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Short communication

Miniaturization of batch- and flow-type chemiluminescence detectors in capillary electrophoresis

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Abstract

Glass and PTFE tubes as detection cells were put in small light-tight boxes to achieve miniaturization of batch- and flow-type chemiluminescence detectors for capillary electrophoresis. These light-tight boxes which included a detection cell and a photosensor module were successfully designed. In the batch-type detector using a glass tube as a detection cell, the influences of a repeated injection of sample and a reagent volume of the detection cell on chemiluminescence intensity were examined in detail. By using 3.8 mm I.D. glass tube including 400 μl chemiluminescence reagent solution, the chemiluminescence peaks were reproducibly observed for the repeated injection experiment up to the eight injection with each run time of 3.0 min. Dansyl-Trp was determined over the range $3 \cdot 10^{-8}$–$1 \cdot 10^{-5}$ *M* with the detection limit of 0.43 fmol ($S/N=3$). In the flow-type detector using a PTFE tube as a detection cell, both ends of the PTFE tube were connected to three-way joints; a chemiluminescence reagent solution was delivered into the cell and a capillary was inserted through one of the joints while an electrode was inserted through the other one. Dansyl-Trp was determined over the range $1 \cdot 10^{-7}$–$1 \cdot 10^{-5}$ *M* with the detection limit of 1.3 fmol ($S/N=3$). By using the compact flow-type detector, a mixture of dansyl-amino acids was separated and detected in micellar electrokinetic chromatography mode.
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1. Introduction

Over the past decade, capillary electrophoresis (CE) has been shown to be a powerful and efficient analytical separation technique. One of the major areas of study is the development of sensitive detection methods. Adsorption and fluorescence detections are the most commonly used as on-column optical detection modes, due to the extremely small sample zone and capillary dimension in CE. More sensitive detection can be realized when the analytes can be used in conjunction with laser-induced fluorescence [1], amperometric [2], or radiometric [3] detection.

An alternative sensitive detection scheme is the use of chemiluminescence (CL) [4]. CL reactions generally lack selectivity, however, the CL detection system combined with separation methods can offer excellent analytical selectivity and sensitivity. CL

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has already been shown to be a highly sensitive detection method in both flow-injection analysis and high-performance liquid chromatography [5–7]. Recently, the applicability of CL detection in CE has been successfully demonstrated. Various CL reagents, such as luminol [8,9], acridinium [10], peroxyoxalate [11,12], and Ru(II) complex [13], have been utilized. We have also reported various types of CE–CL detection methods, including batch-type [14–16] and the flow-type CL detection cells [8,11,17]. Other research groups have developed excellent CE–CL detection systems for analyses of a trace amount of metal ions [18–21].

The CL detection featured a high sensitivity, a wide determinable range of sample concentration, and an inexpensive apparatus and reagent. Additionally, miniaturization of the analytical system must be predominant merit in CL detection, because the CL detector does not need any light source and spectroscopy. We could combine the microchip CE with CL detector [22,23] on the concept of μ-total analytical system. We also examined miniaturization in batch- and flow-type detection cells for an ordinary CE [24,25]. However, miniaturization of CL detector including a detection cell and a photosensor module does not seem to be sufficiently examined and realized up to date.

In this study, in order to miniaturize the CL detector in CE, we designed small light-tight boxes for the batch- and flow-type CL detectors which included a detection cell and a photosensor module. These CL detectors were very simple and compact as shown in Fig. 1 in comparison with other detectors such as adsorption and fluorescence which necessitated a light source and spectroscopy. Also, we examined the influences of a repeated injection of sample and a reagent volume of the cell in the batch-type detector as well as the influences of a flow-rate and an inner diameter of the cell in the flow-type detector.

