Talanta 85 (2011) 906–912

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

High throughput method for the analysis of cetrizine hydrochloride in pharmaceutical formulations and in biological fluids using a tris(2,2′-bipyridyl)ruthenium(II)–peroxydisulphate chemiluminescence system in a two-chip device

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ARTICLE INFO

Article history:
Received 2 January 2011
Received in revised form 28 April 2011
Accepted 28 April 2011
Available online 6 May 2011

Keywords:
Microfluidics
Two-chip device
Cetrizine
Chemiluminescence
Tris(2,2-bipyridyl)ruthenium(II)
Ammonium peroxodisulphate

ABSTRACT

A fast, economic and sensitive chemiluminescence (CL) method has been developed for the analysis of cetrizine hydrochloride (CET) in pharmaceutical formulations and in biological fluids. The CL method is based on the oxidation of tris(2,2′-bipyridyl)ruthenium(II) (Ru (bipy)$_3^{2+}$) by peroxydisulphate in a two-chip device. Up to 180 samples can be analysed per hour, consuming only minute quantities of reagents. Three instrumental setups were tested to find the most economical, sensitive and high throughput setup. In the first setup, a continuous flow of sample and CL reagents was used, whereas in the second setup, a fixed volume (2 μL) of (Ru (bipy)$_3^{2+}$) was introduced into a continuous infusion of peroxydisulphate and the sample. In the third design, a fixed volume of sample (2 μL) was injected while the CL reagents were continuously infused. Compared to the first setup, a 200% signal enhancement was observed in the third setup. Various parameters that influence the CL signal intensity, including pH, flow rates and reagent concentrations, were optimized. A linear response was observed over the range of 50 μg L^{-1} to 6400 μg L^{-1} (R^2 = 0.9959) with RSD values of 1.1% (n = 15) for 1000 μg L^{-1}. The detection limit was found to be 15 μg L^{-1} (S/N = 3). The amount of consumed sample was only 2 μL, from which the detected amount of CET was found to be 6.5 × 10^{-14} mol. This procedure was successfully applied to the analysis of CET in pharmaceutical formulations and biological fluids.

1. Introduction

CET (±-[2-(4-[(4-chlorophenyl) phenylmethyl]-1-piperazinyl) ethoxy] acetic acid, dihydrochloride) is a second generation anti-histaminic drug used for the symptomatic treatment of seasonal allergic rhinitis. It selectively inhibits histamine H1-receptors. This drug cannot pass through the blood–brain barrier and therefore does not cause sedation [1].

A literature survey reveals a variety of analytical methods for the analysis of CET in various samples. Spectrophotometric techniques have been reported for the determination of CET in raw material and in dosage forms [2,3]. However, these methods suffer from high detection limits and the lack of automation. High performance liquid chromatography (HPLC) [1,4–9], capillary electrophoresis (CE) [7,10,11], liquid chromatography–mass spectrometry (LC–MS) [12] and liquid chromatography–tandem mass spectrometry (LC–MS/MS) [13–16] have all been reported for the analysis of CET in pharmaceutical formulations and in biological fluids. The sensitivity, estimated sample throughput (based on the total run time) and type of samples analysed were compared in Table 1. Detection limits as low as 3 ng mL^{-1} have been reported for the analysis of CET in human plasma using CE [7]. The low detection limit was obtained using a UV detection system (λ = 200 nm). CE is considered an environmentally friendly technique because only a small volume of chemicals is required for analysis. The total migration time is 9 min, in addition to the washing time between runs (3 min). This total run time reduces the sample throughput to only 5 samples per hour. MS-based methods are the most sensitive among the analytical methods, and 1.0–0.5 ng mL^{-1} [12,14–16] have been reported as the limits of quantification. Additionally, these methods are highly selective. Some methods can be used for enantioselective analysis of CET, whereas others have been developed to separate seven process-related substances in tablets. However, all of these techniques are based on highly sophisticated instruments, and their associated operating costs are high. Moreover, the sample throughput is modest or even very low. Fast, economical and sensitive

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doi:10.1016/j.talanta.2011.04.072

Table 1
Comparison of the analytical performance of the proposed method with other reported methods for determination of cetrizine in pharmaceuticals and biological samples.

