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Analysis of fexofenadine in pharmaceutical formulations using tris (1,10-phenanthroline)–ruthenium(II) peroxydisulphate chemiluminescence system in a multichip device

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ABSTRACT: A simple, rapid and sensitive method has been developed for the analysis of fexofenadine (FEX) in pharmaceutical formulations, using a tris(1,10-phenanthroline)–ruthenium(II) [$Ru(phen)_3^{2+}$] peroxydisulphate chemiluminescence (CL) system in a multichip device. Various parameters that influence the CL signal intensity were optimized. These included pH, flow rates and concentration of reagents used. Under optimum conditions, a linear calibration curve in the range 0.05–5.0 µg/mL was obtained. The detection limit was found to be 0.001 µg/mL. The procedure was applied to the analysis of FEX in pharmaceutical products and was found to be free from interference from concomitants usually present in these preparations. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: microfluidics; multichip; fexofenadine; chemiluminescence; tris(1,10-phenanthroline)–ruthenium(II); ammonium peroxodisulphate

Introduction

(±)-2-[4-{1-hydroxy-4-[4-(hydroxydiphenylmethyl) piperidino] butyl}phenyl]-2-methylpropanoic acid (FEX; Fig. 1) is a selective H1-receptor antagonist having antihistaminic and anti-inflammatory properties. The drug cannot pass through the blood–brain barrier and therefore does not cause sedation (1,2).

Several methods have been reported for the analysis of FEX in biological fluids and pharmaceutical formulations. HPLC and capillary electrophoresis separation techniques coupled to a UV detection system have been reported (2–6). However, these methods are not very sensitive and in many cases the run time is very long, reaching up to 60 min. Methods based on HPLC coupled to a fluorescence (FL) detection system have also been reported (1,7). Although this method is selective and very sensitive (detection limit 1 ng/mL), a large sample volume was used (100 µL) and the total run time was long (13 min).

Mass spectrometric methods for the analysis of FEX have also been developed. The sensitivity was found to be comparable to the FL method (8,9). However, these methods are very costly and are only justifiable for the analysis of complex samples that require extensive separation, such as biological fluids. Pharmaceutical formulations are usually less complex and therefore a simpler method should be used.

Few methods for the quantitative determination of FEX using spectrophotometric techniques have been reported (10,11). These methods suffer from high detection limits and lack of automation. Therefore, there is a need for a fast, simple, sensitive and economical method, especially for a busy quality control laboratory or in-process control.

Chemiluminescence (CL) has been shown to be a powerful analytical technique with a high sensitivity, wide linear range, rapid and reproducible means of detection and simpler instrumental set-up. Currently, the most commonly used technique for CL methods is flow-injection analysis (12). However, the major drawback of flow-injection-based CL techniques is the excessive consumption of expensive reagents, as high flow rates are required to realize a useful analytical signal (13).

A CL method based on microfluidics can eliminate this problem or reduce it significantly. 'Microfluidics' refers to any device where fluids can be driven in a network of µm-sized channels etched into a solid substrate (14). Using micromachining, a network of channels is fabricated in a planar substrate that can perform sample injection, processing, pretreatment and separation. Flow of fluids through the channels can be achieved hydrodynamically, using a syringe pump, or electro-osmotically, by applying an electrical field along the channels (15). The miniaturized techniques use µL to pL volumes of solvents compared to mL commonly used in the standard methods; hence, they reduce chemical consumption by a factor of 1000–10 000 000. This leads

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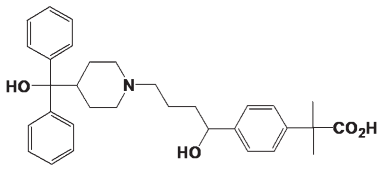

Figure 1. Chemical structure of fexofenadine.

to the generation of minute hazardous wastes and reduces the cost of analysis. Additionally, microfluidics has many unique advantages afforded by the reduced dimensions compared to standard methods. The reduction in the reaction vessel dimensions leads to a high degree of control and sensitivity, better selectivity and reduced analysis time (16–17).

