

# Production of Monodispersed Water-in-Oil Emulsions Using Polymer Microchannels

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**ABSTRACT:** Monodispersed water-in-oil (W/O) emulsions were prepared using new fabricated acrylic polymer microchannel (MC) plates. Acrylic plate MC-A1 was 20  $\mu\text{m}$  wide, 10  $\mu\text{m}$  deep, and 120  $\mu\text{m}$  long; and MC-A2 was 40  $\mu\text{m}$  wide, 20  $\mu\text{m}$  deep, and 120  $\mu\text{m}$  long with respect to MC sizes. Both MC plates had a terrace length of 30  $\mu\text{m}$ . Monodispersed water-in-triolein emulsions with average diameters of 42 to 60  $\mu\text{m}$  (MC-A1) and 62 to 98  $\mu\text{m}$  (MC-A2) were prepared successfully using tetraglycerin condensed ricinoleic acid ester (CR310) as a surfactant. The CV for droplets produced was less than 10%, demonstrating monodispersity. When we used decane (which is less viscous than triolein) as the continuous phase and CR310 and hexaglycerin pentaoleate (PO500) as surfactants, the average diameters and CV of the droplets became smaller, and the pressure ranges for stable droplet formation were much wider than for the water/triolein system. For comparison, a silicon MC plate (MC-Si) with dimensions similar to the polymer MC-A1 was tested and exhibited little significant difference in the average diameter and CV of droplets between MC-A1 and MC-Si.

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**KEY WORDS:** Acrylic polymer microchannel, microchannel emulsification, monodispersed water-in-oil emulsions, silicon microchannel.

Emulsification is an important process in the food, cosmetics, and pharmaceutical industries, and mechanical emulsification methods such as colloid milling and high-pressure homogenization are commonly used to prepare emulsions (1). However, there are some disadvantages in using mechanical emulsification processes, such as increased temperature and the energy loss caused by mechanically stressing dispersed- and continuous-phase mixtures. Additionally, the droplet size distribution is usually polydispersed in mechanically processed emulsions.

Nakashima *et al.* (2) have developed a membrane emulsification technique to produce monodispersed emulsions. Emulsion droplets are formed by forcing the to-be-dispersed phase into the continuous phase through a microporous membrane having a relatively uniform pore size. To date, the membrane emulsification technique has been applied to drug delivery systems (3) and in the food industry (4). We

(5) proposed a microchannel (MC) emulsification method using microfabricated MC arrays on a single-crystal-silicon plate, in which the to-be-dispersed phase breaks through the MC arrays and forms monodispersed droplets in the continuous phase. This MC emulsification method has been successfully used on a laboratory scale to prepare monodispersed water-in-oil (W/O) or oil-in-water (O/W) emulsions, solid lipid particles, and polymeric particles (6–9). This method has received much attention because of its ability to produce microspheres with a small CV (less than 10%) in droplet size. However, single-crystal-silicon MC arrays manufactured by photolithography and anisotropic wet-etching processes involve both high raw material and manufacturing costs. Moreover, silicon MC arrays have poor alkaline resistance. Therefore, the usability of silicon MC arrays is limited.

If use of MC emulsification is to become widespread, less expensive materials and less specialized methods for device fabrication are needed. Elastomeric micromolding was first developed at Bell Laboratories in 1974 when researchers developed a technique of molding soft materials from a lithographic master (10). Recently, Whitesides and coworkers (11,12) have summarized soft lithography used in microfluidics. Several researchers also have reported the use of MC plates microfabricated with polymethylmethacrylate, polycarbonate, and polydimethylsiloxane as chips for DNA analysis (13), capillary gel electrophoresis analysis (14), and cell handling (15). In those cases, the general MC sizes are on the order of tens or hundreds of micrometers (16).

Acrylic resin is a high-grade plastic that surpasses other plastics in terms of transparency and weatherability. In addition, this molding material is well known for the ease with which it can be processed. The costs of the substrate material are on the order of 0.2–2 cents  $\text{cm}^{-2}$ , whereas single-crystal-silicones are on the order of 0.9–1.2 dollars  $\text{cm}^{-2}$  (17). In this study, we used acrylic resin-based MC plates to prepare monodispersed emulsions. These results were compared with silicon MC emulsification.

## MATERIALS AND METHODS

**Chemicals.** Triolein (purity >60%; Wako Pure Chemical Industries, Osaka, Japan), decane (purity 99%; Wako Pure Chemical Industries), and mixtures of triolein/decane (80:20 and 60:40, w/w) were used as the continuous phase. MilliQ water

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(Millipore, Milton, MA) was used as the dispersed phase. For the conventional emulsification method, the maximal stability of W/O emulsion is obtained at a low hydrophilic-lipophilic balance (HLB) value (1). From our previous study, tetraglycerin condensed ricinoleic acid ester (trade name: CR310, HLB < 1) is suitable for preparing the water-in-triolein emulsion, and hexaglycerin pentaoleate (trade name: PO500, HLB = 4.9) is suitable for the water-in-decane emulsion (7). Therefore, we used CR310 and PO500 (Sakamoto Yakuhin Co., Ltd., Osaka, Japan) as the surfactants in the present study. Both surfactants were dissolved into the continuous phase at 3 wt% concentration. A sodium hydroxide solution (Sigma-Aldrich-Japan Co., Ltd., Tokyo, Japan) of  $0.1 \text{ mmol L}^{-1}$  was used in the acrylic and silicon plate anti-alkalinity experiment. Octadecyltriethoxysilane was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan) and used for surface modification of the silicon and the glass plates. All materials were reagent grade and were used without further purification.

