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ORIGINAL PAPER

Ultrasensitive Study of Gatifloxacin Based on Its Enhancing Effect on the Cerium (IV)-Sodium Hyposulfite Chemiluminescence Reaction in a Micellar Medium

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Abstract A sensitive and rapid flow-injection chemiluminescence (CL) method has been developed for the determination of gatifloxacin in pharmaceutical preparations and biological samples. The method is based on the enhancing effect of gatifloxacin on CL emission generated by the interaction of Ce (IV) in sulphuric acid and sodium hyposulphite ($Na_2S_2O_4$) sensitized by sodium dodecyl benzene sulfonate (SDBS). Strong CL emission was observed when gatifloxacin was injected into the Ce (IV) in sulphuric acid and $Na_2S_2O_4$ solution incorporated with SDBS in a flow-cell. Several experimental parameters affecting the CL reaction were investigated and optimized systematically. Under the optimum conditions, it was found that the CL intensity is proportional to the concentration of gatifloxacin in the range of 1.12×10^{-11}–4.40×10^{-9} g mL^{-1} with a co-relation coefficient of 0.9994. The limit of detection was found to be 4.87×10^{-12} g mL^{-1} and the relative standard deviation (RSD, $n=7$) was 1.8% for 4×10^{-8} g mL^{-1} of GFLX. The proposed method offers higher sensitivity, wide linear range and better stability without requiring sophisticated instrumentation. Thus, the proposed method has been successfully applied to the determination of gatifloxacin in pharmaceuticals, serum and human urine.

Keywords Chemiluminescence · Gatifloxacin · Sodium hyposulphite · Sodium dodecyl benzene sulphonate · Flow-injection

Introduction

Gatifloxacin [(±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid] (GFLX) is the fourth generation of a new class of synthetic antibacterial fluoroquinolone agents. It is a novel 8-methoxy broad spectrum fluoroquinolones (Fig. 1) with an improved gram-positive, gram-negative and anaerobe coverage compared with other agents. It is effective in a range of clinical infections, including community-acquired pneumonia, acute exacerbations of chronic bronchitis, acute sinusitis and genitor urinary tract infections [1, 2]. It exhibits enhanced activity against clinically relevant pathogens, including such common respiratory pathogens such as *Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydia pneumoniae, Moraxella catarrhalis,* and *Legionella* [3]. GFLX has been evaluated in the treatment of adults with a wide range of infectious diseases in numerous clinical trials conducted in many countries. Clinical cure ratios in all trials of patients treated with GFLX were about 90% or higher. Like other new fluoroquinolones, GFLX has a dual mechanism of action, inhibiting both bacterial DNA gyrase and topoisomerase IV. Therefore, the extensive need

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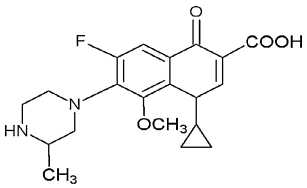
Fig. 1 Structure of gatifloxacin



of GFLX for clinical and pharmacological study require fast and sensitive analytical techniques for the determination of its presence in biological and pharmaceutical preparations.

Various methods have been reported in the literature for determination of GFLX such as spectrophotometry [4–8], spectrofluorometry [9–11], HPLC [12–14], liquid chromatography [15], and thin layer chromatography [16], fluorimetry [17], capillary electrophoresis [18], and chemiluminescence (CL) determination [19–21]. Chemiluminescence combined with flow-injection (FIA-CL) method has been widely used for the quantitative estimation of pharmaceutical compounds in recent years because of its promising advantages of low detection limit, wide linear dynamic range, relatively simple, high sensitivity and inexpensive instrumentation [22–25].

To the best of our knowledge there is no report for the determination of gatifloxacin using Ce (IV)-$Na_2S_2O_4$ system sensitized by sodium dodecyl benzene sulfonate (SDBS). Thus, in this study, we proposed a new flow-injection chemiluminescence method which has the advantages of high sensitivity, a wide linear range, improved selectivity, fast and convenience for the determination of GFLX based on Ce (IV)-$Na_2S_2O_4$ system in presence of SDBS. It has been observed that CL intensity was intensified when gatifloxacin were introduced in the flow stream of Ce (IV)-$Na_2S_2O_4$-SDBS system because the interaction between Ce (III) (after reduction of Ce (IV)) ions and GFLX. Detail possible mechanism of this study has been discussed. The degree of enhancement was linearly related to the amount of GFLX added. Under optimum conditions, successful determination of trace level of GFLX in pharmaceutical preparations and biological samples was found with satisfactory results.

