Simple and Sensitive Detection Cell for Capillary Electrophoresis-Chemiluminescence Analysis Using Peroxyoxalate Reagent

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Simple and convenient batch-type detection cells were developed for the capillary electrophoresis with chemiluminescence detection using peroxyoxalate reagent. A fused-silica capillary was directly inserted into the cell, and a grounding electrode was also done into it. The cell could be easily combined with capillary electrophoresis without any complex construction and expensive implements. A mixture of Dnsyl-amino acids was satisfactorily separated. Dnsyl-Trp was detected with the detection limit of 1 x 10^{-8} M by use of a glass cuvette-type cell.

The applicability of chemiluminescence (CL) detection in capillary electrophoresis (CE) has recently been successfully demonstrated. Various CL reagents, such as luminol,[1-5] acridinium,[6] peroxyoxalate,[7-10] and Ru(II) complex,[11] have been utilized. Particularly, peroxyoxalate reagent which can detect fluorescent and fluorescent-labeled compounds seems to be one of the most useful and attractive CL analyses. Because the labeling for fluorescence methods have been widely investigated, and the technique usefully applied for CE-fluorescence detection.

We have reported the CE-CL detection methods using peroxyoxalate reagent, including flow-[7,8,10] and batch-type[12] CL detection cells. The previous batch-type cell had several merits for measurement.[12] However, an optical fiber which the cell needed must be set up there elaborately to the capillary with a small space.

In this paper, novel batch-type cells were proposed for the CE-CL detection method using peroxyoxalate reagent. They were glass cuvette- and Teflon tube-type cells. The CL light generated at the capillary outlet was directly detected by a photosensor module (PM) (Model H5783, Hamamatsu) without an optical fiber. The present cells were clearly simpler and more convenient than any other detection cells in the CE-CL detection methods.[1-12]

Schematic diagrams of CL detection cells were shown in Fig. 1 (the whole system of the CE-CL detection had been shown in the previous papers[8,11]). The glass cuvette-type cell has an inner diameter of 5 mm and the inner volume of about 0.7 ml. A capillary and a platinum wire as grounding electrode were inserted into the cuvette through the upper silicone rubber. The Teflon tube-type cell has the same inner diameter and volume as the glass cuvettte-type one. In the Teflon tube-type, an electrode was inserted into the tube through the lower silicone rubber, but a capillary was done into it through the upper one. Both of the detection cells also worked as outlet reservoir including an electrolyte solution (a migration buffer). The cells were put just in front of PM.

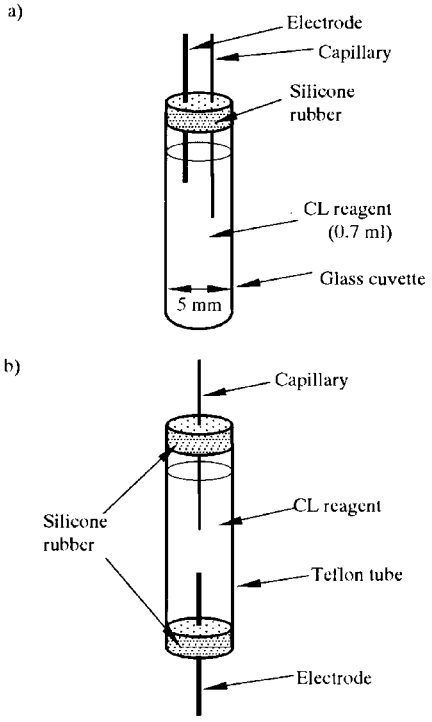

Figure 1. Schematic diagrams of CL detection cells. a) Glass cuvette-type and b) Teflon tube-type.

A high voltage (15 kV) was applied to the electrodes using a DC power supply (Model HCZE-30PNO. 25, Matsusada Precision Devices Co. Ltd.). A fused-silica capillary of 40 cm length x 50 μm I. D. (GL Sciences Inc.) was used. A mixture of 40 ml of 2 mM bis[2-(3,6,9-trioxadecanyloxycarbonyl)-4-nitrophenyl] oxalate (TDPO) acetonitrile solution and 280 µl of 30 wt% H_2O_2 was added into the cells. Dnsyl (Dns) amino acids were used here as model samples mainly because of commercial availability. The samples were dissolved in 0.1 M tris-borate buffer (pH 7.0) which was used as a migration buffer. A sample was introduced into the capillary by gravity method for 20 s at a height of 15 cm. The sample was migrated in the electrolyte solution toward the CL detection cell and mixed with reagents. The resulting CL at the capillary outlet was directly detected by the PM which was equipped in CL detector (Model EN-21, Kimoto Electric, Inc.). The output from the detector was fed to an integrator (Chromatopac C-R6A, Shimadzu) to produce electropherograms.

