Peak Formation Due to Chemiluminescence Reaction through the Collapse of Laminar Flow Liquid–Liquid Interface in a Microreactor

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A peak signal due to a chemiluminescence (CL) reaction in a microreactor consisting of a microchannel without sample plug formation is presented. The CL reaction of luminol–hydrogen peroxide–Cu(II) was used in this study. A CL profile, including a peak signal, was observed by the collapse of the liquid–liquid interface based on laminar flow in the microchannel. We examined CL profiles for various flow rates of reagents and detection points in the microreactor. The data obtained were taken into consideration, together with the residence times and diffusion lengths. On the basis of on the measurements of peak height, Cu(II) was determined over the range of 20 nM–0.1 mM with a detection limit of 20 nM (S/N = 3).

Chemiluminescence (CL) reactions have been well characterized in a variety of species for over a century. CL detection has many advantages in various applications, as demonstrated in FIA,[1] HPLC,[2] and capillary electrophoresis (CE).[3–5] These include: (1) high detection sensitivity, in particular, a detection limit down to the single-molecule level can be accomplished in CE, which is comparable with that achieved using laser-induced fluorescence; (2) a wide linear range of the response signal, which is beneficial for quantitative analyte determination; (3) inexpensive reagent and apparatus as well as easy and rapid measurement; and (4) no light source or spectroscopes are needed, allowing the instrument configuration to be very simple.

Over the past decade, miniaturized chemical analysis systems, commonly referred to as micro-total analysis systems (μ-TAS),[6] have been reported in various fields. A microreactor comprising a microchannel[7,8] has several features for chemical reactions including: (1) rapid heat exchange and mass transfer which cannot be achieved using a conventional batch system; (2) laminar flow which can be obtained in a micro-fluidic system; and (3) large specific surface area to volume ratio. Some microreactors have been shown to have potential applications for analyzing small amounts of compounds in conjunction with very limited volumes.

Several groups have tried to apply the CL reaction in microchannels fabricated in microreactors for quantitative analyses. For example, Cr(III)[9] and epinephrinein[10] were detected with CL reactions of luminol and lucigenin, respectively. It should be emphasized that most CL detection systems in microreactors need a sample injection device outside a microreactor. The use of a sample injector forms a sample plug in a microchannel. The sample plug leads to peak formation in the CL profile as an output. The observation of a peak signal is very useful for quantitatively detecting analytes that participate in the CL reaction. However, a sample injection device for making a sample plug sometimes needs additional parts such as sample loops, injection valve, carrier line, and a delivery system, necessitating laborious procedures to handle them. Furthermore, any mechanism to mix a sample plug with a CL reagent requires additional devices to be fabricated in the microreactor.

In this study we propose to induce a peak signal due to a CL reaction in a microchannel fabricated within a microreactor without the sample plug formation. An interesting CL profile, including a peak signal, was observed through the collapse of the liquid–liquid interface based on laminar flow in a microchannel. That is, the laminar flow results in the liquid–liquid interface separation of the two reagents. Stopping the flow results in the collapse of the interface at the detection point thereby inducing mixing of the reagents, leading to the CL reaction and to give a CL peak. We examined the CL profiles for various flow rates of reagents and at different detection points in the microreactor. The observation of the CL peak in the CL profile was applied for the quantitative analysis of Cu(II).

Figure 1 shows an illustration of the microreactor used in this study, incorporating a microchannel (300-μm width × 200-μm depth) made of polymethylmethacrylate.[7,8] Reagent 1 was a mixture of 1.0 mM luminol and 20 nM–0.1 mM Cu(II) (pH 10.8 phosphate buffer), which included sodium tartrate (20 times the Cu(II) concentration) as a masking reagent. Reagent 2 was 1.0 mM hydrogen peroxide (pH 10.8 phosphate buffer). The two reagents were joined at the junction point and subsequently fed to the microchannel. CL was detected at four detection points (1.0-mm length each along the channel), as shown in Figure 1, with a photomultiplier tube located under the microreactor. The detection points were named Point 1, 2, 3, and 4 from the junction point on the microreactor.

First, we examined the CL profiles for luminol–hydrogen peroxide–Cu(II) at detection Point 1 (Experiment 1). The results obtained are shown in Figure 2. Reagents 1 and 2 were delivered at the same flow rates by the respective syringe pumps. The flow rates were adjusted to be 50, 20, and 10 μL min^{-1}, respectively,

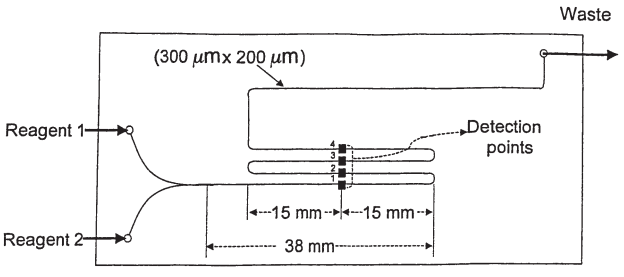

Figure 1. An illustration of the microreactor used in this study consisting of a microchannel. The figure shows the four detection points used in the study.

