New Developed Analytical-Chemiluminometric (Sensitized) Continuous Flow Injection Analysis, for Determination of Ciprofloxacin; Application to Pharmaceutical Preparation

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Abstract

A newly developed analytical method characterised by its speed and sensitivity for the determination of ciprofloxacin via the improvement of the chemiluminescence system for luminol-H_2O_2-OH^--ciprofloxacin via continuous flow injection analysis. The method is based on the indirect chemiluminescence-reaction (Sensitized) via the improvement of the amount of photons released through the oxidation of luminol-H_2O_2-OH^- in the presence of ciprofloxacin as a sensitizer for the reaction. Linear dynamic range for the chemiluminescence-emission vs. ciprofloxacin concentration was 0.01-1 mmol.L^{-1} while C.O.D was 96.64%. The L.O.Q was 203 ng/sample. L.O.D. (S/N=3) = 6.01ng/sample from the stepwise dilution for the minimum concentration of lowest concentration in the linear dynamic ranged of the calibration graph. R.S.D.% for n=10 was <1% for ciprofloxacin with concentration of 0.5 mmol.L^{-1}. the method was applied successfully for the determination of ciprofloxacin in four pharmaceutical drugs. Using paired t-test it was shown that there were no significant difference between the two methods and on that basis the new method can be accepted as an alternative analytical method.

Keywords: Chemiluminescence, flow injection analysis, ciprofloxacin, fluoroquinolones.

1-Introduction

Ciprofloxacin (CPLX) [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazinyl)-quinolone-3-carboxylic acid] is one of the third generation members of synthetic fluoroquinolone antibiotics which exhibits a greater intrinsic antibacterial activity and a broader antibacterial spectrum[1].

This synthetic antibiotic has been widely used in the treatment of urinary and respiratory tract infections with good localized action on infected sites, and also in gastrointestinal disease[2]. In addition, it has found off-label use as an alternative drug for the treatment of gonorrhea, salmonella, and yersinia infections[3,4]. In consequence, attention has been paid to their determination in various biological fluids. A variety of techniques such as spectrophotometry[5,6], spectrofluorometry[7,8], chromatography, high-performance liquid chromatography (HPLC)[9,10], capillary electrophoresis (CE)[11,12], etc., have been proposed for the determination of the CPLX.

Chemiluminescence (CL) is the light emission derived from a chemical reaction in which chemically excited molecules decay to the ground state and emit photons[13]. In terms of the production mechanism of light emission, the CL technique can be divided into direct CL and indirect CL, in which the chemical energy is transferred from a product C^* in an excited state to a fluorophore (F)[14], for sensitized the weak CL[15,16] or insitu fluorescence[17,18], as illustrated in Fig.(1)[19].

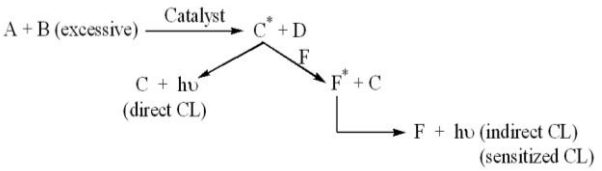


Fig.(1) The main mechanisms leading to a chemiluminescence reaction: A, analyte, B, oxidant, C.D, product, F, fluorophore, *, the excited state, hυ, luminescence.

The limited selectivity of CL reactions can be overcome by coupling with flow technique including flow injection analysis (FIA)[20,21] as the most widely used technique. Analytical procedures applying CL methods combined with FIA have some advantages such as sensitivity, rapidity, ease and simple instrumentation, which has been frequently used for the analysis of pharmaceutical

compounds[22,23]. A method for the determination of ciprofloxacin, norfloxacin and ofloxacin was described based on the enhancement by these compounds for the weak CL from tris (2,2`bipyridyl) ruthenium(II)-Ce(IV) in sulfuric acid medium[24-26].

The peroxy nitrous acid system, obtained by acidified H_2O_2 plus nitrite, was proposed for the determination of CPLX[27] in pharmaceutical preparations. A CL-FIA method based on the sensitizing effect of fluoroquinolones including ofloxacin, norfloxacin, ciprofloxacin and lomefloxacin on the CL reaction of cerium (IV)-sulfite was described for determination of fluoroquinolones in pharmaceutical preparations[28].

The use of $Ru(bipy)_3$-$^{2+}$Ce(IV)-sulfuric acid system[29] was described for the determination of fluoroquinolones by enhancement of weak CL. Based on the CL reaction of Ce(IV)-SO_3^{2-}-sensitized by Tb^{3+}-CPLX, a CL method has been described for the determination of CPLX in human serum and urine sample [30].

The main purpose of this work is to establish a simple, sensitive and rapid CL method for the determination of CPLX. The researchers found that CL reaction of luminol-H_2O_2-OH^- could be sensitized by CPLX. This CL method combined with flow injection technique has been applied for the determination of CPLX in pharmaceutical preparations with satisfactory results.

2- Experimental

2-1 Chemicals

All chemicals used were of analytical reagent grade. Deionized water was used throughout this work. CPLX stock standard solution ($C_{17}H_{18}FN_3O_3$, 331.346, SDI, 100 mmol.L^{-1}) was prepared by dissolving 3.858 g/100 ml distilled water. A stock solution of luminol (BDH, 10 mmol.L^{-1}): 0.17716g in 100 ml of 0.1 mol.L^{-1} Na_2CO_3. A 100 mmol.L^{-1} H_2O_2 solution (BDH, 30%, 1.1 g.ml^{-1}, 100-volume) was prepared by dilution of 5.15 ml in 500 ml distilled water. Standarized against standard solution of $KMnO_4$ (100 mmol.L^{-1}).

