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ARTICLES

Flow-Injection Determination of Cephalosporin Antibiotic Cefixime in Pharmaceutical Formulations with Luminol-Diperiodatoargentate(III) Chemiluminescence Detection

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Abstract—A simple and sensitive flow injection chemiluminescence method was developed for the determination of cefixime trihydrate (**CFX**) based on its enhancing effect on chemiluminescence (**CL**) of luminol-diperiodatoargentate(III) (Ag(III) complex) in an alkaline solution. A linear CL response for CFX from 0.005 to 3 mg/L ($y = 566.3x + 12.1$, $R^2 = 0.9998$, $n = 10$) was achieved with limits of detection and quantification of 0.001 ($S/N = 3$) and 0.003 mg/L ($S/N = 10$), respectively, relative standard deviations (**RSDs**) from 1.0 to 3.4%, and injection throughputs of 90/h. The pharmaceutical samples containing CFX were extracted by liquid-liquid extraction utilizing diethyl ether, analyzed, and satisfactory results were achieved with recoveries of 98 to 105% and RSDs of 1.6 to 3.6% ($n = 4$). The samples were also analyzed with the reported spectrophotometric method, and results assessed by applying statistical tests were not different significantly. The interference effects of excipients commonly found in formulations, anions, and cations were also evaluated. Chemiluminescence mechanism was proposed by application of UV-Vis spectrophotometry.

Keywords: flow injection analysis, chemiluminescence, luminol, diperiodatoargentate(III) complex, cefixime trihydrate

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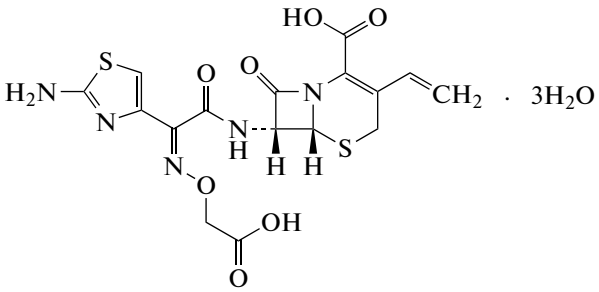
Cefixime trihydrate (**CFX**; $C_{16}H_{21}N_5O_{10}S_2$, Mw. 507.5 g/mol, Scheme 1) is a third-generation semisynthetic oral cephalosporin antibiotic with an antibacterial spectrum against various gram-positive and gram-negative bacteria [1, 2]. It melts at a temperature of 220–250°C, has solubility in alcohol and white colored powder [3]. The half-life of CFX is around 3–4 h, and it is eliminated by renal and biliary tracts within 24 h after ingestion. Approximately 50% of the absorbed drug is excreted in urine without changing [4]. According to reports, CFX can be used to treat otitis media, pneumonia, pharyngitis, laryngitis, gonorrhea, bronchitis, and tonsillitis [5]. The described mechanism works by inhibiting an enzyme called transpeptidase which is involved in the establishment of bacterial cell walls building [6].



Scheme 1. Chemical structure of cefixime trihydrate.

Many analytical techniques have been described in the literature for the determination of CFX in drugs alone or in conjunction with other drugs in pharmaceuticals and biological fluids. These techniques

include UV-Vis spectrophotometry [7–10], spectrofluorimetry [11–13], Fourier-transform infrared spectroscopy [14], electrochemistry [15–17], capillary electrophoresis [18, 19], high performance thin layer chromatography (**HPTLC**) [20], HPTLC and high performance liquid chromatography (**HPLC**) coupled with ultraviolet [21–23], diode array [24, 25], photodiode array [26], mass spectrometry [27], and tandem mass spectrometry [28] detection. Although these techniques are effective, they have some disadvantages: they are time-consuming, expensive, require considerable reagent consumption, lengthy process, and low sample throughput. Therefore, it is necessary to establish simple, selective, and sensitive methods to detect small amounts of CFX in diverse samples.

The term chemiluminescence (**CL**) refers to the light emitted by chemical reactions in the ultraviolet, visible, and/or near-infrared regions [29]. The flow injection (**FI**) technique combined with CL detection has been described for the quantitation of various analytes in different samples (such as pharmaceutical, environmental, clinical, food, and biological matrices), utilizing various CL reagents along with inorganic oxidizing agents [30, 31]. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedion) is the most widely used CL reagent [32] which undergoes oxidation in an alkaline solution to generate electronically excited 3-aminophthalate which then emits blue luminescence at λ_{max} of 425 nm on de-excitation. Various oxidants have been combined for its oxidation including periodate, hydrogen peroxide, hypochlorite, and permanganate. When hydrogen peroxide is used, the reaction is catalyzed by transition metal ions which involve Fe(II), Co(II), Cu(II), Cr(III), and Ni(II) and complexes, such as haemoglobin and peroxidases, which imparts high sensitivity in detecting the above-mentioned metal ions. Also, several transition metals with unusually high oxidation numbers, such as Cu(III), Ag(III), and Ni(IV), form highly stabilized chelate compounds with polydentate ligands, e.g., periodate (IO_4^-) [33, 34], which have been subjected to a variety of analytical applications in recent years. One such complex is diperiodatoargentate(III) that has found many applications under acidic and/or basic conditions for the determination of various analytes in biological, pharmaceutical, and environmental matrices [35].

