Chemiluminescence on a Microchip

Xing-Zheng WU,^{*†} Mari SUZUKI,^{*} Tsuguo SAWADA,^{**} and Takehiko KITAMORI^{***}

*Department of Materials Science and Engineering, Faculty of Engineering, Fukui University, Fukui 910-8507, Japan

**Department of Applied Chemistry, the University of Tokyo, Hongo, Tokyo 113-8656, Japan

***Kanagawa Academy of Science and Technology, Kawasaki 213-0012, Japan

(Received September 2, 1999; Accepted November 8, 1999)

Recently, there has been an increasing tendency toward miniaturizations or microfabrications of a chemical or analytical system on a glass chip. For example, many separations based on liquid chromatography, micellar electrokinetic chromatography, electrochromatography, and electrophoresis have been performed on chips with sizes of several centimeters.¹⁻¹⁰ DNA sequencing on a microfabricated chip was also reported.¹¹ Usually, micro channels with different patterns are designed and fabricated on the chips. A series of chemical or analytical operations such as introduction and transportation of reagent solutions, mixing and reaction, separation, and detection of the interested chemical species, are carried out on the chips. In addition to low sample consumption, cheap unit prices and disposability for the miniaturized chemical or analytical system, it also has distinctive properties inherent to the small dimension range. For example, diffusion is an efficient process for mixing fluids in the microchannels.¹²

The fluorescence method is the main detection tool for the miniaturized systems in the chips. Although fluorescence detection is highly sensitive, it is usually performed with a laser excitation and microscopic collection. Thus it requires complicated and precise optical arrangements. Recently, a thermal lens microscope has also been used for ultra sensitive detection of non-fluorescent species in a micro channel.¹³ In these cases, the detection systems are orders of magnitude larger than the chips, thus reducing the benefits of the miniaturization.¹⁴ Therefore, sensitive detective methods that are easily integrated to the chips are desirable. So far, only electrochemical detection has been integrated onto a chip.¹⁴

The chemiluminescence (CL) method is known to be one of the most sensitive detection methods. It has been widely used for analysis of metal ions and immunoassays.^{15,16} Since no light source is required in CL measurements, the instruments for CL method are much simpler than those for optical methods such as the fluorescence method. The CL method has the potential to be integrated onto a chip for the detection. Also, the micromachining technique could fabricate desirable reaction manifolds for CL reaction. However, few reports about CL on a chip have been reported so far.¹⁷ This might be due to the following reasons. Most of the experiments on the chips employ electroosmotic flow as a driving force to transport solutions in the micro channels. Electroosmotic flow is surely a suitable means for fluid control in the micro channels. However, it can be applied

only to aqueous solutions or mixtures of water and a few polar solvents.¹⁸ Moreover, it requires no gaseous components to obtain a stable electroosmotic flow. On the other hand, many CL experiments such as those using bis(2,4,6-trichlorophenyl)oxalate (TCPO) are carried out in nonaqueous solvents.¹⁹ Even for aqueous CL systems such as the most used luminol and H₂O₂ CL system, gaseous products such as N₂ and O₂ are usually involved.²⁰ Accordingly, the applications of CL to the chip with electroosmotic driving force are relatively limited.¹⁷ In this work, we investigate CL on the chip with a gravity flow as a driving force. Not only aqueous CL of luminol-H₂O₂ system but also nonaqueous CL of bis(2,4,6-trichlorophenyl)oxalate (TCPO)-H₂O₂ on the chip have been successfully demonstrated.

Experimental

Chemiluminescence reagents and preparation of solutions

Luminol and TCPO were dissolved in 0.1 M Na₂CO₃ buffer solution (pH 10.2)²¹ and ethyl acetate, respectively. Their concentrations were 10⁻⁴ M. Fluorescent dye perylene was added in the TCPO solution, its concentration was 10⁻⁴ M. A stock solution of 10⁻² M H₂O₂ was made by diluting 31% H₂O₂ with water. In the experiments using electroosmotic flow, the stock solution of H₂O₂ was diluted to desirable concentrations with the Na₂CO₃ buffer solution. In the luminol CL experiments using the gravity flow, the stock H₂O₂ solution was diluted with water. In TCPO CL experiments, the stock solution of H₂O₂ was diluted with acetonitrile. All chemicals were used as received from Wako Chem. Corp. (Japan).

