Development of Tube Radial Distribution Chromatography Based on Phase-Separation Multiphase Flow Created via Pressure Loss

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A tube radial distribution chromatography (TRDC) method based on phase-separated multiphase flow created through phase transformation via temperature change had been developed. These systems typically required a temperature-controlling device containing a water bath and a stirrer. Herein, we proposed a novel TRDC system without a cooling device, where the phase transformation was achieved via pressure loss in a capillary tube of 50 µm inner diameter and 550 cm length. Model analytes were successfully separated with the developed TRDC system, which provided a simplified platform and helped to clarify the operating principle of TRDC based on phase transformation in a capillary tube.
Introduction

Open-tubular capillary chromatography is a unique separation method featuring a simple device, easy operation, small sample and solvent volume, and low cost.\textsuperscript{1,2} However, only a few novel concepts concerning capillary chromatography have been proposed in this century. In one such study, wide-bore hydrodynamic chromatography\textsuperscript{3,4} was performed using an open capillary tube composed of fused silica. Diffusive and non-diffusive analytes showed quite different elution behaviors in this capillary tube under laminar flow conditions. Micellar electrokinetic capillary chromatography is a separation mode typically used in capillary electrophoresis, where the micelle acts as a pseudo-stationary phase during chromatographic separation.\textsuperscript{5,6}

Phase-separation multiphase flow is created with a two-phase separation mixture, such as a water/acetonitrile/ethyl acetate ternary mixed solution,\textsuperscript{7} through phase transformation by changing the temperature and/or pressure.\textsuperscript{8,9} The two-phase mixed solution changes from homogeneous to heterogeneous including upper and lower phases in a batch vessel through phase transformation. On the other hand, when the mixed solution is fed into a microspace, such as a capillary tube, changing the temperature, phase-separation multiphase flow generates, having a liquid-liquid interface. Under certain conditions, an annular flow consisting of an inner and outer phases can be observed as one of phase-separation multiphase flow; we call it tube radial distribution flow (TRDF). Novel capillary chromatography has been performed based on the specific TRDF; we call the separation method tube radial distribution chromatography (TRDC).\textsuperscript{10-12} In the TRDC, the outer phase acts as a pseudo-stationary
phase during chromatographic separation.

To date, TRDC has been performed through phase transformation caused by temperature change, and the system requires a temperature controlling device including a water bath and a stirrer. In this study, we proposed a novel type of TRDC system without any cooling device, where the annular flow, TRDF, is created through pressure loss in a capillary tube. Model analytes were successfully separated with the TRDC system developed herein.

Experimental

Reagents

Purified water obtained from an Elix 3 UV water purification system (Millipore Co., Billerica, MA) was used. All reagents were commercially available and of analytical grade and include 1-naphthol, 1-naphthalenesulfonic acid (1-NS), 2,6-naphthalenedisulfonic acid (2,6-NDS), 1,3,6-naphthalenetrisulfonic acid (1,3,6-NTS), Eosin Y, perylene, acetonitrile, and ethyl acetate from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Fused-silica capillary tubes with an inner diameter of 50 µm and an outer diameter of 375 µm were purchased from GL Science Co. (Tokyo, Japan). In order to maintain the absorption sensitivity constant that depends on a capillary inner diameter or light length and hold the same length of sample zone in a capillary tube, we determined a capillary inner diameter to be 50 µm in this paper.
Figure 1 shows a schematic diagram of the TRDC system developed by combining a solution delivery pump (LC-20AD, Shimadzu; Kyoto, Japan), sample injector (0.2 µL, Valco Instruments Co. Inc. TX), open-tubular capillary tube (separation capillary length, 350 cm; effective length for separation, 330 cm) as a separation column, and UV/Vis detector (875-CE, Jasco Co.; Tokyo, Japan). A capillary tube 550 cm in length was connected to the outlet of the detector to provide sufficient pressure loss. The ternary solution of water/acetonitrile/ethyl acetate (volume ratio of 3:8:4, respectively) was used as the eluent at a fixed flow rate (1.0 µL min⁻¹) under laminar flow conditions. The model analyte solution (0.2 µL) that was dissolved with the eluent was injected into the system, separated using the capillary tube at a room temperature, and detected by the spectrophotometric detector at 254 nm.