A limited number of FI-CL methods for CFX determination have been reported in the literature. Du and Li [36] reported an FI-CL assay for thirteen cephalosporin antibiotics including CFX in standard solutions based on the reaction of luminol–Cu^{2+} with the limit of detection (**LOD**) of 0.8 ng/mL. Manganese(II) and cobalt(II) produced positive interference on the CL emission intensity due to their similar effect to that of copper(II). He and He [37] reported an analytical method for CFX estimation in capsules based on its enhancement effect on CL reaction of N-bromosuccinimide–fluorescein in the flow mode with LOD of 0.048 mg/L. Yousaf et al. [38] have employed the CL reaction of diperiodatoargentate(III)–rhodamine 6-G for the determination of CFX in pharmaceutical formulations. The method has been claimed to be fast and sensitive with an injection throughput and LOD of 180 injections/h and 3.0×10^{-3} mg/L, respectively.

In this work, a simple and sensitive CL assay has been developed for the determination of CFX based on its enhancement effect on luminol–Ag(III) complex reaction in an alkaline medium. This method was utilized for CFX determination in pharmaceutical formulations, and a brief discussion on the CL reaction mechanism is presented in the following section.

EXPERIMENTAL

Reagents and solutions. Analytical grade chemicals/reagents were obtained from BDH Chemicals (Poole, UK) and Merck (Darmstadt, Germany), and deionized water (0.0167 μS/cm, Elga, Purelab Option, UK) was used for cleaning and preparing solutions throughout this work. Utilized glassware was washed with detergent followed by rinsing multiple times with deionized water. They were then dipped in a bath of 10% HCl_{aq} (v/v) for 24 h and again rinsed several times with deionized water. For spectrophotometric study, a double beam UV-visible spectrophotometer (UV–1700, Pharmaspec, Shimadzu, Japan) was utilized.

Cefixime trihydrate stock solution (50 mg/L) was prepared by weighing an accurate quantity from its bulk in deionized water, diluting to 100 mL in a volumetric flask, sonicating for about 5 min to ensure complete dissolution, and storing at 4°C. Working standard solutions were prepared from this stock solution in deionized water. Pure CFX compound was received as a gift from Nexus Pharma (Karachi, Pakistan).

Potassium and sodium hydroxide stock solutions (2.0 M each) were prepared by weighing appropriate quantities (11.22 and 8.0 g, respectively) from their bulks, dissolving in deionized water, diluting to 100 mL in volumetric flasks, and standardizing against primary standard potassium hydrogen phthalate reagent in the presence of phenolphthalein indicator (0.1% in 60% C_2H_5OH). Working standard solutions were prepared by taking a series of aliquots from stocks and diluting with deionized water when needed.

Diperiodatoargentate(III) complex (Ag(III) complex) was synthesized in the laboratory from Ag(I) in alkaline solution as suggested in [39]. The complex solution was characterized by UV-visible spectrophotometry which indicated absorption peaks at 362 and 252 nm in KOH solution (1.0 mM). The complex concentration was calculated by absorbance measurement at 362 nm (molar absorptivity (ε) was 1.26×10^4 L/(mol cm)) [33]. This solution was stable for

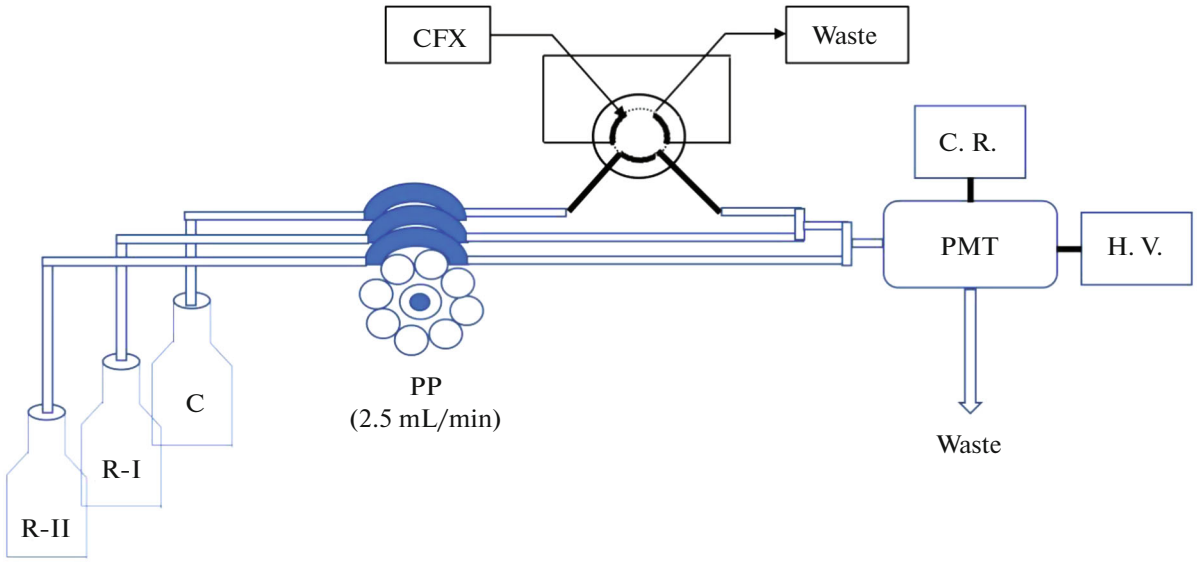


Fig. 1. Flow injection-chemiluminescence manifold utilized for CFX assay: C—(carrier) deionized water; R-I—150 μM Ag(III) complex in 0.01 M KOH; R-II—5 μM luminol in 0.075 M NaOH; sample loop volume, 60 μL; PMT voltage, 800 V; PP—peristaltic pump; H.V.—high-voltage power supply; C.R.—chart recorder.

about three months, and fresh Ag(III) complex solutions were arranged on a daily basis when needed.

