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Control of drug loading efficiency and drug release behavior in preparation of hydrophilic-drug-containing monodisperse PLGA microspheres

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Abstract We prepared monodisperse poly(lactide-*co*-glycolide) (PLGA) microspheres containing blue dextran (BLD)—a hydrophilic drug—by membrane emulsification technique. The effects of electrolyte addition to the w_2 phase and significance of the droplet size ratio between primary (w_1/o) and secondary ($w_1/o/w_2$) emulsions during the preparation of these microspheres was examined. The droplet size ratio was evaluated from the effect of stirring rate of the homogenizer when preparing the primary emulsion. The drug loading efficiency of BLD in these microspheres increased with stirring rate. It increased to approximately 90% when 2.0% NaCl was added to the w_2 phase. Drug release from these microspheres was slower than that when they were prepared without electrolyte addition. Despite the very high efficiency drug release was gradual because BLD was distributed at the microspheres core. Relatively monodisperse hydrophilic-drug-containing PLGA microspheres with controlled drug loading efficiency and drug release behavior were prepared.

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1 Introduction

Monodisperse microspheres prepared from biodegradable polymers such as poly(lactide-*co*-glycolide) (PLGA) are of significance in research on drug delivery system (DDS), for example, in research on constant drug loading efficiency and drug release behavior. The solvent evaporation method is a standard method used to prepare biodegradable polymer microspheres. Such microspheres are prepared as polydisperse particles because the prepared emulsion is stirred mechanically [1–3]. Currently, a technique for preparing monodisperse PLGA particles is under development in order to further increase the potential of DDS research [4, 5]. Kondo and coworkers proposed a simple technique for preparing monodisperse PLGA particles by using Shirasu porous glass (SPG) membrane emulsification [4]. Furthermore, the author prepared monodisperse PLGA microspheres containing rifampicin (anti-tuberculosis drug) as a hydrophobic model drug and then carried out an in vivo evaluation of these microspheres [6–8]. Monodisperse PLGA microspheres containing a hydrophilic drug were also prepared by using this membrane emulsification technique [9]. The most important factor in the preparation of hydrophilic-drug-containing biodegradable polymer particles is the control of the drug loading efficiency or drug release behavior of the prepared microspheres. When suitable particles are used in the fabrication of a DDS, a high drug loading efficiency and slow drug release are achieved. The main factors involved in the preparation of microspheres having high drug loading efficiency are the viscosity of the w_1 (inner water) phase, molecular weight or concentration of the polymer, and osmotic pressure between the w_1 and the w_2 (outer water) phase [10–12]. In a previous study, we determined a new factor that influences the drug loading efficiency of a hydrophilic drug in

the conventional solvent evaporation method [13]. In the present study, we examined whether this factor is applicable to the membrane emulsification technique in order to control the drug loading efficiency of the prepared hydrophilic-drug-containing monodisperse PLGA microspheres. The concentration and molecular weight of the polymer to increase the viscosity of the o (oil) phase cannot be determined easily in the membrane emulsification technique. This is because the primary (w_1/o) emulsion can easily cause clogging of the pores of the used membrane, resulting in a delay or halt in the reaction process. This clogging occurs due to the passing of the primary (w_1/o) emulsion through the uniform pores. It is necessary to examine the osmotic pressure between the w_1 and the w_2 phases to investigate the drug loading efficiency in the membrane emulsification technique. The effect of the addition of NaCl as an electrolyte to the w_2 (outer water) phase in the membrane emulsification technique was also examined in this study. The effect of the addition of NaCl on the preparation of hydrophilic-drug-containing biodegradable polymer microspheres using the membrane emulsification technique was previously reported by Liu et al., where single poly(lactide) (PLA) microspheres and PLA/PLGA microspheres were used [14, 15]. These studies reported that the addition of NaCl to the w_2 phase caused an increase in the drug loading efficiency of the microspheres because the NaCl concentration controlled the osmotic pressure between the w_1 and the w_2 phases. Further, the drug release behavior of the prepared monodisperse microspheres was gradually controlled by increasing the NaCl concentration [15]. These results are of considerable interest for the fabrication of a DDS formulation. However, these reports did not discuss the source of an interesting effect. Namely, these reports did not confirm the actual site of the drug in the microspheres by the control of the osmotic pressure between the w_1 and the w_2 phase. In the present study, the subject demonstrated by the degree of the color of the used model drug on the optical images and discussed about the effect. These results are discussed in this paper.

2 Materials and methods

2.1 Materials

Poly(lactide-*co*-glycolide), (PLGA7505; lactide:glycolide = 75:25; M_w: 5,000) purchased from Wako Pure Chemical Industries, Japan, was used as the biodegradable polymer in this study. Blue Dextran 2000 (BLD) purchased from Amersham Biosciences Corporation was used as the hydrophilic model drug that was to be loaded in the PLGA microspheres. Sunsoft 818H purchased from Taiyo Kagaku Co., Ltd., Japan, was used as the oil-soluble surfactant in the preparation of the water-in-oil (w_1/o) emulsion. Poly(vinyl alcohol) (PVA) (degree of polymerization: 500, saponification: 86–90 mol%) purchased from Wako Pure Chemical Industries was used as the dispersant of the w_2 (outer water) phase in the preparation of the water-in-oil-in-water (w_1/o/w_2) emulsion. Polyethylene glycol (PEG) 20000 purchased from Wako Pure Chemical Industries was added to the PVA aqueous solution as a co-dispersant. Other chemicals were of reagent grade.

