Chapter 3

Preparation of Lipid Microspheres on a Micron-Scale Using Microchannel Emulsification and Solvent Evaporation

3.1 Introduction

A solvent evaporation method has been extensively studied as a potential one to prepare biodegradable polymer (O'Donnell and McGinity, 1997; Cleland, 1998) and lipid (Reithmeier *et al.*, 2001) based misrospheres (MS) as colloidal carriers for drug delivery systems (DDS). The MS preparation by solvent evaporation can be achieved by diffusion of the organic solvent in emulsion droplets into the continuous phase and its evaporation at the continuous phase fluid/air interface, as illustrated in Fig. 3.1 (O'Donnell and McGinity, 1997). In particular, lipid MS prepared using triglycerides as the matrix material have received much attention in recent years due to their excellent biocompatibility (Reithmeier *et al.*, 2001).

The MS size distribution of emulsions greatly influences the size distribution of MS prepared through solvent evaporation. Emulsions produced using conventional emulsification devices exhibit considerable polydisperse. It is therefore difficult to prepare MS with narrow sizes distributions by solvent evaporation of the emulsions. Membrane emulsification and subsequent solvent evaporation allowed the preparation of relatively monodisperse, biodegradable polymer MS with coefficients of variation lower than 30% (Shiga *et al.*, 1996; Ma *et al.*, 1999). A novel microchannel (MC) emulsification proposed by Kawakatsu *et al.* (1997) can produce monodisperse MS with coefficients of variation below 5% (Kobayashi *et al.*, 1999). We have reported successful monodisperse MS production by MC emulsification using different liquid and solid triglycerides (Kawakatsu *et al.*, 1997; Sugiura *et al.*, 2000; Kobayashi *et al.*, 2001).

In the present chapter, we attempted to prepare micron-scale lipid MS with narrow size distributions by applying the MC emulsification and solvent evaporation techniques. We also investigated the effects of the organic solvent type, the surfactant type, and the lipid concentration in the dispersed phase on the MC emulsification and subsequent solvent evaporation processes.

