Short Communication

Separation of Dansyl-DL-Amino Acids Through Tube Radial Distribution Chromatography by Using a Commercially Available HPLC System with a Capillary Tube Manufactured for GC as a Separation Column

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Abstract
Dansyl-DL-amino acids were separated by tube radial distribution chromatography (TRDC) comprising a commercially available HPLC system, where open-tubular capillary tubes manufactured for capillary GC were used as the separation column. Initially, the separation performance of GC capillary tubes in TRDC was assessed by separating a model analyte mix of 1-naphthol and 2,6-naphthalenedisulfonic acid by using water/acetonitrile/ethyl acetate solutions (3:8:4 volume ratio, organic solvent-rich and 4:3:1 volume ratio, water-rich) as the eluent and high-polarity and non-polarity capillary tubes. Next, enantiomer separation of dansyl-DL-methionine and dansyl-DL-valine was examined using a water/acetonitrile/ethyl acetate solution (8:2:1 volume ratio, water-rich) containing 1.0 mM β-cyclodextrin and non-polarity capillary tubing. The D- and L-enantiomers were separated and detected in this order through interaction between amino acids and β-cyclodextrin by tube radial distribution flow in the TRDC system.

Keywords: Phase separation multiphase flow; Tube radial distribution chromatography; HPLC system; Enantiomer separation; Dansyl amino acids

1. Introduction
Conventional multiphase flow, which is also termed immiscible multiphase flow, is generated by mixing water and a hydrophobic organic solvent solution in a tube to provide a kinetic liquid-liquid interface in the flow [1-3]. We have studied a new type of multiphase flow in a microspace, called phase separation multiphase flow, which contrasts with immiscible multiphase flow [4,5]. Two-phase separation mixed solutions, such as a ternary water/hydrophilic/hydrophobic organic mixed solution, are separated through a phase transformation by changing the temperature and/or pressure into upper and lower phases in a batch vessel. When a homogeneous solution of the mixed solutions is delivered into a microspace and the temperature and/or pressure are/is changed, a phase transformation occurs, leading to a phase separation multiphase flow. We are especially interested in stable annular flow possessing inner and outer phases provided by the phase-separation multiphase flow [6,7]. Such specific microfluidic behavior and flow are called the tube radial distribution phenomenon and tube radial distribution flow (TRDF), respectively [4,6,8]. We have developed capillary...
Chromatography based on TRDF, where the inner and outer phases in the annular flow work as mobile and pseudo-stationary phases in chromatography, respectively. We refer to this new type of chromatography as tube radial distribution chromatography (TRDC) [4-6]. In general, TRDC requires specific instruments and treatments for microfluidic flow. However, we have previously proposed a new model of TRDC using a commercially available HPLC system comprising a pump, an injector and a detector [11].

In this study, to further develop the applicability of TRDC we tried to perform enantiomer separation, which is one of the most difficult tasks in analytical science, by TRDC using a commercially available HPLC system. Additionally, as a first attempt, an open-tubular capillary tube manufactured for capillary GC was connected between the sample injector and detector as a separation column in the TRDC system. Although we have used various types of open-tubular capillary tubes as separation columns, such as fused-silica (untreated and inactivated), PTFE, polyethylene and stainless tubes [4,6,12], GC open-tubular capillary tubes have not been used in the TRDC system. We have previously separated dansyl-DL-amino acids by using a home-made TRDC system equipped with a PTFE tube [13]. In this study, because many kinds of GC capillary tubes with various inner wall characteristics are commercially available, we examined their performance in TRDF and TRDC by using model GC capillary tubes to find future possible systems through separation of dansyl-DL-amino acids [13].

2. Experimental

2.1. Reagents and materials

Water was purified using an Elix 3 UV system (Merck, Darmstadt, Germany). All reagents were commercially available and of analytical grade. 1-Naphthol, 2,6-naphthalenedisulfonic acid (2,6-NDS), Eosin Y, perylene, acetonitrile, ethyl acetate and β-cyclodextrin were purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan). Dansyl-DL-methionine, dansyl-L-methionine and dansyl-DL-valine were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Open-tubular capillary tubes (100 μm i.d.) manufactured for capillary GC, such as high-polarity (Rtx-Wax; Shimadzu Co., Kyoto, Japan) and non-polarity (CBP-1; Shimadzu Co.), were used as the separation columns.

