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Colloids and Surfaces B: Biointerfaces 54 (2007) 173–178
COLLOIDS AND SURFACES B
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Incorporation of water-soluble drugs in PLGA microspheres

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Received 22 May 2006; received in revised form 3 October 2006; accepted 7 October 2006
Available online 26 October 2006

Abstract

Poly(lactide-*co*-glycolide) (PLGA) microspheres containing blue dextran, as a model of water-soluble drugs, were prepared from $w_1/o/w_2$ emulsions by using a microhomogenizer and a solvent evaporation method. Effects of preparation conditions, such as, concentration of poly(vinyl alcohol) (PVA) in w_2 phase, viscosity of inner soluble water phase, volume ratio of oil phase to w_1 phase in primary emulsion, PLGA concentration in oil phase, and molecular weight or composition of PLGA, upon the properties of PLGA microspheres containing water-soluble drugs were examined. Concentration of poly(vinyl alcohol) (PVA), the dispersant dissolved in w_2 phase of secondary emulsion did not show any effects on the final particle size. On the other hand, volume ratio of oil phase to water one in primary emulsion affected the final particle size, which seemed to be related to the local PLGA concentration in w_1/o emulsions. That is, the particle size increased as the volume ratio of w_1 phase against oil phase, w_1/o (v/v), increased. The loading efficiency, however, was not affected by the volume ratio of w_1/o (v/v), but affected by blue dextran concentration in w_1 phase. Higher loading efficiency was observed in PLGA microspheres prepared from w_1 phase containing lower concentration of blue dextran. Blue dextran solution (inner water phase) with the lower viscosity may result in the lower leakage ratio of blue dextran during the preparation procedure. Increases in concentration and molecular weight of PLGA made particle size larger.
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Keywords: PLGA; Microspheres; Solvent evaporation; Viscosity of primary emulsion; Particle size; Loading efficiency

1. Introduction

Microspheres composed of biodegradable polymers have widely been studied as drug carriers in the field of drug delivery system (DDS). Once-a-month injectable poly(lactide-*co*-glycolide) (PLGA) microspheres containing leuprolide acetate have been reported by Ogawa et al. [1]. Water-in-oil-in-water (w/o/w) emulsion method has been used to incorporate water-soluble drugs like peptides, proteins, sugar, and vaccines in PLGA microspheres. Drug loading efficiency and particle size of microspheres are not completely controlled, although they have been reported to be related to stirring rate in secondary emulsion preparation, viscosity of oil phase, polymer concentration, and osmotic pressure [1–4]. For example, when microspheres were prepared using a stirrer, such as ultrasonic homogenizer, the obtained particles were polydisperse, which affected the drug loading ratio, and drug release behavior from the microspheres. Recently, preparation technique of monodisperse biodegradable polymer microspheres by membrane emulsification technique with SPG (Shirasu Porous Glass) membrane was proposed by Kondo et al. [5]. Also, in our experimental group, monodisperse PLGA microspheres containing rifampicin (RFP), a hydrophobic anti-tuberculosis drug, were prepared with the technique [6,7]. But when membrane emulsification method is used to prepare microspheres containing water-soluble drugs from w/o emulsions, each microsphere should contain different amount of drug molecules in it, since it takes more than 10 h for the permeation of w/o emulsions through SPG membrane. To overcome this problem, we have used a microhomogenizer as an emulsification apparatus in this research article. In this research article, we will present the factors affecting microsphere size, size distribution, drug-loading efficiency of PLGA microspheres containing water-soluble drugs.

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doi:10.1016/j.colsurfb.2006.10.019

2. Materials and methods

2.1. Materials

Biodegradable polymer, poly(lactide-*co*-glycolide), PLGA-5005 (lactide:glycolide = 50:50, M_w: 5000), PLGA5010 (lactide:glycolide = 50:50, M_w: 10,000), PLGA5015 (lactide:glycolide = 50:50, M_w: 15,000), PLGA7505 (lactide:glycolide = 75:25, M_w: 5000), PLGA7510 (lactide:glycolide = 75:25, M_w: 10,000), and PLGA7520 (lactide:glycolide = 75:25, M_w: 20,000) were purchased from Wako Pure Chemical Industry, Japan, and all of them were stored at −80 °C prior to use. Blue Dextran 2000 purchased from Amersham Biosciences Co. was used as a water-soluble model drug. Sunsoft 818H offered from Taiyou Kagaku Co. Ltd., Japan was used as an emulsifier dissolved in oil phase. Poly(vinyl alcohol) (PVA) with the degree of polymerization of 500 and saponification of 86–90 mol% purchased from Wako Chemical Industry, was used as a dispersant in outer water phase. Other chemicals were of the reagent grade.