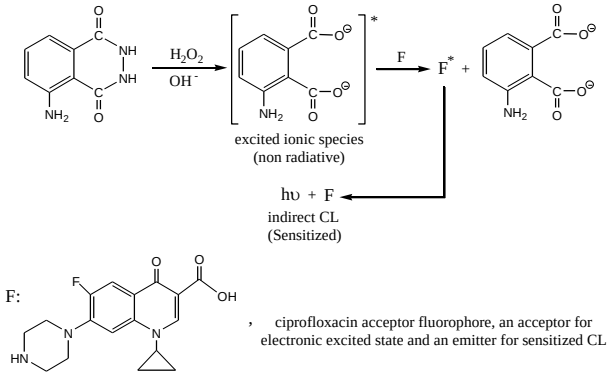
2-2- Apparatus and Manifold

The flow system used for the determination and CL detection of CPLX, shown schematically in Fig.(2). A peristaltic pump: three channels, variable speed (Ismatec, Switzerland). The drug solution was injected through the six-way injection valve (Rheodyne, U.S.A) with a sample loop (0.7 mm i.d., teflone, variable length) which allows mixing of the sample with the reactants (Luminol/OH^--H_2O_2) in the flow cell (100 µL. glass). The emitted light was measured by a photomultiplier tube (RCA 931A (Great Britain)). The photomultiplier was operated at (0-1.6) kV, provided by a stable power supply (JOBIN YVON-France). The signal was amplified by amplifier (nA-PA) (united detector technology, U.S.A). The emitted light signal was recorded by a Kompen Sograph Model C1032 recorder (Siemens, Germany, Range (1-500) mV or (1-500) Volt). Peak height was measured for each signal. UV spectra were measured with an UV-Vis. (CARY 100 conc) spectrophotometer (Japan).

2-3 Methodology

The whole manifold system for ciprofloxacin determination via chemiluminescence: Luminol-hydroxide ion-hydrogen peroxide-ciprofloxacin shown in Fig.(2). The manifold system is composed of three lines: first line supplied with hydrogen peroxide (5 mmol.L^{-1}) at 2.5 ml.$min.^{-1}$, while the second line is for luminol solution 5×10^{-3} mmol.L^{-1} in sodium hydroxide solution at 10 mmol.L^{-1} at 2.4 ml.$min.^{-1}$ flow rate. Both line meet at a junction (methyl methacrylate-Y-junction); with an outlet for reactant product (i.e. Lu-OH^--H_2O_2). That gives no CL before the arrival to the reaction cell. The third line represent the carrier stream (distilled water) leading to the injection valve, which allows the use of 83µl and a flow rate of 2.3 ml.min^{-1} (loop length 21.5 cm with 0.7 mm I.D). Both outcoming lines meet at the CL reaction cell inlets (flat spiral). The cell is well protected with a black non transparent leather, keeping both the PMT and the CL cell in a close attachment. At the reaction cell a light is emitted through the oxidation of luminol molecule by hydrogen peroxide in alkaline medium and in the

presence of ciprofloxacin molecule as a sensitized as a result of recieving CL energy released from the excited ionic species from the oxidation product of luminol. The proposed suggested mechanism for energy transfer from excited electronic state (non radiative step) to an acceptor fluorophore (ciprofloxacin) which releases light (does not participate in chemical reaction) i.e addition of energy transfer with the shift of CL from the blue to a longer wave length. That was proved practically and spectroscopically as follows[19,30]:



3- Results and discussion

3-1- Spectroscopic Study of Chemiluminescence System

Fig.(3 a,b,c,d) shows the various spectrum obtained for CPLX, Lu-OH^- or Lu-OH^--H_2O_2, Lu-H_2O_2-CPLX and Lu-OH^--H_2O_2-CPLX it shows the disappearance of both absorption maxima of luminol. It is expected that it might be attributed to the total consumption of luminol solution by the effect of the occurance of CL and the formation of non radiative excited ionic species. Also the appearance absorption maxima at 380 nm which might be attributed to the absorption of CPLX molecule and this proves that it does not share in CL but it is an acceptor to CL energy liberated from ionic species.

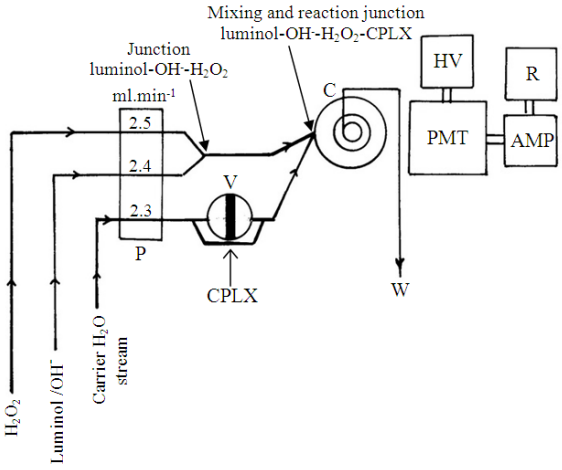


Fig.(2) Schematic diagram of flow injection CL analysis system. P, peristaltic pump; V, injection valve; C, flowing cell; PMT, photomultiplier tube; AMP, amplifier; HV, high voltage; R, recorder; W, waste.