Method	Dynamic range ($\mu g\,mL^{-1}$)	LOD/LOQ ($\mu g\,mL^{-1}$)	Sample throughput (h^{-1})	Type of sample analysed	Ref.
Spectrophotometric	2.5–22	0.1328/–	–	Bulk drug, tablets and syrups	[2]
Spectrophotometric	1.0–16	0.0285/–	–	Bulk drug and pharmaceutical preparations	[3]
HPLC	1.25–10.0	0.1/–	12	Capsules	[1]
HPLC	0.01–0.5	0.005/–	12	Human serum	[4]
HPLC	2.5–200	–/2.5	1.5	Rat plasma	[5]
HPLC	1–4	0.1/0.34	2	Oral solution and tablet formulations	[6]
HPLC	0.01–1.00	0.005/–	10	Human plasma	[7]
HPLC-FL	0.025–2.00	0.005/–	3	Human serum	[8]
HPLC	1–11	0.012/0.038	6	Solid dosage forms	[9]
CE	0.01–1.00	0.003/–	4	Human plasma	[7]
CE	1.0–50.0	0.5/–	8	Human plasma	[10]
CE	2–50	0.6/2.0	6	Tablets	[11]
LC–MS	0.0025–0.250	–/0.01	4	Tablets	[12]
LC–MS/MS	0.001–1.00	–	30	Human sodium heparin plasma	[13]
LC–MS/MS	0.001–0.	–/0.001	5	Human plasma	[14]
LC–MS/MS	0.0005–0.500	–/0.0005	30	Human plasma	[15]
LC–MS/MS	0.0005–0.3	–/0.0005	5	Human plasma	[16]

analytical methods are therefore required, especially for certain types of analyses, such as in-process control, dissolution testing and investigation of uniformity of contents.

Chemiluminescence (CL) has been shown to be a powerful analytical technique. It allows the detection of analytes at low concentrations and over a wide dynamic range. This ability is partly due to the chemical excitation at occurs during CL reaction. Because there is no excitation light source in this analytical technique, the background signal is relatively low and the need to subtract scattered light arising from an excitation source is eliminated. This allows for highly sensitive detection without the use of expensive instrumentation [17].

Due to its simplicity, low cost, high sensitivity and selectivity, CL-based detection has become an attractive tool in combination with many analytical techniques such as HPLC [18,19], (CE) [20], flow injection analysis [21] and microfluidics [22–27].

A number of CL detection-based microfluidics systems have been reported; most of these methods use continuous infusion as a method of sample introduction to the microfluidic system. For instance, atropine and pethidine were determined on a chip using ($Ru(bipy)_3^{2+}$) and cerium (IV) sulphate CL system. Detection limits were found to be 3.8×10^{-9} mol L^{-1} and 7.7×10^{-8} mol L^{-1} for atropine and pethidine, respectively, and sample throughput was 60 samples per hour [22]. Som-Aum et al. [23] developed a method for the determination of Cr(III) and total chromium using a dual channel chip and a continuous negative pressure pumping system. Chlorpheniramine maleate was analysed using ($Ru(phen)_3^{2+}$) peroxydisulphate CL system in a multi-chip device [24]. Continuous flow of the sample was introduced to the multi-chip device. Up to 72 runs can be carried out in an hour with a detection limit of 5.49×10^{-8} mol L^{-1}. Rapid determination of chemical oxygen demand in water was carried out using a potassium dichromate CL system. The CL system used discrete microdroplet sampling of CL reagent to reduce reagents consumption and enhance the reproducibility of the assay [25]. Tyrrell et al. [26] developed a microfluidic system for the analysis of copper in water samples using 1,10-phenanthroline hydrogen peroxide system. Under optimized conditions, the detection limit was 20 $\mu g\,L^{-1}$. Initially, a sample injector was used. However, in order to simplify the setup, the injector was removed.

Continuous flow is usually used as the sample introduction technique because of its simplicity compared to the fixed volume injection technique. Fix volume injection technique requires a more complex instrumental setup, and reports are available in which fixed volume injection has been used. For example, analysis of uric acid in human serum and urine samples were carried out without enzyme, using luminol and ferricyanide. The detection limit obtained was 0.5 mg L^{-1} using 3 μL of sample, and 60 samples could be analysed in an hour [27].

In this paper, we propose a ($Ru(bipy)_3^{2+}$) peroxydisulphate CL system in a two-chip device for the determination of CET in pharmaceutical formulations and biological fluids. This method is based on the enhancement of ($Ru(bipy)_3^{2+}$)–peroxydisulphate CL system by CET. To the best of our knowledge, this is the first CL method reported for the analysis of CET. Additionally, this method exhibits high sensitivity, low reagent consumption and a sampling frequency of 180 per hour.

2. Experimental

2.1. Reagents

All reagents used were analar grade and were used without further purification. Ammonium peroxodisulphate and potassium dihydrogen phosphate were purchased from Kanto Chemical Co. (Tokyo, Japan). Tris(2,2′-bipyridyl)ruthenium(II) chloride ($Ru(bipy)_3^{2+}$) was purchased from Aldrich (Gillingham, UK). CET was a gift from quality control laboratory – Ministry of Health (Muscat, Sultanate Oman). Ultrapure water was obtained from a Milli Q water system (Millipore) and was used for the preparation of solutions.