2.2. Preparation of blue dextran-loaded PLGA microspheres

PLGA and Sunsoft 818H were dissolved in dichloromethane (DCM). Blue dextran solution as an inner water phase was poured into the solution. Water-in-oil (w_1/o) emulsion was prepared with a microhomogenizer (NS-310E; Microtec Nition Co. Ltd.) by stirring at 10,000 rpm for 90 s in a 50 ml screw capped tube. Prepared primary emulsion was subsequently added to 190 ml of 1.00% (w/v) PVA solution, and the emulsion was stirred at 10,000 rpm for 90 s to obtain water-in-oil-in-water (w_1/o/w_2) emulsion. Emulsion was poured into 100 ml of distilled water so as to prevent coagulation of the emulsion droplets. Then, microspheres were prepared by solvent evaporation with stirring at 250 rpm for 6 h at room temperature.

2.3. SEM observation of microspheres

The prepared blue dextran-loaded PLGA microspheres were redispersed in distilled water. A droplet of the suspension was placed on the aluminum sample stage, and was dried for 1 day in a vacuum desiccator. The gold sputtering was performed with an ion sputtering device (JFC-1100, JEOL Ltd.). The observation of microspeheres was carried out with a scanning electron microscope (SEM, JSM-6060LA, JEOL Ltd.).

2.4. Measurements of particle size distribution

The volume-averaged diameter and size distribution of the microspheres were measured with a light scattering particle sizer (Mastersizer/E, Malvern, Inc.). Size distribution was evaluated with the span value, defined as expression (1):

$$\text{Span} = \frac{D_{90\%} - D_{10\%}}{D_{50\%}} \quad (1)$$

where $D_{N\%}$ ($N = 10$, 50, 90) means that the volume percentage of microspheres with diameters up to $D_{N\%}$ is equal to $N\%$. The smaller the span value is the narrower the size distribution.

2.5. Measurements of loading efficiency of blue dextran in PLGA microspheres

Four milliliters of DCM were added to the dried blue dextran-loaded PLGA microspheres to completely dissolve PLGA. The blue dextran was precipitated by centrifugation, and the supernatant was removed. After removing DCM by drying for 1 day in a room atmosphere, 5.00 ml of distilled water was added. Concentration of blue dextran was spectrophotometrically measured at 620 nm.

2.6. Measurements of viscosity of primary emulsion

One milliliter of the prepared primary emulsion was set on corn plate type viscometer (VISCOMETER, TV-20, TOKIMEC) device. Measurements of the viscosity were carried out at 100 rpm at 25 °C.

3. Results and discussion

3.1. Effects of PVA concentration on size of blue dextran-loaded PLGA microspheres

Effects of PVA concentration dissolved in outer water phase on the microsphere size were examined. As summarized in Table 1, PVA concentration was changed between 0.50 and 2.50% (w/v), with the concentrations of other materials kept constant. The average diameters of blue dextran-loaded PLGA microspheres were almost 6.0–7.0 μm, independent of PVA concentration, although the stability of emulsion and microsphere size are usually affected by the concentration of polymer molecules adsorbed on the surfaces [8]. This effect is considered to be related to the steric hindrance of polymer molecules stabilizing the emulsion surfaces by adsorption, which inhibits the coagulation of emulsions and microspheres. Span values as an indicator of particle size distribution were also almost constant ones, around 2.0, in all samples, implying that coagulation of emulsions or microspheres did not occur in all samples. The changes in particle size of sample 2 during solvent evaporation were studied. The average diameters of 200 droplets were measured every 1 h during solvent evaporation using optical microscopic photographs. The results were shown in Fig. 1. The droplet size of the secondary emulsion (w_1/o/w_2) was over 20 μm at time 0. Just after solvent evaporation started, the particle size was drastically reduced by the shrinkage of droplets, and it was maintained almost constant about 6.0 μm. In other words, the coagulation of droplets which influences the particle size of final microspheres did not occur during solvent evaporation. In addition, the particle size of microspheres was controlled in the initial 1 h from solvent evaporation started. From SEM photographs shown in Fig. 2, it is clear that micorspheres

Table 1
Preparation of blue dextran-loaded PLGA microspheres with various concentration of PVA

