Flow-Injection Chemiluminescence Determination of Fleroxacin in Pharmaceutical Preparations and Human Urine

Hanwen Sun1 and Liqing Li2

1College of Chemistry and Environmental Science, Hebei University, Key Laboratory of Analytical Science and Technology of Hebei Province, Baoding, 071002, China
2Department of Chemistry, Taishan University, Taian, 271021 China

Abstract

Quality control of drug fleroxacin dosage, its monitoring in biological fluids, and research of drug’s metabolism and action are an important analytical task. A new chemiluminescence (CL) reaction system was established for the determination of fleroxacin (FLX). The trivalence dysprosium-sensitized CL emission mechanism was investigated by comparing the fluorescence emission with CL spectra. The CL spectra of FLX-KMnO4-Na2S2O3-H2P04 system are from the narrow characteristic emission of Dy3+ at 482 and 578 nm (F → H13/2, F → H11/2) through the energy transfer from the excited SO2* to analyte, followed by intramolecular energy transfer from analyte* to Dy3+. The optimum conditions for CL emission were investigated and optimized. The relationships between the relative CL intensity and the concentration of the studied analyte have good linearity. The detection limit for FLX was 3.0×10^{-6} g/mL. The relative standard deviation is 2.0% for 11 determinations of FLX at 2.0×10^{-7} g/mL. The proposed CL system has been successfully applied for the determination of FLX in the injections and urine sample with satisfactory result.

Keywords: Chemiluminescence; Fleroxacin; Pharmaceutical preparations; Human urine

Introduction

Fleroxacin [FLX, 6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid] is a new fluoroquinolone antibiotic that exhibits strong bactericidal activity against a wide range of Gram-negative and Gram-positive bacteria [1]. Cullmann et al. reviewed the chemistry, microbiology, toxicology, pharmacokinetics, clinical efficacy and safety of FLX [2]. The mechanism of action of FLX is based primarily on the inhibition of bacterial DNA topoisomerase II (DNA gyrase). Quality control of drug dosage, its monitoring in biological fluids, and research of drug’s metabolism and action are an important analytical task. Therefore, it is necessary to establish sensitive analytical technique.

Several methods have been described for the determination of FLX either in pure form, in dosage forms or in biological fluids [3-10]. High-performance liquid chromatography (HPLC) with fluorescence detection has been developed for the measurement of FLX in rat plasma using a solid-phase extraction column [11], and FLX in serum [12]. Capillary electrophoresis (CE) and HPLC have the advantage of high separation capability suitable for components determination, and disadvantage of lower sensitivity.

The chemiluminescence (CL) method shows the advantages of simplicity, rapidity and high sensitivity, and has been applied extensively for the analysis of pharmaceutical compounds [13,14]. Chemiluminescence sensors are important tools in analytical chemistry due to their high sensitivity and selectivity [15-18]. A critical review was presented for the use of acidic solutions of potassium permanganate to generate CL during the oxidation of both organic compounds and inorganic species [19]. The CL reactions of potassium permanganate and reducer have been studied extensively. Among them, sodium thiosulfate is a classical reducer and has been used with potassium permanganate to detect some pharmaceutical compounds, but CL emission from the redox reaction of potassium permanganate and sodium thiosulfate is not significant enough. For cerium(IV)-sulfite CL system, the reduction–oxidation reaction between Ce(IV) and sulfite shows a weak peak. Recently, Chen and Fang reviewed flow injection technique for biochemical analysis with CL detection in acidic media [20]. New recently, a new CL method is reported for the determination of fluoroquinolone derivatives based on the enhancement of CL of luminol-hydrogen peroxide-gold nanoparticles system by fluoroquinolones [21]. The detection limits of the reported methods for the determination of FLX were at 10^-9-10^-10 g/mL levels.

The main purpose of this work is to develop a new Dy3+ sensitized CL system for the determination of FLX. The proposed method was applied for the determination of FLX in the injectable and urine samples with satisfactory result. The CL mechanism was also described.

Experimental Section

Chemicals and solution

All chemicals used were of analytical-reagent grade. Deionized water was used throughout. FLX was purchased from Institute of Medicinal Biotechnology Beijing, China). The Stock standard solution (5.0×10^{-4} g/mL) for FLX was prepared by dissolving 25.00 mg analyte in 1.5 mL 0.1 M sodium hydroxide, and diluting with deionized water to 50 mL, respectively. The more diluted solutions were freshly prepared by diluting the stock solution with deionized water.

A Dy3+ stock solution, 1×10^{-2} M, was prepared by dissolving 373 mg Dy2O3 in 15.0 mL HCl (11.6 M) at 95°C, evaporating the solution to be almost dry, then diluting it to 100 mL with water. Stock KMnO4 solution (5×10^{-2} M) and Na2S2O3 solution (2×10^{-3} M) were prepared daily and diluted as required. The working solutions of Na2S2O3, H2SO4, H2P04, HNO3 and HC1 were prepared daily and diluted as required.

