Flow Injection Chemiluminescence Determination of Neomycin in Pharmaceutical Formulations

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Abstract

A simple flow injection chemiluminescence method was proposed for the determination of neomycin in commercial pharmaceutical formulations. The chemiluminescence reaction is based on the enhancing effect of neomycin on the chemiluminescence reaction between luminol-hydrogen peroxide catalysed by potassium ferrocyanide in the Triton X-100 alkaline media. Neomycin solution was injected into a water carrier stream and then mixed with hydrogen peroxide in the presence of Triton X-100 solutions. The reaction mixture was then merged at a Y-piece with a reagent stream consisting of potassium ferrocyanide and luminol in alkaline solution. The elicited chemiluminescence intensity was measured by a photomultiplier tube operated at a voltage of 880 V. The linear detection range was found to be 0.1–14 µg mL^{-1}. The relative standard deviation for 12 replicates was found to be 2.90% at 4 µg mL^{-1} of neomycin standard solution with the detection limit of 0.01 µg mL^{-1}, whilst the sample throughput of 152 h^{-1}. The proposed FI-CL method was successfully applied for the determination of neomycin in pharmaceutical formulations. The method validation has been compared versus spectrophotometric method.

1. Introduction

Neomycin (Fig. 1), produced from the fermentation of *Streptomyces fradiae*, is widely used in many variety of pharmaceutical applications [1]. Due to its advantage properties likes; water-soluble which belongs to the group of aminoglycoside antibiotics, it is mainly used in the treatment of topical infections via the inhibition of the growth of both Gram positive and Gram-negative-bacteria. However, neomycin has a narrow therapeutic range, and can be led to potentially toxic similar to others aminoglycosides, and may cause ototoxicity and nephrotoxicity [2, 3].
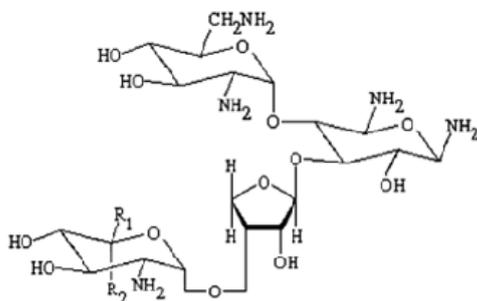
Fig. 1. Chemical structures of neomycin.

Direct UV detection of neomycin cannot be easily performed because the lack of a strong UV absorbing chromophore in the molecule. The official methods for neomycin analysis in pharmaceuticals, food or tissues normally employ biologically assays based on various detection procedures, which determine the total antibiotic activity in a sample compare to the reference standard [3, 4]. The alternative methods based on HPLC as a separation procedure have employed with several different strategies to overcome the lack of chromophore for UV-Vis spectrophotometric detection. Pre-column [5, 6] and post-column chemical derivatisation have also been reported [7]. However, derivatisation has the associated limitations of varying derivatisation efficiencies and reagent instabilities that compromise method ruggedness. HPLC procedures have been connected to other detection systems to vanquish the lack of chromophore, for example; pulse electrochemical detection [2, 8, 9], mass spectrometry [10], indirect fluorescence detection [11], evaporative light scattering detection [12, 13] and integrated pulsed amperometric detection [14]. A thin-layer chromatography-densitometric method [15] and capillary electrophoresis with indirect UV detection [16, 17] have also been described recently. However, these methods are time consuming, with low detectability and sometime not sensitive enough due to the lower sample injection required and short optical path-length for on- capillary detection [18]. Therefore, a simple, rapid and reliable analysis method is required.

