

Capillary Chromatography Based on Tube Radial Distribution of Ternary Mixed Solvents: Construction of the Phase Diagram and the Separation Performance

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Open-tubular capillary chromatography using a ternary solvent mixture consisting of water–hydrophilic–hydrophobic organic solvent as a carrier solution has been developed. When the ternary carrier solution is fed into the capillary tube, the carrier solvents are radially distributed and generate inner and outer phases in the tube. The outer phase functions as a pseudo-stationary phase in chromatography. In this study, investigations proceeded with reference to the solubility curves on the phase diagram of the ternary mixed solvents at 5 and 20°C. Model analytes, 1-naphthol and 2,6-naphthalenedisulfonic acid (2,6-NDS), were examined with ternary water–acetonitrile–ethyl acetate solvent mixtures as carrier solutions and a fused-silica capillary tube (50- μm inner diameter). They were separated in this order with organic solvent-rich carrier solutions, while, in the reverse order with the water-rich carrier solutions at a tube temperature of 5°C.

Key words: capillary chromatography, tube radial distribution, ternary mixed solution, phase diagram

1. Introduction

Since the nineteenth century, microfluidic flow has been known to exhibit interesting and useful physical and hydrodynamic phenomena such as electroosmotic flow and laminar flow. The electroosmotic flow in a capillary tube promotes capillary electrophoresis¹⁻³⁾ and capillary electrochromatography^{4,5)}, whereas the laminar flow conditions enable hydrodynamic chromatography⁶⁻⁸⁾. Our group reported the tube radial distribution phenomenon of carrier solvents in 2009⁹⁻¹¹⁾, which we

call the “tube radial distribution phenomenon” (TRDP) for convenience. When the ternary mixed solvents of water–hydrophilic/hydrophobic organic solvent mixtures are delivered into a microspace such as a microchannel or a capillary tube under laminar flow conditions, the solvent molecules are radially distributed in the microspace and generate inner and outer phases.

The TRDP creates a phase interface or a kinetic liquid-liquid interface in a microspace. We are currently investigating the TRDP from the viewpoint of chromatography^{12,13)}, extraction¹⁴⁾, mixing¹⁵⁾, and

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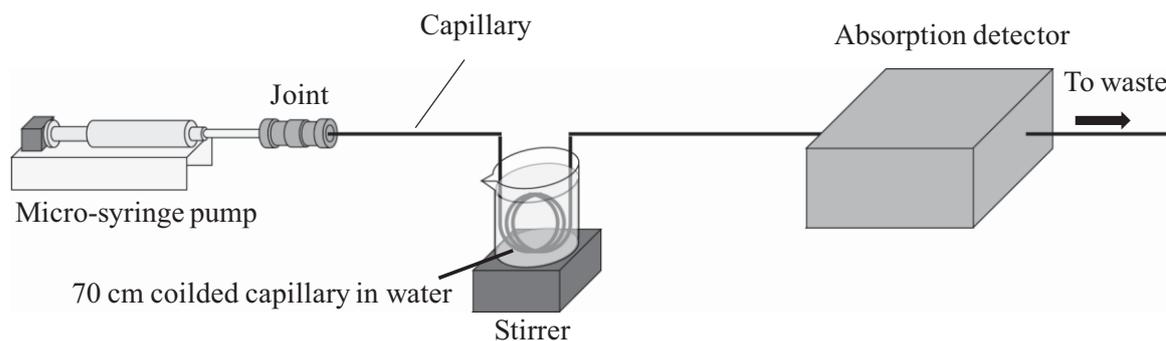


Fig. 1. Schematic diagrams of open-tubular capillary chromatographic system (TRDC system).

chemical reaction space¹⁶). A capillary chromatography system in which the outer phase acts as a pseudo-stationary phase under laminar flow conditions has been developed based on the TRDP. We call it “tube radial distribution chromatography” (TRDC)^{9,12,13}.

