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Study of types and mixture ratio of organic solvent used to dissolve polymers for preparation of drug-containing PLGA microspheres

Fuminori Ito [a,b,c,*], Hiroyuki Fujimori [b], Hiroyuki Honnami [b], Hiroyoshi Kawakami [a], Kiyoshi Kanamura [a], Kimiko Makino [b,c,d]

[a] Department of Applied Chemistry, Graduate School of Urban Environmental Sciences, Tokyo Metropolitan University, 1-1 Minami-ohsawa, Hachioji, Tokyo 192-0397, Japan
[b] Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan
[c] Center for Drug Delivery Research, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan
[d] Institute of Colloid and Interface Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan

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ABSTRACT

The effects of the types and the ratios of various organic solvents used as a mixtures to dissolve poly (lactide-co-glycolide) (PLGA) by using a solvent evaporation method, a technique used to prepare polymer particles, were carefully studied in order to investigate their advantages in developing drug delivery system (DDS) formulations for the prepared microspheres. The particle size and drug loading efficiency of drug-containing PLGA microspheres were found to be dependent on the types of solvent used due to the interfacial tension between the organic solvent and water phase. The drug loading efficiency of monodisperse microspheres prepared by using a membrane emulsification technique employing organic solvents and high interfacial tension for dissolving the PLGA was increased in a controlled manner. The organic solvents with high interfacial tension in the water phase used for the preparation of polymer particles by means of the solvent evaporation method were found to be suitable in terms of improvement in the properties of DDS formulations.

1. Introduction

Particles composed of poly (lactide-co-glycolide) (PLGA) have been used in various fields in life sciences, such as biomedicine, bioscience, biomaterial, and drug delivery systems (DDS) [1–3]. Such particles are generally fabricated by means of solvent evaporation, solvent diffusion, coacervation, spray drying etc. [4–5]. With regard to the preparation of drug-containing PLGA microspheres by using a solvent evaporation method, we have been examining the control of the particle size, monodispersity, drug loading efficiency, and drug releasing behaviors of the microspheres [6–9]. To begin with, the physicochemical factors that must be controlled during the preparation of drug-containing PLGA microspheres include the viscosity of the prepared emulsion [6], ratio of the size of the droplets in w_1/o and $w_1/o/w_2$ emulsions [7] and addition of polyethylene glycol (PEG) as a co-dispersant [8]. We have established the Shirasu porous glass (SPG) membrane emulsification technique for preparing drug-containing monodisperse PLGA microspheres [8–9]. In these examinations, the organic solvent used in the oil phase was almost dichloromethane (DCM). Some advantages and properties of other organic solvents, such as acetone and ethyl acetate, which are used to prepare PLGA particles, have been reported [10–12]. In this study, the properties of drug-containing PLGA microspheres prepared using acetone, ethyl acetate, dichloromethane, and chloroform were carefully evaluated. In addition, the properties of drug-containing PLGA microspheres prepared by varying the mixture ratio

* Corresponding author. Address: Department of Applied Chemistry, Graduate School of Urban Environmental Sciences, Tokyo Metropolitan University, 1-1 Manami-ohsawa, Hachioji, Tokyo 192-0397, Japan. Tel./fax: +81 4 2677 2830.
E-mail address: fuminoito@spice.ocn.ne.jp (F. Ito).

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of dichloromethane and chloroform were examined. The drug-containing PLGA microspheres prepared by using various organic solvents or by varying the mixture ratio were possessed several advantages. From the data obtained, it was examined whether or not the microspheres could be applied to an already established membrane emulsification technique. The information obtained from these examinations is reported.

2. Experimental

2.1. Materials

PLGA7505 (poly (lactic-co-glycolide), 75:25, Mw 5000), which was purchased from Wako Pure Chemical Industry, Japan, was stored at −80 °C prior to use. Anti-tuberculosis drug, rifampicin (RFP), which was purchased from SIGMA, was used as a hydrophobic model drug to be loaded into the PLGA microspheres. Blue dextran (BLD), which was purchased from Amersham Biosciences Corporation, was used as a hydrophilic model drug to be loaded into the PLGA microspheres. Polyvinyl alcohol (PVA) with a degree of polymerization of 500 and saponification of 86–90 mol% and purchased from Wako Pure Chemical Industry, Japan, was used as a dispersant for the prepared particles and emulsion. Polyethylene glycol (Wako Pure Chemical Industry, Mw 20,000) was used as a co-dispersant to be added into the PVA solution. Sunsoft 818H, which was offered by Taiyou Kagaku Co., Ltd., Japan, was used as an emulsifier dissolved in the oil phase. The other chemicals used were of the highest grade commercially available.

2.2. Preparation of RFP/PLGA microspheres by using the conventional solvent evaporation method

PLGA (500 mg) and RFP (50 mg) were dissolved with the prescribed organic solvent (10 mL) in a 10.0-mL screw-cap tube. After mixing in a vortex mixer, the solution was used in the oil phase. This oil phase was poured into 190 mL of a continuous phase of PVA solution in a 200-mL beaker and was homogenized at 10,000 rpm by using a micro homogenizer (NS-310E; Microtec Nition Co. Ltd.) to prepare an oil-in-water (o/w) emulsion. The prepared oil-in-water (o/w) emulsion was transferred into a 300-mL beaker containing 100 mL of PVA solution. Solvent evaporation was performed at room temperature at 250 rpm for 4 h to prepare the polymer microspheres. The polymer microspheres were collected by centrifugation for 10 min at 1500 rpm. These microspheres were washed thrice in the following manner: the separation between the water phase and the particles by performing centrifugation at 1500 rpm for 3 min, re-dispersion of particles with fresh distilled water and then re-centrifugation.