Nowadays, flow injection (FI) analysis has proved to be preferable for pharmaceutical analysis and other fields due to the advantages of its low reagent and sample consumption, high sampling frequency and ease of automation [19]. Numerous reports have appeared on the use of chemiluminescence (CL) detection in FI for the determination of organic and inorganic species, and the majority of these investigations have been based on the oxidation of luminol by hydrogen peroxide [20]. There is no uncertainty that the combination of sensitive chemiluminescence (CL) detection together with the FI method could ameliorate the performance of this technique substantially for the determination of pharmaceutical or clinical sample. It obviously shows the advantages of instrument simplicity (no monochromator required), rapidity in signal detection (normally 0.1-10s), sensitivity, selectivity and ease of use, over the other

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detection systems.

The present paper describes the simple flow injection procedure in conjunction with chemiluminescence detector (FI-CL) for the sensitive determination of the total content of neomycin in commercial pharmaceutical formulations.

2. Experimental

2.1. Reagents

All reagents were of analytical reagent (AR). The mixture of 0.05% Triton X-100 and 2 mmol L^{-1} hydrogen peroxide (H_2O_2) used as a carrier solution which was prepared daily by pipetting 25 mL of 1% Triton X-100 (Fisher Scientific, UK.) and 4 mL of 250 mmol L^{-1} H_2O_2 (Merch, Germany) to a 500 mL volumetric flask and diluted to the mark with de-ionized water. The reagent stream consisting of 35 μmol L^{-1} potassium ferrocyanide ($K_4Fe(CN)_6$; Ajax, Australia) and 1.25 mmol L^{-1} luminol (Fluka, Switzerland) in 0.45 mol L^{-1} sodium hydroxide (NaOH; Lab-Scan, Ireland) solution was prepared daily by pipetting 1.75 mL of 0.01 mol L^{-1} $K_4Fe(CN)_6$ stock solution and 62.5 mL of 10 mmol L^{-1} luminol stock solution into a 500 mL volumetric flask and diluted with 0.45 mol L^{-1} NaOH. Water purification system using a compact ultra-pure water system (18.2 MΩ, Millipore, France) was used for all solution preparations.

2.2 Standard Solution and Sample Preparation

Neomycin sulfate was obtained from Sigma, Stock standard solution of neomycin (2000 μg mL^{-1}) was prepared by dissolving 0.1570 g of neomycin sulfate in 50 mL of deionised water, and was kept in a sealed amber container in a refrigerator at 4 ^oC when not in use. Working standard solution of neomycin was prepared daily by making appropriate dilution of the neomycin stock solution with deionised water and protected from light.

Commercial pharmaceutical formulations (Synalar®-N, Supralan-N cream, Flunalar-N cream) were purchased from local drug stores in Chiang Mai Province Thailand. About 1.0000 g of the each sample was accurately weighed into a glass tube. Then 6.25 mL of chloroform was added. The mixture was shaken manually before adding 5.0 mL of milli-Q water. The final mixture was mixed again for 1 min with a vortex followed by centrifugation at 3000 rpm for 3 min. The upper layer was filtered with 0.45 μm nylon filters and the appropriate concentration of sample solution was obtained by dilution with water [17].

2.3 Apparatus

The FI manifold used in all experiments is illustrated in Fig. 2. The experimental setup consisted of a two-channel peristaltic pump with rate selector (Minipuls 3, Gilson, France), a sample injection valve (Type 50, Rheodyne Inc., CA, USA) and PTFE connection tubing (0.5 mm i.d.). The chemiluminescence signal was monitored in a custom built flow-through luminometer, which consisted of a flat spiral glass flow cell (glass tubing i.d. 1.5 mm, spiral coil diameter 25 mm) mounted flush against a photomultiplier tube (PMT) (Thorn-EMI 9828SB, Electron Tubes Ltd, UK). The operational potential for the PMT was provided by a stable power supply (Thorn-EMI model PM20D, Electron Tubes Ltd, UK). The flow cell, PMT and voltage divider were encased in light tight housing. The detector output was recorded on a PC (Pentium IV) via a USB/RS-232 interfaced to the detector, with a multimeter (UNI-T, UT60F, Hong Kong) used to determine the peak maximum.

