Simple and Convenient Cell for Chemiluminescence Detection in Capillary Electrophoresis

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Most capillary electrophoresis(CE)-chemiluminescence(CL) detection methods reported so far have involved variations of a postcapillary reactor to mix reagents.¹ The reactors require the insertion of one capillary (separation capillary) into another larger capillary (reaction/detection capillary). The procedures are manually intensive and cumbersome, and it is difficult to reproducibly control the reagent concentrations at the reactor. Furthermore, the reagents must be mostly passed through the larger capillary with an extremely small flow rate by high performance, and very expensive, pumps. Although the CL detection system combined with CE can offer excellent analytical selectivity and sensitivity, this method is still problematic.

In a previous paper², we designed a batch-type CL detection cell using an optical fiber. The capillary was well-set up straightly to the optical fiber with a small distance. The CL light generated at the capillary outlet was transported by the optical fiber to a photomultiplier tube. In this paper, a simpler and more convenient batch-type cell was developed without an optical fiber for CE, to which only a fused-silica capillary and a grounding electrode were inserted. Luminol CL was adapted for use with this detection cell. The cell was put just in front of a photosensor module (PM). The CL light generated at the capillary was directly captured by the PM. The cell could be easily combined with CE without any complex construction and the CE-CL detector was operated for measurements without any tedious procedures.

Experimental

A schematic diagram of the CL detection cell is shown in Fig. 1. The detection cell was made of Teflon and the inner volume was about 8 ml. One of the faces of the cubic cell was equipped Pylex glass (3 mm thick-

ness). A fused-silica capillary of 50 cm length×75 µm i.d. (GL Sciences Inc.) and a platinum wire as a grounding electrode were fixed to the detection cell. That is, the cell also worked as an outlet reservoir including an electrolyte solution (a migration buffer). The distance between the capillary outlet and the inner face of the glass was kept at about 3 mm. As analytes emerged from the capillary, they reacted with reagents to produce visible light.

A high voltage (12 kV) was applied to the electrodes using a DC power supplier (Model HCZE-30PNO.25, Matsusada Precision Devices Co. Ltd.). Luminol and a H₂O₂ CL system were used together with microperoxidase used as a catalyst. Luminol, isoluminol isothiocyanate (ILITC)-labeled compounds, H₂O₂, and the catalyst were dissolved in a 10 mM phosphate buffer solution (pH 10.8), which was used as a migration buffer. The catalyst was added to the inlet reservoir and H₂O₂ to the outlet reservoir or the detection cell. Sample injections were performed by gravity for 10 s at a height of 20 cm. A sample was migrated in the migration solution toward the CL detection cell and mixed with reagents. The resulting CL at the capillary outlet was directly captured by the PM, which was equipped in the 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.

Results and Discussion

An electropherogram of luminol was obtained under the conditions of 4 µM microperoxidase and 400 mM H₂O₂ (in Fig. 2). It clearly showed high sensitivity and high resolution efficiency. A luminol sample was injected repeatedly 15 times, without any treatments, such as capillary washing or reagent exchange. The peaks did not change; the heights showed good reproducibility of relative standard deviation, 3.4%.

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Fig. 1 Schematic diagram of the CL detection cell.

The calibration curve of luminol was examined. The obtained curve featured a determinable range, 5×10^{-10} – 1×10^{-6} M ($r=0.999$); the detection limit ($S/N=3$) was 5×10^{-10} M (15 amol); the theoretical plate numbers were 20000 – 30000. The detection limit was approximately 5 or 6 orders of magnitude lower than those obtained with absorbance. Zare *et al.*³ have reported an excellent design for a CE-CL detector, and derived the following data: the detection limit of luminol was 2.0×10^{-8} M (500 amol), and the theoretical plate number was 10000 – 20000. The present system is one of the most sensitive for luminol among any other CE-CL detection systems reported before.^{2–6} A mixed sample of glycine, glycyglycine, and glycyglycyglycine, which were labeled with ILITC, was subjected to the present CE-CL detector. The obtained electropherogram is shown in Fig. 3. The electropherogram indicates complete base-line separation.

Judging from the results, it was found that the cell could be easily combined with CE, and the system was operated without any tedious procedures. Also, the system featured an extremely high sensitivity and resolution. The performance must be one of the most excellent ones among the CE-CL detection methods that have been reported to date.

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Fig. 2 Electropherogram of luminol. Luminol concentration was 5.0×10^{-9} M.

Fig. 3 Separation of ILITC-labeled compounds. The sample concentration was 10^{-7} M order. Peak identification: 1, hydrolyzed ILITC; 2, ILITC; 3, glycyglycyglycine; 4, glycyglycine; 5, glycine.

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