Preparation of Micron-Scale Monodisperse Oil-in-Water Microspheres by Microchannel Emulsification

Isao Kobayashi[a,b], Mitsutoshi Nakajima[a,b,*], Hiroshi Nabetani[a], Yuji Kikuchi[a], Atsushi Shohno[c], and Kazumi Satoh[c]

[a]National Food Research Institute, Tsukuba, Ibaraki 305-8642, Japan, [b]University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan, and [c]Science University of Tokyo, Tokyo 162-8601, Japan

ABSTRACT: Micron-scale monodisperse oil-in-water (O/W) microspheres (MS) were prepared using a novel microchannel (MC) emulsification technique. The characteristics of the MS preparation and the O/W-MS prepared were studied. Soybean oil and medium-chain triacylglycerol (MCT) were used as the dispersed phase, and physiological saline was used as the continuous phase. Silicon MC with 1 to 3 μm-equivalent channel diameters were employed. A novel MC module was devised to easily recover the O/W-MS prepared. The effects of the channel shape on the behavior of MS formation, on the MS size, and on the distribution were investigated. An MC with a terrace at the MC outlet stably yielded micron-scale monodisperse O/W-MS; the MS had diameters of about 5 μm, and their coefficients of variation were below 9%. Monodisperse food-grade O/W-MS with diameters of about 4 μm could be obtained by using polyglycerol fatty acid ester as the surfactant. The size and size distribution of the recovered O/W-MS remained almost constant over 60 d, demonstrating their long-term stability.

Paper no. J9850 in *JAOCS 78*, 797–802 (August 2001).

KEY WORDS: Food-grade microspheres, medium-chain triacylglycerol, microchannel emulsification, micron-scale microspheres, monodisperse oil-in-water microspheres, stability.

Microspheres (MS) are emulsion droplets or solid microparticles dispersed in a continuous phase that are widely utilized in foods, cosmetics, pharmaceuticals, and chemicals. They are produced by mechanical emulsification techniques that apply shear force, such as mixing, colloid milling, and homogenization (1). Formation of monodisperse MS is highly desirable because MS with a narrow size distribution generally have better physical and qualitative stability. However, these techniques make it difficult to control the size distribution of the MS, yielding polydisperse MS. A fractionation technique proposed by Bibette is available for purifying polydisperse MS to obtain monodisperse MS (2).

Nakashima *et al.* (3) developed a membrane emulsification technique that has great potential for continuously preparing monodisperse MS. It uses a porous glass membrane through which the dispersed phase is forced into the continuous phase by applied pressure. This technique prepares MS with much less mechanical stress and lower energy consumption than conventional methods (4). Suzuki *et al*. (5,6) proposed a membrane emulsification technique combined with preliminary emulsification for higher productivity. This membrane emulsification enables the formation of monodisperse MS on a submicron or micron scale since the glass membrane has relatively uniform submicron or micron pores. However, the membrane emulsification has difficulty preparing monodisperse MS with a coefficient of variation less than 10% because of the pore size distribution of the glass membrane.

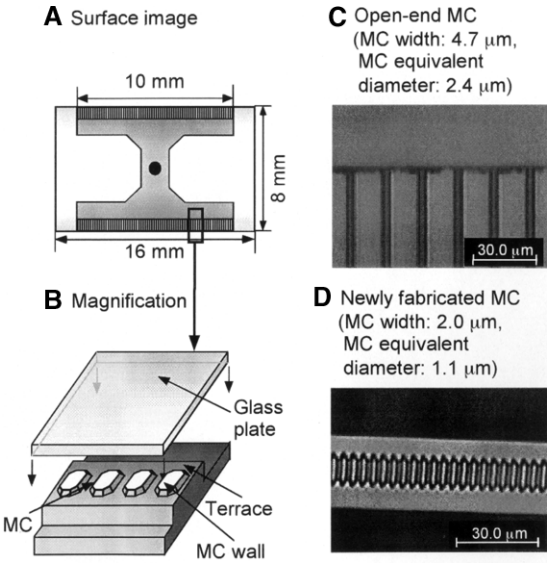
We have proposed a novel microchannel (MC) emulsification technique to form monodisperse MS using a silicon MC array fabricated precisely in a single-crystal silicon substrate by micromachining technology (7). The MC array was originally developed by Kikuchi *et al.* (8,9) to observe the flow characteristics of human blood. This technique has advantages such as a real-time visual observation of the emulsification process and a better understanding of the emulsification behavior, which lead to improved operating conditions. Both monodisperse oil-in-water (O/W)- and water-in-oil (W/O)-MS with average diameters of 20 to 50 μm and coefficients of variation below 5% have been formed by this technique (7, 10–12). However, the formation of monodispersed MS with diameters smaller than 10 μm is commonly desired in dispersed systems, so a smaller MC is required.