Vortex (Touch Mixer model 232, Fisher Scientific, U.S.A.) and Centrifuge (Centurion 1000 series, Labquip, England) were used

to prepare sample solution. All samples were filtered through 0.45 μm Nylon syringe filters before FI-CL analysis. A double beam spectrophotometer (Hitachi U2001, Japan) was also employed for the determination of neomycin with reference method [20] by measuring the absorbance at 521 nm.
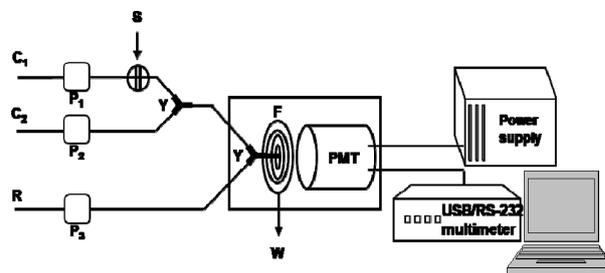
Fig. 2.   Schematic diagram of the chemiluminescence FI manifold: C_1, H_2O; C_2, 2 mmol L^{-1} H_2O_2 and 0.05% Triton X-100; R, 35 μmol L^{-1} $K_4Fe(CN)_6$ and 1.25 mmol L^{-1} luminol in 0.45 mol L^{-1} NaOH; P_1–P_3, peristaltic pump; S, Sample injection valve; Y, PTFE connection tubing; F, flat spiral glass flow cell; W, waste; and PMT, photomultiplier tube.

2.4 Procedures

Using the FI-CL manifold as shown in Fig.2, the peristaltic pump was employed to propel the reagent and carrier streams, a 100 μL aliquots of standard or sample solution containing neomycin was injected manually into a water carrier stream (flow rate 2.5 mL min^{-1}) and mixed with 2 mmol L^{-1} H_2O_2 in the presence of 0.05% Triton X-100 solution (flow rate 3.5 mL min^{-1}), the reaction mixture was then merged at a Y-piece with a reagent stream (flow rate 1.5 mL min^{-1}) consisting of 35 μmol L^{-1} $K_4Fe(CN)_6$ and 1.25 mmol L^{-1} luminol in 0.45 mol L^{-1} NaOH solution. The flow of combined reaction mixture at 7.5 mL min^{-1} was passed through a flat spiral coil flow cell, where the elicited CL intensity was measured by PMT operated at a voltage of 880 kV. The output of the PMT which was proportional to the CL intensity was monitored continuously. The FI-CL signals corresponding to peak heights were plotted versus various concentrations of neomycin.

3. Results and discussion

3.1 Manifold Design

The FI-CL manifold used for the determination of neomycin (Fig. 2) was designed to provide different reaction conditions for enhancing the CL signal generated by the reaction between luminol-H_2O_2 catalysed by $K_4Fe(CN)_6$ in the presence of Triton X-100 alkaline condition. Maximum CL intensity was obtained when the sample was injected to water carrier stream and then mixed with 2 mmol L^{-1} H_2O_2 in the presence of 0.05% Triton X-100 solution. The reaction mixture was then merged with a reagent stream consisting of 35 μmol L^{-1} $K_4Fe(CN)_6$ and 1.25 mmol L^{-1} luminol in 0.45 mol L^{-1} NaOH solution before reaching the detector.

3.2 Optimization of Experimental Variables

A series of experiments were conducted to establish the optimum experimental variables that give the highest CL sensitivity which was then used for the determination of neomycin in real samples. The conditions for the determination of neomycin were optimized by studying the influences of the various parameters, such as sample and reagent volumes, reagent and carrier flow rate, and reagent concentrations of respective

measurements.

