Enhanced Silver Nanoparticle Chemiluminescence Method for the Determination of Gemifloxacin Mesylate using Sequential Injection Analysis

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Summary: A sequential injection analysis (SIA) with chemiluminescence detection has been proposed for the determination of the antibiotic gemifloxacin mesylate (GFX). The developed method is based on the enhancement effect of silver nanoparticles (Ag NPs) on the chemiluminescence (CL) signal of luminol-potassium ferricyanide reaction in alkaline medium. The introduction of gemifloxacin in this system produced a significant decrease in the CL intensity in presence of (Ag NPs). The optimum conditions for CL emission were investigated. Linear relationship between the decrease in CL intensity and concentration was obtained in the range 0.01-1000 *n*g mL^{-1}, (r = 0.9997) with detection limit of 2.0 pg mL^{-1} and quantification limit of 0.01 *n*g mL^{-1}. The relative standard deviation was 1.3 %. The proposed method was employed for the determination of gemifloxacin in bulk drug, in its pharmaceutical dosage forms and biological fluids such as human serum and urine. The interference of some common additive compounds such as glucose, lactose, starch, talc and magnesium stearate was investigated, and no interference was found from these excipients. The obtained SIA results were statistically compared with those obtained from a reported method and did not show any significant difference at confidence level 95%.

Keywords: Silver nanoparticle; Gemifloxacin mesylate; Luminol- potassium ferricyanide system; Sequential injection analysis; Chemiluminescence

Introduction

Gemifloxacin (Fig. 1) is a synthetic broad-spectrum antibacterial agent for oral administration. It is chemically known as (R, S)-7[(4Z)-3-(aminomethyl-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo1,8-naphthyridine-3-carboxylicacid. Gemifloxacin is a compound related to the fluoroquinolone class of antibiotics and it is available as mesylate salt. It is used for the treatment of respiratory and urinary tract infections. This antibiotic has a broad spectrum of activity against gram-positive and gram-negative bacteria [1].

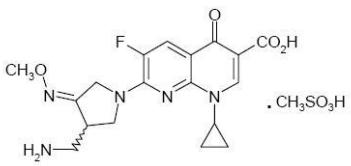

Fig. 1: Chemical structure of gemifloxacin mesylate.

Several methods have been reported for the determination of GFX including high performance liquid chromatography [2-7], liquid chromatography /mass spectrometry [8, 9], high performance thin layer chromatography [7], spectrophotometry [10-13], voltammetry [14, 15], Spectrofluorimetry [16], chemiluminescence [17] and potentiomerty [18].

In recent years, nanotechnology is seen as the way of the future. A lot of scientists think that it will bring a lot of benefits in all areas of life. Much attention was given for using silver nanoparticles or gold nanoparticles, etc. in pharmaceutical analysis. The objective of using nanotechnology in pharmaceutical analysis is the evaluation of pharmaceutical drugs in very small scales, increase the efficiency of the analytical methods and cut down the cost of analysis.

This study describes the use of Ag NPs for enhancing the CL signal of luminol-ferricyanide system in alkaline medium. This CL intensity could be clearly decreased in presence of GFX. The decrease in CL signal is proportional to GFX concentration. Thus, a novel and sensitive sequential injection CL analysis using luminol-potassium ferricyanide system in presence of Ag NPs was proposed for determination of GFX in bulk drug, in its pharmaceutical dosage forms and in biological fluids.

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Results and Discussion

Optimization Studies

Selection of Potassium Ferricyanide as Oxidizing Agent

Various oxidants including potassium ferricyanide, potassium permanganate, potassium periodate and hydrogen peroxide were carefully examined to select the most suitable oxidizing agent. No CL signal was recorded on using potassium permanganate and potassium periodate, while on using hydrogen peroxide or potassium ferricyanide ,CL signals were obtained. Potassium ferricyanide gave higher CL intensity signal rather than that of hydrogen peroxide. Therefore, luminol/potassium ferricyanide CL system in alkaline medium was selected and the effect of luminol and potassium ferricyanide concentrations was further investigated and optimized.