*Corresponding author: Hanwen Sun, College of Chemistry and Environmental Science, Hebei University, Key Laboratory of Analytical Science and Technology of Hebei Province, Baoding, 071002, China, E-mail: hanwen@hbu.edu.cn

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Apparatus

The FI system, as shown in Figure 1, was a MPI-B flow-injection chemiluminescence 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 (120μL), which is automatically operated by a computer equipped with a software for operation system of MPI-B flow injection analysis. 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).

PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. The signal from the CL reaction was recorded. Fluorescence spectra were recorded with RF-5301PC spectrophotometer (Shimadzu, Japan) for the study of the fluorescence characteristics.

Procedure

The injection sample of FLX was made of 20 bottles of FLX injection selected from same group randomly. The working solutions were directly diluted with water. Human urine was kindly provided by healthy volunteers. No further pre-treatment was required for urine samples.

As shown in Figure 1, all solutions were continuously pumped into the manifold. A 120μL mixture of analyte solution and Na2S2O3 solution was injected into a mixed stream of KMnO4 and Dy3+ solutions. The mixed solution was transferred into the CL flow cell, and gave rise to an intensive CL signal immediately. The CL signal produced in the flow cell was recorded. Calibration graphs were constructed by plotting the intensity (peak height) of the CL signal versus the concentration of analyte.

Results and Discussion

Choice of sensitizers and CL system

Both KMnO4-S2O32- and MnO2-S2O32-FLX systems could only produce weak CL emission, respectively. The effects of various fluorescence compounds, such as rhodamine 6G, rhodamine B, eosin and fluorescein, on CL emission were investigated. No enhancing effect was observed clearly. Based on the fluorescence properties and fluorescein, on CL emission were investigated. No enhancing produce weak CL emission, respectively. The effects of various CL system was higher than that of Tb3+-sensitized fluorescence characteristics.

Effect of KMnO4 concentration on detection

In this CL system, KMnO4 was used as the oxidant. The KMnO4 concentration influences the sensitivity. Therefore, the dependence of the KMnO4 concentration on the CL intensity was investigated for 1.0×10^6 g/mL analyte. The CL intensity increased with increasing KMnO4 concentration from 0.5×10^4 to 2.5×10^4 M, and decreased obviously in range of 2.5×10^4–1.0×10^3 M. The KMnO4 concentration of 2.5×10^4 M was selected with the maximum CL intensity.

Effect of sodium thiosulfate concentration on detection

The effect of sodium thiosulfate concentration over the range of 5×10^-5–5×10^-4 M on CL emission was examined for 1.0×10^6 g/mL analyte. The maximum CL emission was obtained under the sodium thiosulfate concentration of 7.5×10^-3 M.

Interference studies

The influence of some common excipients used in drugs was investigated for the determination of 4.0×10^-7 g/mL analyte by injected sample volume of 120μL and flow rate of 3.0 mL/min for all solutions were used, the highest emission was obtained along with greater precision and economy in the use of reagents.

Effect of acidic medium on detection

The kind and concentration of the acid used in the reaction has a very significant influence on the CL emission intensity. Therefore several acids, such as HCl, H2SO4, HNO3, H3PO4 and H6P4O13, were added in FLX–KMnO4–Na2S2O3 solution to test the effect of acidic medium on the CL signal, respectively. The highest and stable emission was observed in H3PO4 medium for FLX–KMnO4–Na2S2O3 system, and the optimal concentration was 1×10^-5 M.

Effect of sample volume and flow rate on detection

As shown in Figure 1, when the mixed solution flowed into the cell, the CL reaction took place. The role of sample volume and flow rate is critical, for instance, if sample volume and flow rate were too small or too large, CL maximum could not be obtained. Whan the...
reagent mixing to peak maximum only 3 s was needed for Dy 3+-FLX-Na2S2O3-
zero again.

and relative standard deviation (RSD) for detection of hour. Under the optimum conditions described above, the linearity
of Dy3+-FLX-Na2S2O3-KMnO4-H6P4O13 system were investigated The CL
in Table 1.

in order to improve the veracity. The experimental results are listed
investigated. The calibration graph was consists of five parts for FLX
concentration that produces a peak with a height three times of the level
of baseline noise, and the limit of quantification (LOQ) was calculated
comparing with the CL emissions obtained using analyte solution alone
or analyte with foreign species added. A substance was considered no
interference if the variation of the CL intensity was <±5%. The results
indicate that 100-fold magnesium stearate, sucrose, dextrin, galactose,
fructose, starch, lactose, 60-fold glucose, 50-fold sodium benzoate,
polyglycol, and 20-fold sodium citrate did not interference for the
transfers in the CL systems. Since Dy3+ forms the chelate with analyte,
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Kinetic characteristics of CL reaction

The chemiluminescence kinetic characteristics of the reactions
of Dy3+-FLX-Na2S2O3-KMnO4-H6P4O13 system were investigated The CL
intensity–time profile of the system is presented in Figure 2.

It was found that the reaction rate in solution was very fast, from
reagent mixing to peak maximum only 3 s was needed for Dy3+-FLX-
Na2S2O3-

KMnO4-H6P4O13 system, and it took 9 s for the signal to return to
zero again.

