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Double Spiral Detection Channel for on-chip Chemiluminescence Detection

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Abstracts

In this paper, we introduced a three layered microchip that consists of a double spiral channel design for chemiluminescence (CL) detection and a passive micromixer to facilitate the mixing of reagents. The design with two overlapping spiral channels doubled the CL intensity emitted from the reaction as compared to the design with a single spiral channel. The addition of the passive micromixer improved the signal by 1.5 times. Luminol-based chemiluminescence reaction was used for the characterization of the device. This microchip was successfully tested with the determination of L-cysteine and uric acid.

Keywords: chemiluminescence, lab-on-chip, micro total analysis system, luminol, cobalt, L-cysteine, uric acid

1 Introduction

Chemiluminescence (CL) detection methods do not require an excitation light source. Hence, compared to fluorescence detection where an excitation source is required, CL detection could be realized in a more compact system for portable applications. Such systems are often called micro total analysis system (μ TAS) or labs on a chip (LoC). The CL signal solely emits from the chemical reaction. There are limited molecules species that are chemiluminescent. Consequently, background interferences can be reduced. There are many CL systems such as luminol, acridinium compounds, coelenterazine, dioxetanes and luciferase. These systems have various applications in immunoassays, receptor assays, DNA probes and biosensors [1]. Mangru and Harrison first introduced the use of CL detection in microchip-based capillary electrophoresis, in which horseradish peroxidase catalyzed reaction of luminol with peroxide was used as a post-separation detection scheme [2]. Following this, CL detection was popular in various LOC applications.

The main drawbacks of CL reactions are the rapid reaction time and the fast decay of the emitted luminescent signal. Hence, the reactants need to be mixed quickly, followed immediately by its detection. The signal is emitted solely from the reaction. Hence, the intensity of the signal is not as bright as in fluorescence, in which there is an excitation light source. Therefore, a sensitive optical detection system such as a standard fluorometer or a photon-multiplier tube (PMT) is needed to capture this immediate light emission. These drawbacks posed many challenges in implementing CL detection system in μ TAS/LoC devices.

Micromixers are used in μ TAS/LoC devices to assist mixing of fluids in the microchannels. Simple serpentine mixing channels were often used in the microchip designs [3-5]. Mixing in these devices relied entirely on molecular diffusion of the reactants. The flow rate needs to be kept at a low value to minimize convective transport. Mei *et al.* showed that having staggered herringbone micromixer (SHM)

in the microchip improved the sensitivity of luciferase assay by three times [6]. Hence, having a proper micromixer design in the microchip may be important to facilitate maximum mixing efficiency, followed by maximum reaction efficiency prior to the detection of CL signal. The length of the micromixer is also important to improve the quality of fluid mixing. According to Lin's work [7], a similar SHM requires 60 mm in length to achieve a mixing efficiency of 95%. Williams *et al.* also gave an in-depth analysis of a SHM, in which mixing was found to be a function of Peclet number in the mixer [8]. Lok *et al.* showed that the length for a reaction channel is longer than the mixing channel for luminol CL assay [9]. This extended length needed is likely due to the delay in CL emission.

In flow injection analysis (FIA), the flow cell is coupled close to the photon detector. Nozaki *et al* first introduced a setup of a coiled Teflon tube packed horse radish peroxidase-immobilized gels in front of a PMT for the fast determination of hydrogen peroxide [10]. Kida *et al* expanded this idea to a silicon-glass flow cell containing spiral channel for the similar chemistry [11]. Zhang *et al* also introduced another variant of the spiral flow cell design [12]. Terry *et al* created an intricate sinusoidal channel for better coupling to photon detector [13]. These works aims to maximize CL detection in flow cell.