Effect of Luminol and Potassium Ferricyanide Concentrations

To investigate the influence of luminol and potassium ferricyanide concentrations on the CL signal, various concentrations in the range of $1.0x10^{-5}$-$1.0x10^{-1}$ mol L^{-1} for both reagents were investigated. As shown in Fig. 2, it was found that the CL intensity showed significant increase at $2.0x10^{-3}$ and $1.0x10^{-2}$ mol L^{-1} for luminol and potassium ferricyanide, respectively. Therefore, these concentrations were chosen for further studies.

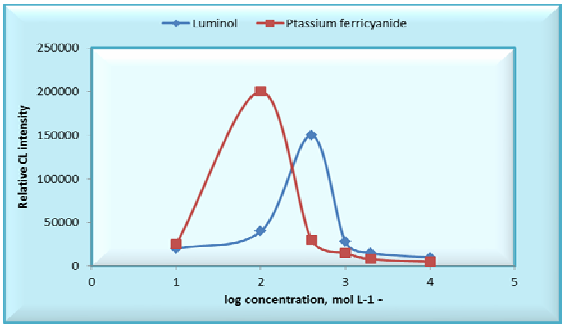

Fig. 2: Effect of luminol and potassium ferricyanide Concentration on CL intensity, for luminol concentration (Ag NPs $5.0x10^{-3}$ mol L^{-1} and potassium ferricyanide $1x10^{-2}$ mol L^{-1}) and for potassium ferricyanide concentration (Ag NPs $5.0x10^{-3}$ mol L^{-1} and luminol $2.0x10^{-3}$ mol L^{-1})

Optimization of Alkaline Medium

Due to the significant effect of alkaline medium on luminol/ferricyanide CL system, the effect of ammonium hydroxide, sodium carbonate and sodium hydroxide in the range $1.0x10^{-3}$-$1.0x10^{-1}$ mol L^{-1} were investigated. As shown in Fig. 3, it was found that the use of $1.0x10^{-2}$ mol L^{-1} sodium hydroxide gave a sharp CL signal while in the cases of ammonium hydroxide and sodium carbonate a significant decrease in CL signal was observed. Therefore, $1.0x10^{-2}$ mol L^{-1} sodium hydroxide was used in this method.

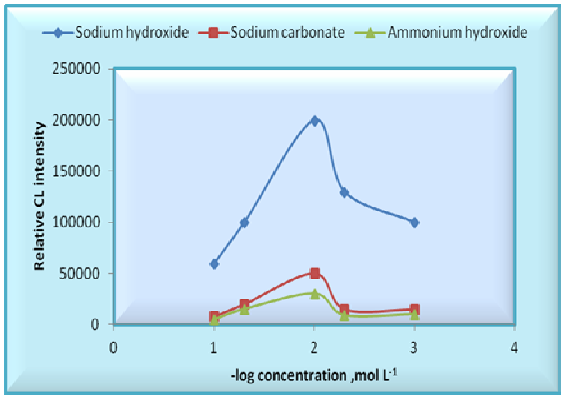

Fig. 3: Effect of sodium hydroxide, ammonium hydroxide and sodium carbonate concentration on CL intensity of luminol-potassium ferricyanide system (Ag NPs $5.0x10^{-3}$ mol L^{-3}, potassium ferricyanide $1x10^{-2}$ mol L^{-1} and luminol $2.0x10^{-3}$ mol L^{-1}).

Effect of Silver Nanoparticles Concentration

Ag NPs can be greatly affecting the CL intensity of luminol-ferricyanide system. The influence of Ag NPs concentration was investigated over the concentration range $1.0x10^{-3}$-$1.0x10^{-1}$ mol L^{-1}. As shown in Fig. 4 the CL intensity was sharply increased on using $5.0x10^{-3}$ mol L^{-1}. Hence, $5.0x10^{-3}$ mol L^{-1} was selected in further studies.

