Anal. Chem. **2008,** *80,* 9817–9821

Chemiluminescence Detector with a Serpentine Flow Cell

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We present a new chemiluminescence detector, with solution channels that have been machined into a Teflon disk and sealed with a sapphire window. The configuration of the flow cell can be conveniently modified by replacing the Teflon disk. A comparison of some existing and novel designs, using the chemiluminescence reaction of morphine with acidic potassium permanganate and the bioluminescence reaction of ATP with the commercially available "BacTiter-Glo" reagent, has revealed that a serpentine channel allows greater quantities of light to be captured than a spiral channel, due to more efficient mixing of the analyte and reagent solutions within the cell.

Chemiluminescence detection has been used extensively in procedures based on flow injection analysis (FIA) and sequential injection analysis (SIA) methodology,[1−5] due in part to the excellent sensitivity and wide calibration ranges that have been obtained for diverse classes of analytes. Moreover, the instrumentation is simple, essentially comprising a reaction vessel or conduit with a transparent surface, mounted against a photodetector. The emission of photons from a chemiluminescence reaction is transient and occurs at a rate that is dependent on both the kinetics of the chemical reaction and the physical processes of solution mixing. For the greatest sensitivity, the instrument manifold and the flow cell should be configured to maximize the emission and detection of light when the reacting mixture passes through the cell.[2] For relatively fast chemiluminescence reactions, such as the oxidation of organic molecules by acidic potassium permanganate[6] or tris(2,2′-bipyridine)ruthenium(III),[7] the analyte and reagent solutions should merge at (or close to) the point of detection. In addition, the dead volume should be minimized to ensure rapid rinsing of the cell between analyses.

A variety of chemiluminescence detection cells have been described.[8−17] The most commonly used configuration consists of a coil of glass or polymer tubing (normally 0.5−1.0 mm i.d.) mounted against a photomultiplier tube within a light-tight container. Solutions merge at a T- or Y-shaped junction shortly before entering the coil.[8,9] However, this design has several limitations: (i) the walls of the tubing are curved, and therefore most of the surface is not flat against the photodetector window, (ii) polymer tubing is often translucent rather than totally transparent, (iii) mixing is initiated before the reacting mixture enters the coil, and (iv) the internal diameter of the tubing is limited by availability.

Alternatively, in the *fountain* flow cell, the reacting mixture enters the center of an open, shallow cylindrical space and drains into a ring-shaped well around the edge, which contains the outlet hole.[13,18] This configuration allows a greater volume of solution to be in contact with a flat surface facing the photodetector. Similarly, detection cells with spiral channels have been created by etching or machining channels into polymer blocks or chips.[19−23] Another design, referred to as the *bundle cell*,[16] consists of a bundle of PTFE tubing packed into a plastic cuvette. This configuration was found to be 50% more efficient than a "spiral cell" composed of a coil of the same PTFE tubing. However, the first turn of the coil used in that particular study had a diameter

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10.1021/ac801842q CCC: $40.75
Published on Web 11/17/2008

of 1 cm, leaving a relatively large empty space in the center of the coil compared to similar flow cells constructed by other researchers. Nevertheless, that study[16] highlighted the potential of repeated sharp deviations in a narrow conduit to improve mixing efficiency.

In this paper, we present a detector designed to maximize both the generation and transmission of light from chemiluminescence reactions. The key component of the new detector is a thin Teflon disk with grooves machined into one side. The Teflon disk is placed against a sapphire (Al_2O_3) window to form a flow channel with a flat transparent wall for efficient transmission of light to the photomultiplier tube. The channel configuration can be conveniently modified by replacing the Teflon disk, and we have explored a novel *serpentine* design that incorporates 114 reversing turns to enhance mixing efficiency.[24]

EXPERIMENTAL SECTION

Chemiluminescence Detectors. The general design of the new "GloCel" chemiluminescence detector (Global FIA, Fox Island, WA) is depicted in Figure 1.