2. Experimental

2.1. Reagents

All of the reagents used were of commercially available special grade. Ion-exchanged water was distilled for use. Amino acids labeled with 5-(dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride) (Dns) (Dns-Ala, Dns-Val, Dns-Trp, Dan-Met, Dns-Lys, and Dns-Gly) were purchased from Sigma (St. Louis, MO, USA), Bis[2-(3,6,9-

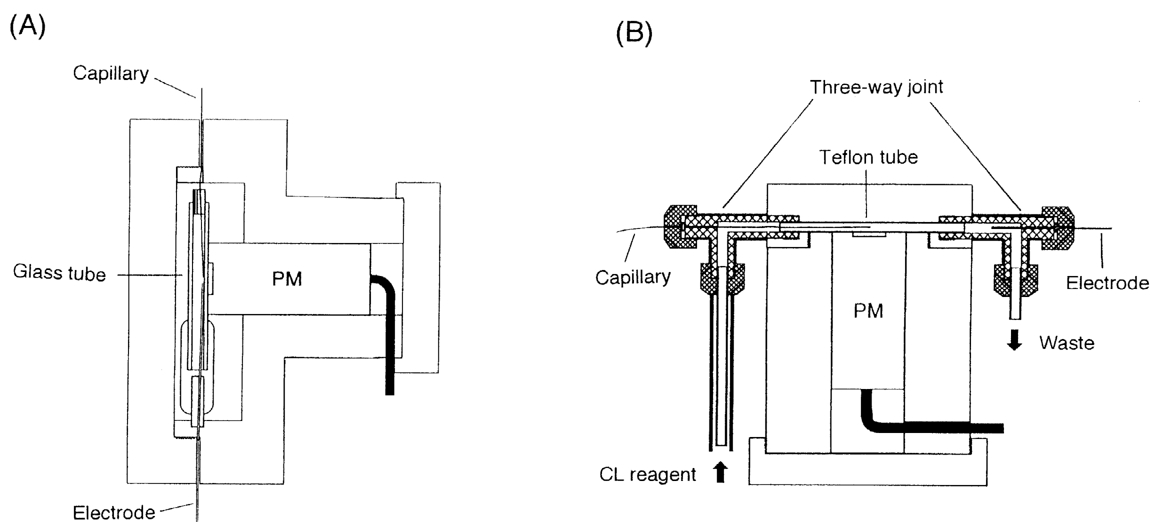

Fig. 1. Schematic diagrams of (A) batch-type and (B) flow-type CL detectors.

trioxadecanyloxycarbonyl)-4-nitrophenyl]oxalate (TDPO) was received from Wako (Osaka, Japan).

2.2. CL detectors

Schematic diagrams of CL detectors including a detection cell and a photosensor module are shown in Fig. 1. In the batch-type (Fig. 1A), a glass tube was used as a detection cell. A capillary was inserted into the tube through the upper silicone rubber and an electrode was passed through the lower PTFE union joint. The detection cell also worked as outlet reservoir including CL reagent solution. The cell was put just in front of a photosensor module. The detection cell had a simple construction, so that it was easily shielded to a light together with a photosensor module with polyacetal resin (black color), as shown in Fig. 1A.

In the flow-type (Fig. 1B), PTFE tube as a flow detection cell and photosensor module were enclosed in a small light-tight box made of polyacetal resin (black color). Both ends of the PTFE tube were connected to the three-way joints. CL reagent was delivered to the cell and a capillary was inserted through one of the joints, while an electrode was inserted through another one.

2.3. Apparatus and analytical procedure

A new capillary (40 cm×50 μm I.D. fused-silica) was treated with 0.5 *M* NaOH for 10 min and then washed with distilled water. A definite high voltage was applied to electrodes using a d.c. power supplier (Model HCZE-30PNO. 25, Matsusada Precision Devices). 0.1 *M* Tris–borate buffer solution (pH 7.0), except for 0.1 *M* NaOH–borate buffer solution (pH 8.3) containing 150 m*M* sodium dodecylsulfate (SDS) in micellar electrokinetic chromatography (MEKC), was used as a migration buffer solution. Sample injection was performed by gravity for 20 s at a height of 15 cm. A sample was migrated in the capillary toward the CL detection cell in the batch- or flow-type detector, and then mixed with CL reagent (4000 μl of TDPO acetonitrile solution+94 μl of 30% (w/w) H_2O_2 aqueous solution). The resulting CL at the capillary outlet was detected by a photosensor module which was connected to an instrument (Model EN-21, Kimoto Electric).

