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Short communication

3D-printed and CNC milled flow-cells for chemiluminescence detection

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ABSTRACT

Herein we explore modern fabrication techniques for the development of chemiluminescence detection flow-cells with features not attainable using the traditional coiled tubing approach. This includes the first 3D-printed chemiluminescence flow-cells, and a milled flow-cell designed to split the analyte stream into two separate detection zones within the same polymer chip. The flow-cells are compared to conventional detection systems using flow injection analysis (FIA) and high performance liquid chromatography (HPLC), with the fast chemiluminescence reactions of an acidic potassium permanganate reagent with morphine and a series of adrenergic phenolic amines.

1. Introduction

For chemiluminescence detection involving fast chemical reactions, the analyte and the reagent must be reproducibly and efficiently mixed shortly prior to entering (or even within) a detection zone that permits the transfer of emitted light to a photodetector [1–5]. The most commonly used chemiluminescence flow-cells designed for this purpose comprise a flat coil of glass or polymer tubing connected to a T or Y-piece at which the reactant solutions merge and enter the coil [1–8]. However, the recent fabrication of chemiluminescence flow-cells by etching or milling channels into polymer materials [9–16] have enabled more reproducible construction, the introduction of features such as reversing turns to enhance mixing efficiency, and the exploration of alternative configurations and materials, in efforts to transfer a greater proportion of the emitted light to the photodetector. In this paper, we exploit these recent advances in construction techniques to develop a polymer flow-cell that divides the analyte stream towards two separate zones for reaction with two different chemiluminescence reagents. We also examine, for the first time, the feasibility of creating chemiluminescence detection flow-cells using a simple 3D-printing technique. We evaluate these novel analytical devices in direct comparison with conventional approaches to flow-cell construction and flow-splitting using the fast chemiluminescence reactions of permanganate with various phenolic amines (morphine, octopamine, synephrine, tyramine, and hordenine), utilizing flow injection analysis (FIA) and high performance liquid chromatography (HPLC) methodology.

2. Experimental methods

2.1. Flow injection analysis

The instrument manifold was constructed as previously described [13], comprising a Gilson Minipuls 3 peristaltic pump (John Morris Scientific, Balwyn, VIC, Australia) with bridged PVC pump tubing (1.02 mm i.d.; DKSH) and Valco six-port injection valve (SGE, Ringwood, VIC, Australia) with 70 μL injection loop. With each change of flow-cell, the housing was re-sealed and left for 40 min to avoid the temporary increase in baseline signal that was observed under the most sensitive settings. All tubing entering and exiting the detector was black PTFE (0.76 mm i.d., Global FIA, Fox Island, WA, USA). The output signal from the detector was

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recorded with an e-corder 410 data acquisition system (eDAQ, NSW, Australia).

2.2. High performance liquid chromatography

Analyses were carried out on an Agilent Technologies 1260 series liquid chromatography system, equipped with a quaternary pump, solvent degasser system and autosampler (Agilent Technologies, Victoria, Australia), using a Reversed Phase Hypersil GOLD chromatography column (100 mm × 4.6 mm i.d., 5 μm; ThermoFisher Scientific, Victoria, Australia) for the separation of the four phenolic amines, with an injection volume of 20 μL and a flow rate of 1 mL min^{-1}. Isocratic elution was performed with 98% solvent A: deionised water adjusted to pH 2.15 with trifluoroacetic acid and 2% solvent B: methanol [17]. The column eluate (1 mL min^{-1}) and acidic potassium permanganate reagent (1 mL min^{-1}) merged at a confluence point located immediately prior to the detection zone(s). An analog to digital interface box (Agilent Technologies) was used to convert the signal from the chemiluminescence detector.

2.3. Flow-cells

2.3.1. Polymer tubing

Flow-cell A was a coil of polymer tubing, which has been commonly used in chemiluminescence studies and adopted in several commercially available detectors [8]. In our case, the flow-cell was constructed by mounting a 3 cm diameter coil of transparent PTFE-PFA tubing (0.8 mm i.d.; DKSH, Queensland, Australia) onto a thin board (3.5 cm × 5 cm), using Blutac™. The tubing at the centre of the coil passed through a small hole in the board and was connected to a plastic barbed T-piece by slipping silicon tubing over both parts.