The objective of this paper was to prepare micron-scale monodisperse O/W-MS by this MC emulsification technique. We investigated the effects of the channel shape on MS size and distribution and the behavior of MS formation using three different MC. MC emulsification for food-grade O/W-MS was also carried out, and the characteristics of the MC emulsification and the long-term stability of the recovered O/W-MS were also studied.

EXPERIMENTAL PROCEDURES

Materials. Soybean oil (Wako Pure Chemical Ind., Osaka, Japan) and medium-chain triacylglycerol [MCT, MCT-7; fatty acid residue composition, 75% caprylic acid (C8:0) and 25% capric acid (C10:0)] (Taiyo Kagaku Co. Ltd., Mie, Japan) were used as the dispersed phase. Physiological saline (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) was used as the continuous phase. Polyoxyethelene (20) sorbitan monooleate [Tween 80; hydrophilic-lipophilic balance (HLB), 15.0] (Wako Pure Chemical Ind., Osaka, Japan) and poly-

*To whom correspondence should be addressed at National Food Research Institute, Kannondai 2-1-12, Tsukuba, Ibaraki 305-8642, Japan.
E-mail: mnaka@nfri.affrc.go.jp



SCHEME 1

glycerol fatty acid ester (PGFE, pentaglycerol monolaurate with 65% purity; Sun soft A-121E; HLB, 12) (Taiyo Kagaku Co. Ltd.) were used as the surfactants.

Microchannel (MC). Scheme 1 is a schematic depiction and images of the silicon MC plate. The MC plates measure $8 \times 16 \times 0.5$ mm and have a 1 mm-diameter hole in the center. The MC walls are precisely fabricated on two arrays of 40 μm height terraces along the longer side edges by photolithography and anisotropic etching processes (8,9). The MC plate was tightly attached onto a flat glass plate, and the MC array was formed between the MC plate and the glass plate. The channel shape is determined by the MC width (W), height (H), and terrace length (TL) (Scheme 1B). Schemes 1C,D show an open-end MC and a newly fabricated MC, the two types of MC employed in this study. The open-end MC plate has an open space at the MC outlet formed by cutting in a mechanical process, which allows easy dispersion of O/W-MS into the bulk continuous phase. The newly fabricated MC plates consist of MC with and without the terrace at the MC outlet and have the well outside the MC outlet (Scheme 1D). Table 1 presents the dimensions of the MC plates and the numbers of channels employed. All the new MC have the same MC wall height of 1.2 μm. The equivalent diameter of the MC is calculated as follows:

TABLE 1
Microchannel (MC) Types Designed on Silicon MC Plates

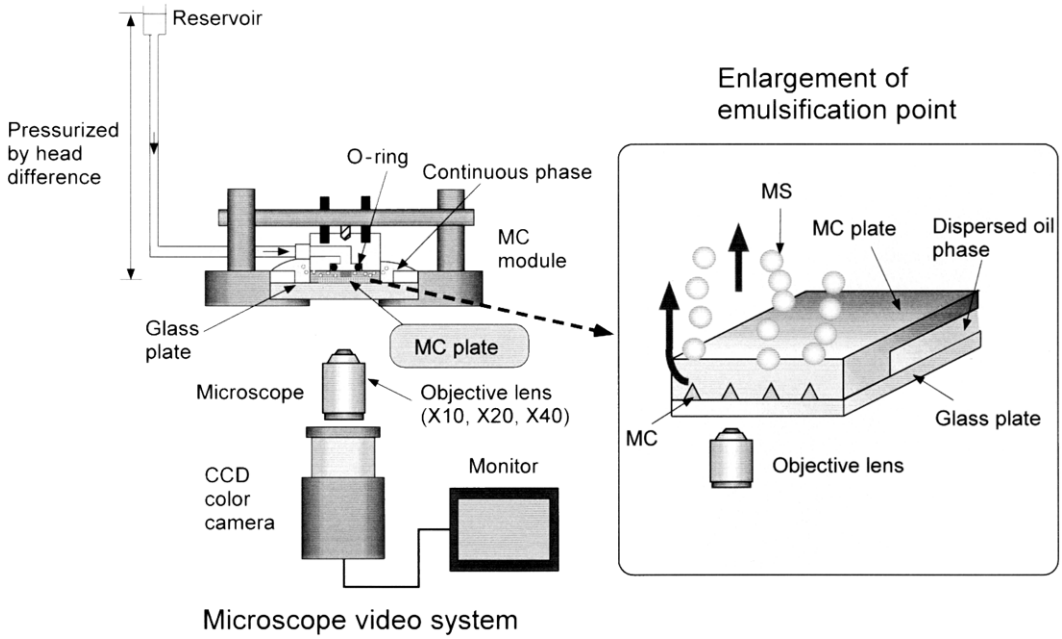
MC type	*W*–*H*–TL[a] (μm)	MC equivalent diameter (μm)	Total channel number
Open-end MC	4.7–2.2–0	2.4	4600
SMC-A1	2.0–1.2–0	1.1	4800
SMC-A4	4.7–1.2–0	1.7	1200
SMC-B1	2.0–1.2–6.9	1.1	4800
SMC-B2	3.2–1.2–6.9	1.5	2400
SMC-B3	4.0–1.2–6.9	1.6	1600
SMC-B4	4.7–1.2–6.9	1.7	1200

[a]*W*, MC width; *H*, MC height; TL, terrace length.

$$D_{eq} = (A/L) \times 4 \quad [1]$$

where D_{eq} is the MC equivalent diameter in μm, A is the area of the MC cross section in μm^2, and L is the circumferential length of the MC cross section in μm.

Experimental setup. Scheme 2 depicts the experimental setup and the flow mechanism through the channels in the module. The composition of the system used in this study is



SCHEME 2

similar to that described previously (7). The MC plate was inverted and attached to a glass plate in the newly designed MC module. This module allows O/W-MS to disperse into a bulk continuous phase open to the air and to be easily recovered with a pipette. The emulsification process was monitored through an inverted metallographic microscope (MD-300EF; Nikon Co., Tokyo, Japan) and a CCD color camera (KP-C550; Hitachi, Tokyo, Japan). Magnifications of 500 to 2000× were possible by changing the magnification of the objective lens. The process was recorded with a video recorder (EV-PR2; Sony Co., Tokyo, Japan).

Effect of MC shape on MC emulsification and MS size. To ensure a sufficient supply of a surfactant at the interface, a surfactant concentration exceeding the critical micellar concentration (CMC) was employed. Soybean oil with 1.5 wt% Tween 80 was used as the dispersed phase, and saline was used as the continuous phase. The MC module was initially filled with continuous phase. A dispersed phase was fed into the module by lifting a reservoir filled with the disperse phase. The applied pressure of the dispersed phase was slowly increased. The dispersed phase intruded into the space between the MC plate and the glass plate through the center hole of the plate. When the applied pressure reached the breakthrough pressure, the dispersed phase broke through the channels and O/W-MS formation commenced. The O/W-MS dispersed in the bulk continuous phase were recovered with a pipette. All experiments were carried out at room temperature, approximately 25°C.

Preparation of micron-scale food-grade O/W-MS. We considered the various conditions necessary for formation of stable O/W-MS using food-grade surfactants. PGFE containing 65% pure pentaglycerol monolaurate was chosen as a food-grade surfactant. MCT have been attracting interest for foods and pharmaceuticals because of their unique physical and metabolic properties (13). In particular, MCT are rapidly absorbed and utilized energy sources. Thus, soybean oil and MCT were used as the dispersed phase, and saline with 0.5 wt% PGFE was used as the continuous phase.

