Phase Separation and Collection of Annular Flow by Phase Transformation

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A polyethylene glycol/citrate mixed solution was fed into a single channel of a Y-type micro-channel on a microchip as an aqueous two-phase system. A phase separation multi-phase flow with a liquid–liquid interface was generated due to a phase transformation. An annular flow, one of the flow types in the phase separation multi-phase flow, was observed through bright-field microscopy. The flow consisted of citrate-rich inner and polyethylene glycol-rich outer phases. We attempted to separate and collect the two phases in the single channel into two separate Y-type channels. When the pressure losses for the separated channels were not very different, we observed symmetric flow in the Y-type channel. When the pressure losses were quite different, the polyethylene glycol-rich phase with higher viscosity was selectively distributed to the separated channel with lower pressure loss. Thus, the polyethylene glycol-rich phase was successfully and intentionally collected from the chosen Y-type channel via the creation of annular flow in the single channel.

Keywords Phase separation multi-phase flow, polyethylene glycol, trisodium citrate, Y-type micro-channel, tube radial distribution flow

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and collected the two phases by using a separate Y-type channel, by taking advantage of different pressure losses that were intentionally produced in the two separate channels by tubes of different lengths and diameters.

**Experimental**

**Reagents and materials**

Water was purified using an Elix 3 UV purification system (Millipore Co., Billerica, MA). All reagents were commercially available and of analytical grade. PEG (MW, 6000), trisodium citrate (citrate), and Rhodamine B were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). A microchip fabricated with a Y-type micro-channel (Microchemical Technology, Kanagawa, Japan) was used, wherein the channel was 100 $\mu$m wide and 40 $\mu$m deep. The Y-type micro-channel is illustrated in Fig. 1(a) and the microchip setup in a microchip holder is shown in Fig. 1(b).

**Viscosity measurement**

A homogeneous solution of the PEG/citrate system was converted in a batch vessel to a heterogeneous solution comprising two phases (upper PEG-rich and lower citrate-rich) by controlling the temperature. The viscosities of the mixture solution as well as the upper and lower solutions were measured with a viscometer (HAAKE RheoScope 1; Thermo Scientific, Sydney, Australia).

**Generation of different pressure losses in the separate Y-type channels**

Two kinds of PTFE tubes, Tube X with a 500-$\mu$m inner diameter (25.4 cm constant length) and Tube Y with a 250-$\mu$m inner diameter (different lengths), were connected to two separate micro-channels in the Y-type microchip to provide different pressure losses. The length of Tube Y was varied from 2.5 to 37.5 cm in 2.5 cm intervals to produce 15 different lengths.

**Results and Discussion**

**Phase diagram of PEG/citrate mixed solution and viscosities of the solutions**

The phase diagram of the PEG/citrate mixed solution was examined at temperatures of 4, 25, and 40°C (Fig. 2). The solubility curves in the diagram indicate the boundary between the homogeneous and heterogeneous solutions including the two phases. The plots on the curves in Fig. 2 are cloud points.
that were obtained through observation when a citrate solution was added to a PEG solution in a glass vessel. The components of PEG/citrate mixed solutions A – C are A, 12.0 wt% PEG and 8.5 wt% citrate; B, 10.0 wt% PEG and 10.0 wt% citrate; and C, 5.0 wt% PEG and 12.5 wt% citrate. After mixed solutions A – C were heated from 25 to 40°C, the homogeneous solutions turned heterogeneous with upper (PEG-rich solution) and lower (citrate-rich solution) phases. The volume ratios of the heterogeneous solutions were estimated through visual observation in a glass vessel, since Rhodamine B (red) was mostly distributed in the upper (PEG-rich solution) phase. The upper to lower volume ratio was 2:1 for solution A, 5:7 for solution B, and 1:3 for solution C. The viscosity of the mixed solution B at 25°C was 6.36 mPa·s. The viscosities of the upper and lower phases for solution B at 40°C were 13.2 and 1.41 mPa·s, respectively.

Control of two-phase flow in the TRDF with PEG/citrate mixed solution

We examined the phase distribution in the two-phase system with a Y-type micro-channel. Solution B in Fig. 2 was delivered from the single channel to the separated Y-type micro-channels at 40°C at a flow rate of 0.2 μL min⁻¹. When the Tube Y length was 2.5 – 22.5 cm (9 different length tubes), we observed a symmetric annular flow, as shown in Fig. 4(a), but when the length was 25.0 cm, the kinetic liquid-liquid interface became unstable. When the Tube Y length was 27.5 – 37.5 cm (5 different length tubes), the PEG-rich phase with higher viscosity moved to the separated channel with lower pressure loss, where Tube X was connected, and the citrate-rich phase with lower viscosity moved to the separated channel with higher pressure loss, where Tube Y was connected, as shown in Fig. 4(b). The experimental data clearly showed that a difference in pressure losses between the two separate channels led to phase separation and enabled collection of the two phases in the single channel into the separated channels.

The apparent pressure losses caused by Tubes X and Y were estimated by the Hagen-Poiseuille equation, as follows:

\[ Q = \frac{\pi a^4}{8\mu} \frac{\Delta p}{L} \]

where \( Q \) is the flow rate, \( a \) is the radius, \( \Delta p \) is the loss of pressure, \( \mu \) is the viscosity, and \( L \) is the tube length. The calculation was performed using the viscosity of a PEG/citrate mixed solution at 25°C and assuming a flow rate of 0.1 μL min⁻¹ to obtain the apparent pressure loss. The pressure loss for Tube X (500 μm inner diameter and 25.4 cm length) was about 0.11 kPa. The loss for Tube Y (250 μm inner diameter and 27.5 cm length) that enabled phase collection as shown in Fig. 4(b) was about 1.83 kPa. When the ratio of the apparent pressure losses was 17 times or more, the higher-viscosity PEG-rich phase moved to the separated channel with the lower pressure loss, as shown in Fig. 4(b).

These results indicate that the homogeneous mixed solution was separated into two phases in the micro-channel, and these phases could then be intentionally separated in the Y-type channels on the microchip. This is the first study to report this phenomenon, which has the potential to be useful for flow extraction or separation of samples with extremely small volumes (less than several hundred microliters).

Fig. 2 Phase diagrams of a PEG/citrate mixed solution. The components of PEG/citrate mixed solutions A – C are A, 12.0 wt% PEG and 8.5 wt% citrate; B, 10.0 wt% PEG and 10.0 wt% citrate; and C, 5.0 wt% PEG and 12.5 wt% citrate.

Fig. 3 Bright-field images of microfluidic flow in a Y-type micro-channel for solution C (PEG 5 wt% and citrate 12.5 wt%) containing 0.1 mM Rhodamine B at flow rate of 1.0 μL min⁻¹. (a) 25 and (b) 40°C.
Conclusions

Phase separation multi-phase flow, namely TRDF, was experimentally demonstrated for the first time using a PEG/citrate mixed solution as the ATPS. When the mixed solution was fed into the micro-channel, TRDF was observed for the inner citrate-rich and outer PEG-rich phases. The PEG-rich phase was successfully and intentionally separated and collected from the chosen Y-type channel with the two-phase system. A Y-type channel on a microchip is the simplest channel structure for collecting two separated phases in TRDF under certain conditions. This technique has potential for the flow extraction or separation of extremely small-volume samples.

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