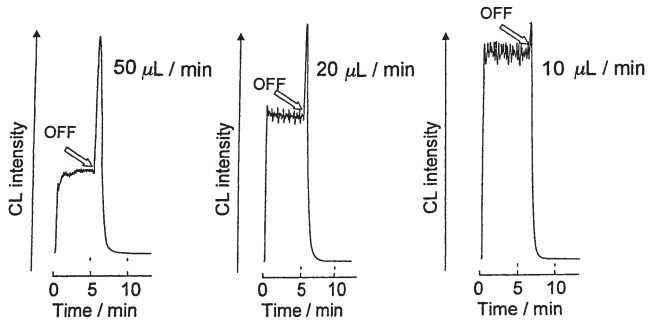

Figure 2. CL profiles obtained from Experiment 1 using two reagents. Reagent 1 was a mixture of 1.0 mM luminal, 50 μM Cu(II), and 1.0 mM sodium tartrate (pH 10.8 phosphate buffer); Reagent 2 was 1.0 mM hydrogen peroxide (pH 10.8 phosphate buffer). The CL profiles were obtained at Point 1. "OFF" indicates the time when the syringe pumps were turned off.

and the Reynolds numbers were calculated roughly to be 9.7, 3.7, and 1.9. Reagents 1 and 2 were delivered at appropriate flow rates to generate laminar flow in the microchannel, creating a liquid–liquid interface. We could observe stable CL intensity (baseline CL intensity) due to the CL reaction at the liquid–liquid interface. The baseline CL increased with decreasing flow rates or increasing reaction time (the corresponding residence times from the junction point to Point 1 were 0.9, 2.2, and 4.2 s, respectively). The liquid–liquid interface likely became fuzzy because of molecular diffusion with increasing residence times, which would make the mixing between Reagent 1 and 2 gradual. When the flow of both reagents was turned off, we could promptly observe a CL peak by the collapse of the laminar flow liquid–liquid interface at the detection point in the microchannel.

Next, we examined the CL profiles at Points 1–4 at a constant flow rate of 50 μL min^{-1} similar to Experiment 1 (Experiment 2). Analogous CL profiles comparable to those shown in Figure 2 (data not shown) were observed. The CL peak heights from the zero-line CL intensity (correspond to CL disappearing-line) were almost the same in both Experiments 1 and 2. That is, even though the conditions were different, the maximum CL intensities were constant. This might be due to a definite mixing state between Reagents 1 and 2 in the microchannel. The relative baseline CL intensity was plotted against the residence time

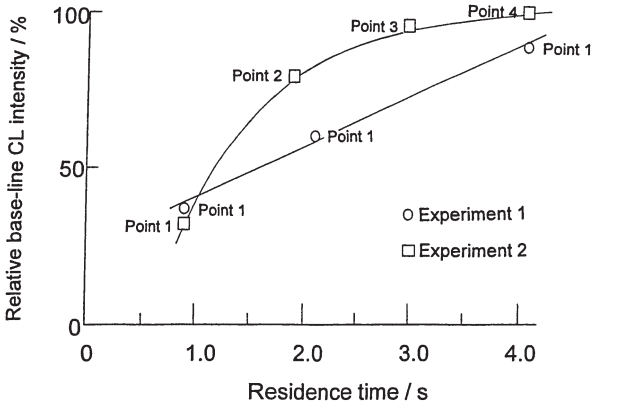

Figure 3. Plots of relative baseline CL intensity versus the residence time for Experiment 1 (○) and Experiment 2 (□) using the two reagents. Detection points were at 1–4.

(Figure 3). The relative baseline CL intensity indicates the percentage of the baseline CL intensity to peak height from the zero-line CL intensity. As shown in Figure 3, the relative baseline CL intensities of Experiment 2 were greater than those of Experiment 1. This indicates that passing the curves of the microchannel in Experiment 2 enhanced the mixing of the reagents. The diffusion length of the molecules is calculated by the following equation; $T = L^2/D$ (T = residence time, L = diffusion length, and D = diffusion coefficient; ca. 1×10^{-5} cm s^{-1}). The diffusion length of the molecules was estimated to be ca. 64 μm at the residence time of 4 s. It should be note that the baseline CL intensity became large, and the CL peak due to a collapse of the interface was not observed around 64-μm diffusion length in both Experiment 1 and 2, in spite of the 300-μm width of the microchannel. As mentioned above, a definite mixing state between Reagent 1 and 2, or an optimum mixing state, might exist for bringing about a constant and maximum CL intensity (peak height from zero-line CL intensity) in the CL profile. Future research will focus on the investigation of detailed relationships between diffusion, reagent concentration, chemical reaction, and the CL property.

Finally, we examined the relationship between the Cu(II) concentration and CL peak height from the baseline CL at a flow rate of 50 μL min^{-1} at Point 1. Cu(II) was determined over the range of 20 nM–0.1 mM with a detection limit of 20 nM (S/N = 3). The relative standard deviation was within 5%. In a previous report,[11] we developed an FIA system using the luminol CL reaction for determining metal ions; Cu(II) was analyzed over the range of 100 nM–50 μM with a detection limit of 100 nM. It was confirmed that observing a CL peak through the collapse of the liquid–liquid interface was useful for the quantitative analysis of Cu(II).

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