2.2. Reagents and standard drug solutions

A stock solution of $Ru(bpy)_3^{2+}$ (2.5×10^{-3} mol L^{-1}) was prepared by dissolving 0.0187 g of tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate in deionized water and diluting it to 10.0 mL. The oxidant, peroxydisulphate, was prepared by dissolving 0.0043 g in deionized water and diluting it to 25.0 mL to give a 0.75×10^{-3} mol L^{-1} stock solution.

A stock standard solution (100 mg L^{-1}) of CET was prepared separately by dissolving 10 mg of pure drug in 100 mL of deionized water. The stock solution was kept in a cold, dark place. Working solutions were prepared daily by appropriate dilutions of the stock solution with 0.2 M phosphate buffer pH 7.

2.3. Pharmaceutical formulations

Tablet samples were prepared by crushing 10 tablets that had been previously weighed out and then dissolving an amount of the powder equivalent to 10 mg of CET in water. The solution was placed in an ultrasonic bath for 30 min and then filtered. Appropriate volumes were measured and transferred to volumetric flasks.

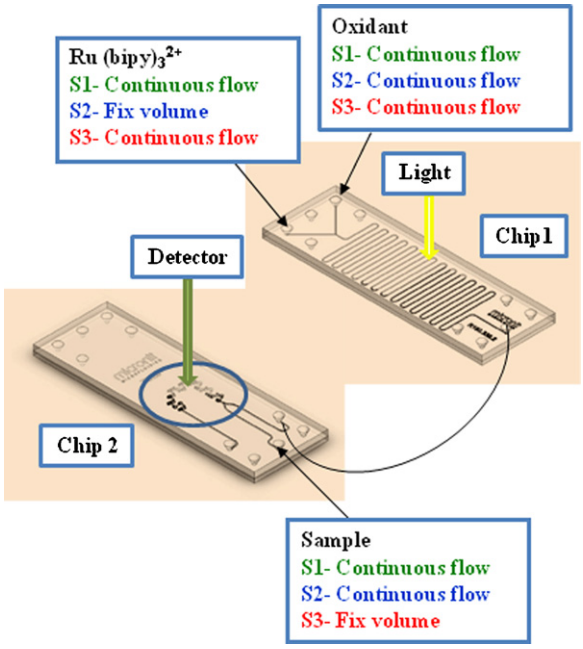

Fig. 1. Three instrumental setups were compared – S1: the CL reagents and sample were both continuously pumped; S2: a fixed volume of Ru (bipy)$_3^{2+}$ was injected, and an oxidant and the sample were continuously pumped; S3: a fixed volume of sample was injected, and Ru (bipy)$_3^{2+}$ peroxydisulphate was continuously pumped; for other experimental conditions, see Table 2.

The contents were then filled to the mark with 0.2 M phosphate buffer pH 7.

Syrup solutions were prepared by diluting an amount of the syrup equivalent to 10 mg in a volumetric flask with 0.2 M phosphate buffer pH 7.

2.4. Biological fluids

The serum or urine samples were spiked with a known amount of CET. Samples were then vortexed for 20 s, followed by the addition of 3 mL methylene chloride three separate times. The contents were vortexed again for one minute, and the phases were separated by centrifugation for 5 min at 3200 revolutions per minute (rpm). The aqueous layer in each tube was discarded, and the organic portion was transferred to a 25 mL tube and evaporated to dryness under a stream of nitrogen at 50 °C [28]. The residue in each tube was reconstituted in an appropriate volume of 0.2 M phosphate buffer pH 7 and vortexed for 30 s. A fixed amount (2 μL) was finally injected directly into the two-chip device.

2.5. Apparatus

Serpentine and teardrop microfluidic chips, fluidic connect 4515 and fused silica capillary were from Micronit (Netherlands), Syringe pumps were from Basi Bee (USA) and Harvard Apparatus (USA), and the detector was a photomultiplier tube (PMT, H7155-2, Hamamatsu, Japan) connected to a PC via a Counting Unit (C8855, Hamamatsu, Japan). The pH meter was from Hanna (Romania).

2.6. Two-chip setup

The two-chip setup used is shown in Fig. 1. Using fluidic connect 4515 and silica capillaries, chip 1 (internal volume 6 μL) was connected to syringe pumps. A torch was placed on top of this chip to catalyse the oxidation of the Ru (bipy)$_3^{2+}$ to Ru (bipy)$_3^{3+}$. The chip was connected to chip 2 (internal volume 2 μL) via a silica capillary (I.D. 150 μm, 20 cm long); the sample was infused and detection took place every 20 s. 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.

2.7. Procedure

Three instrumental setups were used as illustrated in Fig. 1.