2.2 Preparation of BLD/PLGA microspheres using SPG membrane emulsification technique

The standard procedure for preparing BLD/PLGA microspheres using the SPG membrane emulsification technique is shown in Table 1. First, 500 mg of PLGA and 100 mg of Sunsoft 818H were dissolved in 6.0 ml of dichloromethane (DCM). The resultant solution was used as the o phase. Then, 4.0 ml of the BLD solution that formed the w_1 phase was poured into the o phase contained in a 20-ml screw-capped tube. The w_1/o emulsion was prepared by using a microhomogenizer (NS-310E; Microtec Nition Co., Ltd.). The prepared w_1/o emulsion was immediately injected into the oil tank that is part of the apparatus (Ise Chemical Corporation) used in the SPG membrane emulsification technique. The preparation of the w_1/o/w_2 emulsion using the SPG membrane emulsification technique was initiated by streaming nitrogen gas into the o phase. To prepare an emulsion having the same droplet size, membrane emulsification was carried out under constant nitrogen pressure (0.03–0.04 kg/cm^2). BLD/PLGA microspheres were prepared by stirring the resultant oil phase at 250 rpm using an impeller-type stirrer (BL 1200, Shinto Scientific Co., Ltd.) for 4 h at room temperature.

Table 1 Standard procedure for preparation of BLD/PLGA microspheres using the SPG membrane emulsification technique

Sample	#
w_1 (inner water) phase	
Blue dextran conc.% (w/v)	1.25
Distilled water (ml)	4.00
o (oil) phase	
PLGA 7505 conc.% (w/v)	8.33
Sunsoft 818H conc.% (w/v)	1.67
DCM (ml)	6.00
w_2 (outer water) phase	
PVA conc.% (w/v)	1.00
Distilled water (ml)	290
PEG20000 conc.% (w/v)	0.017
Total volume (ml)	300
SPG membrane (μm)	5.25

2.3 Collection of prepared BLD/PLGA microspheres

The prepared BLD/PLGA microspheres were collected by centrifugation for 10 min at 1000 rpm. These samples were washed three times in a 10-ml screw-capped tube by the following steps: (1) centrifugation at 1000 rpm for 3 min using a centrifugal separator (KA-1000, KUBOTA Co.), (2) exclusion of a supernatant, and (3) centrifugation after redispersion with fresh distilled water.

2.4 Measurement of yield of prepared microspheres

After the termination of the membrane emulsification process, the w_1/o emulsion that could not pass through the SPG membrane was collected in a Petri dish. The collected emulsion was dried in a vacuum desiccator for 1 day in order to completely remove DCM and water. The yield of the prepared microspheres was calculated by Eq. 1.

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

where w_i is the initial weight of the materials required for the preparation of the w_1/o emulsion and w_t the weight of the materials that could not pass through the SPG membrane.

2.5 Observation of microspheres using optical microscope

A droplet of a suspension containing the prepared BLD/PLGA microspheres was placed on a glass slide and sealed with a glass cover. The microspheres were observed and photographed by using an optical microscope (OM: OM, BH-2, Olympus Co.).

2.6 Scanning electron microscopy (SEM) observation of microspheres

A droplet of the suspension containing the prepared microspheres was placed on an aluminum sample stage and dried for 1 day in a vacuum desiccator. Platinum sputtering was carried out by using an ion-sputtering device (Auto Fine Coater, JFC-1600, JEOL Ltd.). PLGA microspheres were observed and photographed by using a scanning electron microscope (JSM-6060LA, JEOL Ltd.).

2.7 Measurements of particle size

The average diameters and the value of the coefficient of variation (CV) for the prepared microspheres were calculated by using a sample of 200 particles observed in the photographs captured using the scanning electron microscope or those captured using the OM. The values of CV were calculated by using Eq. 2.

$$\text{CV} = \sigma/d_p \times 100 \quad (2)$$

where σ is the standard deviation and d_p the average particle diameter obtained from the SEM or OM images. A low value of CV indicates uniformly sized particles.

2.8 Measurement of loading efficiency of BLD in PLGA microspheres

4.0 ml of DCM was added to the dried BLD/PLGA microspheres in a Petri dish. The solution was then exposed to a field of ultrasonic waves in order to dissolve the polymer completely. The sample was then transferred to a 10-ml glass tube. BLD was precipitated by centrifugation, and the supernatant was removed. After removing the DCM by drying the precipitate for 1 day, 5.0 ml of distilled water was added to the resulting solution. The concentration of BLD was measured at 620 nm using a spectrophotometer (V-660, JASCO Co.).