3.2.1 Effect of Photomultiplier Tube Voltage

The effect of the PMT voltage was investigated in the range of 750 to 930 V (Fig. 3). For optimization, 20 μg mL^{-1} neomycin standard solution was used. Initially, neomycin standard solution was injected into water carrier stream which was merged with a 1×10^{-3} mol L^{-1} H$_2$O$_2$ in the presence of 0.05% Triton X-100 solution, the reaction mixture was then merged with a reagent stream consisting of 75 μmol L^{-1} K$_4$Fe(CN)$_6$ and 0.4 mmol L^{-1} luminol in 0.4 mol L^{-1} NaOH solution (flow rate of each stream was 2 mL min^{-1}). The potential of the power supply was increased stepwise and the CL signal was measured after the injection of neomycin solution at each potential step. The noise from the background current was also measured at each potential step. As expected, the detector signal increased with the quantity of applied electrode potential. However, high noise signal was observed for the applied voltage exceeding 930 V. The signal-to-noise ratio reached a maximum value at 880 V was then selected for all subsequent experiments.
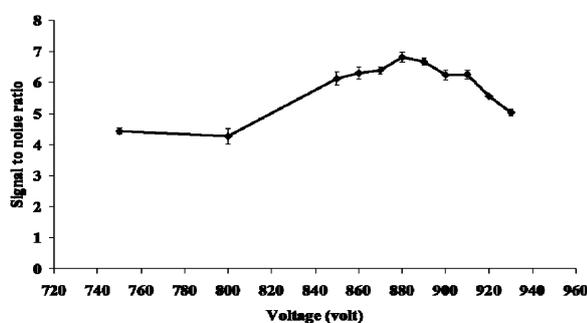
Fig. 3.   Effect of photomultiplier tube voltage (mean value ± standard deviation obtained for three measurements of each point are drawn). Conditions: Triton X-100, 0.05%; H$_2$O$_2$, 1×10^{-3} mol L^{-1}; K$_4$Fe(CN)$_6$, 75 μmol L^{-1}; luminol, 0.4 mmol L^{-1}; NaOH, 0.4 mol L^{-1}; sample volume 100 μL; neomycin standard 20 μg mL^{-1}; flow rate of each stream, 2 mL min^{-1}.

3.2.2 Effect of K$_4$Fe(CN)$_6$ Concentration on the CL Intensity

The effect of K$_4$Fe(CN)$_6$ concentration on the increment of CL intensity was examined from 5 to 45 μmol L^{-1} (Fig. 4). The signal to noise ratio was at the maximum when the concentration of K$_4$Fe(CN)$_6$ was 35 μmol L^{-1}. Beyond this concentration the signal to noise ratio decreased gradually. Thus, the concentration of K$_4$Fe(CN)$_6$ at 35 μmol L^{-1} was chosen as optimum for consequent experiments.

3.2.3 Effect of Luminol Concentration on the CL Intensity

The effect of the concentration of luminol on the CL intensity was investigated over the range of 0.60 to 1.35 mmol L^{-1} when the NaOH concentration in the reagent stream was fixed at 0.40 mol L^{-1}. It was found that the signal to noise ratio reached a maximum value when luminol concentration was 1.25 mmol L^{-1} (Fig. 5), and this concentration was used throughout the experiments.

3.2.4 Effect of NaOH Concentration on the CL Intensity

Alkaline media is one of the key factors that influence neomycin detection. By varying NaOH concentration (plus 1.25 mmol L^{-1} luminol solution) in 0.2 to 0.6 mol L^{-1} concentration range, it can be observed that maximum signal to noise ratio was