	Sample				
	1	2	3	4	5
Inner water phase					
Blue dextan concentration (% (w/v))	5.00	5.00	5.00	5.00	5.00
Distilled water (ml)	2.00	2.00	2.00	2.00	2.00
Oil phase					
PLGA 7505 concentration (% (w/v))	6.25	6.25	6.25	6.25	6.25
Sunsoft concentration (% (w/v))	0.94	0.94	0.94	0.94	0.94
DCM (ml)	8.00	8.00	8.00	8.00	8.00
Outer water phase					
PVA concentration (% (w/v))	0.50	1.00	1.50	2.00	2.50
Distilled water (ml)	190	190	190	190	190
Primary emulsion					
Stirring rate (rpm)	10,000	10,000	10,000	10,000	10,000
Stirring time (s)	90	90	90	90	90
Total volume (ml)	200	200	200	200	200
Particle size (μm)	7.61	6.20	7.60	7.67	7.42
Span	2.16	1.15	1.97	2.29	1.79
Loading efficiency of blue dextran (%)	8.66	21.7	17.8	17.2	16.9
Viscosity of w_1/o emulsion (mPa s)	1.54	1.54	1.54	1.54	1.54

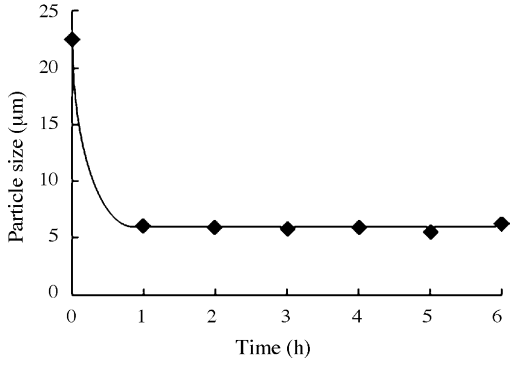

Fig. 1. Changes in size of emulsion droplets during solvent evaporation procedure.

prepared under the conditions shown in Table 1 have almost constant particle sizes, spherical shapes and smooth surfaces. It is clear that 0.50 or 1.00% (w/v) of PVA solution is proper to prepare microspheres. Also, in Table 1, the loading efficiency of blue dextran was shown. When the 1.00% PVA solution (w/v) was used, the maximum loading efficiency was obtained. The loading efficiency of hydrophilic drugs in PLGA microspheres was highly dependent on PVA concentration. When PVA concentration in w_2 phase is too high, PLGA microspheres having surface layers with high water contents and high wettability are prepared, since PVA remains in the surface layers of the microspheres [9]. Then the loading efficiency seems to be lower in samples 3–5 than that in sample 2.

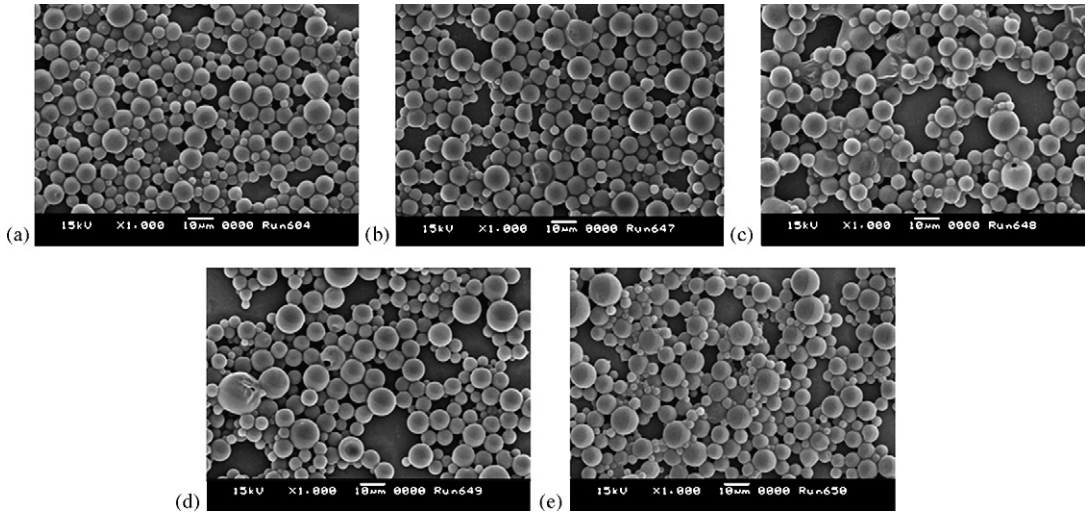

Fig. 2. SEM photographs of blue dextran-loaded PLGA microspheres prepared by using PVA solutions with various concentrations. PVA concentration (% (w/v)); (a) 0.50, (b) 1.00, (c) 1.50, (d) 2.00, and (e) 2.50.