2.9 Release study of BLD/PLGA microspheres

The release study of BLD/PLGA microspheres was carried out by a previously described method [9, 16]. Briefly, 20 mg of the prepared BLD/PLGA microspheres was added to 5.0 ml of a phosphor buffer solution (PBS, pH: 7.4; ionic strength; 0.154 M) in a 10.0-ml screw-capped tube. The sample was dispersed for exactly 30 s using a voltex mixer (Test tube mixer, TTM-1, SIBATA Co.). The screw-capped tube containing the dispersed solution was soaked in a water bath shaker (Shaking baths SB-13, AS ONE Co.) at 37°C. The shaker was moved at a rate of 60.0 times per 1 min. After 1 day, the screw-capped tube was removed from the water bath shaker. The dispersed solution was precipitated at 2000 rpm for 5 min using a centrifugal separator, and the supernatant was measured at 620 nm using a spectrophotometer. The PBS was replaced with 5.0 ml of fresh PBS solution daily. The release study was carried out at $n = 3$ for 14 days.

3 Results and discussion

3.1 Effect of droplet size of w_1/o emulsion on preparation of BLD/PLGA microspheres in membrane emulsification technique

Previously, the author confirmed that the ratio of the droplet size between the primary (w_1/o) and the secondary ($w_1/o/w_2$) emulsions directly affected the drug loading efficiency of the prepared microspheres [13]. When the

ratio of the droplet size between the w_1/o and $w_1/o/w_2$ emulsions was higher, the drug loading efficiency was higher. First, in order to control the drug loading efficiency of the prepared microspheres, we determined whether the factor mentioned earlier, which was also reported in a previous paper, was applicable to the membrane emulsification technique. The w_1/o emulsions with various droplet sizes were made to pass through a membrane having a pore size of 5.25 μm. The droplet size was controlled by the stirring rate of the microhomogenizer. A high stirring rate resulted in small-sized droplets [17]. According to the standard specifications listed in Table 1, the stirring rates of the microhomogenizer for the preparation of the w_1/o emulsion were selected as 3500, 10000, 20000, and 30000 rpm. Each sample was placed in the microhomogenizer for 90 s. The following droplet sizes (d_{pd}) of the prepared w_1/o emulsion for different stirring rates were obtained from the evaluation of the size of 200 droplets from the optical images: 5.02 (3500 rpm), 2.84 (10000 rpm), 2.33 (20000 rpm), and 2.02 μm (30000 rpm). It can be observed that a higher stirring rate resulted in smaller w_1/o emulsion droplets. Each w_1/o emulsion droplet that was prepared was passed through a membrane having a pore size of 5.25 μm. The value of the critical pressure, i.e., the initial value of nitrogen pressure required to initiate the membrane emulsification process, affects the properties (such as the viscosity and concentration) of the o phase or w_1/o phase. However, the critical pressure in every sample was 0.03–0.04 kgf/cm^2, independent of the droplet size of the w_1/o emulsion. Figure 1 shows the results of change in the particle size (d_p) and CV of the prepared BLD/PLGA microspheres with a change in the stirring rate during the preparation of the w_1/o emulsion. The CV for every sample is below 20%, which led to the formation of relatively monodisperse BLD/PLGA microspheres. The particle size of every sample was approximately 10.0 μm, which was twice the pore size of the membrane used. Similar results were obtained in a previous study [9]. The droplet sizes of the emulsion formed by membrane emulsification were three times the pore size of the membrane used. The final size of the microspheres prepared by solvent evaporation after membrane emulsification was lesser than the droplet size because of the shrinking of droplets during solvent evaporation. As observed from Fig. 1, the particle size and CV of the prepared microspheres do not appear to affect the stirring rate of the microhomogenizer in the preparation of the w_1/o emulsion. The particle size of the microspheres prepared by the two-step emulsion preparation method depends on the stirring rate during the preparation of the $w_1/o/w_2$ emulsion. The size of the microspheres prepared by the membrane emulsification technique did not change due to the change in the stirring rate in the preparation of the w_1/o emulsion because it passed through uniform pores. Since the w_1/o emulsion passed through a membrane with a pore size of 5.25 μm, the ratio of the droplet size between the w_1/o and the $w_1/o/w_2$ emulsions can be expressed as $5.25/d_{pd}$. Figure 2 shows the results of the drug loading efficiency (denoted using open square) and yield (denoted using open diamond) of the prepared microspheres against the ratio of the droplet size between the w_1/o and the $w_1/o/w_2$ emulsions ($5.25/d_{pd}$). The yield and drug loading efficiency increased with $5.25/d_{pd}$. This result was the same as that obtained by the conventional solvent evaporation method [13], and it can also be applied to the membrane emulsification technique. A lower ratio leads to greater clogging of the pores when the w_1/o emulsion is passed through them, which in turn results in a low yield and drug loading efficiency. During the preparation of microspheres containing a hydrophilic drug, the BLD and PLGA are added to the w_1 and o phases. A sample with $5.25/d_{pd} = 1.05$, which is approximately equal to the droplet

