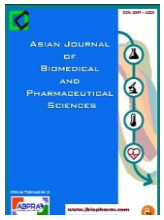
Asian Journal of Biomedical and Pharmaceutical Sciences
ISSN : 2249 - 622X



RESEARCH ARTICLE

Received on: 29-12-2013
Accepted on: 21-02-2014
Published on: 15-03-2014

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QR Code for Mobile users

Conflict of Interest: None Declared !

Chemiluminescence Determination of Some Fluoroquinolones Using NBS-Luminol System

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Abstract

A new, simple, rapid and sensitive batch chemiluminescence (CL) method for determination of six fluoroquinolones (ciprofloxacin, gatifloxacin, levofloxacin, lomefloxacin HCl, ofloxacin and sparfloxacin) is proposd. The method is based on the CL generated during the oxidation of luminol by N-bromosuccinimide (NBS) in alkaline medium. The determination of the studied drugs is based on their inhibiting effect on the emission intensity of NBS-luminol chemiluminescent reaction. The effect of analytical variables on this CL system is discussed. The study was validated according to ICH guidelines. Under the optimum experimental conditions, the linear range is 50 to 400 ng/ml for ciprofloxacin, 50 to 600 ng/ml for gatifloxacin, lomefloxacin HCl and sparfloxacin and 25 to 400 ng/ml for levofloxacin and ofloxacin and the detection and quantitation limits for the studied drugs were not more than 4.88 and 14.80 ng/ml, respectively. The proposed method has been applied to detect the studied drugs in their pure forms and in different pharmaceutical formulations. The possible mechanism of the CL reaction was discussed.

Keywords: Batch chemiluminescence; Fluoroquinolones; N-bromosuccinimide; Luminol; Pharmaceutical formulations.

Cite this article as:
Gamal A. Saleh, Hassan F. Askal, Ibrahim H. Refaat, Fatma A. M. Abdel-aal. Chemiluminescence Determination of Some Fluoroquinolones Using NBS-Luminol System. Asian Journal of Biomedical and Pharmaceutical Sciences; 04 (29); 2014. 39-49.

1. INTRODUCTION

In recent years, extreamly sensitive analytical techniques based on chemiluminescence (CL) and bioluminescence systems have received considerable attention. In contrast to spectrophotometry and fluorimetry, the absence of strong background light levels in CL methods reduces noise signals and leads to improved detection limits (better sensitivity) and wide linear dynamic ranges. Moreover, the absence of the light excitation source leads to a low cost and simple operation of the instruments resulting in a simple, robust and cost-effective apparatus. As an extensively used brominating and oxidizing reagent, N-bromosuccinimide (NBS) has been successfully introduced into CL reaction [1-3]. In aqueous solutions, its oxidizing properties were attributed to hypobromous acid generated by its hydrolysis [3]. NBS has been used extensively as brominating and oxidizing agent for organic compounds and has been used for the CL reaction of some compound such as ammonia, isoniazide, pyrogallol and hydrazone [1, 3-6]. It was reported also that the oxidation of luminol by NBS in alkaline medium was a chemiluminescent reaction [5, 7]. Various methods have been reported in the literature for the analysis of the studied drugs including spectrophotometry [8-14], spectrofluorometry [15-20], atomic absorption [21-22], high performance liquid chromatography (HPLC) [23-30], thin-layer chromatography [31-32], capillary electrophoresis [33-35] and voltametry [36-37].

In the present work, it was found that NBS could oxidize luminol to produce strong CL radiation in alkaline medium and the CL intensity could be greatly inhibited by the presence of the studied fluoroquinolones (FQs) Table 1. The decreased CL intensity is proportional with the concentrations of the studied drugs in certain ranges. Based on the observations, a novel and sensitive inhibition CL method was developed for the rapid determination of the studied drugs. The method is simple, rapid and inexpensive and applied to detect the drugs in their pure forms and in their different pharmaceutical formulations. The possible inhibition mechanism of the studied drugs on NBS-luminol system was discussed briefly.

2. EXPERIMENTAL

2.1. Materials and reagents

All solvents and chemicals used were of analytical grade. Ciprofloxacin (Egyptian International. Pharmaceutical Industries Co., E.I.P.I.CO.), lomefloxacin HCl (Alkan Pharma Co. 6 October City, Egypt), ofloxacin (Hoechst AG, Frankfurt, Germany), gatifloxacin (Bristol-Myers Squibb Pharmaceutical Co., Cairo, Egypt), levofloxacin (Al-Pharonia Pharmaceutical Co., Alexandria, Egypt) and sparfloxacin (Global Napi Pharmaceuticals, Egypt) were obtained as gifts and were used as supplied. Pharmaceutical formulations containing these drugs were purchased locally. Ciprofloxacin IV infusion® (Amriya Pharm. Ind. , Alexandria, Egypt) labeled to contain 200 mg ciprofloxacin lactate/100 ml, ciprocin eye drops® (Egyptian Int. Pharmaceutical Industries Co. , E.I.P.I.Co.) labeled to contain 3 mg ciprofloxacin HCl /ml, ciprofar tablets® (Pharco Pharmaceuticals, Alexandria , Egypt) labeled to contain 250 mg ciprofloxacin HCl /tablet, lomoxen tablets® (Egyptian Group for Pharmaceutical Industries Co. , Egypt) labeled to contain 400 mg lomefloxacin HCl /tablet, orchacin eye drops® (Kahira Pharm. and chem. Ind. Co. , Cairo, Egypt) labeled to contain 3 mg lomefloxacin /ml, ofloxacin tablets® (Sedico Pharmaceutical Co. , 6 October City, Egypt) labeled to contain 200 mg ofloxacin /tablet,occufloxin eye drops® (The Nile Co. for Pharmaceuticals and Chemical Industries) labeled to contain 3 mg ofloxacin /ml, Tymer eye drops® (Jamjoom Pharmaceuticals, Jeddah, Saudi Arabia) labeled to contain 3 mg gatifloxacin / ml, Levanic tablets® 500 mg (Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt) labeled to contain 500 mg levofloxacin / tablet, Tavanic IV infusion® (Aventis, Zeitoun, Cairo, Egypt) labeled to contain 500 mg of levofloxacin / 100 ml and Sparatec tablets® **(**Unipharma - El Obour City Cairo – Egypt**)** labeled to contain 200 mg Sparfloxacin / tablet.