A syringe pump (Model 11; Harvard Apparatus Inc., Boston, MA) was employed to feed the dispersed phase. The dispersed phase flow rate through the channels was regulated at 5 μm/h. All the other emulsification processes and conditions are the same as described in the above section.

Measurement and analytical method. The MS formation was analyzed from the video images recorded with a microscope video system. The size and number of the MS were measured with a Coulter counter (ZM; Beckman Coulter, Inc., Fullterton, CA) after diluting the recovered emulsion with physiological saline. The average diameter and coefficient of variation (CV) of the MS were then calculated from the data obtained. The CV, expressed as the following equation, was used to present the monodispersity of the MS:

$$CV = (\sigma/D_{av}) \times 100 \quad [2]$$

where CV is the coefficient of variation as a percentage, σ is the standard deviation in μm, and D_{av} is the average MS diameter in μm.

The interfacial tension between two phases was measured with an automatic interfacial tensiometer (PD-W; Kyowa Interface Science Co., Japan) using the pendant drop method.

RESULTS AND DISCUSSION

Effect of MC shape on MC emulsification and MS size. Figure 1 demonstrates the behavior of O/W-MS formation using an open-end MC. O/W-MS formation commenced through the channels at a breakthrough pressure of 3.60 kPa. The D_{av} and CV of the prepared MS were 6.58 μm and 16.2%, respectively. This open-end MC enabled us to obtain micron-scale MS by MC emulsification. The ratio of the average MS diameter to the MC equivalent diameter was 2.7, which was smaller than the ratio of 3.25 or 5.0 in membrane emulsification (4,14). This might be attributable to the unique channel shape. However, the CV of the MS was more than three times that of the MC emulsification reported (7,10–12), indicating less monodispersity. Consequently, it was difficult to prepare

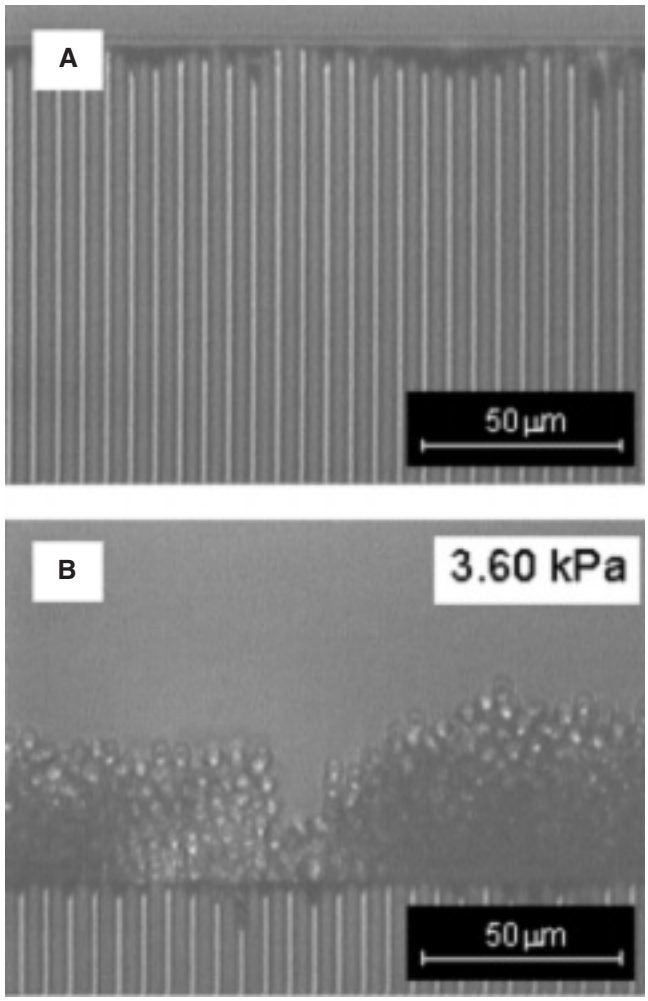


FIG. 1. Microsphere (MS) formation using open-end microchannels (MC) (D_{eq} = MC equivalent diameter = 2.4 μm); (A) filled with continuous phase; (B) breaking through MC and MS formation.

micron-scale monodisperse O/W-MS with a CV less than 10% using the open-end MC.

