Chemiluminescence Detection in Microchip Capillary Electrophoresis

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Chemiluminescence detection was used in microchip capillary electrophoresis. Peroxyoxalate chemiluminescence reaction was used, and dansyl amino acids were successfully separated and detected. The present system had advantages in rapid run time (within 40 s), small (several 10 pL) and accurate sample injection method, and simplification and miniaturization of detection device.

In the past several years, micro total analysis system (μ-TAS) has received much attention. Since Harrison et al. demonstrated the feasibility of a chemical analysis system on a small glass substrate using electrokinetic phenomena,[1,2] capillary electrophoresis (CE) integrated on microchip (microchip CE) has been much studied. Laser-induced fluorescence (LIF) detection has been the most commonly used in microchip CE.[3-6] However, LIF detection essentially necessitates a large laser light source and spectroscope. The requirement not only leads the instrumentation to expensiveness but also reduces benefits of miniaturization.

Chemiluminescence (CL) does not need any light source, so that CL could be one of the most attractive detection methods in μ-TAS. We have successfully demonstrated that the ordinary CE with CL detection system was applicable to the separation and determination of small amounts of metal ions, metal complexes, dyestuffs, proteins, alkaloids, and oligopeptides.[7-10] Several CL reagents, such as luminol,[8] peroxyoxalate,[7,10] and ruthenium (II) complex[9] were used.

We have tried to apply CL to a detection method in microchip CE for miniaturization and simplification. Harrison et al. reported CL detection using luminol CL reaction for microchip-based capillary electrophoresis.[11] They used devices of comparatively complicated design; intricate channels, five reservoirs, double-T injector, junction of two channels for mixing, and CL reagent flow at detection area. We will report here microchip CE-CL detection using peroxyoxalate CL reaction. The present system features simpler device construction in comparison to Harrisons' one; only two main channel, four reservoirs, cross-shaped injector, and absence of the junction of channels for CL reaction. The simplification was achieved on the basis of our original know-how which was previously presented as a batch-type CL detector for CE.[10] Bis[(2-(3,6,9-trioxadecanyloxycarbonyl)-4-nitrophenyl]oxalate (TDPO) reaction was used with H_2O_2, and dansyl amino acids were separated and detected in the microfabricated device.

Figure 1 shows schematic diagram of microchip CE-CL detection device. Microchips made of quartz were obtained from Shimadzu Corporation. The microchip consists of two pieces of 28 mm x 11 mm plate: the bottom plate (1.0 mm thickness) has two microchannels of 20 μm deep and 50 μm wide, while the cover plate (0.5 mm thickness) has *ca.* 1 mm diameter holes drilled into it, to facilitate access to the microchannels and serve as reservoirs (P1–P4) in Fig. 1. The channel lengths are as follows: P1 to intersection 7.5 mm; P2 to intersection 7.5 mm; P3 to intersection 7.5 mm; and P4 to intersection 21.5 mm (which corresponds to effective separation length). Four pieces of silicon rubber tubes (i.d. 2.0 mm, length ~5 mm) were attached to a cover plate to form larger reservoirs for the electrolyte solution. Pt wires were inserted into these reservoirs. A photosensor module (Model H5783, Hamamatsu Photonics, Inc., Hamamatsu, Japan) was located under the P4. A computer-controlled system was used to apply and switch the potential on the device reservoirs.

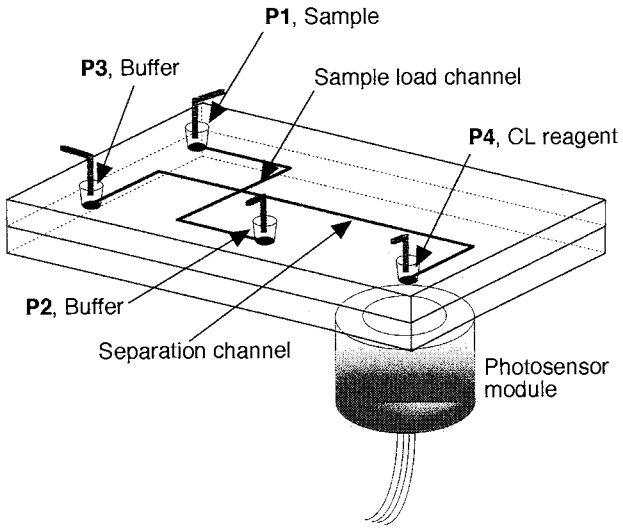

Figure 1. Schematic diagram of microchip CE-CLD system.