3. Results and discussion

3.1. Repeated injection experiment in batch-type CL detector

As the detection cell of the glass tube had simple construction, the cell and the photosensor module were easily shielded to a light with polyacetal resin (black color) to achieve miniaturization of a batch-type CL detector, as shown in Fig. 1A.

The following preliminary experiment was done: The effect of the distance between the tip of capillary and the end of electrode in a cell on CL performance was examined in the batch-type CL detector. The peak height and the theoretical plate number of Dns-Trp were measured as a function of distances of 2, 10, and 20 mm. The peak height and the plate number did not change under these conditions. The experimental data indicated that we do not need any especial attention with respect of the positionings of the capillary and the electrode.

Since the batch-type CL detector does not need expensive pumps and complex joints and lines, the detection device is simply made. However, there are two important matters to be examined in miniaturization of the batch-type detector; a repeated injection of sample and a reagent volume in the detection cell. First, the repeated injection was examined by use of Dns-Trp as a model sample.

The sample of Dns-Trp was injected repeatedly without any treatments such as capillary washing and reagents exchange. The experiments were carried out in run times of 2.5 and 10.0 min, respectively. The CL intensities were plotted against number of injections and total run time (Fig. 2). The intensities or peak heights gradually decreased in both experiments. The cause of the decreasing CL might be thought as follows: (1) reaction products between sample and CL reagent; (2) a migration buffer solution eluted from the capillary into the cell, and (3) electrochemical reaction with a grounding electrode in the cell. As shown in Fig. 2b, in both experiments of each run time of 2.5 and 10.0 min, the CL intensities decreased with the same decreasing degree. Judging from the data the cause of (1) would be negligible for the decreasing CL intensity.

We also examined the relationship between total run time and the CL intensity by use of a capillary of

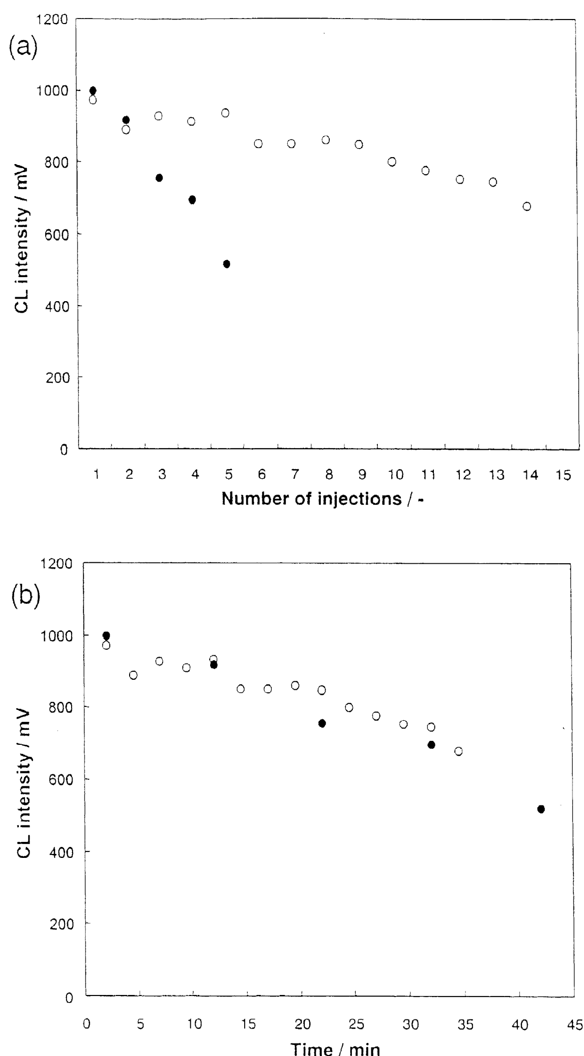

Fig. 2. The CL intensity of Dns-Trp as a function of (a) number of injections and (b) total run time for the repeated injection experiment. (○) 2.5 min and (●) 10 min run times. Conditions: Capillary: 40 cm×50 μm fused-silica; applied voltage: 18.5 kV; migration buffer: 100 m*M* Tris–borate (pH 7.0); CL reagent volume: 400 μl; sample concentration: $6.0 \cdot 10^{-6}$ *M* Dns-Trp.

