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Production and Characterization of Monodispersed Oil-in-Water Microspheres Using Microchannels

Isao KOBAYASHI,[1,2] Mitsutoshi NAKAJIMA,[1]* Jihong TONG,[1] Takahiro KAWAKATSU,[1] Hiroshi NABETANI,[1] Yuji KIKUCHI,[1] Atsushi SHOHNO[2] and Kazumi SATOH[2]

[1] *National Food Research Institute, Ministry of Agriculture, Forestry and Fishers, Kannondai, 2-1-2, Tsukuba, Ibaraki 305-8642, Japan*

[2] *Department of Industrial Chemistry, Faculty of Engineering, Science University of Tokyo, Kagurazaka, 1-3, Shinjyuku, Tokyo 162-8601, Japan*

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Oil-in-water (O/W) microspheres (MS) were produced using microchannels (MC) of different channel size and type formed on a single-crystal silicon substrate, and the fundamental properties for the MS generation were studied. Sunflower oil containing sorbitan monolaurate as surfactant was used. MC of 5.3 μm in equivalent diameter gave an average 20 μm diameter of the produced O/W-MS. The coefficient of variation of the MS was below 2% with the surfactant concentration less than 0.5 wt%. The average diameter and the coefficient of variation of the MS changed little over 60 days, indicating high stability against coalescence. Horizontally setting the MC of 8.5 μm similar to that of 5.3 μm, the monodispersed MS were difficult to produce because of their coalescence and expansion near the channels. Changing MC from horizontal to vertical type, the monodispersed MS could be produced continuously by utilizing the buoyancy.

Keywords: monodispersed microspheres, silicon microchannels, vertical microchannel, stability, buoyancy

Microspheres (MS), which are emulsion cells or solid microparticles dispersed in a continuous phase, have been utilized in various industries such as foods, cosmetics, pharmaceuticals, etc. Mechanical and physical emulsification methods have been used in various industrial fields of mixers, colloid mills, high-pressure homogenizers, and ultrasonicators (Sherman, 1968; Tsuji, 1976; Katoh, 1995). The physical and qualitative stability of the MS may depend on their size and size distribution. However, with these methods it is difficult to control these two factors because the produced MS are polydispersed. The size distribution is usually broad if the MS size is large. To obtain a narrower size distribution, a stronger shearing stress is needed, which makes the MS size smaller. Also, the emulsification using mixers and high-pressure homogenizers easily incorporates bubbles into a system, inducing the oxidation and denaturation of the MS components (Kawakatsu *et al.*, 1998).

In addition to the above mechanical homogenizers, a membrane emulsification method that produces a monodispersed MS was proposed (Nakashima *et al.*, 1993). This utilizes a glass porous membrane in which a disperse phase passed through a membrane into a continuous phase. The size and size distribution of the MS can be controlled by the membrane pore size. This method has already been applied to industrial production of low-fat margarine (Katoh, 1993; 1995). A membrane emulsification method combined with preliminary emulsification was also proposed for higher productivity (Suzuki, 1996; Suzuki *et al.*, 1996; 1998). The membrane method is somewhat more attractive than previous methods because of its simplicity and lower energy consumption. Nevertheless, it was reported that the MS size distribution tended to be greater when the emulsification was done with membrane having a micrometer-level pore size (Nakashima *et al.*, 1990). Therefore, to produce MS several tens of micrometers in diameter, membranes having a uniform pore size of micrometer-level need to be developed.

Recently a system was developed in which flow characteristics of human blood were investigated using standard microchannels (MC) which were formed on the surface of a single-crystal silicon substrate by semiconductor technology (Kikuchi *et al.*, 1992; 1994). The behavior of micrometer-sized materials on the silicon MC plate can be observed using the microscope video system. A novel MC emulsification method was proposed by which it is possible to produce and observe the monodispersed oil in water (O/W) MS using the standard MC (Kawakatsu *et al.*, 1997). The MC and membrane emulsification methods are similar in principle. Advantages of the MC emulsification method are as follows: a visual observation of the channels during processing, a good understanding of the MS production phenomena involved in the processes and improved operating conditions. It was reported that the average diameter of the produced O/W-MS was about 20 μm and was almost independent of the sodium lauryl sulfate (SDS) concentration and the pressure when standard MC (about 5 μm in equivalent diameter) was used in the triolein/ SDS/ water system (Kawakatsu *et al.*, 1998).