Doubly distilled water was used. NBS solution (5×10^{-3} M) was used by daily dissolving 0.2225 g of NBS (Merck) in water and diluting to 250 ml with water. Luminol solution (1×10^{-3} M) was prepared by dissolving 0.0177 g of luminol (Merck) in phosphate buffer (pH= 10) and diluting to 100 ml with the buffer. The minimum number of dilution steps possible was used for preparation of more dilute solutions.

2.2. Instruments

A schematic diagram of the batch CL system is shown in Fig. 1. It consists of Shimadzu RF-5301 spectrofluorophotometer **(**Kyoto, Japan) for chemiluminescent measurements after removing of the excitation source before its use. The slit width of emission monochromator was set at 5 nm.

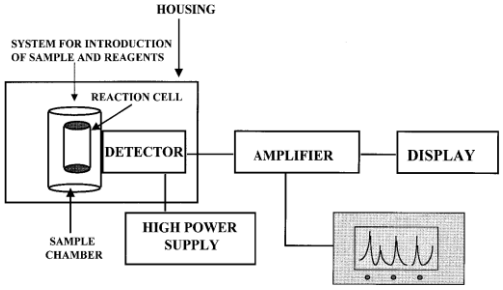


Fig. 1. Schematic configuration of a basic luminometer

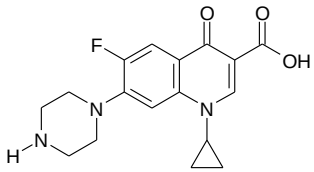
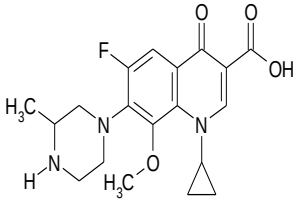
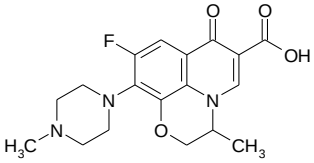
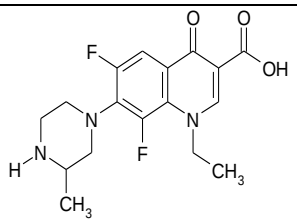
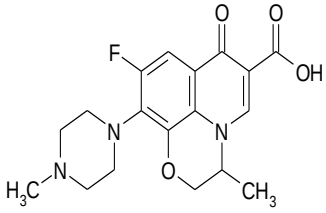
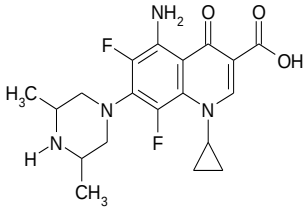
Name	Chemical structure	Nomenclature	Generation
Ciprofloxacin (Cip.)		1-cyclopropyl- 6-fluoro- 4-oxo- 7-piperazin- 1-yl- quinoline- 3-carboxylic acid	2nd
Gatifloxacin (Gat.)		1-cyclopropyl-6-fluoro- 8-methoxy-7-(3-methylpiperazin-1-yl)- 4-oxo-quinoline-3-carboxylic acid	4th
Levofloxacin (Lev.)		(2S)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.0^{5,13}]trideca-5,7,9(13),11-tetraene-11-carboxylic acid	3rd (S-enantiomer of ofloxacin)
Lomefloxacin HCl (Lom. HCl)		1-ethyl-6,8-difluoro-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	2nd
Ofloxacin (Ofl.)		7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.0^{5,13}]trideca-5,7,9(13),11-tetraene-11-carboxylic acid	2nd (racemic mixture)
Sparfloxacin (Spr.)		5-amino-1-cyclopropyl-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	3rd

Table 1:- The names, chemical structures, nomenclature and generations of the studied fluoroquinolones

2.3. Preparation of standard solution

Stock solution of each fluoroquinolone containing 1 mg/ml was prepared in methanol except Lom. HCl was prepared in double distilled water. Working standard solutions containing 0.5-6 µg/ml were prepared by suitable dilution of the stock solution with double distilled water. The stock and working solution must be freshly prepared.

2.4. General procedure

Accurately measured one milliliter portions of the working standard solution of the studied FQs were transferred into 10-ml calibrated flasks. One milliliter of NBS solution containing 5×10^{-3} mol $l^{-1}$was added to each flask. Then the volume was completed with double distilled water. The reaction was allowed to proceed for 5 min. at room temperature [25 ± 5 ºC]. Two milliliter of the resulting mixture was pipetted into the cuvette and then one milliliter of 100 µM luminol solution was added. The emission intensities of the resulting solutions were measured at room temperature against reagent blank treated similarly at 425 nm. The concentration of the standard was quantified by the relative decreased CL intensity.