2.2. TRDC system

Figure 1 shows a schematic diagram of the TRDC system, which comprises a commercially available HPLC system. Separation column, open-tubular capillary tubes (100 μm i.d. and 350 cm length); eluent solution, a water/acetonitrile/ethyl acetate mixed solution containing or not containing 1.0 mM β-cyclodextrin; flow rate, 10 μL min⁻¹; analyte injection volume, 2 μL; cooling temperature, 5 °C; and detection wavelength, 254 nm. Besides the separation column, PEEK tubing was used in all other parts of the TRDC system.

Fig. 1. Schematic diagram of the TRDC system, which comprises a commercially available HPLC system. Separation column, open-tubular capillary tubes (100 μm i.d. and 350 cm length); eluent solution, a water/acetonitrile/ethyl acetate mixed solution containing or not containing 1.0 mM β-cyclodextrin; flow rate, 10 μL min⁻¹; analyte injection volume, 2 μL; cooling temperature, 5 °C; and detection wavelength, 254 nm. Besides the separation column, PEEK tubing was used in all other parts of the TRDC system.

length) was connected between the sample injector and the detector in the HPLC system [11]. The capillary tube temperature was controlled by submerging the tube into a beaker of water maintained at a fixed temperature (5 °C) while stirring. The ternary mixed solution of water/acetonitrile/ethyl acetate containing or not containing 1.0 mM β-cyclodextrin [13] was delivered as the eluent solution at a flow rate of 10 μL min⁻¹. The analyte solution (2.0 μL) was injected and separated through the tube and detected with the spectrophotometric detector at 254 nm.

2.3. Fluorescence microscope-CCD camera system

We set up the separation capillary tube on the fluorescence microscope-CCD camera system with a size equivalent to that used in the TRDC system. The tube temperature was controlled with a thermostate. The fluorescence in the tube was monitored at approximately 300 cm from the tube inlet using a fluorescence microscope (BX51; Olympus, Tokyo, Japan) equipped with a Hg lamp, optical filters (U-MWU2; 330–385 nm excitation filter and >420 nm emission filter) and a CCD camera (JK-TU53H; Toshiba, Tokyo, Japan). The eluent solution contained 0.1 mM perylene and 1 mM Eosin Y. The eluent solution was delivered into the tube at a flow rate of 10 μL min⁻¹ using the delivery pump.

3. Results and discussion

3.1. TRDF observation in a GC capillary tube

In our previous paper [14] we presented a phase diagram of the water/acetonitrile/ethyl acetate mixed solution, including solubility curves at 20 and 5 °C (Fig. 2). Compositions of the ternary water/acetonitrile/ethyl acetate homogeneous solutions (with volume ratios of 3:8:4 for the organic solvent-rich solution and 4:3:1 for the water-rich solution), as eluent solutions, were
positioned near the solubility curve at 20 °C in the phase diagram. A homogenous solution (single phase) at 20 °C changed to a heterogeneous solution (two phases) at 5 °C through a phase transformation in the batch vessel.

In contrast, when the ternary mixed solutions were delivered into the capillary tubes (high-polarity and non-polarity GC capillaries), changing the temperature from room temperature to 5 °C at a flow rate of 10 μL min⁻¹ enabled observation of TRDF in the tube using a fluorescence microscope-CCD camera system. TRDF was clearly observed at 5 °C; the obtained fluorescence photographs are shown in Fig. 3. Blue and green curves (Fig. 3) are fluorescence profiles for perylene and Eosin Y, respectively, which were obtained by computer software estimation. Delivery of the organic solvent-rich solution into the high-polarity capillary tube generated an organic solvent-rich major inner phase (perylene, blue) and a water-rich minor outer phase (Eosin Y, green). In contrast, delivery of the water-rich solution into the non-polarity tube generated a water-rich major inner phase (Eosin Y, green) and an organic solvent-rich minor outer phase (perylene, blue). The configuration of inner and outer phases in TRDF has been discussed previously by us in terms of the viscous dissipation principle and linear stability analysis [9]. These results confirm that phase-separation multiphase flow or TRDF occurs in the open-tubular capillary tube manufactured for GC equipped with a commercially available HPLC system under the present conditions. In addition, the combination of an organic solvent-rich solution and non-polarity capillary tube as well as a water-rich solution and high-polarity tube gave a certain degree of unstable TRDF that frequently included slug flow.