- *Setup 1 (S1)*: Two syringe pumps were used: the first was for the analyte and the second was for CL reagents, Ru (bipy)$_3^{2+}$ and peroxydisulphate. Initially, the CL reagents were infused into chip 1 at a flow rate of 20 μL min^{-1}. After 10 s, the analyte was infused for 25 s. A maximum signal was obtained within a few seconds. The average height of all of the points between 20 and 35 s was used in the calculations.
- *Setup 2 (S2)*: Three syringe pumps were used: the first syringe pump was used for injecting a fixed volume of Ru (bipy)$_3^{2+}$ (2 μL), the second was for the sample and the third was for the peroxydisulphate solution. The sample was infused at a flow rate of 5 μL min^{-1} while the peroxydisulphate solution was infused in chip 1 at a flow rate of 20 μL min^{-1}. The Ru (bipy)$_3^{2+}$ (2 μL, 20 μL min^{-1}) was injected every 70 s. The average height of all the points was used in calculations.
- *Setup 3 (S3)*: Two syringe pumps were used, the first was used for injecting a fixed volume of sample (2 μL), and the second was used to inject CL reagents, Ru (bipy)$_3^{2+}$ and peroxydisulphate. Initially, the CL reagents were infused in chip 1 at a flow rate of 20 μL min^{-1}. The sample was injected every 20 s. The average height of all the points was used in calculations.

In all the measurements, three replicates were carried out for every sample analysed, and the CL signal intensity was measured after subtracting the background signal.

3. Results and discussion

Using the micro-photochemical reactor chip 1, Ru (bipy)$_3^{2+}$ was mixed with peroxydisulphate to produce Ru (bipy)$_3^{3+}$. The resultant solution was pumped chip 2 to mix with CET solution; detection took place at chip 2. Three instrumental setups were tested and evaluated to obtain the most economical, sensitive and high throughput setup (Fig. 1). Good sensitivity was obtained when setup S1 was used. However, a 10-s washing time between successive runs was required for the signal to return to the baseline signal, and this step reduced the sample throughput to 80 samples per hour. When the second setup S2 was used, 5 μL min^{-1} was the maximum flow rate at which the sample could be injected; higher flow rates resulted in back flow of the Ru (bipy)$_3^{2+}$ solution, which dramatically affected reproducibility. As a result, the sensitivity was drastically decreased and the run time was increased to 70 s. This increased run time reduced the sample throughput to 45 per hour. However, for setup S3, in which a fixed volume (2 μL) of the sample was injected, higher flow rates of both the oxidant and the Ru (bipy)$_3^{2+}$ solutions were possible than for S1 and S2 and no back pressure was produced. It has been previously reported for the determination of copper that the main difference between the continuous flow rate setup and the fixed volume setup is in the data presentation and that no differences in analytical performance were observed [26]. However, this was not the case in the current experiment. A 200% enhancement in the CL signal was observed when setup S3 was used compared to S1 (continuous flow), although the flow rate and other experimental conditions were the same between the two setups. This enhancement in S3 was observed at various flow rates, which showed that the fixed

Table 2
Experimental conditions, run time, reagents consumption and the sensitivity of the three experimental setups used.

	Setup 1	Setup 2	Setup 3
$(Ru(bipy)_3^{2+})$ (mmol L^{-1})	2.5	2.5	2.5
Peroxydisulphate (mmol L^{-1})	0.75	0.75	0.75
CET (mg L^{-1})	1	1	1
Flow rate of $Ru(bipy)_3^{2+}$ (μL min^{-1})	20	Fix volume injected (2 μL) at 20 μL min^{-1}	20
Flow rate of Peroxydisulphate (μL min^{-1})	20	20	20
Flow rate of CET (μL min^{-1})	20	5	Fix volume injected (2 μL) at 20 μL min^{-1}
$Ru(bipy)_3^{2+}$ consumption per run (μL)	11.7	2.0	6.7
Peroxydisulphate consumption per run (μL)	11.7	23.3	6.7
Total reagents consumption per run (μL)	35	31.2	13.3
Sample consumption per run (μL)	11.7	5.8	2.0
Washing time (s)	10	10	0
No. of sample per hour	80	45	180
Reagents consumed per hour (mL)	2.8	1.4	2.4
Relative signal intensity CET solution 1 mg L^{-1} (%)	100	20	200

volume injection afforded higher signal intensity than the continuous flow setup. This result was mainly because the residence time in this setup is very short (<1 s). Therefore, better mixing occurs when a plug of the sample is mixed with a stream of the CL reagents compared to when a stream of sample flow is pumped into the microfluidics channel and mixed with a stream of CL reagents. The enhancement in the signal intensity was also associated with a decrease in the run time (20 s only, Fig. 2) because the sample plug is injected directly to the detection chip. As a result, the sample throughput is increased to 180 per hour. Table 2 summarizes the experimental conditions for each instrumental setup used and compares the three setups in terms of reagent consumption and analytical performance. It is obvious from the table that S3 is superior over the other instrumental setups (S1 and S2) in terms of reagent consumption and sensitivity. Thus, setup 3 was chosen for this work.