A limited number of analytical methods using CL as a detection system for microfluidics have been reported for the determination of active ingredients in pharmaceutical samples, e.g. atropine and pethidine were determined on a chip using a tris(2,2′-bipyridine)–ruthenium(II) and cerium(IV) sulphate CL system. Detection limits were found to be 3.8×10^{-9} and 7.7×10^{-8} mol/L for atropine and penthidine, respectively, and sample throughput was 60 samples/h. However, the system was not tested on real samples (18). Analyses of uric acid in human serum and urine samples were carried out without enzyme, using luminol and ferricyanide. A detection limit of 0.5 mg/L was obtained and the relative standard deviation (RSD) was 4.42% (19). Recently, chlorpheniramine maleate was analysed using a $Ru(phen)_3^{2+}$ peroxydisulphate chemiluminescence (CL) system in a multichip device (13).

Here we developed a multichip set-up in which chip 1 was used as the microphotochemical reactor. In this chip, $Ru(phen)_3^{2+}$ was mixed with peroxydisulphate to produce $Ru(phen)_3^{3+}$. The resultant solution was pumped to meet FEX solution in chip 2. A strong CL signal was then obtained when FEX reacted with $Ru(phen)_3^{2+}$. FEX possesses a tertiary amine and it is well known that such compounds are capable of enhancing the $Ru(phen)_3^{2+}$–peroxydisulphate CL (13). Therefore, this system was utilized to develop an analytical method for the assay of FEX in pharmaceutical products. To the best of our knowledge, this is the first CL method reported for the analysis of FEX. The method is sensitive, simple, fast, versatile and cost-effective. The detection limit was found to be 0.001 µg/mL, which is comparable to the most sensitive reported methods. However, the sample throughput in the developed method is 13 times higher and up to 60 runs can be carried out in 1 h, consuming only 20 µL 2.5 mmol/L of $Ru(phen)_3^{2+}$ per run. Moreover, the simple instrumentation used makes the whole set-up portable and it can be fixed in a short time.

Experimental

Reagents

Ammonium peroxodisulphate and potassium dihydrogenphosphate were purchased from Kanto Chemical Co. (Tokyo, Japan). Tris(1,10-phenanthroline)–ruthenium(II) chloride [$Ru(phen)_3^{2+}$] was purchased from Aldrich (Gillingham, UK). FEX was a gift from the Quality Control Laboratory, Ministry of Health, Muscat, Sultanate of Oman. Ultrapure water was obtained from a MilliQ water system (Millipore) and was used for the preparation of solutions.

Standard drug solutions

A stock standard solution (5000 ppm) of FEX was prepared separately by dissolving 25 mg pure drug in a 5 mL volumetric flask in deionized water. The stock solution was kept in cold dark place. Working solutions were prepared daily by appropriate dilutions.

Sample preparation

Tablet samples were prepared by crushing 10 tablets previously weighed out and dissolving an amount of powder equivalent to 30 mg FEX. The solution was placed in an ultrasonic bath for 30 min and then filtered. An appropriate volume was measured and transferred to volumetric flasks. The solution was then diluted in phosphate buffer, pH 7.

Apparatus

Serpentine and teardrop microfluidic chips, Fluidic Connect 4515 and fused silica capillary, were from Micronit (The Netherlands). Syringe pumps were from Basi Bee (USA). The detector was a photomultiplier tube (PMT; H7155-2, Hamamatsu, Japan) connected to a PC via a Counting Unit (C8855; Hamamatsu). A pH meter (Hanna HI18314, Romania) was used. The official USP 33N 28 HPLC procedures for the analysis of the two tablet formulations was performed using a Breez 2 system (Waters Corp., Milford, MA, USA) equipped with a UV-visible detector and controlled using Breeze software (20).

