Capillary Electrophoresis with Chemiluminescent Detection for Luminol Using Potassium Ferricyanide as a Catalyst

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Absorption and fluorescence detectors have been most commonly used for capillary electrophoresis (CE), which is performed with on-capillary detection. On the other hand, several groups have studied CE with a chemiluminescence (CL) detector.¹⁻⁴ This system can offer excellent analytical selectivity and sensitivity.⁵ We have applied several CL reagents to CE with a CL detector, such as luminol,⁶ peroxyoxalate,^{3,7} and Ru(II) complex.⁸ Luminol and isoluminol isothiocyanate-labeled compounds were analyzed using CE with a CL detector and microperoxidase as a catalyst.⁶ However, microperoxidase is expensive, and sometimes has problems in a capillary due to its large molecular size.

In this study, we examined the electrophoretic behavior of ferricyanide ion in a capillary by use of a batch-type detection cell. On the basis of the obtained information, we proposed and developed CE with a CL detector using potassium ferricyanide, instead of microperoxidase, as a catalyst for determining luminol or its derivative. A repeated injection analysis of luminol was achieved by using a flow-type CL detection cell.

Experimental

All of the reagents used were of commercially available special grade. Ion-exchanged water was distilled for use. Luminol, potassium ferricyanide, and H₂O₂ were purchased from Nacalai Tesque, Inc.

An apparatus of CE with a CL detector was made while referring to previous work.⁶⁻⁸ A high voltage (12 kV) was applied to electrodes using a DC power supply (Model HCZE-30PNO.25, Matsusada Precision Devices Co. Ltd.). A fused-silica capillary of 50 cm length and 75 μ m i.d. (GL Sciences Inc.) was used. Luminol was used as a model sample. The sample was dissolved in a 10 mM phosphate buffer (pH 10.8), which was used as a migration buffer. Sample injections were performed by siphoning from a height of 20 cm for 10 s. The sample of luminol was migrated toward the CL detection cell, batch- or flow-type, and then mixed with the reagent. The resulting CL at the capillary outlet was captured by a photomultiplier tube (PM) equipped in a CL detector (Model EN-21, Kimoto Electric, Inc.).

The concept of a batch-type CL detection cell was originally proposed by us in a previous paper.⁶ The detection cell was made of Teflon, which had a 4 cm outer diameter, 2.5 cm

height, and an 8 ml inner volume. An optical fiber (core diameter of 2 mm), a capillary, and a platinum wire as a grounding electrode were fixed to the cell. That is, the detection cell also served as an outlet reservoir. The optical fiber was set up straight to the capillary with a distance of 0.3 mm between them. When the sample emerged from the capillary, it reacted with the reagent at the capillary outlet to produce visible light. In the batch-type cell, potassium ferricyanide (25 mM) as a catalyst and H₂O₂ (10 mM) were added to the inlet and outlet reservoirs, respectively. Usage A: the catalyst was added to the inlet reservoir and H₂O₂ was added to the outlet reservoir or the CL detection cell, and in reverse Usage B: H₂O₂ was added to the inlet reservoir, and the catalyst to the outlet reservoir.

A schematic diagrams of the CE system with the flow-type CL detection cell is shown in Fig. 1. The phosphate buffer (pH 10.8) containing 25 mM potassium ferricyanide was fed through a Teflon-tube by siphoning at 15 cm height. A sample of luminol was migrated in a migration buffer containing 10 mM H₂O₂ to the CL detection cell, and was then mixed with the catalyst at the tip of the capillary to produce visible light. The light was directly captured by a PM without an optical fiber.

Results and Discussion

Much information concerning reagent concentration, mixing order, mixing method, *etc.* has been reported for CL detection in conjunction with FIA and HPLC for establishing a highly sensitive and reproducible system. However, there seems to have been a paucity of information about CL detection in CE, until now. Luminol CL generally requires that luminol or its derivatives be mixed with H₂O₂ and a catalyst. If the three components are not introduced as separate streams, the peroxide is destroyed by the catalyst before reacting with luminol or its derivatives, and no CL is observed. In order to provide useful information on connecting the CE system with the CL detector, the way to mix H₂O₂ and the catalyst was examined by the two usage procedures (Usages A and B) described above, using batch-type CL detection.

No CL signal was observed when potassium ferricyanide was added to the inlet reservoir (Usage A). In the present system, the velocities (toward the capillary outlet) of electroosmotic flow and migration of ferricyanide ion were estimated to be *ca.* +0.13 and -0.32 cm s⁻¹, respectively. Therefore, the anion could not be introduced into the CL detection cell from the

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Fig. 1 Schematic diagram of the CE with a CL detector using a flow-type CL detection cell.

Fig. 2 Effect of the H_2O_2 concentration on the CL intensity of luminol. Conditions: capillary, 50 cm length of 75 μm i.d.; applied voltage, 12 kV; migration buffer, 10 mM phosphate buffer (pH 10.8) containing H_2O_2 ; catalyst, 25 mM potassium ferricyanide; and sample, 1×10^{-7} M luminol.

capillary, which might result in the absence of CL. In Usage B, when potassium ferricyanide was added to the outlet reservoir, luminol showed a relatively high sensitivity. However, the CL signal decreased markedly in repeated injection analysis, and finally disappeared. The ferricyanide ion must migrate from the outlet reservoir to the inlet reservoir. The electrophoretic behavior of the anion led to a decreasing CL signal of luminol

in repeated injection analysis. Luminol and H_2O_2 in the migration buffer reacted with ferricyanide ion in the capillary, which migrated from the outlet reservoir. Visible light appeared in the capillary, and then the light became less intense when the sample zone of luminol reached the capillary outlet in the batch-type cell.

The flow-type CL detection cell could improve the defect of the decreasing CL signal. Luminol migrated in the migration buffer containing H_2O_2 always reacted with fresh potassium ferricyanide at the tip of capillary which was delivered through Teflon-tube by siphoning. The distance between the end of capillary and the electrode was *ca.* 8 cm. There was no problem concerning the distance for electrophoresis because of the buffer containing sufficient electrolytes. The effect of the H_2O_2 concentration on the CL intensity was examined (Fig. 2). The maximum CL intensity was observed at around 10 mM H_2O_2 , and the concentration was used for this study. The effect of the potassium ferricyanide concentration on the CL intensity was also examined (the data not shown), and a concentration of 25 mM was recommended. Figure 3 shows the relationship between the height of the vessel containing the potassium ferricyanide solution and the CL intensity. It was found that the height or the flow-rate influenced the CL intensity; and a height of 15 cm was used in this study.

The electropherogram of luminol was examined under the optimized analytical conditions; the obtained result is shown in Fig. 4. A repeated injection analysis of luminol was achieved by use of a flow-type CL detection cell. The relative standard deviation of 1×10^{-7} M luminol was within 3% ($n = 10$). We examined the calibration curve of luminol; the determinable

Fig. 3 Relationship between the height of the vessel containing potassium ferricyanide solution and the CL intensity of luminol. The experiments were carried out under the conditions described in Fig. 2, except for 10 mM H_2O_2 .

range of luminol was 8.0×10^{-9} – 1.0×10^{-6} M (correlation coefficient, 0.999). Daddo and co-workers reported an excellent design for CE with CL detection, and derived the detection limit of luminol to be 2.0×10^{-8} M.⁴ The present system seems to become one of the most promising ways in CE with a CL detector.

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Fig. 4 Electropherogram of luminol. The experiments were carried out under the conditions described in Fig. 3.