There are rooms for improvement by having a better micromixer and maximizing the area exposed to the photon detector in μ TAS/LoC. The light intensity from the CL reactions is dependent on the amount of excited molecules. Therefore, this intensity can be increased by increasing the concentration of reactants in a given volume or increasing the reaction volume at a given concentration of reactants. A stronger light signal can be generated by using a larger reaction volume. Using these principles, the reaction volume exposed to the photon detector can be increased by overlapping two spiral channels. The two spiral channels can be fabricated on two layers of the same microchip, which they are connected by a via hole. Further improvements can be achieved by having a mirror to reflect the scattered light back to the detector and a more sensitive detector to record the weak signal.

Additional setup such as lenses, optical fiber and photonic crystal fiber [14] could also be used to enhance the transfer of the emitted light to the detector.

In this paper, we used a luminol-based CL system for detection purpose. CL with luminol (5-aminophthalhydrazide) is a well characterized reaction system that have been useful for various applications such as monitoring of metals and other pollutants in water [15, 16], immunoassays, DNA analysis [17] and dating of human remains [18]. During this reaction, luminol is oxidized to 3-aminophthalate ions with the aid of a catalyst or co-oxidant under alkaline aqueous conditions, producing water, nitrogen gas and light (wavelength=425 nm). Cobalt(II) ions are known to be more effective catalysts for this reaction compare to Cu(II), Ni(II), Fe(III), Mn(II) and Fe(II) ions [19].

The aim of present study is first to verify that a double spiral channel design would improve the strength of the CL intensity emitted from the reaction. Second, a passive micromixer is added to this double spiral channel design further improve the CL signal. L-cysteine and uric acid determination are demonstrated in the new microchip design.

2 Materials and Methods

2.1 Microchip design

Three microchip designs A, B and C were investigated in this work, Figure 1. The devices consisted of three layers. These microchips had three inlets to provide versatile reagents input into the system.

Design A consisted of a double spiral channel to maximize area for optical detection. Design A measured 40 mm in length, 30 mm in width and 3 mm in thickness. Design B consisted of a passive micromixer for fluids mixing and a single spiral design for optical detection. Design C consisted of a passive micromixer for fluid mixing and a double spiral design for optical detection. Both designs B and C

measured 50 mm in length, 30 mm in width and 3 mm in thickness. The purpose of having design A and C was to verify the effect of having a micromixer on the efficacy of the CL reaction. The purpose of having design B and C was to evaluate the sensitivity of single spiral channel and double spiral for optical detection.

The double spiral design was formed by a clock-wise spiral channel in the middle layer, which was overlapped by an anti-clockwise spiral channel in the bottom layer. Figure 2 shows the three-dimensional schematic layout of design A containing the double spiral design. The channels were 1 mm in width and 0.6 mm in depth. These two spirals overlaid exactly each other leaving no gap between the channels. The spiral channels occupied a full circular area with a diameter of 25 mm, forming a liquid reservoir of approximately 295 μL .

The passive micromixer included in designs B and C, was adopted from Tan *et al's* work [20]. The micromixer served as a reaction channel to mix the reagents prior to detection. The channel dimension of this micromixer is 1.8 mm in width and 0.4 mm in height. The staggered herringbone microstructures are designed as pleats of 0.5 mm in width and 0.2 mm in height on the bottom of the channel. The height of the pleats is half of the height of the channel. The channel is serpentine to reduce footprint size of the microchip. The total length of this channel is 64 mm. The details of the effectiveness of this micromixer was investigated in our laboratory but had not been reported. Early studies indicated that the mixing channel required a mixing length of 56 mm to achieve mixing efficiency of 90% at 20 $\mu\text{L}/\text{min}$. Further investigations were made with luminol-peroxide CL, showing that the channel (acting as micro-reactor) required as minimum length of 99.4 mm for CL detection [9]. Since this particular mixer uses chaotic advection as the principle of mixing, a higher flow rate may enhance the quality of mixing [21]. This implies that a higher flow rate could be set in the microchips to provide a shorter residence time and in turn a rapid analytical time for each sample.