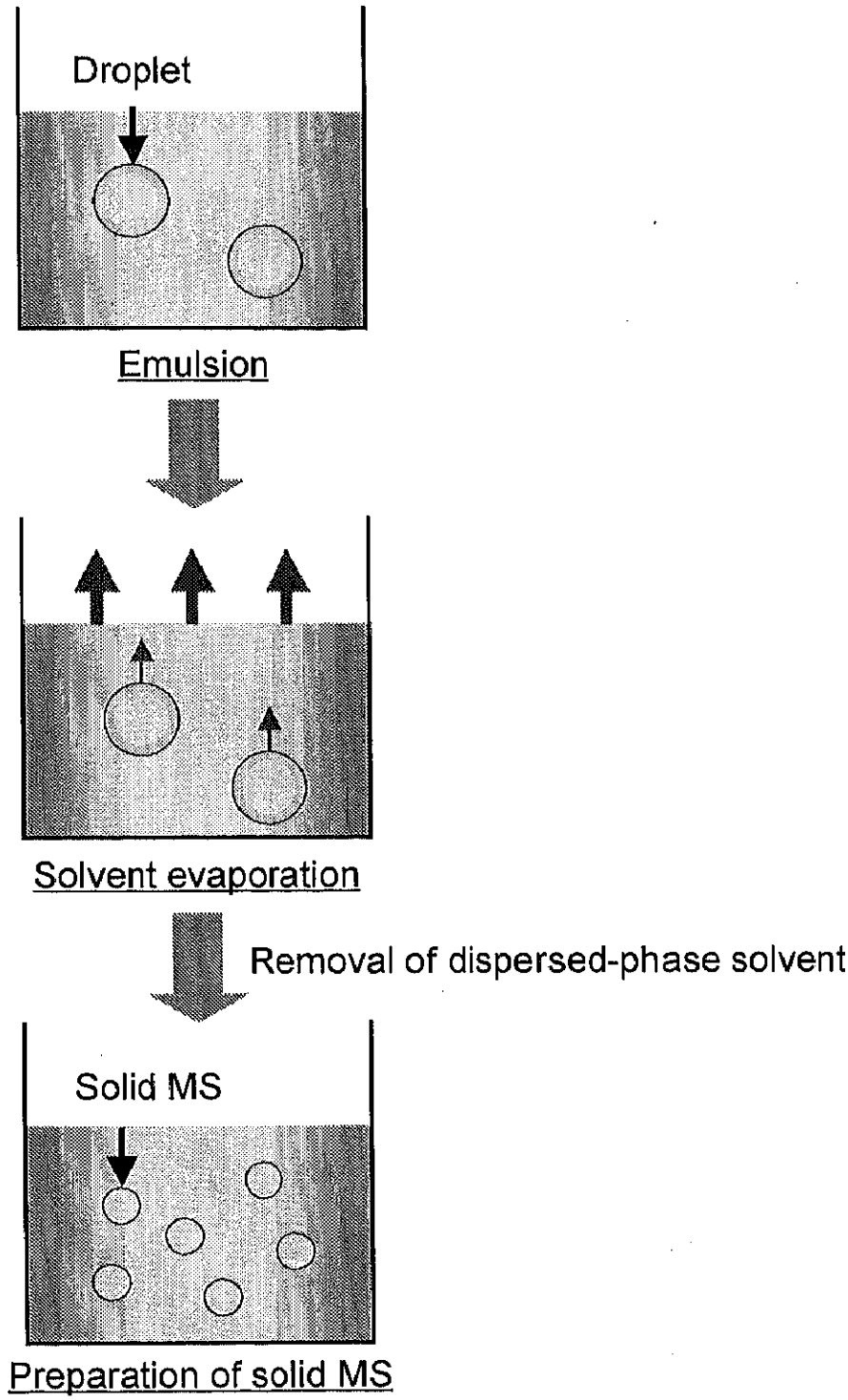

Fig. 3.1 Schematic of solvent evaporation process.

3.2 Materials and Methods

Materials

Ethyl acetate and hexane (Wako Pure Chemical Ind., Osaka, Japan) were used as the organic solvents in the dispersed phase. They have lower toxicity than the organic solvents that were generally used in the solvent evaporation method, e.g., methylene chlorides. Tripalmitin (Wako Pure Chemical Ind., Osaka, Japan), which is the lipid matrix, was dissolved into the organic solvent under given concentrations at 35°C. MilliQ water (Millipore Co., Massachusetts, USA) was used to prepare all of the continuous phase solutions. Sodium dodecyl sulfate [SDS, hydrophilic lipophilic balance (HLB): 40] (Wako Pure Chemical Ind., Japan) and pentaglycerol monolaurate [PGM, >65% purity; Sun soft A-121E; HLB: 12] (Taiyo Kagaku Co., Ltd., Mie, Japan) were dissolved in the continuous phase at a concentration of 1.0 wt.% as the surfactants. PGM was of food additive grade and all the other chemicals were of reagent grade.

Experimental setup

The silicon cross-flow MC plate, depicted in Fig. 3.2, measures 24 mm×24 mm×0.4 mm, with two 1.5-mm diameter holes as the inlet and outlet to flow the continuous phase. A total of 600 channels of uniform size is precisely fabricated on the two terrace lines with a 100-μm height and a 14-μm length. The MC used in this chapter has a 12-μm channel width and a 2-μm channel depth, and the terrace at the channel outlet has a 32-μm terrace width and a 25-μm terrace length. The hydrophilic surface of the MC plate is achieved by oxidization process and subsequent 0.1N nitric acid treatment. Details of the cross-flow MC plate are described in previous papers (Kawakatsu *et al.*, 1999; Sugiura *et al.*, 2001). Figure 3.3 schematically illustrates the experimental setup (Kawakatsu *et al.*, 1997, 1999), which consists of a MC module, two syringe pumps (505U, Watson-Marlow Ltd., , UK) to feed the dispersed phase and the continuous phase, and a microscope video system. The microscope video system developed by Kikuchi *et al.* (1992) allows visual