The MC plate was cut at a line 25 μm off the MC outlet for fabrication of the SMC-A (small-sized microchannel, without terrace) and SMC-B (small-sized channel, with terrace) series. Uniform MC with and without a terrace at their outlets could then be fabricated.

We first investigated the behavior of micron-scale MS formation using the SMC-A1 and SMC-A4 without terraces at the MC outlet. Microscopic observations found that the SMC-A series yielded uniformly sized MS from each channel. However, the MS size depended on the site of the channels, resulting in the formation of differently sized MS in a plate and the recovery of polydisperse MS. As a result, micron-scale monodisperse O/W-MS were prepared with difficulty by using MC without terraces.

We investigated the behavior of micron-scale MS formation using SMC-B1, SMC-B2, SMC-B3, and SMC-B4 with terraces at the MC outlet. The images of the MS formation process using SMC-B4 are presented in Figure 2. We found that all the channels used in the SMC-B series yielded uniformly sized O/W-MS, which differed from the case of MC without terraces. A microscopic observation of the prepared MS also revealed the monodispersity of these MS (Fig. 2C). Figure 3 shows the effect of the MC equivalent diameter on the average MS diameter and the CV. The prepared O/W-MS had average diameters of 4.6 to 5.2 μm and coefficient of variations of 7 to 9% for all the MC. This result verifies the monodispersity of the MS since the CV were less than 10%, which is the index of monodispersity. The increase in the MS diameters between the smallest and largest MC was about one-third that in the MC equivalent diameters, indicating that the change in the MC equivalent diameter had a slight effect on the MS diameter. This result can be explained by the MC width and the shape around the MC outlet and is discussed later. The above results demonstrated that the SMC-B series is capable of excellent performance in preparing monodisperse MS compared to MC without terraces.

We also investigated the effect of the applied pressure on the behavior of O/W-MS formation using all of the above MC. The MC efficiency, defined as the ratio of the number of channels forming MS to their total number on a plate, increased with applied pressure over the breakthrough pressure for the open-end MC. Subsequently, the MC efficiency increased without larger MS formation up to about 70%. However, the size distribution of the prepared MS using SMC-A series remained broader than that using the SMC-B series. The change of the applied pressure affected the MS size more at higher pressures than the breakthrough pressure for the SMC-A series without terraces. When the SMC-B4 was employed, MS sizes were not changed in a range of 1.4 to 5.4 kPa, showing that monodisperse MS were obtained. Larger MS were formed at some channels at pressures higher than 5.4 kPa. The MC efficiency increased with the applied pressure, and the maximal MC efficiency without larger MS formation reached about 50% at 5.4 kPa. The MS formation rate increased with the applied pressure in a similar manner to the MC efficiency, achieving up to 3 droplets per second for each channel on average. The maximum MC efficiency and the MS formation rate may depend on the channel shape, e.g., MC width, MC depth, MC length, and terrace length. Optimization of the channel shape would be required to improve the MC efficiency and MS formation rate.

The shape around the MC outlet plays an important role in the behavior of MS formation in MC emulsification. The effect of the channel shape on the behavior of MS formation can be considered on the basis of the above results. The open-