2.3. Preparation of RFP/PLGA microspheres by using the SPG membrane emulsification technique

PLGA (500 mg) and RFP (50 mg) were dissolved with dichloromethane (DCM) or chloroform (CR) in a 10.0-mL screw-cap tube. After mixing in a vortex mixer, the solution used in the oil phase was injected into an oil tank used for Shirasu porous glass (SPG) membrane emulsification. The apparatus was soaked into 190-mL of PVA solution (continuous phase) containing PEG20000 0.026% solution in a 200-mL beaker, and it was precisely wetted between the continuous and dispersion phases of the SPG membrane by gentle stirring for 1 h. The membrane emulsification technique was initiated by streaming nitrogen gas into the oil phase through the pores in the SPG membrane. After the membrane emulsification was completed, the prepared oil-in-water (o/w) emulsion was transferred to a 300-mL beaker containing 100 mL of PVA solution. Solvent evaporation was performed at room temperature at 250 rpm for 4 h. After these suspensions were passed through a 22-μm sieve, monodisperse polymer microspheres were collected by performing centrifugation for 10 min at 1500 rpm. The microspheres were washed thrice in the following manner: re-dispersion of the particles with fresh distilled water after separation from the water phase and then re-centrifugation.

2.4. Preparation of BLD/PLGA microspheres

In the conventional solvent evaporation method, 500 mg of PLGA and 100 mg of Sunsoft 818H were dissolved in a solvent of dichloromethane (DCM) or chloroform (CR) in a 10-mL screw-cap tube. Blue dextran (BLD) solution in an inner-water phase was poured into the solution in the oil phase. A water-in-oil (w_1/o) emulsion was prepared by using a micro homogenizer (NS-310E; Microtec Nition Co. Ltd.) and by stirring at 30,000 rpm for 90 s in a 20-mL screw-capped tube. Subsequently the generated droplets were further homogenized by using an ultra sonic homogenizer (UH-50, SMT Co.) for 90 s. The prepared w_1/o emulsion was subsequently added to 290 mL of 1.00% (w/v) PVA solution, and the emulsion was stirred at 10,000 rpm for 90 s to prepare a water-in-oil-in-water ($w_1/o/w_2$) emulsion. Then, BLD/PLGA microspheres were prepared by solvent evaporation by stirring at 250 rpm for 4 h at room temperature.

In the SPG membrane emulsification technique, 500 mg of PLGA and 100 mg of Sunsoft 818 H dissolved with dichloromethane (DCM) or chloroform (CR) were used in the oil phase. Blue dextran solution in an inner-water phase (water phase 1) was poured into this oil phase. A water-in-oil (w_1/o) emulsion was prepared by using a micro homogenizer at 30,000 rpm for 90 s and further homogenized by using an ultra sonic homogenizer for 90 s in a 20-mL screw-cap tube. The prepared w_1/o emulsion was immediately transferred into an oil tank used for SPG membrane emulsification. The SPG membrane emulsification technique was initiated by streaming nitrogen gas into the oil phase in a 290 mL of 1.00% (w/v) PVA solution containing PEG20000 of 0.017% solution. After the $w_1/o/w_2$ emulsion was prepared by using the membrane emulsification technique, the BLD/PLGA microspheres were prepared by means of solvent evaporation at 250 rpm for 4 h at room temperature. The prepared microspheres were washed thrice in the following manner: re-dispersion of particles with fresh distilled

water after separation from the water phase and then re-centrifugation.

2.5. Measurement of the yield of the prepared microspheres

The extracted microspheres that could not pass through the 22-μm sieve were collected by filtration using a filter paper, and they were dry-weighed thoroughly. The yield of the microspheres is calculated from Eq. (1).

$$\text{Yield} = \frac{w_i - w_e}{w_i} \times 100 \quad (1)$$

where w_i denotes the initial weight of the microspheres, and w_e denotes the weight of the microspheres that could not pass through the 22-μm sieve.

2.6. SEM observations of the microspheres

A droplet of the suspension of the prepared microspheres was placed on an aluminum sample stage and was dried for 1 day in a vacuum desiccator. Platinum sputtering was performed using an ion sputtering device (Auto Fine Coater, JFC-1600, JEOL Ltd.). Microscopic observations of the microspheres were performed by means of scanning electron microscopy (SEM, JSM-6060LA, JEOL Ltd.). The images of the particles were observed 1000 times.

2.7. Measurements of the particle sizes

Two techniques were adopted for the measurement of the particle sizes of the prepared microspheres. The volume-averaged diameter of the microspheres and their size distribution were measured by using a light-scattering particle sizer (Malvern 3601, Mastersizer/E, Malvern, Inc.). The size distribution was evaluated on the basis of a Span value defined as follows.

$$\text{Span} = \frac{D_{90\%} - D_{10\%}}{D_{50\%}} \quad (2)$$

where $D_{N\%}$ (N = 10, 50, 90) demonstrates that the volume percentage of microspheres with diameters up to $D_{N\%}$ is equal to N%. A small Span value implies a narrow size distribution.