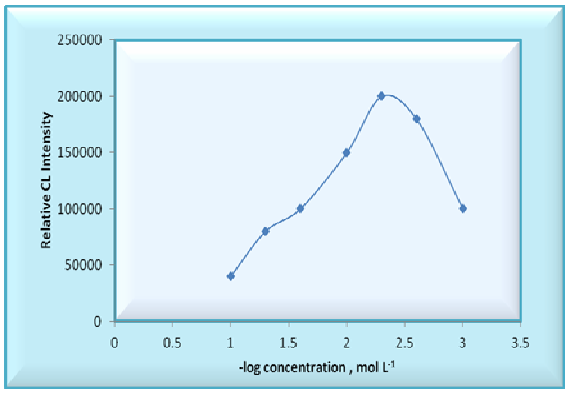

Fig. 4: Effect of Ag NPs concentration on the CL signal of luminol-potassium ferricyanide system (luminol $2.0x10^{-3}$ mol L^{-1} and potassium ferricyanide $1x10^{-2}$ mol L^{-1}).

Optimization of Aspirated Volumes of Sample and Reagents

The main critical parameter which should be carefully optimized in SIA-CL detection was the aspirated volume of sample and reagents. Computer-aided simplex method was used by varying the volume of sample and CL reagents. The optimum aspirated volume for luminol, Ag NPs and potassium ferricyanide was 50, 50 and 60 µL, respectively and 30 µL for GFX sample. The time was extended to about 45 s for complete flushing through the holding cell with carrier in between analysis cycles. Also, the effect of flow rate on CL intensity of luminol/ ferricyanide system in presence of Ag NPs was investigated in the range of 10-150 µL s^{-1}. It was noticed that the CL intensity was increased with the increase of flow rate. As shown in Fig. 5, the optimum flow rate was found to be 120 µL s^{-1} which was used for further studies.

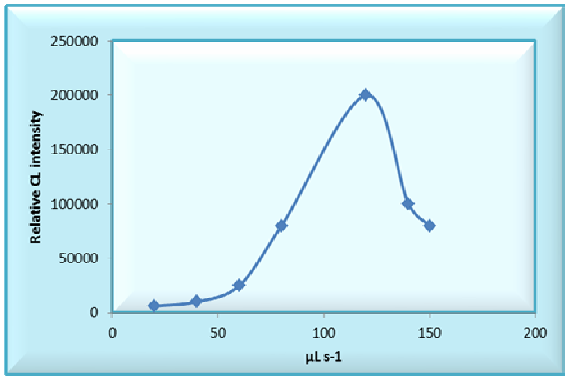

Fig. 5: The influence of flow rate on the relative CL intensity. Conditions; 50 µL of $2.0x10^{-3}$ mol L^{-1} luminol; 50 µL of 5.0 $x10^{-3}$ Ag NPs mol L^{-1} and 60 µL of $1.0x10^{-2}$ mol L^{-1} ferricyanide

SIA Control Program

A SIA control program was utilized to perform all calibration measurements and experimental analysis of GFX. Also, the proposed program was performed for the determination of tested drug in its dosage forms and biological fluids. Table-1 showed the typical steps of program. The single cycle takes about 45s therefore, the sample throughput of 80 h^{-1} can be recorded.

Characterizations

Ag NPs- luminol- ferricyanide system was employed for the determination of GFX. The presented results in Table-2, clarified that the measurable linear concentration range was 0.01-1000 *n*g mL^{-1}, (r = 0.9997) with lower limit of detection of 2.0 pg mL^{-1} and quantification limit of 0.01 *n*g mL^{-1}. The regression parameters were calculated from the calibration graph. The reproducibility of the proposed method was tested over the range 0.01-1000 *n*g mL^{-1} test solutions and the relative standard deviations were less than 5% indicating that the proposed method was suitable for routine analysis of the investigated drug.