Dns-Gly and -Lys were dissolved in 0.1 M (M = mol dm^{-3}) Tris-H_3BO_3 buffer (pH 7.0) as migration electrolyte, and the sample was diluted with the buffer for use. CL reagent was a mixture of 1 mL of 4 mM TDPO in acetonitril and 21 μL of 30 wt% H_2O_2 aqueous solution. The solutions added to each reservoir are indicated in Figure 1.

Sample plug formation was achieved by applying 600 V for 20 s to P1, with P2 held at ground. At this process, 400 V was also applied to P3 and P4 to prevent sample spreading out to the separation channel. After the intersection was fully filled with the sample, separation was effected with 500 V applied to P3, with P4 grounded. Also, 360 V was applied to P1 and P2 to hinder sample in the sample load channel leaking to the separation channel. The ingenious procedure enabled one to inject a definite and small sample volume (several 10 pL). The sample allowed separation was mixed with CL reagent in P4 to produce visible light. The light was detected with the photosensor module. The output from the module (operated at 900 mV) fed an amplifier (Model EN-21, Kimoto

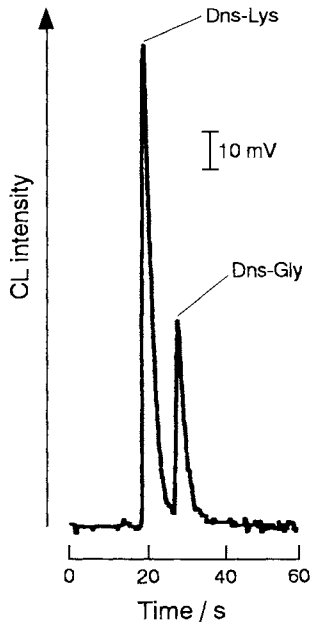

Figure 2. Electropherogram of Dns-Lys and Dns-Gly. Concentrations of each dansyl amino acid were $4x10^{-4}$ M. Solutions added to each reservoir were represented in Figure 1. Applied voltages to each reservoir were described in the text.

Electric, Inc., Osaka, Japan) connected to an integrator (C-R6A, Shimadzu, Inc., Kyoto, Japan) to produce electropherograms.

Figure 2 shows an electropherogram of dansyl amino acids. Dns-Gly and -Lys were successfully separated and detected within 40 s. Very fast run time could be achieved due to extremely short separation length on the microchip. Slight peak tailings in the electropherogram are attributed to the lifetime of the present CL reaction. Theoretical plate numbers for the dansyl amino acids were typically 300–600. As compared with an ordinary CE system, the present microchip CE-CL detection system has remarkably advantages as follows: rapid run time; small sample volume; accurate injection volume; and simplification and miniaturization of detection device.

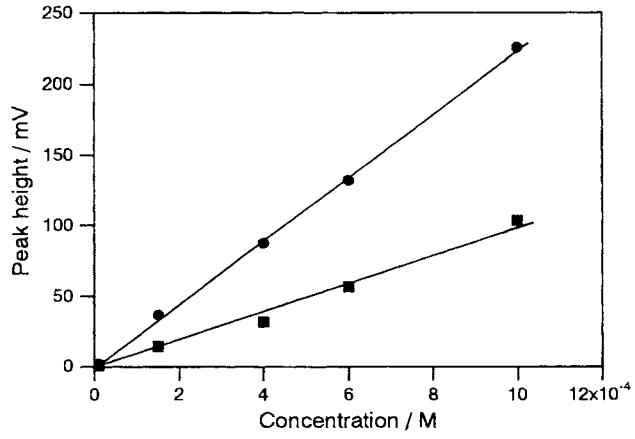

Figure 3. Calibration curves of dansyl amino acids. ●, Dns-Lys; ■, Dns-Gly. The experiment was performed under the same conditions as in Figure 2.

Calibration curves of Dny-Gly and -Lys are shown in Figure 3. Linear correlation could be obtained between concentration and peak height for both the compounds. The detection limit (S/N = 3) for Dns-Lys was $1x10^{-5}$ M, which was higher than those obtained in the ordinary CE with CL detection system. Several nL of sample volume was commonly injected in ordinary CE while several 10 pL of sample volume was injected in microchip CE. CL intensities are significantly dependent on reagent volumes. The extremely small injection volumes in microchip CE are considered to be the reason for the less sensitive results.

In conclusion, microchip CE-CL detection was constructed by use of peroxyoxalate CL reaction. The separation and detection system was simple and miniature since CL detection needs neither light source nor complex spectroscopes. The size of the detection device matches with that of microfabricated separation and reaction devices, bringing reality the laboratory-on-a-chip concept.

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