obtained at 0.45 mol L^{-1} (Fig. 6), this concentration was chosen as the optimum for consequent experiments.
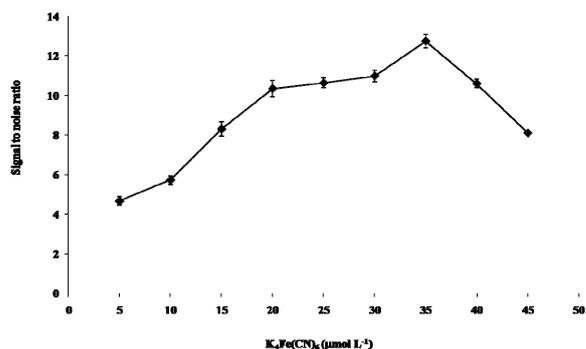
Fig. 4.   Effect of K$_4$Fe(CN)$_6$ concentration on CL intensity of the system (mean value ± standard deviation obtained for three measurements of each point are drawn). Conditions: Triton X-100, 0.05%; H$_2$O$_2$, 1×10^{-3} mol L^{-1}; luminol, 0.4 mmol L^{-1}; NaOH, 0.4 mol L^{-1}; sample volume 100 μL; neomycin standard 20 μg mL^{-1}; flow rate of each stream, 2 mL min^{-1}; PMT, 880 V.
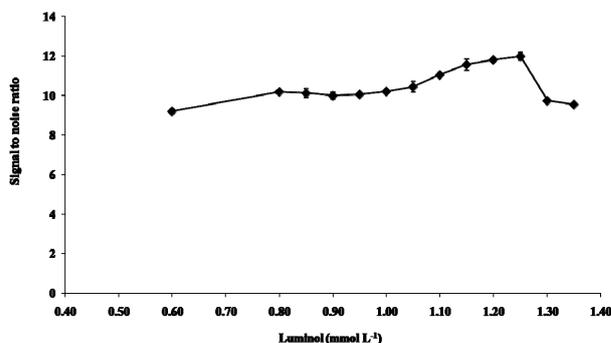
Fig. 5.   Effect of luminol concentration on CL intensity of the system (mean value ± standard deviation obtained for three measurements of each point are drawn). Conditions: Triton X-100, 0.05%; H$_2$O$_2$, 1×10^{-3} mol L^{-1}; K$_4$Fe(CN)$_6$, 35 μmol L^{-1}; NaOH, 0.4 mol L^{-1}; sample volume 100 μL; neomycin standard 20 μg mL^{-1}; flow rate of each stream, 2 mL min^{-1}; PMT, 880 V.
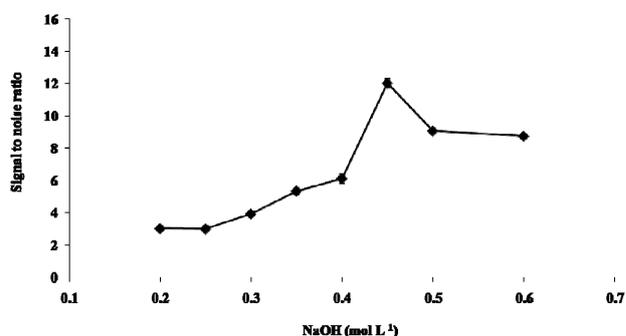
Fig. 6.   Effect of NaOH concentration on CL intensity of the system (mean value ± standard deviation obtained for three measurements of each point are drawn). Conditions: Triton X-100, 0.05%; H$_2$O$_2$, 1×10^{-3} mol L^{-1}; K$_4$Fe(CN)$_6$, 35 μmol L^{-1}; luminol, 1.25 mmol L^{-1}; sample volume 100 μL; neomycin standard 20 μg mL^{-1}; flow rate of each stream, 2 mL min^{-1}; PMT, 880 V.

