Microfluidic Inverted Flow of Ternary Water/Hydrophilic/Hydrophobic Organic Solvent Solution in a Y-Type Microchannel and a Proposal of the Response Microfluidic Analysis through the Experiment

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Two solutions that are individually fed at the same flow rate into two separate microchannels of a microchip, combine to form a single channel (a Y-type microchannel). This flow is either parallel for immiscible solutions or initially parallel, but then becomes homogeneous through diffusion, for miscible solutions. However, a new type of microfluidic behavior in a Y-type microchannel that was neither parallel nor homogeneous flow has been observed using, for example, water/acetonitrile (3:4.5, v/v) and acetonitrile/ethyl acetate (3.5:4, v/v) mixed solutions. Each mixed solution was marked with distinctive dyes and delivered at the same flow rate into a Y-type microchannel under laminar flow conditions. In the single channel, the two phases were initially observed to flow in parallel, but then apparently swapped to flow on the opposite wall while retaining parallel flow with a slight change in the components of the two phases. We have named this type of laminar flow “microfluidic inverted flow” for ternary water/hydrophilic/hydrophobic organic solvent mixed solutions. The inverted flow of a ternary water/acetonitrile/ethyl acetate system was examined in detail under various flow conditions. We also proposed a concept of response microfluidic analysis based on such microfluidic inverted flow.

Keywords Microfluidic inverted flow, ternary water/hydrophilic/hydrophobic organic mixed solution, Y-type microchannel, response microfluidic analysis

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Introduction

The development of micro total analysis systems (μ-TASs) that include microchip or microfluidic device technology is an interesting aspect of analytical science.1–4 Microfluidic solvents exhibit various fluidic behaviors in microchannels. Their flow patterns have been examined by varying the channel configuration and solvent flow rate, and introducing specific obstacles into the microchannels. The fluidic flow of solvents in microchannels is related to separation, diffusion, and solute reactions. Information regarding solvent microfluidic flow and interface formation is important and useful in designing not only μ-TASs, but also sensing devices and microreactors.4–6

Various types of aqueous/organic mixed solvents are used in dissolution, cleaning, preservation, and separation, and as reaction solvents. However, to our knowledge, the use of ternary water/hydrophilic/hydrophobic organic solvents has not been examined in detail. We previously reported that ternary water/hydrophilic/hydrophobic organic solvent solutions were two-phase separation mixed solutions, and that when the ternary mixed solutions were fed into a microspace under laminar flow conditions, a phase transformation occurred, leading to a liquid-liquid interface.3,10 This microfluidic behavior is called “phase separation multiphase flow”.11,12

In this study, the mixing behavior of ternary water/hydrophilic/hydrophobic organic solvent solutions was investigated using a Y-type microchannel,11,14 mainly using a ternary water/acetonitrile/ethyl acetate solution. For example, two mixed solutions, water/acetonitrile and acetonitrile/ethyl acetate, were individually fed into two separate microchannels of a microchip at the same flow rate, which then combined to form a single channel in the Y-type microchannel, giving a ternary mixed solvent in the single channel. Interesting and unique microfluidic behavior was observed in the single channel, wherein the two phases were initially observed to flow in parallel, but then apparently swapped to the opposite wall while retaining parallel...
flow. We have named this specific fluidic behavior “microfluidic inverted flow” and proposed a concept of the response microfluidic analysis based on the inverted flow.

**Experimental**

**Chemicals and materials**

Water was purified using an Elix 3 UV system (Millipore Co., Billerica, MA). All reagents used were of analytical grade and obtained commercially. Perylene, Eosin Y, acetonitrile, ethyl acetate, 1,4-dioxane, and chloroform were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Trichloro(octadecyl)silane (ODS) was purchased from Sigma-Aldrich (Tokyo, Japan). A microchip fabricated with a Y-type microchannel made of glass (Microchemical Technology, Kanagawa, Japan) was used, wherein each channel was 100 μm wide × 40 μm deep (Fig. S1(a), Supporting Information). The microchip was set up in a microchip holder (Fig. S1(b), Supporting Information).

**Fluorescence photographs and profiles**

The microchip was also set up with a fluorescence microscope–charge-coupled device (CCD) camera system (Fig. S2, Supporting Information) to confirm microfluidic flow of the solvent solutions. For example, a water/acetonitrile mixture containing 1.0 mM Eosin Y and an acetonitrile/ethyl acetate mixture containing 0.1 mM perylene were fed at the same flow rate into the separate channels of the Y-type microchannel using a microsyringe pump. The fluorescence was monitored near the combining point and in the single channel using a fluorescence microscope (BX51; Olympus, Tokyo, Japan) equipped with a Hg lamp, a filter (U-MWU2; excitation, 330 – 385 nm; emission, combining point and 3 cm from the combining point in the single channel).