In the present paper, monodispersed O/W-MS was produced and characterized by means of the standard MC plate on the basis of the previous work (Kawakatsu *et al.*, 1997). The effects of the surfactant concentration and the pressure on

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E-mail: mnaka@nfri.affrc.go.jp

production behavior, average diameter and standard deviation of the MS were investigated. The breakthrough pressure was evaluated by correlating with the interfacial tension between two phases measured at each surfactant concentration. MS stability on coalescence was evaluated from the time course of the average diameter and the coefficient of variation. From the results of the O/W-MS production experiments using a vertical MC plate, the possibility of the several tens of micrometer-sized monodispersed O/W-MS production was confirmed.

Materials and Methods

Materials High-oleic sunflower oil (triolein, >90% purity, Nippon Lever B.V., Tokyo) was used as the oil phase, and water filtrated through an ultrafiltration membrane (Nihon Millipore Co., Tokyo) was used as the water phase. Sorbitan monolaurate (HLB=8.6; Wako Pure Chemical Ind., Osaka) was used as surfactant in the oil phase, considering food grade and use in the oil phase. The surfactant concentration in the oil phase ranged from 0.1 wt% to 1.5 wt%. For preliminary study of long-term stability of MS, 300 mg/*l* sodium azide aqueous solution was used as the water phase to prevent bacteria from propagating in the system.

Microchannels (MC) The detailed structure of the silicon MC plate is shown in Fig. 1. The size was 15 mm×15 mm×0.5 mm, and the walls were manufactured on a 60 μm high terrace. MC were formed by tightly covering the silicon plate with an optically-flat glass plate. Two silicon MC plates with MC equivalent diameters of 5.3 μm and 8.5 μm were used for the MC emulsification study (Fig. 1 (c), (d)). The MC equivalent diameter was calculated as follows:

$$D_{\mathrm{eq}}=(A/L)\times 4 \tag{1}$$

where D_{eq} is the MC equivalent diameter [m], A is the area of MC cross section [m^2] and L is the circumferential length of MC cross section [m], respectively.

Apparatus for MS production Detailed description of the MS production using the standard horizontal MC plate was reported previously (Kawakatsu *et al.*, 1997; 1998). Figure 2 shows a diagram of the apparatus for the MS production using the vertical MC plate. The produced O/W-MS were expected to float up as a result of their buoyancy in the vertical MC apparatus. The MC and their images can be observed on a monitor through an inverted metallographic microscope (MS-511B, Seiwa Optical Industrial Co., Tokyo) and a 3CCD color camera (Hitachi Electric Co., Tokyo). The images recorded with a video cassette recorder (Sony Co., Tokyo) are used to measure the diameter of the produced MS on a personal computer (Power Macintosh 8500/120, Apple Computer Inc., USA).

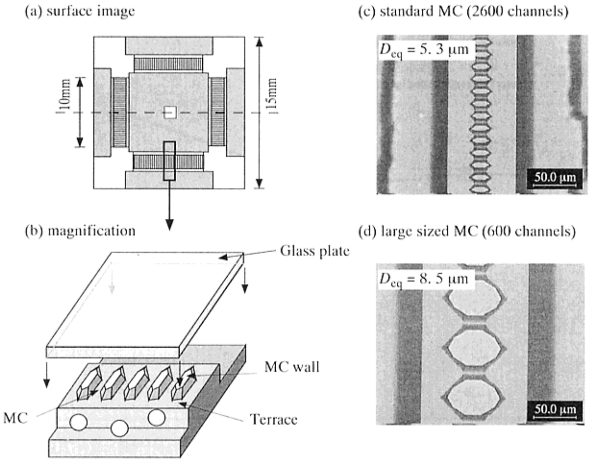

Fig. 1. Schematic diagram and images of silicon microchannels (MC) plate: (a) surface image, (b) magnification, (c) standard MC (2600 channels), (d) large sized MC (600 channels).