To set the path of solution flow within the cell, a groove was machined into one side of a Teflon disk (Figure 2). Inlet port or ports (at the center of the disk) and an outlet port (at the end of the groove) were drilled through the disk. A sapphire window served as the transparent top surface of the flow cells. The spiral flow cell (Figure 2a) had a channel width and depth of 0.040 in. and a total volume of 275 μL. The single-inlet and double-inlet serpentine cells (Figure 2, parts b and c) had channel widths of 0.030 in. and depths of 0.035 in. The serpentine design contained 114 turns. The volume of these cells was 245 and 235 μL, respectively. After the cell was fastened together by screwing in the black PEEK cell cap, a photomultiplier module (Electron Tubes model P30A-05, ETP, Ermington, NSW, Australia) or photon counting module (Electron Tubes model P23252) was inserted into the holder, and the nut and ferrule were tightened to hold the module against the flow cell window and prevent external light from entering the detector. Cell caps were machined to accept two or three standard 1/4−28 fittings for the single- and double-inlet flow cells, respectively.

A "fountain" flow cell was constructed by replacing the Teflon disk with a spacer ring, forming a shallow open cylindrical space. It should be noted that this configuration does not contain the deep ring-shaped well described by Ruzicka and co-workers.[13,18] A conventional flow cell was constructed by mounting a coil (3 cm diameter) of transparent PTFE tubing (0.8 mm i.d.; DKSH, Caboolture, Queensland, Australia) on a thin metal sheet (3.5 cm

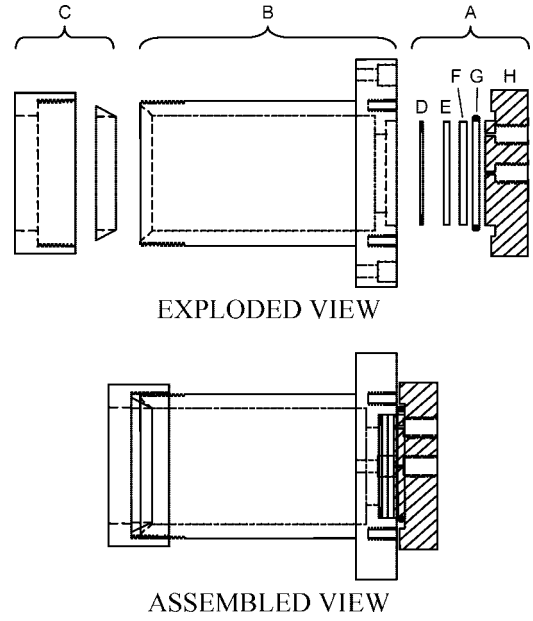

Figure 1. Chemiluminescence detector consisting of (A) flow cell, (B) main body to house the photomultiplier tube (PMT) module and position it against the flow cell window, and (C) nut and ferrule to lock in and light-seal the PMT module. Flow cell components: (D) flat gasket, (E) sapphire window, (F) Teflon disk with machined channels, (G) light-seal "O"-ring, and (H) back plate (single-inlet design shown), which is fastened to the main detector body to seal the flow cell.

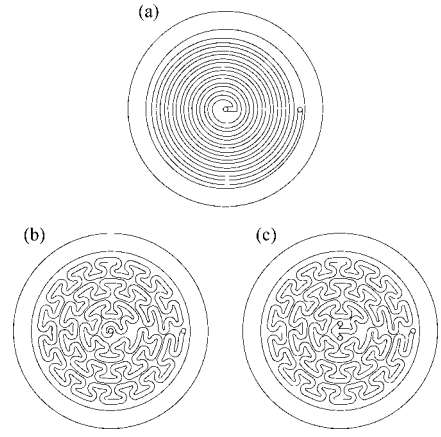

Figure 2. Teflon disks with machined channels: (a) spiral, (b) serpentine, and (c) double-inlet serpentine.

× 6 cm). The tubing at the center of the coil passed through a small slit in the sheet. The photomultiplier module was mounted flush against the coil, and the components were encased in a light-tight housing.

Instrument Manifolds. The FIA manifold (Figure 3a) was constructed from a Gilson Minipuls 3 peristaltic pump (John Morris Scientific, Balwyn, Victoria, Australia) with bridged PVC pump tubing (1.02 mm i.d.; DKSH), black manifold tubing (0.76 mm i.d., Global FIA), and Valco six-port injection valve (SGE, Ringwood, Victoria, Australia) with 70 μL injection loop.