2.2 Microchip fabrication

Polymethyl methacrylate (PMMA) sheets of 1-mm thickness was obtained from Ying Kwang Acrylic Trading (Singapore). PMMA, a thermoplastic was chosen as the substrate material to fabricate the microchip because it is transparent, up to 92% for visible light (3 mm thickness). Thus, this material minimally absorbs the light emitted from the chemiluminescence (CL) reaction. Its low glass transition temperature of 85 to 165 °C, make its fabrication simple. The layout of these microfluidic devices were designed using CorelDraw (Corel Co., Canada). A commercial CO₂ laser system (Universal M-300, Universal Laser Systems Inc., Arizona, USA) was used to transfer the design to the device substrate. The 1-mm thick PMMA sheet was engraved to create the microchannels and cut to create the microchip parts by laser ablation. These parts were then thermally bonded under low pressure and at a temperature of 165 °C. Metal tube connectors were attached to the microchip, using epoxy glue to provide the inlets and outlets. Figure 3 shows the fabricated microchip design A.

2.3 Regent for chemiluminescence

All reagents were analytical grade. Luminol was obtained from Fluka (Gillingham, Dorset, UK). Sodium carbonate (NaHCO₃) and cobalt(II) nitrate (Co(NO₃)₂·6H₂O) was obtained from Ajax Finechem (Australia). Anhydrous sodium bicarbonate (Na₂CO₃) was obtained from GCE Laboratory Chemicals (Germany). 30% (v/v) hydrogen peroxide (H₂O₂), potassium hexacyanoferrate(II) (K₄Fe(CN)₆), potassium hexacyanoferrate(III) (K₃Fe(CN)₆), cetyltrimethylammonium bromide (CTAB) and sodium hydroxide (NaOH) pellets were obtained from Scharlau (Australia). L-cysteine, potassium persulfate (K₂S₂O₈), and uric acid were obtained from Sigma Aldrich (US).

A solution of 0.1 M sodium carbonate and 0.2 M sodium bicarbonate were created by dissolving their salts in ultrapure water (18.2 MΩ/cm). They were mixed together to form pH 10.0 carbonate buffer. Luminol was dissolved in this pH 10 buffer solution and filtered with disposable syringe filter

(0.22 μm), giving a stock concentration of 1 mM. A stock solution of 1mM cobalt(II) nitrate in deionised water was made, and diluted accordingly for later use. The solution of 30% (v/v) hydrogen peroxide was diluted to 3% (v/v) in this experiment in deionised water and kept in ice before use.

The experimental protocol for L-cysteine determination was adopted from Waseem *et al.* [22], with minor modifications. Potassium persulfate was used instead of sodium persulfate. For uric acid determination, the experimental conditions was adopted from Han *et al.* [23]. Sodium hydroxide pellets were dissolved in deionised water to make up to concentration of 0.6 M. A mixture of 0.7 mM luminol, 0.075 M Potassium hexacyanoferrate (II) and 0.7 mM CTAB was created by dissolving their salts in this 0.6 M NaOH solution. Potassium hexacyanoferrate (III) was dissolved in deionised water to a concentration of 0.1 mM. L-cysteine, potassium persulfate and uric acid were dissolved in deionised water to create 1 mM individual stock solution. All the reagents were prepared fresh before the experiments. They were diluted to appropriated concentrations. These exact concentrations would be stated later in results and discussions (3.1, 3.2 and 3.3).

2.4 Light detection and data analysis

A photon detector, H7467, with a microcontroller and RS-232C interface was obtained from Hamamatsu (Japan). The programmable syringe pump, KD Scientific KDS 250 was purchased from Microdialysis Infusion Pump (Massachusetts, US). The software Analysis 3.4 was obtained from Veriner Software.

A lens holder was made to house the microchip, a plano-convex lens and the photon detector, Figure 4. The lens holder was made of 2 mm PMMA. The plano-convex lens had a focal length of 30 mm. The lens was used to focus the light emitted from CL reaction to the photon detector. A backing mirror was used to reflect the scattered light at the back of the microchip back to the detector. The box was covered by black paper to reduce background light. Figure 4A shows the side view of the arrangement of the backing mirror, microchip, plano-convex lens and photon detector. Figure 4B shows

the front view of the alignment of the microchip to the circular detection window of the holder. Figure 4C shows the photograph of the lens holder without the backing mirror and black paper cover.