3.2. Chromatograms of model mixed analytes

A model mixed analyte solution (1-naphthol and 2,6-NDS, 1.0 mM each) was subjected to the TRDC system, where the open-tubular capillary tubes composed of either high-
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polarity or non-polarity were connected as a separation column to a commercially available HPLC system. The compounds were separated by the separation principle of TRDC as follows [4,6]: 1-naphthol and 2,6-NDS were separated in this order with the organic solvent-rich solution, where the water-rich outer phase worked as a pseudo-stationary phase. Conversely, 2,6-NDS and 1-naphthol were separated in this order with the water-rich solution, where the organic solvent-rich outer phase worked as a pseudo-stationary phase. The obtained chromatograms are also shown in Fig. 3.

3.3. Enantiomer separation by the TRDC system

Phase diagrams for the ternary mixed solvents of water/acetonitrile/ethyl acetate containing 1.0 mM β-cyclodextrin in a vessel were examined at temperatures of 5 and 20 °C. The solubility curves were similar to those obtained when β-cyclodextrin was not present (Fig. 2). However, 1.0 mM β-cyclodextrin was previously shown to not dissolve in ternary mixed solvent solutions when the water component ratio is less than ca. 32 volume % [13]. In this study, a homogeneous solution of the water/acetonitrile/ethyl acetate mixture (volume ratio of 8:4:1) including 1.0 mM cyclodextrin was used as an eluent solution for separation of dansyl-DL-amino acids according to our previous paper [13]. Non-TRDF and TRDF were observed when the solution was delivered into the capillary tube (non-polarity) at 20 and 5 °C, respectively (Fig. 4).

Next, dansyl-DL-methionine was examined by the TRDC system using the ternary mixed solvents containing β-cyclodextrin. The obtained chromatograms are shown in Fig. 5. Enantiomer separation with the ternary mixed solvents including cyclodextrin was observed to be similar to that of the chromatograms obtained for the model analytes. The D- and L-enantiomers were separated and detected in this order. The elution order was also confirmed by data for dansyl-L-methionine as an analyte. Dansyl-D-methionine has been reported [15] to have a stronger interaction with β-cyclodextrin as a chiral selector when compared with that of dansyl-L-methionine. The interaction between dansyl-D-methionine and β-cyclodextrin must alter the distribution of the D-enantiomer from the outer phase (organic solvent-rich) to

![Fig. 4. Fluorescence photographs of a water/acetonitrile/ethyl acetate mixed solution containing 1.0 mM β-cyclodextrin. Fluorescence photographs were observed under the conditions of a water/acetonitrile/ethyl acetate solution at a 8:2:1 volume ratio, 10 μL min⁻¹, 1.0 mM Eosin Y and 0.1 mM perylene, and at 20 °C and 5 °C. The arrows indicate the laminar flow direction.](image)

![Fig. 5. Chromatograms of dansyl amino acids obtained by the TRDC system with a water/acetonitrile/ethyl acetate mixed solution (8:4:1 volume ratio) containing 1.0 mM β-cyclodextrin. Other conditions used are described in Fig. 1.](image)
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the inner phase (water-rich), leading to an earlier elution time of the D-enantiomer than the L-enantiomer. That is, the stronger interaction or binding between the dansyl amino acid and cyclodextrin, the larger the hydrophilicity of the complex and distribution to the water-rich inner phase. Separation performance for the enantiomers in the TRDC system is illustrated in Fig. 6 [13]. Dansyl-DL-valine was also separated using the presented method (Fig. 5); however, the elution order in the figure is postulated because we have not determined the order of elution by using single isomers.

4. Conclusion
We have separated dansyl-DL-amino acids with the TRDC system successfully, which comprises a commercially available HPLC system with a pump, an injector and a detector. Separation was achieved by using capillary tubing manufactured for GC, which was connected between the injector and the detector in the HPLC system. Enantiomer separation was attained successfully by combining a commercially available HPLC system and open-tubular capillary tubing for GC. The obtained data provide important insights for developing outstanding practical capillary HPLC applications. Because there are many different types of GC capillary tubing commercially available, our future efforts will continue to examine the performance of GC capillary tubes in TRDF and TRDC.

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