25 μm I.D., and the obtained data were compared with the data obtained by 50 μm I.D. capillary. The CL intensity of 50 μm I.D. capillary gradually decreased as mentioned above, while the CL intensity of 25 μm I.D. capillary was almost constant. Therefore, the cause of (3) may be deleted.

On the assumption of that electroosmotic flow does not change between 25 and 50 μm I.D. capillary, the volume of the migration buffer solution eluted from the capillary when using 25 μm I.D. capillary reduces to 25%, compared with when using 50 μm I.D. capillary. We have not known the exact reason for the decreasing CL intensity, however, the migration buffer solution eluted from the capillary (the cause of (2)), which led to change in composition of solution in the cell, seemed most likely reason for the decreasing CL.

3.2. Influence of reagent volume in batch-type CL detector

CL reagent volume in the cell was also very important parameter in miniaturization of the batch-type detector. Various reagent volumes, 600 and 400 μl using 3.8 mm I.D. glass tube, as well as 240 and 80 μl using 2.6 and 1.5 mm I.D. glass tube, respectively, were examined for the repeated injection experiment with each run time of 3.0 min (Fig. 3). The CL intensity was almost constant up to the eight injection with 600 and 400 μl reagent volume, while it decreased from the beginning with 240 and 80 μl. The degree of the decreasing CL increased with decreasing of the reagent volume.

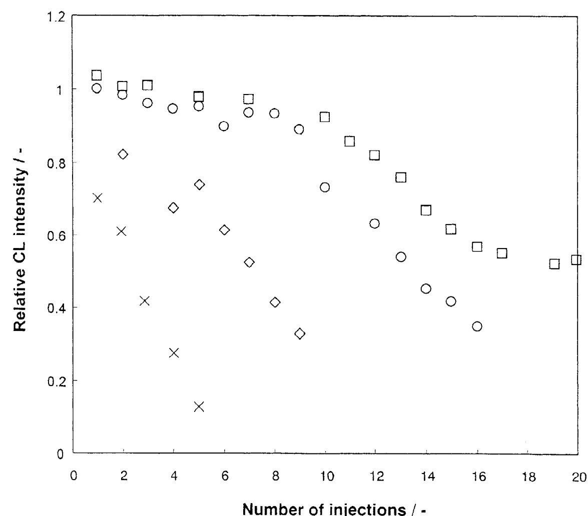

Fig. 3. The effect of CL reagent volume on CL intensity. (□) 600 μl; (○) 400 μl; (◇) 240 μl; and (×) 80 μl. Conditions: Capillary: 40 cm×50 μm fused-silica; applied voltage: 18.5 kV; migration buffer: 100 m*M* Tris–borate (pH 7.0); sample concentration: $1.0 \cdot 10^{-5}$ *M* Dns-Trp.

This phenomenon is not consistent with the above consideration that the migration buffer solution eluted from the capillary into the cell may cause the lowering of CL intensity in the repeated injection experiment.

3.3. Separation and determination of amino acids in batch-type CL detector

A mixture sample of Dns-amino acids (Dns-Trp, Dns-Gly, and Dns-Lys) was subjected to the CE with the present batch-type CL detector using 3.8 mm I.D. glass tube including 400 μl CL reagent solution. These dansyl-amino acids were separated and detected within 3 min; Dns-Lys, Dns-Trp, and Dns-Gly were migrated in this order. Dns-Trp was determined over the range $3\cdot10^{-8}$–$1\cdot10^{-5}$ M with the detection limit of 0.43 fmol (S/N=3). The present CE–CL detector showed about ten times higher sensitivity than the previous CE–CL detector [15] using the detection cell equipped with an optical fiber.

3.4. Analytical conditions in flow-type CL detector

A PTFE tube as a flow detection cell is small in itself, compared with the detection cell of a glass tube in the batch-type. Therefore, we tried to enclose the detection cell of a PTFE tube to both ends of which two three-way joints were connected and the photosensor module in a light-tight box for miniaturization. We could design the compact flow-type CL detector, as shown in in Fig. 1B, on the basis of know-how have been obtained through our research of the CE–CL detection systems [8,11,13–17].