Experimental

Reagents and Materials

All chemicals were of analytical reagent grade and were used without further purification. Distilled deionised (DI) water (Millpore, MilliQ Water System, USA) was used throughout. GFLX and $Ce(SO_4)_2{\cdot}4H_2O$ were purchased from Sigma-Aldrich (St. Louis, USA). SDBS and cetyltrimethylammonium bromide (CTAB) were purchased from Sigma Aldrich Corporation (Steinheim, Germany). Sodium dodecyl sulphate (SDS) was purchased from Fluka chemical company (Switzerland). Stock solutions (1.0×10^{-3} g mL^{-1}) of GFLX were prepared by dissolving appropriate amount of GFLX in 0.005 mol L^{-1} H_2SO_4 and working standard solutions were prepared by appropriately diluting the stock standard solution with 0.005 mol L^{-1} sulphuric acid solutions. The Ce (IV) solution (2.0×10^{-2} mol L^{-1}) was prepared by dissolving the $Ce(SO_4)_2{\cdot}4H_2O$ in 0.08 mol L^{-1} sulphuric acid. The sodium hyposulphite solution (1.5×10^{-2} mol L^{-1}) was freshly prepared by dissolving sodium hyposulphite in DI water. SDBS stock solution (0.01 mol L^{-1}) was prepared by dissolving SDBS in DI water daily before experiment.

Apparatus

The flow system used in this work is schematically shown in Fig. 2. Two peristaltic pumps (Model 404, Ismatec, Zurich, Switzerland) were used to convey all solutions at the same flow rate. One pump delivered $Na_2S_2O_4$, SDBS and sample solutions while another pump conveyed H_2O and Ce (IV). $Na_2S_2O_4$ and SDBS solution stream was entered with the sample solution in a Rheodyne (Model 7125, Cotati, CA, USA) six-way injection valve with a loop which was mixed with Ce (IV) solution stream at the T-piece. PTFE tubing (0.8 mm i.d.) was used throughout the manifold to carry all components. 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

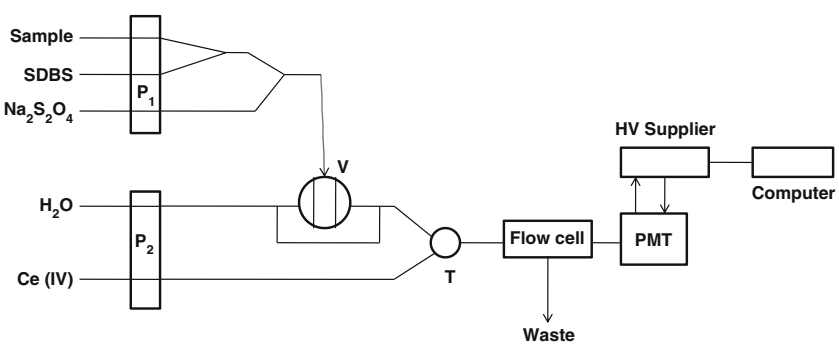


Fig. 2 Schematic diagram of the FIA-CL manifold employed for the quantitative estimation of GFLX. P_1, P_2: peristaltic pumps; V: six-way valve; T: Y- pieces

detecting and recording the chemiluminescence and fluorescence 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.

Analytical Procedure

The mixture of sample, $Na_2S_2O_4$ and SDBS 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 stream was transferred into the flow cell in the fluorimeter, accompanying the increase of CL intensity. The CL signal produced in the flow cell was recorded. As mentioned above, GFLX was found to enhance the CL signal of the Ce (IV)–$Na_2S_2O_4$–SDBS system strongly. Determination of GFLX was based on the net CL intensity changes from the with and without GFLX sample solution.

Sample Preparation

The average tablet weights were calculated from the weight of each of 10 tablets. An accurately weighed portion of each homogenized sample containing 400 mg of GFLX (Gatizone) were transferred separately into 1,000 mL calibrated dark flask containing 500 mL of water and dissolved in ultrasonic bath for 20 min and diluted with DI water up to the mark. The dissolved sample was filtered through millipore membrane filter paper and diluted with to obtain the appropriate concentration for 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 4,000 rpm. A known amount of GFLX was added into the protein free serum and then diluted to 50 mL with DI water in order to obtain a concentration of GFLX in the range of linearity. A healthy volunteer was administered 200 mg GFLX and then after 12 h the real urine sample were collected. No further pretreatment was needed for urine samples except proper dilution in order to make the concentrations of the drug within the working range and then GFLX was measured by the standard addition method.