The TRDP appears through phase separation from homogeneous solution to heterogeneous solution including two phases with pressure and temperature changes. The phase separation with associated changes forms an upper and lower phase in a batch vessel under the control of gravity. At the same time, the phase separation introduces TRDP, including inner and outer phases in a micro-flow where is under non-control of gravity and laminar flow conditions¹⁷). During the TRDP and TRDC investigation, we considered that the solubility curves on the phase diagram must be useful tools for investigation. In this study, various component ratios of the ternary mixed solvents on the phase diagram including solubility curves were used as carrier solutions in the TRDC, and the obtained chromatograms are considered together along with the data provided through the solubility curves.

2. Experimental

2.1 Reagents and capillary tube

Water was purified using an Elix 3 UV purification system (Millipore Co., Billerica, MA). All

reagents used were commercially available and were of analytical grade. 1-Naphthol, 2,6-NDS, Eosin Y, perylene, acetonitrile, and ethyl acetate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The fused-silica capillary tube (50- μ m inner diameter) was purchased from GL Science (Tokyo, Japan).

2.2 TRDC system

A schematic diagram of the present capillary chromatography system (TRDC system) comprised an open-tubular fused-silica capillary tube (120-cm total length and 100-cm effective length), microsyringe pump (MF-9090; Bioanalytical Systems, Inc., West Lafayette, IN), and absorption detector (modified SPD-10AV spectrophotometric detector; Shimadzu Co., Kyoto, Japan) (Fig. 1). The tube temperature was controlled by dipping the capillary tube in water maintained at a definite temperature (5 or 20°C) in a beaker with stirring. Water–acetonitrile–ethyl acetate solvent mixtures were used as carrier solutions. Analyte solutions including 1-naphthol and 2,6-NDS (1 mM each) were prepared using the carrier solutions.

The analyte solution was introduced directly into the capillary inlet using the gravity method (20 s \times 30 cm height). After analyte injection, the capillary inlet was connected through a joint to a microsyringe. The syringe was set on the microsyringe pump. The carrier

solution was fed into the capillary tube at a definite flow rate ($0.2 \mu\text{L min}^{-1}$) under laminar flow conditions. On capillary absorption detection (254 nm) was performed using the detector.

3. Results and Discussion

3.1 Phase diagram for ternary solvents

A phase diagram for the ternary mixture of water–acetonitrile (hydrophilic organic solvent)–ethyl acetate (hydrophobic organic solvent) was examined in a batch vessel at temperatures of 5 and 20°C. The obtained phase diagram is shown in Fig. 2.

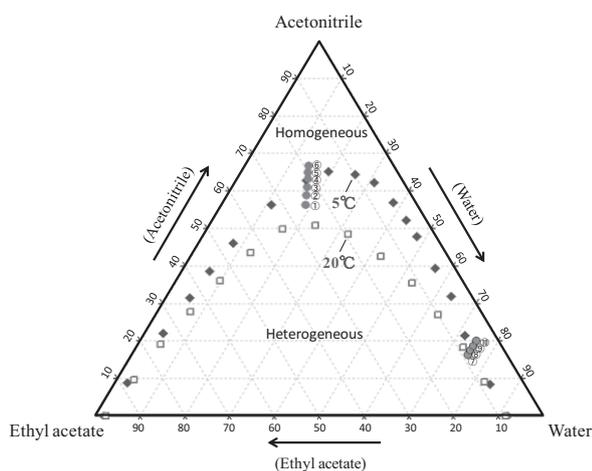


Fig. 2. Phase diagram for water–acetonitrile–ethyl acetate mixture at 5 and 20°C. The curves expressed with symbols of \blacklozenge and \square in the diagram indicate the solubility curves at 5 and 20°C, respectively. The symbols of \bullet show the component ratios of the solution 1-10 that gave the chromatograms as shown in Fig. 4.