2.3.2. 3D-printing

A Pro-Jet HD 3000 3-D Modeler System (3D Systems, Rock Hill, SC, USA) was used for the 3D-printing of flow-cells B to D, using Visijet EX200 UV Curable Acrylic Plastic and Visijet S200 Wax Support Material, which provides adhesion to the build platform within the 3D-printer and is used to produce supports required to build a model (i.e., the support material filled the flow channels within the cell) [18]. The initially evaluated designs were based on our previous fabrication of simple spiral-channel flow-cells by high-precision milling (with dimensions shown in Fig. S1) [14].

After each layer of material is deposited in the build chamber, the part is exposed to UV light, which cures the liquid to a solid polymer. The support material was removed from the flow-cell channels by placing the part in an oven at 70 °C to melt the wax, and then ultrasonication in an oil bath at 60 °C for 15 min. For flow-cells C and D, in which the channels were sealed during the printing process by additional layers (0.1 or 0.2 mm, respectively), the oil and warm water were also propelled through the cells using a syringe to expel residual wax from the channels. Alternatively, flow-cell B was printed only to the top of the cell channels walls. The channels were cleaned and later sealed by applying a transparent epoxy-acetate film as described in the following section. Flow-cell measurements are shown in the Electronic Supplementary Information.

2.3.3. CNC milling

Flow-cells E–J were created from transparent polycarbonate or white Acetal using a Datron M7 HP milling machine (Datron AG, Mühltal, Germany). The channels (0.8 mm × 0.8 mm) were milled with a 0.5 mm flat end mill (Datron AG). Prior to fabrication, a model of each flow-cell was drawn using the AutoDesk Inventor (Autodesk Inc., San Rafael, USA) and the models were converted into machine code using EdgeCAM software (Pathtrace, Ashford, Kent, UK). To seal the channels, the surface of the flow-cell was prepared by wet sanding (600 grit sandpaper) and polishing (1 μM alumina optic fiber polishing sheets). UV-curable epoxy (Norland Optical Adhesive 68, Norland Products, NJ, USA) was placed between two sheets of acetate, which were compressed to evenly spread the epoxy. The sheets were then pulled apart, leaving a thin film of uncured epoxy on each sheet. One sheet was used to evenly coat the surface of the chip with epoxy; the second sheet was then attached to the chip surface and the epoxy was cured for ≥ 1 h, with a UV lamp (365 nm, 60–80 mW/cm^2). In an alternative approach to seal the channels, we used universal optical sealing tape (Fisher Scientific, UK). The tape was simply cut to size, pealed from the backing material, and applied to the flow-cell surface. For all milled and 3D-printed flow-cells, the fitting holes and threads were milled using the Datron M7, and solution lines were connected to the flow-cells using Ministac fittings (Aviaquip, Victoria, Australia).

2.3.4. Flow-cell holder

A purpose built flow-cell holder (Fig. S2 in Electronic Supplementary Material) was milled from two blocks of 6061 aluminum (78 mm × 65 mm × 9.5 mm) using the Datron M7. A mirror (50 mm × 50 mm × 3 mm) was slotted into a recess milled into the back of the first block, and was held in place with an acrylic backing plate (78 mm × 65 mm × 5 mm) that was screwed onto the block. A rectangular hole was milled into this same block to fit the cut-down 3D-printed flow-cells. Three channels were milled at the top of this hole for the flow-cell inlet and waste line fittings. A hole was milled into the second block to hold the PMT against the detection zone of the flow-cell.

2.4. Chemicals and reagents

Deionised water and analytical grade reagents were used unless otherwise stated. Morphine (supplied by the Opiate Division, GlaxoSmithKline; Victoria, Australia), octopamine, synephrine, tyramine and hordenine (Sigma-Aldrich; NSW, Australia) were prepared at 1 mM in deionised water and diluted as required. Potassium permanganate was obtained from Chem-Supply (SA, Australia); methanol and sulfuric acid were obtained from Merck (Victoria, Australia); trifluoroacetic acid, sodium thiosulfate and sodium polyphosphate were from Sigma-Aldrich. The permanganate reagent was prepared by dissolution of potassium permanganate (1.9 mM) in 1% (m/v) sodium polyphosphate and adjusting to pH 2.5 with sulfuric acid and then adding sodium thiosulfate (0.6 mM), using a small volume of a 0.1 M solution [19].