Design of quartz glass chip

Figure 1 shows the design of the glass chip. The chip was composed of three quartz glass plates (the cover, middle, and bottom plates). Each plate was a 4×6 cm rectangle; the thickness were 350, 100, and 500 μm, respectively. A highly focused and intensified excimer laser beam was shone on the middle plate to pierce the channel part, and then the beam was scanned to inscribe a 'Y' shape channel pattern. The micro channels were made inside the glass chip by sandwiching the middle plate between the top and the bottom plates. Three small holes 1 mm in diameter, were mechanically bored on the top glass for two inlets and one drain. These three plates were laminated by using optical contact, that is, the plates were polished to an optically smooth and flat (λ/10) finish, then

[†] To whom correspondence should be addressed.

Fig. 1 Illustration of the experimental setup for CL on a chip.

laminated together in an oven at 800°C without any adhesive. The micro channels formed in the glass chip were 150 μm wide and 100 μm deep. Distance from the inlet holes to the cross point of the 'Y' shape micro channel was 3 cm, and that from the cross point to the drain hole was 1 cm (Fig. 1).

Chemiluminescence measurement

A photomultiplier tube (PMT, R4220, Hamamatsu, Japan) with a bias voltage of -900 V was used for measuring CL. As shown in Fig. 1, the PMT was placed above the glass chip. About 5 mm of the micro channel from the cross point was exposed to the PMT. The other parts of the micro channel were covered with pieces of black tape. The chip and PMT were placed in a light-tight box. The signal from the PMT was detected by an electrometer, and was recorded and treated with a smoothing program in a personal computer.

Introduction and transportation of the reagents solutions

In the gravity flow method, three capillaries (i.d.: 200 μm , o.d.: 360 μm , length: 65 cm) were coupled to the three holes in the chip by three sheets of rubber film, which were closely pressed onto the chip with an aluminum holder. The two inlet capillaries were immersed into the luminol (or TCPO) and H_2O_2 solution vials, respectively. Waste of the CL reaction was drained out from the micro channel through the drain capillary. The vials were placed in a 50 cm high position for about 1 min, then moved to the same horizontal level as the end of the drain capillary for about 1 min. This operation was repeated for several times.

In the electroosmotic flow method, three small vials about 0.2 ml in volume for two inlets and one drain, were directly glued to the chip around the three holes, respectively. The two inlet vials were filled with the solutions of luminol and H_2O_2 (in the Na_2CO_3 buffer solution), respectively. The drain vial was filled with the Na_2CO_3 buffer solution. In each vial, a Pt electrode was immersed. Polarity of the Pt electrodes in the inlet vials was '+', and that in the drain vial was '-'. An electric voltage about 1–3 kV was applied between the '+' and '-' electrodes for a certain period, then, the electric voltage was switched off. The switch on and off operations of the electric voltage were repeated.

Results and Discussions

As stated above, so far most experiments on chips have used the electroosmotic flow as the driving force. It was also tried in this

Fig. 2 Typical CL signals of luminol- H_2O_2 system. Concentration of luminol was 10^{-4} M , and concentration of H_2O_2 in A), B), C) were 10^{-5} , 10^{-6} , 10^{-7} M , respectively. Arrows 'S' mean that the flows were stopped (the height difference was 0 cm), and 'F' flowed (the height difference was 50 cm).

work. However, experimental results showed that it was difficult to obtain reproducible CL signals because of the following reasons. Firstly, electrogenerated chemiluminescence (ECL)²² of luminol was observed when an electric voltage was applied to the electrode immersed into the vial for luminol solution which was glued on the chip. This ECL interfered with the measurement of CL from the luminol CL reaction. Secondly, a gaseous product N_2 was produced during the model CL reaction.^{20,23} This resulted in unstable electric currents, and thus unstable electroosmotic flow during the experiments.