**Hydrophobic-modified microchannel**

A hydrophobic-modified microchannel was prepared as follows: a 1 wt% ODS toluene solution was fed into the microchannel with a microsyringe pump at a flow rate of 1 μL min⁻¹ for approx. 5 min. The channel was then sufficiently washed with toluene fed from both ends of the channels at a flow rate of 50 - 100 μL min⁻¹. The channel was then washed with chloroform and the microchip heated at 150 °C for 30 min to afford a hydrophobic-modified microchannel. The experiments in this study were carried out by using an untreated microchannel if without any description of a hydrophobic-modified one for use.

**Results and Discussion**

**Conventional microfluidic flow in Y-type microchannel**

The microfluidic behavior of two combined solutions was examined in a Y-type microchannel on a microchip using the fluorescence of dyes Eosin Y (green) and perylene (blue). The two dye solutions were fed into the separate channels at the same flow rates. Combinations of two solutions, including water and water, acetonitrile and acetonitrile, ethyl acetate and ethyl acetate, water and acetonitrile, and acetonitrile and ethyl acetate solutions (all miscible) were examined. Each of these combinations exhibited initial parallel flow that gradually became homogeneous flow upon mixing in the single channel through a collapse of the liquid–liquid interface by molecular diffusion. For example, the parallel and homogeneous flow of water and acetonitrile solutions is shown in Fig. 1(a). Immiscible solutions of water and ethyl acetate showed a clear liquid–liquid interface and produced stable parallel flow in the Y-type microchannel (Fig. 1(b)), which was indicative of immiscible multiphase flows with a liquid–liquid interface.

**Microfluidic inverted flow in Y-type microchannel**

The two mixed solutions to be combined, water/acetonitrile and acetonitrile/ethyl acetate, were fed into the two separate channels. In the single channel, the combined mixture showed a specific fluidic behavior. The two phases in the single channel were initially observed to flow in parallel, but then apparently swapped over to opposite walls while retaining parallel flow. This distinctive feature in the Y-type microchannel was named “microfluidic inverted flow”. The example of water/acetonitrile and acetonitrile/ethyl acetate mixed solutions is shown in Fig. 1(c). As mentioned above, only the ternary mixed solvent of water, acetonitrile, and ethyl acetate produced microfluidic inverted flow. This information is useful for determining the creation of microfluidic inverted flow in subsequent sections.

**Effects of flow rates and channel lengths on microfluidic inverted flow**

Figure 2 shows the effects of flow rate on microfluidic...
Fig. 2 Effect of flow rate on microfluidic inverted flow in a Y-type microchannel. Conditions: solutions as described in Fig. 1(c).

Fig. 3 Component ratios on the phase diagrams of water/acetonitrile/ethyl acetate mixed solution and microfluidic inverted flow in a Y-type microchannel. (a) Typical component ratio that resulted in inverted flow and (b) typical component ratio that did not result in inverted flow. Dotted curves indicate the solubility curve (upper region, homogeneous; lower region, heterogeneous). The two mixed solutions were fed at the same flow rate (total flow rate in the single channel, 10 μL min⁻¹).
inverted flow in a Y-type microchannel. Fluorescence photographs were captured at the mixing point and in the single channel at distances of 1, 2, and 3 cm from the mixing point using flow rates of 5, 10, 20, and 100 μL min⁻¹. As the flow rate increased, we observed various stages of inverted flow in the channel. However, the progress status of the inverted flow depended on the residence times under laminar flow, which are written below every photograph in Fig. 2. That is, the progress of the inverted flow proceeded with the residence times in the microchannel. The inverted flow in a long single channel (12 cm) in a Y-type microchip was also examined; the inverted flow was even observed in the long and bent microchannel (Fig. S3, Supporting Information).

Effect of the ternary mixed solution composition on the phase diagram and microfluidic inverted flow

The water/acetonitrile and acetonitrile/ethyl acetate mixed solutions were delivered into and combined in the Y-type channel to obtain microfluidic inverted flow. We examined the inverted flow using different component ratios of the mixed solutions in the phase diagram of the ternary water/acetonitrile/ethyl acetate system. For example, when water/acetonitrile (2:3, v/v) and acetonitrile/ethyl acetate (1:4, v/v) were fed into the microchannels to obtain a ternary water/acetonitrile/ethyl acetate solution (1:2:2, v/v) in the single channel, we observed inverted flow (Fig. 3(a)). However when water/acetonitrile (4:1, v/v) and acetonitrile/ethyl acetate (3:2, v/v) were fed into the microchannels to obtain a ternary water/acetonitrile/ethyl acetate solution (2:2:1, v/v) in the single channel, inverted flow was not observed (Fig. 3(b)).