3.2.5 Effect of H_2O_2 Concentration on the CL Intensity

The dependence of signal to noise ratio on H_2O_2 was examined in the 0.5 to 2.5 mmol L^{-1} concentration range. The maximum signal to noise ratio can be observed when the H_2O_2 concentration was 2 mmol L^{-1} (Fig. 7). Therefore 2 mmol L^{-1} was chosen as the optimum H_2O_2 concentration.
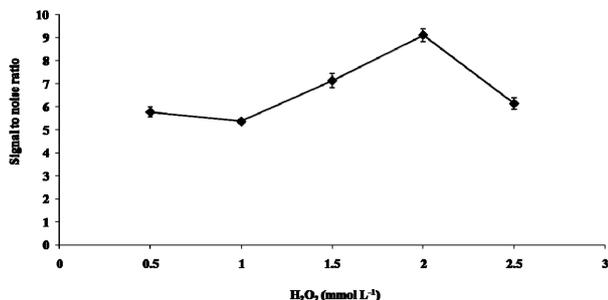
Fig. 7. Effect of H_2O_2 concentration on CL intensity of the system (mean value ± standard deviation obtained for three measurements of each point are drawn). Conditions: Triton X-100, 0.05%; $K_4Fe(CN)_6$, 35 μmol L^{-1}; luminol, 1.25 mmol L^{-1}; NaOH, 0.45 mol L^{-1}; sample volume 100 μL; neomycin standard 20 μg mL^{-1}; flow rate of each stream, 2 mL min^{-1}; PMT, 880 V.

3.2.6 Effect of Triton X-100 Concentration on the CL Intensity

The influence of Triton X-100 on the CL intensity was studied in the range of 0.0005 to 0.30%. The maximum CL intensity was obtained (Fig. 8) when the concentration of Triton X-100 was 0.05%. Thus, 0.05% Triton X-100 was chosen for consequent research work.
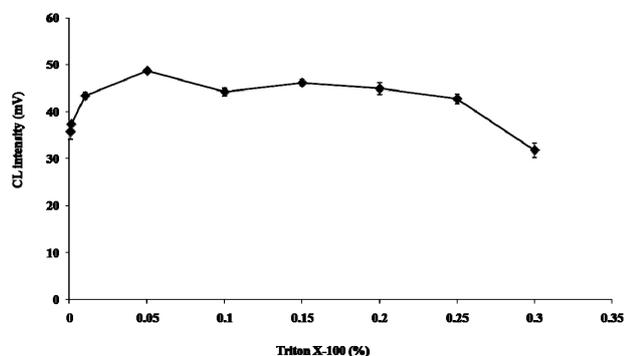
Fig. 8. Effect of Triton X-100 concentration on CL intensity of the system (mean value ± standard deviation obtained for three measurements of each point are drawn). Conditions: H_2O_2, 2 mmol L^{-1}; $K_4Fe(CN)_6$, 35 μmol L^{-1}; luminol, 1.25 mmol L^{-1}; NaOH, 0.45 mol L^{-1}; sample volume 100 μL; neomycin standard 20 μg mL^{-1}; flow rate of each stream, 2 mL min^{-1}; PMT, 880 V.

3.2.7 Effect of Surfactant

Surfactants have the violent effect on the CL system, their effect may be formation of micelle, changing dielectric constant of the solution, facilitation of dissolubility, build up the stability, improvability acid-alkalescence of the solution, etc. [21]. Apart from Triton X-100, the effect of cetyl trimethyl ammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) on the signal of neomycin was also studied. Various concentrations of CTAB or SDS, (0.005-0.3% surfactant) were dissolved in 2 mmol L^{-1} H_2O_2 carrier solution. It was found that SDS could

steeply decrease the analyte signal when the concentration was increased stepwise, whereas CTAB showed no effect on the CL intensity and gave CL intensity lower than Triton X-100. Therefore, Triton X-100 was used for surfactant in following studies.

3.2.8 Effect of Sensitizers

Based on the observation that some of the fluorescing compounds can be utilized for energy transfer in the CL reactions with an enhancement of the intensity [22, 23], difference fluorophores were examined for obtaining maximum yields in CL intensity. 0.05 to 2 μg mL^{-1} of the studied sensitizers, such as rhodamine-B, fluorescein and quinine sulfate, by dissolving in water stream. It was found that all sensitizers had little or no effect on CL intensity. Therefore, it is not necessary to use these sensitizers for enhancing the intensity of the neomycin intensity.