Using the programmable syringe pump, the CL reagents and samples were pumped into the inlets (A, B and C) of the microchip via teflon tubing at various flow rates. The flow rate was set to be 1 or 0.5 mL/min on the syringe pumps, equivalent to 3 or 1.5 mL/min in the main channel. CL light intensity emitted from the reaction was captured by the photon detector. This detector was connected directly to a personal computer via serial interface. The data was captured by a customized Q-basic routine that integrated the data over a time period of 30 ms or 50 ms. Each data point was collected over a period of 10 seconds. These data points were exported to the software Analysis 3.4 for further processing. The photon counts numbers was averaged and converted to per ms interval for analysis. All the experiments and readings were conducted in a dark room to reduce background noise.

Between each experiment, the microchip was flushed with deionised water, then 1 M HCl and finally deionised water again. HCl was used to remove any accumulated chemicals in the chip. Each experiment was repeated at least 5 times. The average values were calculated and outliers were filtered. The error bars in the figures represented standards deviations.

3 Results and discussions

3.1 Effect of cobalt(II) ions on CL intensity

The light intensity emitted from the luminol-peroxide CL reaction was measured against different concentration of cobalt(II) ions in designs A, B and C. The solution of 1 mM luminol in carbonate buffer, various concentrations of cobalt(II) ions from 0.1 nM to 0.1 mM and 1% v/v hydrogen peroxide were introduced via inlet A, B and C of the microchip, respectively. The flow rate was set to be 3 mL/min. The

corresponding Reynolds number and Peclet number are 4.55×10^{-2} and 4.2×10^5 respectively. The light emitted from the CL reaction was captured by the photon detector and analysed further.

Figure 5A shows the long exposure captured photograph of microchip design C, with luminol-peroxide CL, catalysed by $10 \mu\text{M}$ cobalt(II) ions. A bluish glow appeared after 9.54 mm from the entrance of the microchip. This glow maintained uniformly throughout the microchannel. Figure 5B shows the effect of the concentrations of cobalt(II) ions on CL intensity in microchip A, B and C. In general, the CL intensity produced by different concentrations of cobalt(II) ions for design C was the highest, followed by design A, then design B. The background noises for these experiments were approximately 40 ± 5 counts. The concentration of cobalt(II) ions at 0.1 nM gave very poor data reading in all microchip designs and was taken out from further analysis. For all microchip designs, a linear relationship was obtained between cobalt(II) concentration and the CL intensity at low concentration of between 1 nM and $1 \mu\text{M}$. For design A, the first order fitting function of the curve is $I_A = 3930c + 132$, $R^2 = 0.998$; for design B, the first order fitting function of this curve is $I_B = 2640c + 116$, $R^2 = 0.997$; for design C, the first order fitting function of this curve is $I_C = 5960c + 362$, $R^2 = 0.997$. I_x and c represent the CL intensity in counts and the concentration of cobalt(II) ions in μM respectively. The limits of detection (LOD) of design A, B and C were 0.7 , 0.5 and 0.1 nM , respectively.

Comparing the calibration curves of designs A and C, the gradient obtained in design C is 1.5 times steeper than design A. Design A is a microchip with a double spiral channel design for the detection window. In design A, the reagents depends on passive diffusion, the bulk reagents is not well-mixed when it enters the detection window. To cater to passive diffusion of the reagents, a lower flow rate may be set but this will result in a longer analytical time. An alternative is to design a longer channel to allow time for diffusion for higher flow rate, but this will make the microchip footprint larger. A CL

reaction is on-going process, and reactants will be exhausted quickly, if mixing is not done acceptably well, CL intensity will diminish, a weaken signal will then be recorded.