The average diameter and the value of coefficient of variation (CV) for the prepared microspheres were calculated for 200 particles on the obtained SEM image. The value of CV is calculated by using Eq. (3).

$$\text{CV} = \frac{\sigma}{D_p} \times 100 \quad (3)$$

where σ denotes the standard deviation, and D_p denotes the average particle diameter obtained by SEM observations. A low value of CV corresponds to uniform-sized particles.

2.8. Measurement of the interfacial tension between the organic solvent and water phase

Distilled water and organic solvent at 1:1 ratio per volume of the solution were stored for 3 h on the desk in a 500-mL beaker. A certain amount of the water phase in the solution was then extracted. The preparative oil phase was measured at 25 °C by using an interfacial tension meter (Tensiometer K12, Krüss GmbH, Germany).

2.9. Determination of the partition coefficient of RFP between the organic solvent and water

For 5 mg of RFP, dichloromethane (DCM) and chloroform (CR) were mixed in a stepwise manner up to the prescribed volume. Distilled water (5.0 mL) was added into these solutions and mixed with a vortex mixer. The samples were then stirred 60 times/min for 40 h at 25 °C in a water bath shaker. After shaking, the samples were stored on the desk for 1 h at 25 °C, and the supernatant used to measure the amount of RFP partitioned into the water phase was spectrophotometrically investigated at 475 nm.

2.10. Measurements of the drug loading efficiency in the PLGA microspheres

During the measurements of the drug loading efficiency of RFP in the microspheres, 10 mg of RFP/PLGA microspheres were dissolved in 5.0 mL of chloroform in a 10.0-mL screw-cap tube. The RFP concentration in the solution was spectrophotometrically measured at 475 nm. By combining this RFP concentration and the initial ratio of the composition for the preparation of the particles, the loading efficiency of RFP in the microspheres was calculated. The measurements were performed at n = 3.

For the measurement of the loading efficiency of BLD in the PLGA microspheres, 4 mL of DCM was added into the dried blue dextran-loaded PLGA microspheres and soaked in a field of ultrasonic waves to dissolve the PLGA completely. Blue dextran was precipitated by performing centrifugation, and the supernatant was removed. After extracting the DCM by drying for 1 day in a room atmosphere, 5.0 mL of distilled water was added. The concentration of blue dextran was spectrophotometrically measured at 620 nm.

2.11. Release study

In the release study of the RFP/PLGA microspheres, 10 mg of the prepared microspheres was dispersed in 5.0 mL of phosphate buffer solution (PBS) having a pH of 7.4 and an ionic strength of 0.154 M in a 10-mL screw-cap tube. The dispersion solution was stirred 60 times/h at 37 °C. After 1 h, the dispersions were centrifuged for 5 min at 2000 rpm, and the supernatant was spectrophotometrically measured at 475 nm. The PBS was changed every hour after performing the measurements. The measurements were performed for a total of 6 h by sampling every hour.

In the release study of the BLD/PLGA microspheres, 20 mg of the prepared microspheres were dispersed in 5.0 mL of PBS having a pH of 7.4 and an ionic strength of 0.154 M in a 10-mL screw-cap tube. The dispersion solution was stirred under the same conditions (60 times/h at 37 °C) as those for the RFP/PLGA microspheres. After 1 day, the dispersion solution was centrifuged for 5 min at 2000 rpm. The supernatant was then

spectrophotometrically measured at 620 nm. The PBS was changed every day after performing the measurements. The measurements were performed for a total of 10 days by sampling every day.

3. Result and discussion

3.1. Preparation of the RFP/PLGA microspheres by using the conventional solvent evaporation method along with various organic solvents

RFP/PLGA microspheres prepared using various organic solvents such as acetone (AC), ethyl acetate (EA), dichloromethane (DCM) and chloroform (CR) were examined, as shown in Table 1. The coagulum in the reaction system after solvent evaporation was not entirely presented. The yields of RFP/PLGA microspheres prepared in this experimental series were 100% because the particles were selected entirely by centrifugation. The particle size and the drug loading efficiency of the microspheres appeared to show dependency on the type of organic solvent used. As a reference examination, the value of an interfacial tension between the solvent used and water phase was measured, as shown in Table 1. The values of interfacial tension of dichloromethane (DCM) (20.4) and chloroform (CR) (31.4) were relatively higher than those of acetone (AC) (0) and ethyl acetate (EA) (6.78). The interfacial tension between acetone (AC) and water phase was not entirely presented. Apparently, the particle size of the microspheres increased with the interfacial tension in the water phase. The solvent with a low interfacial tension with water phase produced smaller-sized droplets during the preparation of the emulsion. The PLGA microspheres were generated by the solvent evaporated from the smaller-sized emulsion droplets. On the basis of this principle, Kawashima et al. prepared PLGA nanospheres with particle sizes of the order of 200 nm [10]. The water phase and PLGA solution (oil phase) dissolved in dichloromethane (DCM) and acetone (AC) were mixed, and generated the infinitesimal droplets of emulsion for a split second during the stirring. Then, the 200-nm PLGA nanospheres were produced by the diffusion of the organic solvent into the water phase (solvent diffusion method) [10]. This method is an interesting technique used for the preparation of nanospheres composed of the polymer. However, the drug-containing polymer nanospheres produced by using this technique exhibit poor drug loading efficiency because of absence of interfacial tension between the oil phase of the dissolved drug and the water phase. In fact, the drug loading efficiency of the RFP/PLGA microspheres prepared by using acetone (AC) was approximately 15.0%, as shown in Table 1. Thus, the drug loading efficiency of the RFP/PLGA microspheres is poor in comparison with that of the other microspheres prepared by using the other solvents. The low drug loading efficiency of the microspheres was a result of further transition to the water phase of the drug because of the better miscibility of water and the oil phase. The drug loading efficiency of the microspheres obtained from ethyl acetate with an interfacial tension of 6.78 was lower than that of the microspheres obtained from dichloromethane (DCM) and chloroform (CR). Ethyl acetate is preferred over dichloromethane and chloroform as a solvent because it is safe for humans as well as environment-friendly [11]. At high boiling points (76.7 °C) and low interfacial tension (6.78), the smell of the solvent in the reaction system remains after solvent evaporation. The smell appears to originate from the water phase and not from the prepared microspheres. Therefore, the low drug loading efficiency of the microspheres (41.9%) is due to the transition to the water phase of the drug dissolved in the oil phase. The value of the interfacial tension between water and the oil phase indicates that the type of organic solvent used in the solvent evaporation method affects the particle size and drug loading efficiency of the prepared PLGA microspheres.

