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Distribution of Fluorescent Dyes Dissolved in Ternary Mixed Solvent in a Microchannel under Laminar Flow Conditions

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We observed chromatographic separation in an open capillary tube with a water–hydrophilic–hydrophobic organic mixture carrier solution. The separation is thought to be based on the tube radial distrubution of aqueous and organic carrier solvents in the capillary tube. In this study, in order to obtain information concerning the solvent distribution, we delivered a carrier solution containing fluorescent dyes into a microchannel (100 μm in width × 40 μm in depth) and monitored the tube radial distribution of the dyes using a fluorescence microscope. When an organic solvent-rich carrier solution (water–acetonitrile–ethyl acetate; 3:8:4 volume ratio) containing perylene and Eosin Y was fed into the channel, perylene and Eosin Y were distributed around the center and near the inner wall of the microchannel, respectively. This observation consists with the specific solvent distribution proposed for the chromatography system.

We have developed a capillary chromatography system using an open capillary tube made of fused-silica, polyethylene, or poly(tetrafluoroethylene), and a water–hydrophilic–hydrophobic organic mixture carrier solution.[1,2] Using this tube radial distribution chromatography (TRDC) system, chromatography separation was achieved under laminar flow conditions. To date, various mixtures of hydrophilic and hydrophobic analytes have been separated using TRDC. The separation was performed in the capillary tubes without using any packing agents and monolithic columns[3] or applying high voltage.[4]

The separation behavior in the TRDC system was explained in our previous papers as follows.[1,2] First, aqueous and organic solvents in the carrier solution are dispersed nonuniformly in a specific flow in the capillary tube under laminar flow conditions, generating an organic solvent-rich phase and a water-rich phase in the capillary tube. A major inner phase is formed around the center of the tube and away from the inner wall, while a minor outer or capillary wall phase is generated near the inner wall. An organic solvent-rich carrier solution generates an organic solvent-rich inner phase, while a water-rich carrier solution results in a water-rich inner phase. The tube radial distribution of the solvent molecules in the carrier solution is thus caused by the flow in the capillary tube. Consequently, the analytes that are delivered through the capillary tube are distributed between inner and outer phases, undergoing chromatographic separation under laminar flow conditions. In the TRDC system the order of analyte elution times was also easily changed by altering the solvent component ratios in the carrier solution.

So far the tube radial distribution in the TRDC system has been supported by experimental data using polymer particles as analytes[5] and phenylboronic acid or iminodiacetic acid-modified fused-silica capillary tubes.[6] Here we tried to obtain new information concerning the solvent distribution by using a fluorescence microscope-CCD camera. An aqueous–organic solvent carrier solution containing fluorescent dyes, perylene and Eosin Y, was delivered into a microchannel incorporated in a microreactor. The dyes dissolved in the carrier solution must be distributed based on their hydrophilic or hydrophobic nature in the channel, if the tube radial distribution of the solvent molecules in the carrier solution is caused by the flow as we propose.

Water was purified using an Elix UV 3 system (Millipore). All reagents used were commercially available and were of special analytical grade. Perylene, Eosin Y, acetonitrile, and ethyl acetate were purchased from Wako Pure Chemical Industries. A microreactor made of quartz, incorporating a straight microchannel line (100 μm in width × 40 μm in depth; 40 mm length) was purchased from Michrochemical Technology. Carrier solutions of a water–acetonitrile–ethyl acetate mixture were prepared with the volume ratios of 3:8:4 and 15:3:2, respectively, as typically reported for the TRDC system.[1,2,5,6] The organic solvent-rich carrier solution contained 0.1 mM perylene and 1 mM Eosin Y, while the water-rich carrier solution contained 1 mM Eosin Y (perylene was little dissolved in the solution). The carrier solution was delivered into the microchannel through a poly(tetrafluoroethylene) tube (500 μm i.d. and ca. 30 cm length) at a flow rate of 0.8 μL min^{-1} using a microsyringe pump (MF-9090; Bioanalytical System).

