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Short communication

Simultaneous operation of plural separation modes in capillary electrophoresis with a chemiluminescence detector possessing a micro-space area for reaction/detection

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Abstract

Analysis of α-amino acids, proteins, and phenolic compounds was simultaneously performed using three capillaries in capillary electrophoresis with chemiluminescence detection, taking advantage of the micro-space area for reaction/detection at the tip of the capillary. The three capillaries included usual, polymer-containing, and sodium docley sulfate (SDS)-containing migration buffers for separation. The eluted samples from the capillaries, which were inserted into the chemiluminescence detection cell, were mixed with chemiluminescence reagent at the tips of the capillaries to generate visible light. The specific micro-space area for reaction/detection at the tips of the capillaries enabled the simultaneous operation of the three separation modes in the present system.

Keywords: Chemiluminescence detection; Detection, electrophoresis; Instrumentation; Amino acids; Proteins; Phenolic compounds

1. Introduction

Chemiluminescence (CL) detection has been developed to provide a simple analytical instrument for not only flow injection analysis (FIA) and HPLC [1–3], but also capillary electrophoresis (CE) and microchip CE [4,5], as CL detection does not require a light source or spectroscopes. We also have studied CE with CL detection, and developed various types of interface devices between the CE and CL detection equipment, namely CL detection cells [6]. Many kinds of samples, including metal ions, metal complexes, α-amino acids, peptides, proteins, saccharides, nucleic acids, phenolic compounds, and liposomes, can be analyzed using the CE with CL detection system. In order to make separation of such compounds possible, we have introduced several separation modes other than capillary zone electrophoresis into the CE with CL detection system, such as capillary isoelectric focusing [7], micellar electrokinetic chromatography [8,9], and capillary gel electrophoresis [10].

Our past research into CE–CL detection, published in more than 40 related papers [6], has revealed a very significant feature of the CE-CL detection system. While absorption and fluorescence detection is performed in an on-capillary manner, CL detection is brought about by end-capillary (post column) detection. Thus, the CE–CL detection system possesses a very interesting and useful "micro-space area" for reaction/detection at the tip of the capillary outlet.

In the present study, we proposed taking advantage of the micro-space area for the simultaneous operation of multiple separation modes in the CE–CL detection system using multiple capillaries and corresponding migration buffers. The CL detection in CE was performed with end-capillary detection at the capillary outlet. The micro-space area for reaction/detection at the tip of the capillaries successfully led to the simultaneous operation of multiple separation modes in the CE–CL detection system.

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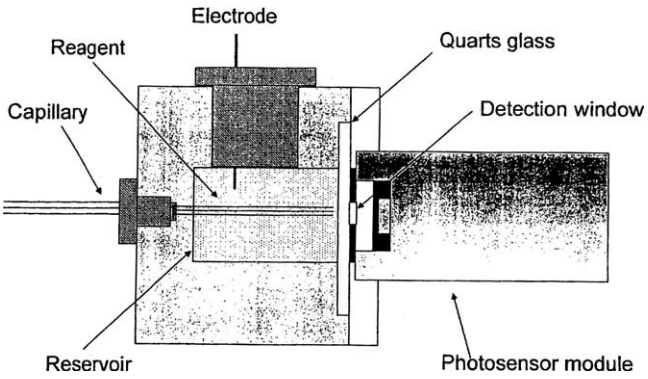

Fig. 1. A schematic diagram of the CL detection cell into which the three capillaries were inserted. Capillaries; 50 cm × 50 μm i.d. of fused-silica, applied voltage; 15 kV, CL reagent; acetonitrile solution containing 2 mM TDPO and 200 mM H_2O_2, and detection window; 0.3 cm diameter.

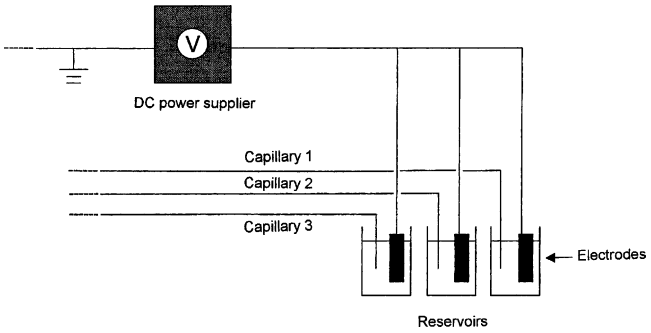

Fig. 2. A schematic diagram of the three inlet reservoirs in which the three capillaries and electrodes were inserted. Capillaries; 50 cm × 50 μm i.d. of fused-silica, applied voltage; 15 kV, and migration buffers; a 100 mM Tris–borate buffer (pH 7.0), a 100 mM Tris–borate buffer (pH 8.4) containing 0.1% (w/w) carboxymethylcellulose and 1 mM EDTA, and a mixture of a 10 mM phosphate buffer (pH 8.0) containing 2 mM SDS–acetonitrile (ratio buffer–acetonitrile is 1:1, v/v) for capillaries 1, 2, and 3, respectively.