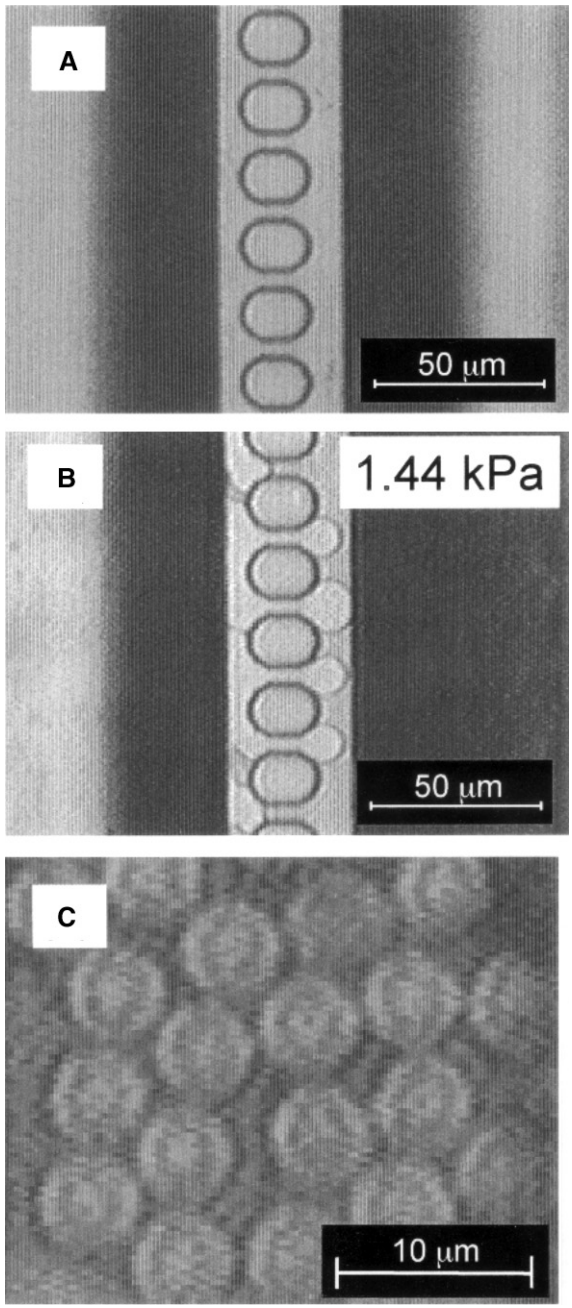


FIG. 2. MS formation using SMC-B4 (D_{eq} = 1. 7 mm); (A) filled with continuous phase; (B) breaking through MC and MS formation; (C) monodisperse oil-in-water MS. For abbreviations see Figure 1.

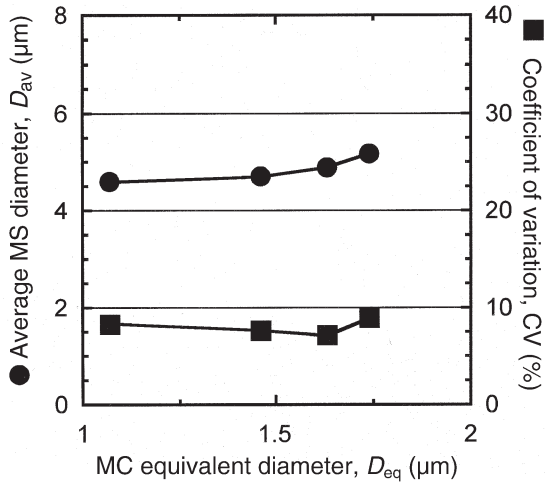


FIG. 3. Effect of MC equivalent diameter (SMC-B) on the average MS diameter, D_{av} (μm), and coefficient of variation, CV (%). For abbreviations see Figure 1.

end MC has no terrace or well outside the MC outlet (Scheme 1C). In general, the MS size and distribution critically depend upon the MC equivalent diameter for MC without a terrace at the MC outlet. However, the size distribution of the MC outlet becomes broader in the open-end MC because of microchipping caused by diced cutting, as illustrated in Figure 1. This broader size distribution of the MC outlet would lead to reduced monodispersity of the MS. Although the MS prepared with the open-end MC exhibited less monodispersity, a stable O/W-MS formation was observed that differed from that seen in the SMC-A series without terraces. The bulk space outside the MC outlet in the open-end MC facilitates the detachment of the MS from the MC outlet by buoyancy. Furthermore, the microchipping at the MC outlet might provide an effect similar to a terrace. We consider that these contributed to the stable MS formation and the minimal effect of the applied pressure on the MS size.