Design C is a microchip with the same double spiral channel design for the detection window, but has an extra passive micromixer. In design C, the micromixer homogenizes the reagents before entering the detection window. Therefore, for the same concentration of cobalt(II) ions and high flow rate setting, the CL intensity emitted from design C is much brighter. This result is consistent with the work reported by Mei *et al.* and Wang *et al.* [6, 24], where the authors used mixers to improve the sensitivity of their assays.

However, the length of the mixing channel cannot be too long, because the reagents may have been exhausted before they enter the detection window. In this work, the mixer channel is kept at 64 mm (2.1). CL still maintains its glow uniformly until it makes its exit, as shown in Figure 5A. The channel length of the micromixer has to been optimized to provide both fast and good mixing. Compared to the traditional SHM [25], the compacted serpentine micromixer has a smaller footprint, which can reduce overall size of an integrated microchip. The micromixer uses the principle of chaotic advection for rapid mixing; this permits the use of higher flow rate settings on the microchip. Compared to Mei *et al.* [6], a flow rate of 1 $\mu\text{L}/\text{min}$ was used, the flow rate used here was three thousand times higher. However, it was important to note that this flow rate setting was limited by the diffusion coefficient (D) of the reagents used. Lower flow rate setting will be advised for molecules with low D .

Comparing the sensitivity of the microchip between design B and C, the gradient of the line equation in design C is twice in design B. Both designs B and C have a passive micromixer. Design B only has a single spiral channel in the detection window. The detection volume for design C is twice of design B. Thus, the intensity given off by the CL reactions also doubles in design C for the same concentration of cobalt(II) ions. This result shows that a double spiral channel can improve the sensitivity of CL reaction

twice for the same concentration of cobalt(II) ions. This design with two overlapping spiral channels connected with a vial hole, may be useful to replace the conventional single spiral design in continuous flow cell in FIA. The total effective circular area covered the current flow cell in FLA is intensified twice with the double spiral design. The size of the flow cell in FIA can also be reduced without reducing the quality of the light signal captured by the current single spiral design.

Compared to Mei *et al.* and Wang *et al.*'s works [6, 24], although our work looks similar with a difference only in the location of the return spiral. There is actually a vast difference. In their works, the clockwise and anti-clockwise return spiral requires a gap in between the channel. The double spiral is fabricated in a single layer. This design is predetermined due to the restriction in microfabrication technology. Hence, the final effective detection volume is only 50% of the circular area formed by the spiral. This is of equivalence to the microchip design B in our work. PMMA is highly transparent (2.2), so the amount of lateral light loss due to the extra layers of PMMA is a minimum. This explains the reason why CL intensity for design C is nearly double of design B for the same cobalt(II) concentrations.

The CL sensitivity of the microchip increases by increasing the detection volume. However, this detection volume is limited by the footprint of the microchip and the amount of reagents used. A larger microchip needs to be fabricated to accommodate a bigger detection volume, but if the microchip is too large, the stem may no longer be considered as a μ TAS/LoC. The reagents may be exhausted in the long channel before the batch reaches the exit outlet. A compromise between CL sensitivity and detection volume needs to be established for optimal detection.

The reagents and the sensitivity of the photon detector limit the upper detection limit of this microchip. If the concentration of cobalt(II) ions is very high, the reaction is instantaneous. The reagents are exhausted quickly before the bulk reagent reaches the detection window. It is recommended to do series dilution for determination of high concentration of cobalt(II) ions in the sample. When the CL

intensity is too bright (~ 5 millions counts), the photon detector reaches a photon saturation and cannot detect higher reading. Reducing the integration time interval, may help to prevent the detector from reaching its saturation limit.

In this work, a backing mirror is used to reflect the CL light emitted from the back face of the microchip back to the detector. According to Mohr *et al.* [26], the effectiveness of the backing mirror is about 18 to 99% depending on the width of the channel. Hence, the effectiveness of the backing mirror is limited. Perhaps, putting two detectors on both sides of the microchip will capture the CL intensity more effectively. Dual detectors system may be worth exploring in the future.