Results and Discussion

Kinetic Characteristics of the CL Reaction

In order to get an idea about the sensitizing effect of GFLX on the Ce (IV)-$Na_2S_2O_4$-SDBS CL reaction, the emission spectra of Ce (IV)-$Na_2S_2O_4$-SDBS and Ce (IV)-$Na_2S_2O_4$-SDBS-GFLX were examined with a static injection method, using the solution consisted of 9.0×10^{-4} mol L^{-1} Ce (IV) in 0.08 mol L^{-1} H_2SO_4, 8.5×10^{-4} mol L^{-1} $Na_2S_2O_4$, and 2.5×10^{-3} mol L^{-1} SDBS and the typical CL curve is shown in Fig. 3. From Fig. 3, it was observed that the CL reaction is rapid and showed markedly enhanced CL intensity in the presence of GFLX, and the value of enhancement is proportional to the concentration of GFLX added. Therefore, using this property, GFLX can be determined sensitively with this CL method.

Possible CL Reaction Mechanism

In order to discuss the CL reaction mechanism, the fluorescence spectra for the solution of Ce (IV), Ce (III), GFLX and the mixture of Ce (IV) and GFLX were investigated at the same excited wavelength and the FL curves are shown in Fig. 4. It was observed that fluorescence emission spectra for Ce (III) and GFLX were obtained at 365 and 493 nm respectively (Fig. 4, curve 1 and 2). The fluorescence spectra for the mixture solution of 3.5×10^{-4} mol L^{-1} $Ce(SO_4)_2$ in 0.08 mol L^{-1} H_2SO_4 acid solution and 4.2×10^{-7} g mL^{-1} GFLX solution (Fig. 4, curve 3) is very similar to that of Ce (III) (Fig. 4, curve 1), and does not show significant fluorescence emission between the wavelength range 420–540 nm, while the GFLX solution gives obvious fluorescence emission in this range (Fig. 4, curve 2). Moreover Ce (IV) is non-fluorecent [26]. Consequently, it appears that the luminophore is attributed to the Ce (III) ions or the complex formed between Ce (III) and GFLX, not to the GFLX alone. It is known that in any sensitized CL system the key interme-

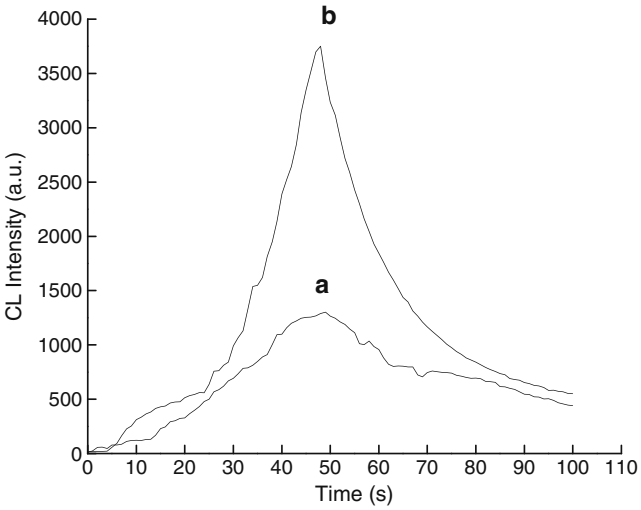


Fig. 3 Kinetic curves of the CL reaction. (**a**) Ce (IV)-$Na_2S_2O_4$-SDBS; (**b**) Ce (IV)-$Na_2S_2O_4$-GFLX-SDBS. Conditions: GFLX, 4.40×10^{-9} g mL^{-1}; Ce (IV) (in 0.08 mol L^{-1} H_2SO_4), 9.0×10^{-4} mol L^{-1}; $Na_2S_2O_4$, 8.5×10^{-4} mol L^{-1}; SDBS, 2.5×10^{-3} mol L^{-1}; flow rate, 3.0 mL min^{-1}