3. Results and discussion

3.1. Selection of chemiluminescence reactions

For the FIA comparisons of flow-cells, we selected the widely used permanganate and morphine in acidic solution as an example of the many fast chemiluminescence reactions between oxidizing agents and organic analytes [3,20–23]. The rate (and therefore the chemiluminescence intensity) of this reaction can be enhanced by a preliminary partial reduction of permanganate to create a stable, high concentration of Mn(III) in the reagent solution [19,24]. The operation of the assembled FIA instrument manifold and light-tight detector housing (initially containing a traditional coiled tubing flow-cell 'A') were verified by preparing a calibration using the permanganate reagent and morphine standards ranging from 1×10^{-9} M to 1×10^{-5} M (Fig. S3). Each morphine solution was injected (five replicates) into a water carrier, which merged

with the permanganate reagent at 3.5 mL min^{-1} per line. The HPLC comparisons in this study were performed using the same permanganate reagent, but with a series of phenolic amines (octopamine, synephrine, tyramine and hordenine [25]) that can be rapidly separated under predominantly aqueous conditions. We have previously detected these compounds with the enhanced potassium permanganate chemiluminescence reagent [24] and these chemiluminescence systems were included in our previous investigation into the design and fabrication of detection flow-cells [16].

3.2. Exploration of 3D-printed flow-cells

Three flow-cells (denoted here as B, C and D) were 3D-printed from UV-curable acrylic plastic and a wax support material using a Pro-Jet HD 3000 3D Modeler. This device prints the material in layers, which we considered to be better suited for the production of water-tight flow-cells than the hashed-line printing of many other devices. These initially evaluated designs were based on our previous fabrication of simple spiral-channel flow-cells by high-precision milling (flow-cell dimensions shown in Fig. S1) [14]. Flow-cell B was printed in this design and its channels were sealed using a transparent epoxy-acetate film. The channels of flow-cells C and D were sealed by additional 3D-printed layers (with a total thickness of 0.1 and 0.2 mm, respectively).

A comparison of chemiluminescence intensity from the reaction of morphine (1×10^{-6} M) and the permanganate reagent within these flow-cells, using FIA methodology, showed that flow-cell B gave a larger signal than either C or D (by 19% and 29%, respectively). We attributed these differences to (i) the thinner width and the greater transparency of the detection window in flow-cell B, and (ii) the significant staining of flow-cells C and D (Fig. S4), presumably due to the formation of Mn(IV) [26] on the traces of the wax support left in their channels of flow-cells C and D. It was difficult to completely remove the wax by pumping solution through the channels from inlets to outlet.

At this time, we were limited to 3D-printing with a translucent polymer material, and based on our previous studies of chemiluminescence flow-cells milled from transparent polycarbonate and white Acetal polymers (both sealed with transparent epoxy-acetate films) [14], and the considerable differences in intensity observed from chemiluminescent reactions performed in clear, black, and white microtiter plates [27], we assumed that a significant portion of the emitted light was lost through other surfaces of the 3D-printed flow-cells. Drawing on previous studies that showed addition of a backing mirror can increase the transfer of chemiluminescence from flow cells or microfluidic chips to a photodetector [28,29], we constructed a reflective aluminum housing that held the flow-cell between a mirror and the PMT (Fig. 1 and Fig. S2). The 3D-printed flow-cell B, and two cells that were milled from transparent polycarbonate (E) and white Acetal (F), respectively [14], were trimmed to a smaller rectangle size to fit the holder. During these investigations, we also examined an alternative approach to seal the channels of the flow-cells, where optical sealing tape (normally used to seal microplates for real-time PCR) was simply removed from its backing material and applied to the flow-cell surface. The seal was sufficiently strong to prevent any solution leaking from the channels under the pressures encountered during normal operating conditions of the flow-cells and no difference in chemiluminescence intensity was observed between identical flow-cells sealed with epoxy-acetate or the optical sealing tape. However, the epoxy-acetate approach, although more time consuming, was cheaper and was therefore used for the flow-cells subsequently constructed in this study.