2.5. Procedure for pharmaceutical preparations

***** *Procedure for Tablets*

Twenty tablets were weighed, finely powdered and mixed thoroughly; and an accurately weighed amount

is obtained from powdered tablets equivalent to 100 mg of the drugs and transferred to a 100 ml volumetric flask. The content of the flask was mixed with about 80 ml of methanol except lomefloxacin HCl was mixed with 80 ml of distilled water. After 15-20 min of sonication, the volume of the solution was made to mark with methanol; after gentle shaking, the solution was filtered, and the first portion of the filtrate was discarded. The rest was collected and used as stock sample solution of 1 mg/ml. The stock solution is diluted with double distilled water to the range of the concentrations used in the standard calibration curve. Then the general procedure was followed.

** Procedure for Eye drops*

One milliliter of the drops was transferred into 100 ml volumetric flask and completed to the mark with methanol to obtain a solution of 30 µg/ml. The stock solution was diluted with double distilled water to the range of the concentrations used in the standard calibration curve. Then the general procedure was followed.

** Procedure for I.V. infusions*

One milliliter of the infusion of levofloxacin or ciprofloxacin was transferred into 100 ml volumetric flask and complete the volume with methanol to obtain stock solution of concentration 50 µg/ml and 20 µg/ml for lev. and cip. respectively. The stock solution was diluted with double distilled water to the range of the concentrations used in the standard calibration curve. Then the general procedure was followed.

3. RESULTS AND DISCUSSION

A series of experiments were conducted on lomefloxacin HCl as an example to establish the optimum analytical conditions for the inhibition of CL intensity of NBS-luminol system by the studied drugs.

3.1. Condition optimization of the CL system

3.1.1. Selection of oxidant and the effect of NBS concentration on the CL intensity

Various oxidants, including permanganate, iodate, periodate, cerric sulphate, ammonium persulphate, hydrogen peroxide, dichromate, NCS or NBS were used for the CL reaction of the studied drugs. In the presence of luminol the most significantly decreased CL signal was recorded when NBS was used as an oxidant in basic medium. Therefore, a procedure based on the inhibition effect of the studied drugs on NBS-luminol CL reaction was proposed. NBS was chosen as optimum and the effect of its concentration on the decreased CL intensity was further examined from 0.5×10^{-4} to 7×10^{-4} M. The results showed that at concentration lower than 5×10^{-4} M there was a decrease in the decreased CL intensity and higher this concentration there is a plateau. Thus, 5×10^{-4} M was selected as optimum concentration of NBS throughout this research as shown in Fig. 2.

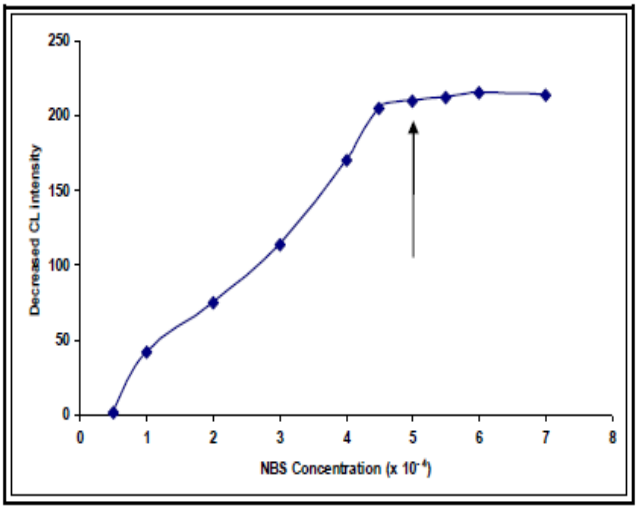


Fig.2: Effect of NBS concentration on the decreased CL intensity Lomefloxacin HCl is 200 ng/ml.

3.1.2. Effect of luminol concentration on the CL intensity

The effect of luminol concentration on the decreased CL intensity was examined over the range of 10 to 150 µM. It was found that the decreased CL intensity reached a maximum value when luminol concentration was 100 µM. Thus the luminol concentration of 100 µM was chosen as optimum concentration for consequent research work as shown in Fig.3.

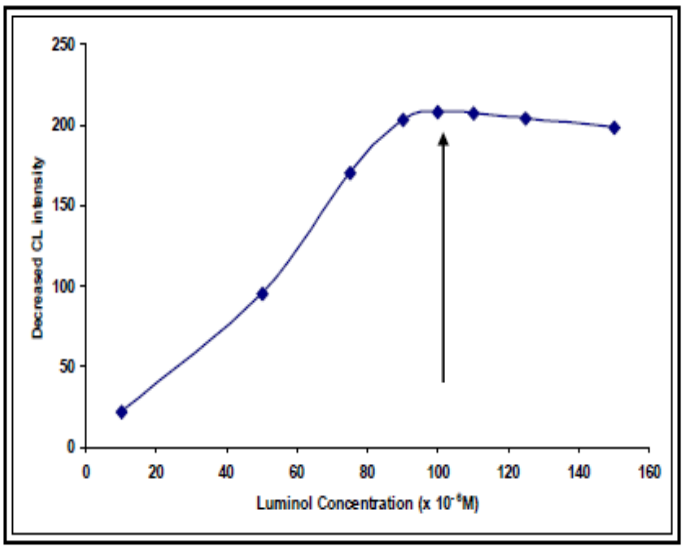


Fig.3: Effect of luminol concentration on the decreased CL intensity Lomefloxacin HCl is 200 ng/ml

3.1.3. Effect of diluting solvent

The effect of various solvents of different polarities and hydrogen bonding capacities including water, acetonitrile, DMF, ethanol, isopropanol or methanol on the decreased CL intensity was studied. Water was

found the best solvent used to give the higher decreased CL intensity, thus it was used in all subsequent experiments for all of the studied FQs (Fig. 4).