Table 1
Preparation of RFP/PLGA microspheres by using various organic solvents in the conventional solvent evaporation method.

Sample	1	2	3	4
PLGA7505% (w/v)	5.00	5.00	5.00	5.00
RFP% (w/v)	0.50	0.50	0.50	0.50
AC (mL)	10.0	0	0	0
EA (mL)	0	10.0	0	0
DCM (mL)	0	0	10.0	0
CR (mL)	0	0	0	10.0
PVA% (w/v)	1.00	1.00	1.00	1.00
Distilled water (mL)	190	190	190	190
(In solvent evaporation) (mL)	(100)	(100)	(100)	(100)
Total volume (mL)	300	300	300	300
Homogenizer (rpm)	10,000	10,000	10,000	10,000
Time (s)	90	90	90	90
Particle size (μm)	3.31	4.27	6.49	7.06
Span	0.37	1.90	0.87	0.91
Drug loading efficiency (%)	14.7	41.9	68.2	90.4
Interfacial tension between solvent and water (mN/m)	0	6.78	20.4	31.4

3.2. Preparation of the RFP/PLGA microspheres by varying the ratio of CR and DCM in the conventional solvent evaporation method

From the results presented in Table 1, it is observed that an organic solvent with high interfacial tension is desirable in terms of the drug loading efficiency into the microspheres. In order to obtain further information regarding the characterization of the prepared microspheres, the effect of the solution mixture of DCM (20.4) and CR (31.4) with higher interfacial tension in the water phase is also examined, as shown in Table 2. The ratios of DCM and CR are selected as 10:0, 8:2, 6:4, 5:5, 4:6, 2:8 and 0:10. The homogenization rate and time required by the homogenizer to prepare the emulsion in the entire reaction were set to 10,000 rpm and 90 s, respectively. The yields of all the RFP/PLGA microspheres are 100% in this series of experiments. The particle sizes and values of Span of all the samples after the preparation of the microspheres are 6.0–7.0 μm and 0.90–1.80, respectively. These values imply that the ratio of mixture of DCM and CR does not affect the particle size and monodispersity of the prepared microspheres. The drug loading efficiency of the microspheres increased with the volume of CR per 10 mL in

the oil phase. A mixture of the two organic solvents with almost equal values (20.4 and 31.4) of interfacial tension at hydrophobicity does not appear to affect the particles sizes, but it affects the drug loading efficiency of the prepared microspheres. The drug loading efficiencies in the microspheres prepared in ratios of 2.00:8.00 for DCM and CR exhibit significantly high values (samples 10 and 11). The value of interfacial tension between the oil and water phase in the mixture solvent DCM and CR might increase with the volume of CR per oil phase of 10.0 mL. On further examination of the drug loading efficiency of the microspheres prepared using a solvent or a solvent mixture, the partition coefficient of RFP between the organic solvent and water is determined, as shown in Table 3. The data related to acetone are not listed because the interfacial tension between the organic solvent and water phase could not be obtained, as observed from the data on sample 1 provided in Table 1.

The value of *log* P_{ow}, an indicator of the partition coefficient in the case of ethyl acetate (EA), was lower than that obtained in the case of the other samples, thereby indicating the affinity with the water phase. In the case of the mixture of CR and DCM as samples i–vii, the value of *log* P_{ow} increased with the volume of CR; these values indicated that the rate of transition of the drug to the water phase decreased with increase in the volume of CR. Fig. 1 illustrate the transition to the water phase of a drug (RFP) dissolved in an organic solvent (oil phase) after reaction termination. These samples were extracted from the water phase after shaking for 40 h and storing for 1 h. The lower portion of the image presents the ratio of DCM and CR. The image clearly reveals the degree of transition of RFP to the water phase from the oil phase. The degree of red color of the RFP in the water phase gradually lightens with increase in the volume of CR in the oil phase, as shown in Fig. 1. Further, in the case of ethyl acetate (EA), whose interfacial tension (6.78) in the water phase is lower than that of DCM or CR, a remarkable transition of RFP to the water phase is illustrated. The organic solvent with high interfacial tension between the oil and water phases in the solvent evaporation method might be beneficial in terms of promoting the drug loading efficiency. From these examinations, using organic solvents with high interfacial tension for the preparation of polymer microspheres by means of the solvent evaporation method can enhance the drug loading efficiency.