**MC plates and their anti-alkalinity.** Acrylic (resin grade: Parapet GH-S, MFR-10; Kuraray Co., Ltd., Kurashiki, Japan) MC plates were manufactured by the injection molding method. Figure 1A shows a schematic diagram of the cross-flow MC module. In the MC plate, there is a continuous-phase flow path with two holes at either end of the path as an inlet and an outlet for the continuous phase. At each sidewall of the path, an array of regularly sized micro-slits was fabricated. A transparent, flat glass or acrylic plate was tightly affixed to the MC plate to cover the top of the slits and form the array of channels. The emulsions were produced by forcing the to-be-dispersed phase through the channels into the continuous phase. Figure 1B depicts an enlargement of the channels. The MC

structure is identified in terms of width, depth, and length of the channels, and terrace length (flat area outside of the channel). Table 1 lists the dimensions of the acrylic MC plates (MC-A1, MC-A2) that were used, and of the silicon MC plate (MC-Si) that was included for comparison.

To produce hydrophobic plate surfaces, the silane coupler method was performed as follows. Silicon and glass plates were immersed in toluene containing 10% octadecyltrichlorosilane for 30 min and heated at  $110^\circ\text{C}$  for 1 h. They were then rinsed with toluene to wash out unreacted materials (18).

The acrylic and silicon plate anti-alkalinity experiments were carried out by soaking both plates in sodium hydroxide solution. The surface images of the MC plates were taken by a JSM-5600 LV scanning electron microscope (SEM; JEOL Co., Ltd., Tokyo, Japan).

**MC emulsification system and microscope video system.** The MC emulsification system (Fig. 1C) consisted of a syringe pump to feed the continuous phase, a reservoir for the to-be-dispersed phase, an emulsion recovery bottle, and an MC module. The emulsification process was observed on a monitor through an inverted metallographic microscope (TS-V; Chuo Precision Industrial Co., Ltd., Tokyo, Japan) and a charge-coupled device (CCD) camera (HV-C20M; Hitachi Denshi Co., Ltd., Tokyo, Japan). The images recorded by an attached D-VHS digital recorder (HM-DR10000; Victor Co., Ltd., Yokohama, Japan) were used, along with a personal computer, to measure the average diameter of the droplets.

**Measurement and analytical method.** Image-processing software (Winroof; Mitani Co., Ishikawa, Japan) was used to determine the average droplet size and size distribution from

