Note

Post-column detection for capillary zone electrophoresis*

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(First received June 28th, 1988; revised manuscript received August 16th, 1988)

Ultraviolet absorption[1], fluorescence[2], potential gradient[3–5], conductivity[6], mass spectrometric[7] and carbon fibre electrochemical[8] detectors have been used in capillary zone electrophoresis. Post-column detection is widely used in liquid chromatography because it is highly selective, and if it could be used in capillary zone electrophoresis it would be particularly useful in the medical and pharmaceutical fields. In this paper, we propose a post-column detection method for this purpose. As a certain pressure is generated at the outlet side of the capillary column due to the mixing of reagents with the effluent, a closed system for capillary electrophoresis is proposed that uses three pumps and two mixing parts for post-column detection.

EXPERIMENTAL

A schematic diagram is shown in Fig. 1. Fluoroethylene–propylene (FEP) tubing was supplied by Shimadzu (Kyoto, Japan). A pyrex glass capillary tube was drawn with a glass drawing machine (GDM-1; Shimadzu). These tubes were used as columns for capillary electrophoresis. Column media were supplied by pump 1 (Microfeeder, MF-2; Azuma Denki Kogyo, Tokyo, Japan) at a very low flow-rate of 0.1–2 μl/min. For post-column reaction, buffer and fluorescent reagent were supplied by pump 2 (FLC-A700; JASCO, Tokyo, Japan) and pump 3 (MF-2), respectively. A mixture of 0.1 M disodium hydrogen phosphate and sodium hydroxide solution (pH 11) containing 0.5% ethylene glycol was used as a buffer at a flow-rate of 0.15–0.40 ml/min.

The design of the four-way tetrafluoroethylene connector (Tuff connector; Gasukuro Kogyo, Tokyo, Japan) is shown in Fig. 2A. The end of the capillary column was inserted about 5 mm into a tetrafluoroethylene tube (0.5 mm I.D.). As the outside

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* Part of this work was presented at the *6th Symposium on Capillary and Isotachophoresis, December 11–12th, 1986, Nagoya, Japan.*
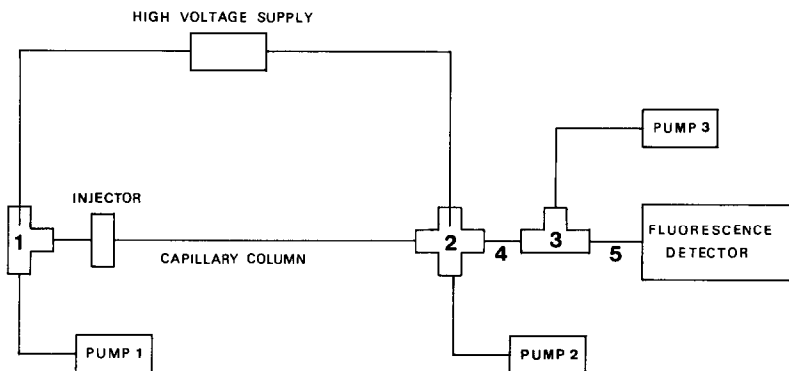
Fig. 1. Schematic diagram of post-column detection for detection in capillary electrophoresis. 1 = Positive terminal; 2 = four-way connector for earth terminal and mixing of column media and buffer; 3 = three-way connector for mixing with fluorescent reagent; 4 and 5 = PTFE tubes (0.5 mm I.D.) of length 5 and 70 cm, respectively. Column medium, alkaline buffer solution and fluorescent reagent were supplied by pumps 1, 2 and 3, respectively.

diameter of the FEP tubing used as the column was more than 0.5 mm, a fused-silica capillary (*ca.* 15 mm × 250 μm I.D. × 340 μm O.D.) (Scientific Glass Engineering, North Melbourne, Australia) was inserted in the end of the column, as shown in Fig. 2A. Detailed designs of the positive terminal and three-way connector are shown in Fig. 2B.