Table 3
Determination of partition coefficient for RFP between the organic solvent and water.

#	i	ii	iii	iv	v	vi	vii	viii
Water (mL)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
DCM (mL)	5.0	4.0	3.0	2.5	2.0	1.0	0	0
CR (mL)	0	1.0	2.0	2.5	3.0	4.0	5.0	0
EA (mL)	0	0	0	0	0	0	0	5.0
RFP (mg)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
log P_{ow}	1.9	2.0	2.2	2.3	2.4	2.6	3.0	0.9

DCM, dichloromethane; CR, chloroform; EA, ethyl acetate.

3.3. Preparation of the RFP/PLGA microspheres by changing the ratio of CR and DCM in the SPG membrane emulsification technique

The characterization of the microspheres prepared by changing the ratio of CR and DCM in the membrane emulsification technique is also examined, as shown in Table 4. The ratios of volume in DCM and CR per 10.0 mL is selected to be 10.0:0, 8.00:2.00, 6.00:4.00, 5.00:5.00, 4.00:6.00, 2.00:8.00 and 0:10.0, which are identical to the values in the previous experimental series (Table 2). The pore size of the SPG membrane used in the experimental series was to 3.63 μm. The critical pressure was 0.02–0.03 kgf/cm^2, which is identical to that in the previous data [8–9], in which the affection for interfacial tension or the ratio of mixture solvent for water of used organic solvent was absent. The yield of the prepared microspheres was 100% because the oil phase passed completely through the pores of membrane under all conditions. The particle size appeared to be independent of the ratio of DCM and CR, as observed in the conventional solvent evaporation method,

Table 2
Preparation of RFP/PLGA microspheres by varying the ratio of CR and DCM in the conventional solvent evaporation method.

Sample	5[a]	6	7	8	9	10	11[b]
PLGA7505% (w/v)	5.00	5.00	5.00	5.00	5.00	5.00	5.00
RFP% (w/v)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DCM (mL)	10.0	8.00	6.00	5.00	4.00	2.00	0.00
CR (mL)	0.00	2.00	4.00	5.00	6.00	8.00	10.0
PVA% (w/v)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Distilled water (mL)	190	190	190	190	190	190	190
(In solvent evaporation) (mL)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Total volume (mL)	300	300	300	300	300	300	300
Homogenizer (rpm)	10,000	10,000	10,000	10,000	10,000	10,000	10,000
Time (s)	90	90	90	90	90	90	90
Particle size (μm)	6.49	6.97	6.84	6.60	7.01	6.61	7.06
Span	0.87	1.18	1.75	0.90	0.91	1.12	0.91
Drug loading efficiency (%)	68.2	70.9	71.9	72.4	77.1	90.4	90.4

[a] Sample 5 is the same as sample 3 in Table 1.
[b] Sample 11 is the same as sample 4 in Table 1.

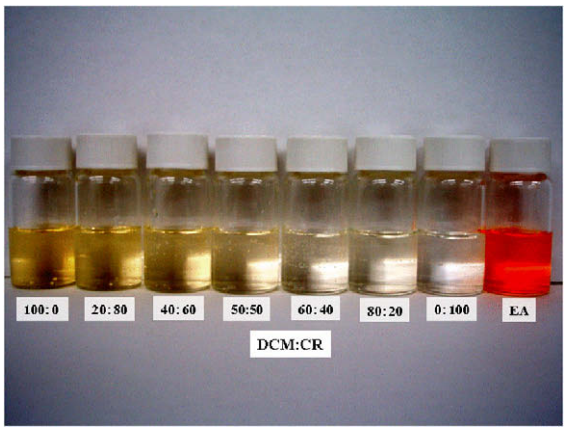

Fig. 1. Image illustrating the transition of RFP dissolved in organic solvent into the water phase.

