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Evaluation of coloured materials in microfluidic flow-cells for chemiluminescence detection☆

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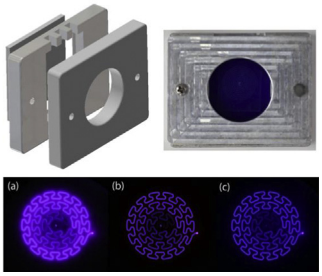
HIGHLIGHTS

- Chemiluminescence flow-cells machined in different coloured polymers compared.
- Polymer colour has a significant influence on transfer of light to the photodetector.
- Dependent on colour and opacity of polymer and spectral distribution of the emission.

GRAPHICAL ABSTRACT



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ABSTRACT

Recent advances in the construction of chemiluminescence flow-cells has included high precision milling of channels into a range of different polymer materials, in efforts to maximise the transfer of light from the chemical reaction to the photodetector. However, little is known of the extent that the colour of polymer materials will influence this transfer. This may become increasingly important as chemiluminescence detection zones are integrated with other operations within microfluidic devices or micro total analysis systems (μTAS). Herein, we compare microfluidic flow-cells fabricated from five polymer sheets (clear, white, black, red, blue), using two flow-cell designs (spiral and serpentine), two modes of photodetection, and four chemiluminescence reactions that provide a range of different emission colours. The direct transfer of light from the reaction within the white flow-cell channel to the photodetector made only minor contributions (10%–20%) to the measured intensity, with the majority of the measured light first interacting with the polymer material into which the channels were machined. The extent that the emitted light was absorbed or reflected by the coloured polymer materials was dependent on not only the properties of the polymer, but also the spectral distribution of the chemiluminescence. The changes in chemiluminescence intensities from absorption of light by the flow-cell materials can be accompanied by distortion of the spectral distribution.

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1. Introduction

Chemiluminescence can provide highly sensitive detection of analytes of importance in clinical, agricultural and industrial applications, including some compounds that can be difficult to detect by other approaches [1–6]. The instrumentation required to measure the emission of light is relatively simple, and amenable to portable analytical devices, but precise chemiluminescence measurements require reproducible mixing of two or more reactant solutions [7,8]. Moreover, chemiluminescence reactions with fast kinetics place even greater demands on the efficiency of solution mixing [9]. For these reasons, chemiluminescence has been widely applied in flow-based analytical systems, such as flow injection analysis [10–12], high performance liquid chromatography [13–16], and microfluidic devices [17–19], where the eluate/carrier stream containing the analyte(s) can be merged with the reagent(s) under highly controlled conditions within a simple flow manifold.

Chemiluminescence detection flow-cells have traditionally comprised a coil of glass or translucent polymer tubing that can be mounted flush against the circular window of a photomultiplier tube [7,8,15,20]. Conventionally, a T- or Y-piece is used to merge the solutions prior to the central entrance of the coil. For fast chemiluminescence reactions, the distance between the points of confluence and detection must be very short, to ensure that the transient emission reaches its maximum intensity as the reacting mixture moves through the coil in front of the photodetector [9].

This simple and effective coiled-tubing design has remained the most popular approach for several decades [15], but in recent years, researchers have exploited engraving/machining [9,21–26], 3D printing [27,28] and other microfabrication techniques [29–33] to not only create flow-cells with more reproducible construction, greater mixing efficiency, and superior transfer of light to the photodetector, but also integrate chemiluminescence detection zones into microfluidic chips. These approaches enable a wide range of materials to be utilised, but most flow-cells and microfluidic chips for chemiluminescence detection are constructed from transparent polymers [22,23,30–32,34–36], sometimes with a reflective backing to direct more light towards the photodetector [25,31,32,34]. Previous experiments from our research group have shown that flow-cells constructed by machining channels into an opaque white polymer (and sealed with a thin transparent film) transferred light of approximately 4-fold greater intensity to the photodetector than a transparent polymer flow-cell of the same design that was placed on a mirror, due to the light lost through the sides of the clear polymer [25]. Similarly, Stieg and Nieman reported that the chemiluminescence intensity from a flow-cell constructed by stacking white polymer plates decreased by up to two-fold when the back plate was replaced by a dark-brown material, and that the change was most prominent with lower channel depths [37]. These studies indicate that reflective materials for all surfaces other than that facing the photodetector may be ideal [25,37], but little is known of the extent that channel walls fabricated from coloured materials will influence the transfer of light to the photodetector. We anticipate that this will become increasingly important as chemiluminescence detection zones are integrated with other analytical operations within complex microfluidic devices [17–19]. Moreover, the smaller volumes of sample and reagent within typical microfluidic devices compared to conventional flow-analysis systems reduce the chemiluminescence intensity considerably [25], placing even greater demands on detector efficiency.