Procedure of O/W-MS production The MC module is initially filled with the water phase, and the oil phase is pressed into the module by lifting the reservoir. The oil phase flowed into the module and filled the space between the MC plate and the glass plate through the hole in the center of the MC plate, then reached the entrance of the MC. The operating pressure was slowly increased, and when the pressure reached a certain value, the oil phase began to break through the channels, and the generation of O/W-MS took place. This pressure is called breakthrough pressure. The produced MS were kept in the module, and their coalescence stability was evaluated from the time course of the average diameter and the coefficient of variation of the MS. All the experimental runs were done at room temperature.

Measurement and analytic method The applied pressure for MS processing was calculated on the basis of the density of high-oleic sunflower oil used in this study (911 kg/m^3) and the head difference. The MS production rate was measured from the videotape recorded. An image processing software (MAC SCOPE, Mitani Co., Fukui) was used to measure the MS diameter. Over 200 particles were counted for the calculation of the average diameter and the standard deviation of the MS. The coefficient of variation represented as the following equation was utilized to investigate the degree of the monodispersion of MS (Yano *et al.*, 1988).

$$\alpha=(\sigma_{\mathrm{p}}/D)\times 100 \tag{2}$$

where α is the coefficient of variation [%], D_{p} is the average MS diameter [m] and σ is the standard deviation [m].

The interfacial tension between two phases was measured with an automatic interfacial tensiometer (PD-W, Kyowa

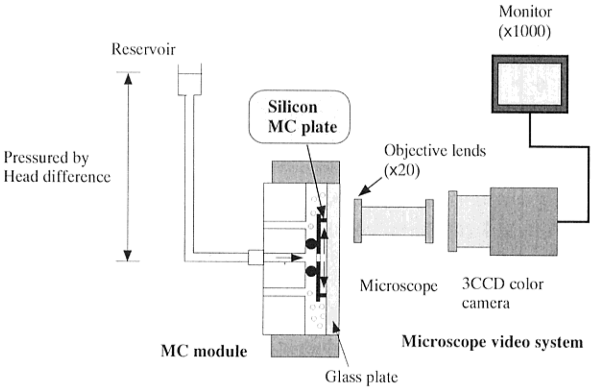

Fig. 2. Apparatus for microspheres (MS) production, a vertical MC setup.

Interface Science Co., Saitama) adopting the pendant drop method (Andreas *et al.*, 1938).

Results and Discussion

Effect of surfactant concentration on O/W-MS production The effect of the surfactant concentration on the O/W-MS production was studied using sorbitan monolaurate dissolved in triolein. The standard MC plate was used, which had an equivalent diameter of 5.3 μm. The relationship between the breakthrough pressure and the surfactant concentration is shown in Fig. 3. The pressure value shown is the average of several measurements. The distribution of breakthrough pressure measured at each surfactant concentration was less than 5% compared to its average value. The breakthrough pressure decreased with the increase in the surfactant concentration below 1.0 wt%, and became constant when the surfactant concentration exceeded 1.0 wt%. The relationship between the interfacial tension and the breakthrough pressure will be discussed later. The average diameter and the coefficient of variation for MS at the breakthrough pressure in several surfactant concentrations in triolein with sorbitan monolaurate/ water system are shown in Fig. 4. The average diameter was around 20 μm which was also obtained using SDS as surfactant (Kawakatsu *et al.*, 1998). When the surfactant concentrations were less than 0.5 wt%, the coefficients of variation were below 2% indicating high monodispersity of the produced MS. In the case of 0.3 wt% sorbitan monolaurate, all the produced MS were monodispersed, and continuous outflow of the oil phase was not obtained at all. When the surfactant concentrations were less than 0.2 wt% and more than 0.5 wt%, continuous outflow of the oil phase and the production of irregular-sized MS also occurred at some portions of channels where the oil phase broke through. Moreover, under the surfactant concentrations between 0.7 and 1.0 wt%, the coefficients of variation became very large compared with the other surfactant concentrations and the diameters of the produced MS differed among the channels, although monodispersed MS were produced at each channel on a MC plate. In this case, the size of MS tended to become slightly smaller at the channels where the production rate was rapid. With those surfactant concentrations, it was therefore difficult to obtain the monodispersed MS at all the channels where the oil phase broke through. This is probably due to the wetting of the MC plate and the interfacial tension of the two liquid phases. From these results, the optimum surfactant concentration was found to be 0.3 wt% for monodispersed MS. Thus, we used triolein with 0.3 wt% sorbitan monolaurate as the oil phase for the following studies.

