Chapter 2

Preparation of Monodisperse Oil-in-Water Emulsions with Micron-Scale Droplets Using Microchannel Emulsification

2.1 Introduction

Emulsions, with one of two immiscible liquids dispersed as small droplets in the other, generally have droplets with diameters somewhere between 0.1 and 100 μm. The size and size distribution of emulsion droplets are most important parameters in characterizing any emulsion. The physical and qualitative stabilities and other major physicochemical properties of emulsion-based products are determined by their droplet size and size distribution (McClements, 1999). Stability and resistance to creaming of emulsions depend on their droplet size (McClements, 1999; Mason *et al.*, 1996). Precisely size-controlled emulsions with a narrow droplet size distribution lead to their better stability and facilitate control of their properties. It is therefore important to reliably control and predict the droplet size and size distribution of emulsions. However, emulsions produced by conventional emulsification devices (e.g., colloid mills, rotor-stator systems, high-pressure homogenizers) are of considerably polydisperse over the above-mentioned droplet sizes (McClements, 1999; Karbstain and Schubert, 1995).

Emulsification devices, which can produce monodisperse emulsions, have received much attention over the last ten years. Membrane emulsification enables the production of size-controlled relatively monodisperse emulsions with coefficients of variation greater than 10% (Nakashima *et al.*, 1991, 2000; Joscelyne and Trägårdh, 2000). A novel microchannel (MC) emulsification, proposed by Kawakatsu *et al.* (1997), have successfully produced monodisperse emulsions with average diameters of 10 to 100 μm and coefficients of variation below 5% (Kawakatsu *et al.*, 1999; Kobayashi *et al.*, 1999; Sugiura *et al.*, 2002). A smaller MC is required to produce monodisperse emulsions with micron-scale droplets, which include promising applications in various industries, such as foods, cosmetics, pharmaceuticals and chemical industries.

The primary objective of this chapter was to prepare monodisperse oil-in-water (O/W) emulsions with micron-scale droplets by MC emulsification. The effect of the channel shape on the droplet formation behavior was investigated using three different small-sized MCs. The long-term stability of the obtained O/W emulsions was also studied.

2.2 Materials and Methods

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 [Tween80; hydrophilic lipophilic balance (HLB), 15.0] (Wako Pure Chemical Ind., Osaka, Japan) and polyglycerol monolaurate [PGM, >65% purity; Sun soft A-121E; HLB, 12] (Taiyo Kagaku Co. Ltd., Mie, Japan) were used as the surfactants.

Silicon MC plate

Figures 2.1a and b depict the silicon MC plate used in this chapter. The MC plate measures 8 mm × 16 mm × 0.5 mm and has a 1mm-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 a process of photolithography and anisotropic etching (Kikuchi *et al.*, 1992). 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 channel width (W), depth (H), interval (I), and terrace length (L_T) (Fig. 2.1b). Figures 2.1c and d show an open-end MC and a newly fabricated MC. The open-end MC plate has an open space at the channel outlet formed by cutting in a mechanical process, which allows easy dispersion of formed emulsion droplets into the bulk continuous phase. The newly fabricated MC plates consist of MCs with and without the terrace at the channel outlet and have the well outside the channel outlet (Fig. 2.1d). Table 2.1 presents the dimensions of the MC plates and the numbers of channels employed. All the new MCs have the same channel depth of 1.2 μm. The channel equivalent diameter was calculated as follows:

$$d_{eq} = (A/L_W) \times 4 \quad (2.1)$$

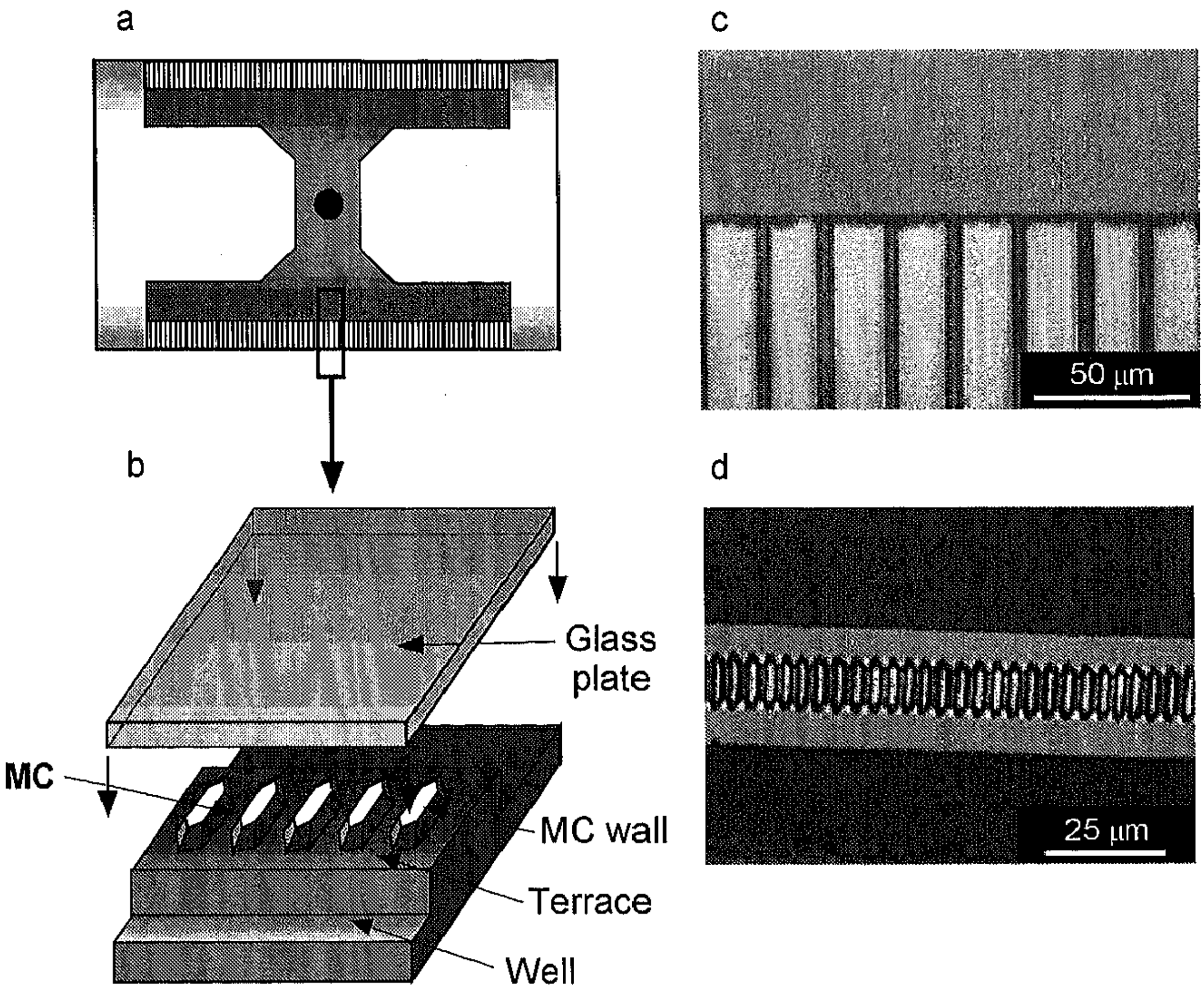

Fig. 2.1 Schematic diagram and photographs of silicon MC plate.
(a) Surface image; (b) magnification; (c) open-end MC; (d)SMC-B1.

Table 2.1 Silicon MC plates used in this chapter.

MC plate	Channel width W (μm)	Channel depth H (μm)	Terrace length L_T (μm)	Channel interval[a] I (μm)	Total channel number (-)
Open-end MC	4.7	2.2	-	6.1	4600
SMC-A1	2.0	1.2	-	4.0	4800
SMC-A4	4.7	1.2	-	16.0	1200
SMC-B1	2.0	1.2	6.9	4.0	4800
SMC-B2	3.2	1.2	6.9	8.0	2400
SMC-B3	4.0	1.2	6.9	12.0	1600
SMC-B4	4.7	1.2	6.9	16.0	1200

[a]Channel interval is defined as the length between the center of neighboring channels.

where d_{eq} is the channel equivalent diameter in m, A is the area of the channel cross section in m^2 and L_W is the wetted perimeter of the channel cross section in m.

Experimental setup

Figure 2.2 depicts the experimental setup and the flow mechanism through the channels in the module. The composition of the system used in this chapter is similar to that described previously (Kawakatsu *et al.*, 1997). The MC plate was inverted and attached onto a glass plate in the newly designed MC module. This module enables formed emulsion droplets to disperse into a bulk continuous phase open to the air and to be easily recovered with a pipette. The MC 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).

MC emulsification

Effect of channel shape on MC emulsification and droplet diameter: To ensure a sufficient supply of a surfactant at the interface, a surfactant concentration exceeding the critical micelle concentration (CMC) was employed. Soybean oil with 1.5 wt.% Tween80 was used as the dispersed phase, and saline was used as the continuous phase. The MC module was initially filled with a 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 exceeded the breakthrough pressure (Kawakatsu *et al.*, 1998), the dispersed phase broke through the channels and droplet formation commenced. The oil droplets dispersed in the bulk continuous phase were recovered with a pipette. All experiments were carried out at room temperature, approximately 25 °C.