3.2.9 Effect of Flow Rate and Injection Volume

The flow rate is an important parameter in FI-CL method as the time taken to transfer the excited product into the flow cell is critical for maximum collection of the emitted light [24]. The flow rate of the reagent solution was optimized in order to obtain satisfactory CL intensity. The flow rate is conveniently controlled by the peristaltic pump. The flow rates of three streams were studied over the range 1.0-3.5 mL min^{-1}. It was found that the best CL intensity was obtained at the flow rates of 2.5 mL min^{-1} for water carrier stream, 3.5 mL min^{-1} for 2 mmol L^{-1} H_2O_2 in the presence of 0.05% Triton X-100 solution and 1.5 mL min^{-1} for reagent stream that consisting of 35 μmol L^{-1} $K_4Fe(CN)_6$ and 1.25 mmol L^{-1} luminol in 0.45 mol L^{-1} NaOH solution.

It is necessary to optimize the injection volume to achieve the desired sensitivity. Since the amounts of sample injected into the FI system should be sufficient to permit effective CL reaction. The influence of the sample or standard volume on the CL intensity was investigated by injecting the standard neomycin solution (20 μg mL^{-1}) with varying volumes in the range of 50–500 μL. It was found that the CL intensity continued to be gradually increased with increasing injection volume up to 500 μL but injection volume at 100 μL produced sharper peak, smooth base line and rapid sample throughput. Therefore, a volume of 100 μL was selected for all experiments.

3.3 Analytical Application

3.3.1 Analytical Figures of Merit

Under the optimum conditions for the determination of neomycin, the calibration graph was found to be linear over the range of 0.1–14 μg mL^{-1}. The regression equation was I = 3.8144C+40.306 (C is the neomycin concentration, μg mL^{-1}) with a correlation coefficient (r^2) of 0.9957. The detection limit of the method was found to be 0.01 μg mL^{-1} (calculated at three times the standard deviation of the noise) and the relative standard deviation for 12 samples at 4 μg mL^{-1} was 2.90%. The sample throughput for neomycin was found to be 152 h^{-1}.

3.3.2 Effect of Interferences

In order to assess the possible analytical application of the proposed method to the analysis of neomycin in pharmaceutical formulations, the influences of some possible interferences from exipients and preservatives in samples were investigated. The effect of common exipients and preservatives normally used in pharmaceutical formulations were examined by preparing the

synthetic mixtures containing 4 µg mL^{-1} neomycin standard solution in the presence of different concentrations of common exipients (propylene glycol, polyethylene gycol, carboxymethyl cellulose and methylcellulose) and preservatives (methylparaben and propylparaben). The effect of interference was not count to be significant if it caused a relative error of less than 5% at 4 µg mL^{-1} of neomycin. The tolerable concentrations are shown in Table 1. It can be seen that all interferences had no effect on the quantitative analysis of neomycin even through they are present at a 100 times (exipients) to neomycin. Therefore common excipients in formulations did not interfere with the proposed FI-CL method.

3.3.3 Analysis of Pharmaceutical Formulations

In order to evaluate the accuracy of the method, three anticeptic creams were analyzed by the proposed FI-CL procedure under optimum experimental conditions. The spectrometric method [20] was used as reference method to identify neomycin in creams. The comparison between FI-CL and spectrometric method results were shown in Table 2. The spectrophotometric results were found to be statistically indistinguishable from the FI-CL results at 95 % confidence level by the Student t-test (*t-values* 2.18 - 2.58) as regards to accuracy and precision [25].

The average recovery of each neomycin drug measured by the proposed method, after spiking three different amounts of neomycin in each individual samples. The recoveries were in the range 101.67-103.12%.

The proposed FI-CL method was successfully applied to the determination of neomycin in a commercial pharmaceutical preparations. Unlike the reference method [20] was based on the oxidation of studied drugs by a known excess of potassium permanganate in acidic medium and estimating the unreacted permanganate with amaranth dye in the same acid medium at 521 nm, although these methods provided near LOD (0.009 µg mL^{-1}) but the reaction seems to be suitable in a very limited concentration range (5-10 µg mL^{-1}) and time consuming.

