

Preparation of Monodisperse Water-in-Oil-in-Water Emulsions Using Microfluidization and Straight-Through Microchannel Emulsification

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ABSTRACT: Water-in-soybean oil-in-water (W/O/W) emulsions with an internal water phase content of 10–30% (vol/vol) were prepared by a two-step emulsification method using microfluidization and straight-through microchannel (MC) emulsification. A straight-through MC is a silicon array of micrometer-sized through-holes running through the plate. Microfluidization produced water-in-oil (W/O) emulsions with submicron water droplets of 0.15–0.26 μm in average diameter ($d_{\text{av,w/o}}$) and 42–53% in CV ($\text{CV}_{\text{w/o}}$) using tetraglycerin monolaurate condensed ricinoleic acid esters (TGCR) and polyglycerin polycondensed ricinoleic acid esters (PGPR) as surfactants dissolved in the oil phase. The $d_{\text{av,w/o}}$ and viscosity of the W/O emulsions increased with an increase in internal water phase content. Straight-through MC emulsification was performed using the W/O emulsions as the to-be-dispersed phase and polyoxyethylene (20) sorbitan monooleate (Tween[®] 80) as a surfactant dissolved in the external water phase. Monodisperse W/O/W emulsions with $d_{\text{av,w/o/w}}$ of 39.0–41.0 μm and $\text{CV}_{\text{w/o/w}}$ below 5% were successfully formed from a straight-through MC with an oblong section (42.8 \times 13.3 μm), using the TGCR-containing systems. The $d_{\text{av,w/o/w}}$ of the monodisperse W/O/W emulsions decreased as the internal water phase content increased because of the increase in viscosity of the to-be-dispersed phase. Little leakage of the internal water droplets and no droplet coalescence or droplet breakdown were observed during straight-through MC emulsification.

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KEY WORDS: Droplet formation, internal water phase content, microfluidization, monodisperse emulsion, oblong straight-through microchannel, straight-through microchannel emulsification, viscosity, water-in-oil-in-water emulsion.

Water-in-oil-in-water (W/O/W) emulsions consist of oil droplets dispersed within an external water phase, with the droplets themselves containing smaller internal water droplets. Their double-compartment structure can be used to encapsulate substances within the internal water droplets or to reduce the fat content of emulsion-based products (1). The features of W/O/W emulsions have received a great deal of attention in the pharmaceutical, cosmetics, food, and chemical industries. In particular, their potential pharmaceutical applications include

use as vehicles for prolonged delivery systems of hydrophilic drugs, e.g., anticancer and peptide drugs (2,3). An effective way to improve their stability and to facilitate control of their properties is through the preparation of monodisperse W/O/W emulsions. The two-step stirring emulsification method, which prepares a water-in-oil (W/O) emulsion in the first emulsification step and a W/O/W emulsion in the second, is most widely used to prepare W/O/W emulsions (2). However, the resultant W/O/W emulsions have a wide droplet size distribution and low encapsulation efficiency.

Membrane emulsification (4), which can produce emulsions with minimum CV of about 10%, can be used to prepare relatively monodisperse W/O/W emulsions (5). W/O/W emulsions have been prepared by forcing a premixed W/O emulsion into the external water phase through a microporous membrane of a narrow size distribution. W/O/W drug emulsions prepared by this technique have actually been administered in clinical trials (6,7).

Microchannel (MC) emulsification (8) allowed us to prepare monodisperse emulsions with CV below 5% using a channel array with a slit-like terrace microfabricated on a silicon plate. Size-controlled monodisperse emulsions with droplet diameters of 3–90 μm (9,10) have been successfully prepared by this technique. A two-step emulsification method including MC emulsification as the second step can be used to prepare monodisperse W/O/W emulsions with high encapsulation efficiency (11,12). These W/O/W emulsions were prepared by forcing a W/O emulsion containing internal water droplets on a 10- μm scale into the external water phase through the channels.