Luminol stock solution (5 mM) was prepared by weighing precise quantity (88.6 mg) of the compound, namely, 3-aminophthalhydrazide (International Laboratory, USA), in 25 mL of 0.1 M sodium hydroxide solution, sonicating for about 20 min, making up to 100 mL with 0.1 M NaOH, and storing at 4°C. A working solution (5 μM) was prepared by diluting 0.1 mL of the stock solution to 100 mL with NaOH solution (0.075 M).

Organic compounds (Tween-80, glucose, glycerol, magnesium stearate, starch, and sucrose) stock solutions (100 mg/L) and various anions (nitrate, phosphate, sulfate, bicarbonate, and chloride) and cations (iron, magnesium, calcium, potassium, and sodium) stock solutions (500 mg/L) were made by dissolving precise quantity of each from their commercially available respective compounds and/or their salts in deionized water. It should be noted that Mg-stearate is slightly soluble in hot water (8 mg per 100 mL at 50°C) and hot ethanol. During the preparation of Mg-stearate solution (100 mg/L), a few drops of ethanol were added for complete solubility. Working standards of the above-mentioned chemical substances were made by taking known aliquots from each stock solution, diluting with deionized water, and they were utilized for interference study.

Apparatus and procedure. The proposed FI-CL manifold utilized for CFX assay is given in Fig. 1. In order to connect all the flow injection analysis components and for making an injection loop, polytetrafluoroethylene flow tubing (0.8 mm i.d., Fischer Scientific Loughborough, UK) was used. Similarly, for the propulsion of all the solutions, a peristaltic pump (Ismatec, Glattbrugg-Zurich, Switzerland) was used. An injection valve (Rheodyne 5020, Anachem, Luton, UK) was used to inject CFX standard/sample solutions (60 μL) into deionized water using as a sample carrier stream which was connected and combined with Ag(III) complex solution (150 μM R-I in 0.01 M KOH) *via* a T-piece. This stream containing Ag(III) complex and CFX solutions is merged with luminol (5 μM R-II in 0.075 M NaOH) stream in a spiral glass flow cell (1.5 i.d., 18 mm dia) which is positioned directly in-front of an end window photomultiplier tube (**PMT**, electron tubes Ruislip, UK) connected to a Burle HV power supply (type PF1053, USA) operating at 800 V. A chart recorder (BD40, Kipp & Zonen, Delft, Holland) was used to record CL intensity signals on a strip.

Preparation of sample. CFX capsules were purchased from a local pharmacy. In order to achieve a gross sample, the contents of the weighed three tablets were ground into a fine powder. After dissolving an appropriate amount of homogeneous powder in 10 mL of deionized water, the solution was sonicated for approximately 15 min. Then, CFX was extracted from the sample (aqueous sample) by diethyl ether in a ratio of 4 to 10 mL, which eliminates all water-soluble interferences (if any) in the excipient. A nitrogen stream was used to evaporate diethyl ether at 34°C, and the remaining material was dissolved in deionized water to arrange a 55 mg/L solution.

Spectrophotometry method. By comparing with spectrophotometry [10], the applicability of the pro-

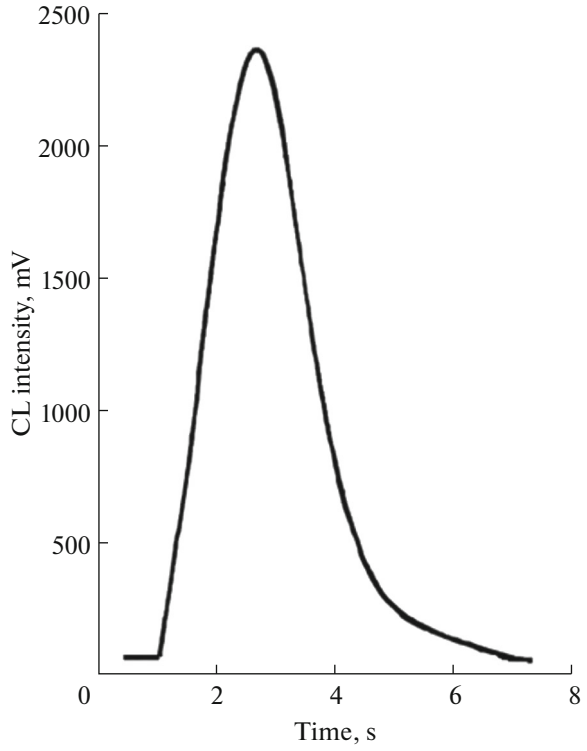


Fig. 2. Kinetic curve for luminol–Ag(III) complex–CFX chemiluminescence reaction in a batch mode. Conditions: 0.5 mL of 5 μM luminol in 0.075 M NaOH, 0.5 mL of 150 μM Ag(III) complex solution in 0.01 M KOH, and 0.5 mL of 1.0 mg/L aqueous CFX solution, PMT voltage of 600 V, chart recorder speed of 5.0 mm/s.

posed method for determining CFX was verified. For this reason, several CFX working standard solutions (0.25, 0.5, 1.0, 2.5, 5.0, and 7.5 mg/L) were made from CFX stock solution (50 mg/L). Then, 1.0 mL of Fe(III) solution (2.0 mM) and 3.0 mL of 1,10-phenanthroline solution (6 mM) were added to each volumetric flask containing the aforementioned standard solutions, stirred well, heated on a water bath at 90°C for 20 min followed by dilution up to 25 mL by deionized water. The final product (orange-red ferroin complex) was monitored at 510 nm using a double beam spectrophotometer equipped with quartz cuvettes (10 mm). Similarly, pharmaceutical formulations containing CFX were analyzed, the concentration of CFX was calculated from the calibration curve, and the results were compared with the proposed FI-CL method.