As shown in Fig. 3, combining the two mixed solutions (homogeneous) could lead to heterogeneous solutions with upper and lower phases in a batch vessel. However, the 1:2:2 (v/v) ternary water/acetonitrile/ethyl acetate solution produced inverted flow in the single channel on a microchip, while the 2:2:1 (v/v) ternary water/acetonitrile/ethyl acetate solution showed only parallel flow.

Furthermore, we examined other component ratios in the phase diagram to create inverted flows in the ternary water/acetonitrile/ethyl acetate system. The results are shown in Fig. 4(a). We also examined the inverted flow using other ternary mixed solutions systems, namely water/acetonitrile/chloroform and water/1,4-dioxane/ethyl acetate, for their component ratios on the phase diagrams (Figs. 4(b) and 4(c)). We tentatively concluded that the following conditions were required for inverted flow: (i) Ternary mixed solutions should be heterogeneous including two homogeneous phases, and (ii) the amount of water in the ternary solution should be less than 35 vol% for water/acetonitrile/ethyl acetate, 25 vol% for water/acetonitrile/chloroform, and 35 vol% for water/1,4-dioxane/ethyl acetate.

Fluorescence photographs and profiles of microfluidic inverted flow

Fluorescence photographs and profiles of Eosin Y and perylene in the single channel were obtained based on the emissions of Eosin Y (550 nm, green) and perylene (440 nm, blue) (Fig. 5). Fluorescence photographs and profiles gradually changed during the inverted flow process in the single channel. Differences were observed in the color densities of the photographs and intensities of the profiles of the two dyes in the channel before and after inverted flow. This showed that
inverted flow coincided with a change in the solvent compositions. Therefore, the two mixed solutions were not the same after inversion, with the changes in their component ratios shown in Fig. 5.

**Influence of the inner wall characteristics on inverted flow**

We examined the effects of the pH on inverted flow by adding a buffer solution to the ternary buffer/acetonitrile/ethyl acetate solutions. The buffer solutions were prepared as follows: pH 2.0, 10 mM HCl-KCl buffer; pH 3.0, 10 mM NaOH-KCl buffer; pH 4.0, 10 mM acetic acid-sodium acetate buffer; pH 6.0 – 8.0, 10 mM Na₂HPO₄–NaH₂PO₄ buffer; pH 9.0, 10 mM glycine-NaOH buffer; pH 10.0, 10 mM Na₂CO₃–NaHCO₃ buffer; and pH 11.0, 10 mM Na₂HPO₄-NaOH buffer.

The above ternary buffer/acetonitrile/ethyl acetate solutions were examined for inverted flow using component ratios H, I, L, M, and Q, as shown in Fig. 4, which had led to inverted flow in the ternary water/acetonitrile/ethyl acetate solutions, and component ratios N, R, and U, as also shown in Fig. 4, which had not produced inverted flow. For component ratios H, I, L, M, and Q, inverted flow was observed with all buffers (pH 2.0 – 11.0)/acetonitrile/ethyl acetate mixed solutions. In contrast, component ratios N, R, and U showed no inverted flow for buffer (pH 2.0 – 6.0)/acetonitrile/ethyl acetate mixed solutions, while inverted flow was observed for buffer (pH 7.0 – 11.0)/acetonitrile/ethyl acetate mixed solutions (Fig. 6).

We considered inverted flow for component ratios N, R, and U with respect to the hydrophobicity of the solutions and inner wall. The fused-silica inner wall possessing silanol groups (pKₐ, approx. 3 – 4) might be relatively hydrophobic toward the ternary mixed solutions with buffer pH <6 due to non-dissociation of the silanol groups, while the inner wall might be relatively hydrophilic toward the ternary mixed solutions with buffer pH >7 due to dissociation of the groups. That is, the water-rich phase at the liquid-liquid interface near the inner wall that has larger interfacial tension than the organic solvent-rich phase might move along the relatively hydrophilic inner wall with buffer pH >7, leading to inverted flow.

We examined inverted flow using a microchip possessing a hydrophobic-modified inner wall channel. We did not observe inverted flow in the hydrophobic-modified microchannel for all component ratios that had provided inverted flow in the untreated microchannel (Fig. 4). The modified inner wall was much more hydrophobic than the untreated inner wall. The inner wall characteristics must play an important role to create the microfluidic inverted flow.