We recently proposed a novel straight-through MC emulsification (13) to resolve the low-throughput problem of monodisperse emulsions in a MC emulsification. A straight-through MC is a silicon array of micro-through-holes (13). The present laboratory-scale instrument has a throughput capacity of monodisperse emulsion droplets of 1–10 mL/h and is currently being scaled up. Oblong straight-through MC exceeding a threshold aspect ratio were suitable for preparing monodisperse emulsions with droplet diameters of 30–50 μm and CV below 5% (13,14). This technique has been applied to the preparation of monodisperse food-grade oil-in-water (O/W) emulsions (15).

The aim of this study was to prepare monodisperse W/O/W emulsions containing internal water phase droplets on a submicron scale by the two-step emulsification method

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using microfluidization in the first emulsification step and straight-through MC emulsification in the second. We also studied the effects of varying the internal water phase content and surfactant type on the preparation characteristics of W/O emulsions (microfluidization) and W/O/W emulsions (straight-through MC emulsification).

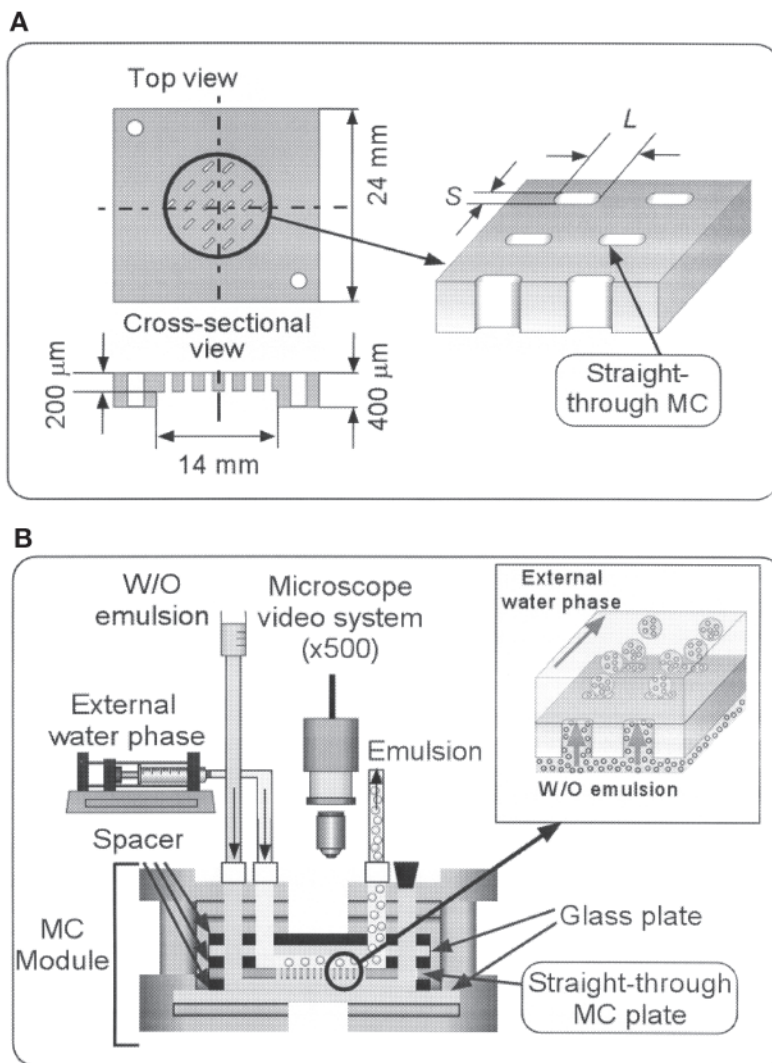
EXPERIMENTAL PROCEDURES

Materials. Refined soybean oil was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The soybean oil used had a saponification value of 190, an iodine value of 130, an acid value below 0.2, and a phospholipid content below 10 mg/kg (catalog value). Milli-Q water was used to prepare all of the water-phase solutions. D(+)-Glucose was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Tetraglycerin monolaurate condensed ricinoleic acid esters (TGCR, HLB <1) and polyglycerin polycondensed ricinoleic acid esters (PGPR, HLB <1), both of food grade, were provided by

Sakamoto Yakuhin Kogyo Co. Ltd. (Osaka, Japan). Polyoxyethylene (19) sorbitan monooleate (Tween[®] 80, HLB = 15.0), which was of reagent grade, was purchased from Wako Pure Chemical Industries Ltd.

