### Investigation of Specific Microfluidic Flow with Two-phase Separation Mixed Solvent Solutions and Application to Flow Technology

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### Abstract

Specific microfluidic flows with two-phase separation mixed solvent solutions, such as ternary water-hydrophilic/hydrophobic organic solvent, water-surfactant, water-ionic liquid, and fluorocarbon/hydrocarbon organic solvent mixtures, have been reported as a new type of multiphase flow. When homogeneous mixed solvent solutions are fed into a microspace, the solutions change from homogeneous to heterogeneous solutions featuring a liquid-liquid interface via phase transformation by changing the pressure and/or temperature. This type of flow is called "phase separation multiphase flow" (PS-MPF) and differs from conventional immiscible multiphase flows. PS-MPFs show droplet, slug, parallel, and annular flows under different conditions. In particular, the annular flow in a PS-MPF, which generates an inner and outer phase, is interesting and useful. This flow is called a "tube radial distribution flow" (TRDF). PS-MPFs were examined in detail primarily with a ternary water-hydrophilic/hydrophobic mixed solvent solution through fluorescence photographs, the construction of phase diagrams, and flow conditions to discuss their creation mechanism. TRDFs with various two-phase separation mixed solvent solutions were also constructed, and the formation and configuration of inner and outer phases in the TRDFs were then discussed through hydromechanics, including dimensionless numbers and viscous dissipation principles. The outline of the PS-MPF has been clarified from various viewpoints of the first research stage. Furthermore, new technologies were developed based on a TRDF in a PS-MPF; for example, a capillary chromatographic system, a microreactor, and unit microoperation systems referring to separation, extraction, and mixing on a microchip. These were well matched to the Lab-on-a-Chip concept.

**Keywords** Phase separation multiphase flow (PS-MPF), two-phase separation mixed solvent solution, tube radial distribution flow (TRDF), capillary chromatography, microreactor, Lab-on-a-Chip

### 1. Introduction

A fluidic flow in a tube can typically be classified into one of two groups: homogeneous single-phase flows, which do not have a liquid–liquid interface [1,2], and immiscible multiphase flows, which feature a liquid–liquid interface [3]. Electroosmotic and laminar flows generated in a microspace are classified as homogeneous single-phase flows. In the last century, capillary electrophoresis [4] and hydrodynamic chromatography [5] were developed by manipulating electroosmotic flows and laminar flows, respectively. Various types of immiscible multiphase flows that lead to the creation of liquid–liquid interfaces using water and hydrophobic organic solvents have been reported [6–9]. These reports show droplet, slug, parallel, or annular flows depending on the employed conditions.

We have recently developed a method of generating a multiphase flow using two-phase separation mixed solvent solutions [10–14]. These new types of multiphase flows are

called "phase separation multiphase flows" (PS-MPFs), and they \*Corresponding author. Tel.: +81 75 462 4636.

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differ from conventional immiscible multiphase flows. We can

also observe droplet, slug, parallel, and annular flows under different conditions in a PS-MPF. We are particularly interested in annular flows generated in PS-MPFs. The specific microfluidic phenomenon and flow are termed the "tube radial distribution phenomenon" (TRDP) and "tube radial distribution flow" (TRDF), respectively [10–14].

Since 2009, we have experimentally and theoretically investigated PS-MPFs from the viewpoints of creation mechanism clarification and functional appearance with the ultimate goal of technical application [10–16]. Related data and considerations are reviewed in this paper.

### 2. Experimental information [13]

Water and reagents: Water was purified with the Elix 3 UV water purification system (Millipore Co., Billerica, MA, USA). All reagents used were commercially available and of analytical grade. Capillary tubes: Fused-silica, high-density polyethylene (PE), polytetrafluoroethylene (PTFE), and tetrafluoroethylene-perfluoroalcoxyethylene (PTFE-PFAE) copolymer capillary tubes were purchased from GL Science (Tokyo, Japan), Natume Co. (Tokyo, Japan), Yasaka Industries, Inc. (Tokyo, Japan), and Iwase Co. (Tokyo, Japan), respectively.