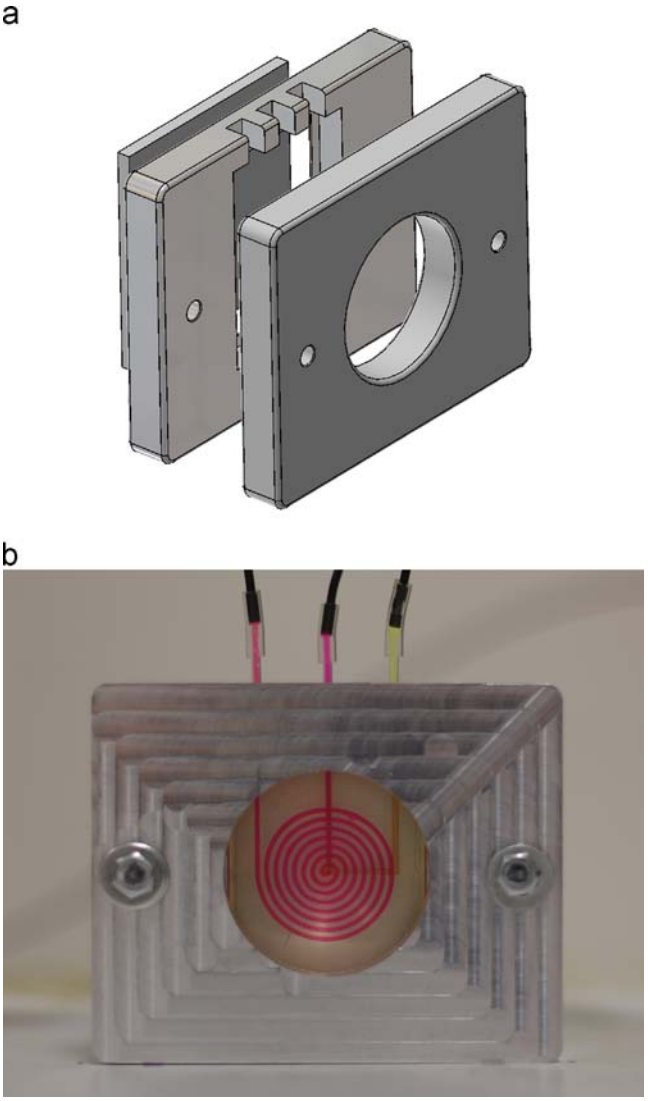

Fig. 1. (a) A three-dimensional model of the custom built reflective flow-cell housing. (b) A photograph of the housing containing a 3D-printed flow-cell (see also Fig. S2), with channels containing dyes to clearly show their position.

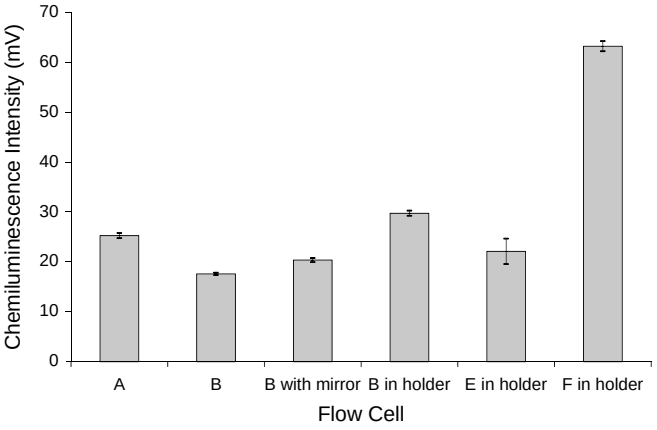

Fig. 2. Comparison of chemiluminescence detection flow-cells for the reaction of 1×10^{-6} M morphine with the permanganate reagent, using flow injection analysis. Flow-cells: A: coiled tubing, B: 3D-printed, E: transparent polycarbonate milled, and F: white Acetal milled. B, E and F were all sealed with an epoxy-acetate film. RSD for five replicate injections were less than 1%, with the exception of flow-cell E (average RSD ~3%). Error bars show $\pm 2\sigma$.

The intensity of chemiluminescence detected from flow-cell B was improved when a mirror was positioned behind the flow-cell, and further enhanced when the flow-cell and mirror were placed inside the aluminum holder (Fig. 2). Moreover, the signal obtained from the 3D-printed flow-cell in the purpose-built reflective holder was greater than that obtained from the traditional coiled tubing flow-cell A.