Multichip set-up

The multichip set-up used is shown in Fig. 2. Using Fluidic Connect 4515 and silica capillaries, chip 1 (internal volume, 13 µL) and chip 3 (internal volume, 6 µL) were connected to syringe pumps. A torch was placed on top of chip 3 to catalyse the oxidation of the $Ru(phen)_3^{2+}$ to $Ru(phen)_3^{3+}$. Chip 1 was used to prepare and pump the reagent mixture, while chip 3 was used for pumping the sample. The two chips were connected to chip 2 (internal volume, 2 µL) via a silica capillary (i.d. 150 µm, length 20 cm) where detection took place. The PMT was placed on top of chip 2 and its position was optimized to collect the maximum CL signal. The detection chip was placed in the dark.

Procedure

Two syringe pumps were used, the first for the buffer and the analyte and the second for the CL reagents, $Ru(phen)_3^{2+}$ and peroxydisulphate. Initially, the CL reagents were infused in chip 1 at a flow rate of 20 µL/min for each reagent (total flow rate, 40 µL/min). After 10 s, the buffer and the analyte were infused for 40 s. Maximum signal was reached within a few seconds. The average of all the points between 35 and 50 s was calculated and then the average of four runs was used in the calculations. In all the measurements, the CL signal intensity of the analyte was measured after subtracting the background signal.

Result and discussion

Optimization

The various parameters that may influence the CL signal intensity were optimized. These parameters are discussed below.

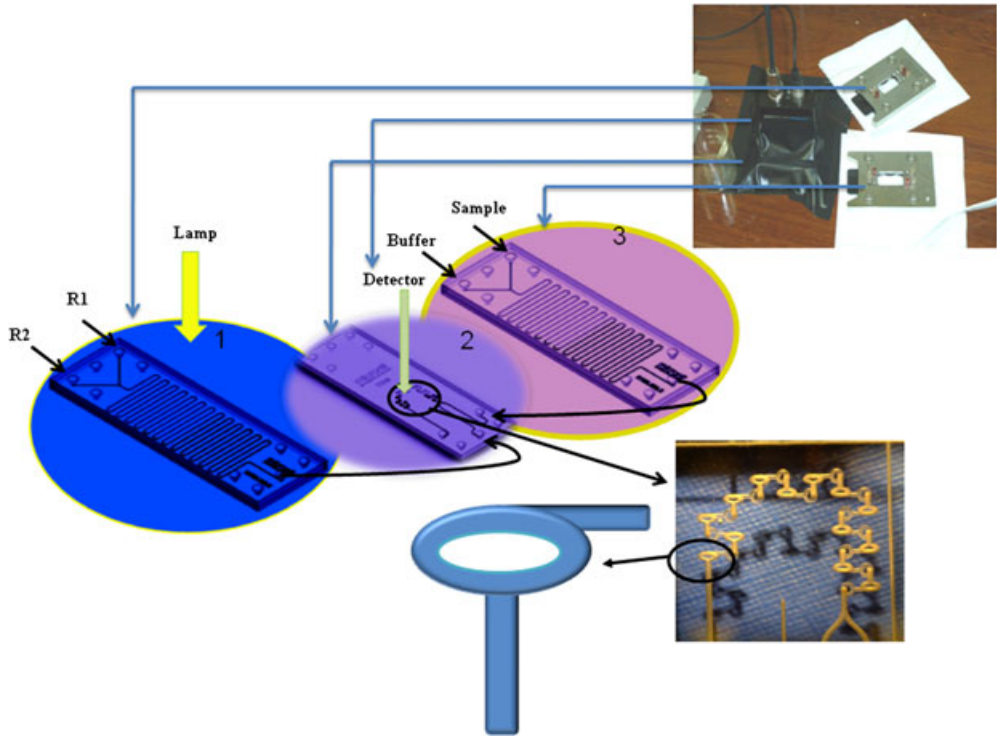

Figure 2. Multichip set-up used. R1, $Ru(phen)_3^{2+}$; R2, peroxydisulphate. Chip 1 is used as a photoreactor, chip 2 is the detection chip and chip 3 is for sample preparation. Insets: (top) multichip set-up; (bottom) teardrop chip. The schematic is for a single mixing unit in a teardrop chip.