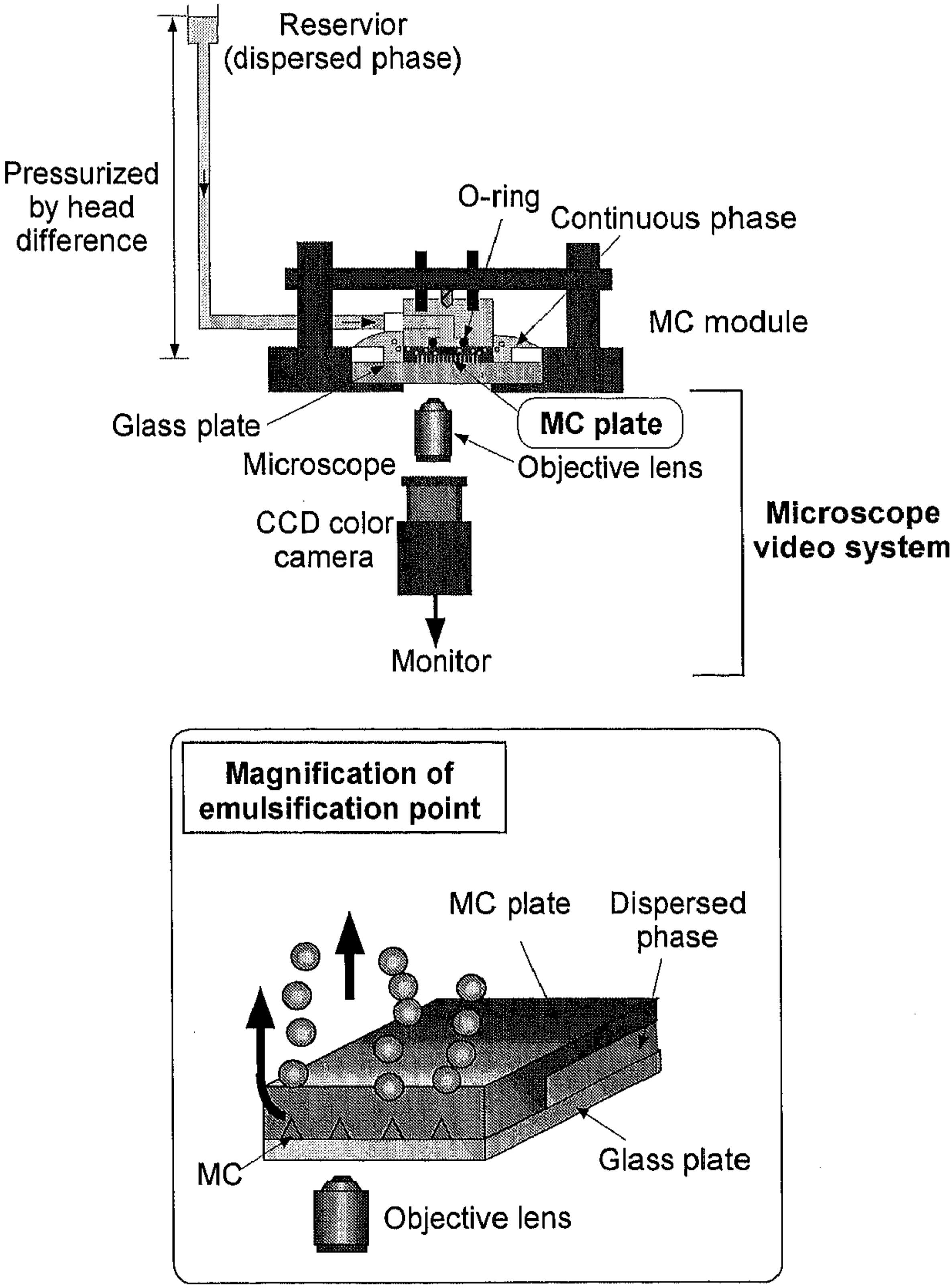

Fig. 2.2 Experimental setup for MC emulsification.

Preparation of food-grade O/W emulsions with micron-scale droplets: We consider the various conditions necessary for stable preparation of O/W emulsions using food-grade surfactants. PGM was chosen as a food-grade surfactant. MCT have been attracting interest for foods and pharmaceuticals because of their unique physical and metabolic properties (Heydinger and Nakhasi, 1996). In particular, MCT are a rapidly absorbed and utilized energy source. Soybean oil and MCT were thus used as the dispersed phase, and saline with 0.5 wt.% PGM was used as the continuous phase.

A syringe pump (Model 11, Harvard Apparatus Inc., Massachusetts, USA) 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.

Measurements and analysis

The droplet formation behavior from the channels was analyzed from the video images recorded with a microscope video system. The size and number of the formed droplets were measured with a Coulter counter (ZM, Beckman Coulter, Inc., California, USA) after diluting the recovered emulsion with physiological saline. The number-average droplet diameters and coefficients of variation of the resultant O/W emulsions were then calculated from the data obtained. The coefficient of variation expressed as the following equation was used to present the monodispersity of the emulsions:

$$CV = (\sigma / d_{av}) \times 100 \qquad (2.2)$$

where CV is the coefficient of variation as a percentage, σ is the standard deviation in m, and d_{av} is the average droplet diameter in m.