**Response of microfluidic inverted flow to pH**

The response of microfluidic inverted flow to the pH was examined using the experimental device shown in Fig. 7(a). The pH 6.0 buffer/acetonitrile mixed solution (4:1, v/v) and

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**Fig. 5** Fluorescence photographs and profiles in a single channel during inversion of flow. Conditions: solutions as described in Fig. 1(c). Fluorescence photographs (a), (b), and (c) were observed at distance points of around 1, 2, and 3 cm from the combining point, respectively.

**Fig. 6** Effect of buffer solution pH in the ternary buffer/acetonitrile/ethyl acetate solutions on microfluidic inverted flow. (a) Buffer (pH 2.0 – 6.0)/acetonitrile/ethyl acetate mixed solutions and (b) buffer (pH 7.0 – 11.0)/acetonitrile/ethyl acetate mixed solutions. ○ Inverted flow and ●, non-inverted (parallel) flow. The two mixed solutions were fed at the same flow rate (total flow rate in the single channel, 10 μL min⁻¹).
pH 7.0 buffer/acetonitrile mixed solution (4:1, v/v), containing 1.0 mM Eosin Y, were separately combined with an acetonitrile/ethyl acetate (2.3, v/v) mixed solution containing 0.1 mM perylene at the same flow rate (total flow rate in the single channel, 10 μL min⁻¹) using a four-way cock to obtain the ternary buffer/acetonitrile/ethyl acetate solution (4:3:3, v/v; R in Fig. 4) in the single channel. As shown in Fig. 7(b), when the pH 6.0 buffer-containing solution was used, inverted flow was not observed, but when the pH 7.0 buffer-containing solution was used, inverted flow was observed; non-inverted flow and inverted flow were alternately observed by switching the cock. The experimental data for the pH in microfluidic inverted flow provided useful information for the development of a new type of responsive device of pH based on this specific microfluidic behavior.

**Computer simulation**

The microfluidic inverted flow in a Y-type microchannel was computer-simulated using both the volume of fluid method (VOF) and the species transport method (ST) (Fluent program; ANSYS, Inc., Canonsburg, USA). The VOF method simulates a multiphase flow with a liquid–liquid interface based on an assumption of the interface viscosity, density, surface tension, and contact angle. The ST method simulates mixing at the molecular level from viscosity, density, molecular weight, and diffusion coefficients, but without considering the interface. These methods were performed simultaneously in this study.

The simulation conditions were as follows. The mixed solutions were water/acetonitrile (3:4.5, v/v) and acetonitrile/ethyl acetate (3.5:4, v/v). The microchannel was 5000 μm long, 100 μm wide, and 40 μm deep. The contact angles of 5° and 20° with the inner wall expressed the hydrophilic and hydrophobic nature of the inner wall with respect to the solution, respectively. The linear velocity was 0.083 m s⁻¹ (20.0 μL min⁻¹), the outlet pressure was atmospheric pressure (1.0 × 10⁵ Pa), and the total mesh number was 12400. The diffusion coefficients of water, acetonitrile, and ethyl acetate were estimated to be 2.11 × 10⁻⁹, 1.47 × 10⁻⁹, and 1.19 × 10⁻⁹ m² s⁻¹, respectively, from the Stokes–Einstein equation. The capillary was filled with water in advance.

The combination of the VOF and ST methods for a multiphase flow model based on mixing at the molecular level was adopted as a model simulation for microfluidic inverted flow that incorporated the liquid–liquid interface and mixing of solvent molecules. Microfluidic behavior in the microchannel with hydrophobic and hydrophilic inner walls was obtained in the simulation under the conditions described above, as shown in Fig. 8. These images were generated 0.02, 0.04, 0.06, 0.08, and 0.1 s after flow had started. The hydrophilicity (blue) or hydrophobicity (red) of the mixed solutions is expressed using 10 color gradients from blue to red or red to blue. The color gradient bars are also shown in Fig. 8.

As shown in Fig. 8, for the hydrophobic inner wall (contact angle, 20°), the two mixed solutions, water/acetonitrile (blue) or water-rich and acetonitrile/ethyl acetate (red) or organic solvent-rich, were delivered along the inner walls at which the solutions were introduced. Inverted flow was not observed in the images showing the cross-sectional view. In contrast, for the hydrophilic inner wall (contact angle, 5°), the water/acetonitrile (blue) or water-rich phase at liquid–liquid interface near the inner wall that has larger interfacial tension than the organic solvent-rich phase moved along the relatively hydrophilic inner wall, leading to inverted flow. That is, moving of the water-rich phase along the inner wall toward the opposite side was simulated as an initial behavior of inverted microfluidic flow in the image of Fig. 8 with the hydrophilic inner wall.