Although it improved the signal intensity, the holder did not entirely remove the deleterious loss of light through the translucent polymer flow-cell material. This was evident when comparing the signal from flow-cell B with that of the milled flow-cells (placed into the same holder): a lower signal was obtained using the transparent flow-cell E, and a considerably larger signal was obtained using the white flow-cell F (Fig. 2). These effects were visually corroborated by continuously merging a higher concentration of morphine with the permanganate reagent in each flow-cell (Fig. 3).

3.3. *Milled flow-cell with two detection zones*

Our recent interest in the simultaneous application of multiple chemiluminescence detection systems with distinct selectivities [30,31] led us design flow-cell G, where the analyte solution was split towards two separate serpentine-channel detection zones on a single white Acetal chip (Fig. 4(a) and Fig. S5). In the case of post-column chemiluminescence detection, splitting the flow within the detection cell (rather than at a T-piece between the column and two separate detectors), reduces the extra-column volume, thus minimizing post-column peak broadening in multiplexed detection systems. Moreover, this approach also demonstrates the potential for multiplexed chemiluminescence detection within portable fluidic devices. A black plastic bar was added to minimize crosstalk between detection zones, and when assembled in the light-tight housing, black felt was packed between the two PMTs. For visualization purposes, an additional flow-cell H was milled from polycarbonate with the same design (except that the black bar was not included) (Fig. 4(b)).

Water was initially pumped through the cells at 3.5 mL min^{-1} per line, and the solutions from the two waste outlets was collected and measured to confirm an even distribution of solution flow. A visual examination of the transparent flow-cell H using the chemiluminescent oxidation of luminol, with and without the addition of rhodamine B to generate two different emission colors (Fig. 4(b)), confirmed the absence of backflow of either reagent