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

2.3 Results and Discussion

Effect of channel shape on MC emulsification and droplet diameter

Figure 2.3 demonstrates the formation behavior of O/W emulsion droplets using an open-end MC with a 2.4 μm-channel equivalent diameter. Droplet formation commenced through the channels at a breakthrough pressure of 3.60 kPa. The average droplet diameter and coefficient of variation of the prepared O/W emulsion were 6.58 μm and 16.2%, respectively. This open-end MC enabled us to obtain an O/W emulsion with micron-scale droplets by MC emulsification. The ratio of the average droplet diameter to the channel equivalent diameter was 2.7, which was smaller than the ratio of 3.25 or 5.0 in membrane emulsification (Schröder *et al.*, 1998; Katoh *et al.*, 1996). This might be attributable to the unique channel shape. However, the coefficients of variation of the prepared emulsion was more than three times that of the MC emulsification reported (Kawakatsu *et al.*, 1997, 1998; Kobayashi *et al.*, 1999; Sugiura *et al.*, 2000), indicating their less monodispersity. Consequently, it was difficult to prepare a monodisperse O/W emulsion with micron-scale droplets and a coefficient of variation below 10% using the open-end MC.

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

The droplet formation behavior using the SMC-A1 and SMC-A4 without terraces at the channel outlet was first investigated. Microscopic observations found that the SMC-A series yielded uniformly sized oil droplets from each channel. However, the droplet diameter of the prepared O/W emulsion depended on the site of the channels (Fig. 2.4), resulting in the formation of differently sized oil droplets in a plate and the recovery of polydisperse emulsions. As a result, monodisperse O/W emulsions with micron-scale droplets were prepared with difficulty by using MC without terraces.

The droplet formation behavior using SMC-B1, SMC-B2, SMC-B3, and SMC-B4

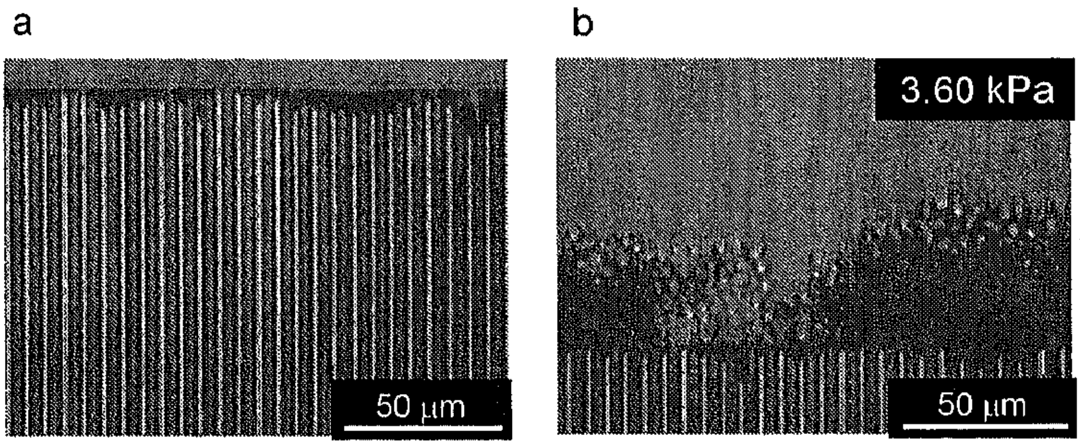

Fig. 2.3 Droplet formation using open-end MC (d_{eq} = MC equivalent diameter = 2.4 μm). (a) Filled with continuous phase; (b) breaking through MC and droplet formation.