as shown in Table 2. The value of CV, an indicator of monodispersity, gradually reduced with increase in the volume of CR. The value of CV for the RFP/PLGA microspheres prepared using CR alone (sample 18) was 5.70%, indicating the high monodispersity of the drug-containing PLGA microspheres. The SEM photographs of the RFP/PLGA microspheres prepared by varying the ratios of DCM and CR (samples 12, 15, 17, and 18) are illustrated in Fig. 2. These images indicate the gradual and uniform production of microspheres with increase in the volume of CR. The effect of the solvent mixture in an oil phase in the membrane emulsification technique has been examined in the previous paper [13]. As reported in this paper, the monodispersity of the prepared emulsion droplets depends on the viscosity of the solvent used. In the membrane emulsification technique, the degree of wetting in the dispersion phase on the hydrophilic membrane significantly depends on the monodispersity of the prepared emulsion droplets [14]. The oil phase with low interfacial tension in the water phase shows affinity (wetting) to the hydrophilic membrane and can not successfully generate droplets from the uniform pores of the membrane. Hence, emulsion droplets with a broad distribution were obtained in the membrane emulsification technique [15–16]. Basically, the oil phase selected for the membrane emulsification technique with a hydrophilic membrane should be selected a solvent with high interfacial tension for the water phase. This implies that in the membrane emulsification technique involving a hydrophilic membrane, the confined oil phase alone cannot successfully produce monodisperse microspheres. To overcome this issue, the droplet swelling technique, which swells the minute hydrophilic droplets into monodisperse hydrophobic droplets, was established [16–17]. This technique initially prepares monodisperse emulsion droplets of micron sizes in the hydrophobic oil phase by means of the membrane emulsification technique. Subsequently, the small sized droplets in the hydrophilic oil phase are prepared using a homogenizer. The monodisperse emulsion droplets containing the hydrophilic oil phase are produced by mixing these droplets. In addition, the monodisperse hydrophilic emulsion droplets are prepared by successfully controlling the streaming of nitrogen gas during membrane emulsification. The organic solvent with high interfacial tension and used in the membrane emulsification technique is advantageous for the preparation of monodisperse PLGA microspheres containing drugs. In Table 4, the drug loading efficiency is gradually increased with the volume of CR in the oil phase of 10 mL, as shown in the results in Table 2. In particular, the drug loading efficiency of sample 18 prepared using CR alone exhibited the maximum value (67.5%). Similar to the result in Fig. 1, the transition to the water phase of the dissolved drug in the oil phase is controlled during membrane emulsification. The lower drug loading efficiency of PLGA microspheres produced using the membrane emulsification technique as compared to that of the conventional solvent evaporation method is attributed to the production period of emulsion droplets in the membrane emulsification technique. By shortening the production of emulsion, microspheres with higher drug loading

Table 4
Preparation of RFP/PLGA microspheres by varying the ratio of CR and DCM in the SPG membrane emulsification technique.

Sample	12	13	14	15	16	17	18
SPG pore size (μm)	3.63	3.63	3.63	3.63	3.63	3.63	3.63
PLGA7505% (w/v)	5.00	5.00	5.00	5.00	5.00	5.00	5.00
RFP% (w/v)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DCM (mL)	10.0	8.00	6.00	5.00	4.00	2.00	0
CR (mL)	0	2.00	4.00	5.00	6.00	8.00	10.0
PVA% (w/v)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PEG20000% (w/v)	0.026	0.026	0.026	0.026	0.026	0.026	0.026
Distilled water (mL)	190	190	190	190	190	190	190
(In solvent evaporation) (mL)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Total volume (mL)	300	300	300	300	300	300	300
Particle size (μm)	6.10	7.00	5.96	7.40	6.20	6.50	5.70
CV (%)	15.6	10.8	10.6	9.70	9.60	8.60	5.60
Yield (%)	100	100	100	100	100	100	100
Drug loading efficiency (%)	57.3	61.0	61.4	62.0	61.3	64.8	67.5
Critical pressure (kgf/cm^2)	0.02–0.03	0.02–0.03	0.02–0.03	0.02–0.03	0.02–0.03	0.02–0.03	0.02–0.03

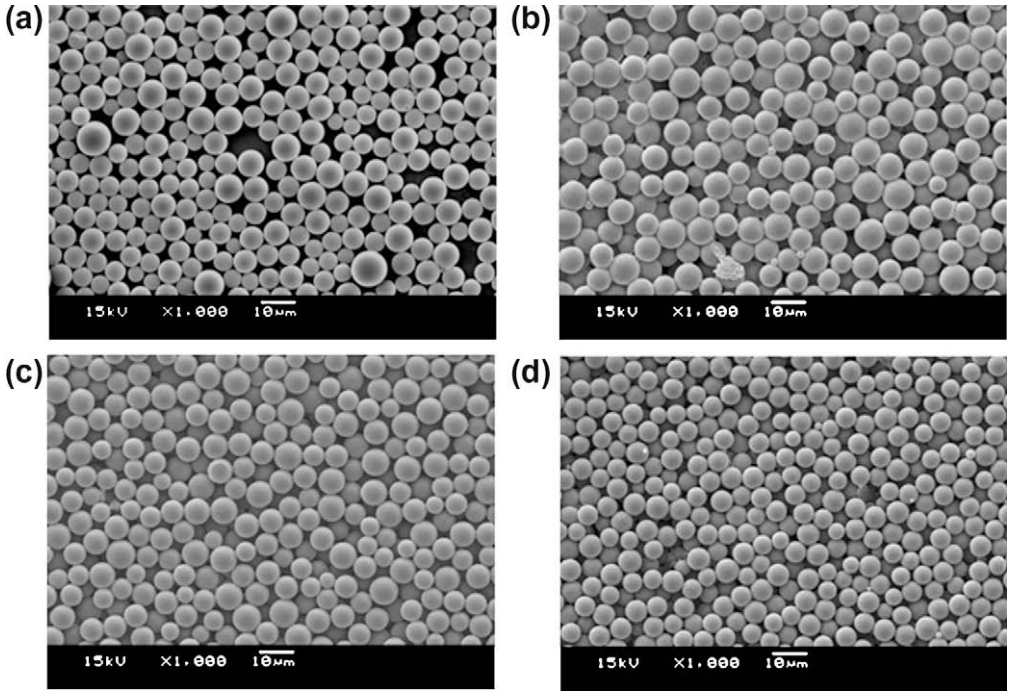

Fig. 2. SEM images of the RFP/PLGA microspheres prepared by using the membrane emulsification technique. (a) Sample 12, (b) sample 15, (c) sample 17, (d) sample 18.

efficiency can be prepared. From these examinations, it can be concluded that the organic solvent with the high interfacial tension in water used in the preparation of drug-containing PLGA microspheres by using the membrane emulsification technique has the advantage that the monodisperse microspheres have high drug loading efficiency.