The SMC-A series has no terrace at the MC outlet in the newly fabricated, uniformly sized MC, while the SMC-B series has terraces at the MC outlet (Table 1). In the SMC-A series, the dispersed phase passes through the MC and inflates spherically in the well part due to interfacial tension. Droplet detachment starts when the diameter of the dispersed phase reaches a certain value, resulting in droplet formation. However, the SMC-A series without terraces could not satisfy the above conditions necessary for stable MS formation. This leads to difficulties with monodisperse MS preparation, i.e., the applied pressure greatly affects the MS diameter. On the other hand, the dispersed phase in the SMC-B series passes through the MC and inflates on the terrace in a disk-like shape. A droplet is then formed by the shear from the interfacial tension (12). The existence of a terrace caused instability of the interface, and promoted the shearing of the interface and droplet formation. Hence, the SMC-B series yielded monodisperse O/W-MS because of its specific mechanism of MS formation. We consider that the shearing is significantly stronger than the other external forces over a relatively wide range of applied pressure. The MS diameter started to become larger at very high pressures. As a result, this MC enabled the stable formation of monodisperse MS in the range described above.

The effect of the MC width on the MS size can also be explained as follows. The MS size depends on the volume of the dispersed phase, which inflates on the terrace in a disk-like shape, since the dispersed phase on the terrace is squeezed into the well just before MS formation. In addition, the terrace length largely influences the volume of the dispersed phase inflating on the terrace. This suggests that the effect of the MC width on the MS size becomes less significant as the terrace length increases. The effect of the terrace length is likely to be significant in the SMC-B series with terraces, leading to a reduced effect of MC width on MS size. We therefore consider that the MC width slightly affected the MS diameter in the SMC-B series with terraces at the MC outlet.

The above results indicate that the shape at the MC outlet significantly affects MS formation, and that the SMC-B series contributed to the preparation of micron-scale monodisperse MS.

Preparation of micron-scale food-grade O/W-MS. Figure 4 shows the MS size and distribution of food-grade O/W-MS prepared using SMC-B4 with PGFE as surfactant. Stable MS formation was observed in both cases using soybean oil and MCT as the dispersed phase, and the D_{av} of the food-grade O/W-MS prepared were 3.7 to 3.8 μm, which is about 1 μm smaller than those of about 5.0 μm obtained using the other surfactant. There was no difference except for the kind of surfactants. The interfacial tension of the system using PGFE was 5.1 to 5.8 mN/m, higher than that using Tween 80, which was 0.65 mN/m. The different kinds of surfactant affected the adsorption kinetics on the interface, resulting in a change of MS size. We considered that these might contribute to a decrease in the MS size. The CV of the MS ranged from 6 to 7%, which verifies the monodispersity of the food-grade MS. We achieved stable formation of monodisperse food-grade

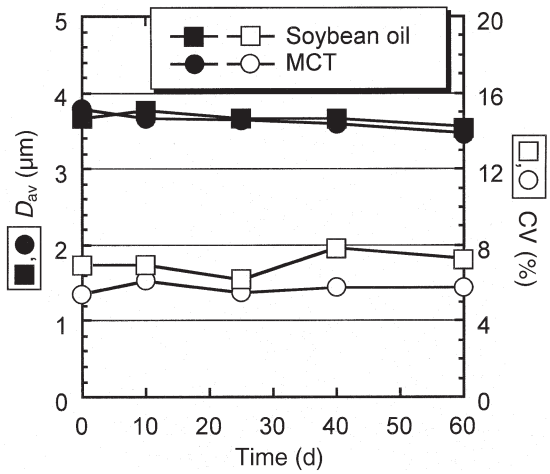


FIG. 4. Time courses of the D_{av} and CV of oil-in-water-MS recovered using SMC-B4 with pentaglycerol monolaurate as surfactant. MCT, medium-chain triacyglycerol (75% caprylic acid, and 25% capric acid). For abbreviations see Figure 1.

O/W-MS on a micron scale by utilizing a food-grade surfactant, PGFE. This result is important for future applications of MC emulsification to foods and pharmaceuticals. The prepared O/W-MS were recovered and stored in a sample bottle and then used to investigate the stability of the MS.

