Fabrication of Microfluidic Cells with Liquid Core Waveguide and 1,1'-Oxalydiimidazole Chemiluminescence Detection

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ABSTRACT

We have developed simple and inexpensive microfluidic cells using liquid core waveguide (LCW) principles and rapid 1,1'-oxalyldiimidazole derivative chemiluminescence (CL) reactions. A micro-mixing tee was connected directly to polymer tubing used as the microfluidic cell to obtain proper mixing rates of CL reagents and analytes at pH 6.5, and syringe pumps delivered an appropriate range of flow rates (6.0–25.0 μl/sec). The length of LCW in a typical microfluidic cell needed to obtain the optimum CL efficiency for each PAH was 2.0 cm. With this configuration, very low concentrations of PAHs were quantified. The microfluidic cells devised in this research enable us to solve several problems (e.g, design of detection channels for complicated CL reactions, larger size than those for absorbance or fluorescence detection, high cost, limited applications) associated with previous products that have used detections based on other, less efficient, CL reactions. (e.g., luminol, peroxyoxalate).

Keywords: microfluidic cell, 1,1'-oxalyldiimidazole (ODI), chemiluminescence, liquid core waveguide (LCW), micro-total analytical system (μ-TAS)

1. Introduction

Since the first introduction of capillary electrophoresis (CE) on a chip in 1993 [1], development of devices has grown enormously because of the advantages of these miniaturized devices, including rapid separation of complex sample mixtures, portability, reagent/solvent economy, low cost, and broad applications in diverse fields such as biochemistry, genetics, and medical diagnostics. A variety of detection systems (*e.g.*, absorbance [2], fluorescence [3], chemiluminescence (CL) [4], electrochemical [5], mass spectrometry [6]) capable of determining detection limits as low as possible without impacting the quality of separation have been developed because the overall performance of CE devices is extraordinarily influenced by the properties of the detection system.

To develop micro-total analytical systems (μ-TAS), many research groups [7-10] have begun using CL (e.g., lumonol, peroxyoxalate) detection systems recently because the devices do not require a light source. Peroxyoxalate CL (PO-CL) detection systems for μ-TAS have been developed because they have excellent sensitivity and selectivity with a wide dynamic range [7]. However, development of μ-TAS with PO–CL detection has been limited because the light emitted from the complex CL reactions is extraordinarily dependent on the physicochemical properties of the organic solvents used [11, 12]. In addition, due to the lower solubility and rapid hydrolysis of oxalate esters in aqueous solution [13], it is hard to develop PO–CL detection systems for μ-TAS capable of quantifying aqueous analytes. One other disadvantage of PO–CL detection systems is that the kinetics of the PO–CL reaction pathway selected to design CE devices on a chip are so slow at present that extra flow elements are needed to observe the reaction in a time window at maximum emission intensity [14, 15]. An analytical separation system with extra flow elements would generally be larger than a corresponding CE microchip device, but not have better resolution due to band broadening [14, 15].

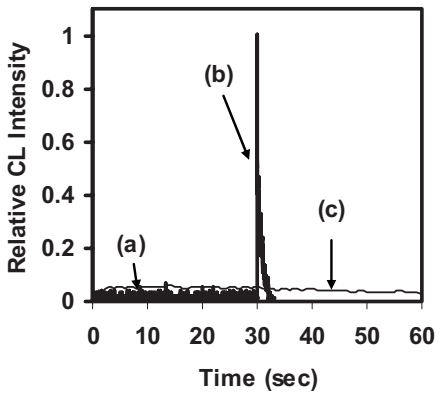

Fig. 1 Comparison between ODI derivative CL and PO–CL reactions in final solvent mixtures containing a large water volume (acetonitrile: water: ethyl acetate = 27.5:60.0:12.5) at pH 6.5. (a) noise, (b) ODI derivative CL reaction, (c) PO-CL reaction. Reagents: [1-Aminopyrene] = 37.5 nM and [H_2O_2] = 10.0 mM in water at pH 6.5, [TCPO] = 0.20 mM in organic solvent mixture (acetonitrile:ethyl acetate = 1:1), [4MImH] = 1.0 mM in aqueous acetonitrile containing 40% (v/v) water at pH 6.5 (figure adapted from [18]).

