Analytical Performance of Capillary Electrophoretic System with UV/CL or FL/CL Dual Detector

Kazuhiko Tsukagoshi,* Kaori Sawanoi, Masayuki Kamekawa, and Riichiro Nakajima
Department of Chemical Engineering and Materials Science, Faculty of Engineering, Doshisha University, Kyotanabe 610-0321

(Received June 13, 2003; CL-030538)

The capillary electrophoretic system with UV/CL or FL/CL dual detector was developed, taking advantage of the batch-type CL detection cell and the CL reaction of luminol-hydrogen peroxide or the flow-type CL detection cell and the CL reaction of peroxyoxalate–hydrogen peroxide. In each system, the UV or FL detection was carried out with on-capillary, followed by the CL detection with end-capillary. These systems could offer easily, rapidly, and simultaneously informations due to absorption and CL as well as those of FL and CL, respectively.

Capillary electrophoretic (CE) system has received much attention as a powerful separation instrument in the fields of not only analytical chemistry but also pharmaceutical chemistry and medicine. Absorption and fluorescence (FL) have been commonly adopted as a detection manner in CE system, owing to the very small dimension (<100 μm i.d.) of capillary into which the very small amount of sample migrates. These detection manners were carried out with on-capillary.

Also, chemiluminescence (CL) has been shown to be attractive and useful detection manner in FIA, HPLC, and CE system.[1–4] The CL detection in CE system is brought about with end-capillary (post column reaction), because a sample eluted from the capillary must be mixed with CL reagent at the tip of capillary to induce CL. We have developed various types of CL detection cells for CE system, including batch- and flow-types.[5] In this study, we tried for the first time to develop the CE system with ultraviolet absorption (UV)/CL dual detector or FL/CL dual detector. Sample was first analyzed by UV or FL detection with on-capillary, followed by the CL detection with end-capillary.

All reagents used were commercially available and analytical special grade. Ion-exchanged water was distilled before use. The batch-type CL detection cell is shown in Figure 1. The flow-type CL detection cell was similar to that reported in the previous paper.[6] They were enclosed in small light-tight boxes together with photomultiplier tubes to make compact CL detectors.

In the CE system with UV/CL dual detector, the batch-type CL detection cell was used, and luminol and isoluminol isothiocyanate (ILITC) were analyzed as a model. A fused-silica capillary of 75 μm i.d. and 75-cm length was used; 50-cm length for UV detection (280 nm) and 75-cm length for CL detection. A 10-mM phosphate buffer (pH 10.8) containing 4-μM microperoxidase was prepared as a migration buffer. A 10-mM phosphate buffer (pH 10.8) containing 400-mM hydrogen peroxide was put in the CL detection cell (ca. 0.8-mL volume). A modified spectrophotometric detector of Shimadzu Co. SPD-6A was used for absorption detection.

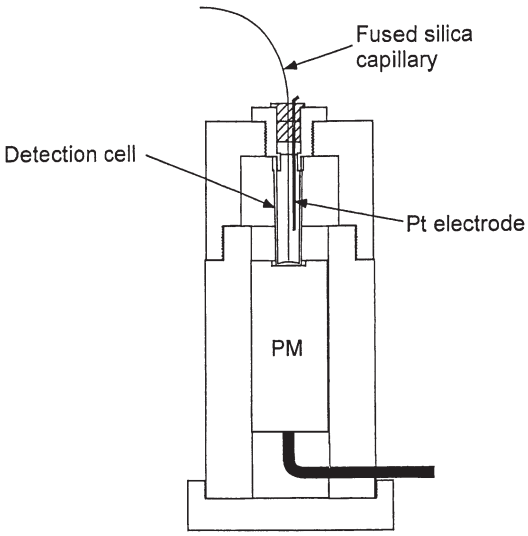

Figure 1. The schematic diagram of the CL detector including the batch-type CL detection cell and photomultiplier tube.

In the CE system with FL/CL dual detector, the flow-type CL detection cell was used and dansyl α-amino acids were analyzed as a model. A fused-silica capillary of 50 μm i.d. and 80-cm length was used; 50-cm length for FL detection (ex. 330 nm and em. 560 nm) and 80-cm length for CL detection. A 100-mM borate buffer (pH 7.0) was prepared as a migration buffer. An acetonitrile solution containing 2-mM bis[2-(3,6,9-trioxadecanyloxycarbonyl)-4-nitrophenyl] oxalate (TDPO) and 220-mM hydrogen peroxide as a CL reagent was fed to the tip of capillary outlet at a flow rate of 25 μl min^{-1} with a syringe pump. A modified fluorescence detector of Shimadzu Co. RF-535 was used for FL detection.

In the CE system with UV/CL dual detector, luminol was detected at ca. 12 and 18 min with UV and CL, respectively, and it was determined over the range of 1×10^{-6}–5×10^{-4} M and 1×10^{-9}–1×10^{-7} M with UV and CL detections. Although the CL signal of luminol was also observed with enough CL intensity at higher concentrations than 1×10^{-7} M, the CL intensity did not increase in proportion to the luminol concentration.