Effect of Foreign Substances

The influence of some additive excipients and common species such as amino acids, sugars and some common cations was examined. To evaluate the interferences a test solution of 0.01 µg mL^{-1} of GFX was treated with appropriate foreign substance to contain ≈ 1.0 mg mL^{-1}. The mean peak heights were compared with those obtained with pure 0.01 µg mL^{-1} analyte solution. The tolerable level was defined as the amount of foreign species that produce an error not exceed 5% in determining the tested drug. The obtained results of tolerated concentration level for studied interferences were presented in Table-3. From the presented results the proposed method demonstrated good selectivity for the determination of the investigated drug in its pharmaceutical formulations and biological fluids.

Method Validation

Method validation was carried out with respect to linearity, lower limit of detection, quantification limit, accuracy, precision, and robustness according to ICH guidelines [19].

Linearity

After optimizing the experimental parameters, the proposed SIA-CL method was successfully applied for evaluation of the linear concentration range. The decrease in CL signals was plotted as a function of the tested drug concentrations. Twelve standard solutions were subjected to SIA-CL detection. The regression analysis was calculated using least square method. The results obtained showed that the proposed SIA-CL method exhibits a linear concentration range at 0.01-1000 *n*g mL^{-1}.

Limit of detection, LOD

Signal-to-noise ratio was performed to evaluate the lower limit of detection of GFX. It is the concentration of GFX that has a CL signal equals two times that of blank signal (S/N=2). The recorded signals showed that the limit of detection was 2.0 pg mL^{-1}.

Table-1: The control program of Ag NPs- luminol- potassium ferricyanide
SIA-chemiluminescence detection of GFX

Device	Command	Parameter	Action
Loop Start (#) 1			
Next sample	Counter clockwise		
Peristaltic pump	Delay (s)		
Detector	Peristaltic pump off		
Syringe pump	ON	% 50	
Syringe pump	Valve position IN	25	
Syringe pump	Set flow rate (µL s^{-1})		
Syringe pump	Aspirate (µL)		
Multiposition valve	Delay until done	120	
Syringe pump	Set valve position	1500	
Syringe pump	Set flow rate (µL s^{-1})		Pump filled with carrier
Multiposition valve	Aspirate (µL)	3	
Syringe pump	Set valve position	120	
Syringe pump	Set flow rate (µL s^{-1})	50	
Multiposition valve	Aspirate (µL)	2	(Luminol 2.0x10^{-3} mol L^{-1})
Syringe pump	Set valve position	120	
Syringe pump	Set flow rate (µL s^{-1})	50	
Syringe pump	Aspirate (µL)	5	(Silver nanoparticles 5.0x10^{-3} mol L^{-1})
Multiposition valve	Delay until done	120	
Syringe pump	Set valve position	30	
Syringe pump	Set flow rate (µL s^{-1})		Sample (gemifloxacin mesylate)
Syringe pump	Aspirate (µL)	4	
Multiposition valve	Delay until done	120	
Syringe pump	Detector	60	
PMT	Set flow rate (µL s^{-1})		(Potassium ferricyanide1.0x10^{-2}mol L^{-1})
Syringe pump	Start scan	7	
Syringe pump	Empty	120	
PMT	Delay until done		
Refresh plat	Stop scans		
Loop end			

Table-2: Tolerable concentration level of interferents to 10 *n*g mL^{-1} GFX.