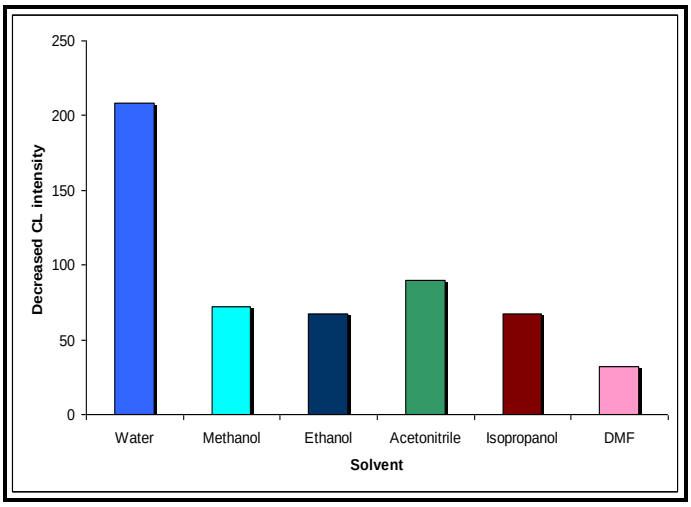


Fig. 4: Histogram indicating the effect of diluting solvent on the decreased CL intensity. Lomefloxacin HCl concentration is 200 ng/ml

A correlation was done between the decreased CL intensities in the tested solvents and their dielectric constants and hydrogen bonding capacities. The dielectric constants gives a rough measure about solvent polarity and hydrogen bonding capacity gives information about the inter-molecular interactions[38]. Results show good positive correlation between the decreased CL intensities and both the dielectric constants and hydrogen bonding capacities as shown in Figures (5, 6).

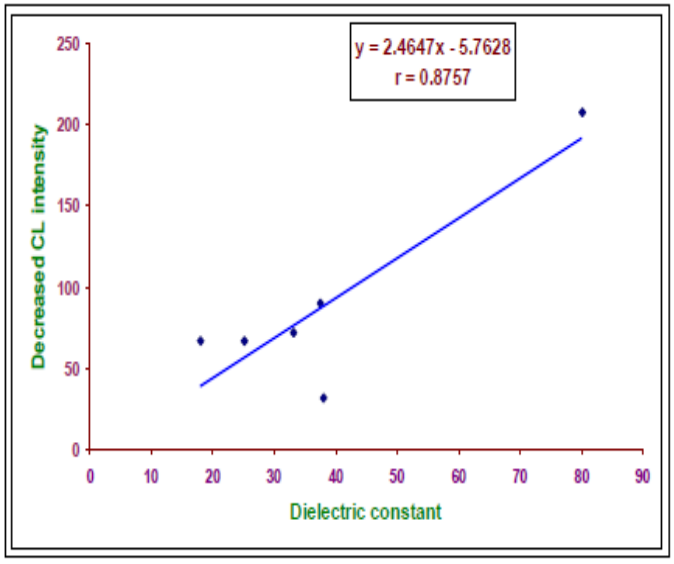


Fig. 5: Correlation between the decreased CL intensities in different solvents and their dielectric constants Lomefloxacin HCl concentration is 200 ng/ml

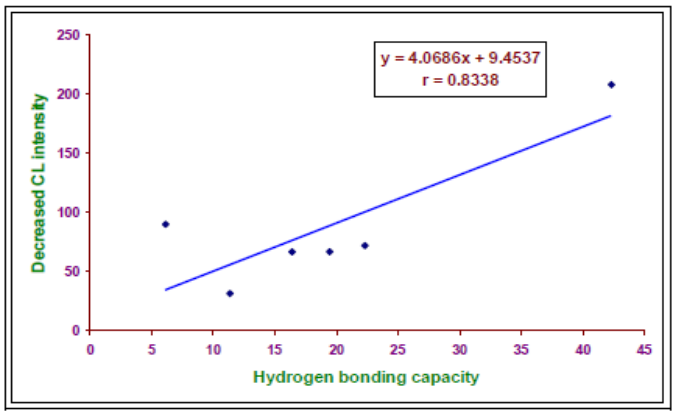


Fig. 6: Correlation between the decreased CL intensities in different solvents and their hydrogen bonding capacities Lomefloxacin HCl concentration is 200 ng/ml

3.1.4. Effect of type and pH of the basic media

The effect of type of basic media for NBS-luminol reaction on the decreased CL intensity was examined by dissolving luminol in different media including 0.05 M NaOH, NaOH/Na_2CO_3 (50mM/50mM), phosphate buffer (either pH 8, 8.5, 9, 9.5, 9.8, 10, 10.2, 10.5, 11 or 12) or borate buffer [39](either pH 8, 8.5, 9, 9.5, 9.8, 10, 10.2, 10.5, 11 or 12). It was found that the decreased CL intensity readings were maximum and reproducible in the presence of phosphate buffer. It was maximum at the pH range 9.8-10.2; hence pH 10 was selected and used for consequent research work as shown in Fig.7.

3.1.5. Effect of buffer concentration

The effect of buffer concentration on the decreased CL intensity was examined from 5 up to 50 mM. It was found that the decreased CL intensity increased with increasing buffer concentration up to 20 mM, but leveled off at higher concentration and then decreased again. Thus a final buffer concentration of 25 mM was used in all subsequent experiments for all of the studied FQs (Fig. 8).