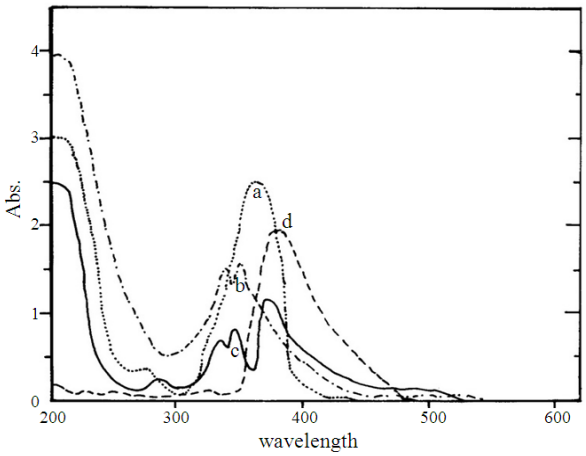


Fig.(3) Absorption spectra for all chemical used in chemiluminescence system for determination of CPLX using optimum experimental parameters: luminol (0.01 mmol.L^{-1}); OH^- (50 mmol.L^{-1}); H_2O_2 (8 mmol.L^{-1}) & CPLX (2 mmol.L^{-1}).
a: Absorption spectra (…) for CPLX in aqueous medium.
b: Absorption spectra (- · - · -) for Lu-OH^- or Lu-OH^--H_2O_2.
c: Absorption spectra (—) for Lu-H_2O_2-CPLX.
d: Absorption spectra (- - -) for Lu-OH^--H_2O_2-CPLX.

3-2-Optimization of Experimental Condition

A series of experiments were conducted to establish the conditions for the production of maximum CL emission for Lu-OH^--H_2O_2-

CPLX. The chemical variables such as concentration of reagents used for the CL reaction and some physical variables, including flow rate, sample volume were investigated, respectively.

3-2-1- Chemical Variables

3-2-1-1- Effect of Luminol Concentration

Series of solutions were prepared for the range of 0-0.1 mmol.L^{-1} in 50 mmol.L^{-1} of NaOH using preliminary concentration of H_2O_2 8 mmol.L^{-1}, 2 mmol.L^{-1} of a choosen concentration of CPLX and a sample volume of 100 µl on the carrier stream of distilled water, each measurement was repeated for three times at a repeatability of <1.5%. Fig.(4) was obtained and it was noticed that 5×10^{-3} mmol.L^{-1} was the optimum and best concentration for luminol.

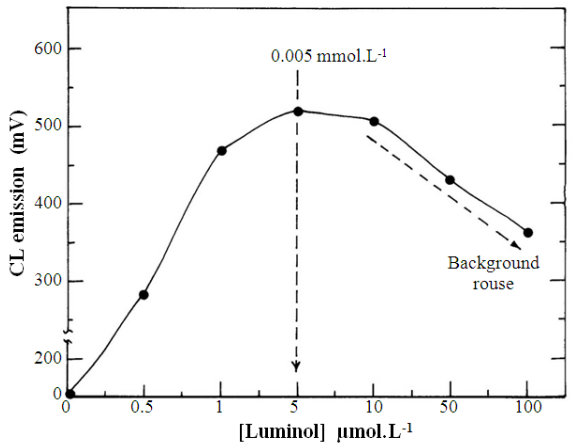


Fig.(4) Effect of variation of luminol conc^{n}. on CL. emission for Lu-OH^{-}-H_2O_2-CPLX.

3-2-1-2- Effect of NaOH Concentration

Since the CL system for CPLX depend on the basic medium of NaOH, and on this basis series of solutions were prepared for 0-100 mmol.L^{-1} using the optimum luminol concentration 5×10^{-3} mmol.L^{-1} and an experimental concentration of H_2O_2 8mmol.L^{-1} and for two variable concentration of CPLX 1, 2 mmol.L^{-1} with 100 µl sample volume. Fig.(5-A) was obtained explaining the increase in the emission of CL light with the increase of NaOH for the range 1-10 mmol.L^{-1}, followed by a decrease in CL light at high concentration of NaOH (i.e. >10 mmol.L^{-1}) examplified by the decrease in peak height (Fig. (5-B)), followed by the broadening in peak maxima and its deformation with a tailing of the peak. Therefore 10 mmol.L^{-1} was choosen as optimal concentration of alkaline medium for the presence of CPLX as a sensitizer.