Comparison of the proposed FI-CL method with the selected earlier reported methods which used for determining neomycin indicated that the proposed method is more sensitive than the high performance liquid chromatographic methods [26, 27] but as sensitive as the spectrophotometric method [20]. The proposed method showed equal accuracy with all selected earlier reported methods as shown in Table 3. Moreover, the proposed method has advantage over other methods in that consumption of sample, reagent and organic solvent also waste generation are tremendously reduced.

4. Conclusions

A simple FI-CL procedure involving reaction between luminol-H_2O_2 catalysed by $K_4Fe(CN)_6$ in the presence of Triton X-100 in alkaline media has been developed for the determination of neomycin in pharmaceutical formulations. The proposed method does not require sophisticated instrumentation, and the methodology is simple, rapid and robust with a high sample throughput and low detection limit. Therefore, it provides a good alternative to the published procedure for the quality control of bulk drugs and their pharmaceutical formulations. Moreover, common reagents were used in small amounts, none of which was a serious problem to the environment. The obtained results are in good agreement with those obtained by the spectrophotometric method.

Table 1 The effect of exipients and preservatives on CL signal of 4.0 µg mL^{-1} neomycin

Interferences	Concentration (µg mL^{-1})	% Relative CL intensity	% R.S.D. (n=3)
Methylparaben	0	100	2.04
	1	100.13	2.76
	2	101.49	2.69
	2.5	104.93	1.28
Propylparaben	0	100	1.57
	0.5	102.61	1.66
	1	97.52	1.41
Popylene glycol	0	100	3.08
	4	99.28	0.44
	40	98.23	0.67
	400	96.71	0.70
Polyethylene glycol	0	100	1.84
	4	100.58	1.42
	40	104.17	0.24
	400	104.76	0.14
Carboxymethyl cellulose	0	100	1.84
	4	99.78	1.12
	40	102.52	0.78
	400	103.48	2.80
Methyl cellulose	0	100	0.67
	4	101.26	0.43
	40	102.17	0.41
	400	104.62	0.23

Table 2 Comparative determination of neomycin in pharmaceutical formulations using the proposed FI-CL method and reference method

Sample	Declared	Amount found (mg) ± SD[a]	
		FI-CL method	Reference method[b]
Supralan-N cream	Each 1 g contains neomycin base 2.89 mg	2.76±2.57	2.93±0.09
Flunalar-N cream	Each 1 g contains neomycin base 3.5 mg	3.57±1.34	3.47±0.06
Synalar®-N	Each 1 g contains neomycin base 3.5 mg	3.35±0.44	3.22±0.07

[a] Standard deviation from three determinations.
[b] Spectrophotometric method [20].

Table 3. Comparison of the analytical figures of merit of the proposed FI-CL method with some earlier reported methods

Techniques	Pharmaceutical compounds	Linear range ($\mu g\ mL^{-1}$)	r^2	% Recovery	LOD ($\mu g\ mL^{-1}$)	LOQ ($\mu g\ mL^{-1}$)	Reference
1. Spectrophotometric method: measurement of the unreacted permanganate with amaranth dyne at 521 nm	Neomycin in pharmaceutical formulation	5-10	0.9989	99.96	0.009	0.029	[20]
2. HPLC using evaporative light scattering detection (LC-ELSD)	Neomycin in sterile pharmaceutical formulation	3-11	0.9999	99.45	0.6	-	[26]
3. HPLC	Neomycin in plasma	0.1-10	0.9986	106.7	-	10 ng mL^{-1}	[28]
4. HPLC determination of aminoglycoside antibiotics	Neomycin in pig feed	0.4-320	> 0.996	94.47	0.15	-	[27]
5. The proposed FI-CL method	Pharmaceutical formulation (neomycin creams)	0.1-14	0.9957	101.67	0.01	-	-

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