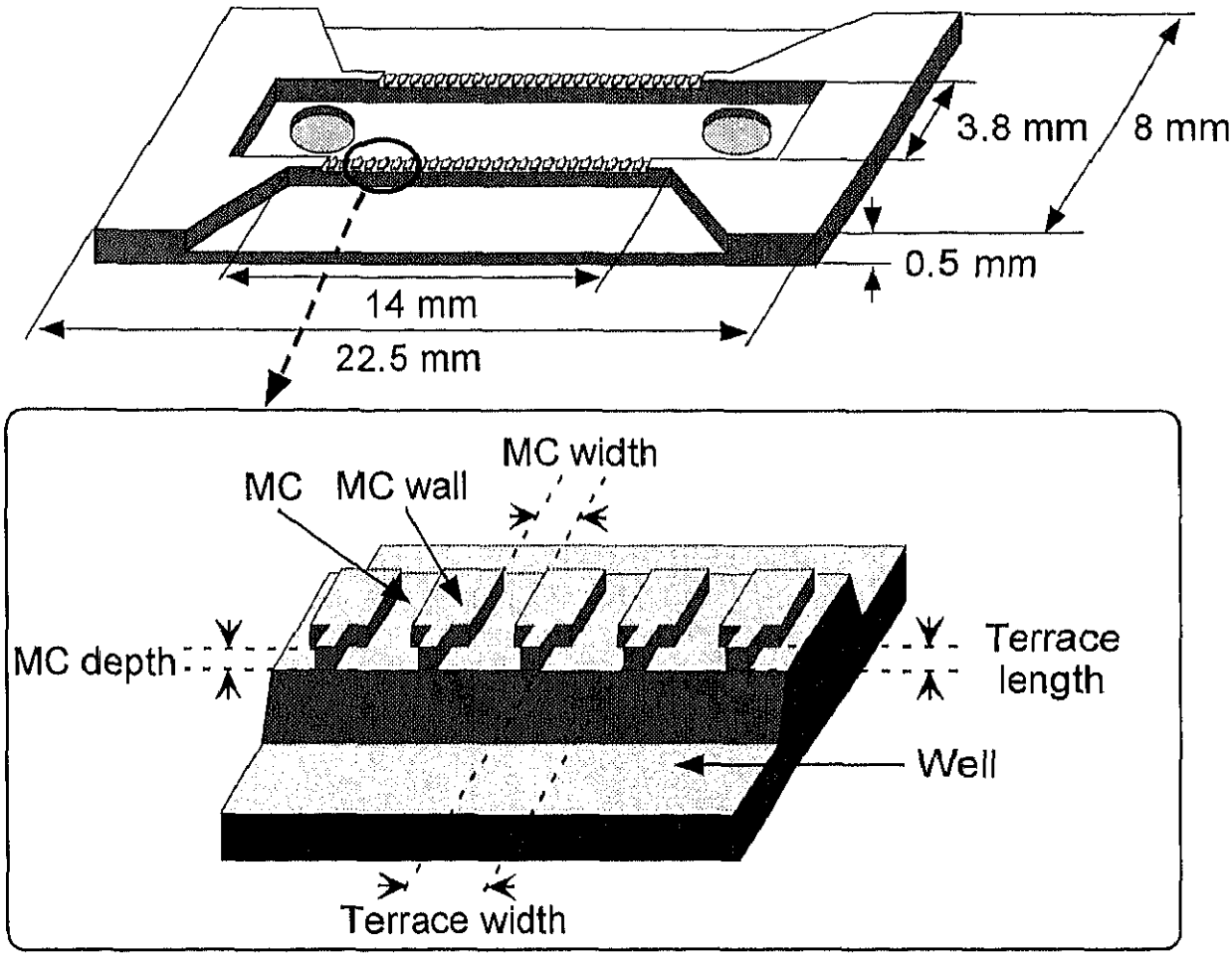

Fig. 3.2 Schematic drawing of silicon cross-flow MC plate.