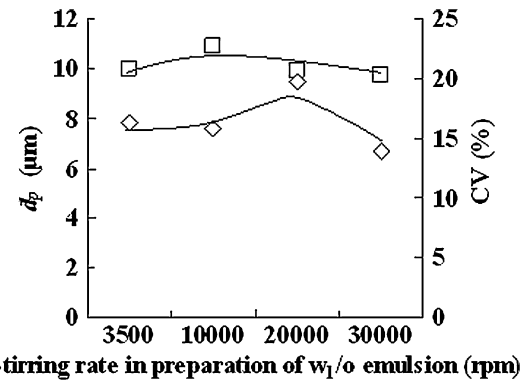

Fig. 1 Results of particle size (d_p) and CV of prepared BLD/PLGA microspheres vs. stirring rate during preparation of w_1/o emulsion. *Open square*: d_p (μm), *open diamond*: CV (%)

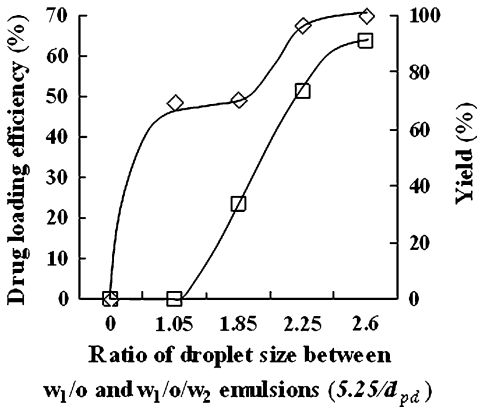

Fig. 2 Results of drug loading efficiency and yield vs. ratio of droplet size between the primary and the secondary emulsions. *Open square*: Drug loading efficiency (%), *open diamond*: Yield (%)

size of the w_1/o emulsion, had a drug loading efficiency of 0% and it clogged the entire w_1 phase. In the case of a sample with 69.1% yield, only the oil phase passed through the pores during membrane emulsification. From Fig. 2, it can be clearly observed that the drug loading efficiencies of the samples are lower than their respective yields. In particular, the drug loading efficiency of the sample with 5.25/$d_{pd} = 2.60$ was 63.8% and its yield was almost 100%. This difference in the drug loading efficiency and yield might be due to the following reasons. (1) All w_1 droplets of the w_1/o emulsion in the oil phase tank mutually coalesced and gradually enlarged to more than 5.25 μm during membrane emulsification, which led to the w_1 phase clogging the pores of the membrane. (2) The loading drug present in the $w_1/o/w_2$ emulsion prepared by membrane emulsification leaked into the w_2 phase before solvent evaporation was carried out. Nevertheless, this long-term reaction in the preparation of the drug-containing PLGA microspheres must have occurred due to the lower drug loading efficiency. Further improvements in the stabilization of the w_1/o emulsion and suppression of the leakage of the drug from the prepared emulsion into the w_2 phase are required. From these results, the high yield and drug loading efficiency were attributed to a large ratio between the w_1/o emulsion droplet size and pore size of the used membrane.

3.2 Preparation of BLD/PLGA microspheres by addition of NaCl to w_2 phase in SPG membrane emulsification technique

The effect of the addition of the electrolyte (NaCl) to the w_2 phase in the membrane emulsification technique was also examined in order to evaluate the control of the osmotic pressure between the w_1 and the w_2 phases. The stirring rate and stirring time of the microhomogenizer during the preparation of the w_1/o emulsion were selected as 30000 rpm and 90 s, respectively. The primary emulsion was prepared by the membrane emulsification technique using a membrane with a pore size of 5.25 μm. The NaCl concentration in the w_2 phase was 0%, 0.50%, 1.0%, and 2.0%. The sample with NaCl concentration of 0% was the same as that mentioned in Sect. 3.1. The values of the critical pressure that initiated membrane emulsification were independent of the NaCl concentration, and they were 0.03–0.04 kgf/cm^2 in every sample. The droplet sizes d_{sp} of the prepared $w_1/o/w_2$ emulsion for every sample are shown in Fig. 3. It can be observed that the droplet sizes of the $w_1/o/w_2$ emulsion decreased with the NaCl concentration. In the previous study, the droplet sizes of the emulsion reduced upon the addition of NaCl because of the adsorption of NaCl on the surface of the prepared droplets [18]. The formation and stability of the emulsion droplets depended on the existence of NaCl and the reduction in the