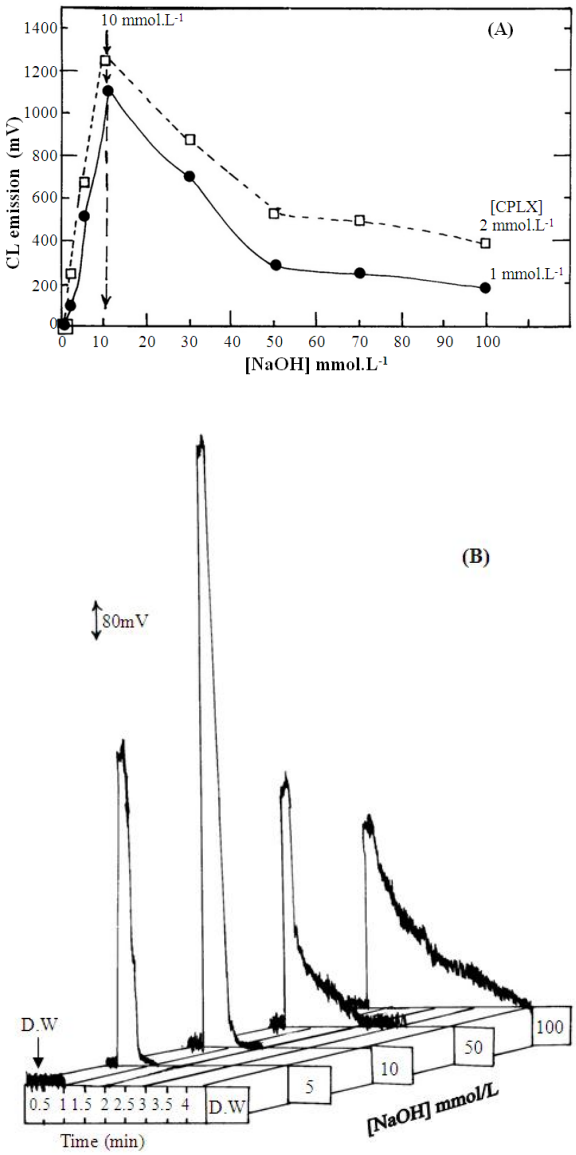


Fig.(5) Effect of variation of sodium hydroxide solution concentration on:
A: Chemiluminescence intensity using 1 & 2 mmol.L^{-1} CPLX.
B: Height and profile of responses using optimum sodium hydroxide 10 mmol.L^{-1}

3-2-1-3- Effect of H_2O_2 Concentration

Using optimum concentration of luminol solution 5×10^{-3} mmol.L^{-1} in 10 mmol.L^{-1} of NaOH and preparing series of diluted solutions of hydrogen peroxide (0-20) mmol.L^{-1}. At sample volume of 100 µL with 2 mmol.L^{-1} of CPLX Fig.(6) was obtained explaining the increase in emission of CL light with the increase of H_2O_2 for 1-5 mmol.L^{-1}, and at concentration > 5 mmol.L^{-1}. It was noticed that

the obtained response was irregular disturbed due to the merging with each other. It is expected that this may be due to the increase of the breakdown of the luminol molecule and the stimulation of constant CL in front of the detector; followed by the occurance of saturation of the PMT. On this basis and on the compromise between the economy of the consumption of chemical material and to obtain high CL intensity. 5 mmol.L^{-1} was choosen as the best concentration of H_2O_2 for the oxidation of luminol in alkaline medium and liberation of ionic species.

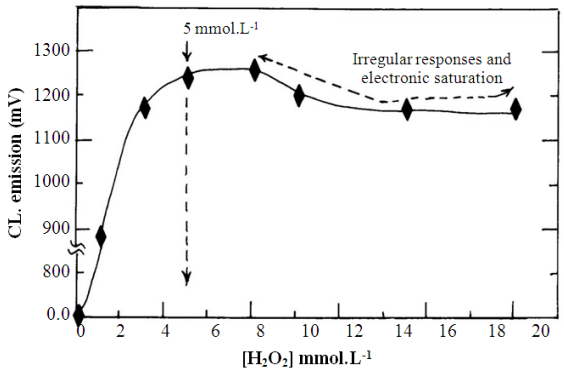


Fig.(6) Effect of variation of hydrogen peroxide on CL. intensity for Lu-OH^--H_2O_2-CPLX system for 100 µl sample volume.

3-2-2- Physical Variables

3-2-2-1- Effect of Flow Rate on CL Emission

Using optimum concentration for CL system with CPLX (2 mmol.L^{-1}) and the injected sample volume of 100 µL at a variable flow rate as tabulated in Table (1).

It was noticed that at low flow rate there is an increase in dilution and dispersion due to the diffusion of light segment in CL cell due to the added volume from reagent solution Lu-OH^--H_2O_2 at the junction point, mixing the reactant, leading to an increase of light segment which might cause an increase in base Δt_B as shown in Fig. (7-A). while at higher speed > 35 (indication approximate), although the effect of physical parameter was not very crucial on the light segment thus obtaining regular responses and sharp maxima, but it is not very high due to the departure of the reactant from measuring cell prior to the completion of CL reaction therefore an indication approximate of 35 was used to obtain a maximum luminescence light and lesser Δt_B as shown in Fig. (7-B).

The time from the departure of sample segment from injection value reaching to the measuring cell takes 21 seconds.

Table (1)
Effect of the variation of flow rate on CL-system: Luminol-OH^--H_2O_2-CPLX.