In the case of the single-inlet cells, a custom-made Y-fitting was screwed into the inlet port. In the case of the conventional approach, a plastic T-piece was connected to the coil by slipping a small piece of silicone tubing (1 mm i.d.; DKSH) over both tubing and fitting. In both cases, the distance from the solution

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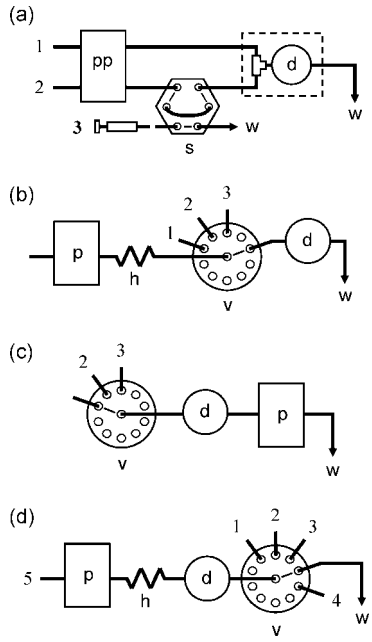

Figure 3. (a) Flow injection analysis, (b) sequential injection analysis, and (c) direct aspiration manifolds for the chemiluminescence detection of morphine. (d) Zone-fluidics manifold for the bioluminescence detection of ATP (pp, peristaltic pump; s, six-port injection valve; d, detector; p, milliGAT pump; h, holding coil; v, multiposition valve; 1−5, sample/reagent solutions; w, waste).

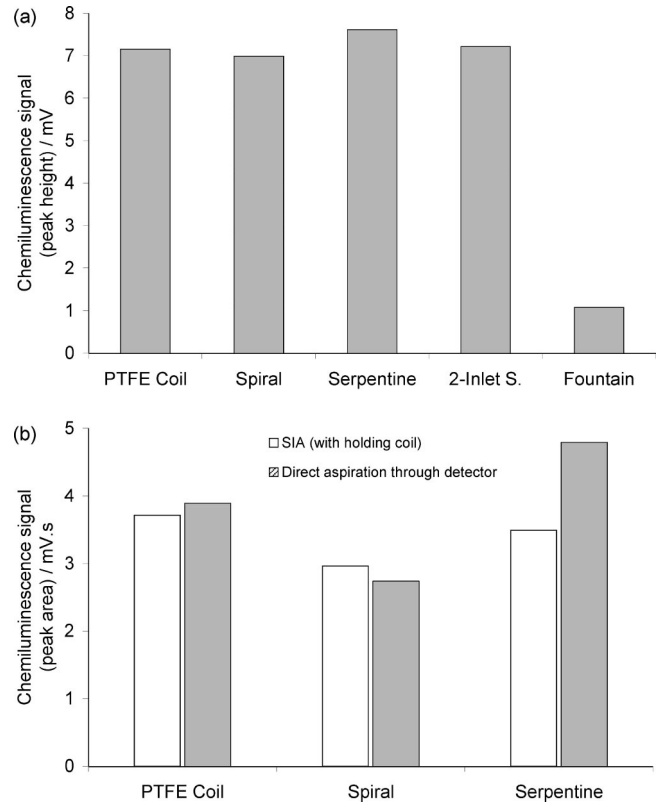

Figure 4. Relative response for the chemiluminescence reaction of morphine (1×10^{-7} M) with acidic potassium permanganate using (a) FIA or (b) SIA methodology.

confluence point to the beginning of the coil was approximately 1 cm. Signal output from the photomultiplier module was documented with a type 3066 chart recorder (Yokogawa Hokushin Electric, Tokyo, Japan).

Other instrument manifolds were constructed using milliGAT pumps (model CP-DSM-GF, Global FIA), a 10-port multiposition valve (model C25Z, Valco), PTFE or PFA tubing (Global FIA), and standard 1/4−28 and 10−32 fittings (Global FIA). Software for instrument control and data acquisition was developed using the LabVIEW platform.

Reagents. The permanganate reagent was prepared daily by dissolving potassium permanganate (Chem-Supply, Gillman, South Australia, Australia) in 1% (m/v) sodium polyphosphate (Aldrich, St. Louis, MO) and adjusting the pH to 2.5 by the dropwise addition of concentrated sulfuric acid (Merck, Kilsyth, Victoria, Australia). Morphine was obtained from GlaxoSmithKline (Port Fairy, Victoria, Australia). The BacTiter-Glo reagent and ATP solutions (Promega, Madison, WI) were stored at −20 °C and prepared fresh daily.