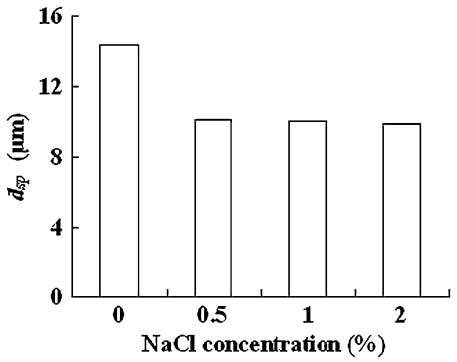

Fig. 3 Result of droplet size (d_{sp}) of secondary emulsion vs. NaCl concentration

emulsion droplet size due to the stabilization of the droplets. In general, the stability of particles depends on the electrostatic hindrance of the electric double layer surrounding the particles and the steric hindrance caused by the adsorption of molecules on the surface of the particles. Because the charge potential of an interface reduces significantly when NaCl is added to the dispersion solution, the stability of the emulsion droplets contributed to the steric hindrance on the surface of the droplets. The emulsion droplets produced by the membrane emulsification technique by the addition of NaCl to the w_2 phase might also be stabilized with a similar tendency. Therefore, the droplet sizes of emulsions might reduce with the addition of NaCl to the w_2 phase. Figure 4 shows the plots of the particle size and CV of the prepared microspheres after solvent evaporation versus the NaCl concentration. From the figure, it can be observed that the particle size and CV increased with the NaCl concentration even after the prepared $w_1/o/w_2$ emulsion was passed through the uniform pores of the SPG membrane. These results indicate that the BLD/PLGA microspheres prepared after solvent evaporation coagulated due to the presence of NaCl in the w_2 phase. Furthermore, the microspheres were larger than

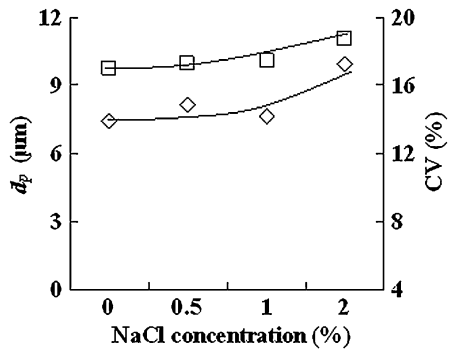

Fig. 4 Results of particle size (d_p) and CV of prepared BLD/PLGA microspheres vs. NaCl concentration. *Open square*: d_p (μm), *open diamond*: CV (%)

those prepared without the addition of NaCl. The coagulation by the addition of NaCl affects the surface potential of particles because of the compression of the electron double layer on the microspheres. Therefore, the coagulation between particles in the presence of NaCl during solvent evaporation is due to the polymer microspheres and not due to the $w_1/o/w_2$ emulsion droplets. The addition of NaCl to the w_2 phase in the solvent evaporation method led to interesting findings with regard to the produced emulsion and polymer microspheres. One is that the existence of NaCl in the prepared emulsion contributed to the stabilization of the formed emulsion droplets. The other is that the presence of NaCl accelerated the coagulation of the formed polymer microspheres. This was not reported in previous papers [14, 15]. In Fig. 4, it can be observed that the values of CV for the prepared microspheres were approximately 14.0–17.0% and relatively monodisperse BLD/PLGA microspheres were prepared despite the presence of NaCl in the w_2 phase. Figure 5 shows a plot of the drug loading efficiency and yield of the prepared BLD/PLGA microspheres versus the NaCl concentration. The yield for every sample was approximately 100%, which confirmed that the emulsion passed almost perfectly through the pores in the o phase. The drug loading efficiency gradually increased with NaCl concentration. In particular, the drug loading efficiency at a concentration of 2.0% increased to approximately 90% (87.1% to be more precise). The control of the osmotic pressure between the w_1 and the w_2 phases was also affected by the addition of NaCl to the w_2 phase. The leakage of the drug in the w_2 phase was also suppressed by this addition. SEM images of the BLD/PLGA microspheres prepared in this experiment are shown in Fig. 6. They are in agreement with the results shown in Fig. 4. It can be clearly observed that the sizes of the BLD/PLGA microspheres in these four images appear to increase gradually as (Fig. 6a < b < c < d) with the NaCl concentration. Furthermore, the size distribution of the BLD/PLGA microspheres increased gradually with the NaCl concentration. The configuration of the prepared microspheres appears to be relatively monodisperse and spherical. From these results, it can be said that monodisperse BLD/PLGA microspheres with a high drug loading efficiency were prepared when NaCl was added to the w_2 phase.

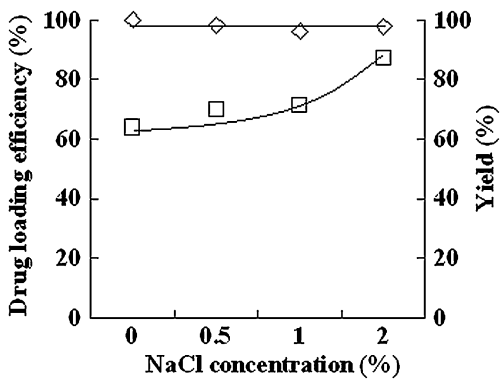

Fig. 5 Results of drug loading efficiency and yield vs. NaCl concentration. *Open square*: Drug loading efficiency (%), *open diamond*: Yield (%)

3.3 Release study of monodisperse BLD/PLGA microspheres prepared by addition of NaCl in SPG membrane emulsification technique