Characterization of O/W-MS The number of channels producing MS was about 30 at the breakthrough pressure, which was very small compared with the total channel number of 2600. Although the effective channel numbers increased with the increase in applied pressure, the

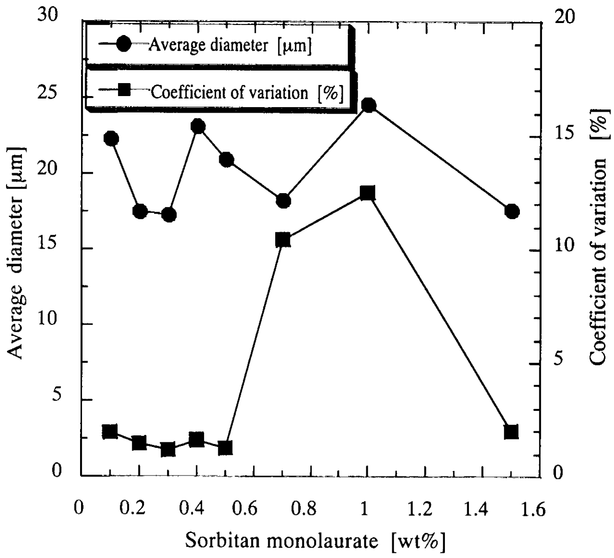

Fig. 4. Size and coefficient of variation for O/W microspheres (MS) at breakthrough pressure for various sorbitan monolaurate concentrations.

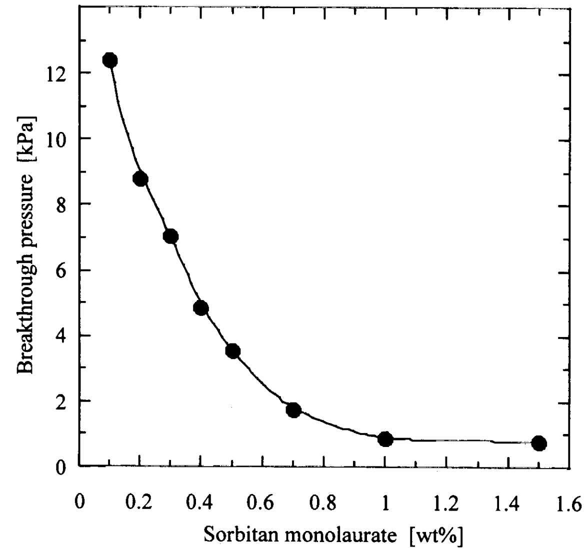

Fig. 3. Effect of sorbitan monolaurate concentration on breakthrough pressure.