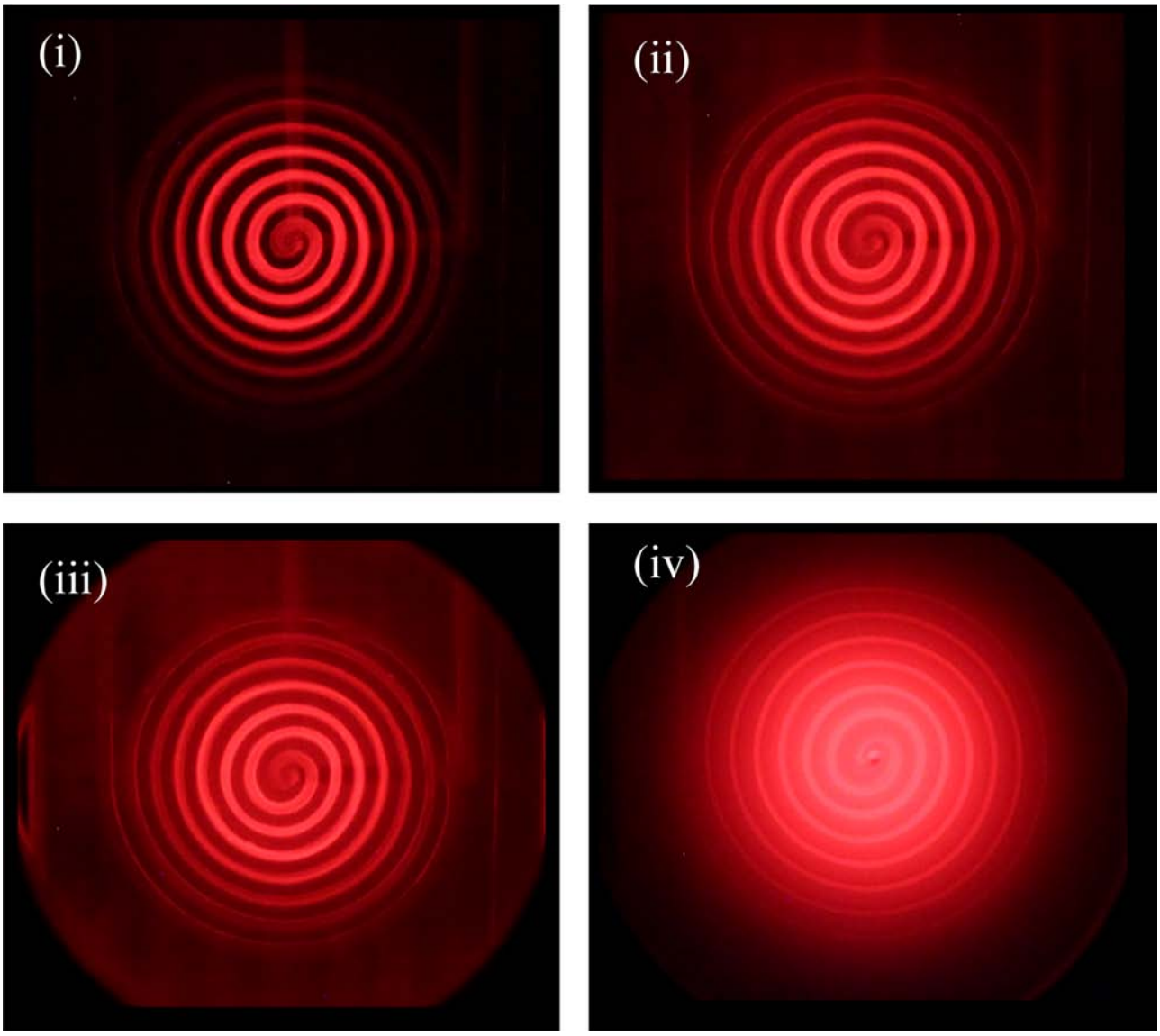

Fig. 3. Photographs of chemiluminescence from the reaction of morphine with the permanganate reagent in (i) flow-cell A, (ii) flow-cell A with a mirror against the back face, (iii) flow-cell A in the purpose-built holder, (iv) flow-cell F in the same holder. The reactant solutions were continuously merged at a flow rate of 3.5 mL min^{-1} per line. A 30 s exposure time was used for each photograph.

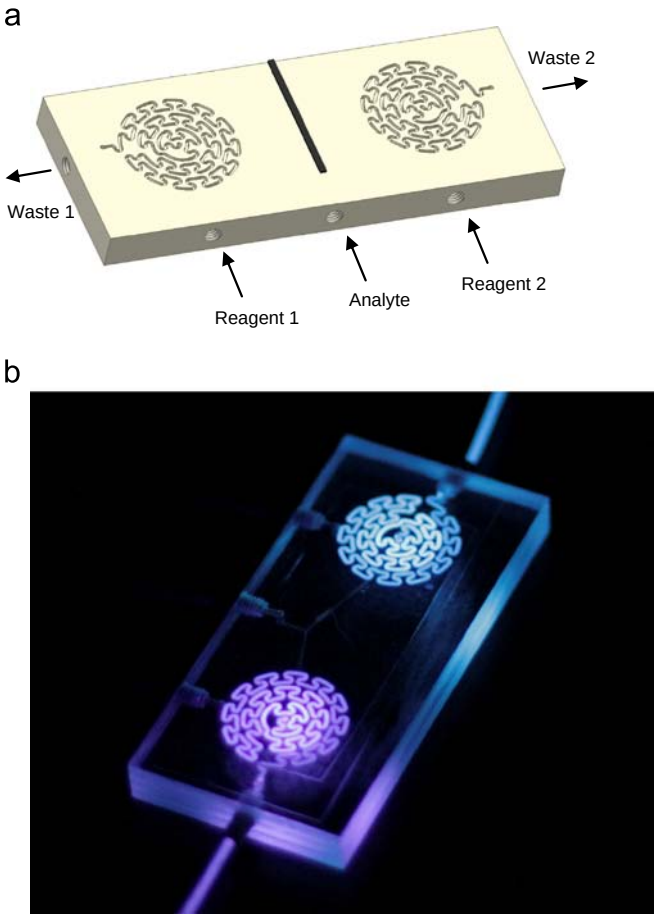

Fig. 4. (a) The design of the dual serpentine detection zone flow-cell G (see Fig. S5 for a transparent view of the model). (b) Photograph of two chemiluminescence reactions in the dual serpentine detection zone flow-cell H (transparent polycarbonate). Central inlet: hydrogen peroxide and potassium hexacyanoferrate(III); Upper reagent inlet: luminol; Lower reagent inlet: luminol with rhodamine B.

into the other inlet channel. In agreement with previous findings using single detection zone flow-cells [14], the white Acetal material resulted in a more intense emission emanating from the detection zone than the transparent polycarbonate material (Fig. S6), and therefore subsequent investigations were conducted using flow-cell G.