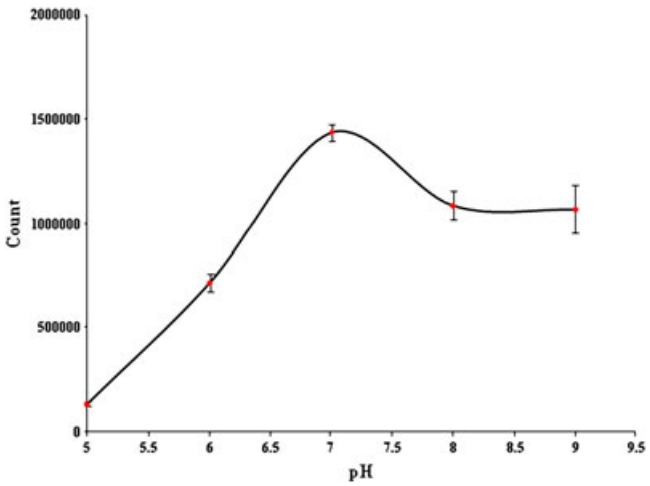

Figure 3. Effect of phosphate buffer pH on CL intensity (-●-). FEX, 1 ppm; Ru $(phen)_3^{2+}$, 2.5 mmol/L; peroxydisulphate, 1.0 mmol/L; flow rates for CL reagents, sample and buffer, 20 µL/min.

Effect of pH

The pH is expected to have an important effect on the CL signal, due to presence of an amine group and a carboxylic acid group in FEX. Using phosphate buffer system, the pH range 5–9 was examined. As shown in Fig. 3, a weak CL was observed at pH 5, in fact no CL signal was observed at pH < 5. On the other hand, the CL emission increases as the pH reached 7, where the maximum signal was obtained. The signal decreased when the pH increased further towards the basic range. At higher pH the reagent $Ru(phen)_3^{3+}$ was consumed by the hydroxide ions, resulting in a decrease of the CL signal obtained due the analyte.

Effect of the flow rate

The $Ru(phen)_3^{2+}$ peroxydisulphate CL system is known to be rapid (21). Therefore, the flow rate applied is critical. A low flow rate would not support the rapid CL system used and would result in low sensitivity, while a high flow rate would not provide sufficient residence time for the reaction to occur.

Initially, the total flow rate of the buffer and the sample (FEX 1 ppm) was varied in the range 3–50 µL/min (total flow rate varied in the range 6–100 µL/min), while the flow rate of the CL reagents was fixed at 10 µL/min. The effect of the sample and buffer flow rates on the CL signal intensity is shown in Fig. 4. It is clear from this figure that the CL intensity increases as the flow rate increases. However, at a flow rate of 20 µL/min the CL signal reaches maximum. Higher flow rates exhibit an insignificant effect on the CL signal. Therefore, the flow rate of the buffer and the sample was fixed at 20 µL/min. Next, the flow rate of CL reagents was varied in the range 3–50 µL/min, while the flow rate of sample and buffer was maintained at 20 µL/min. The CL signal intensity was affected considerably by the change in the flow rate of CL reagents and it increases with the increase of the flow rate. This is because the reaction is rapid and hence residence time does not play an important role in the enhancement of the CL signal. At optimum conditions, the residence time in the detection chip is only 1.5 s. Two other factors play an important role, the amount of CL reagent that passes through the channel and the completeness of the mixing