Microchips: A glass microchip incorporating microchannel lines was acquired from the Institute of Microchemical Technology Co., Ltd. Japan). Fluorescence (Kanagawa, microscope-charge-coupled device (CCD) camera system: the system comprised a fluorescence microscope (BX51; Olympus, Tokyo, Japan), which was equipped with a Hg lamp and a filter (U-MWU2, ex: 330-385 nm, em: >420 nm), and a CCD camera (JK-TU53H; Toshiba, Tokyo, Japan). Capillary chromatographic system: This system consisted of a microsyringe pump (MF-9090; Bioanalytical Systems, Inc., West Lafayette, IN, USA) and an absorption detector (modified SPD-10AV spectrophotometric detector; Shimadzu Co., Kyoto, Japan), a fluorescence detector (modified RF-535 fluorescence detector; Shimadzu Co., Kyoto, Japan), or a chemiluminescence (CL) detector (Model EN-21; Kimoto Electric Co., Ltd., Osaka, Japan) as the main devices.

#### 3. Phase separation multiphase flow

We developed a method of generating a multiphase flow using two-phase separation mixed solvent solutions [10–14], such as ternary water–hydrophilic/hydrophobic organic solvent, water–micelle, water–ionic liquid, and fluorocarbon/hydrocarbon organic solvent mixed solutions. These new types of multiphase flows are called PS-MPFs, and



Fig. 1 Phase diagram of the solubility curves of the ternary water–acetonitrile–ethyl acetate mixed solution. The effect of temperature is demonstrated in the diagram.

they differ from conventional immiscible multiphase flows. In PS-MPFs, a homogeneous two-phase separation solvent solution is fed into a microspace, such as a microchannel on a microchip or the various types of capillary tubes mentioned in the experimental information section, and the solvent solution is then converted into a heterogeneous solution through phase transformation by changing the pressure and/or temperature, leading to the formation of a liquid–liquid interface between the two phases [10–14]. We can observe droplet, slug, parallel, and annular flows under different conditions in PS-MPFs.



Fig. 2 Illustration of PS-MPF creation mechanism with a two-phase separation mixed solvent solution.

	Immiscible multiphase flow	Phase separation multiphase flow (PS- MPF)
Interface formation	Based on mixing of immiscible liquids	Based on phase transformation
Mixed solvent solution	Immiscible solvent solution, e.g., water-hydrophobic organic solvent	Two-phase separation mixed solvent solution, e.g., ternary water- hydrophilic/hydrophobic organic solvent
Fluidic behavior (interfacial tension)	Relatively large (27–43 mN m <sup>-1</sup> ) [6–9]	Relatively small (0.7–3.3 mN m <sup>-1</sup> ) [15]
Flow system	Two flow lines $\rightarrow$ combining device $\rightarrow$ one flow line	Only one flow line
Microspace scale	Inner diameter larger than 250 $\mu$ m [6,7]	Inner diameter larger than 10 µm

Table 1 Comparison of immiscible multiphase flows and phase separation multiphase flows (PS-MPFs).

### 4. PS-MPF creation mechanism

A PS-MPF was observed for the first time with a ternary water–acetonitrile–ethyl acetate mixed solution, which was found to be a novel two-phase separated mixed solvent solution. Here, the generation mechanism for the PS-MPF is explained using the water–acetonitrile–ethyl acetate mixed solution. The phase diagram is shown in Fig. 1. The dotted curves indicate the boundaries or solubility curves between the solution existing in a homogeneous and heterogeneous state. The heterogeneous solution has two phases in a batch vessel: the upper and lower phases. As shown in Fig. 1, the solubility curve expands towards the outer edge of the diagram with decreasing temperature. The curve expands similarly with increasing pressure [12,13].

When the mixed solvent solution, which is labeled "Mixed solvents I" in the phase diagram in Fig. 2, is subjected to changes in temperature and pressure in a batch vessel, the mixture changes from homogeneous to heterogeneous. The phase transformation leads to the generation of upper and lower phases in the batch vessel. Conversely, when the mixed solution is fed into a capillary tube or microchannel, the PS-MPF appears through a phase transformation, and contains inner and outer phases (an annular flow, a TRDF, is pictured as an example in Fig. 2) [12,13].