Electropherograms of a mixture sample of Dns-Trp, Dns-Gly, and Dns-Lys were examined by use of 1.50 and 2.41 mm I.D. PTFE tube (the thickness of both tubes were the same—0.3 mm) as a detection flow cell, and compared with each other. The experiments were carried out with the same flow-rate of CL reagent (the flow-rate was defined as an advancing length of a cross section per min; mm/min). The narrow tube showed better resolution and higher peak height than the other one, probably because the narrow one reduced the dilution of a sample with CL reagent at the tip of capillary.

The effect of the flow-rate of CL reagent on the electropherogram was also examined by use of a mixture sample. It was found from the experiment results that around 65.8 mm/min gave the best resolution and peak height. The flow-rate of 65.8 mm/min was used for this study. Dns-Trp was determined over the range $1\cdot10^{-7}$–$1\cdot10^{-5}$ M with the detection limit of 1.3 fmol (S/N=3). The detection limit obtained by the compact flow-type CL detector was slightly higher than that obtained by the batch-type, maybe due to the vigorous dilution of sample with CL reagent in the flow-type. The flow-type CL detector naturally made the system possible for a continuous repeated injection. A good reproducibility was observed; the relative standard deviation was 4.4% for $1.0\cdot10^{-6}$ M Dns-Trp (n=10).

3.5. Separation and determination of amino acids in flow-type CL detector

When one takes notice of separation modes in the CE–CL detector, capillary zone electrophoresis has been used in almost all works. Only MEKC and capillary isoelectric focusing have been applied as other separation modes in several studies [26,27]. The flow-type detector seemed to be suitable to MEKC than the batch-type detector. Because the flesh CL reagent solution is continuously delivered to a tip of capillary, so that the migration buffer solution including SDS is easily removed from the reaction area. The mixture sample of Dns-amino acids (Dns-Ala, Dns-Val, Dns-Met, Dns-Trp, Dns-Gly, and Dns-Lys) was subjected to the CE with the present flow-type CL detector in MEKC mode. These dansyl-amino acids were base-line separated, as shown in Fig. 4. The order of their migration times was almost the same as that previously reported with absorption detection [28].

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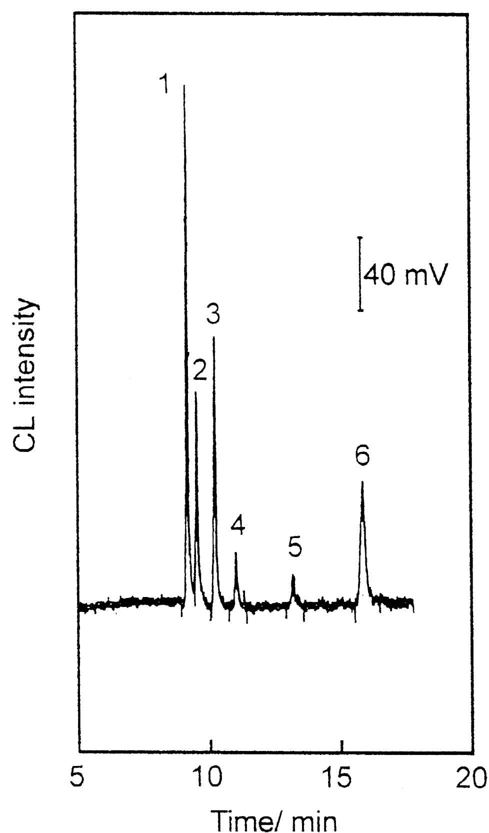

Fig. 4. The electropherogram of the mixture sample obtained using the flow-type CL detector. Peak identification: 1=Dns-Ala; 2=Dns-Gly; 3=Dns-Val; 4=Dns-Met; 5=Dns-Trp, and 6=Dns-Lys. Conditions: Capillary: 40 cm×50 μm fused-silica; applied voltage: 10 kV; migration buffer: 100 m*M* borate buffer (pH 8.3) containing 150 m*M* SDS; CL reagent flow-rate: 65.8 mm/min; sample concentration: $5.0 \cdot 10^{-6}$ M.

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