RESULTS AND DISCUSSION

Kinetics curve of the chemiluminescence reaction. Prior to the FI-procedure of the proposed CL reaction, its kinetic characteristics were studied with the help of a batch procedure. This procedure consists of a quartz cuvette with a capacity of 3.0 mL connected with an injection valve in front of an end window PMT operating at 800 V, utilizing a 2.0 kV power supply and a chart recorder connected with PMT. In the beginning, 0.5 mL of luminol solution (5 μM) was injected followed by introducing a mixture consisting of 0.5 mL of Ag(III) complex solution (150 μM) and 0.5 mL of CFX standard solution (1.0 mg/L). Once the reagents were mixed together, the reaction commenced and reached its maximum in 3 s and declined to baseline in 7.5 s as shown in Fig. 2. The kinetic curve of the CL reaction suggested that it was rapid and sensitive enough for CFX assay.

Optimization of experimental conditions. In order to achieve low LOD, high injection throughput, and wide linear range for CFX detection, the effects of different chemical variables, such as luminol, Ag(III) complex, NaOH, and KOH concentrations, as well as physical variables, such as flow rates, sample volume, and PMT voltage, were examined utilizing CFX standard solution (0.2 mg/L), and all the measurements were performed in triplicate.

Effect of luminol and NaOH concentrations. In the above reaction, luminol concentration has a great influence on the CL emission intensity of CXF determination. The influence of its concentration in the range of 0.1–30 μM was studied. The CL response increased with increasing luminol concentration as shown in Fig. 3a, which also resulted in an increase in background CL emission intensity with a gradual increase in the signal-to-noise ratio (**S/N**). Thus, luminol concentration of 5 μM was chosen as an optimum concentration after baseline analysis, S/N ratio, and sensitivity of the reaction, and it was utilized successively for subsequent experimental work. The concentration of NaOH was also found to affect the CL emission intensity, its effect was examined in the range of 0.01–0.1 M as shown in Fig. 3b. The concentration of 0.075 M produced the maximum CL response, while higher concentrations reduced the CL response. Therefore, the concentration 0.075 M NaOH was chosen as optimum for subsequent studies.

Effect of Ag(III) complex and KOH concentrations. Ag(III) complex acts as a strong oxidant in an alkaline medium and exhibits a reduction potential of 1.74 V. As shown in Fig. 3c, the influence of Ag(III) complex concentration on CFX determination was examined in the range of 1.0–250 μM. The highest and reproducible CL signals were obtained at 150 μM, and higher concentrations resulted in a decrease in the CL signal due to self-absorption. Therefore, Ag(III) complex concentration of 150 μM was selected as optimum. Since Ag(III) complex is very stable in a basic medium, its solution was made in KOH. The influence of KOH concentration in the range of 0.001–0.1 M was also investigated, and the CL intensity remained almost the same over the entire investigated range. However, 0.01 M KOH was chosen for further studies and used subsequently.

Effect of physical parameters. The influence of physical parameters (flow rate, sample injection volume, and PMT voltage) was tested and reported in Table 1. The effect of flow rate is the most important factor because it affects the analytical sensitivity of the system. The flow rate in the range of 0.5–4.0 mL/min was studied, and 2.5 mL/min was selected as optimum due to the maximum CL response. Hence, the flow rate of 2.5 mL/min was used for subsequent studies. Similarly, the influence of sample loop volume in the range of 60–360 μL was tested on the CL intensity. The sample loop volume of 60 μL gave the maximum CL response, and hence was chosen for further studies. As far as PMT voltage is concerned, an increase in PMT voltage (700–1300 V) provided increased CL emission intensities. However, PMT voltage of 800 V was selected as an optimum value due to the fact that it gave maximum signal-to-noise ratio.

Analytical figures of merit. Under the best experimental parameters of CFX (Fig. 4) within the concentration range of 0.005–3 mg/L, the CL emission intensity shows a linear relationship ($R^2 = 0.9998$, $n = 10$) with the regression equation of $y = 566.6x + 12.1$ (where y is the CL intensity in mV, and x is CFX concentration in mg/L), LOD and limit of quantification (**LOQ**) of 1.0 and 3.0 μg/L, respectively. The LOD and LOQ were determined as CFX concentration producing peaks with a height three and ten times the level of the baseline noise, respectively. The relative standard deviation (**RSD**) over the studied range was 1.1–3.1% with the injection throughput of 90/h.

Table 2 describes the analytical figures of merit of the methods previously reported for the detection of CFX compared with the developed method in pharmaceutical and biological samples. As can be seen from the table, except FI-CL method [36], the proposed procedure has the lowest detection limit, sufficient linear range, and comparative injection throughput, which illustrates the applicability of the proposed method for measuring small amounts of CFX in biological fluids.

Interference study. The interference activities of various cations, anions, and excipients present in the CFX sample were checked using blank and 0.1 mg/L CFX. The error of the maximum concentration was less than ±5% and the adoptive concentration of CFX was considered to tolerate any foreign species. Table 3 shows the interference activity of cations, anions, and organic compounds at 1, 10, and 100-fold excess. The results revealed that SO_4^{2-}, HCO_3^-, Cl^-, PO_4^{3-}, NO_3^-, Ca^{2+}, K^+, Mg^{2+}, Na^+, starch, Tween 80, methyl cellulose, sucrose, and Mg-stearate at 100-fold excess did not impart any interference activity. Similarly, 10-fold excess of Fe^{3+} and glucose along with 1-fold vitamin C excess showed no interference. However, 10-fold glycerol excess has an inhibitory effect on the CL emission intensity. Since the solubility and miscibility of glycerol in water and ether are significantly different, the interference of glycerol and other water-soluble substances can be eliminated by dissolving and homogenizing CFX extract with ether.