3.1. Optimization

The various parameters that influence the CL signal intensity were optimized.

3.1.1. Effect of pH

pH is expected to have an important effect on the CL signal due to the presence of an amine group and a carboxylic acid group in CET. Using 0.2 M phosphate buffer system, pHs between 4 and 11 were examined. As shown in Fig. 3, no CL signal was observed at pH values below 5, whereas a weak signal was observed at pH 5. Increasing the pH from 5 to 10 led to an observed increase in CL signal. The maximum CL signal was observed at pH 7. The signal remained constant in the pH range 7–10. Increasing the pH above 10 led to a decreased CL signal. The signal decrease at pH above 10 may be due to competition between the drug and the hydroxyl group for the CL reagent. Thus, a pH of 7 was determined to be optimal for this study.

3.1.2. Effect of the flow rate

The Ru $(bipy)_3^{2+}$–peroxydisulphate CL system is rapid and, consequently, usually has a weak sensitivity in microfluidic systems due to the short residence time and the slow mixing process. However, the sensitivity can be enhanced if a good mixing strategy is employed. In our previous study, we showed that good mixing can be obtained using a teardrop chip design [24]. In the current work, we observed that if a fixed volume of sample is injected, then the sensitivity is further enhanced. Another factor that can improve mixing is the use of high flow rates; this increases the Reynolds number and hence improves mixing. However, high flow rates usually result in excessive consumption of reagents and increase the cost of analysis. In this study, the short run time, made it possible to use high flow rates, thereby improving sensitivity without increasing the consumption of the CL reagent.

Initially, the flow rate at which the sample plug (2 μL of CET, 1 mg L^{-1}) was injected was varied between 10 and 40 μL min^{-1}, and the flow rate of the CL reagents was fixed at 20 μL min^{-1}. The effect of the sample flow rate on the CL signal intensity is shown in Fig. 4. The CL intensity increases as the flow rate increases. However, at a

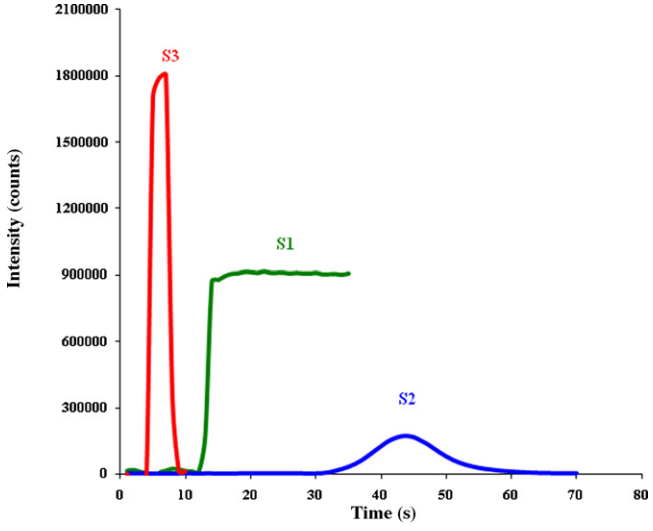

Fig. 2. Comparisons of the signals produced from the three instrumental setups used, S1, S2 and S3; for other experimental conditions see Table 2.

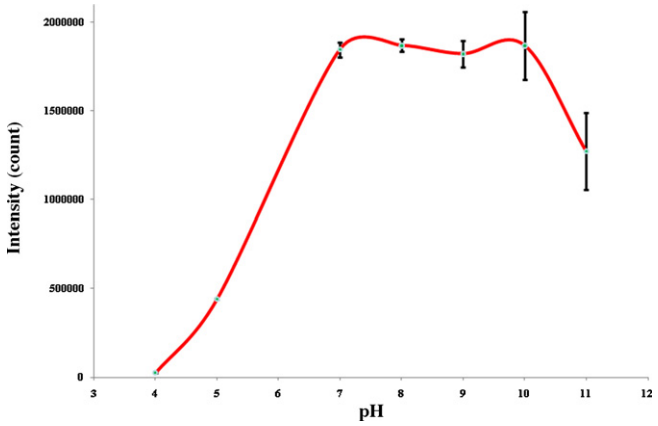

Fig. 3. Effect of 0.2 M phosphate buffer pH on CL intensity ( ). CET (1 mg L^{-1}, 2 μL, 20 μL min^{-1}), Ru $(bipy)_3^{2+}$ (2.5 mmol L^{-1}), peroxydisulphate (0.75 mmol L^{-1}) and flow rates for CL reagents (20 μL min^{-1}).