Peristaltic pump speed (indication approximate)	*Flow rate ml.min.$^{-1}$*			*CL. emission n=3 $\bar{y}$ (mV)*	*Peak base width Δt_B (sec)*	*Estimated Δt_B ^*	*t (sec)*
	H_2O_2	*Luminol/OH^-*	*Carrier stream*				
5	0.9	0.6	0.7	400	60	58.90	39
10	1.0	0.8	0.9	592	54	54.15	36
15	1.4	1.0	1.2	624	50	49.39	33
20	1.8	1.4	1.6	640	44	44.60	30
25	1.9	1.7	1.8	880	39	39.88	27
30	2.1	2.0	1.9	1100	35	35.12	24
35	2.5	2.4	2.3	1230	30	30.36	21
40	3.1	2.9	2.5	1090	24	25.61	15
45	3.4	3.0	2.9	840	21	20.84	12
50	3.8	3.3	3.2	790	18	16.09	9

t= time from the departure of sample segment from injection valve reaching to the measuring cell.

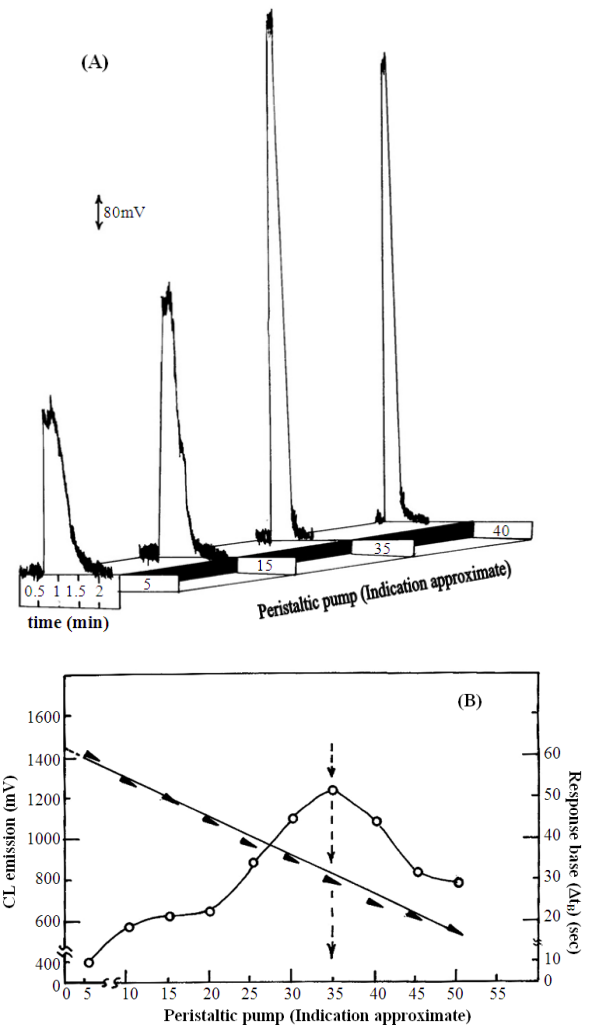


Fig.(7) Effect of variation of flow rate on: A: CL-time profile of chemiluminescene response. B: on emission response (o—o) and on base width (Δt_B) (→→).

3-2-2-2- Effect of Sample Volume

Using the optimum parameters achieved in previous section. Variable sample volumes (70, 80, 83, 90, 100) µL were injected using open valve mode i.e. allowance for continuous purge of sample from the sample loop in the injection valve. The data obtained were plotted as shown in Fig. (8A). showing that the optimum sample volume is 83µL given regular clear chemiluminescence response. Using larger volume i.e > 83µL even though it gave a slight higher response but it was characterized with the width of their peak maxima which was most probably attributed to continuous long time duration of chemiluminescence as shown in Fig.(8B).

3-2-2-3- Effect of Purge Time

Using different purge time for the sample segment i.e. using 3 to 15 seconds allowed time for the carrier to pass through the injection valve in inject mode; and after that allowed time the injection valve is returned to the load position. The volume of sample was 83 µL. Fig.(9) shows the continuation of the increase in emission with the increase of injection time up to 8 seconds. Then followed by a decrease with the increase of injection time. The decrease in emission when using less than 8 seconds was attributed to the incomplete purge of the sample from sample loop in the injection valve. Above 8 seconds the decrease was attributed to the resistance of flow due to the passage through the injection valve.

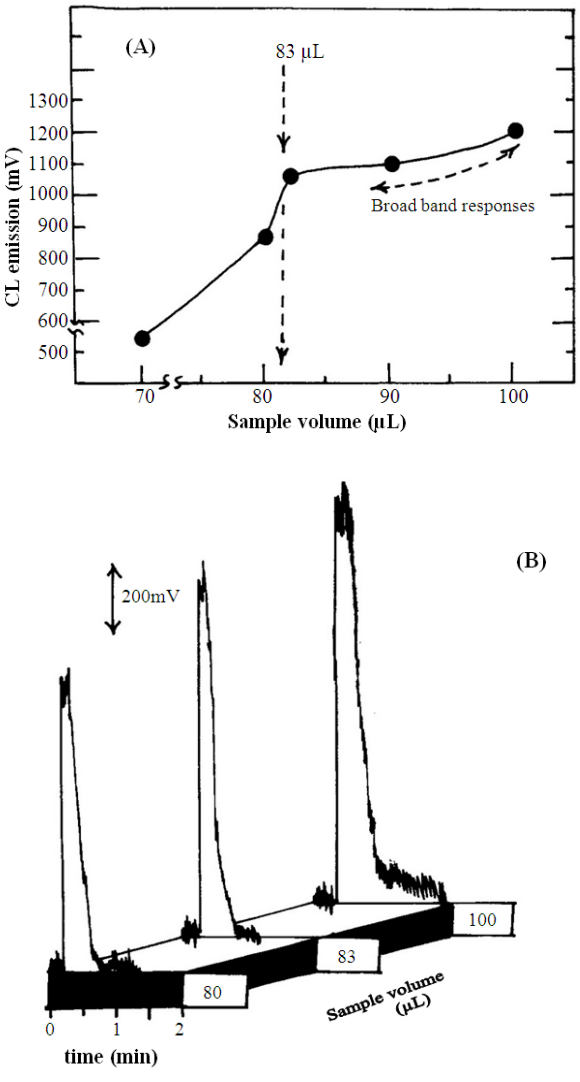


Fig.(8) Effect of variation of sample volume segment of CPLX on: A: Chemiluminescence emission. B: Response profile.