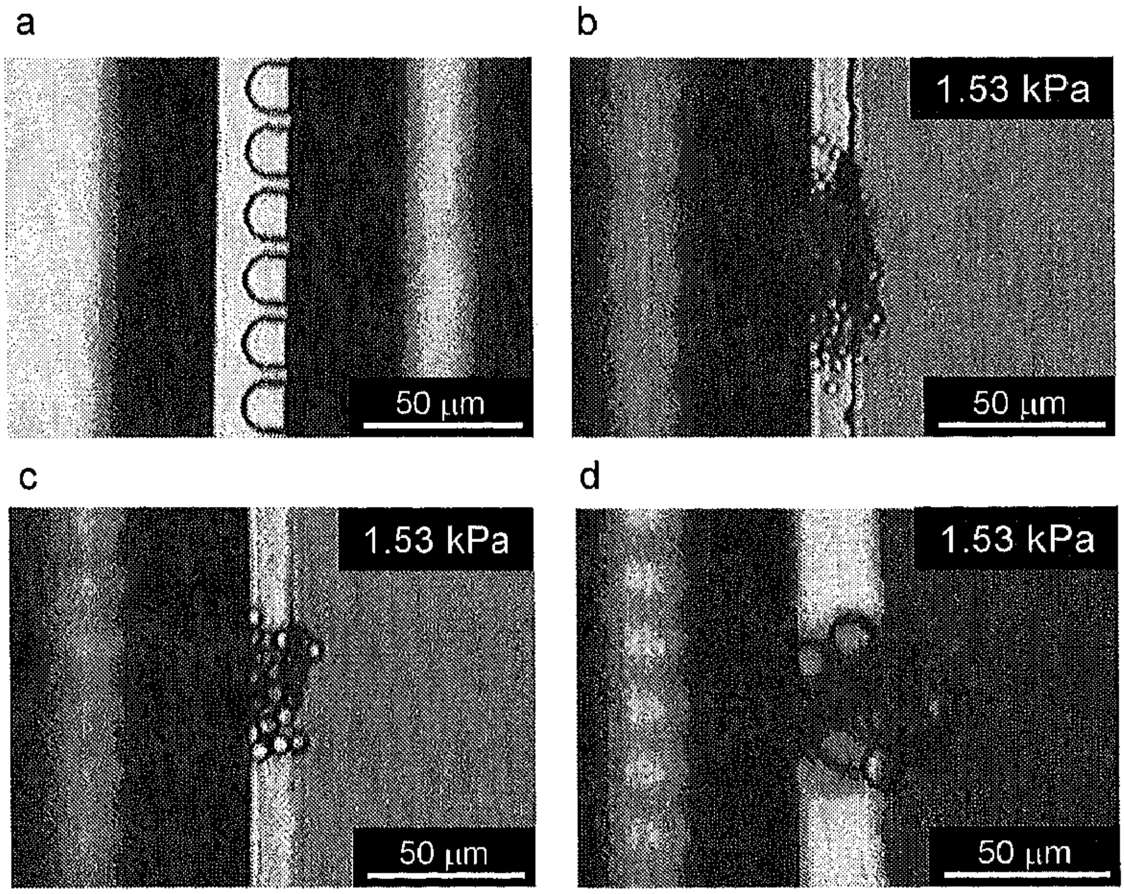

Fig. 2.4 Droplet formation using SMC-A4 (d_{eq} = 1.7 μm). (a) Filled with continuous phase; (b)-(d) O/W emulsion droplets from different channels.

with terraces at the channel outlet was next investigated. The microscopic photographs of the droplet formation process using SMC-B4 are presented in Fig. 2.5. We found that all the channels working in the SMC-B series yielded uniformly sized oil droplets, which differed from the case of MCs without terraces. A microscopic observation of the prepared O/W emulsion also revealed its monodispersity (Fig. 2.5c). The average diameters and coefficients of variation of the emulsions prepared using SMC-B series were presented in Table 2.2. They had average droplet diameters of 4.6 to 5.2 μm and coefficients of variation of 7 to 9% for all the MCs. This result verifies their monodispersity since the coefficients of variation were less than 10%, which is the index of monodispersity. A slight increase in the average droplet diameter of the resultant emulsions was observed as the channel width and channel interval were increased (Table 2.2). The above results demonstrated that the SMC-B series have excellent performance in preparing monodisperse O/W emulsions compared to MCs without terraces.

We also investigated the effect of the applied pressure on droplet formation using all of the above MCs. The channel efficiency, defined as the ratio of the number of channels forming droplets to their total number on a plate, increased with applied pressure over the breakthrough pressure for the open-end MC. The channel efficiency for this MC increased without larger droplet formation up to about 70%. In contrast, the change of the applied pressure in the SMC-A series without terraces greatly affected the droplet diameter of the resultant emulsions. Additionally, the droplet size distribution of the O/W emulsions prepared using SMC-A series remained broader than that using the SMC-B series at all the applied pressures over the breakthrough pressures. The droplet diameters of the emulsions prepared using SMC-B4 were not changed in a range of 1.4 to 5.4 kPa, showing that monodisperse O/W emulsions were obtained. Larger droplets were formed from some channels at pressures higher than 5.4 kPa. The channel efficiency increased with the applied pressure, and the maximum channel efficiency without larger droplet formation reached about 50% at 5.4 kPa. The droplet formation rate increased with the applied pressure in a similar manner to the channel efficiency, achieving up to 3 droplets