RESULTS AND DISCUSSION

Chemiluminescence Detection of Morphine. The reaction of morphine with acidic potassium permanganate was selected for the comparison of different flow cells because it has been used extensively as a method of detection for flow analysis and high-performance liquid chromatography (HPLC)[25] and is a representative example of the relatively fast chemiluminescence reactions between strong oxidizing agents and organic analytes.[6] The emission from this particular reaction reaches maximum intensity within a few seconds under stopped-flow conditions.[26]

With the use of FIA methodology (Figure 3a), morphine standards (1×10^{-8} to 1×10^{-5} M) were injected into a water carrier stream (line 2), which merged with the permanganate reagent (line 1). As shown in Figure 4a, the chemiluminescence intensities obtained using the spiral, serpentine, or double-inlet serpentine flow cells were similar to those obtained using the PTFE coil. The single-inlet serpentine cell gave the greatest signals, generally 6−11% higher than either the coil or spiral cells. The fountain flow cell was found to be inferior in terms of chemiluminescence intensity and precision; the relative standard deviation for 10 replicate injections of 1×10^{-7} M morphine (9.6%) was much greater than that of the other configurations (all <1.5%).

In contrast to the single-inlet cells, where the mixing started at a T- or Y-piece near the inlet, the double-inlet serpentine cell allowed the reaction to be initiated in front of the photomultiplier tube. However, this did not improve the chemiluminescence response. This effect was further explored using the reaction between tris(2,2′-bipyridine)ruthenium(III) and sodium hydroxide, which produced an intense emission of light that was suitable for visual observations (see photograph S-1 in the Supporting Information). When the single-inlet flow cells were used, some light may have been emitted before the reacting mixture entered the inlet hole, but the most intense emission within the flow cell

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occurred near the center. However, in the case of the double-inlet cell, the maximum intensity occurred slightly further away from the center of the flow cell.

We also compared the single-inlet serpentine and spiral configurations with the conventional coil of tubing using SIA methodology (Figure 3b), where mixing involves interdispersion of sequential sample and reagent zones within a single stream, rather than the merging of multiple streams. Deionized water (line 1, 2050 μL, 167 μL/s), morphine (line 2, 500 μL, 20 μL/s), and the permanganate reagent (line 3, 150 μL, 20 μL/s) were aspirated into the holding coil. The flow was then reversed to propel the reacting mixture (167 μL/s) through the detector. The chemiluminescence signals using the PTFE coil, spiral disk, and serpentine disk were compared using two morphine standards (1×10^{-7} and 1×10^{-6} M). As shown in Figure 4b (white bars), the serpentine flow cell gave similar chemiluminescence intensities to the conventional coiled tubing approach, and the signals from the spiral cell were between 17% and 29% lower.

The manifold was then reconfigured (Figure 3c) so that the solutions were aspirated directly through the detector, without reversing the flow of solution. This configuration is well-suited to chemiluminescence detection that involves relatively fast reactions, because it minimizes the mixing of solutions before they enter the flow cell, and therefore a greater proportion of the emitted light can be detected. The permanganate reagent (line 1, 150 μL, 167 μL/s) was aspirated prior to morphine (line 2, 1500 μL, 167 μL/s) and deionized water (line 3, 1000 μL, 100 μL/s). Typical replicate signals for a 1×10^{-7} M morphine standard are shown in Figure 5. Nine standard solutions (between 1×10^{-9} and 1×10^{-5} M) were tested using the three different flow cells. At equivalent concentrations of morphine, the signals obtained with the serpentine flow cell were greater than those from the spiral or coil cells by 75−104% and 7−23%, respectively (Figure 4b).

Bioluminescence Detection of ATP. The spiral and serpentine flow cells were also examined using the bioluminescence detection of ATP with a commercially available BacTiter-Glo reagent (which contains a beetle luciferin and a recombinant luciferase based on the gene from *Photuris pennsylvanica*). In contrast to the rapid flash of light elicited by the reaction of morphine with permanganate, this bioluminescence system produces a sustained glow that can be measured as a near-constant signal for many minutes.

In the Zone-Fluidics manifold (Figure 3d), the flow cell was located as close as possible to the center port of the valve; the tubing between these components had a volume of 15 μL. The sample and reagent solutions were aspirated into the detector, and the flow was stopped for 80 s to provide sufficient time for the emission to reach maximum intensity (Figure 5b). The detector contents were then flushed to waste. Isopropyl alcohol (200 μL) was used to remove any residual ATP from the lines, and the isopropyl alcohol and reaction zones were both segmented by small air zones to minimize interdispersion and carryover (Table 1).

