Tube Radial Distribution Chromatography on a Microchip Incorporating Microchannels with a Three-to-One Channel Confluence Point

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We developed a capillary chromatography system using a phase-separated solvent mixture as a carrier solution—i.e., a water-hydrophilic/hydrophobic organic solvent mixture—which we call “tube radial distribution chromatography” (TRDC). Here, we attempted to apply the TRDC system to a microchip incorporating microchannels with a double T-junction for injection of analyte solution and a three-to-one, narrow-to-wide channel confluence point for tube radial distribution phenomenon (TRDP) at room temperature. A ternary mixed solvent of water, acetonitrile and ethyl acetate was used as a carrier solution. TRDP in the wide microchannel was examined using various flow rates, temperatures, and component solvent ratios. Successful observation was carried out using a fluorescence microscope-CCD camera. Model analytes perylene (hydrophobic) and Eosin Y (hydrophilic) were separated by flowing through the microchannel, without any treatment such as packed columns or coating, at room temperature (25°C).

Keywords Tube radial distribution phenomenon (TRDP), tube radial distribution chromatography (TRDC), tube radial distribution mixing (TRDM), microchannel

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Introduction

The microfluidic behavior of solvents has been investigated since the nineteenth century. Electroosmotic flow and laminar flow contribute to interesting and useful physical or hydrodynamic solvent behaviors in microspaces. Electroosmotic flow was first reported by Reuss, and capillary electrophoresis, micellar electrokinetic chromatography, and capillary electrochromatography were developed by taking advantage of this phenomenon. Laminar flow was reported by Hagen and Poiseuille. Subsequently, Reynolds introduced a dimensionless number, known as the Reynolds number, into laminar and turbulent flow studies. Laminar flow led to the invention of hydrodynamic chromatography, wide-bore hydrodynamic chromatography, and field flow fraction chromatography.

Our group reported the tube radial distribution phenomenon (TRDP) in carrier solvents under microfluidic flow conditions. When a mixture of two phase-separated solvents, such as a ternary mixed solvent (water-hydrophilic/hydrophobic organic solvent), an ionic liquid aqueous solution, or a micellar aqueous solution, is delivered into a microspace such as a microchannel or capillary tube, through the change of temperature and/or pressure, the solvent molecules are radially distributed in the microspace, generating inner and outer phases. The microfluidic flow having a liquid-liquid interface based on TRDP was a new type of multi-phase flow by taking advantage of phase transformation from homogeneous to heterogeneous solution in a microfluidic flow, not using immiscible solvents (e.g., water and oil) solutions as the conventional multi-phase flow. A capillary chromatography system based on TRDP, in which the outer phase acts as a pseudo-stationary phase under laminar flow conditions, has been developed. We call this separation method "tube radial distribution chromatography" (TRDC). The TRDC systems examined were operated using various types of capillary tubes, such as fused-silica and PTEF, without applying high-voltage and specific columns. Also, TRDP has been applied to extraction (tube radial distribution extraction; TRDE), mixing (tube radial distribution mixing; TRDM), and chemical reaction (tube radial distribution reaction; TRDR).

The area of microchip devices is among the most active research subjects in chemical engineering, analytical technology, and separation science, including micro-total analysis systems (μ-TAS) or lab-on-a-chip systems. Microchip electrophoresis has been widely investigated as a rapid small-scale separation method. However, microchip electrophoresis requires a
voltage supplier device and electrodes. Also, microfluidic analysis using capillary chromatography requires specific separation columns such as monolithic or packed channels or tubes. Although they can improve separation performance, including resolution, they are costly, time-consuming and tedious to prepare. Miniaturization on a microchip incorporating microchannels was applied to the TRDC system, as described in our previous paper, but temperature control was required for the process to be successful.

In this study, we attempted to apply the TRDC system to a microchip with a double T-junction for injection of analyte solutions and a three-to-one, narrow-to-wide channel confluence point for TRDP that could generate TRDP at room temperature. That is, an attempt to join TRDM to TRDC was for the first time carried out on a microchip. The microchip TRDC was successfully carried out without any temperature control, high-voltage supplier, and specific-treated channel, such as monolithic or packed channel, thus representing an innovation in the area of lab-on-a-chip or µ-TAS.

**Results and Discussion**

**Preliminary experiments with microchip incorporating a three-to-one channel confluence point**

A microchip with a three-to-one channel confluence point was designed for the preliminary experiments (as microchip B; Fig. S1 (Supporting Information)). A water-acetonitrile solution (3:2 volume ratio) was fed into the center channel (C1), and an acetonitrile-ethyl acetate solution (3:2 volume ratio) was fed into the two side channels (C2 and C3) at 0.4 mL min⁻¹. Consequently, the double T-junction was filled with the analyte solution, as shown in the figure.