Recently, we introduced a new PO–CL reaction pathway called the 1,1'-oxalyldiimidazole (ODI) derivative CL reaction [16-18]. The maximum intensity and time to reach the maximum emission observed in ODI derivative CL reaction are higher and faster than those measured in PO-

CL reactions presently used for μ-TAS. As shown in Fig. 1, another advantage of the ODI derivative CL detection system is that this device can quantify aqueous analytes because the ODI derivative CL reaction to emit light is much faster than the hydrolysis reaction to decompose oxalate esters in aqueous solution. Based on these results, we expect that ODI derivative CL detection systems will solve some of the current problems of μ-TAS that use other CL detection systems.

Several research groups [19-21] have developed analytical systems with liquid core waveguides (LCW) to measure absorbance or fluorescence of products formed from rapid chemical reactions. Also, LCW has been used as a detection cell of analytical systems with luminol CL detection [22, 23].

Using the advantages of rapid ODI derivative CL reactions and LCW systems capable of detecting the CL, we have developed microfluidic flow injection analysis (FIA) systems in the present work.

2. Experimental

Chemical

1-aminopyrene, 9,10-diphenyl anthracene (DPA), perylene, bis(2,4,6-trichlorophenyl) oxalate (TCPO), 4-methyl imidazole (4MImH), and hydrogen peroxide (50%) were purchased from Aldrich. Spectroscopic grade organic solvents (acetonitrile and ethyl acetate) were purchased from Burdick & Jackson. Deionized water of resistivity greater than 17.8 MΩ-cm (Super-Q™ Plus, Millipore) was used to prepare all aqueous solutions.

Measurement of chemiluminescence.

Typical CL reactions were conducted at room temperature (22.0–23.0°C) in a 1 cm fluorescence cell for PO–CL reactions, or in a 1 cm fluorescence flow micro-cell connected to a RX-2000 stop-flow accessory (Applied Photophysics Limited, UK) or homemade microfluidic cell inserted in the sample compartment of a spectrofluorometer (PTI Inc.) for ODI derivative CL reactions. Fresh solutions were prepared daily and kept in the dark. CL intensity *vs.* time was monitored by the spectrofluorometer (i.e., light source off) at the maximum emission wavelength (e.g., 426 nm for 1-aminopyrene, 427 nm for DPA, 468 nm for perylene.

1. PO-CL reaction. A 0.5 ml H_2O_2 solution was added to the cell followed by addition of 0.5 ml each of a 1-aminopyrene solution and an ImH solution. The reaction was initiated by injecting 0.5 ml of TCPO. Each experimental condition was replicated three times. A small magnetic stir bar in the fluorescence cell provided continuous mixing during each experiment.

2. OD4MI–CL reaction.

2.1. Observation of ODI derivative CL using spectro-fluorometer with RX-2000 stop-flow accessory: Equal volumes of 1-aminopyrene and H_2O_2 were inserted in a drive syringe. After TCPO reacted with 4MImH in the other drive syringe for a specified time, both drive syringes were discharged to the micro-cell (silica) inserted in the compartment through a flow line (silica, glass and fluoro-carbon) with constant dead-time (6.0 ms). The mixing ratio between the drive syringes was 1:1, and the observation volume in the microcell was 80 μl.

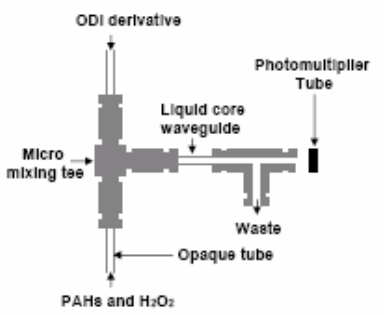

Fig. 2 Microfluidic cell fabricated with LCW and ODI derivative CL detection.

2.2. *Observation of ODI derivative CL using spectro-photometer with homemade microfluidic cell*: 1.0 ml of analyte and H_2O_2 were each added to separate syringes. 1.0 ml of ODI derivative formed from the reaction between 4MIm and TCPO in a beaker was inserted in a third syringe. Using a syringe pump having 4-syringe holders (Harvard, Inc.), flow rates of ODI derivative, analyte (PAHs), and H_2O_2 inserted into the microfluidic cell (Fig. 2) were controlled. Analytes were mixed with H_2O_2 in a tee-connector before being inserted into the microfluidic cell. CL light emitted from the rapid ODI derivative CL reaction in the microfluidic cell was measured using a spectrophotometer with a photomultiplier tube detector. The inner diameter of the opaque tube was 250 μm, whereas inner diameters of the LCWs were 150–750 μm. All materials used to fabricate the microfluidic cell were purchased from VICI Valco Instruments Co. Inc.