Preparation of W/O emulsions using microfluidization. Soybean oil with 5% (w/w) oil-soluble surfactant (TGCR or PGPR) was used as the oil phase. Milli-Q water with 5% (w/w) glucose was used as the internal water phase. A total of 300 mL of the oil–water mixture with a volume fraction of the internal water phase of 10 to 30% (vol/vol) was first emulsified with a rotor/stator homogenizer (Polytron PT-MR 3000; Kinematica AG, Littau, Switzerland) at 5,000 rpm for 5 min to prepare a pre-emulsion. The pre-emulsion was introduced into a microfluidizer (M-110EH; Mizuho Industrial Co. Ltd., Osaka, Japan) at an operating pressure of 140 MPa to prepare a fine W/O emulsion.

Preparation of W/O/W emulsions using straight-through MC emulsification. W/O/W emulsions were prepared using a straight-through MC emulsification instrument (Scheme 1),



SCHEME 1

which consisted of a silicon straight-through MC plate, a module, a liquid chamber to feed the fine W/O emulsion as the to-be-dispersed phase, a syringe pump (Model 11; Harvard Apparatus, Inc., Boston, MA) to feed the external water phase, and a microscope video system to observe the droplet formation behavior (13). The silicon straight-through MC plate, depicted in Scheme 1A, was fabricated using photolithography, deep reactive ion etching, and thermal oxidation processes (13). The plate measured $24 \times 24 \times 0.4$ mm and included two 1.5-mm diameter through-holes at the corners of the plate. A total of 11,225 oblong channels with a 100- μm interval were fabricated in a 10-mm square in the center of a 0.2-mm thick plate. The oblong channels, which were of uniform size, had a longer line (L) of 42.8 μm , a shorter line (S) of 13.3 μm , and a depth of 200 μm . The surface porosity measured in the channel area was 6.4%. The module was initially filled with the external water phase [Mill-Q water with 1% (w/w) Tween 80 and 5% (w/w) glucose]. The straight-through MC plate was degassed in the external water phase by ultrasonication for 20 min, after which time the plate was mounted in the module. The fine W/O emulsion, which was fed into the module by lifting the reservoir, reached the back of the plate. W/O/W emulsion droplets were then formed through the oblong channels in the external water phase.

Measurements and analysis. The volume-weighted average diameter and size distribution of water droplets in the fine W/O emulsions were measured with a laser diffraction particle-size analyzer (Coulter LS 13 320; Beckman Coulter Ltd., Fullerton, CA). The W/O emulsions were dispersed in cottonseed oil (MP Biomedicals Inc., Aurora, OH) before the measurements were taken. Each measurement was repeated at least twice, and the mean values were used. The number-average diameter and size distribution of oil droplets in the W/O/W emulsions were determined using images of 200 droplets taken with the micro-

scope video system. We used image-analyzing software (Win-Roof; Mitani Co. Ltd., Fukui, Japan) to analyze the captured images. The droplet size distributions of the preceding W/O and W/O/W emulsions were expressed as the CV [$\text{CV} = (\sigma/d_{\text{av}}) \times 100$], where CV is expressed as a percentage, σ is the SD in micrometers, and d_{av} is the average droplet diameter in micrometers.

Leakage, coalescence, and breakdown behaviors of the resultant W/O/W emulsion droplets were analyzed by observing them as they existed in the space between the glass plate and the straight-through MC plate.

Interfacial tension measurements were conducted using a fully automatic interfacial tensiometer (PD-W; Kyowa Interface Science Co., Ltd., Saitama, Japan). The viscosity of the oil phase with and without internal water droplets was measured using glass capillary viscometers (SO-200 and SO-350; Shibata Scientific Technology Ltd., Tokyo, Japan).