In the analysis step, the carrier solution was fed into the separation (one wide) and waste channels in the same way as in the preparation step, while the analyte solution was not delivered. At that time, the flow rate of C1 was changed from 4 mL min⁻¹ to 20 mL min⁻¹. The solution was removed from point CW at a flow rate of 4 mL min⁻¹, and the flow rate in the separation channel was estimated experimentally at 16 mL min⁻¹.

**Analytical procedure for TRDC**

The analytical procedure involved the following three steps: 1) preparation, 2) sample loading, and 3) analysis. A schematic illustration is shown in Fig. 2. The analyte (sample) and carrier solutions were delivered into the microchannels from points S and C1, C2, and C3 using the microsyringe pumps.

In the preparation step, a carrier solution (water-acetonitrile, 3:2 volume ratio) was delivered at a flow rate of 20 mL min⁻¹ from point C1, went through the double T-junction and reached the three-to-one channel confluence point. Another carrier solution (acetonitrile-ethyl acetate, 3:2 volume ratio) was similarly delivered at a flow rate of 4.0 mL min⁻¹ from points C2 and C3, reaching the three-to-one channel confluence point. All the channels were filled with the solutions, and were wasted or removed to points CW and W.

In the sample loading step, the analyte solution was delivered via point S at a flow rate of 4 mL min⁻¹ for 10 s, while the carrier solutions were delivered from points C1, C2, and C3 at a flow rate of 4 mL min⁻¹. Consequently, the double T-junction was filled with the analyte solution, as shown in the figure.

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**Experimental**

**Reagents**

Water was purified using an Elix 3 UV system (Millipore Co., Billerica, MA). All reagents were obtained commercially and were of analytical grade. Perylene, Eosin Y, acetonitrile, and ethyl acetate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

**Microchip-TRDC system**

A microchip made of glass was manufactured by Institute of Microchemical Technology Co., Ltd. (Kanagawa, Japan). Figure 1 shows a microchip with microchannels 100 or 300 µm wide and 40 µm deep (microchip A). The microchip-TRDC system comprised a microchip, a microsyringe pump (MF-9090; Bioanalytical Systems, Inc., West Lafayette, IN), and a fluorescence microscope-CCD camera system.

Fluorescence in the microchannel was monitored using a fluorescence microscope (BX51; Olympus, Tokyo, Japan) equipped with a Hg lamp, a filter (U-MWU2, ex. 330 – 385 nm, em. > 420 nm), and a CCD camera (JK-TU53H). Fluorescence photographs mainly consisted of blue and green, because perylene and Eosin Y emit light at 470 and 550 nm, respectively. The photograph data, including on fluorescence intensity, were also expressed as digital data on a computer, and the values were integrated per one second to draw a line, giving chromatograms.

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Fig. 2 Schematic illustration of analytical procedures: (1) preparation, (2) sample loading, and (3) analysis. S, sample (analyte) solution delivery point; C1 - C3, carrier solution delivery points; W, analyte or carrier solution waste point; and CW, carrier solution waste point.

Fig. 3 Fluorescence photographs of solvents containing dissolved fluorescence dyes at the confluence point M, and 0.2 and 4 cm away from the confluence point, in the wide channel in microchip B. (a) Conditions: carrier for C1, water-acetonitrile (3:2 volume ratio) containing 1.0 mM Eosin Y; carrier for C2 and C3, acetonitrile-ethyl acetate (3:2 volume ratio) containing 0.1 mM perylene; flow rate, 4.0 μL min⁻¹ for C1 - C3; volume ratio of water-acetonitrile-ethyl acetate in the wide channel, 20:53:27; temperature, 25°C (room temperature). (b) Conditions: carrier for C1 - C3, water-acetonitrile-ethyl acetate (20:53:27 volume ratio) containing 1.0 mM Eosin Y and 0.1 mM perylene; flow rate, 4.0 μL min⁻¹ for C1 - C3; volume ratio of water-acetonitrile-ethyl acetate in the wide channel, 20:53:27; temperature, 25°C (room temperature).
been observed in the microchannels through phase transformation from homogeneous to heterogeneous solution in the microfluidic flow by changing to the lower temperature about \(15^\circ C\).\(^2,^{10}\) It was concluded that mixing the solvents at the three-to-one channel confluence point leads to TRDP in the microchannel on a microchip TRDC at room temperature. It is still unclear why that the TRDP was observed under the conditions of Fig. 3(a) where a water–acetonitrile and acetonitrile–ethyl acetate solution were mixed in a microchannel, while it was not observed under those of Fig. 3(b) where a water-acetonitrile-ethyl acetate ternary mixed solution was delivered in it, at room temperature. However, the conditions of Fig. 3(a) might require less energy than those of Fig. 3(b) for phase transformation to heterogeneous solution in the center or wide microchannel, generating inner and outer phases.