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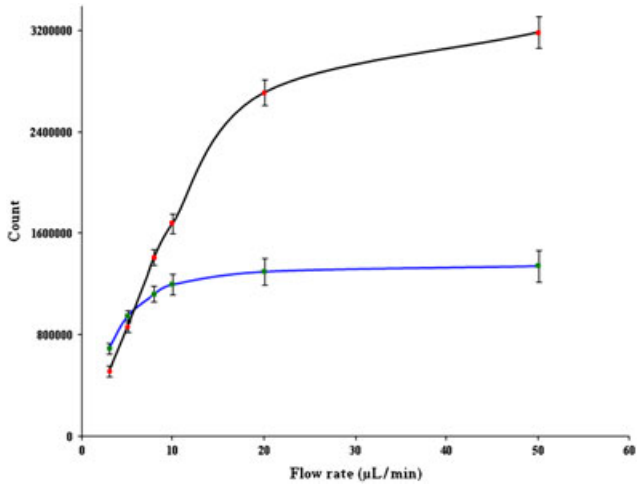

Figure 4. Effect of sample and flow rates on CL intensity (-■-). Effect of $Ru(phen)_3^{2+}$ and peroxydisulphate flow rates on CL intensity (-●-). FEX, 1 ppm; phosphate buffer, pH 7; $Ru(phen)_3^{2+}$, 2.5 mmol/L; peroxydisulphate, 1.0 mmol/L.

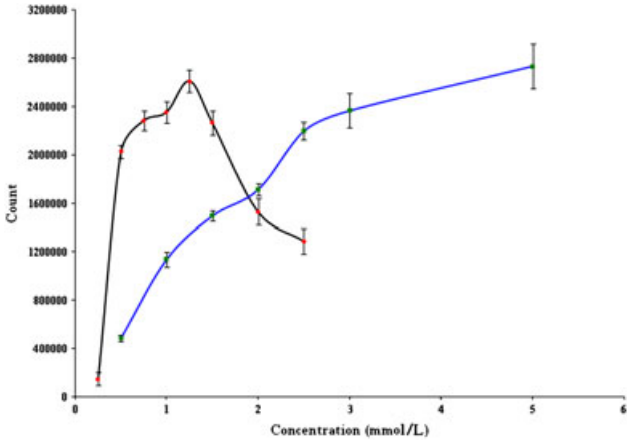

Figure 5. Effect of peroxydisulphate concentration on CL signal intensity (-●-). Effect of $Ru(phen)_3^{2+}$ concentration on CL signal intensity (-■-): flow rate, 20 µL/min for each pump; FEX, 1 ppm; phosphate buffer, pH 7.

between the sample and the CL reagents. Obviously in such systems the better the mixing between reagents and analytes, the higher the CL signal.

Increasing the flow rate results in an increase in the signal, primarily because the CL signal obtained will not be allowed to decay. Recently, we demonstrated the use of teardrop geometry for the enhancement of CL signals (13). The enhancement in signal intensity observed when a teardrop chip was used is due to the enhancement in the mixing processes that occurs in the teardrop chip. Because of the efficiency of mixing in the detection chip, higher flow rates produced higher signals.

The increase in the signal intensity at high flow rates also indicates that photochemical oxidation of $Ru(phen)_3^{2+}$ by peroxydisulphate in chip 1 requires a very short residence time and can occur even if the residence time is only 7.8 s. The optimum flow rate was set at 20 µL/min, although higher flow gives higher CL signal, but this leads to excessive consumption of $Ru(phen)_3^{2+}$.

Effect of CL reagent concentrations

A sharp increase was observed in the CL signal when the concentration of the oxidant changed from 0.25 to 0.50 mmol/L, as shown in Fig. 5. The CL signal then increased slightly as the concentration of the oxidant increased to 1.25 mmol/L, followed by a decrease in the CL signal at higher concentrations. Therefore, a concentration of 1.25 mmol/L of oxidant was used.