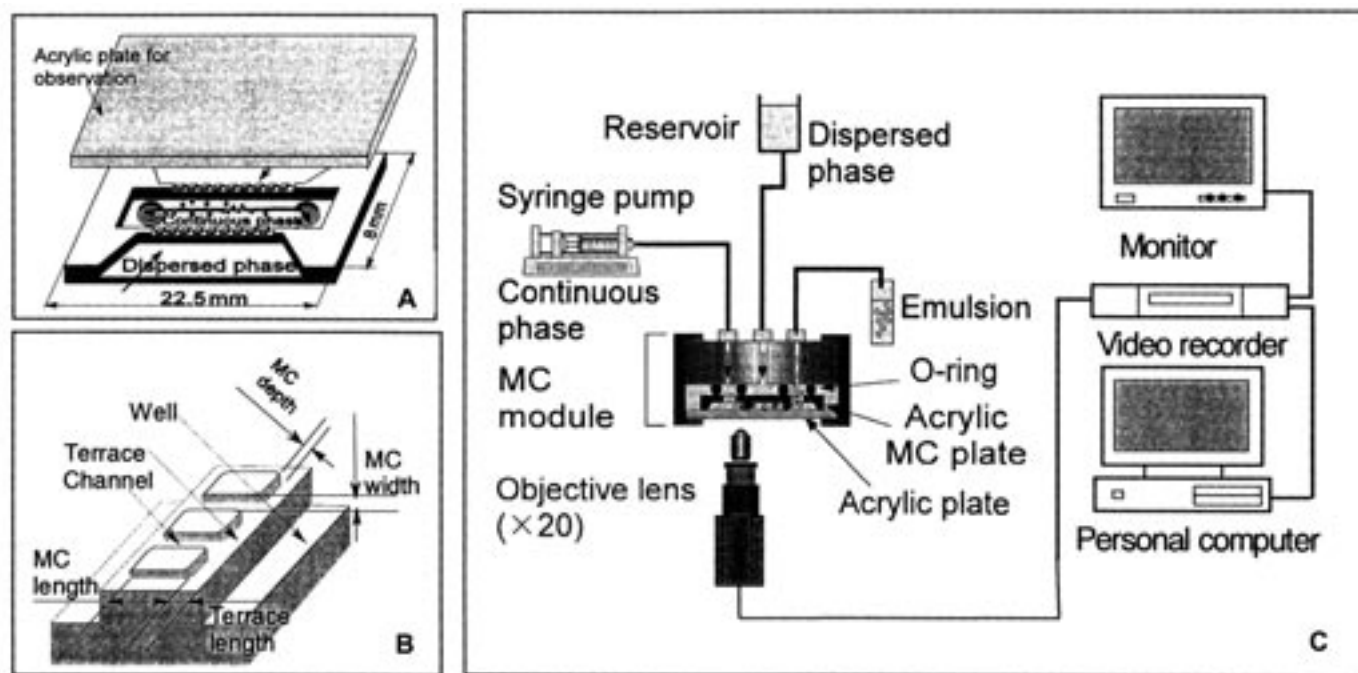


FIG. 1. (A) Schematic diagram of cross-flow microchannel (MC) module; (B) enlargement of channels; (C) MC emulsification system.

**TABLE 1**  
**Dimensions of Microchannel (MC) Plates**

MC plates	Dimensions of MC plate (mm)			Width	Dimensions of MC ( $\mu\text{m}$ )		Terrace length
	Length	Width	Thickness		Depth	Length	
MC-A1	22.5	8.0	1.3	$20.0 \pm 0.9$	10.0	120.0	30.0
MC-A2	22.5	8.0	1.3	$40.0 \pm 1.0$	20.0	120.0	30.0
MC-Si	22.5	8.0	0.5	$16.0 \pm 0.3$	11.0	70.0	30.0

the microscope video system pictures. The CV, which represents the size distribution, was calculated with Equation 1,

$$CV = (\sigma/d) \times 100 \quad [1]$$

where CV is the coefficient of variation (%),  $\sigma$  is the SD ( $\mu\text{m}$ ), and  $d$  is the average diameter ( $\mu\text{m}$ ).

The interfacial tension and contact angles of the water droplet on the surface of acrylic, silicon, and glass plates in the presence of the oil phase were measured using a fully automatic interfacial tensiometer (PD-W; Kyouwa Interface Science Co., Ltd., Saitama, Japan). The viscosities of the continuous phases were measured with a microviscometer (Haake, Karlsruhe, Germany). Each measurement was performed in triplicate, and the mean values and SD were reported. All experiments were carried out at room temperature.

## RESULTS AND DISCUSSION

The channel widths of each MC plate (30 channels along each terrace line, 60 channels in total) were determined by microscopic observation. As shown in Table 1, the width of MC-Si is  $16.0 \pm 0.3 \mu\text{m}$ ; MC-A1 and MC-A2 are  $20.0 \pm 0.9$  and  $40.0 \pm 1.0 \mu\text{m}$ , respectively. Acrylic MC were accurately fabricated with CV of 2–5%, although more precise fabrication was possible for silicon MC.

Figure 2 compares the alkaline resistance of the acrylic and silicon MC plates. The acrylic and silicon MC plates were soaked in  $0.1 \text{ mmol L}^{-1}$  sodium hydroxide solution (pH 10) for 3 d. Significant corrosion occurred on the MC-Si plate surface, as shown in Figure 2B. In contrast, little change was observed on the acrylic MC plate surface (Fig. 2D) even after 30 d of alkali treatment. This demonstrates the strong resistance to alkalinity of acrylic MC plates.

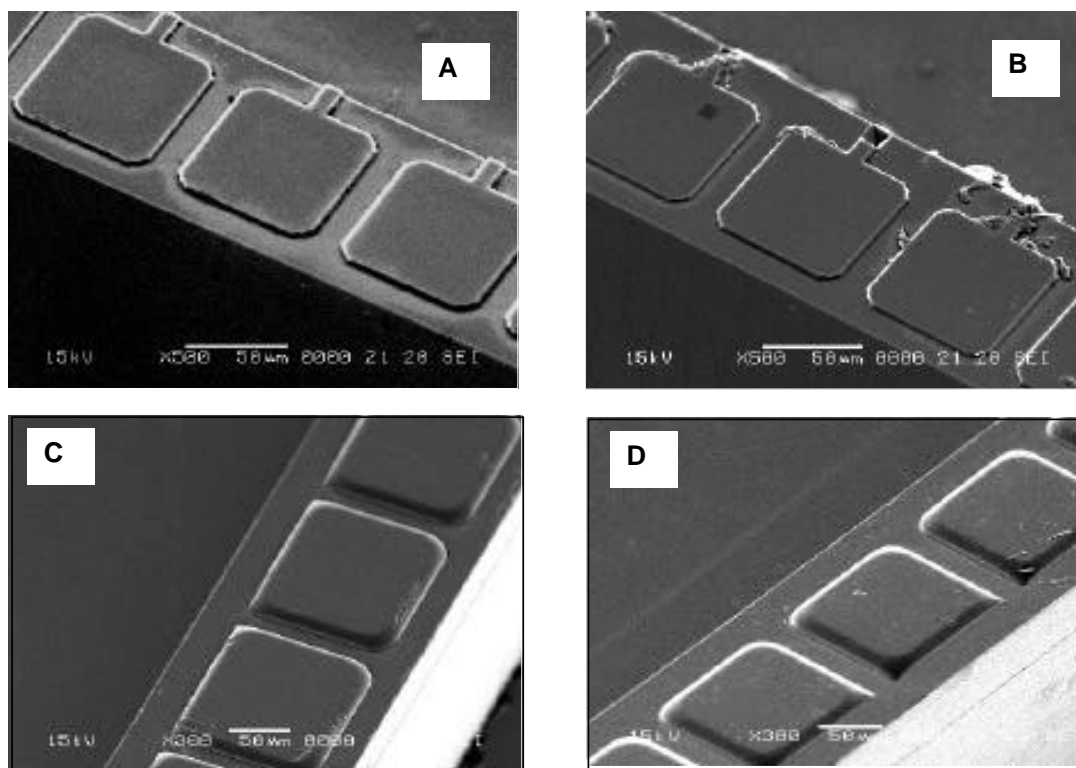
We also measured each contact angle. The contact angle of the water phase droplet on the acrylic plate was  $126.0 \pm 2.1^\circ$ , indicating hydrophobic properties. The contact angles of the water phase droplet on the silicon and glass plates were  $25.0 \pm 0.7^\circ$  and  $27.0 \pm 0.4^\circ$ , respectively, showing hydrophilic properties. These hydrophilic silicon and glass plates were treated with silane coupler reagent (see the Materials and Methods section), after which both surfaces became hydrophobic (silicon plate,  $126.0 \pm 1.5^\circ$ ; glass plate,  $144.0 \pm 1.2^\circ$ ).