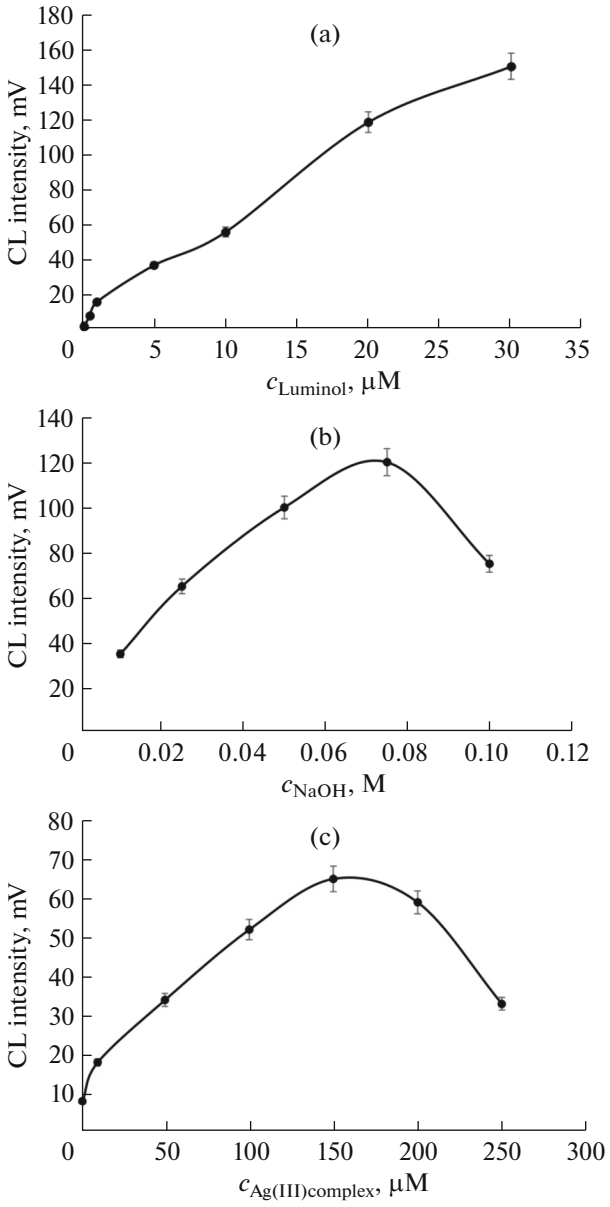


Fig. 3. Optimization of concentrations: (a) luminol, (b) NaOH of luminol stream, and (c) Ag(III) complex. Conditions: 0.2 mg/L CFX; 5 μM luminol in 0.1 M NaOH; 150 μM Ag(III) complex in 0.01 M KOH; sample loop volume of 60 μL; flow rate of 2.5 mL/min; PMT voltage of 800 V. Each optimized parameter was employed in the next optimization study.

Analytical application. The proposed FI-CL method was satisfactorily applied to CFX determination in pharmaceuticals with recovery and RSD values from 98 to 105% and 1.6 to 3.6% ($n = 4$), respectively, and the result was compared with a reported spectrophotometric method [10] which gave values from

Table 1. Effect of physical variables on the measurement of 0.2 mg/L CFX on luminol–Ag(III) complex chemiluminescence system

Parameter	Flow rate, mL/min	Sample volume, μL	PMT voltage, V
Studied range	0.5–4.0	60–360	700–1300
Optimum	2.5	60	800

Each optimized parameter was used in the next optimization study.

91.9 to 110.3% and 1.9 to 3.4% ($n = 4$), respectively. The percent recoveries comparison is given in Table 4. The intra- and inter-day RSD values (in %) for a set of CFX concentrations of 0.05, 1.0, and 3 mg/L, were 3.4, 1.1, and 1.0, and 4.3, 2.2, and 1.9, respectively. In addition, the CL emission intensities of spiked and un-spiked drug samples analyzed using both methods, i.e., the proposed FI-CL method and reported spectrophotometric method, are shown in Table 4. By applying the *F*-test, the calculated *F*-value of 1.21 was obtained, which is lower than the distributed *F*-value ($\alpha = 0.05$, $v_1 = 11$, and $v_2 = 11$) of 2.82 suggesting that the variation between the two method is because of chance and not due to any determinate error. Using the paired Student's *t*-test, the calculated value ($t_{calc} = 1.79$) was less than the tabulated value ($t_{tab} = 2.20$) at a 95% confidence limit. It can be concluded that there is no significant difference between the two methods.

Possible chemiluminescence reaction mechanism. The mechanism of the proposed CL reaction can be explained by the fact that CFX can act as an enhancer of the CL intensity due to redox reaction between luminol (reducing agent) and Ag(III) complex (oxidizing agent). It is possible to measure CFX by the proposed method because it has an enhanced effect on the CL intensity produced because of the oxidation of luminol by Ag(III) complex in an alkaline medium. The above statement can be supported by the fact that adding CFX solution to Ag(III) complex solution prepared in an alkaline medium causes a gradual decrease in the absorption spectrum intensity of Ag(III) complex as shown in Fig. 5 (curves *2*–*5*). As a result, the color given by the product effectively disappeared after 12 min, which may be due to the redox reaction between Ag(III) complex (oxidant) and CFX (reductant).