RESULTS AND DISCUSSION

Preparation of W/O emulsions using microfluidization. Conditions for the preparation of the oil and internal water phases are presented in Table 1. The inner water-phase droplets in the W/O emulsions prepared using the microfluidizer had average droplet diameters ($d_{\text{av,w/o}}$) of 0.16–0.26 μm for TGCR-containing systems and 0.15–0.25 μm for PGPR-containing systems (Table 1). The $d_{\text{av,w/o}}$ value for the TGCR- and PGPR-containing systems gradually increased as the internal water-phase content increased. This result can be explained as follows. The internal water droplets in the W/O emulsion moved around continuously and collided with each other frequently. Droplet–droplet collisions became more frequent with an increase in the internal water-phase content caused by the increase in the number density of droplets per unit volume. The frequency of partial droplet

TABLE 1
Preparation Conditions, Average Diameters and CV of the Resultant W/O and W/O/W Emulsions, and Physical Properties of the Experimental Systems

Experimental system no. ^a	Surfactant in oil phase ^b	Water phase content in W/O emulsion (% vol/vol)	Viscosity of W/O emulsion (mPa s)	$d_{\text{av,W/O}}^c$ (μm)	$\text{CV}_{\text{W/O}}^c$ (%)	$d_{\text{av,W/O/W}}^d$ (μm)	$\text{CV}_{\text{W/O/W}}^d$ (%)	Interfacial tension between the phases (mN/m)
TGCR-0	TGCR	0	60.7 ^e	—	—	43.8 ^f	5.6 ^f	3.4 ^g
TGCR-10	TGCR	10	85.7	0.16	45.4	41.0	4.4	6.6 ^h
TGCR-20	TGCR	20	144	0.19	52.8	39.7	4.0	7.6 ^h
TGCR-30	TGCR	30	205	0.26	47.3	39.0	4.2	9.3 ^h
PGPR-0	PGPR	0	66.2 ^e	—	—	43.2 ^f	5.0 ^f	2.6 ^g
PGPR-10	PGPR	10	86.3	0.15	50.7	43.6	5.8	3.7 ^h
PGPR-20	PGPR	20	144	0.21	42.3	43.9	8.6	4.1 ^h
PGPR-30	PGPR	30	213	0.25	51.2	41.8	6.9	6.3 ^h

^aExternal water phase, Mill-Q water with 1.0% (w/w) Tween 80 and 5.0% (w/w) glucose; oil phase, soybean oil; internal water phase, Mill-Q water with 5.0% (w/w) glucose.

^bTGCR, tetraglycerin monolaurate condensed ricinoleic acid esters; PGPR, polyglycerin polycondensed ricinoleic acid esters. Dissolved at a concentration of 5% (w/w).

^cAverage droplet diameter ($d_{\text{av,W/O}}$) and CV ($\text{CV}_{\text{W/O}}$) of the internal water droplets in the fine W/O emulsions.

^dAverage droplet diameter ($d_{\text{av,W/O/W}}$) and CV ($\text{CV}_{\text{W/O/W}}$) of the oil droplets in the prepared W/O/W emulsions.

^eViscosity of the oil phase with a oil-soluble surfactant.

^fThe O/W emulsions prepared by straight-through MC emulsification.

^gInterfacial tension between the oil phase and external water phase.

^hInterfacial tension between the W/O emulsion and external water phase.