Here, we examine the gravity flow method as a driving force in the micro channel. As shown in Fig. 1, when a height difference exists between the inlet and the drain capillaries, both luminol and H_2O_2 solutions flow into the micro channel. When the height difference is zero, the flows will stop. Figure 2 shows the typical CL signal when the height difference was changed from 50 cm to 0 cm. When the height difference was 50 cm, CL intensity was small. On the other hand, when the height difference was 0 cm, CL intensity increased quickly, and then reached to a maximum. Finally, CL intensity decreased slowly. It is well known that the Reynolds number in a micro channel is much less than that at low flow rates in a conventional tube, and thus a flow in the micro channel is always a laminar flow.¹² When luminol and H_2O_2 solutions flowed in the channel, little mixing occurred between their laminar layers. Since the laminar flow rates were much faster than molecular diffusions, only molecules of luminol and H_2O_2 at the region near interfaces of their laminar layers reacted. Thus, only weak CL was observed. On the other hand, when the flows stopped, luminol molecules diffused to H_2O_2 layer and H_2O_2 molecules to luminol layer. The diffused luminol and H_2O_2 molecules reacted with each other and produced CL. The time at which CL reached to the maximum CL depended on the diffusion coefficients and concentrations of reactants in the aqueous solution. As shown in Fig. 2, when the concentration

Fig. 3 Typical CL signals of TCPO- H_2O_2 system. Concentration of H_2O_2 in acetonitrile and TCPO in ethyl acetate were 10^{-5} M and 10^{-4} M, respectively. Arrows 'S' mean the flows were stopped (the height difference was 0 cm), and 'F' flowed (the height difference was 50 cm).

of luminol was 10^{-4} M, the higher the concentration of H_2O_2 was, the shorter the time at which CL intensity is the maximum was. This is because the higher the H_2O_2 concentration was, the more the luminol molecules reacted during the initial diffusion process.

Figure 2 also shows that the reproducibility of CL signal was not bad when the gravity flow method was used. The repeatability (relative standard deviation) of the maximum CL intensities for solutions of 10^{-6} M H_2O_2 was 7.3% ($n = 7$). The CL maximum intensity was found to be nearly proportional to the H_2O_2 concentration in a range of 10^{-7} M – 10^{-5} M. The detection limit for H_2O_2 was about 10^{-7} M ($S/N = 2$). Since the volume of micro channel exposed to the PMT (detection volume) was about 20 nL, the detection limit of the absolute amount of H_2O_2 was about 2×10^{-15} mol (2 fmol). This detection limit could be lowered by adding metal ions such as Co^{2+} , since metal ions catalyze the CL reaction and thus enhance the sensitivity.²⁰ Also, a more sensitive PMT would decrease the detection limit.

Furthermore, nonaqueous CL of TCPO- H_2O_2 was also investigated. This nonaqueous CL system has been widely used in the CL detection of HPLC. Figure 3 shows the typical CL signals for the nonaqueous CL. Similar to Fig. 2, the maximum CL intensity was also observed when the flow was stopped. When the solutions were flowed in the chip, CL intensity was small. This indicates that the organic solvent in the microchannel was also a laminar flow. On the other hand, the nonaqueous CL decayed faster than the aqueous CL shown in Fig. 2. This is because that the diffusion coefficient of a compound is usually larger in an organic solvent than in aqueous solution,²⁴ i.e., the diffusion of reactants in the nonaqueous solvents are faster than that in aqueous solution.