# 5. Comparison of immiscible and phase separation multiphase flows

Conventional immiscible multiphase flows are usually generated with water and a hydrophobic organic solvent, and their flow systems include two flow lines and a mixing device. Conversely, the novel PS-MPF is generated with two-phase separation mixed solvent solutions, and the flow system consists of a single flow line without any mixing devices. Table 1 summarizes the characteristics of immiscible multiphase flows and PS-MPFs. The interfacial tension of conventional immiscible multiphase flows differs significantly from that of the novel PS-MPFs. The interfacial tension of the PS-MPFs (0.7–3.3 mN m<sup>-1</sup>) [15] is considerably smaller than that of the immiscible multiphase flows (27–43 mN m<sup>-1</sup>) [6–9].

The small interfacial tension leads to a specific stable liquid–liquid interface even in a microspace and at low velocities [13,15,16]. In particular, we are interested in the annular flow



Fig. 3 Typical fluorescence photograph of an annular flow in a PS-MPF, i.e., a TRDF, in a microspace.

generated in an PS-MPF. The solvent molecules are radially distributed through the phase transformation of the two-phase mixed solvent solution, thus generating inner and outer phases that comprise an annular flow (Fig. 3). The corresponding microfluidic phenomenon and flow are termed the TRDP and TRDF, respectively [10–14].

# 6. Annular flow in PS-MPF with various two-phase separation mixed solvent solutions

In our previous paper [14], phase diagrams, viscosities and volume ratios of the two phases (upper and lower) in a batch vessel, and bright-field or fluorescence images were examined using different two-phase separation mixed solvent solutions, water-hydrophilic/hydrophobic including ternary organic solvent mixed solutions (water-acetonitrile-ethyl acetate and water-acetonitrile-chloroform), a water-micelle mixed solution (water-Triton X-100-KCl), water-ionic liquid mixed solutions (water-1-butyl-3-methylimidazolium chloride ([C4mim]Cl)-KOH, water-[C4mim]Cl-K2HPO4, and water-1-ethyl-3-methylimidazolium methylphosphonate  $([C_2mim]MP)-K_2HPO_4),$ and a fluorocarbon/hydrocarbon organic solvent mixed solution (tetradecafluorohexane-hexane). We observed TRDP and TRDF with all of the two-phase separation mixed solvent solutions under certain conditions.

The relationship between TRDF formation and the component ratios of the solvents was examined with the ternary water-hydrophilic/hydrophobic organic solvent mixed solution. Consequently, whether or not a TRDF formed could be discussed based on a dimensionless number, the Weber number, which was derived from the inertial force and the interfacial tension [15].

Furthermore, the differences between the viscosities of the upper and lower solutions of the water–acetonitrile–ethyl acetate, water–acetonitrile–chloroform, water–Triton X-100–KCl, water– $[C_4mim]Cl$ –KOH, water– $[C_4mim]Cl$ –K<sub>2</sub>HPO<sub>4</sub>, and water– $[C_2mim]MP$ –K<sub>2</sub>HPO<sub>4</sub> systems were estimated to be approximately 0.42, 0.49, 440, 1.7, 0.22, and 0.73 mPa s, respectively.

When the difference between the viscosities of the two phases was large (greater than approximately 0.73 mPa s), the phase with the higher viscosity formed as the inner phase regardless of the volume ratio in the TRDF, whereas when the difference was small (less than approximately 0.49 mPa s), the phase with the larger volume formed as the inner phase [14].

#### 7. Viscous dissipation principle [17–19]

The viscous dissipation energy was calculated for the solution component ratios that generated the TRDF [20]. When the difference between the viscosities of the two phases was large (greater than approximately 0.73 mPa s), the phase with the higher viscosity formed as the inner phase in the TRDF regardless of the volume ratio. The distribution pattern or configuration of the two phases is supported by the viscous dissipation principle. In contrast, when the viscosity difference was small (less than approximately 0.49 mPa s), the phase with the larger volume formed as the inner phase in the TRDF. The distribution pattern or configuration of the phases did not always



Fig. 4 Illustration of separation mechanism in a TRDF-based capillary chromatographic system with a ternary mixed solvent solution.

correspond to the viscous dissipation principle.