Interferents	Tolerable level µg mL^{-1}
Na^+, K^+, Mg^{2+}, Cl^-, NO_3^-, NH_4^+ and SO_4^{2-}	1000
Glucose, sucrose, lactose, talc, starch	500
Uric acid, magnesium stearate, citric acid, oxalic acid Adrenaline,	125
dopamine, cystine, histamine, tyrosine and glucosamine	80
Al^{3+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, and Cu^{2+}	25

Table-3: Performance data obtained from the determination of GFX using Ag NPs-luminol - potassium ferricyanide system

Analytical characteristics	Obtained results
Linear range, *n*g mL^{-1}	0.01-1000
Detection limit, pg mL^{-1}	2.0
Quantitation limit, *n*g mL^{-1}	0.01
Intercept on the ordinate Slope	2829.2
%RSD for 0.1 *n*g mL^{-1} (n=12)	44602.1
Correlation coefficient, r	1.3 %
	0.9997

Quantitation Limit, LOQ

In order to determine the LOQ of GFX by the proposed Ag NPs- luminol- ferricyanide SIA-CL method, signal-to-noise ratio equal to 10 was performed and the LOQ was found to be 0.01*n*g mL^{-1}

Accuracy

The accuracy of the proposed Ag NPs-luminol-ferricyanide SIA-CL method was carried out by investigating the tested drug using standard addition method. The obtained results were calculated in terms of mean % recoveries. The calculated % recovery was 99.12±1.4%.

Precision

Intra-day and inter-day precisions were employed to evaluate the proposed Ag NPs- luminol-ferricyanide SIA-CL method. The studies were carried out using three concentrations and three replicates of each concentration. The calculated % RSD values were less than 2% indicating reasonable, repeatability and intermediate precision of the proposed method.

Robustness

The robustness of the proposed SIA-CL method was investigated by introducing small changes on method parameters such as changing the flow rate, aspirate rate of reagents and volume of sample using 120±10 µL s^{-1}, 50±5 µL and 30±5 µL, respectively. These minor changes in experimental operation did not affect the CL intensity.

Table-4: Determination of gemifloxacin mesylate using Ag NPs-luminol-potassium ferricyanide SIA-injection CL detection in pure form, dosage forms and biological fluids

Sample	Taken ng mL^{-1}	Recovery (%)±SD	Reported method [11]	Student's t -test	F-Test	(%) RSD
Pure solution	0.1-1000	99.16±1.3	-	-	-	1.3
Factive®320 mg/ tablet	0.1-1000	99.78±1.1	99.68±0.5	0.22 (2.228)*	4.8(5.05)*	1.1
Urine sample	0.1-1000	99.62±1.8	-	-	-	1.8
Serum sample	0.1-1000	99.02±1.4	-	-	-	1.4

* Figures in parentheses are the tabulated values of t- and F- testes at 95% confidence limit

Table-5: Comparative analytical results relevant to linear concentration range and detection limit between the proposed Ag NPs-luminol potassium ferricyanide
SIA-CL injection method and other reported methods

Method	Linear range (µg mL^{-1})	LOD (µg mL^{-1})	Reference
Proposed SIA-CL method	0.00001-1.0 (0.01-1000 ng mL^{-1})	2.0x10^{-6} (2.0 pg mL^{-1})	-
HPLC-Fluorescence detection	0.025-5.0	1.0x10^{-2}	[2]
HPLC-Mass spectrometry	0.01-5.0	-	[8]
Spectrophotometry	2.0-9.0	-	[11]
Voltammetry	2.47-15.5	9.0x10^{-4}	[14]
Spectrofluorimetry	0.04-0.2	-	[16]
Chemiluminescence	0.001-0.3	7.3x10^{-4}	[17]
Potentiometry	0.049-4854.9		[18]

Analytical Applications

It was evident from the above mentioned results that the proposed method gave satisfactory results for the determination of GFX in pure form. Thus its pharmaceutical dosage form (Factive® 320 mg/tablet) was subjected to the analysis of its GFX content by the proposed enhanced Ag NPs -SIA CL-injection method. The obtained results were presented in Table-4 and statistically compared with those obtained from the reported spectrophotometric method [11] by Student's t-test and F- test [20]. The results did not reveal any significant difference between them at 95% confidence level proving similar accuracy and precision. The content uniformity assay for GFX tablets was investigated and the results were presented as the mean % recoveries ± standard deviation (99.42±0.2%).