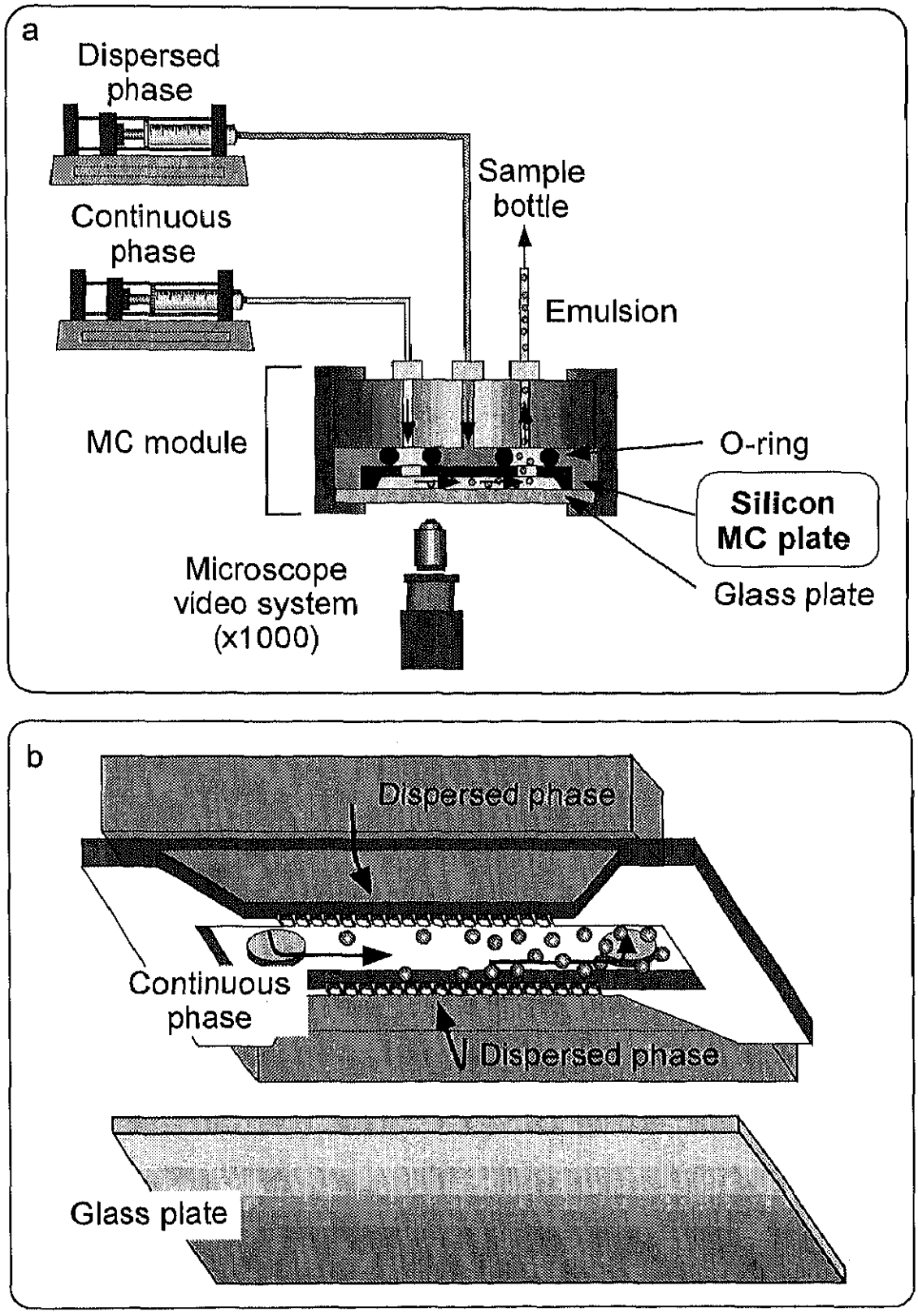

Fig. 3.3 MC emulsification system. (a) Experimental setup; (b) schematic flow in MC module.

observations of the emulsification behavior on a monitor with a total magnification of 1000.