The CL emission peaks of the overall reaction (luminol–Ag(III) complex–CFX) are shown in Fig. 6. The CL emission peaks of only luminol solution, mixture of luminol and Ag(III) complex and mixture of luminol–Ag(III) complex–CFX are shown in curves *1*, *2*, and *3*, respectively. The comparison of curves *2* and *3* clearly shows that CFX improves the CL emission of luminol and Ag(III) complex, while

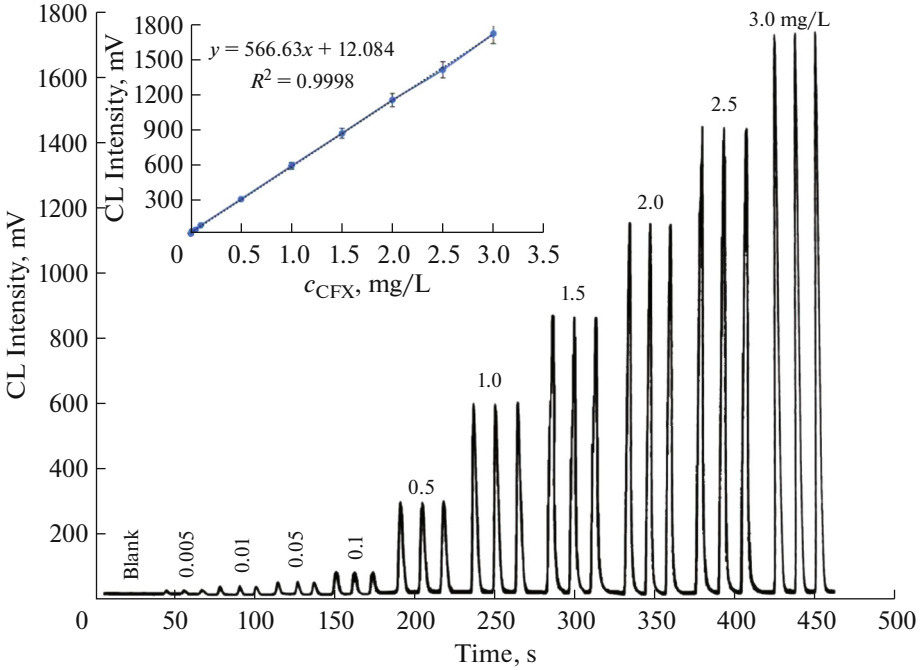


Fig. 4. Chart recorder traces for CFX standards (0.005–3 mg/L) under optimal experimental conditions; inset shows a calibration curve.

Table 2. Analytical characteristics of previously reported methods for CFX determination in pharmaceutical and biological samples

Technique	Reaction/condition	Linear range, μg/mL	LOD, μg/mL	LOQ, μg/mL	Reference
SPEC	AuNPs–Alizarin Red S–CFX, PO_4^{3-} buffer pH 3.0, 640 nm	0.01–0.18	0.0025	–	[9]
SPEC	Fe(III)–CFX–1,10-phenanthroline–acidic medium, temperature of 90°C for 20 min, 510 nm	0.2–10	0.030	0.101	[10]
FLU	Fluorescence quenching of terbium–danofloxacin complex by CFX, Tris buffer pH 6.5, $\lambda_{ex}/\lambda_{em}$ = 347/545 nm	0.0498–0.399	0.01378	–	[13]
AMP	Multi-walled carbon nanotube-supported platinum–tungsten alloy nanoparticle by facile one-step alcohol-reduction process, PO_4^{3-} buffer pH 7.0	0.0045–1.45	0.0027	–	[16]
DPV	Glassy carbon electrode modified with expanded graphene oxide and gold nanowire, then its surface was electro-polymerized with imprinted polymeric layer of polyaniline	0.009–0.43	0.0032	0.01	[17]
CE-PDA	Fused silica capillary (312 mm long × 50 μm i.d.), PO_4^{3-} buffer pH 10, injection time – 10 s, voltage – 25 kV, 254 nm	5.0–200	0.21	0.7	[18]
HPTLC	Silica gel pre-coated plates (2.5 × 10 cm), mobile phase $C_4H_8O_2$–C_3H_6O–CH_3OH–H_2O (5 : 2.5 : 2.5 : 5, v/v/v/v), 270 nm	0.125–0.5 spot^{-1}	0.0184 spot^{-1}	0.0612 spot^{-1}	[20]
HPLC/UV	C-18 column (250 × 4.6 mm, 5 μm), mobile phase C_2H_3N–CH_3OH–$C_2HF_3O_2$ (50 : 50 : 0.1, v/v/v) pH 3.0, flow rate—1.0 mL/min, sample volume—20 μL, 254 nm	4.0–14	0.615	0.78	[21]
RP-HPLC/UV	Waters C-18 column (250 × 4.6 mm, 5 μm), mobile phase PO_4^{3-} buffer pH 2.5–CH_3OH (3 : 1, v/v), flow rate —1.0 mL/min, sample volume—50 μL, 254 nm	10–50	0.61	2.03	[23]
HPLC/DAD	Kromasil 100 C-18 column (15 × 0.46 mm, 5 μm), 2-thiophenecarboxaldehyde is used as a derivatizing reagent for cefixime, mobile phase CH_3OH–HCOOH (70 : 30, v/v), flow rate—1.0 mL/min, sample volume—20 μL, 280 nm	1.0–50	0.132	0.401	[25]
FI-CL	Luminol–copper(II)–CFX CL reaction in alkaline medium	0.001–0.1	0.0008	–	[36]
FI-CL	N-bromosuccinimide–fluorescein–CFX CL reaction in alkaline medium	0.08–5.0	0.048	–	[37]
FI-CL	Diperiodatoargentate(III)–rhodamine 6G–CFX CL reaction in acidic medium	0.01–2.5	0.003	–	[38]
FI-CL	Luminol–diperiodatoargentate(III)–CFX CL reaction in alkaline medium	0.005–3	0.001	0.003	This work