In our first emulsification experiment, the to-be-dispersed phase, triolein, flowed through the channel in a continuous fluid stream, i.e., no droplets were formed. This behavior was expected because of the hydrophobic surface of the acrylic

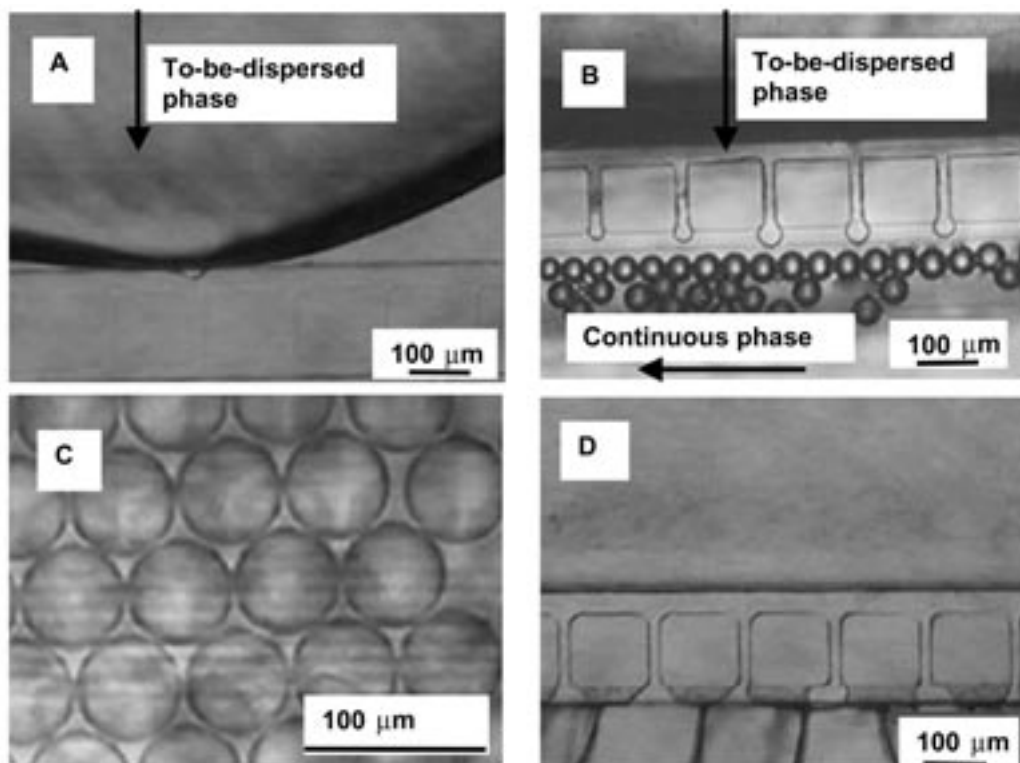
MC. In the MC emulsification process, the MC surface must be wetted by the continuous phase (19). The water-in-triolein emulsion was investigated under the following conditions: 3 wt% of the lipophilic surfactant CR310 was dissolved in triolein and was used as the continuous phase, and water was used as the to-be-dispersed phase; the MC module where the MC-A1 was installed was initially filled with the oil phase. The water phase was pressed into the module by lifting the reservoir, and then flowed close to the channels. As shown in Figures 3A and 3B, when the pressure reached a certain value, the breakthrough pressure (20), the to-be-dispersed phase began to break through the channels and droplet formation took place. Figure 3C shows mono-sized water droplets being created. The droplets were formed stably, and the droplet formation rate increased with increasing pressure over a certain range, called “the pressure range for stable droplet formation.” Over the range, the droplet size became much larger and the to-be-dispersed phase flowed out continuously (Fig. 3D). Under the same experimental condition, a similar emulsification behavior was observed for the emulsion obtained with MC-A2. Sugiura *et al.* (21) proposed a droplet formation mechanism of silicon MC emulsification in which a distorted, dispersed phase is cut off spontaneously into the spherical droplet by interfacial tension at the outside of the terrace end (21). We observed the same emulsification phenomena in the polymer MC emulsification process.