It is interesting and useful to note that the distribution pattern of the TRDF in a PS-MPF can be considered on the basis of the viscous dissipation principle and a previous report of the linear stability analysis [21] in a manner similar to that of annular flows in the conventional immiscible multiphase flow.

# 8. Application of TRDF to capillary chromatography

A TRDF-based open-tubular capillary chromatography system was developed for the separation of analytes [10-14]. Capillary tubes of fused silica, PE, PTFE, and PTFE-PFAE were used. The influence of the tube temperature, tube inner diameter, flow rates, injection volumes, and carrier solvents on the separation performance was examined in detail. The chromatographic system works using the two-phase separation mixed solvent solutions as carriers without applying a high voltage through a method based on capillary electrophoresis and using specific columns, such as packed and monolithic columns, as in conventional capillary chromatography. The separation mechanism illustrated in Fig. 4 is considered for a ternary mixed solution. In an organic solvent-rich ternary system, the organic solvent-rich phase was generated as the inner phase, and the water-rich phase formed as the outer phase (pseudostationary phase). 1-Naphthol and 2,6-naphthalenedisulfonic acid as models are distributed in the inner and outer phases, respectively, according to their respective hydrophobic and hydrophilic natures. Under laminar flow conditions, the linear velocity of the outer phase is very low. Hence, hydrophobic 1-naphthol, which is distributed in the inner phase, elutes with almost the average linear velocity of the laminar flow, whereas hydrophilic 2,6-naphthalenedisulfonic acid, which is distributed in the outer phase, elutes with a velocity lower than the average linear velocity. Consequently, 1-naphthol then and 2,6-naphthalenedisulfonic acid are eluted (Fig. 4).

Various types of analytes, such as organic compounds, amino acids, peptides, proteins, nucleosides, metal ions, metal complexes, fluorescent compounds, lambda DNA, polymer compounds, and optical isomers, were analyzed using the TRDP-based chromatographic system [10–14]. Some systems employed coated capillary tubes, or included additives [13].

### 9. Application of TRDF to microreactor

The TRDF-based microreactor is composed of two tubes with different inner diameters (smaller and larger) connected via a

joint [22]. A water-acetonitrile solution, which contained the biomolecules, was injected into the capillary tube with a smaller inner diameter, while the acetonitrile-ethyl acetate mixture solution, which contained the fluorescence derivatization reagent, was fed into the capillary tube with a larger inner diameter. The biomolecules and the reagent were mixed at the joint, and the chemical reaction between them took place at the liquid-liquid interface generated in the TRDF in the larger tube.

The derivatization reaction of bovine serum albumin (BSA) with fluorescamine (FR) using the TRDF-based microreactor was evaluated and compared with that using conventional batch-based methods [22]. The findings demonstrate that the microreactor was more effective than the traditional techniques: the fluorescence intensity of the FR-derivatized BSA obtained using the micro-reactor was stronger than those obtained with the batch-based methods, which included a homogeneous carrier solution of water–acetonitrile.

# **10.** Application of TRDF to unit microoperation systems on a microchip

#### 10.1 Separation on a microchip

A TRDF technology based on the application of the separation system on a microchip was investigated in a previous study [23]. Chemiluminescence was used as the detection mode. The isoluminol isocyanate (ILITC) and ILITC-labeled biomolecules were used as the model analytes.

Fluorescence images obtained with an organic solvent-rich carrier solution (ternary water–acetonitrile–ethyl acetate, volume ratio of 3:8:4) system were examined in the microchannel (100  $\mu$ m in width and 40  $\mu$ m in depth) in the microchip (3 cm × 7 cm). The carrier solution, which contained fluorescent dyes, was fed into the separated microchannel. An organic solvent-rich major phase that included perylene (blue) was generated in the center of the microchannel as the inner phase.