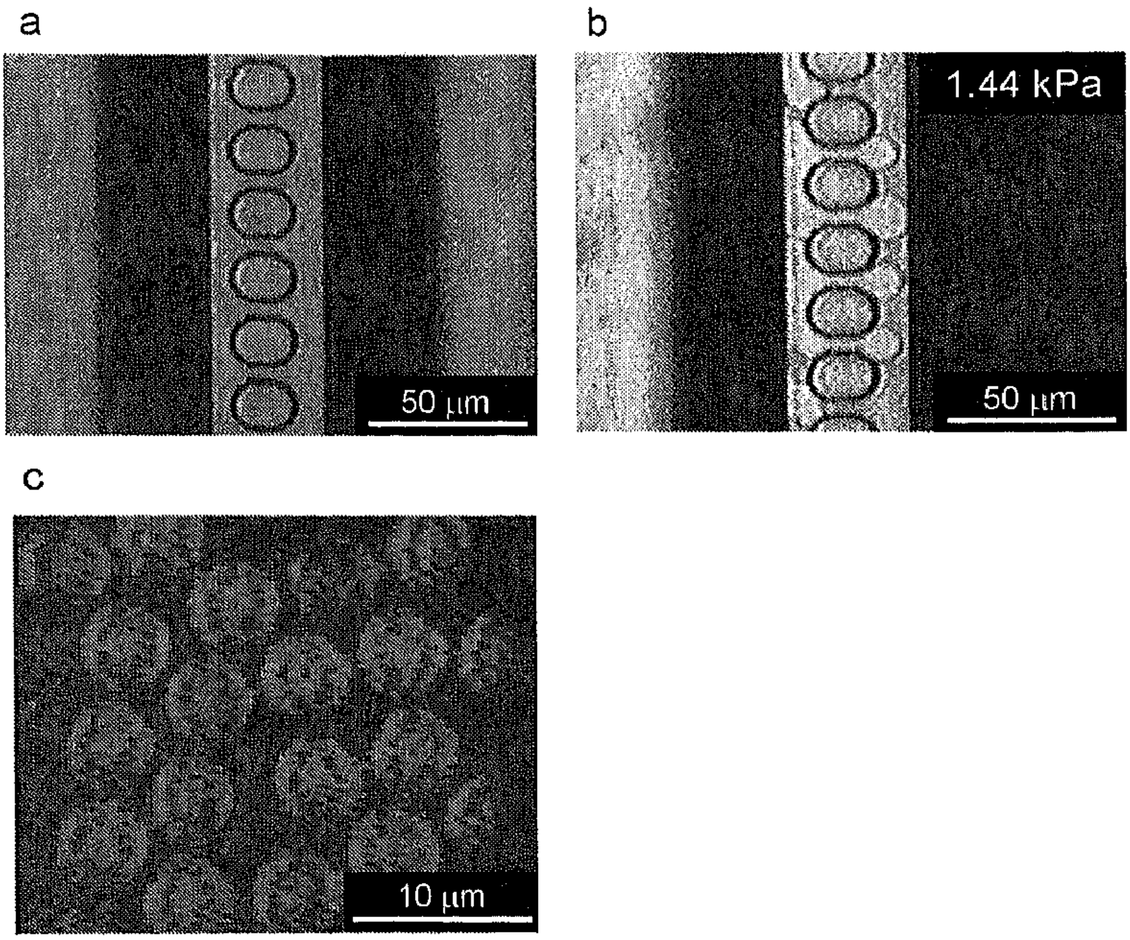

Fig. 2.5 Droplet formation using SMC-B4 (d_{eq} = 1.7 μm). (a) Filled with continuous phase; (b) breaking through MC and droplet formation; (c) monodiserse O/W emulsion droplets.

Table 2.2 Average droplet diameters and coefficients of variation of prepared O/W emulsions.

MC plate	Average droplet diameter d_{av} (μm)	Coefficient of variation CV (%)
SMC-B1	4.6	8.3
SMC-B2	4.7	7.5
SMC-B3	4.9	7.1
SMC-B4	5.2	8.9

per second for each channel on average. The maximum formation rate of uniformly sized droplets from a channel may depend on the channel shape, e.g., channel width, channel depth, channel length and terrace length. Optimization of the channel shape would be required to improve the droplet formation rate, i.e., throughput of emulsion droplets per a plate.

The preceded results show that the shape around the channel outlet plays an important role in the droplet formation behavior in MC emulsification. The droplet formation behavior for each type of the MC was then discussed. The open-end MC has no terrace nor well outside the channel outlet (Fig. 2.1c). The droplet size and size distribution generally depend upon the channel equivalent diameter for MCs without a terrace at the channel outlet. However, the size distribution of the channel outlet becomes broader in the open-end MC because of microchipping caused by diced cutting, as illustrated in Fig. 2.3. This broader size distribution of the channel outlet would lead to reduced monodispersity of the droplets formed. Although the O/W emulsion prepared using the open-end MC exhibited less monodispersity, a stable oil droplet formation was observed that differed from that seen in the SMC-A series without terraces. The bulk space outside the channel outlet in the open-end MC facilitates droplet detachment from the channel outlet by buoyancy. Furthermore, the microchipping at the channel outlet may provide an effect similar to a terrace. We consider that these contributed to the stable droplet formation and the minimal effect of the applied pressure on the droplet diameter.