The average droplet diameter depends mainly on MC depth and terrace length during the MC emulsification process (21). For this reason, in the preliminary comparison of the emulsification behaviors between acrylic and silicon MC plates, we chose a silicon MC plate with the same terrace length and similar MC depth as the MC-A1. Figure 4 depicts the effects of pressure on the average diameter and CV using MC-A1, MC-A2, and MC-Si plates. We first compared the emulsion droplet properties for MC-A1 and MC-Si plates within the same pressure range of 0.8–1.0 kPa. The average diameters were 52 to 60  $\mu\text{m}$  for MC-A1, and 53 to 66  $\mu\text{m}$  for MC-Si; both CV were less than 8%. These results showed that there were no significant differences in properties of the droplets produced between the two MC plates. For the MC-A2 plate, with an applied pressure of 0.3–0.6 kPa, an average diameter of 62 to 98  $\mu\text{m}$  and CV of less than 8% were observed, demonstrating monodispersity.

From Figure 4, we found that the breakthrough pressures of the three MC plates were different; MC-A1, 0.49 kPa; MC-A2, 0.29 kPa; and MC-Si, 0.78 kPa. If we assume that the MC can be regarded as ideal cylinders, the breakthrough pressure can be estimated from the capillary pressure as follows (22):



**FIG. 2.** Scanning electron micrographs of silicon and acrylic plates. (A) Silicon MC (control); (B) silicon MC (after 3 d of alkali treatment); (C) acrylic MC (control); (D) acrylic MC (after 30 d of alkali treatment). For abbreviations see Figure 1.



**FIG. 3.** Photomicrographs of the MC emulsification process in the water/[triolein containing 3 wt% CR310 (tetraglycerin condensed ricinoleic acid ester)] system. (A) To-be-dispersed phase flowed close to the channels; (B) the phase passed through the channels for droplet formation; (C) mono-sized water droplets created; (D) to-be-dispersed phase flowed out continuously through the channels. For abbreviations see Figure 1.

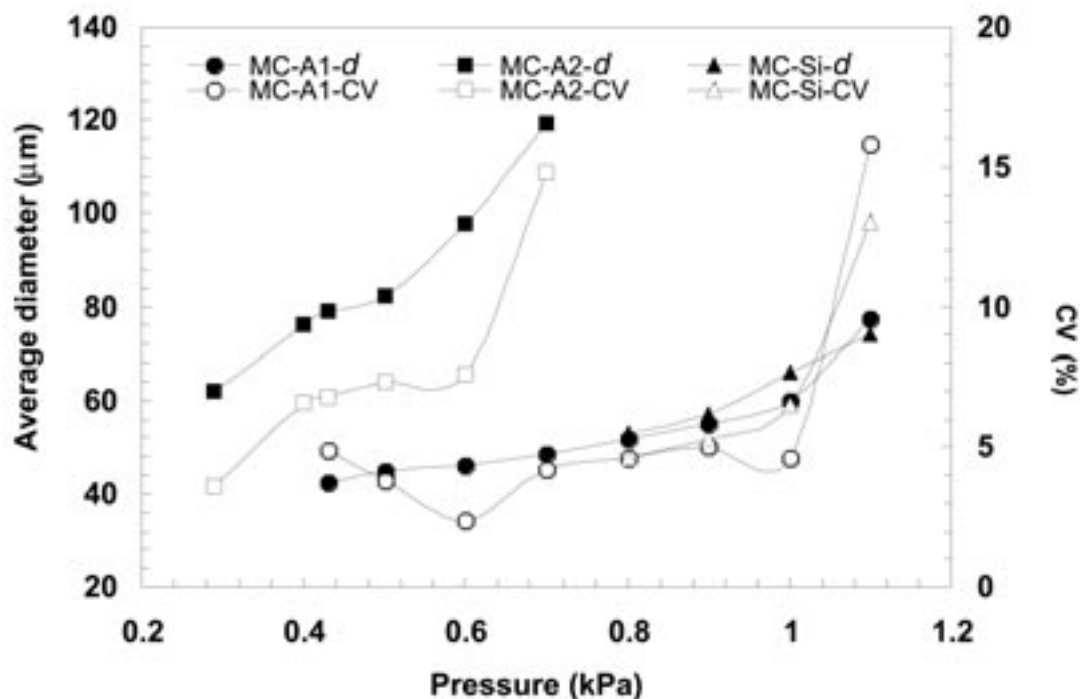


FIG. 4. Effect of pressure on the average diameter ( $d$ ) and CV of formed droplets for the water/triolein system (containing 3 wt% CR310) using MC-A1, MC-A2, and MC-Si plates. For plate dimensions and abbreviations see Table 1; for other abbreviation see Figure 3.