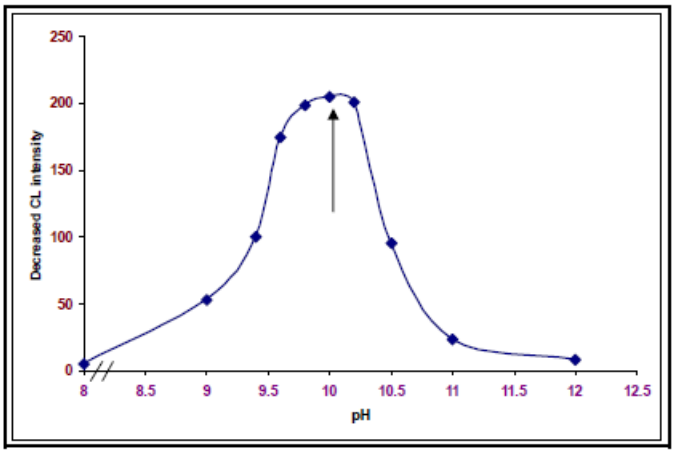


Fig.7: Effect of pH on the decreased CL intensity Lomefloxacin HCl is 200 ng/ml

Drug	Range (ng/ml)	Intercept ± SD[a]	Slope± SD[a]	Correlation Coefficient (r)	Determination coefficient (r^2)	LOD (ng/ ml)	LOQ (ng/ ml)
Cip.	50-400	64.07 ± 0.48	0.47 ± 0.0079	0.9993	0.9987	3.33	10.09
Gat.	50-600	38.40 ± 0.89	0.63 ± 0.0029	0.9998	0.9996	4.70	14.27
Lev.	25-400	21.241 ± 0.59	0.70 ± 0.0093	0.9982	0.9966	2.77	8.39
Lom. HCl	50-600	22.68 ± 0.29	0.46 ± 0.0036	0.9995	0.9991	2.06	6.25
Ofl.	25-400	20.52 ± 0.42	0.83 ± 0.012	0.9995	0.9991	1.70	5.17
Spr.	50-600	62.63 ± 0.55	0.37 ± 0.0051	0.9991	0.9983	4.88	14.80

Table 2:- Summary of quantitative parameters and statistical data using the proposed chemiluminescence method

Drug	Drug Conc. (ng/ml)	Intra-day precision		Inter-day precision	
		Mean ± SD[a]	% RSD	Mean ± SD[a]	% RSD
Cip.	100	109.67 ± 1.21	1.10	110.17 ± 1.17	1.06
	200	160.17 ± 2.64	1.65	160.00 ± 1.55	0.97
	400	255.50 ± 4.23	1.66	260.17 ± 3.60	1.38
Gat.	100	99.83 ± 1.33	1.33	101.00 ± 1.41	1.40
	400	288.17 ± 5.23	1.82	293.83 ± 5.34	1.82
	600	410.50 ± 3.62	0.88	412.17 ± 4.26	1.03
Lev.	100	92.83 ± 1.60	1.73	91.33 ±1.21	1.33
	200	160.67 ± 3.14	1.96	159.50 ± 3.39	2.13
	400	294.50 ± 5.09	1.73	297.00 ± 3.10	1.04
Lom. HCL	100	65.33 ± 1.21	1.85	66.5 ± 1.38	2.07
	400	203.67 ± 4.80	2.03	203.50 ± 4.32	2.12
	600	304.67 ± 2.88	0.94	303.67 ± 5.47	1.80
Ofl.	100	103.50 ± 1.76	1.70	102.33 ± 2.25	2.20
	200	187.67 ± 3.50	1.87	186.50 ± 4.28	2.29
	400	350.83 ± 7.08	2.02	347.33 ± 7.12	2.05
Spr.	100	98.33 ± 2.07	2.10	99.50 ± 2.43	2.44
	400	215.67 ± 2.42	1.12	215.50 ± 3.67	1.70
	600	281.33 ± 1.33	0.47	281.67± 3.70	1.30

Table 3:- Intra- and inter-day precision of of the proposed chemiluminescence method for analysis of the studied FQs.

Drug	Drug Conc. (ng/ ml)	Recovery (%) ± SD[a]	Drug	Drug Conc. (ng/ ml)	Recovery (%) ± SD[a]
Cip.	100	97.88 ± 1.08	**Lom. HCl**	100	98.36 ± 2.53
	200	100.93 ± 1.81		400	97.84 ± 2.60
	400	102.20 ± 1.13		600	101.64 ± 1.04
Gat.	100	97.84 ± 2.12	**Ofl.**	100	100.13 ± 2.12
	400	99.44 ± 2.08		200	100.85 ± 2.11
	600	98.77 ± 0.96		400	99.65 ± 2.14
Lev.	100	100.19 ± 1.73	**Spr.**	100	97.61 ± 2.03
	200	98.81 ± 2.42		400	100.91 ± 2.16
	400	98.54 ± 1.11		600	98.88 ± 1.88

Table 4:- Accuracy of the proposed chemiluminescence method for analysis of the studied FQs.