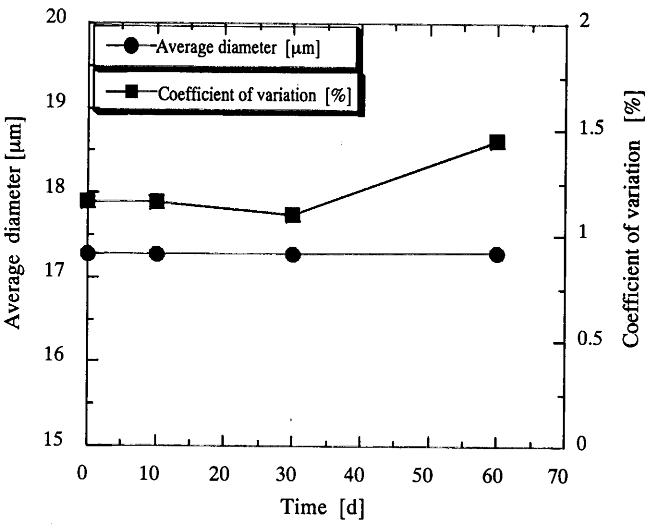

Fig. 5. Time course of average diameter and standard deviation of produced O/W-MS.

maximum proportion of effective channels was only 5%. This is probably due to micro-manufacturing accuracy of the channels or interaction of the channel surface, oil and water at the interface. In the above case, the MS production rate increased up to 126 cells/s with the increase in operating pressure, showing that less efficiency was obtained in this condition. The size of MS was not changed when the operating pressure rose above the breakthrough pressure. The produced MS were gradually packed into the continuous phase, and then were accumulated between the MC plate and the glass plate as monolayer or multilayers. It was a kind of aggregation as the accumulated MS formed a close-packed structure. Hence, the stability on coalescence of the MS was investigated preliminarily by observing the produced MS at regular intervals. The time course of the diameter and the coefficient of variation of the produced MS under the surfactant concentration of 0.3 wt% are shown in Fig. 5. Images of the MS just after production and after 60 days are shown in Fig. 6. As shown in Fig. 5, the average diameter and the coefficient of variation of the MS after 60 days changed little compared to that just after production, indicating that the produced monodispersed MS was stable against coalescence for a long period. This might be mainly attributable to the repulsion between the hydrophilic heads of the surfactant molecules surrounding the MS.

Production of O/W-MS using vertical MC A horizontal MC plate with an equivalent diameter of 8.5 μm was also applied to the production of O/W-MS. The image of this MC with 600 channels is shown in Fig. 7. The coalescence and expansion of the MS occurred near the channels, and the sizes of the MS produced were much larger than those when the standard MC plate was used. Due to their large size, these MS had difficulty leaving the channels and entering the bulk aqueous phase. It was found to be difficult to produce the monodispersed MS using the horizontal MC plate of 8.5 μm in equivalent diameter.

To overcome this problem, it was believed that leaving behavior of the MS from the channels could be improved by utilizing buoyancy generated from the density difference between the oil and the aqueous phases. MS production was attempted using the vertical MC apparatus placed as shown in Fig. 2. The behavior of MS production under the surfactant concentration of 0.3 wt% is shown in Fig. 8. Their buoyancy caused the produced MS to float up. The average diameter and coefficient of variation of produced MS was 31.6 μm and 0.63%, respectively, which shows their monodispersibility.