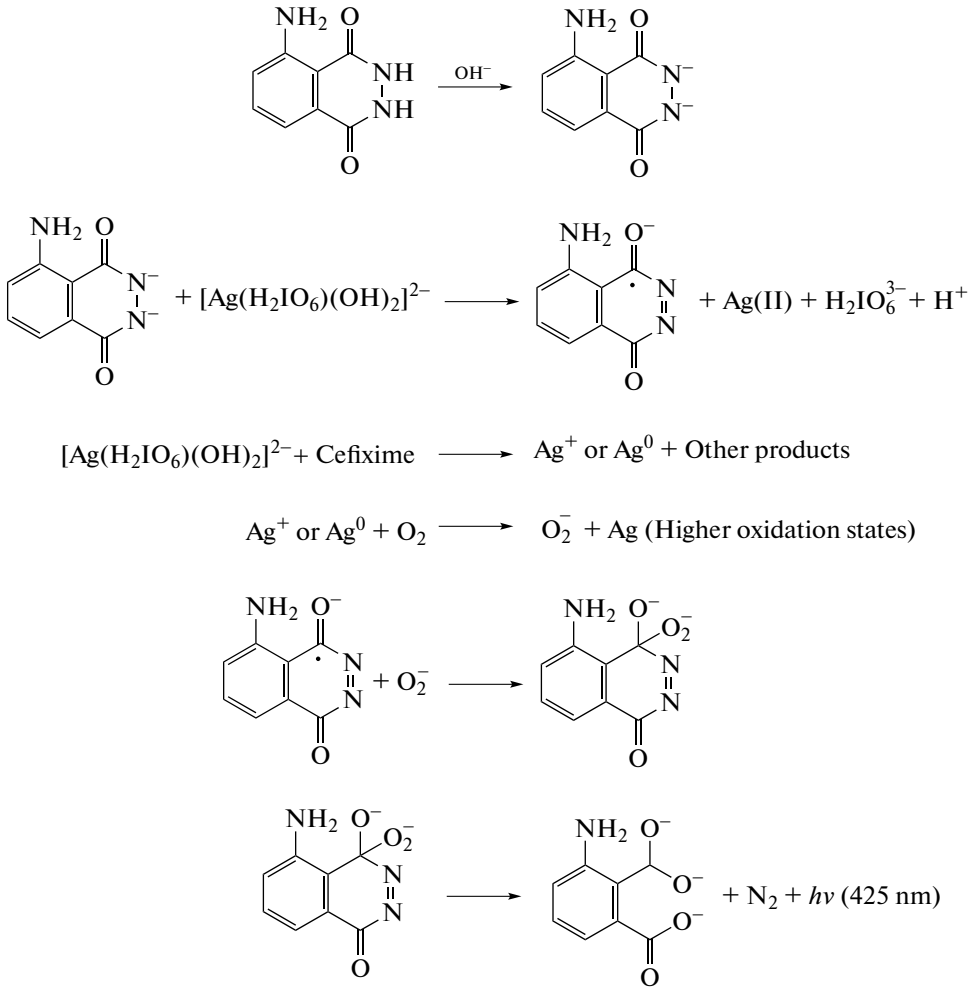
LOD—limit of detection, LOQ—limit of quantification, SPEC—spectrophotometry, FLU—fluorimetry, AMP—amperometry, DPV—differential pulse voltammetry, CE-PDA—capillary electrophoresis-photodiode array detector, HPTLC—high performance thin layer chromatography, HPLC/UV—high performance liquid chromatography/ultraviolet, RP-HPLC-PDA—reversed phase-HPLC-PDA, HPLC/DAD—HPLC/diode array detector, FI-CL—flow injection-chemiluminescence.

Table 3. Interference study of various anions, cations, and organic substances at 1-, 10- and 100-fold excess on the chemiluminescence intensity of CFX (0.1 mg/L)

Chemical species	Tolerable concentration (fold)
HCO_3^-, Cl^-, SO_4^{2-}, PO_4^{3-}, NO_3^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, starch, methyl cellulose, Mg-stearate, sucrose, and Tween-80	100
Fe^{3+}, glycerol, and glucose	10
Vitamin C	1

curve *1* indicates that only luminol solution is not emitting any CL.

Ag(III) complexes for the quantification of various drug samples have been described in conjunction with luminol reagent [40–43]. In the presence of Ag(III) complex, CFX undergoes a redox reaction in which CFX acts as a reducing agent. In addition, silver may be reduced to an oxidation state of zero (Ag^0) or +1. When Ag(I) reacted with dissolved oxygen, superoxide anion ($O_2^{-\bullet}$) or hydroxyl ($OH^{-\bullet}$) radicals were possibly produced [44]. These radicals possibly further oxidize luminol to 3-aminophthalate in electronically excited state which emits light at 425 nm when de-excited [42]. That is related to the indirect determination of CFX. After considering all the above experiments and discussion, the possible reaction mechanism of CL is presented in Scheme 2.