Stability against coalescence of micron-scale prepared O/W-MS. The stability of the monodisperse O/W-MS against coalescence was evaluated by measuring the time course of their D_{av} and CV. The following two systems of monodisperse food-grade O/W-MS were used: soybean oil/saline with 0.5 wt% PGFE and MCT/saline with 0.5 wt% PGFE. While stored in a sample bottle, the recovered O/W-MS gradually floated up due to buoyancy and formed a concentrated cream layer. However, no oil phase appeared, and the cream layer was dispersed easily by light shaking. The size and distribution of the MS before creaming and after dispersing the concentrated cream layer were measured and compared. There was little difference between their D_{av} and CV. This indicates that monodisperse O/W-MS have a high stability against coalescence when they are in contact under creaming conditions. In both systems, the average diameters of the O/W-MS decreased slightly during storage for 60 d as shown in Figure 4. The CV of the O/W-MS also changed little over 60 d. This may be primarily due to the repulsion between the hydrophilic heads of the surfactant molecules surrounding the MS. The recovered O/W-MS thus maintained their monodispersity in the bulk continuous phase for a long period. We clarified that monodisperse O/W-MS recovered on a micron scale have long-term stability against coalescence.

ACKNOWLEDGMENTS

This work was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (MS-project). The authors would like to thank Taiyo Kagaku Co. Ltd. for donating the reagents.

REFERENCES

1. McClements D.J., *Food Emulsions: Principles, Practice, and Techniques*, CRC Press, Boca Raton, 1998, pp. 1–16, 161–184.
2. Bibette J., Depletion Interaction and Fractionated Crystalization for Polydispersed Emulsion Purification, *J. Colloid Interface Sci. 147*:474–478 (1991).
3. Nakashima, T., M. Shimizu, and M. Kukizaki, Membrane Emulsification by Microporous Glass, *Key Eng. Mater. 61/62*:513–516 (1991).
4. 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).
5. Suzuki, K., I. Shuto, and Y. Hagura, Characteristics of Membrane Emulsification Method Combined with Preliminary Emulsification for Preparing Corn Oil-in-Water Emulsions, *Food Sci. Technol. Int. 2*:43–47 (1996).
6. Suzuki, K., I. Fujiki, and Y. Hagura, Preparation of Corn Oil/Water and Water/Oil Emulsions Using PTFE Membrane, *Ibid. 4*:164–167 (1998).
7. 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).
8. Kikuchi, Y., K. Sato, and T. Kaneko, Optically Accessible Microchannels Formed in a Single-Crystal Silicon Substrate for Studies of Blood Rheology, *Microvasc. Res. 44*:226–240 (1992).
9. Kikuchi, Y., K. Sato, and Y. Mizuguti, Modified Cell-Flow Microchannels in a Single Silicon Substrate and Flow Behavior of Blood Cells, *Microvasc. Res. 47*:126–139 (1994).
10. Kawakatsu, T., H. Komori, N. Oda, and T. Yonemoto, Relationship Between Concentration of Surfactant and Pressure for Droplet Creation, and Effect on Droplet Size in Microchannel O/W Emulsification, *Kagaku Kogaku Ronbunshu 24*:313–317 (1998) (in Japanese).
11. Kobayashi, I., M. Nakajima, J. Tong, T. Kawakatsu, H. Nabetani, Y. Kikuchi, A. Shohno, and K. Satoh, Production and Characterization of Monodispersed Oil-in-Water Microspheres Using Microchannels, *Food Sci. Technol. Res. 5*:350–355 (1999).
12. Sugiura, S., M. Nakajima, J. Tong, H. Nabetani, and M. Seki, Preparation of Monodispersed Solid Lipid Microspheres Using Microchannel Emulsification Technique, *J. Colloid Interface Sci. 227*:95–103 (2000).
13. Heydinger, J.A., and K.D. Nakhasi, Medium Chain Triacylglycerols, *J. Food Lipids 3*:251–257 (1996).
14. Katoh, R., Y. Asano, A. Fujita, K. Sotoyama, and M. Tomita, Preparation of Food Emulsions Using a Membrane Emulsification System, *J. Membrane Sci. 113*:131–135 (1996).

[Received December 20, 2000; accepted April 26, 2001]