$$P_{bt} = 4\gamma\cos\theta/D_{eq} \quad [2]$$

where  $P_{bt}$  is the breakthrough pressure (Pa),  $\gamma$  is the interfacial tension (N/m),  $\theta$  is the contact angle, and  $D_{eq}$  is the MC equivalent diameter (m).  $D_{eq}$  was calculated as follows:

$$D_{eq} = (A/L) \times 4 \quad [3]$$

where  $A$  is the area of the MC cross-section ( $m^2$ ) and  $L$  is the circumferential length of the MC cross-section (m). The equivalent MC diameters for MC-A1, MC-A2, and MC-Si were 13.5, 27.0, and 8.3  $\mu m$ , respectively. The interfacial tension between two phases, water and triolein (containing 3 wt% CR310), was measured as  $2.6 \pm 0.2 \text{ mN m}^{-1}$ . Therefore,  $P_{bt}$  were calculated from Equation 2 as 0.45 (MC-A1), 0.23 (MC-A2), and 0.74 kPa (MC-Si), which were in good agreement with the experimental values.

We also investigated emulsification behaviors using different surfactants and continuous phases. Each investigation was carried out using plate MC-A1. We used PO500 as an alternative surfactant, dissolving it in triolein at a concentration of 3 wt%. Experimental results showed that droplets were formed after breakthrough but immediately coalesced at the outside of the terrace end, forming a clearly unstable emulsion. During droplet formation, the newly formed interfaces were occupied by surfactant molecules. The adsorption kinetics of the surfactant determined the stability of droplets against coalescence in all stages of formation and inside the flowing continuous phase after detachment (23). PO500 is a polyglycerin FA ester that is prepared by the esterification of hexaglycerin with six oleic

acids. CR310 is a polyglycerin condensed ricinoleic acid ester that is prepared by the esterification of tetraglycerin with condensed linoleic acid. The molecular structure of CR310 is larger than that of PO500 (24). From this point, we can assume that the membrane layer of CR310 with larger M.W. is thicker than that of PO500. The thicker CR310 membrane would be efficient against coalescence.

We investigated the effect of the viscosity of the continuous phase on polymer MC emulsification. To create continuous phases of different viscosities, triolein, decane, and mixtures of triolein/decane were used (Table 2). PO500 and CR310 at levels of 3 wt% were used as the surfactants. We found that both CR310 and PO500 were suitable surfactants for preparing monodispersed water-in-decane emulsions. The effects of applied pressure on the average diameter and CV of emulsion droplets are shown in Figure 5A. Average droplet

TABLE 2  
Experimental Conditions Used in This Study

Continuous phase	Viscosity (mPa · s, containing surfactant), 25°C	Surfactant <sup>a</sup>
Triolein	78.1 ± 0.6	CR310
Triolein/decane (80:20, w/w)	21.9 ± 0.4	CR310
Triolein/decane (60:40, w/w)	8.1 ± 0.4	CR310
Decane	1.4 ± 0.1	CR310
Decane	1.1 ± 0.1	PO500

<sup>a</sup>CR310, tetraglycerin condensed ricinoleic acid ester; PO500, hexaglycerin pentaoleate. Both surfactants supplied by Sakamoto Yakuin Co., Ltd., Osaka, Japan.

diameters and CV increased and pressure ranges for stable droplet formation decreased as viscosity in the continuous phase increased. In the water/(decane-CR310) and the water/(decane-PO500) systems, the average droplet diameters were 32–37  $\mu\text{m}$  [about 10  $\mu\text{m}$  smaller compared with the water/(triolein-CR310) system], and droplet size distributions were more uniform (CV, ~3%) in the CR310 and PO500 systems. We attribute these observations to the increase in the viscosity of the continuous phase, which may delay detachment of droplets, causing more of the dispersed phase to be supplied during the formation of each droplet, making the droplets larger.

The droplet formation rate, which is the number of droplets produced per minute, is also an important parameter for MC emulsification. Relationships between the droplet formation rate and applied pressure are shown in Figure 5B. The droplet formation rate increased as viscosity in the continuous phase decreased. In the water/triolein system, however, outflow through the channels took place at a pressure of 1.0 kPa, and droplet formation rates were below 40  $\text{min}^{-1}$ . In the water/decane systems, little output difference was observed between the CR310 and PO500 surfactants, droplet formation rates increased significantly with the increase of pressure (180  $\text{min}^{-1}$  at 1.37 kPa), and no outflow was observed under the experimental conditions. We interpreted these results as follows: The continuous phase flow velocity from the well to the terrace became lower as the viscosity of the continuous phase increased, thus increasing the droplet detachment time.

Monodispersed W/O emulsions were successfully prepared by using acrylic MC plates with channel depths of 10 and 20  $\mu\text{m}$ . The droplet average diameters and CV decreased and the pressure ranges for stable droplet formation and droplet formation rate increased with decreasing viscosity in the continuous phase. As compared with the silicon MC, the acrylic MC emulsification showed similar characteristics in terms of average droplet diameter and CV.