The effect of $Ru(phen)_3^{2+}$ concentrations was investigated in the range 0.50–5.0 mmol/L. The results are shown in Fig. 5. Increasing the concentration of $Ru(phen)_3^{2+}$ resulted in significant increase in the CL signal. This was expected, because with an increase in the concentration of $Ru(phen)_3^{2+}$ the total number of emitting species increases. However, it was observed that at concentrations >2.5 mmol/L the reproducibility worsened and chip cleaning took a longer time, therefore 2.5 mmol/L was selected as the optimum concentration of $Ru(phen)_3^{2+}$.

Analytical appraisal

The performance of the proposed method was investigated using the optimized conditions. A linear calibration curve was obtained in the range 0.05–5.0 µg/mL, giving excellent correlation coefficients ($R^2 = 0.9993$) and high precision with RSD values <1%. A definition of the detection limit in this work was based on the analyte concentration that gives the response signal (ΔI_{CL}) three times that of the standard deviation (SD) of the blank signal (22). The detection limit was found to be 0.001 µg/mL, although the detection chip volume is only 2.0 µL. The high sensitivity of the CL system is not only due to the chemical nature of the molecule but also due the enhancement in the mixing process that occurs in the teardrop chip (detector chip) between the CL reagents and the analyte. The mixing mechanism is discussed in detail elsewhere, but briefly, the enhancement is due to three mechanisms, flow folding, chaotic advection and increasing the number of laminates using a multilevel laminating mixer (13). The limit of quantifications was calculated based on the analyte concentration that gives the response signal (ΔI_{CL}) 10 times that of the SD of the blank signal (23).

Interference study

To apply the suggested method to the analysis of FEX in pharmaceutical formulations, the interference of some common chemicals in these samples was investigated by adding these chemicals to a solution containing 0.3 mg/L FEX. The tolerable concentration for interference at the 5% level was >1.5 mg/L for sodium benzoate, methyl parabenzoate and propyl parabenzoate, which are common preservatives found in syrups; it was >200 mg/L for glucose and sucrose.

Analytical applications

The developed method was then successfully applied for the determination of FEX in commercial tablets. Tablets from two manufacturers were analysed. The first tablet contained several additives, such as microcrystalline cellulose, starch, croscarmellose, sodium and magnesium stearate, macrogel 400, titanium dioxide, silica and other components, while the other contained cellulose, starch, croscarmellose, hydroxypropyl methylcellulose, polyethelene glycol, talc and other components. Excellent recoveries were obtained for both samples analysed, as shown in Table 1. This indicates that the proposed method can be used

Table 1. Determination of FEX in pharmaceutical samples by the developed method ($n = 4$)

Formulations	Claimed (mg)	Amount found ± SD (mg)	*t*-test	*F*-test
Tablet A (per tablet)	180.0	181.3 ± 1.3	2.236[a]	1.23[b]
Tablet B (per tablet)	120.0	122.9 ± 4.7	1.380[a]	6.12[b]

[a]Confidence limits at $p = 0.05$ and three degrees of freedom (df) ($t = 3.182$).
[b]Tabulated *F*-value for $p = 0.05$ and $df_1 = df_2 = 3$ (9.28).