The water-rich minor outer phase acted as a pseudostationary phase when the system was under laminar flow conditions. Hence, the hydrophobic free and excess ILITC that was distributed in the organic solvent-rich major inner phase were eluted first, followed by the hydrophilic ILITC-labeled BSA, which was distributed in the water-rich minor outer phase.

### 10.2 Extraction on a microchip

The microfluidic behavior of the ternary mixed carrier solvents of a water–acetonitrile–ethyl acetate mixed solution (volume ratio of 3:8:4) was examined using a microchip that incorporated wide microchannels, which in turn were internally segregated



Fig. 5 Fluorescence photographs of solvents with dissolved fluorescent dyes at (a) the point where the microchannels combined and (b) 3 cm from that point in the wide microchannel. The white dashed line indicates the combination interface between the narrow microchannels and the wide microchannel. The conditions are as follows. Carrier: water-acetonitrile (3:2 v/v) containing 3 mM Eosin Y in the middle narrow microchannel, acetonitrile-ethyl acetate (3:2 v/v) containing 0.15 mM perylene in the side narrow microchannels. Flow rate: 2.0  $\mu$ L min<sup>-1</sup> in the narrow microchannels. The volume ratio of the ternary water-acetonitrile-ethyl acetate mixture in the wide microchannel was 3:8:4.

into three narrow channels (triple-branched microchannel) [24]. The ternary carrier solution containing the fluorescent dyes (hydrophobic perylene (blue) and hydrophilic Eosin Y (green)) was fed into the wide microchannel.

The carrier solvent molecules and the fluorescent dyes were radially distributed in the microchannel, forming major inner (organic solvent-rich, blue) and minor outer (water-rich, green) phases in the wide microchannel. Perylene (blue), which was distributed in the inner phase region, flowed through the narrow central microchannel, whereas Eosin Y (green), which was distributed in the outer phase regions, flowed through the two narrow side microchannels.

The resulting microfluidic behavior of the ternary mixed solvents in the triple-branched microchannel system suggests the potential of a microfluidic extraction system based on a TRDF on a microchip [24].

#### 10.3 Mixing on a microchip

The mixing process of the individual components of a ternary solution (water-hydrophilic/hydrophobic organic solvents mixture) under laminar flow conditions is discussed in this section [25]. The microchip consisted of narrow microchannels that were subsequently combined to form a wide microchannel.

The water–acetonitrile (hydrophilic) mixture, which contained hydrophilic Eosin Y (green), was fed into the narrow central microchannel, and the acetonitrile–ethyl acetate (hydrophobic) mixture, which contained hydrophobic perylene (blue), was fed into the two narrow side microchannels in the microchip. The two mixtures in the narrow microchannels combined in the wide microchannel to generate a ternary mixed solvents system of water–acetonitrile–ethyl acetate, subsequently instigating the tube radial distribution of the solvents. The mixing process in the wide microchannel was observed by monitoring the fluorescence patterns of the green and blue dyes at the point where the microchannels combined and 3 cm from that point in the wide microchannel [25]. As shown in Fig. 5, the green dye that was originally fed into the center microchannel was distributed near the inner side walls of the wide microchannel, whereas the blue dye that was initially fed into the two side microchannels was distributed in the central region of the wide microchannel. Such specific mixing behavior was not observed in two-component solvent systems, such as water–acetonitrile and water–ethyl acetate mixtures.

### **11.** Conclusions

A new type of microfluidic flow, PS-MPE, was developed with various types of two-phase separation mixed solvent solutions. When the homogeneous mixed solutions were fed into a microspace, the solution changed to a heterogeneous solution with a liquid–liquid interface through phase transformation. PS-MPFs were examined primarily with a ternary water–hydrophilic/hydrophobic organic solvent mixed solution to clarify the specific microfluidic behavior.

A TRDF, which is an annular flow in the PS-MPF, was applied to microflow technologies. New types of capillary chromatographic devices and microreactors based on TRDFs were developed. Furthermore, TRDP-based unit microoperation systems on microchips performing various functions, such as separation, extraction, and mixing, were experimentally proposed and shown to be useful for the Lab-on-a-Chip concept.

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