3. Results and Discussion

CL intensity, and thus detection sensitivity, using the microfluidic cell shown in Fig. 2 is influenced by various factors (e.g., CL reagents, flow rates, LCW length, polymer properties of LCW). We investigated the effects of these various factors in order to determine detection limits of PAHs (1-aminopyrene, DPA, and perylene) under optimum conditions. In the present study, solvent, pH, and temperature effects [18] were not examined. Instead, all experiments were conducted at room temperature and a final solution pH of 6.5 using ethyl acetate as the solvent.

Table 1 Effect of H_2O_2

mM	3.33	6.67	10.0	13.33	16.67
[1]Intensity	0.75	1.0	0.83	0.71	0.54

[1] Relative CL intensity measured under each H_2O_2 concentration was normalized with that measured in the present of 6.67 mM H_2O_2. [1-aminopyrene] = 0.03 mM, [TCPO] = 0.17 mM, [4MImH] = 1.70 mM.

Table 2 Effect of TCPO

mM	0.09	0.17	0.34
[1]Intensity	0.34	0.67	1.0

[1] Relative CL intensity measured under each TCPO concentration was normalized with that measured in the present of 0.34 mM TCPO. [1-aminopyrene] = 0.03 mM, [H_2O_2] = 6.67. mM, [4MImH] = 1.70 mM.

Table 3 Effect of 4MImH

mM	0.9	1.7	3.4	5.1	6.8
[1]Intensity	0.76	0.86	1.0	0.77	0.55

[1] Relative CL intensity measured under each 4MImH concentration was normalized with that measured in the present of 3.4 mM TCPO. [1-aminopyrene] = 0.03 mM, [H_2O_2] = 6.67. mM, [TCPO] = 0.34 mM.

3.1 Effects of ODI derivative CL reagents

Tables 1–3 show the effects of ODI derivative CL reagents. ODI derivative molecules were formed from 4MImH and TCPO for 180 seconds in a syringe fixed in a holder of the syringe pump. When the ODI derivative molecules formed were mixed in the microfluidic cell with H_2O_2 and 1-aminopyrene added through the other cell inlet, strong CL intensities were measured for about 30 seconds. Fixed conditions for these experiments were the CL reagent flow rate (12.7μl/sec), length and inner diameter of the LCW (2.0 cm and 750 μm, respectively), and LCW material (PTFE). Based on the results shown in Tables 1–3, the optimum ODI derivative CL reagent concentrations ([H_2O_2] = 6.67 mM, [TCPO] = 0.34 mM, and [4MImH] = 3.4 mM) were determined for quantifying low concentrations of PAHs (e.g., 1-aminopyrene, DPA, perylene).

3.2 Effects of reagent flow rates

As shown in Table 4, ODI CL intensity emitted in the microfluidic cell (length of LCW: 2.0 cm) is dependent on the flow rates of ODI derivative CL reagents.

Table 4 Effects of flow rate of ODI derivative CL reagents

μl/sec	6.5	9.0	16.7	18.3	25.0
[1]Intensity	0.20	0.57	0.80	1.00	0.89

[1] Relative CL intensity measured under each flow rate was normalized with that measured in 18.3 μl/sec. [1-aminopyrene] = 0.03 mM, [H_2O_2] = 6.67 mM, [TCPO] = 0.34 mM, [4MImH] = 3.4 mM.

Table 5 Effects of length of LCW

cm	2.0	7.0	12.2	17.2
[1]Intensity	1.00	0.78	0.52	0.42

[1] Relative CL intensity measured in each length of LCW was normalized with that measured in 2.0 cm. [1-aminopyrene] = 0.03 mM, [H_2O_2] = 6.67 mM, [TCPO] = 0.34 mM, [4MImH] = 3.4 mM, flow rate: 18.3 μl/sec.