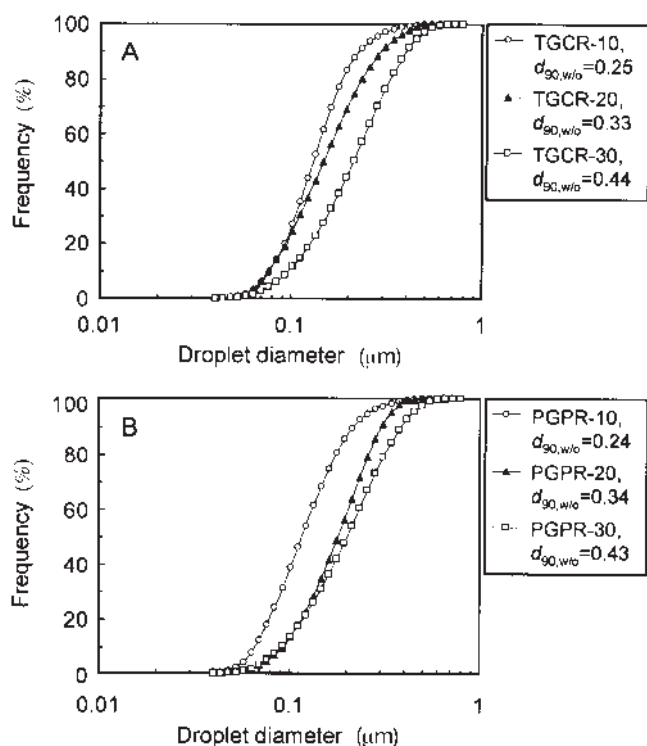


FIG. 1. Droplet size distribution of water-in-oil (W/O) emulsions prepared using a microfluidizer: (A) tetraglycerin monolaurate condensed ricinoleic acid esters (TGCR); (B) polyglycerin polycondensed ricinoleic acid esters (PGPR). See Table 1 for an explanation of the experimental systems.

coalescence just after disruption is believed to have affected the $d_{av,w/o}$ value of the W/O emulsions prepared in this study. The droplet size of the O/W emulsions prepared using microfluidization also tended to increase (16). The CV ($CV_{w/o}$) of the prepared W/O emulsions were between 42 and 53% (Table 1), indicating their polydispersity. In contrast, we should note that the SD were very low, ranging from 0.076 to 0.132 μm . Figure 1 plots the droplet size distributions of the prepared W/O emulsions. The droplet diameters corresponding to 90% (vol/vol) on a relative cumulative droplet size distribution curve, $d_{90,w/o}$, for the prepared W/O emulsions are also presented in Figure 1. The $d_{90,w/o}$ value for each of the prepared W/O emulsions was smaller than 0.5 μm . In addition, droplets with diameters larger than 1 μm hardly existed for any of the experimental systems, verifying that the W/O emulsions prepared in this study contained internal water droplets on a submicron scale. The motion of emulsion droplets is primarily determined by the balance between gravity and Brownian motion. Gravity favors the sedimentation of droplets in a W/O emulsion, whereas Brownian motion favors the random distribution of droplets throughout the entire W/O emulsion. The effect of Brownian motion usually becomes dominant for emulsion droplets on a submicron scale, preventing the sedimentation phenomenon in the W/O emulsion. This can also improve the stability of oil droplets containing internal water droplets in a W/O/W emulsion. We thus believe that the W/O emulsions prepared in this study would be useful as the to-be-

dispersed phase for the preparation of W/O/W emulsions using straight-through MC emulsification.

The viscosities measured for the oil phase and for the prepared W/O emulsions are given in Table 1. The oil phases containing a viscous surfactant (TGCR or PGPR) had viscosities of 60.7–66.2 mPa s, exceeding that of soybean oil (50.4 mPa s). All the prepared W/O emulsions containing low-viscosity water droplets were found to have higher viscosities than those of the preceding oil phases, as presented in Table 1. This result demonstrates a tendency for the viscosity of the fluid system to increase by dispersing the low-viscosity water phase into the high-viscosity oil phase. The emulsion droplets are considered to act as rigid spheres at low shear stresses, since the Laplace pressure difference between the oil phase and the internal water droplets is approximately 0.2 MPa. The Laplace pressure is defined as four times the interfacial tension divided by the droplet diameter. The interfacial tension measured between the oil phase and the internal water droplets was 8.5 mN/m for TGCR and 9.2 mN/m for PGPR. A suspension with rigid spheres has higher viscosity than the pure fluid (1). Moreover, an increase in the number of rigid spheres (the internal water droplets in this study) disturbs the normal flow of the oil phase more, causing greater energy dissipation due to friction. This leads to an increase in viscosity. Thus, the emulsion viscosity increases as the water phase content increases, which can explain the experimental results for both the TGCR- and PGPR-containing systems (Table 1).

