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Short communication

Enhancement of on chip chemiluminescence signal intensity of tris(1,10-phenanthroline)-ruthenium(II) peroxydisulphate system for analysis of chlorpheniramine maleate in pharmaceutical formulations

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ABSTRACT

The effect of detection chip geometry on chemiluminescence (CL) signal intensity of tris(1,10-phenanthroline)-ruthenium(II) peroxydisulphate system for analysis of chlorpheniramine maleate (CPM) in pharmaceutical formulations was investigated. It was observed that the design of the detection chip is very crucial and can play an important role in enhancing the CL signal intensity in this system. The CL signal intensity was enhanced 250% when a teardrop micromixer chip was used, compared to the commonly used serpentine chip geometry. The study was conducted using a multi-chip device. In this device, chip 1 was used to prepare and pump the reagent mixture, whereas chip 3 was used for pumping the sample. The two chips were connected to the teardrop chip (2) via silica capillary where detection took place. Non-linear regression curve fitting of the calibration data revealed that the calibration curves are best described by third order polynomial equation with excellent correlation coefficients ($R^2 = 0.9998$) for the concentration range 7.69×10^{-8} to 5.12×10^{-5} mol L^{-1}. A linear response is also observed over the range 7.69×10^{-8} to 1.28×10^{-5} mol L^{-1} ($R^2 = 0.9996$) and the detection limit was found to be 5.49×10^{-8} mol L^{-1}. The device was successfully used for the analysis of CPM in tablets and a multi-component cough syrup. Results were reproducible with relative standard deviation (RSD) of 0.6–1.1%.

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1. Introduction

Chemiluminescence (CL) detection is one of the most attractive techniques for miniaturized analytical systems. This is because CL is characterized by its simplicity, high sensitivity and better selectivity. Using CL detection system, several on chip analytical systems have been reported [1–16]. Many of these developed systems rely on the selectivity of CL detection, which does not require a pre-separation step [9–16]. This speeds up the analysis and simplifies the instrumental setup. However, many of these CL systems rely on fast chemical reactions to generate a CL signal. This entails difficulties in microfluidic systems due to the short residence time and the slow mixing process. Therefore, various strategies have been implemented in order to enhance the CL signal intensity.

Chromium(III) was analysed in the presence of Cr(VI) in natural water using a luminol and hydrogen peroxide CL system. On chip flow splitting technique was used to reduce the diffusion time and enhance the mixing process; detection limits below 10^{-7} mol L^{-1} were achieved [9].

Tyrrell et al. [10] developed a microfluidic system for the analysis of copper in water samples using 1,10-phenanthroline hydrogen peroxide. The serpentine detection channel was 210 mm long and the shape was optimized such that it can be fitted exactly to the shape of the photomultiplier detector used. Under optimized conditions, the detection limit was 20 μg L^{-1}.

Marle et al. [11] developed a CL system using serpentine chip geometry to detect and quantify hydrogen peroxide in rainwater. The detection limit was improved by 132% when a reflective surface was placed above the chip while the detector was placed underneath.

Here we propose different mixing strategies in an attempt to enhance the sensitivity of the CL signal in a microfluidic system. The strategy is based on the use of teardrop chip geometry as a detection chip for fast CL reactions. This was demonstrated using tris(1,10-phenanthroline)-ruthenium(II) peroxydisulphate CL system in a multi-chip setup for the analysis of (CPM) in pharmaceutical formulations.

CPM is highly effective and widely used for symptomatic relief of common colds and allergy [17]. Several methods have been

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developed for the analysis of CPM, these are based on HPLC with either fluorescence or UV detection system [18–23]. Other methods include capillary electrophoresis with UV detection method [24], HPLC–MS or HPLC–MS/MS [25,26]. However, some of these methods have long analysis time and some are very costly which limits their use. Flow injection analysis CL method has also been developed [17]. The major drawback of flow injection based CL techniques is the excessive consumption of expensive reagents as high flow rates are required to realize useful analytical signal. The multi-chip device described here reduces the time of analysis to 50 s per sample and consume less than 10 μL of each reagent per run making this developed device very attractive for a busy quality control laboratory where high sample throughput is required.