Experimental procedures

The inverted MC plate was tightly attached onto an optically flat glass plate in the MC module, which was initially filled with the continuous phase. The dispersed phase, fed into the module, intruded the space between the MC plate and the glass place. Oil-in-water (O/W)-MS were then formed by forcing the dispersed phase into the continuous phase through the channels. The formed O/W-MS were recovered by the controlled continuous-phase flow. Solvent evaporation of the resultant O/W-MS was conducted under atmospheric pressure and room temperature for 24h.

Measurements and analysis

An analysis of the droplet formation behavior during MC emulsification was carried out using video images taken with the microscope video system. The number-average particle diameters and coefficients of variation of the O/W-MS just after formation from the channels were determined by analyzing images of 200 particles, captured on a personal computer, using an image-processing software (WinRoof, Mitani Co., Ltd, Fukui, Japan). The definition of the coefficient of variation was described in Chapter 2.2. The number-average particle diameter and coefficient of variation of the recovered O/W-MS were measured using a laser diffraction particle size analyzer (SALD-200V-ER, Shimadzu Co., Kyoto, Japan). Measurements were repeated at least two times, and mean values were calculated.

3.3 Results and Discussion

Effect of solvent type on MC emulsification

Two different organic solvents, ethyl acetate and hexane, were examined to investigate the effect of the dispersed-phase solvents on MC emulsification. The

surfactant used in this section was SDS with a marked ability to stably form monodisperse O/W-MS in MC emulsification (Tong *et al.*, 2000). The results are shown in Table 3.1 with the preparation conditions. Figures 3-4a and b depict microscopic photographs of the MC emulsification process using ethyl acetate as the dispersed-phase solvent. Uniformly sized O/W-MS were formed from the channels at a dispersed-phase flow rate of 0.7 ml/h (Fig. 3.4a), although the channel surface was wetted partially by the dispersed phase. The average particle diameter and coefficient of variation of the formed O/W-MS were 10.9 μm and 15.4%, indicating that they have a relatively narrow size distribution. However, a slight increase in the dispersed-phase flow rate resulted in the formation of larger MS with a broad size distribution (Fig. 3.4b). That is, MC emulsification using the ethyl acetate-containing system yields O/W-MS with narrow size distributions in a limited narrow range of the dispersed-phase flow rates. We therefore found that ethyl acetate is not a suitable dispersed-phase solvent for MC emulsification. The hexane-containing system also exhibited the formation of uniformly sized O/W-MS from the channels at a dispersed-phase flow rate of 0.8 ml/h (Fig. 3.4c). The successful MC emulsification without wetting of the dispersed phase on the channel surface was observed under a wide range of the dispersed-phase flow rates. The resultant O/W-MS had the average particle diameter and coefficient of variation of 10.8 μm and 7.1%; they have a narrower size distribution than those formed using the ethyl acetate-containing system. A microscopic photograph of the formed O/W-MS (Fig. 3.4d) confirms their excellent monodispersity.

Difference in the MS formation behavior between the ethyl acetate- and hexane-containing systems can be explained as follows. The hydrophobicity of the dispersed phase significantly affects the O/W-MS formation behavior in MC emulsification. The use of the dispersed phase with high hydrophobicity tends to prevent its wetting on the channel surface, leading to the formation of O/W-MS with narrow size distributions. Hexane has much higher hydrophobicity than ethyl acetate. We consider that low hydrophobicity of ethyl acetate caused partial wetting of the dispersed phase on the channel surface, which results in unstable MC emulsification. The preceded results

Table 3.1 Preparation conditions and results at different organic solvents, surfactants, and lipid concentrations.

Preparation conditions	Solvent (O)	Ethyl acetate	Hexane	Hexane	Hexane	Hexane
	Lipid matrix (O)[a]	None	None	Tripalmitin (1.0 vol.%)	Tripalmitin (1.0 vol.%)	Tripalmitin (0.1 vol.%)
	Surfactant (W)[b]	SDS	SDS	SDS	PGM	PGM
Results	d_{av} (μm)[c]	10.9[f]	10.8	10.7	11.1	10.5
	CV (%)	15.4[f]	7.1	7.1	6.3	5.7
	d_{av} (μm)[d]	-	-	2.8	2.4	1.4
	CV (%)	-	-	17.8	22.3	20.3
	$d_{av,\ calc}$ (μm)[e]	-	-	2.3	2.4	1.5

[a]Dissolved in the dispersed oil phase at given concentrations.