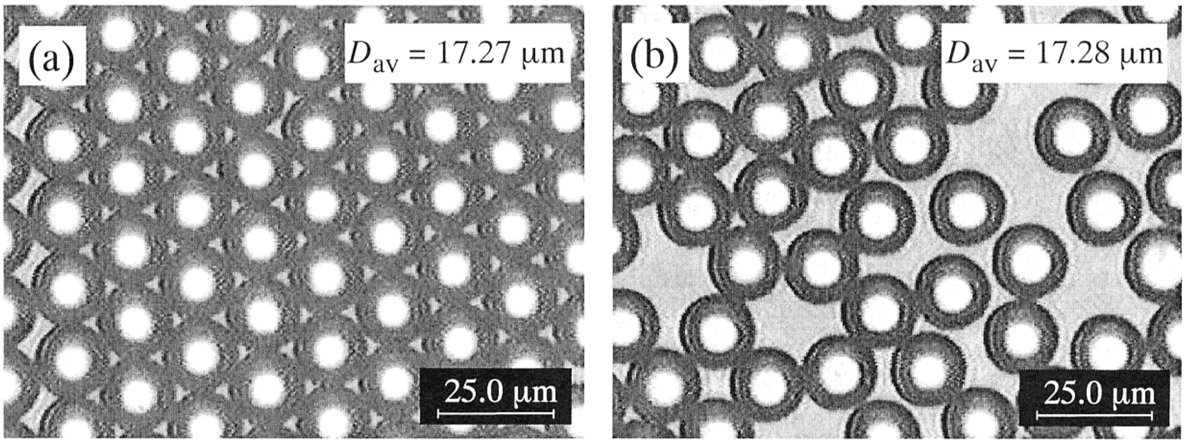

Fig. 6. Images of produced O/W-MS: (a) MS just after production, (b) MS after 60 days; D_{av}, average MS diameter.

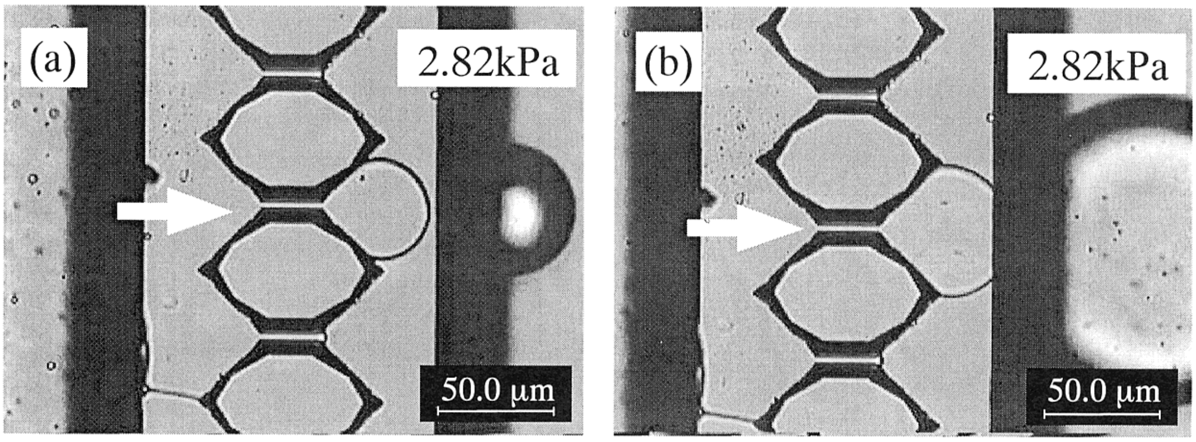

Fig. 7. Behavior of O/W-MS production using MC plate of 8.5 μm in equivalent diameter: (a), (b) coalescence and expansion of MS.

The ratios of the average MS diameter to MC equivalent diameter were determined to be 3.26 and 3.71 in MC plates of 5.3 μm and 8.5 μm, respectively. Although the effective channel number and the production rate increased with increase in operating pressure, less than 10% of the channels worked at most. From the above results, it was found that the vertical MC apparatus is effective for the continuous production of monodispersed MS with several tens of micrometers in size.

Theoretical evaluation of breakthrough pressure In a series of processes in which one phase is pressing the other phase which has initially filled the cylindrical capillary, the radius of curvature reaches a minimum and thus gives maximum pressure difference theoretically when the height of the growing droplet is equal to the radius of capillary pore (Fig. 9). This concept was confirmed experimentally using a single cylindrical capillary (Peng & Williams, 1998). The breakthrough pressure should be higher than the maximum pressure difference (Peng & Williams, 1998). The average pore diameter is substituted for the radius of curvature in Eq. (2), and the breakthrough pressure is represented on the basis of the equation of Young-Laplace as follows (Peng & Williams, 1998):

$$P_{\mathrm{BT}}=4\gamma/D_{\mathrm{p}} \tag{3}$$

where P_{BT} is the breakthrough pressure [Pa], γ is the interfacial tension [N/m] and D_{p} is the average pore diameter [m].