The fluorescence in the microchannel was monitored at approximately 30 mm from the channel inlet using a fluorescence microscope (BX51; Olympus) equipped with an Hg lamp and a filter (U-MWU2, ex 330–385 nm, em. >420 nm) and CCD camera (JK-TU53H, Toshiba). The fluorescence photographs obtained thus were also transformed into line drawings to assess the color depth using a computer. The colors observed on the photographs were divided into red, green, and blue (RGB). The photographs mainly consisted of blue and green, because perylene and Eosin Y emitted light at approximately 470 and 550 nm, respectively. Blue and green color depths were expressed numerically as digital data on a computer and the numbers were finally standardized to the line-drawing data to give fluorescence profiles. Concentration quenching of the fluorescent dyes was not observed on the profiles in this study.

Figure 1 shows the fluorescence photographs and profiles observed for microchannels in which the dye-containing aqueous–organic solvent carrier solutions were delivered. Fluorescence photographs and profiles for the organic solvent-rich carrier solution showed that the hydrophobic perylene molecule (blue) was distributed around the center of the channel and away from the channel inner wall, while the hydrophilic Eosin Y molecule (green) was distributed near the channel inner wall (Figure 1a). On the other hand, fluorescence photographs and profiles showed that Eosin Y was distributed around the center of the channel for the water-rich carrier solution

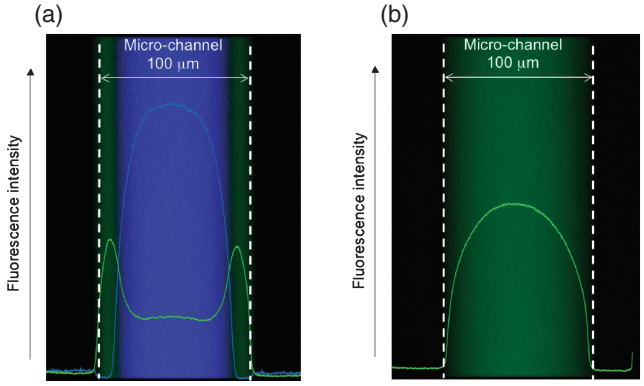

Figure 1. Fluorescence photographs and profiles of the fluorescent dyes dissolved in the aqueous–organic solvent mixture carrier solution at a flow rate of 0.8 μL min^{-1}. (a) 0.1 mM perylene and 1 mM Eosin Y dissolved in the water–acetonitrile–ethyl acetate (3:8:4, v/v/v) mixture and (b) 1 mM Eosin Y dissolved in the water–acetonitrile–ethyl acetate (15:3:2, v/v/v) mixture.

(Figure 1b). We could not obtain any information regarding the behavior of perylene in the water-rich carrier solution, because of its very low solubility in the solution. The tube radial distribution of perylene and Eosin Y present in the carrier solution thus confirmed the partition reversal between organic solvent-rich and water-rich carrier solutions (Figure 1). When the flow of the carrier solution in the microchannel was stopped, the radial distribution of the fluorescent dyes in the channel gradually collapsed, although the fluorescence profiles slightly kept a parabolic curve.

The distribution behavior observed for the fluorescent dyes dissolved in the carrier solution in the microchannel consists with the tube radial distribution of the solvent molecules proposed for the TRDC system. The distribution of perylene and Eosin Y at the center of the microchannel or near its inner wall would support that the aqueous and organic components of the carrier solution are not uniformly dispersed in the microchannel or capillary tube in the TRDC system. Instead, they generate a major inner phase (organic-rich or water-rich) and a minor outer or capillary wall phase (water-rich or organic-rich) in the capillary tube. As a result, the analytes are also distributed between the inner and outer phases depending on their nature and separated by chromatography. We are planning to examine the distribution behavior of analytes in the TRDC system using the fluorescence microscope-CCD camera.

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