Preparation of W/O/W emulsions using straight-through MC emulsification. Six experimental systems with an internal water phase (Table 1) were used to prepare W/O/W emulsions from the oblong straight-through MC. Two other experimental systems without an internal water phase (Table 1) were also used to investigate how the existence of the internal water phase influenced emulsion droplet formation from the oblong straight-through MC. We applied the continuous phase (an external water phase) along the plate surface at average velocities (V_c) lower than 1.2 mm/s, with the pressure of the to-be-dispersed phase (a W/O emulsion or an oil phase) at 1.3 kPa just above the breakthrough pressure. Figure 2A depicts the formation of O/W emulsion droplets from the oblong straight-through MC for the TGCR-0 system without the internal water phase. The to-be-dispersed phase that passed through the oblong channels was cut into monosized oil droplets with an average diameter ($d_{av,o/w}$) of 43.8 μm . This demonstrated successful emulsion droplet formation driven by interfacial tension (17). After droplet formation was complete, the droplets formed were buoyant. Successful droplet formation was also carried out without a continuous-phase flow, indicating spontaneous droplet formation from the oblong channels (15). The prepared O/W emulsion, with a CV ($CV_{o/w}$) of 5.6%, was monodisperse, which was confirmed by a photomicrograph of the formed droplets (see Fig. 3A). The TGCR-0 system containing surfactants in both the oil and water phases was suitable for the successful preparation of a monodisperse emulsion from oblong channels. Therefore, straight-through MC emulsification can be used to prepare W/O/W emulsions.

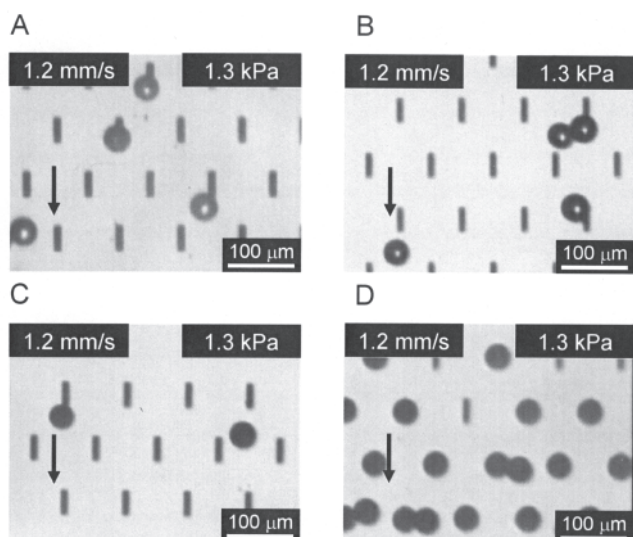


FIG. 2. Photomicrographs of the straight-through microchannel (MC) emulsification process: (A) TGCR-0; (B) TGCR-10; (C) TGCR-20; (D) TGCR-30. See Table 1 for an explanation of the experimental systems; see Figure 1 for other abbreviation.

Figures 2B–D depict the formation of W/O/W emulsion droplets from an oblong straight-through MC for the TGCR-10, TGCR-20, and TGCR-30 systems. For each of these systems, monosized W/O/W emulsion droplets were formed successfully from the oblong channels, both with and without the continuous-phase flow. Photomicrographs of the resultant droplets, shown in Figure 3, demonstrate that their brightness decreased as the water phase content increased. Microscopic observations verified that the volume fraction of the internal water droplets encapsulated in the oil droplets depended on the internal water phase content. Leakage of the internal water droplets was rarely observed during straight-through MC emulsification. In addition, no droplet coalescence or droplet breakdown occurred during the emulsification. The $d_{av,w/o/w}$ and $CV_{w/o/w}$ values for the oil-phase droplets in the prepared W/O/W emulsions are listed in Table 1. Figure 4 plots the size distribution of the oil droplets formed from the oblong straight-through MC. The W/O/W emulsions prepared using the TGCR-containing systems had $d_{av,w/o/w}$ values of 39.0–41.0 μm , which are smaller than the $d_{av,o/w}$ values for the O/W emulsions prepared using the TGCR-0 system. Their $d_{av,w/o/w}$ values also decreased as the internal water phase content increased. The $CV_{w/o/w}$ of the resultant emulsions ranged from 4.0 to 4.4%, demonstrating that monodisperse W/O/W emulsions were obtained. Droplet size distribution curves of a similar slope, plotted in Figure 4A, indicated that there was no significant difference in the size distribution of the oil droplets formed using the TGCR-containing systems. After droplet formation was complete, most of the newly formed W/O/W emulsion droplets (Figs. 2B–2D) were buoyant, whereas some stayed near the channel exit as spherical droplets. They were pushed out by the following to-be-dispersed phase that passed through the channels and only then became detached from the