3.4. Release study of the RFP/PLGA microspheres prepared using DCM or CR alone

The release study of 6 h for RFP/PLGA microspheres and samples 12 and 18 in Table 4, prepared using DCM or CR alone by using the membrane emulsification technique is examined. The amount of release of RFP is plotted in Fig. 3. As shown in Fig. 3, approximately 0.23 mg of RFP is released after 1 h in both the microspheres. After 1 h, the amount of release of RFP from the RFP/PLGA microspheres prepared with CR was slightly higher than that from the microspheres prepared with DCM due to the higher drug loading efficiency of the microspheres prepared with CR. After the initial burst in release, the slow release of RFP from the microspheres prepared with DCM (denoted by symbol ♢) continued upto 5 h and was terminated at 6 h. Against this release behavior, the slow release of RFP from the microspheres prepared by CR (denoted by symbol □) continued upto 6 h after the initial burst in release. Subsequently, almost the same amount of release, i.e., approximately 0.04 mg continued upto 6 h and it continued further. The plot in Fig. 4 shows the release rate of RFP. The release rate of RFP from the

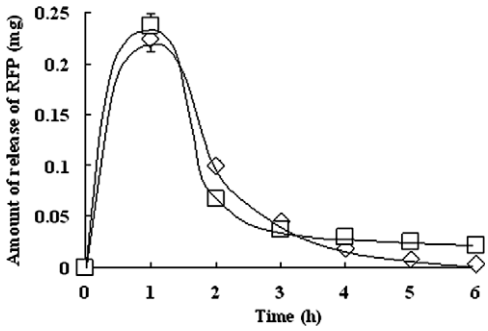

Fig. 3. Plot of the amount of release of RFP from RFP/PLGA microspheres prepared using DCM and CR. ♢, DCM; □, CR.

microspheres prepared using CR alone and having high interfacial tension was precisely controlled as compared to that released from the microspheres prepared using DCM alone, although the drug loading efficiency in the microspheres prepared using CR was higher than 10% of than that in the microspheres prepared using DCM. The amount of drug release distributed at the center of microspheres is more controlled than that near the surface of the microspheres [18]. As a simple explanation for this opinion, a simple model of drug distribution into microspheres is shown in Fig. 5. The drug distributed near the surface of microspheres such as (a) can easily leak into the outer phase because of the distance between the position of the drug in microspheres and the outer phase. The drug distributed to the center of microspheres such

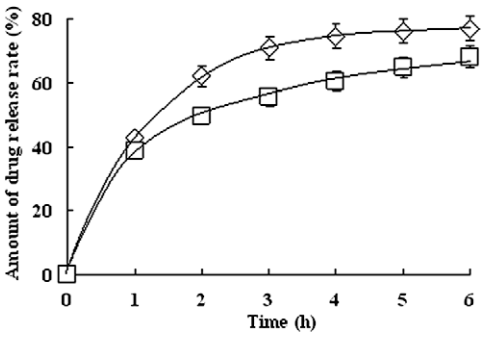

Fig. 4. Plot of the release rate of RFP from RFP/PLGA microspheres produced by using the DCM and CR by using the membrane emulsification technique. ◇, DCM; □, CR.

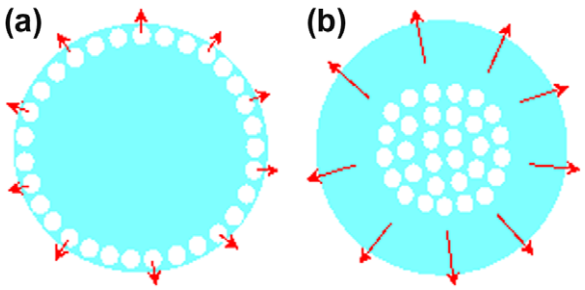

Fig. 5. Simple model of distribution of the drug in the microspheres.

as (b) is slowly released into the outer phase because the distance between the position of the drug in the microspheres and outer phase is relatively large as compared to that of (a). Hence, to control the release of the drug, the drug should be distributed to a nearby region at the center of the particles. However, in general the drug loaded in microspheres or emulsion is located or distributed near the surface of the particles, leading to the initial burst in the release of the drug [7,9,19]. During the preparation of emulsion with CR having the higher interfacial tension for water, most of the drug dissolved by the solvent was deposited near the center of the droplet because the droplets were almost unfamiliar with the water phase. By increasing the distance between the location of the drug in the microspheres and the water phase, a smaller initial release rate or the delayed diffusion of the drug into the water phase was observed. In terms of the drug loading efficiency and drug release behavior, the preparation of microspheres or emulsion containing a drug in a liquid–liquid system is desirable for applying the oil phase with high interfacial tension in the water phase. From these examinations, it can be concluded that for the preparation of biodegradable polymer microspheres for DDS formulation by using the membrane emulsification technique, it is desirable to use organic solvents with high interfacial tension in the water phase.