The curves expressed in the diagram (\blacklozenge : 5 °C and \square : 20°C) indicate the solubility curves. The inside and outside of the curves represent heterogeneous and homogeneous solutions, respectively. The phase diagram shows that each component ratio of the

solvents forms a homogeneous (one homogeneous phase) or heterogeneous (two homogeneous phases) solution. The heterogeneous solutions include the upper (organic solvent-rich solution) and lower (water-rich solution) phases in the batch vessel. The component ratios of the solvents in the solutions 1–10 are plotted in the diagram.

3.2 Chromatograms obtained with tube temperatures of 20°C

The model analytes, hydrophobic 1-naphthol and hydrophilic 2,6-NDS, were examined with the present TRDC system at a tube temperature of 20°C. The ternary mixture of water–acetonitrile–ethyl acetate homogeneous solution 1 (the volume ratio of 3:9:4) and 7 (60:13:7) were used as the organic solvent-rich and water-rich carrier solutions, respectively. The compositions of solvents 1 and 7 were positioned close to the solubility curve at 20°C (Fig. 2). 1-Naphthol and 2,6-NDS were separated in this order with the organic solvent-rich carrier solution, but were not separated with the water-rich carrier solution (Fig. 3).

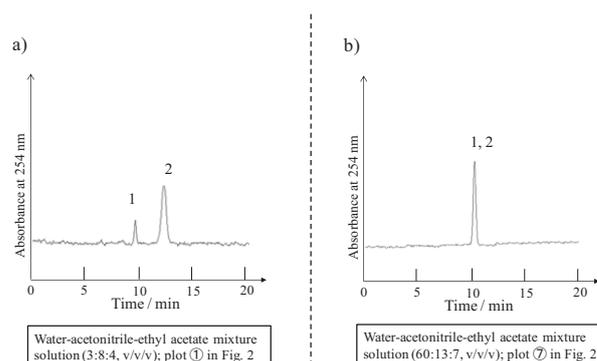


Fig. 3. Chromatograms of 1-naphthol (peak 1) and 2,6-NDS (peak 2) by the TRDC system with tube temperature of 20°C. a) Organic solvent-rich and b) water-rich carrier solution have water–acetonitrile–ethyl acetate mixture volume ratio of a) 3:8:4 and b) 60:13:7, respectively.

3.3 Chromatograms obtained with tube temperatures of 5°C

The analytes were examined with the TRDC system at a tube temperature of 5°C. The homogeneous carrier solutions 1–6 and 7–10 were used as the organic solvent-rich and water-rich carrier solutions, respectively. The obtained chromatograms are shown in Fig. 4. The solvent compositions are described in the figure captions. 1-Naphthol and 2,6-NDS were separated in this order with the organic solvent-rich carrier solution (1–5), but they were not separated with carrier solution 6, for which the solvent composition was positioned outside the solubility curve at 5°C (homogeneous region). With the water-rich carrier solution, 1-naphthol and 2,6-NDS were separated in the reverse elution order with carrier solutions 7 and 8, but they were not separated with carrier solutions 9 and 10, for which solvent compositions were placed outside the solubility curve at 5°C (homogeneous region).

The elution order of the analytes could be changed by altering the component ratios of the

solvents, organic solvent-rich or water-rich, at a tube temperature of 5°C. The experimental data were consistent with the TRDC separation performance and the principles, which were reported previously^{9–11}). With the organic solvent-rich solution, the organic solvent-rich major phase was generated around the middle of the capillary tube as an inner phase, whereas a water-rich minor phase was formed near the inner wall as an outer phase (pseudo-stationary phase). In contrast, with the water-rich solution, the water-rich major phase was generated as an inner phase, whereas the organic solvent-rich minor phase was formed as an outer phase. While undergoing chromatographic separation, the hydrophobic and hydrophilic model analytes were distributed in the inner and outer phases because of their nature.