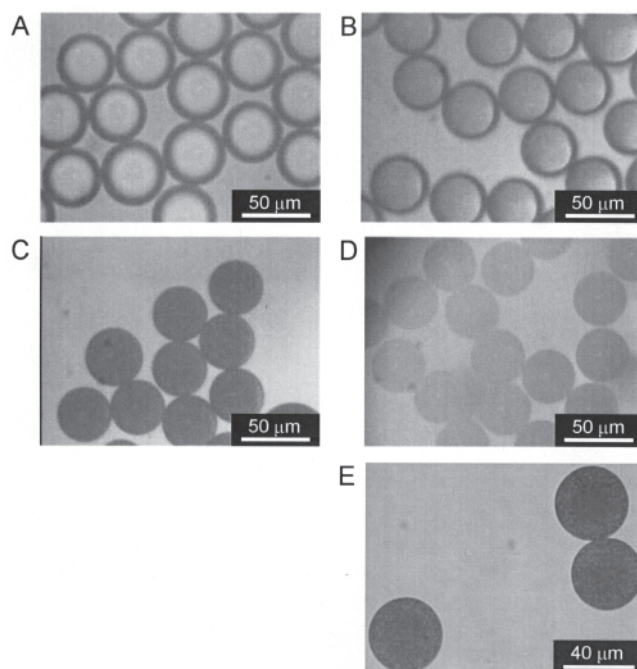


FIG. 3. Photomicrographs of prepared oil-in-water (O/W) and water-in-oil-in-water (W/O/W) emulsions, (A) O/W emulsion, TGCR-0; (B) W/O/W emulsion, TGCR-10; (C) W/O/W emulsion, TGCR-20; (D) W/O/W emulsion, TGCR-30; (E) W/O/W emulsion taken using a microscope with a bright light field mode (DM IRM; Leica Microsystems AG, Wetzlar, Germany), TGCR-30. See Table 1 for an explanation of the experimental systems; see Figure 1 for other abbreviation.

channel exit. This phenomenon occurred more frequently as the water phase content increased. There was no coalescence between the droplets and the to-be-dispersed phase.

The PGPR-0 system demonstrated successful droplet formation, as depicted in Figure 2A. The $d_{av,o/w}$ and $CV_{o/w}$ values for the prepared O/W emulsion were 43.2 μm and 5.0%, respectively. For the PGPR-10, PGPR-20, and PGPR-30 systems, most of the working channels successfully formed monosized W/O/W emulsion droplets. However, large droplets with diameters over 50 μm were also formed in some channels. The $d_{av,w/o/w}$ and $CV_{w/o/w}$ values for the W/O/W emulsions resulting from the PGPR-containing systems (Table 1) were larger than those for the TGCR-containing systems, which was confirmed by their droplet size distribution (Fig. 4). The $d_{av,w/o/w}$ values for the emulsion prepared using the PGPR-30 system were smaller than those for the other PGPR-containing systems. The droplet detachment behavior of the PGPR-containing systems exhibited patterns similar to those described for the TGCR-containing systems.

The results obtained in this section demonstrated that monodisperse W/O/W emulsions containing up to 30% (vol/vol) internal water droplets on a submicron scale were prepared successfully by straight-through MC emulsification, especially when the TGCR-containing systems were used.