2. Experimental

2.1. Reagent

Ammonium peroxodisulfate 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). CPM was a gift from National Pharmaceutical Industries (Muscat, Sultanate Oman). Ultrapure water was obtained from a Milli-Q water system (Millipore) and was used for the preparation of solutions.

2.2. Standard drug solutions

A stock standard solution (5000 mg L^{-1}) of CPM was prepared by dissolving 25 mg of pure drug in a 5 mL volumetric flask in deionzed water. The stock solution was kept in a cold dark place. Working solutions were prepared daily by appropriate dilutions.

2.3. Sample preparation

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

Syrup solutions were prepared by diluting an amount of the syrup in a volumetric flask with phosphate buffer pH 7. Each sample was repeated six times.

2.4. Equipments and instruments

Serpentine and teardrop microfluidic chips, fluidic connect 4515 and fused silica capillary were from Micronit (Netherlands), Syringe pumps were from Basi Bee (USA), the detector was a photomultiplier tube (PMT, H7155-2, HAMAMATSU, Japan) connected to a PC via Counting Unit (C8855, HAMAMATSU, Japan). pH meter (Hanna HI18314, Romania).

2.5. Multi-chip setup

The multi-chip setup used is shown in Fig. 1. Using fluidic connect 4515 and silica capillaries, chip 1 (internal volume 13 μL) and 3 (internal volume 6 μL) were connected to syringe pumps. A torch was placed on top of chip 1 to catalyse the oxidation of the $Ru(phen)_3^{2+}$ to $Ru(phen)_3^{3+}$ by peroxydisulfate. Chip 1 was used to prepare and pump the reagent mixture, whereas chip 3 was used for pumping the sample. The two chips were connected to chip 2 (internal volume 2 μL) via silica capillary (ID 150 μm, 20 cm long) 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 dark.

2.6. Procedure

Two syringe pumps were used; the first one was for the buffer and the analyte while the second was for CL reagents, ($Ru(phen)_3^{2+}$) and peroxydisulphate. Initially, the CL reagents were infused in chip 1 at a flow rate of 10 μL min^{-1} for each reagent (total flow rate is 20 μL min^{-1}). After 10 s, the buffer and the analyte were infused for 25 s. Maximum signal was reached within few seconds.

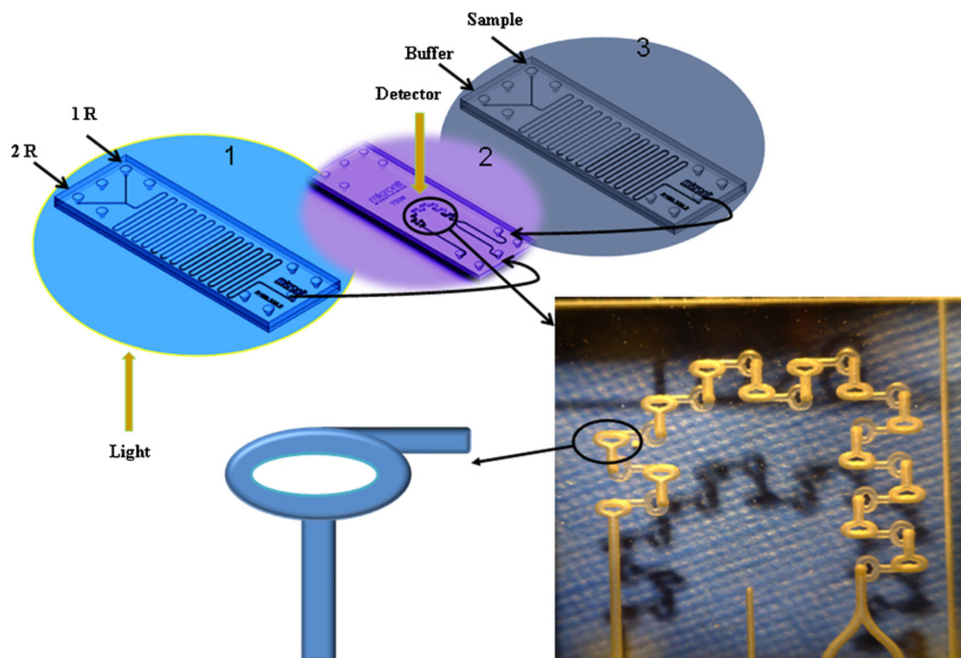

Fig. 1. Multi-chip setup used. R1, $Ru(phen)_3^{2+}$; R2, peroxydisulphate. Chip 1 is used as a photoreactor, chip 2 is the detection chip and chip 3 is for sample preparation. The inset picture is for the teardrop chip and the schematic is for a single mixing unit in a teardrop chip.