Relatively monodisperse BLD/PLGA microspheres with CV approximately in the range of 14.0–17.0% were prepared by adding different concentrations of NaCl (0%, 0.50%, 1.00%, and 2.00%). The drug release behavior of the four microspheres was examined at a period of 2 weeks. The drug release rate of the four BLD/PLGA microspheres is shown in Fig. 7. The drug release rate of the sample (denoted by open diamond) with a NaCl concentration of 0% was approximately 20% by day 1. The slow release behavior of BLD was also observed on day 2 and it continued over the remaining days. Finally, approximately 40% was released by day 14. Because of the high molecular weight of BLD, 100% release of BLD from the microspheres was not possible for 14 days. Three samples (denoted by open square, open triangle, and open circle) with NaCl released approximately 2.0% on day 1. This slow release of BLD continued for 14 days and the final release from every sample was approximately 10.0–13.0%. The results of these plotted curves indicate that drug release from the microspheres prepared with NaCl was more controlled than that from microspheres without NaCl, although the drug loading efficiency of the samples with NaCl was higher than that of samples without NaCl. In addition, the control of drug release gradually increased with the NaCl concentration despite a very high drug loading efficiency. The preparation of microspheres with a slow drug release and high drug loading efficiency is very difficult because a drug in a microsphere is generally distributed near the surface of the particles, which is the main cause of the initial burst. Namely, the microspheres with a high drug loading efficiency exhibited a high release of the drug in the initial stage because the maximum amount of the drug is present near the surface of the particles. Conversely, when the distribution of the drug on a site near the core of the microspheres is greater, the release is slower. From the results shown in Fig. 7, the distribution area (at the site of the core or near the surface) of the drug in the microspheres can be predicted. To demonstrate this, every sample of the prepared BLD/PLGA microspheres was examined using an OM. This is the first study in which the distribution site of a drug in monodisperse biodegradable

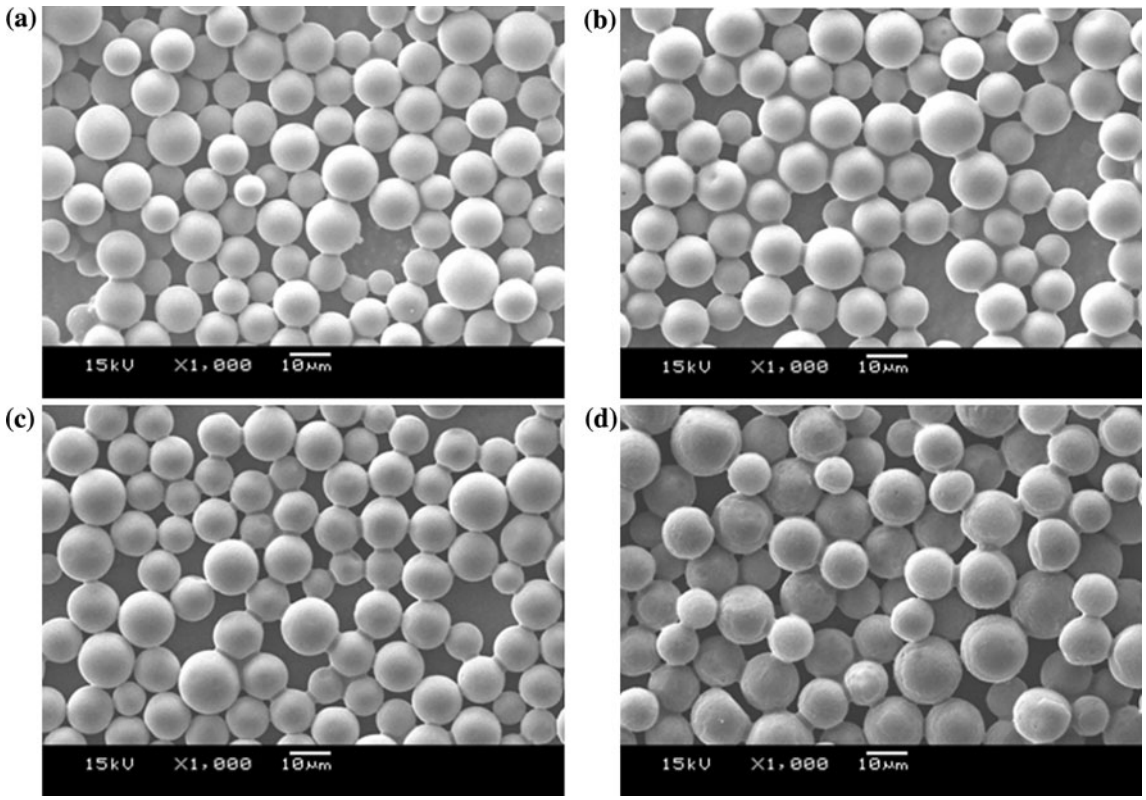

Fig. 6 SEM images of prepared BLD/PLGA microspheres vs. NaCl concentration. (**a**) 0%, (**b**) 0.50%, (**c**) 1.00%, and (**d**) 2.00%