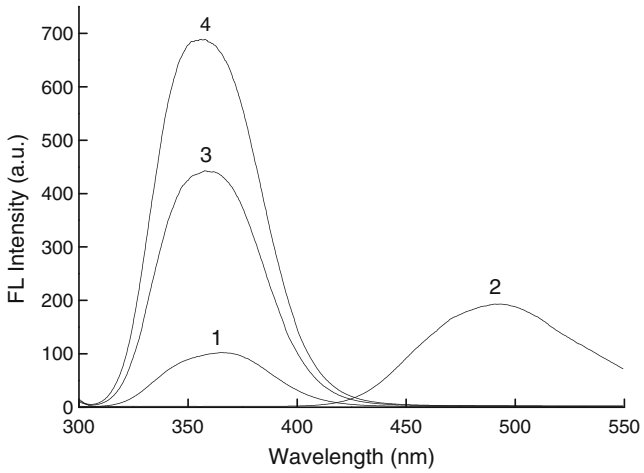


Fig. 4 Fluorescence spectra: 1, Ce (III), λ_{em}=365 nm, λ_{ex}=290 nm; 2, gatifloxacin, 4.2×10^{-7} g mL^{-1}, λ_{em}=493 nm, λex=290 nm; 3, gatifloxacin, 4.2×10^{-7} g mL^{-1}+$Ce(SO_4)_2$ (in 0.08 mol L^{-1} H_2SO_4) 3.5×10^{-4} mol L^{-1}, λ_{em}=357 nm, λex=290 nm; 4, gatifloxacin, 4.2×10^{-7} g mL^{-1}+$Ce(SO_4)_2$ (in 0.08 mol L^{-1} H_2SO_4) 3.5×10^{-4} mol L^{-1}+ SDS 3.0×10^{-3} mol L^{-1}, λ_{em}=358 nm, λex=290 nm

diate frequently involves as a high energetic species in the CL reaction. In the proposed CL system, most probably the $Ce(C_{19}H_{22}FN_3O_4)_2^{3+}$* complex is such a high energetic intermediate species which is sensitized by the surfactant SDBS. As shown in Fig. 4 (curve 4) an enhanced fluorescence spectra of the mixture solution of 3.5×10^{-4} mol L^{-1} $Ce(SO_4)_2$ in 0.08 mol L^{-1} H_2SO_4 solution and 4.2×10^{-7} g mL^{-1} GFLX solution was obtained with the addition of SDBS. Based on the illustration above, the possible mechanism for this CL system can be proposed as follows:

$$S_2O_4^{2-} + 8Ce^{4+} + 5H_2O \longrightarrow 8Ce^{3+}* + 2SO_4^{2-} + 10H^+$$

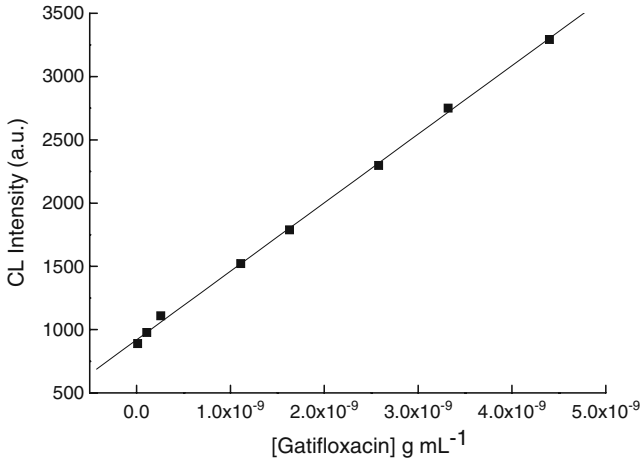


Fig. 5 Calibration curves for determination of GFLX. Conditions: Ce (IV) (in 0.08 mol L^{-1} H_2SO_4), 9.0×10^{-4} mol L^{-1}; $Na_2S_2O_4$, 8.5×10^{-4} mol L^{-1}; SDBS, 2.5×10^{-3} mol L^{-1}; flow rate, 3.0 mL min^{-1}

$$Ce^{3+}* + GFLX \xrightarrow{SDBS} Ce(C_{19}H_{22}FN_3O_4)_2^{3+}*$$

$$Ce(C_{19}H_{22}FN_3O_4)_2^{3+}* \longrightarrow Ce^{3+} + C_{19}H_{22}FN_3O_4^{Ox} + h\nu$$

Or

$$Ce^{4+} + GFLX^{Red} \xrightarrow{SDBS} Ce^{3+}* + GFLX^{Ox}$$

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

Red indicates reduced form and Ox indicates oxidized form.