Scheme 2. The possible chemiluminescence reaction mechanism.

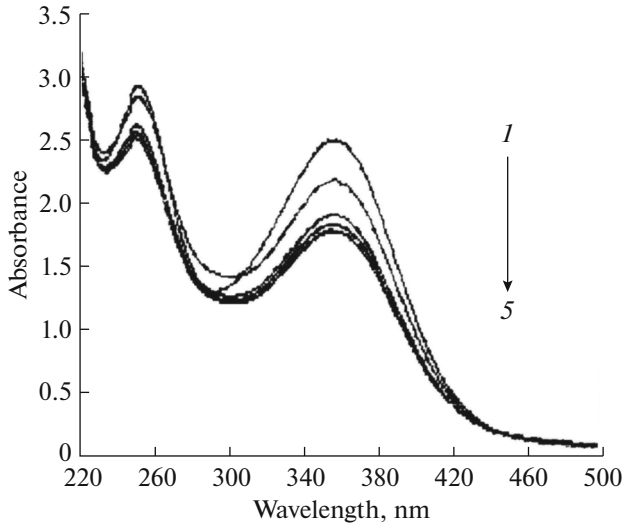


Fig. 5. Spectrophotometric study of Ag(III) complex and CFX: *1*—150 μM Ag(III) complex in 0.01 M KOH, *2*–*5*—mixture of CFX (3.0 mg/L) and Ag(III) complex (150 μM in 0.01 M KOH) after 3, 6, 9, and 12 min time interval, respectively.

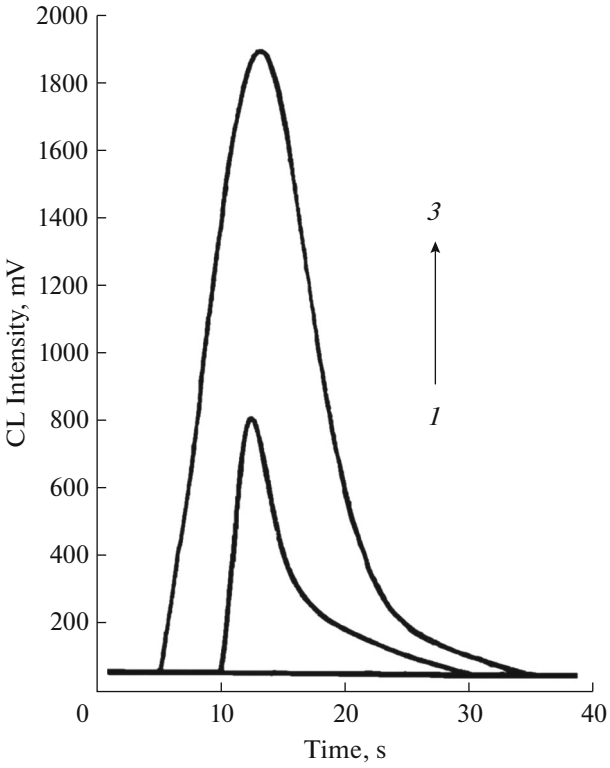


Fig. 6. Chemiluminescence emission peaks of luminol–Ag(III) complex–CFX reaction under flow-mode. Curve *1*: 5 μM luminol in 0.075 M NaOH; curve *2*: 5 μM luminol in 0.075 M NaOH, and 150 μM Ag(III) complex in 0.01 M KOH; curve *3*: 5 μM luminol in 0.075 M NaOH, 150 μM Ag(III) complex in 0.01 M KOH and 3.0 mg/L CFX. Physical parameters: PMT voltage of 800 V, chart recorder speed of 2 mm/s, flow rate of 2.5 mL/min, and sample volume of 60 μL.

CONCLUSIONS

In this study, a simple CL method combined with FI technique was established for the determination of CFX. The feasibility of the proposed method is based on the fact that CFX has an enhancing effect on the CL reaction of luminol–Ag(III) complex. The limit of detection is very low (1 μg/L), and the injection frequency is 90/h. After successful application of the proposed method, it is obvious that there was no interfer-

Table 4. Recovery of CFX from pharmaceutical samples and its comparison with the reported method ($n = 4$)

Sample matrix	Spiked,mg/L	Proposed FI-CL method			Spectrophotometric method [10]		
		found, mg/L	recovery, %	RSD, % ($n = 4$)	found, mg/L	recovery, %	RSD, % ($n = 4$)
Capsule-I	0	0.42	–	2.5	0.43	–	3.4
	0.20	0.65	105	2.3	0.61	97	2.7
	0.40	0.82	100	2.1	0.78	94	2.3
	0.60	1.06	104	1.6	0.97	94	2.1
Capsule-II	0	0.39	–	3.6	0.42	–	2.6
	0.20	0.58	98	2.9	0.57	92	2.5
	0.40	0.82	104	2.7	0.78	95	2.2
	0.60	0.99	100	2.3	0.98	96	1.9
Capsule-III	0	0.41	–	3.2	0.38	–	3.3
	0.20	0.63	103	2.7	0.64	110	3.1
	0.40	0.79	98	2.4	0.83	106	2.6
	0.60	1.04	103	1.9	0.96	98	2.5

F-test value: $F_{calc} = 1.21$, *F*-distributed ($\alpha = 0.05$, $\nu_1 = 11$, $\nu_2 = 11$) = 2.82. Student *t*-test value: $t_{calc} = 1.79$, *t*-distributed (95%) = 2.20.

ence from excipients present in CFX pharmaceutical formulations, and a suitable liquid-liquid extraction procedure was adopted. The CL reaction mechanism was also discussed.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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