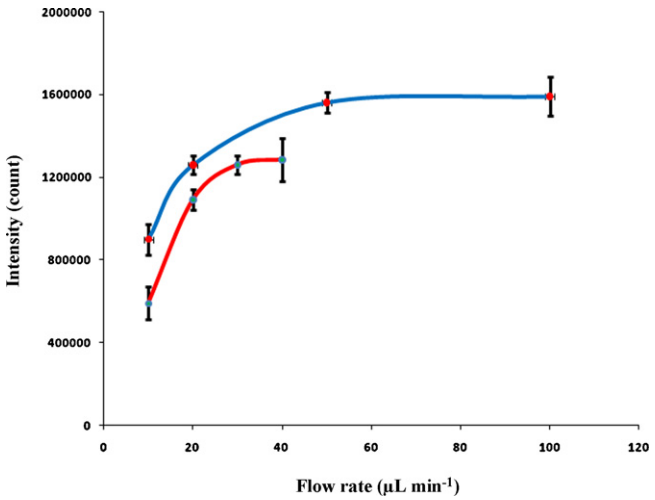

Fig. 4. Effect of sample flow rates on CL intensity ( ). Effect of Ru $(bipy)_3^{2+}$ and peroxydisulphate flow rates on CL intensity ( ). CET (1 mg L^{-1}, 2 μL), $(bipy)_3^{2+}$ (2.5 mmol L^{-1}), peroxydisulphate (0.75 mmol L^{-1}) and 0.2 M phosphate buffer pH 7.

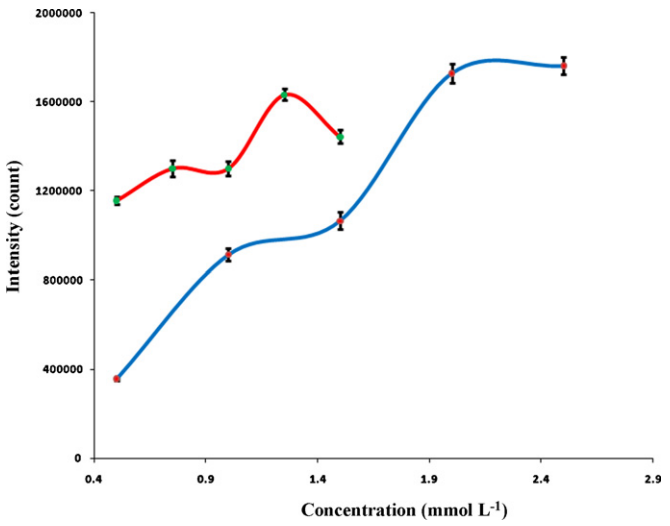

Fig. 5. Effect of peroxydisulphate concentration on CL signal intensity ( ). Effect of $(bipy)_3^{2+}$ concentration on CL signal intensity ( ), flow rate (50 μL min^{-1}), CET (1 mg L^{-1}, 2 μL, 30 μL min^{-1}) and 0.2 M Phosphate buffer pH 7.

flow rate of 30 μL min^{-1}, the CL signal reaches a maximum. Higher flow rates exhibit an insignificant effect on the CL signal. Therefore, the flow rate of the sample was fixed at 30 μL min^{-1}.

Next, the flow rates of the CL reagents Ru $(bipy)_3^{2+}$ (2.5 mmol L^{-1}) and peroxydisulphate (0.75 mmol L^{-1}) were varied from 10 to 100 μL min^{-1}, but the flow rate of the sample (1 mg L^{-1}) was maintained at 30 μL min^{-1}. The CL signal intensity was affected by the change in the flow rate of CL reagents, and it was doubled when the flow rate was increased from 10 to 50 μL min^{-1}; after that, the signal remained constant (Fig. 4). The reaction is fairly rapid, so the residence time does not play an important role in the enhancement of the CL signal. At optimal flow rates, the residence time in the detection chip is shorter than a second. The enhancement of the signal is due to the efficiency of the mixing in the teardrop chip. The signal was further improved and reached maximum when the flow rate was increased to 50 μL min^{-1}.

3.1.3. Effect of CL reagents concentrations

A slight increase was observed in the CL signal when the concentration of the oxidant changed from 0.5 mmol L^{-1} to 0.75 mmol L^{-1}, as shown in Fig. 5. The maximum signal was obtained when the concentration of oxidant was increased to 1.25 mmol L^{-1}. A further increase in the oxidant concentration was accompanied by a decrease in CL signal. Therefore, a concentration of 1.25 mmol L^{-1} of oxidant was used.