Such results show that not only aqueous CL but also nonaqueous CL experiments could be carried out on the chip with a gravity flow as a driving force. The maximum CL intensity was obtained by stopping the flow in the micro channel. When the flow is not stopped, CL reaction efficiency is low since only a little part of the CL reagents located in the interface regions of the two laminar flows takes part in CL reaction. This means that the maximum sensitivity could not be obtained if the CL method was used as a detection method for CE or other separations techniques in the chip, since the stop of

the flow in the micro channel was not desirable in the separation process. In order to solve this problem, a micro mixing technique that could be used in the microchannel is required to be developed. Decrease of the depth of the micro channel is also effective to increase the CL reaction efficiency, since the molecular diffusion-induced CL reaction occurred in the interface regions will become more efficient in a more thinner channel.

References

1. D. J. Harrison, A. Manz, Z. Fan, H. Ludi, and H. M. Widmer, *Anal. Chem.*, **1992**, *64*, 1926.
2. C. S. Effenhauser, A. Manz, and H. M. Widmer, *Anal. Chem.*, **1993**, *65*, 2637.
3. K. Seiler, D. J. Harrison, and A. Manz, *Anal. Chem.*, **1993**, *65*, 1481.
4. S. C. Jacobson, R. Hergenroder, L. B. Koutny, and J. M. Ramsey, *Anal. Chem.*, **1993**, *66*, 2369.
5. D. E. Raymond, A. Manz, and H. M. Widmer, *Anal. Chem.*, **1994**, *66*, 2858.
6. A. W. Moore, S. C. Jacobson, and J. M. Ramsey, *Anal. Chem.*, **1995**, *67*, 4184.
7. F. V. Heeren, E. Verpoorte, A. Manz, and W. Thormann, *Anal. Chem.*, **1996**, *68*, 2044.
8. S. C. Jacobson and J. M. Ramsey, *Anal. Chem.*, **1996**, *68*, 720.
9. S. C. Effenhuser, G. J. M. Bruim, A. Paulus, and M. Ehrat, *Anal. Chem.*, **1997**, *69*, 3451.
10. P. A. Walker, M. D. Morris, M. A. Burns, and B. N. Johnson, *Anal. Chem.*, **1998**, *70*, 3766.
11. D. Schmalzing, A. Adourian, L. Koutny, L. Ziaugra, P. Matsudaira, and D. Ehrlich, *Anal. Chem.*, **1998**, *70*, 2303.
12. Micro Total Analysis System'98, Proceedings of the mTAS'98 Workshop, ed. D. Jed Harrison and Albert van den Berg, Kluwer Academic Publishers.
13. K. Sato, T. Kitamori, and T. Sawada, *Anal. Sci.*, submitted.
14. A. T. Woolley, K. Lao, A. N. Glazer, and R. A. Mathies, *Anal. Chem.*, **1998**, *70*, 684.
15. For example: X.-Z. Wu, M. Yamada, T. Hobo, and S. Suzuki, *Anal. Chem.*, **1989**, *61*, 1505.
16. For example: M. Tobata, M. Totani, and J. Endo, *Anal. Chim. Acta*, **1992**, *262*, 315.
17. D. M. Shakuntala and D. J. Harrison, *Electrophoresis*, **1998**, *19*, 2301.
18. R. S. Sahot and M. G. Khaledi, *Anal. Chem.*, **1994**, *66*, 1141.
19. For example: K. Kobayashi and K. Imai, *Anal. Chem.*, **1980**, *52*, 424.
20. I. Kamiya, "Kagaku Hakkou (Chemiluminescence, in Japanese)", **1972**, Koudansha, Tokyo.
21. D. Price, P. J. Worsfold, and R. F. C. Mantoura, *Anal. Chim. Acta*, **1994**, *298*, 121.
22. A. W. Knight and G. M. Greenway, *Analyst*, **1994**, *119*, 879.
23. C. Matachescu, S. Kulmala, T. Ala-Kleme, and H. Joela, *Anal. Chem.*, **1997**, *69*, 3385.
24. D. E. Gray, "American Institute of Physics Handbook", 3rd ed., **1963**, McGraw-Hill, New York.