Optimization of the Flow-Injection CL Conditions

The factors affecting the CL reaction were studied in order to optimize the operating conditions of the flow system by altering each variable in turn while keeping the others constant and the CL intensity was measured with respect to the reaction variables.

In flow injection analysis, flow rate is an important factor which influences not only analytical efficiency but also the sensitivity of the system. In order to achieve maximum CL intensity, total flow rate of the reagent solution on the CL emission response was investigated in the range of 0.5–4.5 mL min^{-1}. It was observed that the CL intensity was increased as the flow rate increased with maximum CL intensity at 3.0 mL min^{-1} (Fig. S1). Above the flow rate 3.0 mL min^{-1}, however, the CL intensity declined, probably because high flow rates with shorter contact time produced insufficient CL reaction and might lead to the irreproducibility and excess consume of the reagents. Therefore, a flow rate of 3.0 mL min^{-1} was selected as optimum for the whole experiment.

The concentration of acid has a significant influence on this CL reaction because Ce (IV) is readily soluble in acid and becomes stable when dissolved in acid solution. Ce (IV) solutions were prepared in hydrochloric acid, phosphoric acid, nitric acid and sulphuric acid, and the CL intensity was measured for each of them. It was observed that the highest CL intensity was obtained when 0.08 mol L^{-1} sulphuric acids was used to dissolve Ce (IV) (Fig. S2). Thus 0.08 mol L^{-1} sulphuric acids were selected as suitable medium to dissolve Ce (IV) to measure the CL intensity of the system.

Ce (IV) plays an important role for the determination of GFLX in this CL system. In order to investigate the effect of the concentration of Ce (IV) on the CL intensity, Ce (IV) concentration in the range of 1×10^{-5}–3×10^{-3} mol L^{-1} in 0.08 mol L^{-1} sulphuric acid solution was examined. It was found that the maximum CL intensity was obtained at 9.0×10^{-4} mol L^{-1} Ce (IV) (Fig. S3). Thus, 9.0×10^{-4} mol L^{-1} Ce (IV) was used for the whole experiment.

Table 1 Determination of GFLX in pharmaceutical preparations

Sample	Amount found (mg)±RSD[a] (%)		Standard addition method		
	Labeled	Proposed method	Added ($\times10^{-8}$ mg mL^{-1})	Found ($\times10^{-8}$ mg mL^{-1})±RSD[a] (%)	Recovery (%)
Gatizone	400	396.2±0.81	2.00	2.01±0.89	100.5
			4.00	3.94±1.07	98.5
			6.00	5.97±1.65	99.5
			8.00	7.99±1.19	99.9
			10.00	10.11±1.45	101.1

[a] Relative standard deviation for three replicate measurements

Sodium hyposulphite was used as an important reductant in this CL system. The effect of sodium hyposulphite concentration on CL intensity was examined in the range of 2.5×10^{-4}–1.5×10^{-3} mol L^{-1}, and the maximum CL intensity was obtained at 8.5×10^{-4} mol L^{-1} sodium hyposulphite (Fig. S4). The CL intensity was decreased with increasing sodium hyposulphite concentration. Thus, to obtain the highest sensitivity and accuracy, 8.5×10^{-4} mol L^{-1} of sodium hyposulphite was selected as optimum concentration.

Surfactants are often played a vital role to boost up the emission intensities of CL reaction. In the primary investigation, three types of surfactants, SDBS, SDS and CTAB were examined to observe the effect of surfactants on the investigated CL system. Results showed that the intense CL signal was appeared by the use of SDBS as micellar medium. Therefore SDBS was selected for this work. In the proposed CL system, the key intermediate may be cationic Ce^{3+} or cationic complex $Ce(C_{19}H_{22}FN_3O_4)_2^{3+}$. The GFLX can easily be reached the Stern layer of the micelles because a large number of $Ce^{4+}$and Ce^{3+} ions were concentrated in the Stern layer of the SDBS micelles. So the interaction between Ce^{4+} and GFLX might take place which facilitate the energy transfer between the Ce^{3+} ions and GFLX. 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 reproducibility and stability of the system was also achieved in SDBS system. The effect of SDBS concentration was examined in the range of 1×10^{-4}–4×10^{-3} mol L^{-1} and the maximum CL signal was obtained at 2.5×10^{-3} mol L^{-1} SDBS (Fig. S5). Hence, 2.5×10^{-3} mol L^{-1} SDBS was chosen for the whole experiment.