The extremely high sensitivity of the proposed method promoted us to check its applicability to the determination of GFX in biological fluids such as human serum and urine. The obtained results were satisfactorily accurate and precise. The % recoveries were in the range of 98.47-99.02% and 98.15-99.62 % with low %RSD for human serum and urine, respectively.

Comparison of the obtained results of the proposed method with those recorded in previously reported methods [2, 8, 11, 14, 16 and 17] was summarized in (Table-5). All methods are equally accurate and precise but the proposed method has more wide linear concentration range and more sensitive than other reported methods.

Experimental

Materials and Reagents

All reagents were of analytical grade and were used without further purification. Distilled water was used throughout the experiment. Pure grade of gemifloxacin (GFX) and its tablets (Factive® 320 mg /tablet) were kindly supplied by Tabuk Pharmaceutical MFG. Co., Saudi Arabia. 2.0x10^{-3} mol L^{-1} luminol (Sigma Chemical Co.) stock solution was prepared in 100 mL of 1.0x10^{-2} mol L^{-1} sodium hydroxide (WINLAB). Potassium ferricyanide (WINLAB) 1.0x10^{-2} mol L^{-1} was prepared by dissolving 0.0329 g in 100 mL distilled water. Silver nitrate (BDH laboratory supplies, England) 3.0x10^{-3} mol L^{-1} solution was prepared by dissolving 0.0058 g in 100 mL distilled water. Sodium citrate dihydrate (WINLAB) 3.0x10^{-3} mol L^{-1} solution was prepared by dissolving 0.0082 g in 100 mL distilled water. Sodium borohydride (BDH laboratory supplies, England) 2.0x10^{-3} mol L^{-1} was prepared by dissolving 0.0079 g in 100 mL distilled water. Urine samples were obtained from healthy volunteers and serum samples (Multi-Serum Normal, Randox Laboratories, UK) were obtained from commercial sources.

Synthesis of Silver Nanoparticles Ag NPs

The synthesis of Ag NPs was carried out by the reduction of silver nitrate using sodium borohydride as reducing agent in aqueous solution according to the procedure described in the literature [21]. 25 mL of 2.0 x10^{-3} mol L^{-1} sodium borohydride Na BH_4 was added to 70 mL of 3.0x10^{-3} mol L^{-1} silver nitrate dropwise with continuous stirring for 10 min. The mixture was turned yellow which indicated the silver nitrate reduction and the formation of Ag

NPs. 5.0 mL sodium citrate (1.0% w/w) was added to the resultant solution to stabilize Ag NPs. The prepared silver nanoparticles were stirred for 20 min.

Apparatus

SIA system (FIAlab-3500 instrument, USA) comprised of a CAVRO XL 3000 syringe pump volume 2.5 mL (Cavro Scientific Instrument Int., USA) and Vici Valco Cheminer RT® 125-0718 eight-port manifolds. Fluorimetric/Chemiluminescence detector (UIV lamp switched off) equipped with a lab-made CL module with spiral geometry; the photomultiplier tube voltage was 320 V. Autosampler model ALM 3200. The SIA system involved a holding coil (length 70 cm, i.d. 0.8 mm, PTFE tubing volume 1.2 mL). The same tubing was spirally coiled on a 52 mm×52 mm Perspex plate, which substituted the secondary filter in the fluorimeter; this CL module had a central inlet, peripheral outlet and the diameter of the spiral was 24 mm. The SIA unit was PC controlled and data acquisition was performed with (FIAlab for windows version 5.9.321) software. The solution stability monitoring and UV spectrophotometry was performed on an UV-Visible Spectrophotometer Ultrospec (model 2100 pro).

Sample Preparation

Standard Drug Solution

A stock standard GFX solution (100 µg mL^{-1}) was prepared by dissolving 10 mg of pure drug in 100 mL distilled water. Working solutions in the range of 0.01-1000 *n*g mL^{-1} were prepared daily by appropriate dilution.