[b]Dissolved in the continuous water phase at a concentration of 1.0 wt.%.

[c]Average diameter of the MS formed by MC emulsification.

[d]Average diameter of the MS after solvent evaporation.

[e]Calculated average diameter of the MS after solvent evaporation.

[f]Partial wetting of the dispersed phase on the channel surface.

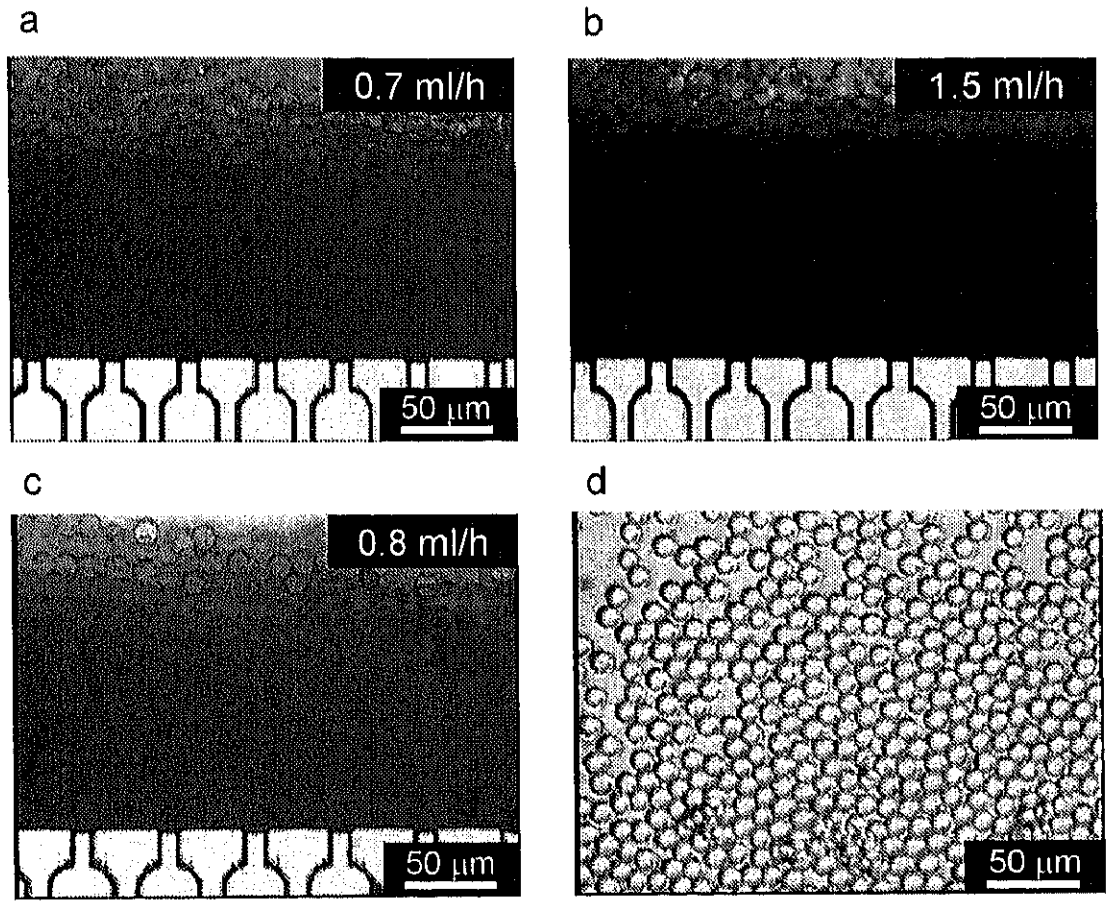

Fig. 3.4 Microscopic photographs of the MC emulsification process. (a), (b) Droplet formation for ethyl acetate / water (1.0 wt.% SDS) system; (c) droplet formation for hexane / water (1.0 wt.% SDS) system; (d) O/W-MS formed for hexane / water (1.0 wt.% SDS) system.

demonstrate that hexane is an appropriate dispersed-phase solvent for forming O/W-MS with narrow size distributions by MC emulsification.