Analytical Features

A calibration curve of CL intensity versus GFLX concentration obtained at the optimum conditions given above is shown in Fig. 5. The linearity for the determination of GFLX was investigated and it can be clearly seen that the CL intensity was increased linearly with the concentrations of GFLX (Fig. 5) in the range of 1.12×10^{-11}–4.40×10^{-9} g mL^{-1} with a regression equation of $I=5.42\times10^{11}C+919$ ($r=0.9994$) where I is the CL intensity and C is the concentration of GFLX (g mL^{-1}). The limit of detection as defined by IUPAC, $C_{LOD}=3$ Sb/m (where Sb is the standard deviation of the blank signals and m is the slope of the calibration curve) was found to be 4.87×10^{-12} g mL^{-1} and the relative standard deviation (RSD) is 1.41% for 5 determinations of 1.4×10^{-7} g mL^{-1} GFLX. Compared with most other reported methods, the proposed method in this study offers

Table 2 Determination of GFLX in spiked human urine and serum samples

Sample	Added (μg mL^{-1})	Found (μg mL^{-1})±RSD[a]	Standard addition method		
			Added ($\times10^{-7}$ μg mL^{-1})	Found ($\times10^{-7}$ μg mL^{-1})±RSD[a]	Recovery (%)
Urine	1.0	0.95±0.85	1.0	0.994±1.35	99.4
			2.0	2.09±0.92	104.5
			3.0	2.93±1.11	97.67
Serum	5.0	5.03±1.29	2.0	2.19±1.15	109.5
			4.0	3.97±1.42	99.25
			6.0	6.03±1.81	100.5

[a] Relative standard deviation for three replicate measurements

high sensitivity, wide linear range and high stability with a rapid and simple procedure for the determination of GFLX in pharmaceutical preparations and biological sample.

Interference Studies

The presence of interfering substances in the real sample might 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 substances and metal ions was investigated by preparing a set of solutions, each one with 1×10^{-7} g mL^{-1} GFLX plus a different concentration of a chemical species to be tested. The results implied that the foreign species did not interfere the determination of GFLX with 1,000 fold for Al^{3+}, Fe^{3+}, Co^{2+}; 500 fold for Na^{+}, K^{+}, NH^{4+}; 200 fold for Cl^{-}, NO_3^{-},SO_4^{2-}, PO_4^{3-}, BrO_3^{-}; 100 fold for fructose, glucose, and 50 fold for sucrose, dextrin, ephedrine, galactose. The results indicate that the proposed method holds good selectivity which can be selectively applied for the determination of GFLX in pharmaceutical preparations, serum and human urine.

Analytical Application of the Proposed Method

In order to evaluate the validity of the proposed method, commercially available pharmaceutical formulations of the sample were analyzed, namely Gatizone (containing 400 mg of GFLX). Results for the determination of GFLX in tablets using the proposed method are summarized in Table 1 which shows that there is no significant difference between the labeled content and that obtained by the proposed method. Recovery studies were also performed on the analyzed samples by standard addition method [27] and found that the overall recoveries were in the range of 98.5–101.1% for GFLX.

The proposed method was applied to the determination of GFLX in sample of serum and human urine. Results of both observations are shown in Table 2. Recoveries of GFLX content in urine and serum samples were 97.67–104.5% and 99.25–109.5% respectively. From the Table 2 it can be seen that the proposed method is responsive and reliable which can be easily performed and affords good precision and accuracy when applied to serum and urine samples.

Conclusions

This work demonstrates that Ce (IV)-$Na_2S_2O_4$ system can be strengthened in the presence of SDBS to detect GFLX. When GFLX was injected to the Ce (IV)-$Na_2S_2O_4$ system incorporated with SDBS, the CL intensity was increased markedly. Under the optimum condition, the CL intensity was proportional to the concentration of GFLX. However, compared to the present FIA-CL method for the determination of quinolones, the proposed method displays satisfactory advantages in terms of sensitivity, simplicity and accuracy. Therefore, the proposed FIA-CL method can be successfully applied for the determination of GFLX in pharmaceutical preparations, spiked human urine and serum samples.

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