In the SMC-A series without terraces at the channel outlet, the dispersed phase, which passed through the channels, inflates spherically in the well part. Droplet detachment initiated at the moment when the diameter of the dispersed phase balanced the cylinder diameter of the channels. However, it is not so easy to transfer the continuous phase to the narrow space between the dispersed phase and the channels (Sugiura *et al.*, 2000), which makes droplet detachment unstable. This unstable droplet formation would be the reason for the great effect of the applied pressure on the droplet diameter. In the SMC-B series with terraces at the channel outlet, the dispersed phase, which passed through the channels, inflates on the terrace in a distorted shape. Interfacial tension-driven droplet formation was then completed by spontaneous transformation of the distorted dispersed phase into spherical droplets (Sugiura *et al.*, 2001). The repeated stable droplet formation process enables to yield monodisperse emulsions from the SMC-B series. The interfacial tension force, acting on the inflating droplets, is significantly stronger than the other external forces over a relatively wide range of applied pressure. We consider that this causes the stable preparation of monodisperse emulsions in the range described above.

The SMC-B series have the same dimensions except for the channel width and channel interval (Table 2.1). The experimental results, shown in Table 2.2, suggest that an increase in the channel width and channel interval of the SMC-B series caused a slight increase in the droplet diameter of the resultant emulsions. The resultant droplet diameter relates to 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 during droplet detachment. Microscopic photographs of the dispersed phase inflating on the terrace for the SMC-B series exhibited that its volume tended to become slightly bigger as the channel width increased (data are not shown). Moreover, the adjacent dispersed phase droplets inflating on the terrace hindered their inflation each other when using the MCs with short intervals such as the SMC-B1 and SMC-B2. Thus, the use of the MC with a short channel width and channel interval decreased the volume of the dispersed phase inflating on the terrace just before droplet detachment, resulting in the reduced droplet diameter. We therefore consider that both the channel width and channel interval slightly affected the droplet diameter in the SMC-B series with terraces at the channel outlet. Sugiura *et al.* (2002) have recently reported that the channel height and terrace length are the dominant parameters for determining the droplet diameter in MC emulsification.

The results in this chapter indicate that the shape at the channel outlet significantly affects droplet formation, and that the SMC-B series contributed to the preparation of monodisperse O/W emulsions with micron-scale droplets.

Preparation of food-grade O/W emulsions with micron-scale droplets

Figure 2.6 shows the droplet size and size distribution of food-grade O/W emulsions prepared using SMC-B4. Stable droplet formation was observed in both experimental systems, and the average droplet diameters of the prepared O/W emulsions were 3.7 to 3.8 μm, which are about 1 μm smaller than those of about 5.0 μm obtained using the other surfactant. There was no difference between the experimental systems except for the kind of surfactants. The interfacial tension of the experimental system using PGM was 5.1 to 5.8 mN/m, higher than that using Tween80, which was 0.65 mN/m. The different kinds of surfactant affected the adsorption kinetics on the interface, resulting in a change of droplet diameter. We considered that these might contribute to a decrease in the droplet diameter. The coefficients of variation of the formed droplets were ranged from 6 to 7%, which demonstrates the monodispersity of the food-grade O/W emulsions. We achieved stable preparation of a monodisperse food-grade O/W emulsion with micron-scale droplets by utilizing a food-grade surfactant, PGM. This result is important for future applications of MC emulsification to foods and pharmaceuticals. The prepared O/W emulsions were recovered and stored in a sample bottle and then used to investigate the stability of the O/W emulsion droplets.

Stability against coalescence of monodisperse O/W emulsions with micron-scale droplets

The stability of the monodisperse O/W emulsion droplets against coalescence was evaluated by measuring the time course of their average diameters and coefficients of variation. The following two experimental systems of monodisperse food-grade O/W emulsions were used: soybean oil/saline with 0.5 wt.% PGM and MCT/saline with 0.5 wt.% PGM. While stored in a sample bottle, the recovered O/W emulsion droplets 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 droplet size and size distribution of the emulsions before creaming and after dispersing the concentrated cream layer were measured and compared. There was little difference