The average of all the points between 20 and 35 s was calculated and then average of five runs was used in the calculations. The CL signal intensity of the analyte was measured after subtracting the background signal.

3. Result and discussion

3.1. Optimization

The chemical system was developed earlier and the effects of various parameters that influence CL signal intensity were reported elsewhere [17]. Here those parameters that may be affected by the change in the instrumental setup were re-optimized and summarized in Table 1.

3.1.1. Effect of chip geometry on CL intensity

The geometry of the detection chip plays a crucial role in enhancing the sensitivity of the CL systems [9,27]. This is because the strength of the CL signal is the result of two concomitant processes, the mixing of the reagent and the reaction rate. If the mixing process is slow relative to the reaction rate, most reagents will react during the mixing process and a maximum signal will not be obtained. On the other hand, if the mixing is completed before significant reaction has occurred, a maximum signal will be achieved. However, mixing in microfluidic chips is relatively slow because it is completely governed by diffusion. The Reynolds number in microfluidics systems is generally very low; to obtain turbulence flow the Reynolds number should exceed 2000 [14]. Moreover, the residence time in these systems is usually very short to allow efficient mixing by diffusion only. To enhance the mixing process and hence the CL signal intensity, various chip geometries have been used, for example, T-shape, flow split and serpentine [13–16,27,28].

In this work we investigated a teardrop chip to enhance the CL signal. A teardrop chip is composed of successively arranged teardrop shaped mixing units in two layers. Hence, the overall path of the mixing units forms a three-dimensional serpentine path (Fig. 1 inset picture and the schematic).

To study the effect of the chip geometry on CL signal intensity, the chip was compared to a serpentine chip. The detector location was optimized to obtain the maximum signal intensity. The use of the teardrop geometry resulted in a significant increase in the CL signal. As can be observed from Fig. 2, a 250% increase in the CL signal was obtained when teardrop geometry was used in the detection chip. 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. Although the same flow folding mechanism is used in a teardrop chip as a serpentine chip, however, the folding occurs more frequently due to the larger number of folding structures (52-fold compared to 25) in the chip. Flow folding enhances mixing and increases the degree of radial mixing by inducing secondary flow due to centrifugal forces as the solution goes around the folding [29,30].

Additionally, two other mechanisms are also encountered in the teardrop chip that enhances the mixing process further. The first mechanism is chaotic advection which is introduced by the

Table 1
Factors included in the optimization of CL reaction of CPM with $Ru(phen)_3^{2+}$ system and the optimum conditions.

Variable	Range studies	Optimum
Flow rate of sample and buffer ($\mu L\,min^{-1}$)	10–100	20
Flow rate of $Ru(phen)_3^{2+}$ and peroxydisulphate ($\mu L\,min^{-1}$)	10–100	20
Concentration of peroxydisulphate ($mmol\,L^{-1}$)	0.5–2.5	1.0
Concentration of $Ru(phen)_3^{2+}$ ($mmol\,L^{-1}$)	0.5–3.5	2.5

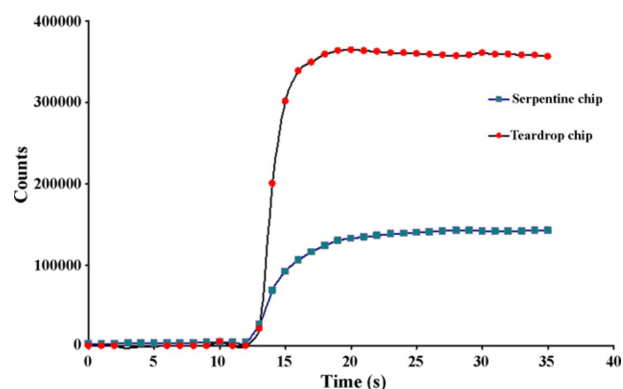

Fig. 2. Effect of chip geometry on CL signal intensity, flow rate 20 $\mu L\,min^{-1}$ for each pump, CPM (800 $\mu g\,L^{-1}$), Phosphate buffer (pH 7), $Ru(phen)_3^{2+}$ (2.5 $mmol\,L^{-1}$), peroxydisulphate (1.0 $mmol\,L^{-1}$).