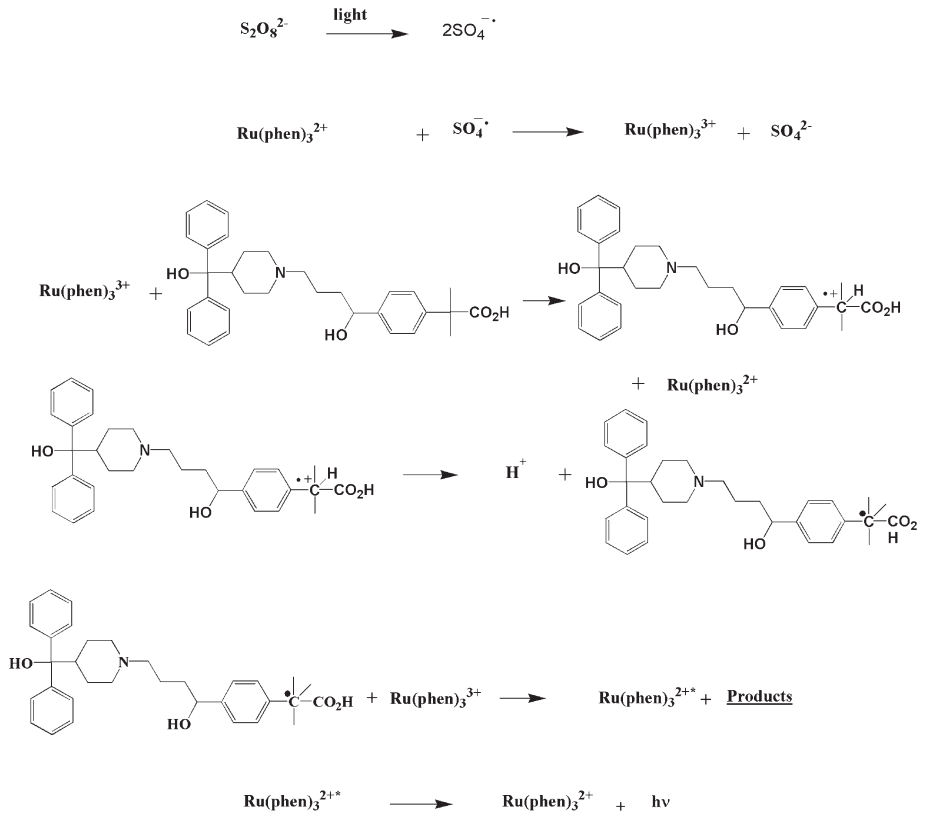

Figure 6. The proposed mechanism for the CL reaction of FEX with $Ru(bpy)_3^{2+}$.

for quantitative analysis of FEX without interference from the other ingredients present in the two samples.

For comparison, the two commercial tablets samples were also determined using USP 33N 28 procedures (20). These determinations were carried out on the same batch of samples. The results obtained were compared statistically using Student's *t*-test and the *F*-test. The experimental values did not exceed the theoretical ones in either test, which indicates that there were no significant difference between the prepared CL method and the standard official method.

Proposed reaction mechanism

$(Ru(bipy)_3)^{3+}$ CL has proved to be a very sensitive detection system for compounds which contain a secondary or tertiary aliphatic amine (27). FEX contains a tertiary amine; thus, the proposed reaction mechanism is presumably similar to that reported previously for amine determination utilizing its CL reaction with $Ru(bipy)_3^{2+}$(23–25). The photochemical cleavage of the peroxydisulphate anion produces the powerful oxidizing agent $SO_4^{\bullet-}$, which oxidizes $Ru(phen)_3^{2+}$ to $Ru(phen)_3^{3+}$. The oxidation of amines produces amine cation radicals which have been reported to possess short lifetimes (26). These cation radicals lose a proton to form neutral amine radicals that possess sufficiently high energy. Collision of these neutral radicals with $Ru(phen)_3^{3+}$ molecules produces excited state $Ru(phen)_3^{2+*}$ molecules, which are believed to be the light-emitting species. These excited molecules emit strong orange light around 620 nm. Figure 6 summarizes the steps of the mechanism of CL reaction of FEX with the $Ru(phen)_3^{2+}$ peroxydisulphate system.

Conclusion

The development of a microfluidics method has been described for the determination of FEX in pharmaceutical preparations, based on the enhancement of the $Ru(phen)_3^{2+}$–peroxydisulphate CL system by FEX. The proposed method was applied successfully to the analysis of FEX in commercial pharmaceutical samples. The use of a multichip system enabled the development of versatile

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analytical system that consumes minute amounts of reagents while preserving higher sensitivities. In addition to the reduced cost, the precision obtained suggests that such techniques would be the right choice to replace currently used techniques for a busy quality control laboratory for the analysis of pharmaceutical products.

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