Tablets Treatment

Ten tablets (Factive® 320 mg/tablet) were finely powdered and weighed. An amount of powder equivalent to 10 mg GFX was dissolved in distilled water and then sonicated for 10 min. The sonicated solution was filtered using membrane filter (pore size 5.0 µm). The working solutions were prepared by serial dilution in the range of 0.1-1000 *n*g mL^{-1}. The proposed SIA-CL method was employed and the nominal content was calculated using calibration graph or the corresponding regression equation.

Urine and Serum Treatment

The samples of human urine and serum were prepared by using spiking technique. 1.0 mL of serum was spiked with GFX standard drug solution to contain 1.48 µg mL^{-1} and deprotinated by adding 1.0 mL of acetonitrile. The prepared solution was centrifuged at 2500 rpm for 10 min to remove any interferent species. The treated sample was diluted with distilled water to obtain a concentration of GFX in the range of 0.1-1000 *n*g mL^{-1}.

For urine samples no further pre-treatment was required. SIA-CL method was employed and the peak heights of CL signal were recorded. The % recovery was calculated by comparing the obtained results in serum and urine with the same concentration of the drug in water.

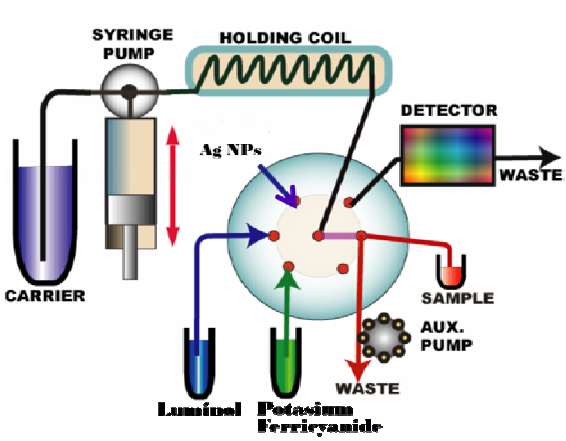

Fig. 6: Schematic diagram of SIA injection system for chemiluminescence determination of gemifloxacin; carrier stream (water); reagent 1 (luminol $2.0x10^{-3}$ mol L^{-1}); reagent 2 (potassium ferricyanide $1.0x10^{-2}$ mol L^{-1}); reagent 3 (Silver nanoparticle $5.0x10^{-3}$ mol L^{-1}; sample (gemifloxacin mesylate).

Procedures

The procedure was carried out using FIAlab-3500 instrument (Fig. 6). All experiments were computer controlled to ensure precise, timing of pump and valve movements. For each experiment all lines were first filled with carrier solution and air bubbles were removed. Prim-port program was used first to fill in the lines connected with the test solution and reagents. The sequence of the aspirated sample and reagents was automatically controlled. Mixture of 50 µL of $2.0 x10^{-3}$ moL L^{-1} luminol, 50 µL of $5.0 x10^{-3}$ mol L^{-1} Ag NPs, 30 µL sample solution and 60 µL of $1.0 x10^{-2}$ mol L^{-1} potassium ferricyanide was aspirated into the holding coil through the eight-way injection valve at a flow rate of 120 µL s^{-1} and then the mixed solution was flushed continuously into the flowthrough cell located in front of detection cell of the photomultiplier tube

(PMT). The emission from the resultant reaction was being monitored and triplicate analysis cycles were carried out per test solution, with the average CL intensity being used for determination or calibration. All measurements were carried out at ambient temperature 25±1˚C.

Calibration

Under the optimum conditions the calibration curve for determination of GFX was obtained in the range of 0.01-1000 *n*g mL^{-1}. The graph related the decrease in CL intensity vs. the concentration of tested drug solutions was plotted at 12 experimental points. The mean peak heights were obtained after triplicate sample aspiration. Conventional linear regression was utilized for fitting the curve.