Triolein solutions with 0.1 wt% to 1.5 wt% sorbitan monolaurate were used as the oil phase, and the standard MC plate with 5.3 μm in equivalent diameter was used in this section. The relationship between the breakthrough pressure and the interfacial tension is shown in Fig. 10. When the MC equivalent diameter (5.3 μm) was substituted for pore diameter in Eq. (3), the theoretical breakthrough pressure was determined to be as follows:

$$P_{\mathrm{BT,t1}}=4\gamma/D_{\mathrm{eq}}=7.55\times10^{2}\gamma \tag{4}$$

where $P_{\mathrm{BT,t1}}$ is the theoretical breakthrough pressure based on the inside of the channel [Pa] and D_{eq} is the MC equivalent diameter [m].

As shown in Fig. 10, there is a great difference between the theoretical and the experimental values. The shape around the

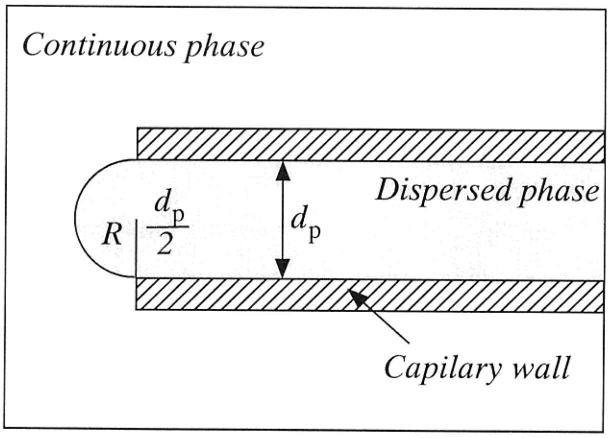

Fig. 9. Model for pressure equilibrium at outlet of capillary.

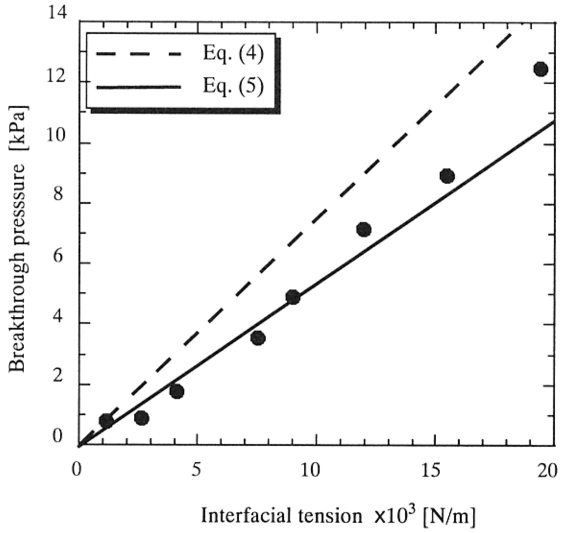

Fig. 10. Relationship between interfacial tension and breakthrough pressure for MS production.