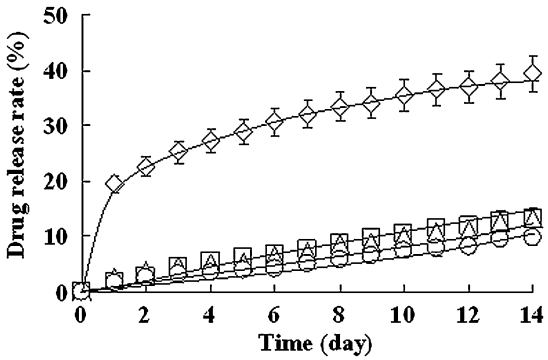

Fig. 7 Release behavior of prepared BLD/PLGA microspheres with changing NaCl concentration. *Open diamond*: 0%, *open square*: 0.50%, *open triangle*: 1.00%, *open circle*: 2.00%

polymer particles has been confirmed practically. The images captured using the OM are shown in Fig. 8. These photographs were captured at similar brightnesses and contrasts. The image in (a) shows a sample (drug loading efficiency: 63.8%) prepared without NaCl with some leakage of BLD (denoted by black circles) and a lower degree of blue stain (color of BLD) in the microspheres. This proves that the BLD is almost completely exposed at the site of the core of the microspheres. The image shown in (a) indicates that BLD from such microspheres can easily cause the initial burst. For the result, the (b) and (c) show OM photographs of the prepared microspheres with NaCl concentrations of 0.5% and 1.0%; these figures show that the degree of the blue stain in the microspheres is greater than that in the case without NaCl, and further, the leakage of BLD from the microspheres decreased. The (d) shows a photograph of the microspheres prepared with NaCl concentration of 2.0%; the degree of the blue stain is greater than that in every other microsphere sample. In addition, the leakage of BLD into the w_2 phase was almost suppressed. Overall, the degree of the blue stain in the microspheres gradually increases with the drug loading efficiency as (a) (63.8%) < (b) (69.8%) < (c) (71.5%) < (d) (87.1%). Conversely, the degree of the blue stain of the leakage (denoted by black circles) from microspheres gradually decreases as (a) > (b) > (c) > (d) with NaCl concentration. These photographs indicate that the addition of NaCl into the w_2 phase controlled the leakage of a drug in the microspheres and loaded more of it at the core of the microspheres. Based on the plot curve shown in Fig. 7 and the photographs shown in Fig. 8, the distribution of BLD in the microspheres after the initial release, especially after 1 day, can be explained by a simple model created on the basis of the results shown in these two figures. This model