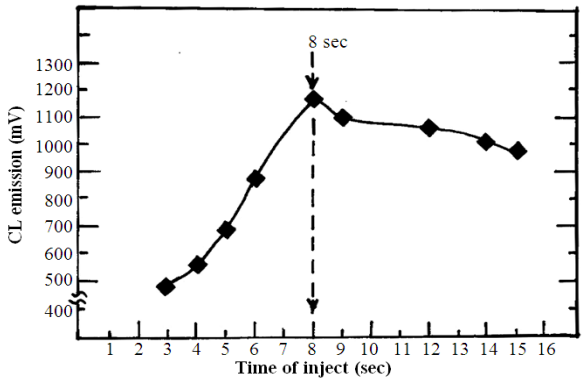


Fig.(9) Effect of variation of injection time on chemiluminescene using optimum parameter.

3-3-Performance of CPLX Measurement System

Fixing all the achieved parameters whether it is physical or chemical. A series of solutions for CPLX 0.01-5 mmol.L^{-1} were prepared, a calibration graph for the variation of CL emission with CPLX for 0.01-1 mmol.L^{-1} as shown in Fig.(10). Above 1 mmol.L^{-1} the value for r will deviate from linearity due to the appearance of two peaks as shown in Fig.(11). The first peak is due to the remaining chemiluminescence that was created from the reaction, while the second peak might be probably due to fluorescence created by the cleavage the molecule of CPLX with the insitu fluorescence created by the cheniluminescence as internal source of radiation. The obtained results were tabulated in Table (2A), using recent advanced statistical treatment as tabulated in Table (2B).

Table (2A)
Summery of calibration graph results for the determination of CPLX using Lu-OH$^-$-H$_2$O$_2$-CPLX.

Measured [CPLX] mmol.L^{-1}	*[CPLX] range for n=10 mmol.L^{-1}*	*y^(mV)=a±S$_a$t+b±S$_b$t [CPLX]mmol.L^{-1} at confidence interval 95%, n-2*	*r, r^2%*	*t$_{tab.}$*	*t$_{cal}$ $= \frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$*
				at 95%, n-2	
0.01-5	0.01-1	222.92±71.67+913.92 ±138.84 [CPLX] mmol.L^{-1}	0.9831 96.64%	2.306<<15.17	

Table (2B)
ANOVA for linear equation results.

Source	*Sum of squares*	*D$_f$*	*Mean square*	*F$_{stat.}$= S$_1^2$ / S$_0^2$*
Regr.	1070600.5	ν_1=1	1070600.5	230.408
Error	37172.374	ν_2=8	4646.5467	
Total	1107772.9	9		

Since: $F_{tab.} = F_{\nu_2}^{\nu_1} = F_8^1 = 5.32 << F_{Stat.} = 230.41$

Therefore it can be concluded that there is a strong relation between variation of CPLX conc. on CL-emission.

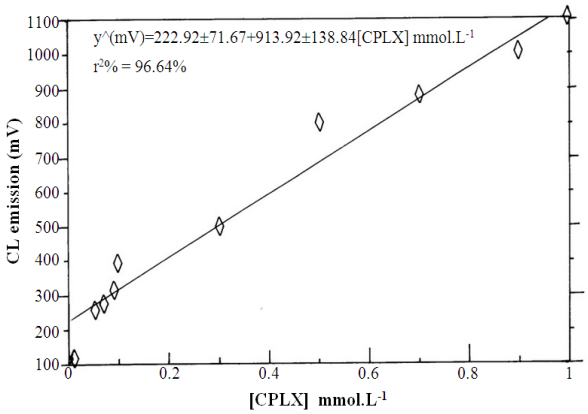


Fig.(10) Effect of variation of [CPLX] on chemilumenescence intensity at optimum parameter Lu (5×10^{-3} mmol.L^{-1}), OH$^-$ (10 mmol.L^{-1}), H$_2$O$_2$ (5 mmol.L^{-1}), flow rate 2.5, 2.4, 2.3 for H$_2$O$_2$, Lu, H$_2$O successively. Sample loop volume of 83µl, allowed permissible time for injection 8 sec.