Variation	% Recovery ± SD[a]					
	Cip.	Gat.	Lev.	Lom. HCl	Ofl.	Spr.
No variation[b]	102.20 ± 1.13	99.44 ± 2.08	98.54 ± 1.11	97.84 ± 2.60	99.65 ± 2.14	100.91 ± 2.16
NBS conc.						
4.5 x 10^{-4} M	98.88 ± 2.24	100.51 ± 1.41	98.06 ± 1.76	98.48 ± 1.16	99.70 ± 2.48	98.67 ± 1.95
5.5 x 10^{-4} M	100.01 ± 2.69	103.62 ± 0.82	101.64 ± 1.39	102.36 ± 1.23	101.56 ± 1.33	101.81 ± 1.52
Luminol conc.						
90 mM	100.80 ± 2.30	100.24 ± 1.73	98.48 ± 0.97	97.49 ± 0.76	98.79 ± 1.63	101.25 ± 2.43
110 mM	99.14 ± 2.52	103.23 ± 2.02	100.21 ± 1.77	99.65 ± 1.99	102.87 ± 1.45	99.90 ± 2.31
pH						
9.8	100.36 ± 2.24	100.77 ± 1.53	99.08 ± 1.25	96.59 ± 1.44	97.79 ± 2.31	100.58 ± 2.23
10.2	102.20 ± 1.31	101.43 ± 1.43	101.46 ± 1.64	96.86 ± 0.99	102.06 ± 1.67	100.13 ± 2.54
Buffer conc.						
20 mM	102.72 ± 1.78	99.84 ± 2.16	99.85 ± 1.19	96.95 ± 1.23	96.98 ± 2.26	99.90 ± 1.93
30 Mm	98.96 ± 2.41	101.43 ± 1.37	99.20 ± 2.37	97.13 ± 0.95	100.91 ± 2.00	99.34 ± 2.17
Variation						
Temperature						
20 ºC	101.85 ± 2.78	101.63 ± 1.98	98.66 ± 2.77	97.58 ± 1.85	99.95 ± 1.14	101.70 ± 1.40
30 ºC	97.39 ± 1.14	100.04 ± 1.83	99.55 ± 1.77	97.67 ± 1.52	101.96 ± 2.43	98.89 ± 2.32
Reaction time						
3 min.	100.80 ± 2.57	102.43 ± 1.95	98.36 ± 2.07	98.66 ± 2.57	99.10 ± 2.45	101.92 ± 1.56
7 min.	98.53 ± 2.29	101.30 ± 2.05	99.85 ± 2.42	97.49 ± 2.67	101.41 ± 2.51	99.56 ± 1.98

Table 5:- Robustness of the proposed chemiluminescence method for analysis of the studied FQs.
[a] Average of six determinations.
[b] Following the general assay procedure conditions.
Drug concentration = 400 ng/ml

Drug	Pharmaceutical formulation	Recovery (%) ± SD[a]	
		Proposed method (n=6)	Official or reported method[c] (n=6)
Cip.	Ciprofloxacin IV infusion®	97.53 ± 1.15, t = 1.83[b] F = 2.57[b]	99.16 ± 1.84
	Ciprocin eye drops®	97.88 ± 1.72, t = 1.68 F = 1.41	99.71 ± 2.03
	Ciprofar tablets®	98.58 ± 1.88 t = 1.62 F = 1.24	100.25 ± 1.68
Gat.	Tymer eye drops®	99.70 ± 2.01 t = 0.64 F = 1.35	99.00 ±1.74
Lev.	Levanic tablets®	98.76 ± 2.15 t = 0.35 F = 1.62	100.77 ±1.91
	Tavanic IV infusion®	100.19 ± 2.33 t = 0.14 F = 4.16	100.34 ± 1.15
Lom. HCl	Lomoxen tablets®	99.80 ± 2.53 t = 0.33 F = 3.16	99.41 ± 1.42
	Orchacin eye drops®	98.72 ± 2.23 t = 0.84 F = 2.09	99.65 ± 1.54
Ofl.	Ofloxacin tablets®	99.12 ± 1.82 t = 1.24 F = 1.78	100.27 ± 1.36
	Ocufloxin eye drops®	98.52 ± 1.78 t = 0.38 F = 1.49	98.95 ± 2.17
Spr.	Sparatec tablets®	97.16 ± 2.20 t = 2.05 F = 2.29	99.37 ± 1.46

Table 6:- Determination of the studied fluoroquinolones in their pharmaceutical formulations by the proposed chemiluminescence method and comparison with the official and reported methods.
[a] Average of six replicates.
[b] Theoretical value for t and F at 95% confidence limit, t = 2.228 and F = 5.053
[c] References (15, 18, 41-44).