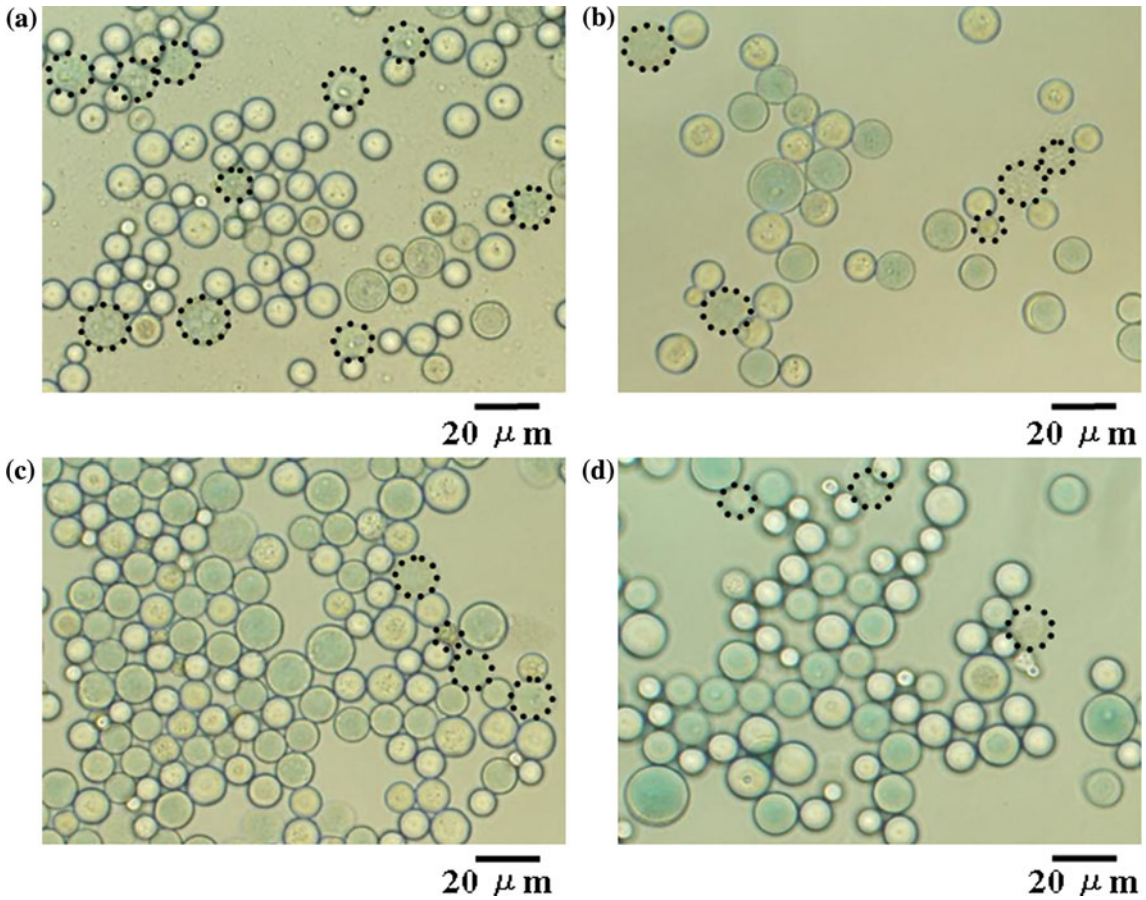

Fig. 8 Optical images of prepared BLD/PLGA microspheres with changing NaCl concentration. (**a**) 0%, (**b**) 0.50%, (**c**) 1.00%, and (**d**) 2.00%

is shown in Fig. 9. As seen from (a), the BLD distributed near the surface of the microspheres without NaCl exhibited a higher release ratio with a shorter distance between the BLD site and the outer water phase. The BLD remaining in the polymer matrix released more gradually with an increase in the molecular weight after 2 days. The samples (denoted by open square, open triangle, and open circle in Fig. 7) with BLD distributed near the core in microspheres with NaCl shown in Fig. 9b exhibited a showed smaller release rate with a greater distance between the BLD distribution site and the outer water phase and a higher molecular weight of BLD. The control site for the distribution of the model drug in the microspheres is of considerable interest from the viewpoint of the controlled release of the drug. In our previous paper, we have reported that it is possible to control the distribution of a drug at the site of the prepared PLGA microspheres by dissolving the PLGA in a solvent [16]. By using a solvent that generates a high interfacial tension with the water phase, the drug can

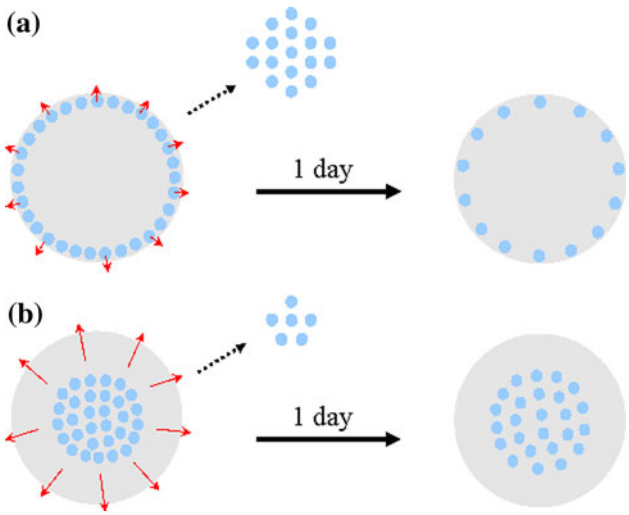

Fig. 9 Simple model for release of BLD in PLGA microspheres. (**a**) BLD/PLGA microspheres without NaCl and (**b**) BLD/PLGA microspheres with NaCl

be forced to distribute near the core of the microspheres. The drug release behavior of the microspheres prepared with a solvent having high interfacial tension was more controlled than that of microspheres prepared with a solvent having lower interfacial tension. As a result, upon the addition of NaCl, BLD was distributed at the site of the core of the microspheres and the release behavior was controlled by the delayed diffusion of BLD in the matrix of PLGA microspheres having higher drug loading efficiency, as indicated by the plots (denoted by open square, open triangle, and open circle) shown in Fig. 7. These results suggest that the microspheres discussed in Sect. 3.1 must have possessed similar properties because they were prepared by simply changing the stirring rate during the preparation of the w_1/o emulsion. From the results obtained in this study, it is clear that hydrophilic-drug-containing monodisperse PLGA microspheres with high drug loading efficiency and slow release behavior can be prepared by the addition of NaCl to the w_2 phase in membrane emulsification; this causes the distribution of the drug at the site of the core in the microspheres.

4 Conclusions

The ratio of the droplet size between the primary (w_1/o) and the secondary ($w_1/o/w_2$) emulsions in the membrane emulsification technique affected the drug loading efficiency in the prepared microspheres. The addition of NaCl as an electrolyte to the w_2 (outer water) phase during the preparation of the $w_1/o/w_2$ emulsion by membrane emulsification affected the droplet size of the formed emulsion, particle size of the prepared microspheres, drug loading efficiency, and drug release behavior. In this paper, a technique for preparing monodisperse hydrophilic-drug-containing PLGA microspheres that are capable of controlling the drug loading efficiency and drug release behavior was proposed.

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