Microchip design C composes of both features of a micromixer and double spiral channel design may be useful for determining analytes at low concentration in applications such as luciferase assay [6], hydrogen peroxide [10], benzoyl peroxide [3], nitrite [4] and glucose determination[27]. Here, design C is then used for later experiment to demonstrate L-cysteine and uric acid determinations.

3.2 L-cysteine determination

L-cysteine is an amino acid that contains a thiol group, and is commonly found in protein. It can undergo redox reactions and has anti-oxidant properties. The determination of concentration of L-cysteine is useful for protein quantification in pharmaceutical practice.

Microchip design C was used to determine low concentration of L-cysteine from 10 pM to 0.1 μ M in luminol CL system. 50 μ M luminol in 0.4 M NaOH buffer, low concentrations of L-cysteine and 1 mM potassium persulfate was introduced into inlets A, B and C of microchip design C. The flow rate was set to be 1.5 mL/min in this experiment. The corresponding Reynolds number and Peclet number are 2.27×10^{-2} and 2.1×10^5 respectively. The CL intensity of the reaction produced in the microchip was captured by the photon detector and subsequently analysed. Figure 6 shows the effects of L-cysteine on

CL intensity in this luminol-persulfate CL system. A linear relationship was observed for the concentration range from 0.1 nM to 0.1 μ M of L-cysteine. The fitting function was $y = 1450x + 319$, where y and x represented the CL intensity in counts and the concentration of L-cysteine in μ M, $R^2 = 0.999$. The background of the experiment was 227 ± 9.2 . The LOD was determined to be 30.7 pM. L-cysteine shows enhancing effect on this luminol-persulfate CL system. L-cysteine also shows comparative enhancement properties in CL intensity in hexacyanoferrate(II)/(III)-catalysed luminol CL reaction in carbonate buffer, luminol–peroxynitrite and luminol-persulfate CL reaction [22, 28, 29].

L-cysteine at low concentrations from 10 pM to 1 mM was also tested in a luminol-peroxide CL system. A solution of 1 mM luminol in sodium carbonate buffer, 3% (v/v) of hydrogen peroxide and 1 μ M of cobalt(II) ions was used in this experiment. The flow rate was kept at 1.5 mL/min. Figure 7 shows the effect of L-cysteine concentration on the CL intensity in this luminol-peroxide CL system. There was an intrinsic CL signal of $18,000 \pm 1,580$ counts, that arose from the luminol-peroxide CL reaction, which is catalysed by cobalt(II) ions. At the concentration of L-cysteine from 10 pM to 10 nM, the CL signal was nearly stagnant at $16,000 \pm 440$ counts. A linearly declined relationship of $y = -117x + 16000$, where y was the CL intensity in counts, x was the concentration of uric acid in μ M, $R^2 = 0.999$, was observed for the concentration range from 10 nM to 0.1 mM. The LOD of this system was determined to be 2.41 nM. L-cysteine was found to inhibit CL signal in luminol-peroxide reaction, catalysed by cobalt(II) ions. Comparatively, L-cysteine also quenched CL intensity in luminol and H_2O_2 , catalysed by mimetic peroxidase (metalloporphyrin) and copper [30, 31]. It was postulated that L-cysteine oxidized cobalt(II) ions to cobalt(III) ions, hence this inhibited the CL reaction.

Our results have shown that for higher concentrations of L-cysteine could be determined by luminol-peroxide CL reaction based on its inhibitory effect. Lower concentrations of L-cysteine could be determined by luminol-persulfate in NaOH buffer based on its enhancing effect. Meanwhile, it would be

interesting to see if the microfluidic system can be used in determining the concentration of L-cysteine-containing analytical samples in the future works.

3.3 Uric acid determination

Uric acid is an important biowaste product that is found in human urine and serum. The determination of uric acid is useful for diagnosis and therapy for a range of disorder such as gout, hyperuricemia and Lesch-Nyhan syndrome. Based on the work of Han *et al.*, CL method could be used for the determination of uric acid on an enhancement CL intensity of luminol-hexacyanoferrate(II)/(III) in the presence of cetyltrimethylammonium bromide (CTAB) [23]. CTAB act as a sensitizer, possess an enhancing effect on this present CL system. This new strategy was adopted and to test whether the design C reported above could be used for uric acid determination.