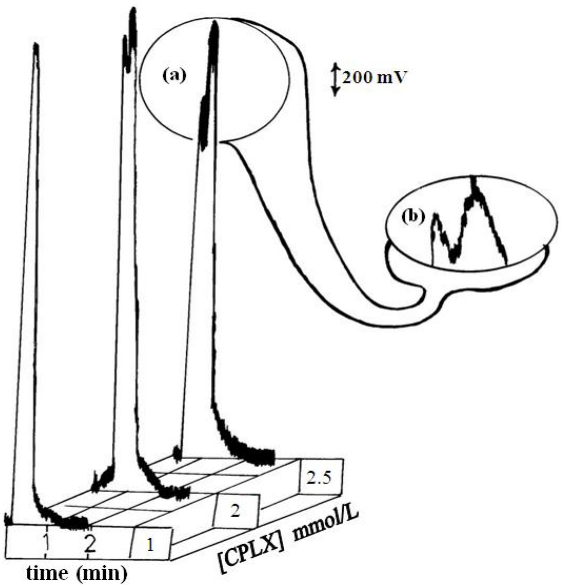


Fig.(11) Effect of concentration variation of CPLX on response and profile.
a) at 1cm/min recorder speed.
b) at 6 cm/min recorder speed.

Limit of detection for CPLX was conducted through four methods as tabulated in Table (3) at injected sample volume of 83µL. Also L.O.Q. was reported.

Table (3)
Limit of detection of CPLX at optimum parameter for Lu-OH^--H_2O_2-CPLX.

Gradual dilution for the minimum conc.	*Based on dilution factor (df)*	*Based on the value of slope* $x = \frac{3S_B}{slope}$	*Linear equation* $y^{\wedge}(mV) = y_B + 3S_B$	*L.O.Q=$y^{\wedge}(mV)=y_B+10S_b$ $S_B=0.58$*
16.01ng	0.873ng	1.05ng	7.16µg	203ng/83µL

df =18.35
x= value of L.O.D. based on slope
S_B = standard deviation of blank solution
y_B= average response for the blank solution (equivalent to intercept in straight line equation
L.O.D = limit of detection
L.O.Q = limit of quantitation

The value of R.S.D% for some selected concentration of CPLX (n=10) tabulated in Table (4).

Table (4)
Repeatability of CPLX results.

[CPLX] $mmol.L^{-1}$	$\overline{y}_i$ *(n=10) mV*	σ_{n-1}	*Repeatability R.S.D.%*	$\overline{y}_i \pm t_{\frac{0.05}{2},9}\frac{\sigma_{n-1}}{\sqrt{n}}$ $(t_{\frac{0.05}{2}}, n-1 = 2.262)$
0.09	312	1.89	0.61	312 ± 1.35
0.5	800	3.28	0.41	800 ± 2.35
0.9	1000	4.15	0.415	1000 ± 2.97

3-4-Analysis of Pharmaceutical Preparation

The CL-method achieved in this work was used for the analysis of CPLX in four different of pharmaceutical preparation and was compared by UV-method via the measurement of λ_{max} at 360 nm. Thirteen tablets were weight, crushed and grinded. 285.8 mg (0.01 $mol.L^{-1}$) from each preparation, dissolved in as little water, followed by filteration to get rid of undissolved material followed by dilution to l00 ml; 1.5ml was drawn to each of five 100 ml volumetric flask followed by the addition of gradual volumes of standard CPLX (0, 0.15, 0.35, 0.55, 0.75) ml at 0.1 $mol.L^{-1}$ to obtain (0.15, 0.3, 0.5, 0.7 and 0.9) $mmol.L^{-1}$. Flask no.1 is the sample flask volume. The measurements were conducted by both methods. Results were mathematically treated for standard addition method. The results were tabulated in Table (5) at confidence interval 95%, and 99%. Paired t-test was used as shown in Table (6). The obtained results indicate clearly that there was no significant difference between newly developed method CL-FIA with the classical UV-method at 95% confidence interval because calculated t value is less than tabulated t value (column 7).

Table (5)
Results for the determination of CPLX in pharmaceutical preparation.

Sample no.	*Commercial name Content Country*	**Confidence interval for average weight* $\overline{w} \pm 1.96\frac{\sigma_{n-1}}{\sqrt{n}}$ at 95% $\overline{w} \pm 2.58\frac{\sigma_{n-1}}{\sqrt{n}}$ at 99% *(g)*	*Sample weight (5.787 mg) equivalent to 0.15 $mmol.L^{-1}$ of the active ingredient (g)*	*Theoretical content for the active ingredient at 95% & 99% for n=∞ (mg)*	*Equation of standard addition curve at 95% for n-2* *$y^{\wedge}=a\pm S_a t+b\pm S_b t x$*	*Practical Conc. ($mmol.L^{-1}$) and what is equivalent of active ingredient (mg)*	*Practical content of active ingredient at 95% & 99% for n=∞ (mg)*	*Efficiency of determination (Rec.%)*
					CL-FIA			
					UV-method			
1	Ciropharm 500mg Pharma International Jordon	0.76752±0.0053 0.76752±0.0069	0.00888	500 ± 3.45 500 ± 4.49	143.36±15.18+1007.58±26.54x	0.140 5.4012	466.84±0.51 466.84±0.67	93.37%
					0.135±0.064+0.906±0.111x	0.143 5.5169	476.84±4.42 476.84±5.82	95.37%
2	Tyflox 250mg Ajanta India	0.35354 ± 0.0052 0.35354 ± 0.0069	0.00818	250 ± 3.68 250 ± 4.88	163.11±15.02+1114.34±26.22x	0.149 5.748	248.45±2.60 248.45±3.43	99.38%
					0.166±0.045+1.22±0.079x	0.146 5.6326	243.45±4.79 243.45±6.31	97.38%
3	Ultraflox 500mg Pharma limited India	0.72139 ± 0.0041 0.72139 ± 0.0054	0.00835	500 ± 2.84 500 ± 3.74	180.41±9.29+1163.72±16.21x	0.149 5.748	496.63±3.64 496.63±4.74	99.33%
					0.25±0.0029+1.68±0.051x	0.153 5.9027	510.03±2.55 510.03±3.36	102%
4	Sipro 750mg Asia Pharma. Industries Syria	0.95204 ± 0.0041 0.95204±0.0054	0.00735	750 ± 3.23 750 ± 4.25	199.18±2.16+1222.54±3.76x	0.156 6.018	779.57±4.44 779.57±5.84	103.9%
					0.341±0.018+1.83±0.032x	0.152 5.86416	759.58±4.06 759.58±5.34	101.28%