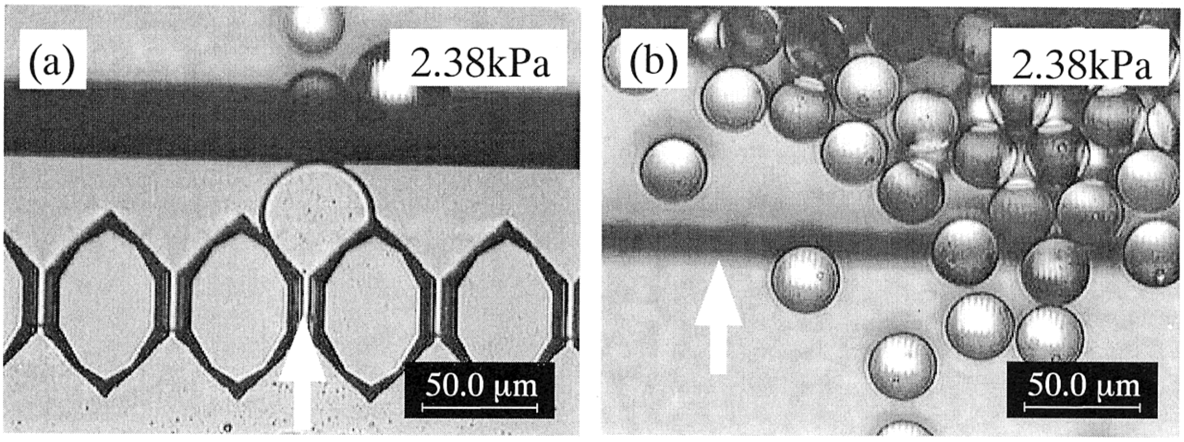

Fig. 8. Behavior of O/W-MS production using vertical MC: (a) Breakthrough of MC and O/W-MS production, (b) monodispersed MS.

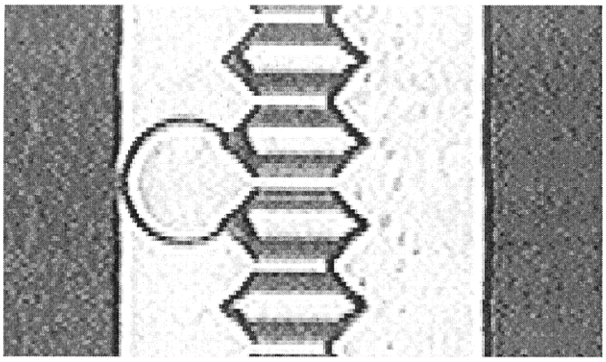

Fig. 11. Image of channel bank for standard MC plate.

channel differs from the cylindrical capillary, which may affect the breakthrough pressure. In MC emulsification, as shown in Fig. 11, the interface attached to the channel-outlet end just before the MS was produced. For this case, the effective equivalent diameter can be determined at the channel-outlet end instead of the MC equivalent diameter. The effective diameter of the standard MC was calculated to be 7.4 μm. If the effective equivalent diameter was substituted for the pore diameter in Eq. (3), the theoretical breakthrough pressure can be established as follows:

$$P_{BT,t2}=4\gamma/D_{eff}=5.41\times10^2\gamma \quad (5)$$

where $P_{BT,t2}$ is the theoretical breakthrough pressure based on the channel-outlet end [Pa] and D_{eff} is the effective equivalent diameter [m].

This theoretical line based on Eq. (5) was found to have relatively good correlation with the experimental value as shown in Fig. 10. The structure around the channel-outlet seems to be an important factor for the breakthrough pressure.

Conclusion

The production and characterization of the monodispersed MS by MC emulsification were investigated using sorbitan monolaurate as surfactant. Using the standard MC of 5.3 μm in equivalent diameter, MS with an average diameter of about 20 μm were obtained. The coefficient of variation depended on surfactant concentration, and its optimum concentration to produce the monodispersed MS with coefficient of variation below 2% and without outflow of the oil phase through channels was 0.3 wt%. The average particle diameter and the coefficient of variation of MS after 60 days changed little compared to those just after production, and the monodispersed MS were stable against coalescence for a long period. A MC of 8.5 μm in equivalent diameter was applied to produce the monodispersed MS. It was found that horizontal MC type gave irregular-sized MS, but the vertical MC made the MS production successful. It was able to continuously produce MS by utilizing buoyancy which allowed them to leave the MC easily; average MS diameter and coefficient of variation were 31.6 μm and 0.63%, respectively. MS size was found to be 3.3–3.7 times the MC diameter. The interfacial tension decreased with the increase in surfactant concentration, causing reduction in breakthrough pressure. It was also found that the breakthrough pressure was well simulated using the size of the channel-outlet end instead of the MC equivalent diameter. Both the MC equivalent diameter and the structure of the channel-outlet were shown to be important parameters for the MS production.

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