Herein, we describe a comparison of flow-cells fabricated from five different coloured polymer sheets (clear, white, black, red, blue), using two flow-cell designs, four different chemiluminescence reactions to provide a range of different emission colours, and two modes of photodetection. Considering the anticipated areas of application, we selected channel widths and depths typical of microfluidic devices, and thus smaller than those considered optimal for conventional flow injection analysis or high performance liquid chromatography [15].

2. Experimental

2.1. Chemicals

Reagents were prepared daily using Milli-Q deionised water unless otherwise stated. Morphine was supplied by SunPharma (Port Fairy, VIC, Australia) and prepared as a 1 mM stock solution and diluted as required. Potassium permanganate was purchased from ChemSupply (Gillman, SA, Australia). Sulfuric acid was obtained from Merck (Bayswater, VIC, Australia). Ofloxacin, cerium(IV) sulfate, luminol, sodium thiosulfate and sodium polyphosphate were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Sodium hydroxide was obtained from Ajax Chemicals (Sydney, NSW, Australia). Rhodamine B was obtained from BDH Chemicals (Poole, England).

The enhanced potassium permanganate reagent was prepared as previously described [38] by dissolving potassium permanganate (1.9 mM) in 1% (m/v) sodium polyphosphate, adjusting to pH 2.5 with sulfuric acid and then adding sodium thiosulfate (0.6 mM). The tris(2,2′-bipyridine)ruthenium(II) ($[Ru(bpy)_3]^{2+}$) reagent was prepared by dissolving $[Ru(bpy)_3]Cl_2.6H_2O$ (Strem Chemicals, MA, USA) (1.0 mM) in 0.05 M sulfuric acid. The reagent was oxidised (on-line) to tris(2,2′-bipyridine)ruthenium(III) ($[Ru(bpy)_3]^{3+}$) by a stream of cerium(IV) sulfate (1.0 mM) in 0.05 M sulfuric acid. Ofloxacin was prepared as a 1 mM stock and diluted as required with 0.05 M sulfuric acid. The luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) reagents were prepared by dissolving the luminol (2.6 mM or 2.08 mM) in 0.1 M sodium hydroxide. The ‘L + RB’ reagent was prepared by adding rhodamine B (1.7 mM) to the 2.6 mM luminol reagent. The sodium hypochlorite reagents (5.6 mM and 0.056 mM) were prepared by dilution of a commercial bleach solution (42 g/L sodium hypochlorite, 9 g/L sodium hydroxide).

2.2. Flow-cell manufacture

Five different acrylic sheets (clear, product number ‘000’; white ‘402’; black ‘502’; red ‘136’ and blue ‘327’) (4.5 mm thick) were purchased from Showcase Plastics (Melbourne, VIC, Australia) and cut into 34 × 46 × 4.5 mm chips, into which channels (0.4 mm wide × 0.2 mm deep) were machined (Fig. 1d–h). Two designs for the reaction zone were used: a spiral (Fig. 1a) and a serpentine comprising 116 reversing turns (Fig. 1b), similar to previous designs [25,26]. Three-dimensional representations of the flow-cells were drawn in SolidWorks 2015 (Dassault Systèmes, S.A., Vélizy, France), and from these models, the machining G-code was generated using NX 10.0 software (Siemens, Munich, Germany). The channels were machined using a Datron M7 HP CNC milling machine (Datron, Mühltal, Germany) with a 0.4 mm diameter, two fluted end mill (Datron) at a spindle speed of 48,000 RPM and linear feed of 200 mm/min in a single pass, with a depth of cut of 0.2 mm. A total of 10 flow-cells were machined (a spiral and serpentine design in each colour), and then sealed with polyolefin adhesive qPCR sealing tape (Sarstedt, Mawson Lakes, SA, Australia).

2.3. Comparison using a photomultiplier tube as the photodetector

A flow injection analysis (FIA) manifold was constructed as