A capillary tube of equivalent size to that used as a separation column in the TRDC system was attached to a fluorescence microscope equipped with a charge coupled device (CCD) camera system. Fluorescence in the capillary tube was monitored at approximately 300 cm from the separation capillary inlet using the 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 CCD camera (JK-TU53H; Toshiba, Tokyo, Japan). The eluent contained 0.1 mM perylene and 1 mM Eosin Y was delivered to the capillary tube at a fixed flow
rate of 1.0 µL min$^{-1}$ using the delivery pump.

Results and Discussion

Effect of pressure loss on TRDF

Fluorescence photographs were taken in the separation capillary tube with and without the capillary tube providing pressure loss after the detection outlet. As shown in Fig. 2, TRDF was not observed without the capillary and was clearly observed with the capillary; TRDF appeared after a constant flow rate and stable microfluidic flow being kept by the plunger pump.

The curves, blue and green, in Fig. 2 showed fluorescence profiles (blue for perylene, hydrophobic and green for Eosin Y, hydrophilic) based on the fluorescence photographs. Linear velocity of solution shows a parabolic curve in a microspace under laminar flow conditions. As the fluorescence intensity is thought to be roughly proportional to the velocity under homogeneous laminar flow conditions, the fluorescence profiles become similar to parabolic curve. In the case of non-TRDF in Fig. 2 (a), the blue and green curves were almost the same parabolic curves, especially near the inner wall, because of their homogeneous flows not having a liquid-liquid interface. On the other hand, in the case of TRDF in Fig. 2(b), the blue curve was almost like parabolic but the green curve had inflection points near the inner wall, and green color was observed near the wall in Fig. 2 (b). That is, the inflection of green curve and green color observation on the fluorescence photograph in Fig. 2 (b) showed the appearance of annular flow or TRDF.

The TRDF system consisted of an inner phase (organic solvent-rich solution) and
The pressure losses were calculated to be $1.5 \times 10^4$ and $4.3 \times 10^5$ Pa without and with the capillary, respectively, based on the Hagen-Poiseuille equation. We reported that the phase transformation of the ternary mixed solution occurred at higher pressure than $3 \times 10^4$ Pa in a batch vessel without temperature changing. It was confirmed here that TRDF based on phase-separation multiphase flow was created through pressure change induced phase transformation.

Separation and injection volume

TRDC was performed on a model mixture of 1-naphthol and 2,6-NDS without and with the capillary tube after the detection outlet and the obtained chromatograms are shown in Fig. 3. Separation was not achieved without the capillary and the analytes were successfully separated with the capillary by TRDC. In the TRDC system, the inner (organic solvent-rich) and outer phases (water-rich) acted as the mobile and pseudo-stationary phases, respectively. The hydrophobic 1-naphthol was first detected and the more hydrophilic 2,6-NDS was detected afterwards because of the increased interaction with the water-rich pseudo-stationary phase, demonstrating the separation principle of TRDC. The analyte injection volume was also examined in the developed TRDC system. As shown in Fig. 4, a volume of 0.2 µL resulted in improved resolution and peak symmetry compared to the 1.0 µL injection. The resolutions were 4.2 and 3.3 for the 0.2 and 1.0 µL injections, respectively, and the 0.2 µL injection volume was recommended for use in the followings.
TRDC through pressure loss

We tried to examine the mixed analytes containing 1-naphthol, 1-NS, 2,6-NDS, and 1,3,6-NTS with the present TRDC. As the obtained chromatogram is shown in Fig. 5, they were successfully separated each other. The elution times were 9.9, 10.8, 13.7, and 16.4 min for 1-naphthol, 1-NS, 2,6-NDS, and 1,3,6-NTS, respectively. The chromatographic separation was, for the first time, performed by introducing the extra capillary tube into the TRDC to create the TRDF. The present TRDC with the extra capillary tube decreased occurrence frequency of air bubble generation in a capillary tube, compared to the TRDC with a temperature-controlling device.