**Fluorescence photographs in the microchannel**

Fluorescence photographs of the organic-solvent-rich carrier solution on microchip A are shown in Fig. 4, together with the conditions. Carrier solutions containing fluorescence dyes were fed into the wide or separation channel. An organic-solvent-rich major phase containing perylene (blue) was generated in the middle of the microchannel as an inner phase in the separation channel. Meanwhile, a water-rich minor phase containing the relatively hydrophilic Eosin Y (green) was formed near the inner wall of the channel as an outer phase. TRDP was clearly observed in the separation channel at room temperature.

The effect of flow rate on TRDP was examined by varying the flow rate for C1 from 15 to 40 \(\mu L \text{ min}^{-1}\) at a constant flow rate of 4 \(\mu L \text{ min}^{-1}\) for C2 and C3. Fluorescence photographs were obtained at point (a) in Fig. 1. Stable TRDP was observed around 20 - 25 \(\mu L \text{ min}^{-1}\) at room temperature (Fig. S2, Supporting Information). The effect of temperature on TRDP was also examined by varying the temperature from 5 to 35 \(^\circ C\); stable TRDP was observed around 20 - 25 \(^\circ C\), or room temperature (Fig. S3, Supporting Information). The effect of the component ratio of the solvent was also examined, using the ratios in the phase diagram (Fig. S4, Supporting Information), stable TRDP was observed around a water-acetonitrile-ethyl acetate ratio of 20:53:27 (volume ratio) (Fig. S5, Supporting Information).

Based on this experimental data, the following analytical conditions were selected as optimum. Carrier for C1, water-acetonitrile (3:2 volume ratio); carrier for C2 and C3, acetonitrile-ethyl acetate (3:2 volume ratio); flow rate, 20 \(\mu L \text{ min}^{-1}\) for C1 and 4.0 \(\mu L \text{ min}^{-1}\) for C2 and C3; volume ratio of water-acetonitrile-ethyl acetate in the wide channel, approximately 20:53:27; temperature, 25 \(^\circ C\) (room temperature).

**Sample injection and chromatogram**

A sample plug was constructed with constant volume at the double T-junction with good repeatability. A fluorescence photograph of the double T-junction is shown in Fig. 5, together with the conditions. Figure 6 shows a chromatogram of the model analytes, perylene and Eosin Y. The hydrophobic perylene was detected first, followed by the relatively hydrophilic Eosin Y. The elution order was reasonable based on the concept of TRDC separation with an organic-solvent-rich carrier solution.\(^2\) Fluorescence photographs are shown along with the chromatogram: perylene (blue) can be seen to be distributed...
widely around the center of the capillary tube (in the inner phase), corresponding to the first peak, while Eosin Y (green) was distributed near the inner wall (in the outer phase), corresponding to the second peak. That is, the relationship between distribution pattern of the solutes or analytes to the inner and outer phases and elution order of the peaks on a chromatogram was for the first time presented with the fluorescence photographs and the chromatogram in Fig. 6, where the carrier solution was delivered in the microchannel along the parabolic flow under the laminar flow conditions.

Fig. 6 Chromatogram of perylene and Eosin Y, taken at point (b) in Fig. 1, and fluorescence photographs at the microchannel (microchip A). Conditions are as described in Fig. 4.

Conclusions
TRDC based on the TRDP was successfully developed on a microchip. A model analyte mixture of perylene and Eosin Y was analyzed using the microchip TRDC system at room temperature without any temperature control. The system was operated without the application of a voltage supplier device, as in microchip electrophoresis, or specific columns such as packed and monolithic columns, as in normal capillary chromatography. We combined previously reported unique TRDP-based separation, TRDC, and mixing procedure, TRDM, on a microchip, which we hope will lead to the development of novel lab-on-a-chip systems.

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Supporting Information

Schematic diagram of microchip B is shown in Fig. S1. Effects of flow rate, temperature, and solvent composition on fluorescence photographs are shown in Figs. S2, S3, and S5, respectively. The phase diagram of a ternary mixed solvent solution is also shown in Fig. S4. These materials are available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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