Drug	Pharmaceutical formulations	Authentic drug added (ng)	Authentic drug found (ng)	Recovery (%) ± SD[a]
Cip.	Ciprofloxacin IV infusion®	0	0	97.53 ± 1.15
		100	99.93	99.93 ± 1.55
		200	199.72	99.86 ± 2.56
		300	300.21	100.07 ± 2.40
	Ciprocin eye drops®	0	0	97.88 ± 1.72
		100	100.17	100.17± 2.61
		200	198.11	99.05 ± 1.93
		300	301.20	100.40 ± 2.12
	Ciprofar tablets®	0	0	98.58 ± 1.88
		100	100.41	100.41 ± 1.68
		200	198.05	99.02 ± 1.01
		300	301.16	100.39 ± 1.82
Gat.	Tymer eye drops®	0	0	99.70 ±2.01
		100	101.22	101.22 ± 1.92
		300	306.97	102.32 ± 1.19
		500	495.97	99.11 ± 1.25
Lev.	Levanic tablets®	0	0	98.76 ± 2.15
		100	100.55	100.55 ± 2.40
		200	205.06	102.53 ± 1.01
		300	296.44	98.81 ± 1.55
	Tavanic IV infusion®	0	0	100.19 ± 2.33
		100	99.85	99.85 ± 2.27
		200	204.17	102.08 ± 1.19
		300	297.27	99.09 ± 2.22
Lom. HCl	Lomoxin tablets®	0	0	99.80 ± 2.53
		100	102.01	102.01 ± 2.26
		300	297.11	99.04 ± 1.42
		500	501.33	100.27 ± 0.27
	Orchacin eye drops®	0	0	98.72 ± 2.32
		100	100.61	100.61 ± 1.20
		300	299.49	100.44 ± 1.36
		500	500.19	100.04 ± 1.06
Ofl.	Ofloxacin tablets®	0	0	99.12 ± 1.82
		100	101.64	101.64 ± 1.98
		200	204.73	102.37 ± 2.26
		300	296.30	98.77 ± 2.43
	Ocufloxin eye drops®	0	0	98.52 ± 1.78
		100	100.56	100.56 ± 2.25
		200	204.08	102.04 ± 2.37
		300	297.09	99.03 ± 2.38
Spr.	Sparatec tablets®	0	0	97.16 ± 2.20
		100	100.20	100.20 ± 1.40
		300	306.39	102.13 ± 1.68
		500	496.31	99.26 ± 1.70

[a] Average of six determinations.

Table 7:- Standard addition method for the assay of the studied FQs using the proposed chemiluminescence method.

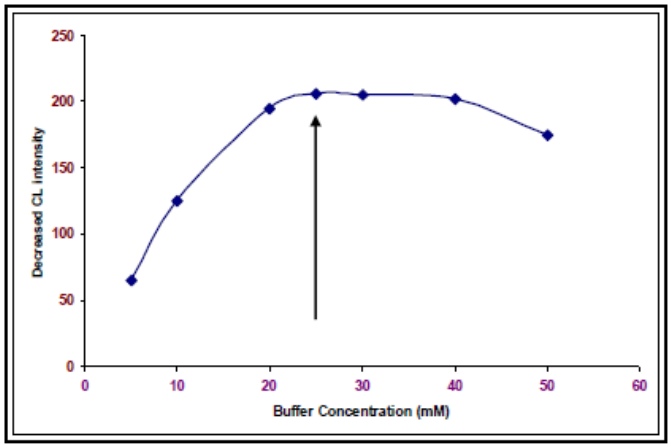


Fig. 8: Effect of buffer concentration on the decreased CL intensity Lomefloxacin HCl concentration is 200 ng/ml

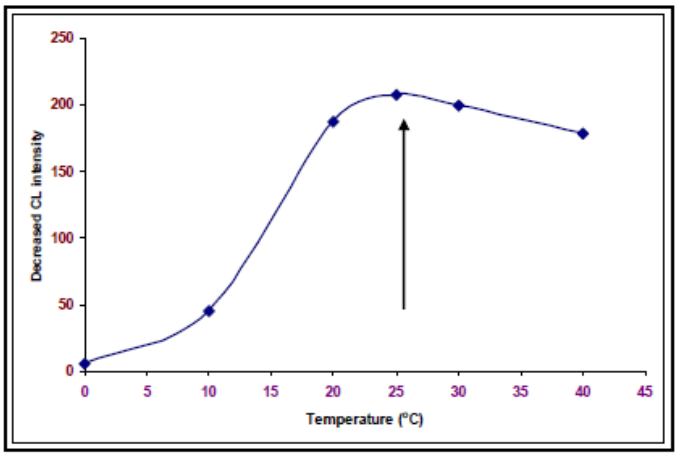


Fig.9: Effect of reaction temperature on the decreased CL intensity Lomefloxacin HCl is 200 ng/ml

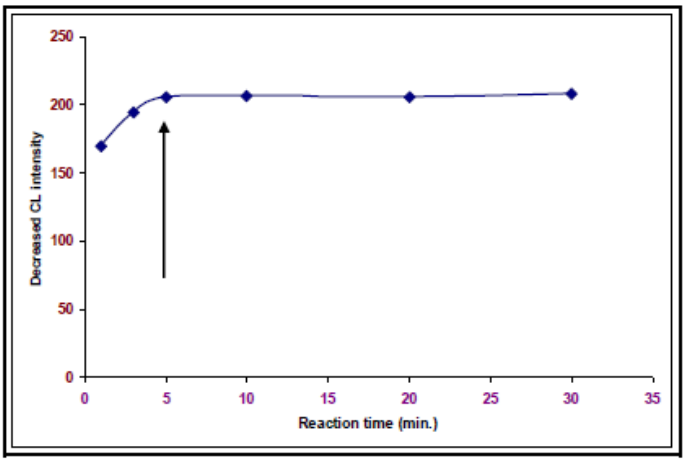


Fig.10: Effect of reaction time on the decreased CL intensity Lomefloxacin HCl concentration is 200 ng/ml

3.1.6. Effect of temperature and time of reaction

The effect of temperature of the reaction on the decreased CL intensity was examined over the range of 0-40 º C. And the effect of time of the reaction on the decreased CL intensity was examined at time interval ranging from 1 minute to 30 minutes.

It was found that the reaction is stable at ambient temperature, but at higher and lower temperatures the decreased CL intensities decreased gradually until completely diminished at 0 ºC and at 40 ºC as shown in Fig.9 and there is no significant difference in the readings after 5 minutes indicating the stability of the products as shown in Fig.10.