The results obtained here imply that computer simulation were useful for investigating microfluidic inverted flow, such as setting the conditions and designing the microfluidic device. In particular, simulation data supported that inverted flow depended on the hydrophilic characteristic and that the inverted flow moved along the inner wall.

**Inverted flow mechanism**

We proposed a hypothesis of concerning the flow mechanism for inverted flow, as illustrated in Fig. 9, from the experimental
and simulation data mentioned above. Figs. 9(a) and 9(b) were correspondent to Figs. 3(a) and 3(b), respectively, where solutions A and B were a water/acetonitrile and acetonitrile/ethyl acetate solution before mixing, respectively, as well as A' and B' were a water-rich solution containing the ternary components and organic solvent-rich solution containing the ternary components after mixing, respectively. In the case of inverted flow (Figs. 3(a) and 9(a)), the solution A' could move along the inner wall through a hydrophilic interaction to the wall, as shown in Fig. 9(a). On the other hand, in the case of non-inverted flow (Figs. 3(b) and 9(b)), the solution A' could not move along the inner wall because of an insufficient hydrophilic affinity to the wall.

**Conclusions**

The mixing behavior of ternary water/hydrophilic/hydrophobic organic solvent solutions was investigated using a Y-type microchannel. These two mixed solutions, water/acetonitrile and acetonitrile/ethyl acetate, were individually fed at the same flow rate into two separate microchannels of a microchip that combined to form a single channel in a Y-type microchannel, affording a ternary mixed solution in the single channel. An interesting and unique microfluidic behavior was observed in the single channel. The two phases in the single channel were initially observed to flow in parallel, but then apparently swapped to the opposite walls while retaining parallel flow. We named this type of laminar flow “microfluidic inverted flow”
for the ternary water/hydrophilic/hydrophobic organic solvent mixed solutions. We proposed a concept of response microfluidic flow analysis based on the inverted flow. The data for the inverted flow, visualized under dye fluorescence, are also expected to be useful for developing a mixing technique to create a phase interface and chemical reaction space in the microspace of a microchip. In addition, the inverted flow process that included unique liquid-liquid and solid-liquid interaction might provide an interesting and novel molecule recognition field in the microchannel in the future.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

References

1. X. Chen and J. Shen, J. Chem. Technol. Biotechnol., 2017, 92, 271.
2. Y. Zhang, S. Ge, and J. Yu, TrAC, Trends Anal. Chem., 2016, 85, 166.
3. D. E. W. Patabadige, S. Jia, J. Sibbitts, J. Sadeghi, and K. Sellens, Anal. Chem., 2016, 88, 320.
4. Y. Li, J. Wang, D. Li, and R. Liu, Int. J. Multiphase Flow, 2018, 98, 139.
5. T. Fu, L. Wei, C. Zhu, and Y. Ma, Chem. Eng. Process., 2015, 91, 114.
6. W. Yang, K. K. Lee, and S. Choi, Sens. Actuators, B, 2017, 243, 292.
7. N. Jinno, M. Murakami, K. Mizohata, M. Hashimoto, and K. Tsukagoshi, Analyst, 2011, 135, 927.
8. M. Murakami, N. Jinno, M. Hashimoto, and K. Tsukagoshi, Anal. Sci., 2011, 27, 793.
9. S. Fujinaga, M. Hashimoto, K. Tsukagoshi, and J. Mizushima, Anal. Sci., 2016, 32, 455.
10. K. Tsukagoshi, Anal. Sci., 2014, 30, 65, and references cited therein.
11. K. Yamada, H. Kan, and K. Tsukagoshi, Talanta, 2018, 183, 89.
12. K. Tsukagoshi, J. Flow Injection Anal., 2015, 32, 89.
13. X. Shi, Y. Xiang, L.-X. Wen, and J.-F. Chen, Ins. Eng. Chem. Res., 2012, 51, 13944.
14. P. Foroozan Jahromi, J. Karimi-Sabet, Y. Amini, and H. Fadaei, Chem. Eng. J., 2017, 328, 1075.
15. N. Jinno, Y. Masuhara, T. Kobayashi, N. Tainaka, Y. Tanigawa, M. Hashimoto, and K. Tsukagoshi, Anal. Sci., 2012, 28, 527.