Kukje pharma, South Korea) and Tavanic tablets (Guju pharma, South Korea) were used. The results are given in Table 1. As shown in Table 1, the LVX 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 can be seen that the recoveries of Levaquin tablets and Tavanic tablets were found to be 99.0-101.5% and 97.3-101.3 % respectively.

### 4. Conclusion

A simple and sensitive FIA-CL method is described for the determination of LVX, based on the sensitizing effect of LVX on the CL of luminol-$H_2O_2$ catalyzed by Cu(II). Under the optimum condition, the CL intensity was proportional to the concentration of LVX. However, the proposed FIA-CL method exhibits satisfactory results and sensitivity for the determination of trace amount of LVX in pharmaceutical preparations which indicates a system is of great analytical potentials.

### Acknowledgement

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# A Flow-injection Chemiluminescence Determination of Levofloxacin

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**Abstract**

**A sensitive chemiluminescence (CL) method using a flow injection analysis (FIA) was investigated to determine levofloxacin (LVX) using luminol-$H_2O_2$ system in the presence of copper(II). It was observed that the CL intensity of the luminol-$H_2O_2$ system is strongly enhanced by the addition of Cu(II) in alkaline condition. On injection of LVX into the luminol-$H_2O_2$-Cu(II) system, the CL intensity is substantially increased. The optimization of working conditions was investigated. Under the optimal conditions, the sensitizing effect of the CL intensity is proportional to the concentration of LVX in the range of $5.6\times10^{-10}$-$2.2\times10^{-7}$ mol $L^{-1}$ (r = 0.9996) with a detection limit (3σ) of $5\times10^{-10}$ mol $L^{-1}$. This method has been successfully applied for the determination of trace amount of LVX in pharmaceutical preparations.**

**Keywords:** Chemiluminescence, Flow injection analysis, Levofloxacin, Copper.

## 1. Introduction

Levofloxacin (LVX) is a synthetic fluorinated quinolone derivative (Fig. 1), which exhibits broad-spectrum in vitro bactericidal activities against Gram-positive and Gram-negative aerobes. They are also found to be active against intracellular pathogens responsible for a typical pneumonia [1,2].

LVX has been used in the treatment of community-acquired pneumonia, acute maxillary sinusitis and acute exacerbation of chronic bronchitis. The pharmacokinetic profile of LVX supports once-daily administration and because of its high tissue distribution, it may be also suitable in bone diseases [2]. It is rapidly and essentially absorbed after oral administration. Patients with urinary, respiratory or cutaneous infections are administered the drug in 500 mg/d doses. LVX undergoes limited metabolism in humans and approximately 87% of an administered dose was recovered as unchanged drug in urine within 48 h, whereas less than 4% of the dose was recovered in feces in 72 h [3].

The few reported methods for the determination of LVX are spectrophotometry [4], capillary electrophoresis with electrochemiluminescence [5] and HPLC with UV detection [6]. Ocana *et al.* [7] reported a flow injection chemiluminescence assay including detection which has been developed and applied to the determination of LVX. To the best of our knowledge there is no report for the determination of LVX using luminol-$H_2O_2$ system catalyzed by Cu(II). It has been reported that decomposition of $H_2O_2$ is relatively fast when metal ions are used as complexes [8].

In this paper a simple and sensitive chemilumine-scence flow system is presented for the determination of LVX using luminol-$H_2O_2$-$CuSO_4$ 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 LVX which produced satisfactory results with lower limit of detection (LOD) compared to the reported flow-injection analysis (FIA) CL method.

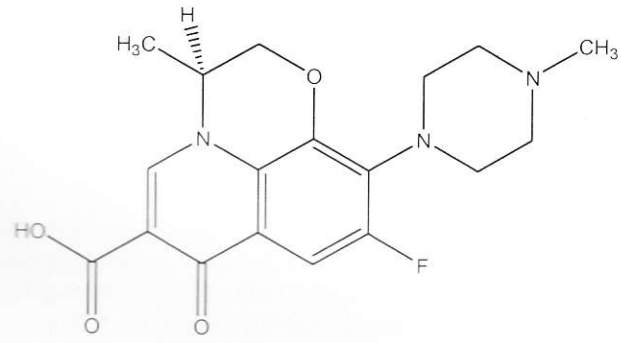


**Fig. 1:** Structure of Levofloxacin.

The LOD of this proposed method with dynamic range proofs the significance of this work. Parameters affecting the reproducibility and CL detection were optimized systematically. Furthermore, the system was applied for the analysis of pharmaceutical preparations.