The distribution coefficient, $K_d$, of four analytes in the upper (organic solvent-rich) and lower (water-rich) phases generated through phase transformation of the ternary solvent (water/acetonitrile/ethyl acetate; 3:8:4 volume ratio) in the batch vessel was investigated. The analyte concentrations in the each phase were estimated by absorption measurements and calibration data.

At a 4:1 volume ratio of the upper and lower phases, the calculation of the capacity factor, $k'$, from the $K_d$ value was straightforward as $k'$ and $K_d$ are related as follows:

$$k' = (C_s V_s)/(C_m V_m) = K_d (V_s/V_m),$$  \hspace{1cm} (1)

where $C$ and $V$ refer to the analyte concentration and phase volume, respectively, and subscripts s and m refer to the stationary and mobile phases, respectively. The $K_d$ and $k'$ values of the upper (organic solvent-rich) and lower (water-rich) phases were determined for the four analytes using the ternary mixed solution of water/acetonitrile/ethyl acetate (3:8:4, volume ratio). The $K_d$ values were 0.033, 1.4, 6.0,
and 380 and the $k'$ values were 0.0082, 0.36, 1.5, and 94 for 1-naphthol, 1-NS, 2,6-NDS, and 1,3,6-NTS, respectively.

The elution order of four analytes in Fig. 5 corresponded to the order of $k'$ values mentioned above. That is, the more hydrophilic analyte having larger $k'$ value distributed better into the water-rich outer phase (pseudo-stationary phase), and then eluted later on the chromatogram. But the peak shape of the fourth eluted analyte of 1,3,6-NTS did not seem to be observed with good reproducible.

Conclusions

A novel type of TRDC system was proposed without any cooling device, where the annular flow or TRDF was created via pressure loss in a capillary tube. Model analytes were successfully separated with the developed TRDC system, providing a simplified platform with which to clarify the principle of TRDC based on phase transformation. The temperature-controlling method must be used directly to the separation capillary tube, while, the pressure loss method can control the phase transformation from the outside of the separation capillary tube. The merit might work for improvement of design in the TRDC system.

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Figure 1. Schematic diagram of the developed TRDC system. Capillary A: fused-silica capillary for separation, 50 µm i.d. and 350 cm length (effective length, 330 cm); capillary B: fused-silica capillary for pressure loss, 50 µm i.d. and 550 cm length; eluent, water/acetonitrile/ethyl acetate mixed solution (3:8:4, volume ratio); flow rate, 1.0 µL min⁻¹; analyte injection volume, 0.2 µL; and detection wavelength, 254 nm.
Figure 2. Fluorescence photographs of the ternary solution in the capillary tube. The photographs were taken of the water/acetonitrile/ethyl acetate solution (volume ratio 3:8:4) at a flow rate of 1.0 µL min\(^{-1}\) (a) without and (b) with capillary B containing 0.1 mM perylene (blue) and 1 mM Eosin Y (green). The arrows in the photograph (b) show the inflection points of the blue curves and the dotted lines show the liquid-liquid interface between the inner (organic solvent-rich) and outer (water-rich) phases.
Figure 3. Chromatograms obtained with the developed TRDC system. (a) Without and (b) with capillary B. The analyte concentrations of 1-naphthol and 2,6-NDS were 0.5 mM. The other analytical conditions are described in Fig. 1.
Figure 4. Effects of analyte injection volumes on the chromatograms. Injection volumes of (a) 0.2 and (b) 1.0 µL. The analyte concentrations of 1-naphthol and 2,6-NDS were 0.5 mM. The other analytical conditions are described in Fig. 1.
Figure 5. Chromatogram obtained for four mixed analytes using the developed TRDC system. Analyte concentrations of 1-naphthol, 1-NMS, 2,6-NDS and 1,3,6-NTS were 0.1 mM. The other analytical conditions are described in Fig. 1.