2. Experimental

All reagents used were commercially available, and of analytical special grade. Ion-exchanged water was distilled for use. The batch-type CL detection cell which was originally developed in our laboratory was used [4]. The migration buffers were prepared for separating α-amino acids, proteins, and phenolic compounds as follows: a 100 mM Tris–borate buffer (pH 7.0), a 100 mM Tris–borate buffer (pH 8.4) containing 0.1% (w/w) carboxymethylcellulose and 1 mM EDTA, and a mixture of a 10 mM phosphate buffer (pH 8.0) containing 2 mM sodium dodecyl sulfate (SDS) and acetonitrile (the volume ratio of the buffer–acetonitrile is 1:1). The three capillaries containing the three kinds of migration buffers were called capillaries 1, 2, and 3, respectively. The three capillaries were inserted into the detection cell as shown in Fig. 1. Mixtures of α-amino acids (dansyl-lysine, dansyl-tryptophan, and dansyl-glycine), proteins (cytochrome *c*, ribonuclease, and lysozyme which were labeled with fluorescein isothiocyanate (FITC)), and phenolic compounds (phenol and 4-chlorophenol which were labeled with dansyl chloride) were used as model samples.

Sample injection was performed by the gravity method (at a height of 30 cm for 15 s). A high voltage of 15 kV was applied simultaneously between the three inlet reservoirs including corresponding migration buffers and the outlet reservoir (the CL detection cell) through the three capillaries (50 cm × 50 μm i.d.). We devised three inlet reservoirs as shown in Fig. 2. The device enabled to operate high voltage and inject samples simultaneously for three capillaries. The peroxyoxalate CL reaction was adopted here, and the CL reagent of an acetonitrile solution containing 2 mM bis[2-(3,6,9-trioxadecanyloxycarbonyl)-4-nitrophenyl] oxalate (TDPO) and 200 mM hydrogen peroxide was added into the detection cell. The model mixture samples migrated in the corresponding capillaries, and then mixed with the CL reagent at the tips of capillaries to induce CL. Visible light was detected by a photosensor module.

3. Results and discussion

In the three previous papers, we analyzed α-amino acids [9], proteins [10], and phenolic compounds [8] by means of respective separation modes using usual, gel-containing, and SDS-containing migration buffers. So that, in this study we selected the three sample groups and the three separation modes as a model by considering the actual results in the previous papers. First, we individually analyzed the model mixture samples of α-amino acids, proteins, and phenolic compounds with capillary 1. Each component in the mixture of dansyl-lysine, dansyl-tryptophan, and dansyl-glycine was well separated. However, the mixtures of cytochrome *c*, ribonuclease, and lysozyme as well as phenol and 4-chlorophenol were not separated by capillary

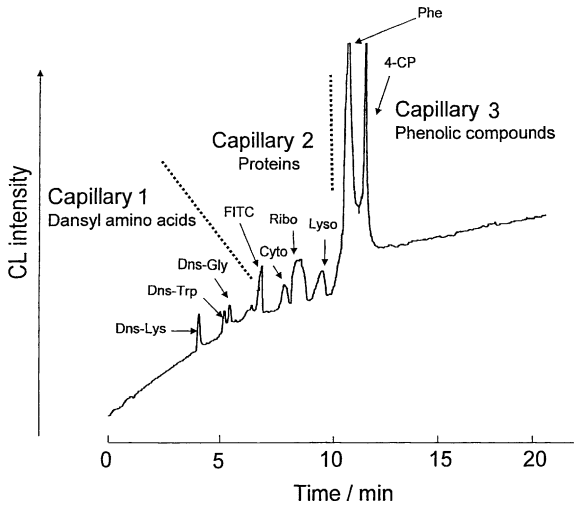

Fig. 3. The electropherogram obtained using the three capillaries. Conditions: capillaries; 50 cm × 50 μm i.d. and of fused-silica, applied voltage; 15 kV, CL reagent; acetonitrile solution containing 2 mM TDPO and 200 mM H_2O_2, and sample concentration; 1.0×10^{-5} M for α-amino acids and phenolic compounds, and 5.0×10^{-5} M for proteins.

1, but were separated by capillaries 2 and 3, respectively. Next, we examined simultaneously the two model mixture samples of α-amino acids and proteins, proteins and phenolic compounds, or α-amino acids and phenolic compounds using the two corresponding capillaries. They were all successfully separated and detected with the present CE with CL detection system (data not shown).

Finally, we tried to analyze the three model mixture samples simultaneously using the three capillaries. The samples were continuously eluted from the corresponding capillaries, and then mixed with CL reagent to generate CL. The CL detection was brought about with end-capillary detection (post-column reaction) at the tips of the capillaries. The obtained electropherogram is shown in Fig. 3. The α-amino acids dansyl-lysine, dansyl-tryptophan, and dansyl-glycine were separated in this order by capillary 1 and detected at around 4–6 min. Next, the proteins cytochrome *c*, ribonuclease, and lysozyme were separated in this order by capillary 2 and detected at around 8–10 min, with the excess fluorescein isothiocyanate detected at ca. 7 min. Subsequently, the phenolic compounds, phenol and 4-chlorophenol, were separated by capillary 3 and detected at around 10–12 min. The reason for the increase in base line with time has not been clear yet. However, the phenomenon might depend on some chemical reaction between CL reagent including TDPO and hydrogen peroxide in the cell and migration buffer from the capillary. In this study we made the detection window small (Fig. 1), changing ca. 0.3 cm from ca. 1 cm, and changed CL reagent in the cell for each experiment in order to decrease the degree of the increasing base line.

The specific micro-space area for reaction/detection at the tip of the capillary outlet enabled the simultaneous operation of multiple separation modes in the CE with CL detection system. The present study has determined some additional benefits of CE with CL detection, such as a quick examination of appropriate separation mode for an unknown sample and a high throughput analysis with various migration buffers.

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