3.4 Consideration through phase diagram and chromatogram

The organic solvent-rich carrier solutions (1–5), for which solvent compositions existed in a large

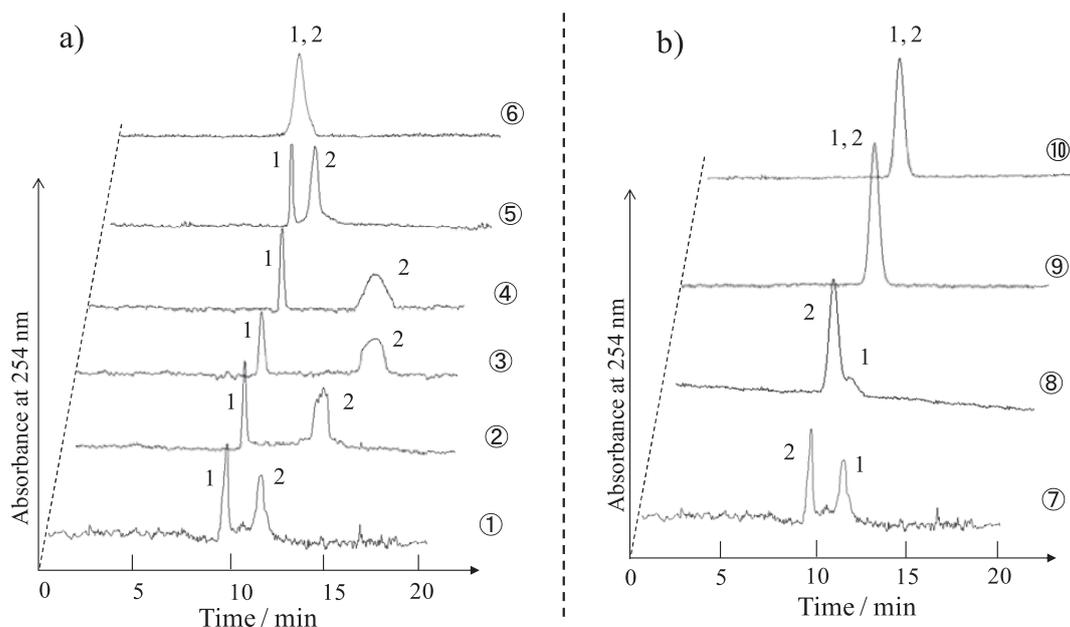


Fig. 4. Chromatograms of 1-naphthol (peak 1) and 2,6-NDS (peak 2) by the TRDC system with tube temperature of 5°C. The water–acetonitrile–ethyl acetate mixture volume ratio of ① 3:9:4, ② 3:10:4, ③ 3:11:4, ④ 3:12:4, ⑤ 3:13:4, ⑥ 3:14:4 in a) organic solvent-rich carrier solutions and ⑦ 60:13:7, ⑧ 60:14:6, ⑨ 60:15:5, ⑩ 60:16:4 in b) water-rich carrier solutions.

domain between the solubility curves of 5 and 20°C as shown in Fig. 2, showed better resolution than the water-rich carrier solutions (7 and 8), for which solvent compositions existed in a narrow domain. It was suggested that separation performance in the TRDC might be related to the data provided through the tie lines on the phase diagram, which will be examined in the near future.

4. Conclusions

The TRDC system in which the outer phase works as a pseudo-stationary phase under laminar flow conditions has been developed based on the TRDP. In this study, the phase diagram including solubility curves at 5 and 20°C was constructed and the component ratios of the solvents near the solubility curves were used as carrier solutions in the TRDC to expand our knowledge of the TRDP and TRDC. Model analytes, 1-naphthol and 2,6-NDS, were separated in this order with organic solvent-rich carrier solutions, while, in the reverse order with the water-rich carrier solutions at a tube temperature of 5°C. The organic solvent-rich carrier solutions, for which solvent compositions existed in a large domain between the solubility curves of 5 and 20°C, showed better resolution than the water-rich carrier solutions, for which solvent compositions existed in a narrow domain. Introducing the phase diagram concept into the TRDC and TRDP will improve ease and clarity of related investigations in the future.

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