The effect of the Ru $(bipy)_3^{2+}$ concentration was investigated in the range 0.50–2.5 mmol L^{-1}. The results (Fig. 5) show that increasing the concentration of Ru $(bipy)_3^{2+}$ from 0.50 to 2.0 mmol L^{-1} resulted in a significant increase in the CL signal, up to 2.0 mmol L^{-1}, after which the signal remained almost constant. Therefore, 2.0 mmol L^{-1} was selected as the optimum concentration of Ru $(bipy)_3^{2+}$.

3.2. Analytical appraisal

Using the optimum experimental conditions described above, a calibration curve for CET was obtained. A linear response was observed over the range of 50 μg L^{-1} to 6400 μg L^{-1} (R^2 = 0.9959). However, when higher concentrations of the standard (up to 15,000 μg L^{-1}) were used, a non-linear increase in the CL signal intensity was observed. The CL signal intensity decreased when a 20,000 μg L^{-1} solution was used. Therefore, no higher concentration was tested. The calibration curves for the concentration range 50 μg L^{-1} to 20,000 μg L^{-1} are best described by third order polynomial equations $\Delta I_{CL} = 1.08 \times 10^3 c^3 - 3.11 \times 10^3 c^2 + 2.00 \times 10^6 c + 92 \times 10^4$, giving excellent correlation coefficients (R^2 = 0.9997), where ΔI_{CL} is the CL intensity and c is the molar concentration of CET. The deviation from rectilinearity in CL methods is a commonly known phenomenon that may be caused by absorption of the emitted radiation by the analyte [29]. The reproducibility of this method was investigated by injecting 1.0 mg L^{-1} standard CET solution 15 times. Excellent reproducibility was obtained with an RSD value of 1.1%, indicating the suitability of this method for routine analysis.

The detection limit was found to be 15 μg L^{-1} (S/N = 3). Sample consumption was only 2 μL, from which the detected amount of CET was found to be 6.5×10^{-14} mol, and the sample throughput was 180 per hour. The limit of quantifications was calculated based on the sample concentration that gives the response signal (ΔI_{CL}) 10 times that of the standard deviation of the blank signal and was found to be 50 μg L^{-1} [21]. The high sensitivity of this CL system is due to the enhancement in the mixing process that occurs in the teardrop chip (detector chip) between the CL reagents and the sample. Additionally, the instrumental setup and the high flow rates of the CL reagents enhanced the signal further.

3.3. Interference study

To apply the suggested method to the analysis of CET in pharmaceutical samples, human serum and urine, the interference of some common chemicals in these samples was investigated by adding these chemicals to a solution containing 0.5 mg L^{-1} CET. The tolerable concentration for interference at the 5% level was higher than 5 mg L^{-1} for sodium benzoate, methyl parabenzoate and propyl parabenzoate, which are common preservatives found in CET syrups; it was over 1000 mg L^{-1} for Na^+, K^+, Ca^{2+}, Cl^-, 200 mg L^{-1} for glucose and sucrose, and 30 mg L^{-1} for CO_3^{2-} and HCO_3^- [30].

3.4. Analytical applications

This method was then successfully applied to the determination of CET in various pharmaceutical and biological samples. Two tablet samples and two syrup samples from different manufacturers were analysed. Excellent recoveries were obtained for all

Table 3
Determination of CET in pharmaceutical formulations ($n = 5$).

Formulations	Claimed (mg)	Amount found ± SD	*t*-Test[a]	*F*-Test[b]
Tablets 1 (per tablet)	10.0	10.0 ± 0.2	1.106	4.00
Tablets 2 (per tablet)	10.0	10.1 ± 0.2	0.134	4.00
Syrup 1 (per 5 mL)	10.0	10.1 ± 0.1	2.011	1.56
Syrup 2 (per 5 mL)	10.0	10.0 ± 0.2	1.124	4.00

[a] Confidence limits at $P = 0.05$ and four degrees of freedom (df) ($t = 2.776$).
[b] Tabulated F-value for $P = 0.05$ and $df_1 = df_2 = 4$ (6.39).

Table 4
Determination of CET in biological fluids by the developed method ($n = 3$).

Sample	Added (mg L^{-1})	Amount found	Recovery (%) ± % SD
Urine sample 1	1.0	1.0	101 ± 1
Urine sample 2	0.05	0.049	98 ± 4
Serum sample 1	1.0	0.97	97 ± 4
Serum sample 2	0.05	0.045	90 ± 4

samples analysed as shown in Table 3, indicating that this method can be used for quantitative analysis of CET without interference from the other ingredients present in these samples.

For comparison, the two commercial tablet samples and two syrup samples were also determined using USP procedures [20]. The results obtained were compared statistically by Student's *t*-test and the *F*-test. The experimental values for the samples did not exceed the theoretical ones in both tests, suggesting there were no significant differences between the prepared CL method and the standard official method.