Effect of surfactant type and lipid concentration on MC emulsification and subsequent solvent evaporation

The MC emulsification and subsequent solvent evaporation characteristics using two different surfactants, SDS and PGM, was first investigated. PGM was selected as the surfactant considering its biocompatibility and marked O/W emulsification ability. A hexane solution with 1.0 vol.% tripalmitin was used as the dispersed phase. The dispersed-phase flow rate was kept at 1.0 ml/h after droplet formation initiated. The stable formation of uniformly sized O/W-MS from the channels for the SDS-containing system verifies that addition of tripalmitin in the dispersed phase did not affect the MC emulsification behavior. Successful MC emulsification that stably yields uniformly sized O/W-MS was also observed for the PGM-containing system. While a nonionic surfactant such as PGM has no strong repulsion with the channel surface, the hydrophilicity of the channel surface can be remained during MC emulsification (Tong *et al.*, 2000). Our result in this section suggests that this idea can also apply to the experimental systems with hexane as the dispersed-phase. The O/W-MS formed for SDS and PGM have the average particle diameters and coefficients of variation between 10.7 to 11.1 μm and 6.3 to 7.1%, which demonstrates their excellent monodispersity. We thus found that MC emulsification for the preceded experimental systems yields monodisperse O/W-MS in which the lipid matrix (tripalmitin) is loaded. In particular, monodisperse O/W-MS formed using the PGM-containing system are a potential precursor for preparing monodisperse lipid MS composed of biocompatible ingredients. The formed O/W-MS were recovered in a sample bottle, and then subjected to solvent evaporation.

The average particle sizes and coefficients of variation of the MS after solvent evaporation are presented in Table 3.1. The average particle diameters of the recovered MS decreased from 10.7 μm to 2.8 μm for SDS and from 11.1 μm to 2.4 μm for PGM

during solvent evaporation. This result indicates that the dispersed-phase solvent (hexane) successfully diffused into the continuous water phase and then evaporated at water/air interface. Microscopic photographs of the MS before and during solvent evaporation (Fig. 3.5) also confirm the decrease of their size by solvent evaporation. A decrease in the average particle diameters of the recovered MS became only minimally after 24 h of solvent evaporation (data are not shown). We believe that solvent evaporation of the recovered O/W-MS was completed within 24 h and then resulted in the preparation of lipid MS. The coefficients of variation of the recovered MS increased from 7.1 to 17.8% for SDS and from 6.3 to 22.3% for PGM during solvent evaporation. The prepared lipid MS have broader size distributions than the O/W-MS formed by MC emulsification, whereas they still had relatively narrow size distributions. It should be noted that the lipid MS prepared in this study do not include MS with considerably larger diameters than their average particle diameters, which is attributable to the unique MS formation process in MC emulsification. The above results in this section revealed that MC emulsification and subsequent solvent evaporation allows the preparation of micron-scale lipid MS with relatively narrow size distributions.

The effect of the tripalmitin concentrations in the dispersed phase on the MC emulsification and subsequent solvent evaporation characteristics was also investigated. The detailed preparation conditions are presented in Table 3.1. The results for 1.0 vol.% tripalmitin in hexane are described in detail in the above section. MC emulsification for 0.1 vol.% tripalmitin in hexane stably yielded O/W-MS with the average particle diameter of 10.5 μm and the coefficients of variation of 5.7%, similarly like those formed for 1.0 vol.% tripalmitin in hexane. Subsequent solvent evaporation of the recovered O/W-MS for 0.1 vol.% tripalmitin in hexane decreased their average particle diameter down to 1.4 μm and increased their coefficient of variation up to 20.3%, resulting in the preparation of lipid MS. It was observed that the solvent evaporation process leads to a significant decrease of the average particle sizes of the MS by 74% for 1.0 vol.% tripalmitin in hexane and 87% for 0.1 vol% tripalmitin in hexane. This result let us to find that the tripalmitin