As the length of tubing between the injection valve and the detector was very short, the emission of light was highly dependent on the efficiency of mixing the sequential sample and reagent zones as they were positioned in the flow cell. Under identical conditions, peak areas obtained using the serpentine flow cell were approximately 65% greater than those obtained with the spiral flow cell. A calibration prepared using the serpentine flow cell and a series of ATP solutions up to 7.6×10^{-10} M was approximately linear ($R^2 = 0.9981$).

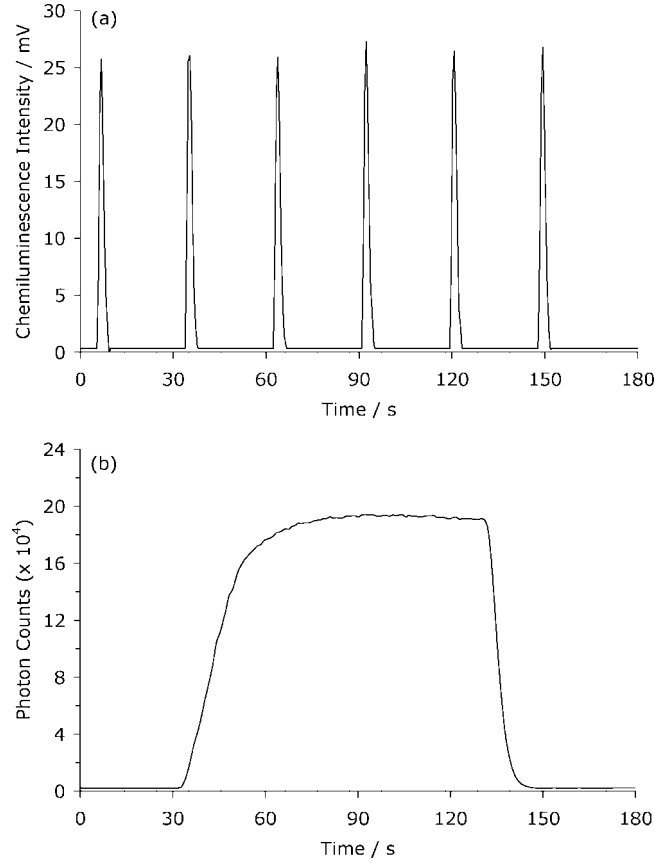

Figure 5. Typical signals for (a) the chemiluminescence reaction of 1×10^{-7} M morphine with acidic potassium permanganate (RSD = 3.1%, $n = 10$) and (b) the bioluminescence reaction of 7.8×10^{-10} M ATP with the BacTiter-Glo reagent (RSD = 3.9%, $n = 3$), using the "GloCel" chemiluminescence detector (with serpentine flow cell) within the instrument manifolds shown in Figure 3, parts c and d, respectively.

Table 1. Sequence for Determination of ATP Using the Manifold Shown in Figure 3d with Bioluminescence Detection

step	solution	instrument line	direction of flow	volume (μL)	flow rate (μL/s)
I	air	1	reverse	15	15
II	isopropyl alcohol	2	reverse	200	15
III	air	1	reverse	15	15
IV	BacTiter-Glo reagent	4	reverse	30	15
V	sample	3	reverse	170	15
VI	BacTiter-Glo reagent	4	reverse	30	15
VII	air	1	reverse	10	15
VIII			pause for 80 s		0
IX	ATP-free water	5	forward (to waste)	1000	15

CONCLUSIONS

This approach to the manufacture of chemiluminescence detectors is an attractive alternative to current practice that enables more reproducible construction, new options for flow cell design, and convenient attachment to flow analysis instrumentation using standard fittings. Several different flow cell designs were examined; the serpentine design was found to be superior to the commonly used

coiled tubing or spiral channel approaches. The advantage of the serpentine flow cell was most evident when solutions were directly aspirated into the detector via the multiposition valve, where up to a 2-fold increase in signal intensity (compared to the spiral flow cell) was observed for the chemiluminescence reaction of morphine with acidic potassium permanganate.

ACKNOWLEDGMENT

The authors thank the Australian Research Council (Linkage Grant) and Deakin University (Central Research Grant scheme) for financial support.

SUPPORTING INFORMATION AVAILABLE

Photographs of chemiluminescence from solutions in the single- and double-inlet serpentine flow cells. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review September 1, 2008. Accepted October 20, 2008.

AC801842Q