Electropherograms of Dns-Trp were examined by use of the two types of the cells. The typical electropherogram is shown in Fig. 2. The two type cells showed almost the same sensitivity and resolution. In order to examine the repro-

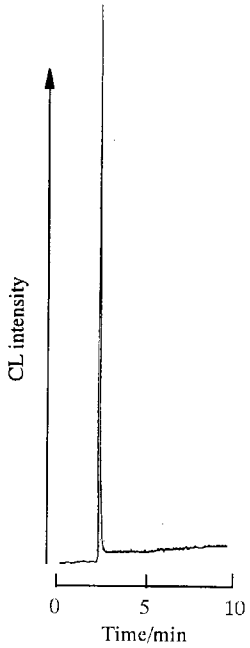

Figure 2. Typical electropherogram of Dns-Try. Conditions: Cell, glass cuvette-type; capillary, 40 cm length × 50 μm I.D.; applied voltage, 15 kV; migration buffer, 0.1 M tris-borate (pH 7.0); CL reagent, 2 mM TDPO and 200 mM H_2O_2; and sample, 1.0×10^{-5} M Dns-Trp.

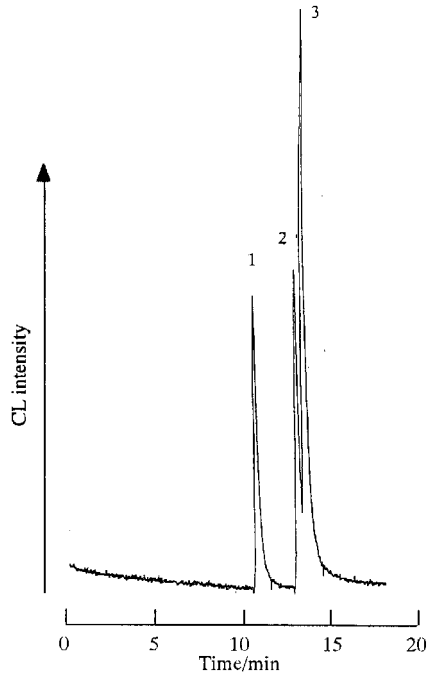

Figure 3. Separation of the mixture of Dns-amino acids(1. Dns-Lys, 2. Dns-Try, and 3. Dns-Gly, each concentration of 3.3×10^{-6} M). The experiment was carried out under the same conditions as described in Figure 2, except for applied voltage of 2.5 kV.

ducibility, the sample of Dns-Trp (1 x 10^{-5} M) was injected repeatedly 10 times, without any treatments such as capillary washing and reagents exchange. In the glass cuvette-type cell, the peak heights did not change until 7th injection but gradually decreased after that. The peak height of 10th injection indicated about 4 % decrease of the peak height of the 1st injection. While, in the Teflon tube-type cell, the peak height did not change at all up to 10th injection. The difference in the reproducibility of peak height seems to depend on the positioning of the capillary and the electrode in the detection cell.

The reproducibility of the Teflon tube-type cell was better than that of the glass cuvette-type cell, as mentioned above. But a bubble formation was observed on the inner wall of the Teflon tube. The hydrophobic property of Teflon is supposed to be a source of the bubbles. The formation often disturbed the measurement of CL intensity. In this study, the separation of the mixture of Dns-amino acids (Dns-Trp, Dns-Gly, and Dns-Lys) and the calibration curve of Dns-Trp were examined by use of the glass cuvette-type cell. The mixture sample of these Dns-amino acids was satisfactorily separated and detected, as shown in Fig. 3. The calibration curve of Dns-Typ featured the determinable range of 1 x 10^{-8} - 1 x 10^{-5} M and the detection limit (S/N=3) of 1 x 10^{-8} M. The theoretical plate number was 12000. The detection limit was approximately 2 orders of magnitude lower than that obtained with fluorescence. The present CE-CL detection system showed about 10 times higher sensitivity than the previous CE-CL detection system[12] using the detection cell equipped with an optical fiber. The direct detection of CL light without the fiber must lead to the improvement of sensitivity.

The present batch detection cells could be easily combined with CE without any complex construction and expensive implements, and the CE-CL detection method was operated for measurement without any tedious procedures and special techniques.

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