3.1.7. Effect of the order of addition

The effect of order of addition of the reactants on the decreased CL reaction was examined. It was found that it has a significant effect on the mixing of the reactants and so affects stability, convenience and method robustness. Different modes were tested and the best order found was the premixing of the drug and NBS before their addition to luminol to facilitate their complete reaction and so enhance the method stability and robustness.

3.2. Analytical method validation

The method was validated according to International Conference on Harmonization guidelines (ICH) on the validation of analytical methods [40]. For the statistical analysis Excel 2003 (Microsoft Office) was used. A 5 % significance level was selected. The developed method was validated for the following parameters:

3.2.1. Linearity and range

As a result of the optimization procedures, the decreased CL intensity was proportional to the concentration of the studied drugs in certain ranges. The results are shown in Table 2.

3.2.2. Precision and accuracy

The precision was well investigated at the levels of repeatability and intermediate precision. The precision was checked at three concentration levels within the specified range. Six replicate measurements were recorded at each concentration level. The results were summarized in Table 3. The calculated % RSD were all below 2.5 % indicating excellent precision of the proposed procedures at both levels of repeatability and intermediate precision. The accuracy was checked at three concentration levels within the specified range. Six replicate measurements were recorded at each concentration level. The results were recorded as percent recovery ± standard deviation Table 4.

3.2.3. Robusteness

The robustness was well checked during the development phase and all the variables affecting the reaction were well studied and optimized as shown in Table 5. It can be easily observed that small variations in NBS concentration, luminol concentration, buffer concentration, temperature and reaction time had no significant effect on the results of the proposed procedures. But it must be stated that the pH of the

reaction should be adjusted to pH 10 ± 0.2 where the reaction is greatly dependant on pH.

3.2.4. Sensitivity

The calculated detection and quantitation limits for the studied drugs were all less than 4.88 and 14.80 ng/ml respectively; indicating good sensitivity of the proposed method as shown in Table 2.

3.3. Application to pharmaceutical dosage forms

According to the procedure detailed in section 2.3, the proposed method was applied to the determination of commercial preparations of the studied drugs and the results are shown in Table 6. The results obtained were validated by comparison with well-established official or reported methods by means of t- and F-tests at 95 % confidence level [15, 18, 41-44]. The recovery tests of standard addition were also carried out on the samples and the obtained recoveries were satisfactory (Table 7).

3.4. Possible mechanism of the CL reaction

The CL emission spectra of the reaction between NBS and luminol was obtained from 380 to 800 nm and showed a maximum at 425 nm [5, 7, 45-46]. The CL spectrum peak is similar to those reported previously for luminol oxidation, and is attributed to the 3-aminophthalate ion. The reaction that occurs is discussed in Fig.11.

In order to get an idea about the reaction product generating the CL, the emission spectra of luminol-NBS CL reaction system in the absence and presence of the studied drugs was further examined. The results showed that the maximum emission appeared at 425 nm for the both reactions, and the relative CL intensity was lower when the studied drugs were presented. It indicated that the CL spectra are independent of the studied drugs and revealed that the luminophor of luminol-NBS-fluoroquinolone system is still 3-aminophthalate, which is the oxidation product of luminol.

There are carboxylic acid group in the molecule structure of the studied drugs which represents the site of reaction between NBS and the studied drugs. The consumption of NBS, the oxidant of luminol-NBS system, led to the decrement of the CL intensity. The reaction between the studied drugs and NBS depends on the ability of NBS to accomplish substitution of the bromine for the hydrogen in reactive aromatic compounds in positions α to double bonds and aromatic rings, and also in positions α to the carbonyl groups [9]. Evidence for bromination reaction is that inspite of the oxidation potential of NBS is nearly the same as N- chlorosuccinamide [NCS]; the later did not react at all, if it is an oxidation reaction NCS must also react.

4. CONCLUSION

The potential application of NBS as organic CL oxidizing and brominating agent has been further demonstrated and extended in this paper. The proposed batch chemiluminescence method is simple, rapid and can be applied for determination of the studied fluoroquinolones in their pure forms and their different pharmaceutical preparations with satisfactory results. As it seen the method is more sensitive and has a wider linear range. This method does not require the use of toxic solvents and does not require the use of complicated instruments and these can be regarded the most important advantages for this method.

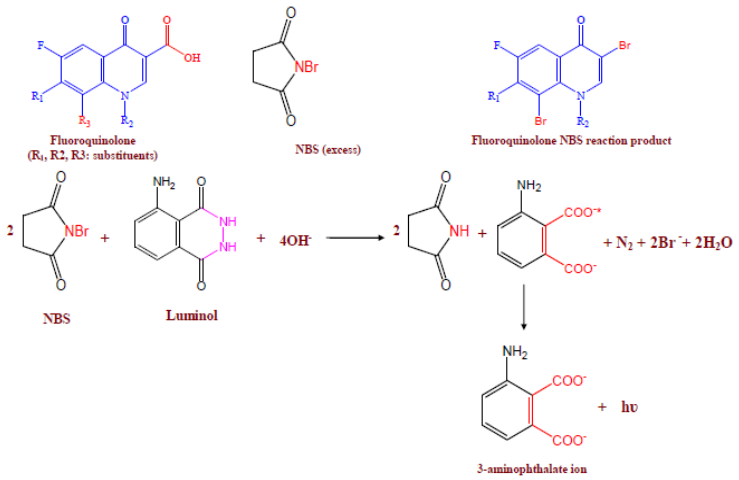


Fig. 11: Suggested reaction mechanism for the proposed chemiluminescence method

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