Conclusion

A new simple sequential injection analysis with chemiluminescence detection based on the catalytic effect of Ag NPs on luminol-potassium ferricyanide system has been developed. The enhanced CL signal was decreased by GFX. The calibration curve was constructed by plotting the decrease in CL intensity *vs.* the concentration of GFX. Linear relationship was obtained over the concentration range 0.01-1000 *n*g mL^{-1}. The proposed method has been applied for the determination of GFX in its pure form, in pharmaceutical preparations and biological fluids. The proposed method has been proved to be fast, inexpensive, highly sensitive and precise.

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References

1. R. C. Owens and P. G. Ambrose, *Clinical Infection Disease Journal.* **41**, 144 (2005).
2. R. R. Nageswara, C. G. Naidu, P. K. Guru, R. Padiya and S. B. Agwane, *Biomedical Chromatography,* **26**, 1534 (2012).
3. L. D. Grunspan, M. Kaise, F. K. Hurtado, T. D. Costa and L. Tasso, *J*
4. *ournal of Chromatography,* **75**, 253 (2012).
5. P. N. Ranjane, S. V. Gandhi, S. S. Kadukar and K. G. Bothara, *Journal of Chromatography,* **71**, 1113 (2010).
6. B. M. H. Al-Haydiya, A. A. Khady and G. A. E. Mostafa, *Talanta,* **83**, 110 (2010).
7. M. Kaiser, L. D. Grunspan, T. D. Costa and L. Tasso, *Journal of Chromatography* B*,* **879**, 3639 (2011).
8. A. R. Rote and S. P. Pingle, *Journal of Chromatography* B, **877**, 3719 (2009).
9. E. Doyle, S. E. Fowles, D. F. McDonnell, R. McCarthy and S. A. White, *Journal of Chromatography* B, **746**, 191 (2000).
10. B. Roy, A. Das, U. Bhaumik, A. K. Sarkar, A. Bose, J. Mukharjee, U. S. Chakrabarty and T. K. Pal, *Journal of Pharmaceutical and Biomedical Analysis,* **52**, 216 (2010).
11. R. R. Ambadas and P. P. Sunita, *E-journal of Chemistry,* **7**, 344 (2010).
12. S. A. M. Ebraheem, A. A. Elbashir and H. Y. Aboul Enein, *Acta Pharmceutica Sinica* B*,* **1**, 248 (2011).
13. M. V. Krishna and D. G. Sankar, *E-Journal of Chemistry*, **5**, 493 (2008).
14. M. V. Krishna and D. G. Sankar, *E-journal of Chemistry,* **5**, 515 (2008).
15. R. Jain and J.A. Rather, *Colloids and Surfaces* A*,* **83**, 340 (2011).
16. A. Radi, A. Khfagy, A. El-Shobaky and H. El-mezayen, *Journal of Pharmaceutical and Biomedical Analysis, In press Accepted* (2012).
17. T. S. E. Kepekci and A. Onal, *Journal of Fluoresce*. **21**, 1001 (2011).
18. N. N. Atia, A. M. Mahmoud, S. R. El-Shabouri and W. M. El-Koussi, *International Journal of Analytical Chemistry*. **2013**, 1 (2013).
19. A. M. Mohaimeed, S. A. Al Tamimi, N. A. Alarfaj and F. A. Aly, *International Journal of Electrochemical Sciences,* **7**, 12518 (2012).
20. ICH *Technical Requirements for Registration of Pharmaceuticals for Human use, Complementary Guidelines on Methodology.* Washington, DC, **13** (1996).
21. J. C. Miller and J. N. Miller, Statistics for Analytical Chemistry 3rd ed. Ellis Horwood-Prentice Hall, Chichester (1993).
22. S. D. Solomon, M. Bahadory, A. V. Jeyarajasingam, S. A. Rutkowsky and C. Boriz, *Journal of Chemical Education*, **8**, 322 (2007).