3.3. Effects of LCW length

Table 5 demonstrates that the length of the LCW should be short to obtain maximum intensities with rapid ODI derivative CL reactions. Tables 4 and 5 together show that flow rates of ODI derivative CL reagents and LCW length in microfluidic cells will be critical factors to quantify low concentrations of fluorophores. For example, if low concentrations of PAHs are quantified with a microfluidic cell using ODI derivative CL detection, flow rates of the reagents (R) should be as fast as possible and the length of the LCW (L) should be as short as possible. Therefore, these two factors have inverse effects on the maximum intensities (I_{max}) emitted by rapid ODI derivative CL reactions in microfluidic cells, as shown by the following simplified equation:

$$I_{\max} \propto \frac{R}{L} \qquad (1)$$

3.4. Effects of other factors

Four types of polymer tubes (e.g., PTFE, FEP, PEEK, ETFE) were used as fabrication materials for the LCW. ODI derivative CL intensities measured with these different LCWs (length: 2.0 cm) were similar. We also measured ODI derivative CL intensities using LCWs with smaller inner diameters than 750 μm (e.g., 150, 250, 500 μm), and found that the CL intensity decreased slightly with decreasing LCW inner diameter using a constant LCW length (2.0 cm) and CL reagent flow rate (18.3 μl/sec).

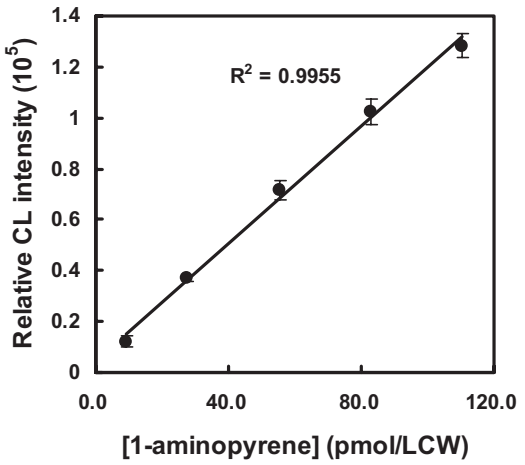

Fig. 3 Calibration curve for 1-aminopyrene obtained from microfluidic cell with ODI derivative CL detection at room temperature. Length of LCW: 2.0 cm, flow rate of ODI derivative CL reagents and 1-aminopyrene: 18.3 μl/sec, [H_2O_2] = 6.67 mM, [TCPO] = 0.34 mM, [4MImH] = 3.4 mM.

Table 6 Detection limits of 1-aminopyrene, DPA, and perylene using microfluidic cell with ODI derivative CL detection.

PAHs	1-aminopyrene	DPA	perylene
pmol/LCW	0.02	17.71	6.62

Length of LCW: 2.0 cm, flow rate of ODI derivative CL reagents and 1-aminopyrene: 18.3 μl/sec, [H_2O_2] = 6.67 mM, [TCPO] = 0.34 mM, [4MImH] = 3.4 mM.

3.4 Quantification of 1-aminopyrene, DPA, and perylene using microfluidic cell with ODI derivative CL detection

With the measurement of CL intensity emitted in the narrow microfluidic cell with ODI derivative CL detection, as shown in Fig. 3, we were able to obtain a calibration curve capable of quantifying low concentrations of 1-aminopyrene. Also, we obtained calibration curves for DPA and perylene (not shown). Sensitivities of DPA and perylene obtained with this system were not as good as that of 1-aminopyrene. Table 6 shows the detection limits (S/N = 3) of those PAHs determined in microfluidic cell with ODI derivative CL detection.

4. Conclusions

We developed a microfluidic cell using the principle of LCW to measure CL intensities emitted from rapid ODI derivative CL reactions. Using this system, low concentrations of 1-aminopyrene, DPA and perylene could be quantified. Based on research results obtained from the FIA system with microfluidic cell and ODI derivative CL detection fabricated in the present work, lab-on-chips and μ-TAS will be able to be developed. We expect that these systems will circumvent several problems (e.g., design of detection channels for complicated CL reactions, larger size than those with absorbance or fluorescence detection, high cost, limited applications) associated with previously reported detection systems (e.g., absorbance, fluorescence, luminal- and PO-CL).

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