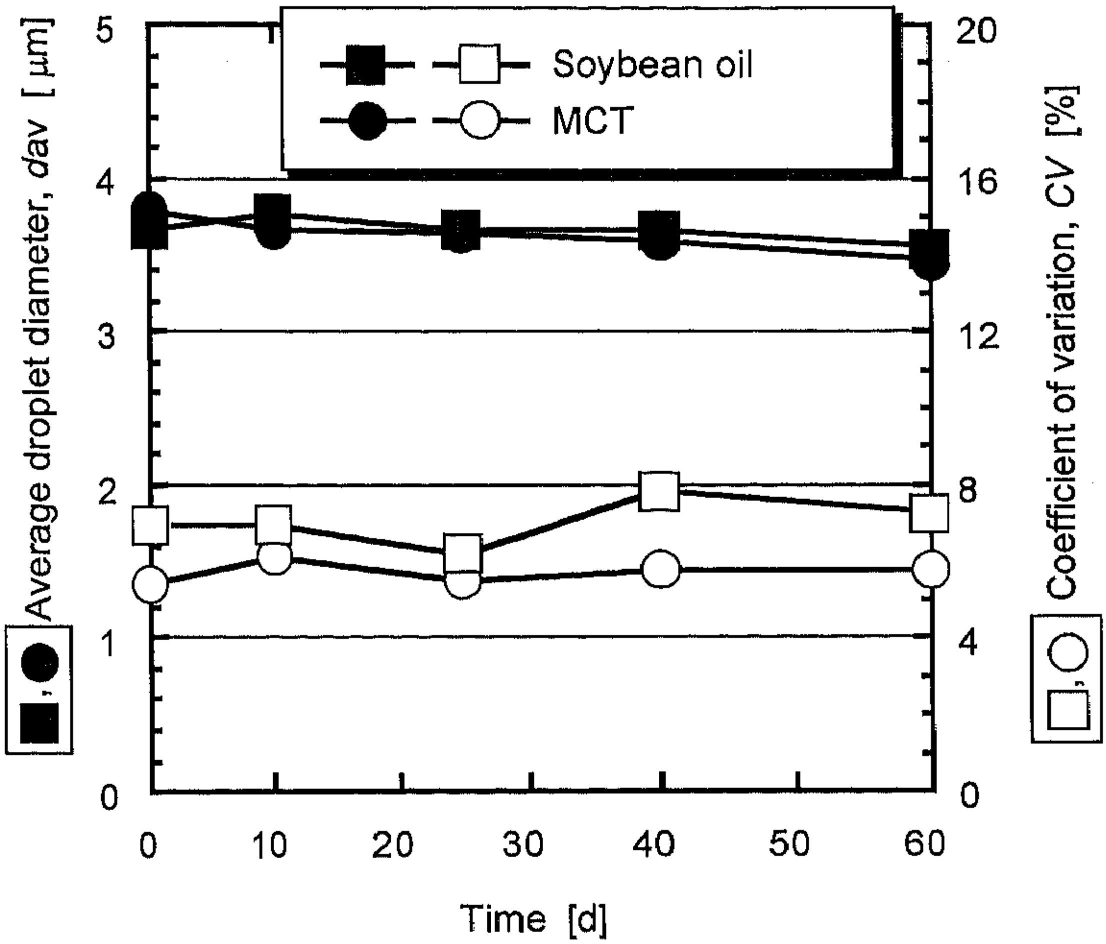

Fig. 2.6 Time course of the average droplet diameter and coefficient of variation of O/W emulsions recovered using SMC-B4 with pentaglycerol monolaurate as surfactant.

between their average diameters and coefficients of variation. This indicates that monodisperse O/W emulsion droplets have a high stability against coalescence when they are in contact under creaming conditions. The average droplet diameters of the O/W emulsions in both experimental systems decreased slightly during storage for 60 d as shown in Fig. 2.6. The coefficients of variation of the droplets also changed little over 60 d. This may be primarily due to the repulsion between the hydrophilic heads of the surfactant molecules surrounding the droplets. The recovered O/W emulsions thus maintained their monodispersity in the bulk continuous phase for a long period. We clarified that the recovered monodisperse O/W emulsions with micron-scale droplets have long-term stability against coalescence.

2.4 Conclusions

In the present chapter, we have demonstrated that monodisperse O/W emulsions with micron-scale droplets can be prepared by MC emulsification using newly designed small-sized silicon MCs. MC emulsification using an open-end MC resulted in the preparation of an O/W emulsion with a broad size distribution due to microchipping at the channels caused by diced cutting. An improved design of MC plate allowed the fabrication of uniformly sized MC with and without a terrace of a 1 to 2 μm-channel equivalent diameter. MCs without terraces at the channel outlet have a difficulty in preparing monodisperse emulsions, since the droplet diameter of the prepared emulsions depended on the site of the channels. MCs with terraces at the channel outlet stably yielded monodisperse O/W emulsions with average droplet sized of about 5 μm and coefficients of variation below 9%. Their droplet diameters were independent of the applied pressure. We thus found that a MC with a terrace at the channel outlet has the potential for preparing monodisperse emulsions with micron-scale droplets.

MC emulsification using a food-grade surfactant (PGM) stably prepared monodisperse food-grade soybean oil in water and MCT in water emulsions with droplet diameters of about 4 μm. The droplet diameter and coefficient of variation of the recovered O/W emulsions remained almost constant over 60 d, which demonstrates their long-term stability against coalescence.