x: [CPLX] $mmol.L^{-1}$
$y^{\wedge}$: Chemiluminescence emission or absorbance in mV
**** : $t_{0.025,\infty} = 1.96$ at 95%, $\alpha = 0.05$***
$T_{0.005,\infty} = 2.58$ at 99%, $\alpha = 0.01$.

Table (6)
Paired t-test results for CL-FIA with classical uv-method using standard addition method for the determination of CPLX in pharmac utical preparation.

Sample no.	A moment found x(mg)±R.S.D at 95%		D	$\bar{xd}$	Standard deviation of the different Sd	$t_{tab.}$ at 95%, n-1	$t = \frac{\bar{xd}\sqrt{n}}{Sd}$ n = 4
	Proposed method (CL-FIA)	UV-method					
1	466.84 ± 0.51	476.84 ± 4.42	-10	0.398	15.31	3.182 >> 0.052	
2	248.45 ± 2.60	243.45 ± 4.71	5				
3	496.63 ± 3.64	510.03 ± 2.55	-13.4				
4	779.57 ± 4.44	759.58 ± 4.06	19.99				

Conclusion

The proposed flow-injection sensitized CL method has a simple, rapid, inexpensive and high sensitivity for the determination of CPLX based on the luminol-OH^--H_2O_2-CPLX system. From the experimental point of view, the manipulation is very simple, and sequential measurement was permitted with high sample frequency, up to 60 samples per hour. The proposed CL method used cheaper instruments and reagents than those of spectrophotometry, fluorometry, HPLC and other CL method with different compounds as sensitizers. The detection limit of the proposed method is better (16.01 ng/83µl) than that of uv-method (0.01 mmol.L^{-1}), other CL methods described in the literature[27-30] in pharmaceutical preparations. The %R.S.D was <1% and good agreements were observed for all samples, which is an indication of satisfactory accuracy of the proposed method. The standard addition method was used to avoid matrix effects. The proposed method and the uv-method were applied to the determination of CPLX in four pharmaceutical preparations to determine recovery. The recovery values for CPLX was in the range of 93.37-103.9%. statistical analysis of the results using student t-test showed no significant difference at p=0.05 between the performance of the two methods as regards to accuracy and precision. The proposed flow-injection CL method can be used for routine determination of CPLX in pharmaceutical preparations and biological fluids.

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الخلاصة

تم تطوير طريقة تحليلية سريعة وحساسة لتقدير سايبروفلوكساسين بوساطة تحسين البريق الكيميائي لنظام: لومينال- بيروكسيد الهيدروجين- هيدروكسيد الصوديوم- سايبروفلوكساسين وباستخدام منظومة الحقن الجرياني. استندت الطريقة على تفاعل البريق الكيميائي غير المباشر (التحسسي) بفعل تحسين كمية الضوء المنبعث من اكسدة اللومينال ببيروكسيد الهيدروجين في وسط قاعدي بوجود سايبروفلوكساسين كمتحسس الى التفاعل. تم الحصول على علاقة لتغير استجابة البريق مع التركيز للسايبروفلوكساسين باستخدام معادلة الخط المستقيم وكان مدى منحني المعايرة (١-٠.٠١) مللي مول.لتر$^{-١}$ ومعامل التقدير (C.O.D) ($\%r^2$ = ٩٦.٦٤%)، اما حد التقدير الكمي (L.O.Q) ٢٠٣ نانوغرام/٨٣ مايكرولتر وبحد كشف (S/N=3) ١٦.٠١ نانوغرام/٨٣ مايكرولتر من التخفيف التدريجي لاقل تركيز في منحني المعايرة. الانحراف القياسي النسبي المئوي (R.S.D% و١٠=n) > ١% لمحلول سايبروفلوكساسين بتركيز ٠.٥ مللي مول.لتر$^{-١}$. طبقت الطريقة لتقدير سايبروفلوكساسين في اربع نماذج من المستحضرات الصيدلانية. اجريت مقارنة بين الطريقة المستحدثة (البريق الكيميائي التحسسي-الحقن الجرياني) والطريقة التقليدية للقياس الطيفي باستخدام نتائج منحني الاضافات القياسية وذلك باخضاعها الى اختبار t- المزدوج وتبين انه لا يوجد فرق جوهري بين الطريقتين وبالامكان استخدام نظام البريق الكيميائي: لومينال-H_2O_2-^-OH-سايبروفلوكساسين كبديل للطريقة التقليدية.