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## REFERENCES

- McClements, D.J., *Food Emulsions: Principles, Practice and Techniques*, CRC Press, Boca Raton, FL, 1999.
- Mine, Y., M. Shimizu, and T. Nakashima, Preparation and Stabilization of Simple and Multiple Emulsions Using a Microporous Glass Membrane, *Colloids Surf. B* 6:261–268 (1996).
- Higashi, S., and T. Setoguchi, Hepatic Arterial Injection Chemotherapy for Hepatocellular Carcinoma with Epirubicin Aqueous Solution as Numerous Vesicles in Iodinated Poppy-seed Oil Microdroplets: Clinical Application of Water-in-Oil-in-Water Emulsion Prepared Using a Membrane Emulsification Technique, *Adv. Drug Deliver. Rev.* 45:57–64 (2000).

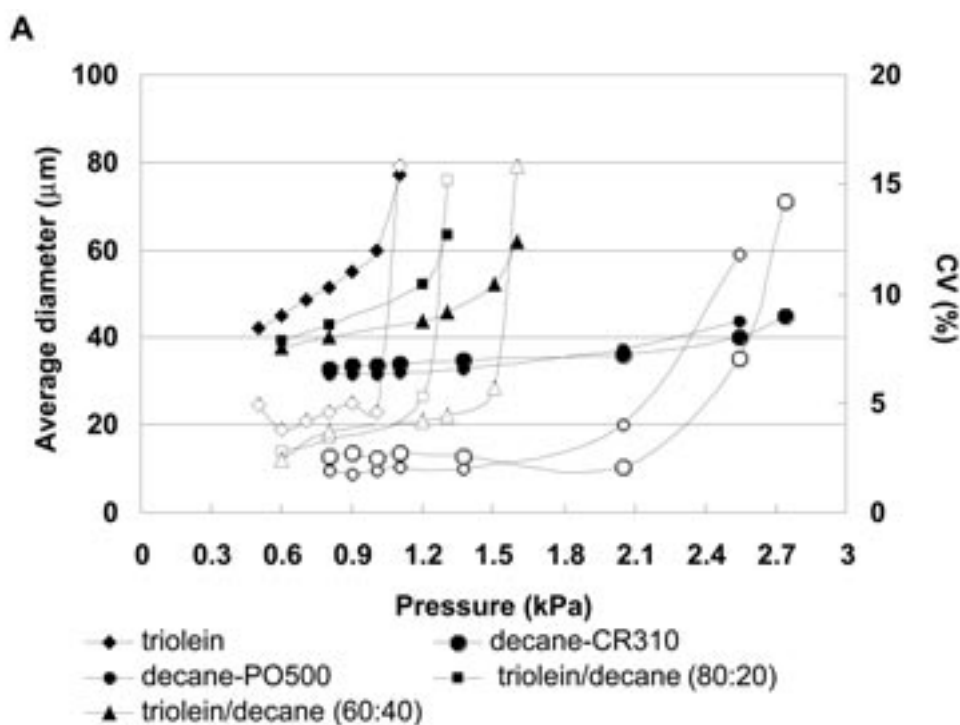


FIG. 5. (A) Effect of applied pressure on the average diameter ( $d$ ) and CV of emulsion droplets using triolein, decane, and mixtures of triolein/decane (80:20, 60:40, w/w) as the continuous phase and CR310 or PO500 as surfactant. (B) Relationship between droplet formation rates and applied pressure in systems using triolein, decane, or mixtures of triolein/decane (80:20, 60:40, w/w) as the continuous phase and CR310 or PO500 as surfactant. Solid symbols: average diameters; open symbols: CV. PO500, hexaglycerin pentaoleate; for other abbreviations see Figure 3.

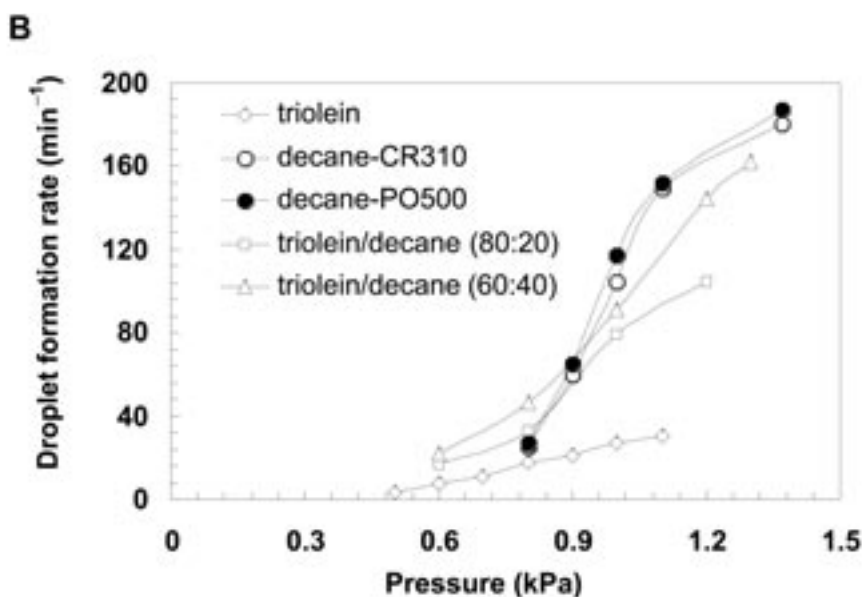


FIG. 5. (cont.)