This method was also applied to the determination of CET in human serum and urine. The results are given in Table 4. Recovery tests were carried out on samples spiked with known amounts of CET. The recoveries for different concentration levels varied from 90% to 101%.

4. Proposed reaction mechanism

The mechanism for CL detection of CET is presumably analogous to that of the $Ru(bipy)_3^{2+}$ systems previously reported for amine determination [21,31,32].

Briefly, in the presence of light, peroxydisulphate is converted, due to photochemical cleavage, to a sulphate radical ($SO_4^{\bullet -}$), which is known to be a powerful oxidizing agent. This powerful oxidizing agent oxidizes $Ru(bipy)_3^{2+}$ to $Ru(bipy)_3^{3+}$. $Ru(bipy)_3^{3+}$ then reacts with the amine group present in cetrizine to produce amine cation radicals, which lose a proton to form neutral amine radicals. This species reduces $Ru(bipy)_3^{3+}$ via a high energy electron transfer reaction to produce the light emitting species $Ru(bipy)_3^{2\bullet +}$. Fig. 6

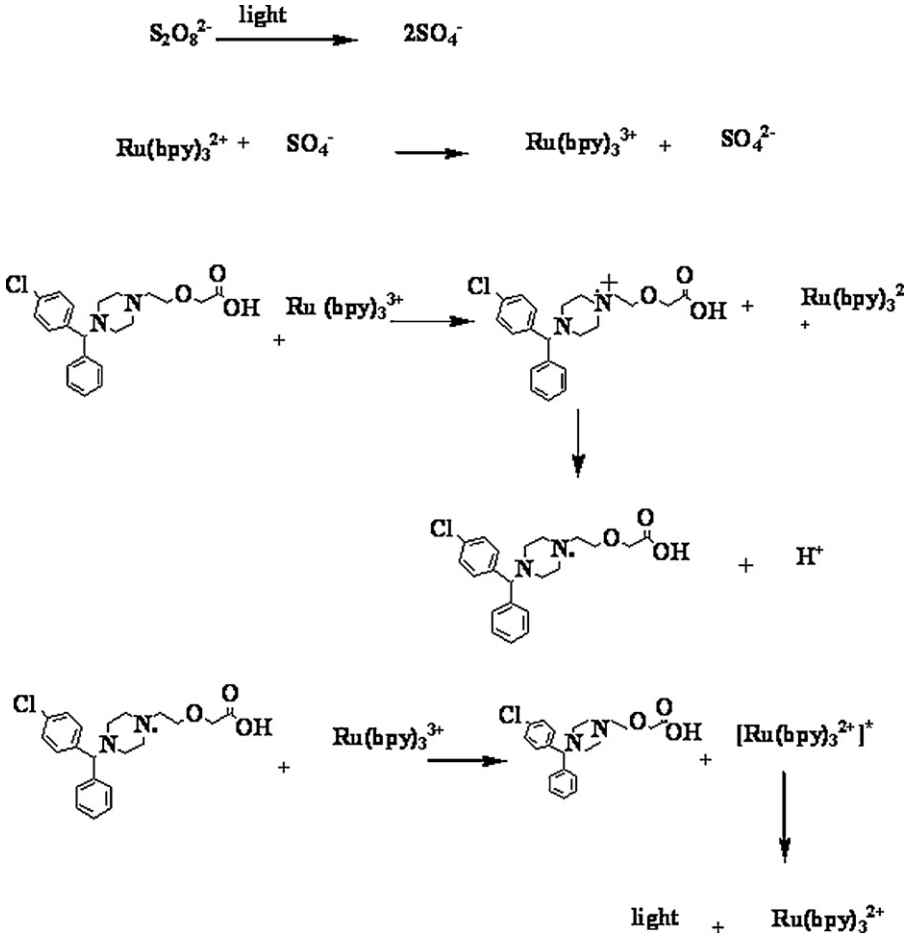

Fig. 6. The proposed CL reaction mechanism.

summarizes the steps of the CL reaction mechanism of CET with Ru $(bipy)_3^{2+}$.

5. Conclusion

A high throughput method for the analysis of CET in pharmaceutical and biological samples has been developed. This method is based on the enhancement of Ru $(bipy)_3^{2+}$–peroxydisulphate CL system by CET. The use of a fixed injection volume, together with high flow rates, enhanced the CL signal significantly. This method was successfully applied to the analysis of CET in pharmaceutical formulations and in biological fluids, without any interference from common contaminants found in these samples. The use of a two-chip system enabled the development of a highly versatile analytical system that consumes minute amounts of reagents while maintaining a significantly high throughput and preserving sensitivity.

Acknowledgments

The project was funded by an H.M. grant (SR/SCI/CHEM/09/01), Sultan Qaboos University. The authors thank the Central Laboratory of Drug analysis, Ministry of Health, Sultanate of Oman.

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