ILITC sample was also analyzed with the CE system with UV/CL dual detector. The obtained electropherograms are shown in Figure 2. The two main peaks were observed at ca. 12 and 15 min with UV detection, and the one main peak was observed at ca. 18 min with CL detection. Judging from their migration times, the first peak in UV detection corresponded

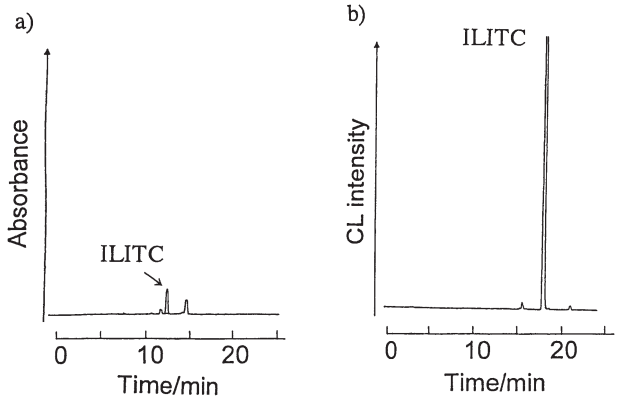

Figure 2. The electropherograms of ILITC sample. a) UV detection and b) CL detection. Conditions: Capillary, 75 μm i.d. and 50 cm for UV and 75 cm for CL detection; applied voltage, 10 kV; migration buffer, 10 mM phosphate buffer (pH 10.8) containing 4 μM microperoxidase; reagent in the outlet reservoir, 10 mM phosphate buffer (pH 10.8) containing 400 mM H_2O_2; and sample concentration, 1.0×10^{-5} M.

to the main peak in CL detection. Through the further experiments, it was found that a component producing the second peak in UV detection did not work as a labeling reagent. That is, ILITC sample included a natural ILITC and an impure component which showed absorption behavior but did not have CL property and labeling performance. The ILITC sample was commercially available, but the impurity may generate during preservation in our laboratory (we would like to close the maker name for the moment). Anyway, the present system quickly and precisely provided the information concerning the impurity or decomposition product through the simultaneous UV/CL detection. The system will be applied to detect a trace amount of compound possessing CL property selectively among many compounds having absorption property. In the future, also, it may be interesting to compare peak shapes obtained using UV detection and those of CL detection.

In the CE system with FL/CL dual detector, a mixture of dansyl α-amino acids was analyzed; dansyl lysine, dansyl tryptophan, and dansyl glycine were separated and detected. The obtained electropherograms are shown in Figure 3. They were detected around 4–5 min with FL and around 6–8 min with CL, respectively. Dansyl tryptophan was determined over the range of 1×10^{-5}–1×10^{-3} M and 1×10^{-7}–5×10^{-4} M with the FL and CL detections, respectively; the CL detection seemed to be about 100 times as sensitive as the FL detection. The system will be also applied to the detection of a specific compound on the basis of the FL performance among many FL compounds all of which are detected with CL.

Novel analytical CE system with UV/CL or FL/CL dual

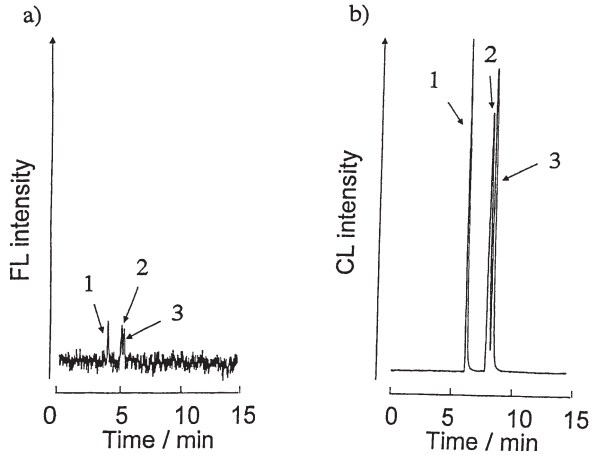

Figure 3. The electropherograms of the mixture sample of α-amino acids. a) FL detection and b) CL detection. 1, Dansyl lysine; 2, dansyl tryptophan; and 3, dansyl glycine. Conditions: Capillary, 50 μm i.d. and 50 cm for FL and 80 cm for CL detection; applied voltage, 20 kV; migration buffer, 100 mM borate buffer (pH 7.0); CL reagent, 2 mM TDPO and 220 mM H_2O_2 acetonitrile solution; and sample concentration, 5.0×10^{-4} M.

detector was successfully demonstrated, taking advantage of the batch-type CL detection cell and luminol–hydrogen peroxide CL reaction or the flow-type CL detection cell and peroxyoxalate–hydrogen peroxide CL reaction. Sample was detected by UV or FL with on-capillary, and successively detected by CL with end-capillary. The system easily, rapidly, and simultaneously gave the information due to both UV and CL detections or that of FL and CL detections, together with the high resolution performance based on capillary electrophoresis.

This work was supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan. The authors also acknowledge the financial support for this research by Doshisha University's Research Promotion Fund.

References

1 M. Yamaguchi, H. Yoshida, and H. Nohta, *J. Chromatogr., A*, **950**, 1 (2002).
2 K. Nakashima, *Bunseki Kagaku*, **49**, 135 (2000).
3 X.-J. Huang and Z.-L. Fang, *Anal. Chim. Acta*, **414**, 1 (2000).
4 K. Tsukagoshi, T. Nakamura, and R. Nakajima, *Anal. Chem.*, **74**, 4109 (2002).
5 K. Tsukagoshi, *Bunseki Kagaku*, **52**, 1 (2003).
6 K. Tsukagoshi, Y. Obata, and R. Nakajima, *J. Chromatogr., A*, **971**, 255 (2002).

Published on the web (Advance View) September 1, 2003; DOI 10.1246/cl.2003.894