- Kato, R., Application of Membrane Emulsification Method for Preparing Food Emulsion, *Shokuhin to kaihatsu* 28:9–13 (1993) (in Japanese).
- Kawakatsu, T., Y. Kikuchi, and M. Nakajima, Regular-Sized Cell Creation in Microchannel Emulsification by Visual Microprocessing Method, *J. Am. Oil Chem. Soc.* 74:317–321 (1997).
- Kobayashi, I., M. Nakajima, J. Tong, T. Kawakatsu, H. Nabetani, Y. Kikuchi, A. Shohn, and K. Satoh, Production and Characterization of Monodispersed Oil-in-Water Microspheres Using Microchannels, *Food Sci. Technol. Res.* 5:350–355 (1999).
- Sugiura, S., M. Nakajima, H. Ushijima, K. Yamamoto, and M. Seki, Preparation Characteristics of Monodispersed Water-in-Oil Emulsions Using Microchannel Emulsification, *J. Chem. Eng. Japan.* 34:757–765 (2001).
- Sugiura, S., M. Nakajima, J. Tong, H. Nabetani, and M. Seki, Preparation of Monodispersed Solid Lipid Microspheres Using a Microchannel Emulsification Technique, *J. Colloid Interface Sci.* 227:95–103 (2000).
- Sugiura, S., M. Nakajima, H. Itou, and M. Seki, Synthesis of Polymeric Microspheres with Narrow Size Distributions Employing Microchannel Emulsification, *Macromol. Rapid Commun.* 22:773–778 (2001).
- Aumiller, G., E. Chandross, W. Tomlinson, and H. Weber, Submicrometer Resolution Replication of Relief Patterns for Integrated Optics, *J. Appl. Phys.* 45:4557–4562 (1974).
- Xia, Y., and G.M. Whitesides, Soft Lithography, *Annu. Rev. Mat. Sci.* 28:153–184 (1998).
- Whitesides, G.M., E. Ostuni, S. Takayama, X.Y. Jiang, and D.E. Ingber, Soft Lithography in Biology and Biochemistry, *Annu. Rev. Biomed. Eng.* 3:335–373 (2001).
- Soper, S.A., A.C. Henry, B. Vaidya, M. Galloway, M. Wabuyele, R.L. McCarley, Surface Modification of Polymer-Based Microfluidic Devices, *Anal. Chim. Acta* 470:87–99 (2002).
- Hong, J.W., K. Hosokawa, T. Fujii, M. Seki, and I. Endo, An Inexpensive PDMS (polydimethylsiloxane) Microchip for Capillary Gel Electrophoresis, *Tenth International Conference on Solid-State Sensors and Actuators* (Transducer '99), Sendai, Japan (1999).
- Thomas, N., A. Ocklind, I. Blikstad, S. Griffiths, M. Kendrick, H. Derand, G. Ekstrand, C. Ellstrom, A. Larsson, and P. Andersson, Integrated Cell Based Assays in Microfabricated Disposable CD Devices, in *Micro Total Analysis Systems*, edited by A. van den Berg, W. Olthius, and P. Bergveld, Kluwer, Dordrecht, 2000, pp. 249–252.
- Stone, H.A., and S. Kim, Microfluidics: Basic Issues, Applications, and Challenges, *AIChE J.* 47:1250–1254 (2001).
- Becker, H., and L.E. Locascio, Polymer Microfluidic Devices, *Talanta*, 56:267–287 (2002).
- Kawakatsu, T., G. Tragardh, C. Tragardh, M. Nakajima, N. Oda, and T. Yonemoto, The Effect of the Hydrophobicity of Microchannels and Components in Water and Oil Phases on Droplet Formation in Microchannel Water-in-Oil Emulsification, *Colloids Surf. A* 179:29–37 (2001).
- Tong, J., M. Nakajima, H. Nabetani, and Y. Kikuchi, Surfactant Effect on Production of Monodispersed Microspheres by Microchannel Emulsification Method, *J. Surfact. Deterg.* 3:285–293 (2000).
- Kobayashi, I., M. Nakajima, J. Tong, T. Kawakatsu, H. Nabetani, Y. Kikuchi, A. Shohn, and K. Satoh, Production and Characterization of Monodispersed Oil-in-Water Microspheres Using Microchannels, *Food Sci. Technol. Res.* 5:350–355 (1999).
- Sugiura, S., M. Nakajima, S. Iwamoto, and M. Seki, Interfacial Tension Driven Monodispersed Droplet Formation from Microfabricated Channel Array, *Langmuir* 17:5562–5566 (2001).
- Peng, S.J., and R.A. Williams, Controlled Production of Emulsions Using a Crossflow Membrane: Part I—Droplet Production from a Single Pore, *Trans. Inst. Chem. Eng.* 76 (A):894–901 (1998).
- Schröder, V., O. Behrend, and H. Schubert, Effect of Dynamic Interfacial Tension on the Emulsification Process Using Microporous, Ceramic Membranes, *J. Colloid Interface Sci.* 202: 334–340 (1998).
- Miyamoto, A., Polyglycerin Fatty Acid Esters, *Fudo Kemicaru* 10:36–38 (1994) (in Japanese).

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