3.5. Preparation of the hydrophilic-drug-containing PLGA microspheres with CR or DCM

Using an organic solvent with higher interfacial tension in the water phase for the preparation of PLGA microspheres containing RFP as a hydrophobic drug has advantages such as high drug loading efficiency and controlled drug release. The characterizations of the microspheres prepared using CR or DCM alone by means of the conventional solvent evaporation method and the membrane emulsification technique are examined, as shown in Table 5. Blue dextran (BLD) is used as the hydrophilic model drug to be loaded in the PLGA microspheres. The pore size of the SPG membrane

Table 5
Preparation of BLD/PLGA microspheres by varying the ratio of CR and DCM in each preparation method for emulsion.

Sample	19	20	21	22
Blue dextran conc.% (w/v)	1.67	1.67	1.25	1.25
Water (ml)	3.00	3.00	4.00	4.00
PLGA7505% (w/v)	7.14	7.14	8.33	8.33
Sunsoft 818H% (w/v)	1.43	1.43	1.67	1.67
DCM (mL)	7.00	0	6.00	0
CR (mL)	0	7.00	0	6.00
PVA% (w/v)	1.00	1.00	1.00	1.00
PEG20000% (w/v)	0	0	0.017	0.017
Distilled water (mL)	290	290	290	290
Total volume (mL)	300	300	300	300
Preparation method of $w_1/o/w_2$ emulsion	Homogenizer	Homogenizer	Membrane emulsification	Membrane emulsification
SPG membrane (μm)	–	–	5.25	5.25
Particle size (μm)	8.23	7.00	9.10	8.80
CV (%)	34.6	29.2	16.6	13.7
Yield (%)	100	100	70.2	73.8
Drug loading efficiency (%)	56.3	59.2	29.8	45.5
Critical pressure (kgf/cm^2)	–	–	0.02–0.03	0.02–0.03

used in the membrane emulsification technique is 5.25 μm. In both the preparation techniques, BLD/PLGA microspheres were prepared by using DCM and CR. The value of CV in microspheres of samples 19 and 20 was approximately 30%, which indicates polydisperse microspheres. The microspheres (samples 21 and 22) prepared by means of the membrane emulsification technique were relatively monodisperse. As mentioned in the previous section, the monodispersity of the microspheres prepared by using CR alone was highly progressed as compared to that prepared by using DCM alone. In samples 19 and 20 prepared by using the homogenizer, the drug loading efficiency of the microspheres prepared by using CR alone is higher than that of the microspheres prepared by using DCM alone, as mentioned in the previous section. In addition, samples 21 and 22 prepared by using the membrane emulsification technique with DCM and CR indicated that the drug loading efficiency of the microspheres prepared by using CR alone was high than that of the microspheres prepared by using DCM alone. The particle size of the microspheres prepared by using CR was smaller than that of the microspheres prepared by using DCM in both the methods. The release behavior of the BLD/PLGA microspheres prepared by using DCM (sample 21) or CR (sample 22) by means of the membrane emulsification technique was preliminarily studied; the plot of the release rate of BLD is shown in Fig. 6. The release rate of the microspheres prepared by using CR were more controlled than that of the microspheres prepared by using DCM, despite the higher drug loading efficiency of the former. From the second day after the initial burst, the slow release of BLD continued up to the tenth day and further. Most of the BLD released from the BLD/PLGA microspheres was released from near the surface of the microspheres [7]. From the release rate on the initial day for each sample, it can be deduced that the BLD in the microspheres prepared by using CR might be distributed at locations near the center or core of the microspheres as compared to than that in the microspheres prepared by using DCM because of the delayed initial release into the water phase. From these examinations, it can be observed that using the organic solvent with the high interfacial tension in the water phase affects the preparation of PLGA microspheres containing hydrophilic drug.

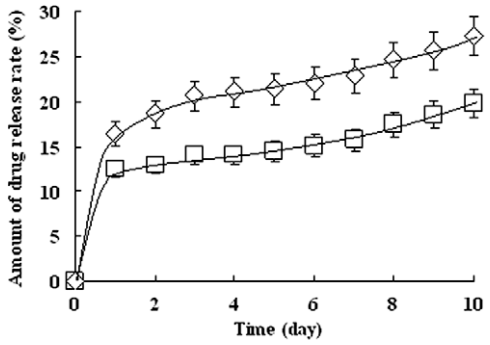

Fig. 6. Plot of the release rate of BLD from the BLD/PLGA microspheres prepared by using the membrane emulsification technique. ◇, DCM; □, CR.

4. Conclusion

The effects of the types and the mixture ratio of the organic solvents in a solvent used to dissolve PLGA for the preparation of drug-containing PLGA microspheres by the solvent evaporation method were examined in the present paper. The using an organic solvent with low interfacial tension in the water phase led to a gradual reduction in the particle size of the prepared PLGA microspheres containing drugs. In the examination of the ratio of CR and DCM with high interfacial tension in the water phase, the drug loading efficiency in the microspheres was observed to gradually increase with the volume of CR in the oil phase. Using the above-mentioned ratio of CR and DCM in the membrane emulsification technique not only increased the drug loading efficiency but also promoted the monodispersity of the microspheres. This knowledge was also applied to the preparation of PLGA microspheres containing BLD as a hydrophilic drug. In conclusion, an organic solvent that is used to dissolve a polymer during the preparation of biodegradable polymer microspheres containing drugs in the solvent evaporation method is desirable to be used a solvent with high interfacial tension for the water phase in terms of the preparation of a DDS formulation.

Acknowledgements

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