overall three-dimensional structure of the chip, while the second is based on increasing the number of laminates using a multi-level laminating mixer [30,31]. This is achieved by flow splitting and recombining. The two fluids entering the inlet ports laminate at the first horizontal junction, producing two side-by-side fluid streams. The fluid flow is split horizontally, and then brought back together vertically, resulting in four streams alternating the two fluids in stage 2. Successive horizontal separation and vertical reuniting of fluid streams increases the number of laminates with each stage and, thus, the contact area between the two fluids. After the third stage, eight laminates exist, with each following stage doubling the number of laminates. The total stages in teardrop chip is 26 and therefore, at the end of the channel the contact area between the two fluids is greatly increased, resulting in an efficient mixing and as a result significant enhancement in the CL signal is obtained. The total residence time for the mixing and for reaction of the CL reagents and the analyte was calculated to be 3 s based on the assigned flow rates (20 $\mu L\,min^{-1}$ for each pump) and the internal volume of the detection chip (2 μL).

It has been reported that some degree of absorbance of the reagents onto the channel walls of the serpentine chip was observed which requires 2–3 min wash out time between the runs [10]. This was also observed here when the serpentine chip was used. This is probably due to the channel length used in the serpentine chip (>21 cm) which requires excessive rinsing. However, this phenomenon was not observed when the teardrop chip was used due to the smaller channel length (<3 cm). The washing time between the runs was only 10 s. Therefore, the sample throughput when a teardrop chip is used can reach up to 72 samples per hour.

3.2. Analytical appraisal

Using the optimum experimental conditions, a calibration curve for CPM was obtained. Non-linear regression curve fitting of the calibration data revealed that the calibration curves for the concentration range 7.69×10^{-8} to $5.12 \times 10^{-5}\,mol\,L^{-1}$ are best described by third order polynomial equations $\Delta I_{cL} = 446.76c^3 - 4837.6c^2 + 655{,}965c + 204{,}346$ giving excellent correlation coefficients ($R^2 = 0.9998$) where ΔI_{cL} is the CL intensity and c is the molar concentration of CPM. A linear response was also observed over the range 7.69×10^{-8} to $1.28 \times 10^{-5}\,mol\,L^{-1}$ ($R^2 = 0.9996$) with RSD values less than 1.5%. The detection limit was found to be $5.49 \times 10^{-8}\,mol\,L^{-1}$.

3.3. Analytical applications

The developed method was then successfully applied for the determination of CPM in commercial tablets and a multi-

Table 2
Determination of CPM in pharmaceutical samples by the developed method ($n = 6$).

Formulations	Claimed (mg)	Amount found ± S.D.	Recovery (%)
Tablets (per tablet)	4.0	4.02 ± 0.02	100.5
Syrup (per 5 mL)	50	50.1 ± 0.6	100.2

component cough syrup. Results are presented in Table 2. The tablet contains several additives such as lactose monohydrate, starch, talc, and magnesium stearate, while the syrup contains preservatives such as methyl parabenzoate and propyl parabenzoate in addition to phenylephrine hydrochloride (100 mg per 5 mL) as an active ingredient and other ingredients. Excellent recoveries were obtained for both samples analysed, indicating that the method is suitable for analysis of CPM without interference from other compounds present in the sample. Additionally the method is very robust and environmentally friendly manifested by the high sampling rate and the minimum amount of waste generated.

4. Conclusion

The detection chip geometry plays an important role in fast CL systems and careful design can enhance the signal intensity significantly. The CL signal was enhanced 250% using teardrop chip geometry for the detection chip when compared to the signal obtained with the commonly used serpentine chip geometry. This is mainly due to the enhancement in the mixing process of the CL reagents with the analyte. The developed multi-chip device is cost effective and generates minute quantities of waste. Up to 72 runs can be carried out in an hour, consuming less than 10 μl of each reagent per run.

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