Using FIA methodology, 1×10^{-6} M morphine was injected into a carrier stream that was pumped into the central inlet and evenly split within the flow-cell to the two detection zones. Initially, the permanganate reagent was pumped into both reagent inlets, followed by inlet 1 only, and then inlet 2 only, with deionised water pumped into the alternative reagent inlet. No significant difference (less than 0.5σ) was observed between the signals (average of 5 replicates) from each detection zone, with or without a chemiluminescence reaction occurring in the adjacent detection zone. Moreover, under these instrumental conditions, no signal was detected using the corresponding PMT when water was pumped into the reagent inlet, even when a chemiluminescence reaction occurred in the adjacent detection zone.

The potential of flow-cell G to be used as a post-column dual chemiluminescence detector was evaluated in comparison to conventional post-column flow splitting at a T-piece to two separate chemiluminescence detectors (containing flow-cells I and J, which each had a single serpentine-channel detection zone identical to the two detection zones within flow-cell G). Using the separation of four phenolic amines and permanganate

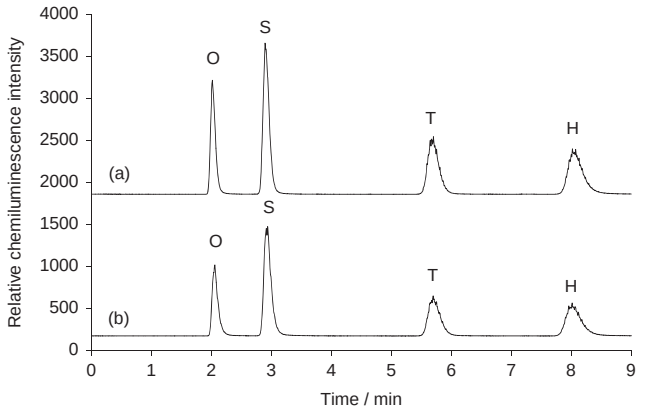

Fig. 5. Comparison of (a) dual detection zone flow-cell and (b) conventional T-piece splitting to two separate flow-cells, for the HPLC separation of phenolic amines (the y-axis of chromatogram 'a' is off-set for clarity only). Peaks: O: octopamine; S: synephrine; T: tyramine; and H: hordenine, with permanganate chemiluminescence detection. In each case, the chromatogram from only one detection zone is shown.

chemiluminescence detection as a model system, similar peak shapes and chromatographic resolution were obtained for both approaches (Fig. 5). The chemiluminescence intensities were slightly higher when the dual detection zone flow-cell was used, but this may in part be due to differences in the positioning of the PMT against the flow-cell. Again, by replacing one permanganate reagent stream (and then the other) with water, only minor crosstalk ($<0.5\%$) was observed between the two adjacent detection zones of flow-cell G.

4. Conclusions

These findings show that 3D-printing is a viable option for the fabrication of chemiluminescence detection flow-cells, which under some circumstances provided greater detection of the emitted light than the conventional coiled tubing approach. It is likely that the 3D-printing of flow-cells using white polymer materials would produce even better performance, comparable with the best flow-cells constructed by high precision milling. The new flow-cell holder significantly improved the transfer of the emitted light from the translucent 3D-printed flow-cells to the photodetector, and was also useful to reproducibly position the PMT on the detection zone and maximize the transfer of light to the photodetector for the white polymer milled flow-cells. During these studies, we also found a simpler (albeit more expensive) approach to seal the flow-cell channels, using commercially available optical sealing tape. The fabrication of flow-cells by milling or 3D-printing of polymer chips provides the opportunity to develop more sophisticated designs than those achievable through the traditional glass or polymer tubing approaches. In this manner, we have successfully created a flow-cell to split the post-column eluate towards two distinct detection zones, providing a simple and highly reproducible flow-manifold for simultaneous detection with multiple complementary chemiluminescence systems.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.03.047.

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