A 2 · 10^{-4} *M* fluorescamine–dioxane solution was mixed with the mixture of effluent and buffer in a T-type three-way connector (1 mm hole) (Gasukuro Kogyo). For fluorescence development, the mixed solution was passed through a coil of PTFE tubing (70 cm × 0.5 mm I.D.). The PTFE tubing was of 1/16 in. O.D. except where stated otherwise. A fluorescence detector (RF-530; Shimadzu) with a 12-μl cell was used without modification. A high-voltage power supply (50 kV and 500 μA) (Matsusada Precision, Kusatsu, Shiga, Japan) and a rotary-type injector[9] were used.

Operational procedure

First pump 1 was started, then pump 2 and finally pump 3. After starting the three pumps, sample was injected with a rotary injector.

To avoid flow backwards into the capillary column, the order of starting the three pumps is important. If alkaline buffer solution does flow into the capillary column, the column should be washed with column medium. If fluorescamine–dioxane solution flows into the capillary column, the column should be washed with an organic solvent such as dichloromethane.

As the fluorescamine–dioxane reagent should be kept dry, it is preferable to fit a stop valve in the line between pump 3 and the three-way connector. After starting pump 3, the stop valve is opened.

RESULTS AND DISCUSSION

The apparatus used consists of three pumps and two mixing parts. To avoid pulsing noise, all the pumps used were of the syringe type. Special care was taken in
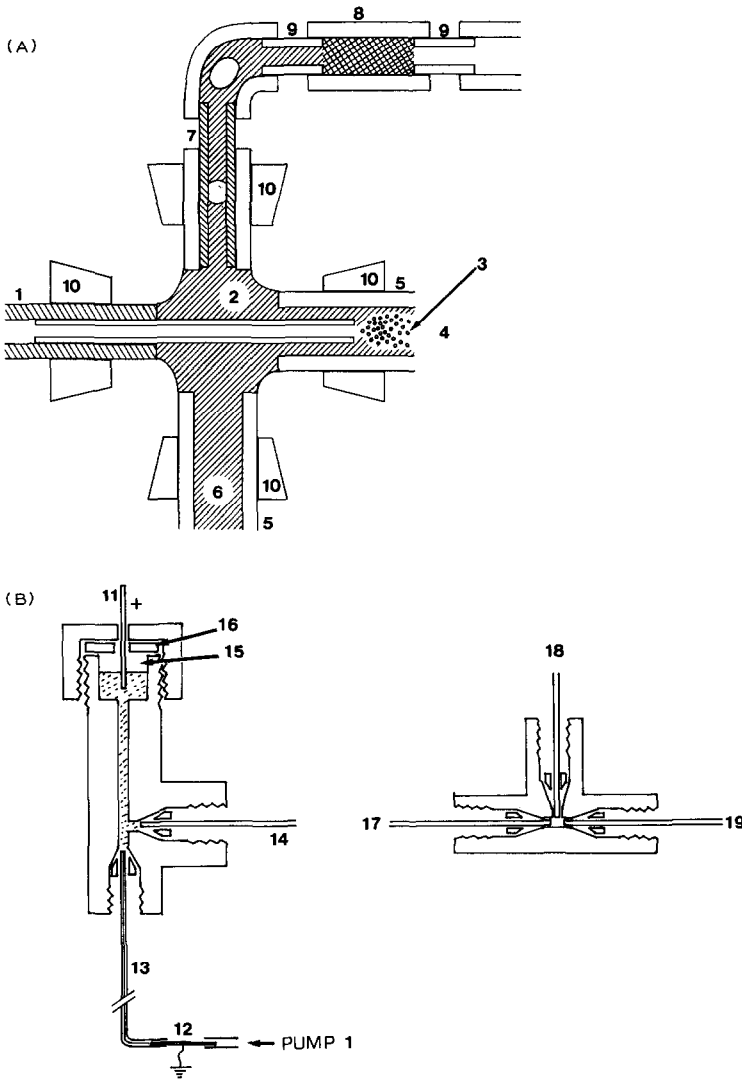
Fig. 2. (A) Mixing part for column medium and buffer at four-way connector. This part corresponds to 2 in Fig. 1. 1 = FEP capillary column; 2 = fused-silica capillary, inserted into the end of the FEP column; 3 = mixing zone; 4 = to fluorescence detector; 5 = PTFE tube (0.5 mm I.D.); 6 = alkaline buffer; 7 = earth terminal (platinum tubing); 8 = flow resistance, PTFE tubing packed with polymer beads; 9 = stainless-steel tubing; 10 = PTFE ferrule. (B) Detailed diagrams of positive terminal (left) and three-way connector (right), corresponding to 1 and 3, respectively, in Fig. 1. 11 = Positive terminal, platinum wire; 12 = platinum tubing (3 cm × 0.3 mm I.D. × 0.7 mm O.D.) connected to earth; 13 = PTFE tubing (2 m × 0.1 mm I.D. × 2 mm O.D.) as an electric resistance; 14 = FEP tubing connecting positive terminal and injector; 15 = space for bubbles; 16 = silicone-rubber septum. The mixtures of column medium and alkaline solution (17) and fluorescent reagent (18) were mixed in the three-way connector, and were led into a coil (19).