Analytical performance of CL system

The proposed CL method can process up to 60 samples per
hour. Under the optimum conditions described above, the linearity
and relative standard deviation (RSD) for detection of FLX were
investigated. The calibration graph was consists of five parts for FLX
in order to improve the veracity. The experimental results are listed in Table 1.

The limit of detection (LOD) was determined as the sample
concentration that produces a peak with a height three times of the level
of baseline noise, and the limit of quantification (LOQ) was calculated
as the sample concentration that produces a peak with a height ten
times the baseline noise [22, 23]. The LOD was 3.0×10⁻⁸ g/mL and LOQ
was 1.2×10⁻⁸ g/mL for the first equations of FLX. The relative standard
deviation was 1.9% for 11 determinations of 6.0×10⁻⁸ g/mL of FLX.
The proposed method has lower LOD than UV spectrophotometry [3],
fluorescence spectrometry [4, 5], voltammetric method [7] and HPLC
[9-12] as well as luminol-hydrogen peroxide-gold nanoparticles CL
method [21]. It is indicated that the proposed CL system has good
linearity, higher sensitivity and precision.

CL mechanism

The chemiluminescence intensity of KMnO4-Na2S2O3-H6P4O13
system is very weak because of the low luminescence efficiency of
SO2⁺ [23]. By introducing a fluorophore whose absorption falls in the
emission range of the excited sulfur dioxide (300–450 nm) [24], the
CL intensity is usually enhanced through energy transfer from SO2⁺ to
the fluorophore [25], Na2S2O3 in acidic medium react to produce
HSO3-, based on this, Dy3+ or analyte was added to the CL system of
KMnO4-Na2S2O3, respectively, but no notable increase in the CL
intensity could be observed. However, when Dy3+ and analyte were
added together to the CL system of KMnO4-Na2S2O3, the CL intensity
was greatly enhanced.

In order to gain a better understanding of the nature of the CL
enhancement, we examined the CL spectra of Dy3+-KMnO4-Na2S2O3-
FLX system by a series of interference filters and the fluorescence
emission spectra of the system, as shown in Figure 3.

The native fluorescence emission of FLX shows broad peak
centers at 438 nm and 445 nm, respectively. When mixing with Dy3+, this wide emission band decreases in intensity greatly while
the narrow emission bands of the Dy 3+ appear at 482 and 578 nm,
respectively [26], which implies that the intramolecular energy
transfer has occurred between analyte and the Dy3+ [27-29]. Meanwhile, it can be concluded that the Dy3+-analyte complex has
been formed.

As shown in Figure 3b, the sensitized CL spectra of Dy3+-KMnO4-
Na2S2O3-FLX system are located at 482 and 578 nm, which is the
characteristic fluorescence spectrum of dysprosium [23], indicating
clearly that the excited Dy3+ is the emitter, and there must be energy
transfers in the CL systems. Since Dy3+ forms the chelate with analyte,

<table>
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<th>Time (h)</th>
<th>Content (×10⁻⁸g/mL)</th>
<th>Added (×10⁻⁸g/mL)</th>
<th>Found (×10⁻⁸g/mL)</th>
<th>Recovery (%)</th>
<th>RSD n=7</th>
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* not detected

Table 2: Recovery experiments for FLX.

Table 3: Determination of FLX in urine samples.
these complexes absorb the energy at the characteristic wavelength of the organic ligand and release it at the characteristic wavelength of the lanthanide due to an energy transfer from the quinoline ligand to the emitting energy level of the metal ion.

An intramolecular energy transfer takes place from SO₄²⁻ to the ligand (analyte) in the chelate (Dy³⁺-FLX) produced in the reaction process. Then, through intramolecular energy transfer from the ligand to Dy³⁺, Dy³⁺-ligand is formed, followed by the narrow characteristic emission of Dy³⁺. The mechanism stated above can be expressed as follows:

\[
S\text{O}_4^{2-} + H^+ \rightarrow HSO_4^-
\]

\[
\text{MnO}_4^- + HSO_4^- \rightarrow \text{MnO}_4^- + HSO_3^-
\]

\[
2\text{HSO}_3^- \rightarrow \text{SO}_2^2- + 2\text{H}^+
\]

\[
\text{SO}_2^2- + \text{[Dy-FLX]*} \rightarrow \text{SO}_4^- + [\text{Dy-FLX}^*]^{1+}
\]

\[
[D\text{y}-\text{FLX}]^{1+} \rightarrow [D\text{y}^2-\text{FLX}]^{1+}
\]

The proposed method can be used for the determination of fleroxacin. An intermolecular energy transfer takes place from SO₄²⁻ to the ligand (analyte) in the chelate (Dy³⁺-FLX) produced in the reaction process. Then through intramolecular energy transfer from the ligand to Dy³⁺, Dy³⁺-ligand is formed, followed by the narrow characteristic emission of Dy³⁺. The CL spectra of Dy³⁺-KMnO₄-SO₄²⁻-analyte systems are from the narrow characteristic emission of Dy³⁺ at 482 and 578 nm. The proposed enhanced CL systems have good linearity, higher sensitivity, precision and potential capability for residue analysis of studied analytes in foods and biological samples. FLX metabolites need to be investigated.

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**References**