The mixture of 0.7 mM luminol, 0.075 M $K_4Fe(CN)_6$ and 0.7 mM CTAB in 0.6 M NaOH solution, 0.1 mM $K_3Fe(CN)_6$ solution, uric acid (10 pM to 0.1 μM) were introduced into inlet A,B and C of the microchip design C respectively. The flow rate was set to be 3 mL/min. The corresponding Reynolds number and Peclet number are 4.55×10^{-2} and 8.1×10^5 respectively. A linear relationship between CL intensity and concentration of uric acid can be obtained in the range between 1 nM and 0.1 μM , Figure 8. The fitting function of this curve was $y = 14353x + 9272$, where y was the CL intensity in counts and x was the concentration of uric acid in μM , $R^2 = 0.999$, with a LOD of 0.3 nM. This system had intrinsic CL emission, with an intensity of $6,300 \pm 223$ counts.

In Han *et al.*'s work, the linear range obtained from the L-cysteine calibration curve, was from 0.71 nM to 0.9 μM [23]. This range was comparable to the results obtained in our present work. At low concentration, uric acid enhanced the CL intensity. High concentrations of uric acid appear to inhibit the CL system. This phenomenon might be caused by to the alternation in the pH buffer as uric acid is acidic.

Kubo *et al.* mentioned that reducing agent such as uric acid is able to give CL with luminol in the presence of a catalyst in an alkaline solution without the need of hydrogen peroxide [28]. Following his work, scientists explore if there are other CL systems that could enhance the determination of uric acid. According to Han *et al.*, it is possible to use the reported chemical procedures to directly measure uric acid concentration without pre-treatment [23]. Thus, our microchip design C should serve as a good analytical tool for uric acid determination.

A similar microchip design based on design C had been fabricated for the determination of vitamin B₁₂ [32]. This shows that design C is viable for luminol-based CL assays for the determination of specific analytes. In this article, we focus on highlighting the introduction of the double spiral as a detection channel. We also determine the effect of adding a passive micromixer prior to the detection channel.

4 Conclusions

CL intensity improved significantly by increasing the area of exposure and addition of a passive micromixer in the microchip design. Linear correlation between CL intensity and the concentration of reactants such as cobalt(II) ions, L-cysteine and uric acid, suggests that this microchip could be useful for analyte determination that involves luminol-based CL detection methods.

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Figures

Figure 1. Schematic layout of the microchips design A, B and C. Design A consists of a double spiral channel system which overlaid each other to provide a full circular detection volume of 25 mm in diameter. Design B consists of a passive micromixer and a single spiral detection channel. Design C consists of a passive micromixer and a double spiral channel system.

Figure 2. Three-dimensional schematic layout of the microchip design A, containing the double spiral channel system. The fluid enters the clockwise spiral channel in the middle layer and goes through the centered via hole and exits through the anti-clockwise spiral channel in the bottom layer.

Figure 3. The fabricated PMMA microchip design A.

A)

B)

C)

Figure 4. A) Side view of the holder arrangement which consisted of microchip, plano-convex lens and H7467 photon detector. B) Front view of the holder arrangement. A, B and C are the inlets. C) Photograph of the lens holder attached to microchip C, without the mirror and black paper cover.

A)

B)

Figure 5. A) Long exposure photograph of microchip design C, with 10 μM cobalt(II) ions catalysing luminol-peroxide CL. B) Effect of cobalt(II) ions on CL intensity in microchip design A ($\diamond$), B($\blacksquare$), and C($\blacktriangle$).

Figure 6. CL intensity versus concentration of L-cysteine in luminol-persulfate system

Figure 7. CL intensity versus concentration of L-cysteine in luminol-peroxide system

Figure 8. CL intensity versus concentration of uric acid