in constructing the four-way connector (Fig. 2A). The flow-rate of the column medium supplied by pump 1 was less than a few microlitres per minute. This column medium was mixed with alkaline buffer at the mixing zone (3 in Fig. 2A) just after it flowed out from the fused-silica capillary tubing. Although the alkaline buffer has a relatively high flow-rate (*e.g.*, 300 μl/min) compared with that of the column medium, it is essential to insert the column end into the 0.5 mm I.D. PTFE tube to avoid band broadening, as shown in Fig. 2A. The bubbles that were generated at the inner wall of the earth terminal were led to the upper part and carried outside after passing through 8 and 9 in Fig. 2A. Only a small fraction of the flow, *ca.* 10 μl/min, consistently came out. This device was also effective in excluding bubbles that were unexpectedly present in the alkaline solution. This device was essential for avoiding detector noise due to bubbles. Bubbles generated at the positive terminal and/or existing in the column medium were also collected (16 in Fig. 2B). To protect pump 1 from damage due to high voltage, a long PTFE tube (13 in Fig. 2B) was used as an electrical resistance. Between pump 1 and the tubing an earthed platinum tube (12 in Fig. 2B) was connected. Column medium supplied by pump 1 flowed through the tubing to the capillary column. There was a small current between 11 and 12 in Fig. 2B. Fluorescence reagent was mixed with the mixture of column medium and alkaline solution inside a simple three-way connector, as shown in Fig. 2B.

A certain pressure was generated at the column end owing to the mixing of reagent and buffer and to the long path from the end of the capillary column to the outlet of the flow cell. Therefore, it was necessary to impose at least an equal pressure at the side of the injector to prevent reverse flow from the column end. For this purpose, a pressurized flow was created by pump 1. Thus the solute was conveyed
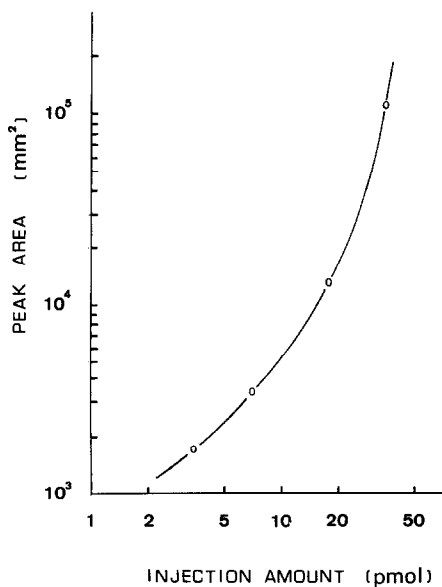

Fig. 3. Calibration graph for putrescine. Column, Pyrex glass capillary tube, (110 cm $\times$ 72 μm I.D.); column medium, 0.2 N lactic acid–0.5% ethylene glycol–0.1% sodium dodecyl sulphonate; applied voltage, 20 kV; flow-rates of pumps 1, 2 and 3, 0.52, 250 and 42 μl/min, respectively.

under a pressurized flow, v(pres), its mobility and electroosmotic flow. The experimental conditions were as in Fig. 3 except for alkaline buffer, 0.40 ml/min, sample 10 pmol putrescine in 0.1 M hydrochloric acid and pressurized flow. When the pressurized flow was varied, $e.g.$, 0.33, 0.52 and 1.04 μl/min, the number of theoretical plates, N, of the solute was 3010, 1020 and 360, respectively. N had the following relationship with v(pres):

$$\log N = A - B \log v(\text{pres})$$

where A and B are constants. Hence v(pres) should be kept as low as possible.