Here we discuss the preceding results using the physical properties (interfacial tension and viscosity) of the experimental

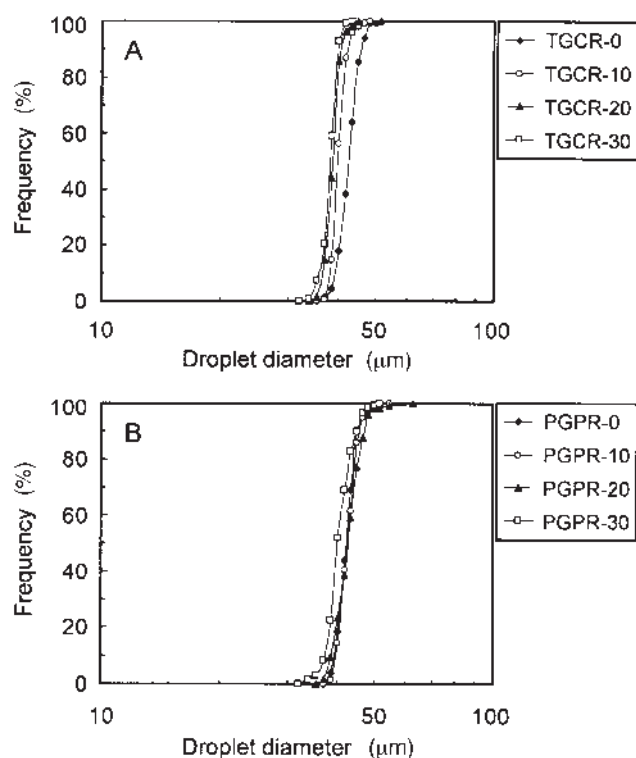


FIG. 4. Droplet size distribution of O/W and W/O/W emulsions prepared using an oblong straight-through MC: (A) TGCR; (B) PGPR. See Table 1 for an explanation of the experimental systems; see Figures 1 and 2 for abbreviations.

systems used in this study. The droplet detachment process in MC emulsification was significantly affected not by interfacial tension but rather by surfactant concentration (18). The adsorption layer of surfactant molecules at the oil–water interface may have become thinner at lower surfactant concentrations and at higher droplet formation rates, which increased the interaction between the to-be-dispersed phase and the MC plate (18). The increase in the total amount of oil-soluble surfactant adsorbing at the W/O interface at higher internal water phase contents led to the decrease in the concentration of surfactant dissolved in the oil phase. This can be explained by the trend in interfacial tension between the W/O emulsion and the external water phase (Table 1) for both the TGCR- and PGPR-containing systems. The adsorption rate of the oil-soluble surfactant at the O/W interface may decrease at a lower surfactant concentration in the oil phase. The W/O/W emulsion droplets stayed near the channel exit more frequently when the internal water phase contents were higher, probably due to the slower adsorption rate of the oil-soluble surfactant at the O/W interface.

The internal water phase content affected the droplet size of the resultant emulsions for the TGCR-containing systems (Table 1). This phenomenon can be explained as follows: The only parameters that could affect the resultant droplet size in this study were the viscosity and the composition of the to-be-dispersed phases used in the straight-through MC emulsification process. This was because we used one oblong straight-through MC, a

to-be-dispersed phase pressure of 1.3 kPa, and the same external water phase for all the experimental systems. The velocity of the continuous phase and the interfacial tension do not affect the resultant droplet size in (straight-through) MC emulsification (13,18). The viscosity of the W/O emulsions prepared using microfluidization was influenced by the internal water phase content. The emulsion droplets formed using the TGCR-containing systems became smaller as the viscosity of the to-be-dispersed phase increased (Table 1). For the PGPR-containing systems, the smallest emulsion droplets were formed using the PGPR-30 system, the one with the highest viscosity. This trend was also observed for the size of the droplets formed by MC emulsification (19,20). The viscosity of the to-be-dispersed phase affected both the droplet detachment time and flow rate of the to-be-dispersed phase from the terrace exit into the well (20). The flow rate of the to-be-dispersed phase had a more significant effect on the resultant droplet size, and a smaller amount of the to-be-dispersed phase flowed into the well with a higher viscosity of the to-be-dispersed phase during the detachment process (20). We therefore believe that the increase in viscosity of the to-be-dispersed phase used in the straight-through MC emulsification process led to the formation of W/O/W emulsion droplets of smaller sizes.

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