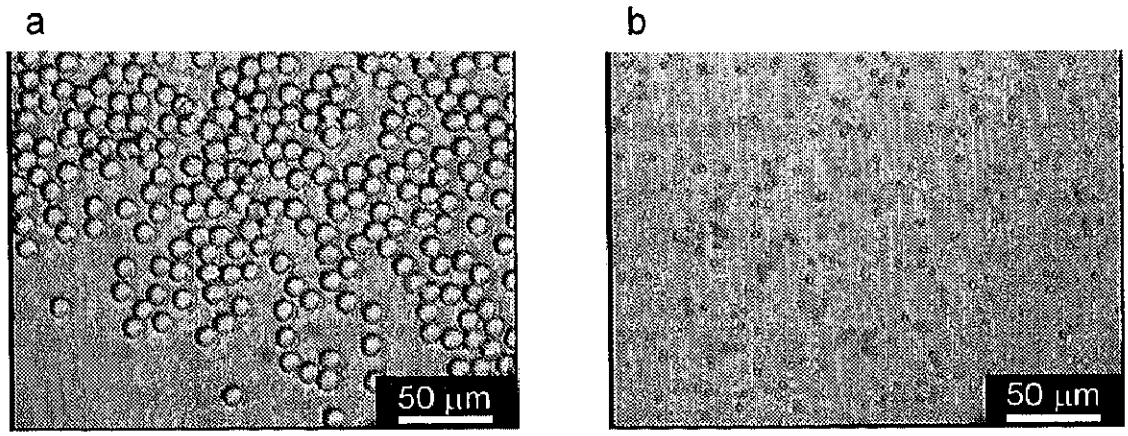

Fig. 3.5 Microscopic photographs of the solvent evaporation process for hexane (1.0 vol.% tripalmitin) / water (1.0 wt.% PGM) system. (a) O/W-MS formed by MC emulsification (b) O/W-MS subjected to solvent evaporation for 12 h.

concentration in the dispersed phase provides a remarkable effect on the average particle sizes of the resulting lipid MS. In contrast, there was no significant difference in their coefficients of variation between 0.1 and 1.0 vol.% tripalmitin in hexane. We calculated the average particle diameter of the lipid MS after solvent evaporation assuming that one dispersed-phase droplet will prepare one lipid MS and that the solvent is completely evaporated. The data in Table 3.1 shows that the calculated average particle diameter of the lipid MS after solvent evaporation corresponds well to the average particle sizes of the prepared lipid MS for both the tripalmitin concentrations in the dispersed phase. The calculated average particle diameter of lipid MS thus enables us to estimate the average particle diameter of the resultant lipid MS after solvent evaporation in the present study. Additionally, size-control of the lipid MS prepared by MC emulsification and subsequent solvent evaporation can be attained by using different tripalmitin concentrations.

3.4 Conclusions

In the present chapter, we have demonstrated that micron-scale lipid (tripalmitin) MS with relatively narrow size distributions were prepared using the MC emulsification and subsequent solvent evaporation processes. Stable MC emulsification for the hexane-containing system demonstrated that hexane is a suitable dispersed-phase solvent for stably forming O/W-MS with narrow size distributions by MC emulsification. In contrast, the use of ethyl acetate with low hydrophobicity as the dispersed-phase solvent caused unstable MC emulsification with partial wetting of the dispersed phase on the channel surface. Solvent evaporation of monodisperse O/W-MS composed of tripalmitin in hexane as the dispersed phase was successfully performed, leading to the preparation of micron-scale lipid MS with relatively narrow size distributions. In particular, lipid MS prepared using the PGM-containing system is considered to be a promising biocompatible material. The average particle diameter of the prepared lipid MS depended on the tripalmitin concentration in the dispersed phase and can be estimated by using their calculated average particle diameter after solvent evaporation.