We examined the effect on N of changing the flow-rate of alkaline buffer from 0.15 to 0.40 ml/min. At more than 0.30 ml/min, N was almost constant. The solute leaving the capillary column took about 20 s to reach the cell of the detector at an alkaline buffer flow-rate of 0.4 ml/min. 20 s was sufficient to complete the reaction between solute and fluorescamine.

A calibration graph for the fluorescamine derivative of putrescine is shown in Fig. 3. The calibration graph is slightly curved, possibly because a certain amount of putrescine had been adsorbed on the inner wall of the capillary column. The detection limit was at the picomole level.

Chromatograms with different pressurized flows are shown in Fig. 4. Both peaks have the same area, although the pressurized flow-rate of in Fig. 2B (2.08 μl/min) was four times that in Fig. 2A. Variation of the pressurized flow in the range 0.33–3 μl/min had no effect on the peak area because the proportion of pressurized flow in the total flow-rate supplied by pumps 1, 2 and 3 was less than 1.5%. The calibration graph was independent on the flow-rate of the column medium and is valid for the experiment using FEP columns.
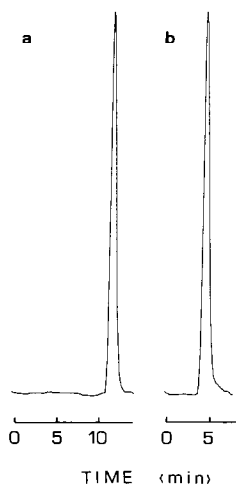

Fig. 4. Chromatograms of putrescine with different pressurized flows: (a) 0.52 and (b) 2.08 μl/min. Sample amount, 3.5 pmol. Other experimental conditions as in Fig. 3.
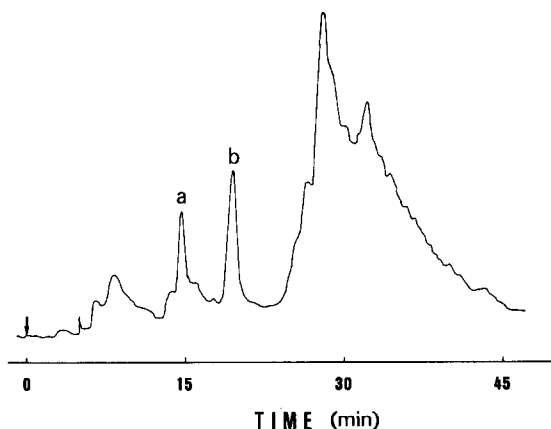
Fig. 5. Separation of groups of (a) free and (b) acetylpolyamines in human urine. Column, FEP (1.5 m × 0.3 mm I.D.); column medium, formic acid–0.01% ethylene cyanohydrin–5% ethylene glycol; applied voltage, 25 kV; current, 147 μA; flow-rates of pumps 1, 2 and 3, 2, 400 and 42 μl/min, respectively.

Application

The method was applied to the determination of polyamines in human urine and organs in rats. A typical example is shown in Fig. 5. The polyamines in human urine are almost always acetylpolyamines[10]. The urine sample was partially hydrolysed with 20% (v/v) hydrochloric acid. Two peaks were obtained, total free polyamines (16 μmol) and total acetyl polyamines (28 μmol). The concentration of total polyamines excreted in human urine in Fig. 5 was 66 μmol/day. Peak a in Fig. 5 belongs to the group of polyamines that have two charges per molecule and peak b one per molecule.

One of the advantages of capillary electrophoresis is that group separation is possible. The relationship between the amount of polyamines in human body fluids and cancer is well established[10–12]. Previous methods for the determination of polyamines have included enzymatic assay and liquid chromatography[12]. The present results indicate that capillary electrophoresis may be a good method for the determination of polyamines.

ACKNOWLEDGEMENT

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