Table 2
Preparation of blue dextran-loaded PLGA microspheres with various amount of blue dextran

	Sample				
	6	7[a]	8	9	10
Inner water phase					
Blue dextran concentration (% (w/v))	2.50	5.00	7.50	10.0	12.5
Distilled water (ml)	2.00	2.00	2.00	2.00	2.00
Oil phase					
PLGA 7505 concentration (% (w/v))	6.25	6.25	6.25	6.25	6.25
Sunsoft concentration (% (w/v))	0.94	0.94	0.94	0.94	0.94
DCM (ml)	8.00	8.00	8.00	8.00	8.00
Outer water phase					
PVA concentration (% (w/v))	1.00	1.00	1.00	1.00	1.00
Distilled water (ml)	190	190	190	190	190
Primary emulsion					
Stirring rate (rpm)	10,000	10,000	10,000	10,000	10,000
Stirring time (s)	90	90	90	90	90
Total volume (ml)	200	200	200	200	200
Particle size (μm)	7.10	6.20	6.30	7.20	6.49
Span	1.74	1.15	1.65	2.04	2.20
Loading efficiency of blue dextran (%)	41.4	21.7	16.0	10.7	10.7
Viscosity of w_1/o emulsion (mPa s)	1.12	1.54	1.11	1.93	1.21
Viscosity of w_1 phase (mPa s)	6.03	22.3	69.7	166	337

[a] Sample 7 is the same as sample 2 in Table 1.

3.2. Influence of blue dextran concentration in inner water phase

The effects of concentration of blue dextran in an inner water phase on its loading efficiency were studied, as shown in Table 2. The amount of blue dextran was changed from 0.05 to 0.25 g with the volume of the inner water phase remains constant, so the viscosity of inner water phase increased as the amount of blue dextran increased. The drug loading efficiency gradually decreased with increasing the concentration of blue dextran. The increase in the concentration of blue dextran may improve coagulation of primary emulsion droplets by the increase in a viscosity of inner water phase, which will accelerate the leakage of inner water phase to outer water phase. On the other hand, the viscosity of inner water phase did not affect the particle size of microspheres. The particle diameter in all samples was kept almost constant to be 6.0–7.0 μm, independent of blue dextran concentration.

3.3. Effects of volume ratio of DCM against water in primary emulsion upon the properties of blue dextran-loaded PLGA microspheres

The effects of the volume ratio of dichloromethane (DCM) solution containing PLGA against distilled water in w_1/o emulsion on the properties of blue dextran-loaded PLGA microspheres were evaluated. The volume of the inner water phase was changed from 1.00 to 5.00 ml, keeping the total volume of w_1/o emulsion to be 10.0 ml, as shown in Table 3. To keep the viscosity of w_1 phase, blue dextran concentration was kept constant. The particle sizes of microspheres increased as the volume ratio of w_1/o (v/v) increased, since the viscosity of oil phase increased as PLGA concentration increased by the decrease of DCM volume. The drug loading efficiency was almost kept constant, independent of the volume ratio of w_1/o (v/v). In this experiment shown in Table 3, blue dextran concentration was kept constant to be 10%. When we have used 2% blue dextran solution, the similar dependency of particle size and loading efficiency on the volume ratio of w_1/o (v/v) was obtained, although the drug loading efficiency was almost 45% (data are not shown), since the loading efficiency increases as blue dextran concentration decreases, as indicated in Table 2.

Particle size seems to be affected by the viscosity of w_1/o emulsion, as shown in Table 3. The viscosity of primary emulsion (w_1/o emulsion) was highly affected by the concentration of PLGA in oil phase. As the viscosity of primary emulsion increases, the coagulation of primary (w_1/o) emulsion during preparation of secondary (w_1/o/w_2) emulsion easily occurs, which makes the diameters of blue dextran-loaded PLGA microspheres larger.

3.4. Effects of the composition, molecular weight and concentration of PLGA on the properties of blue dextran-loaded PLGA microspheres

The effects of molecular weight and composition of PLGA on the properties of blue dextran-loaded PLGA microspheres were examined using six kinds of PLGA, as shown in Table 4. The values of the particle size increased with increasing molecular weight of PLGA, which seemed to be related to the viscosity of w_1/o emulsions. As shown in Table 4, the viscosity of w_1/o emul-

Table 3
Preparation of blue dextran-loaded PLGA microspheres with various volume ratio of DCM against water at 10.0% constant concentration of inner water phase

	Sample				
	11	12[a]	13	14	15
Inner water phase					
Blue dextran concentration (% (w/v))	10.0	10.0	10.0	10.0	10.0
Distilled water (ml)	1.00	2.00	3.00	4.00	5.00
Oil phase					
PLGA 7505 concentration (% (w/v))	5.56	6.25	7.14	8.33	10.0
Sunsoft concentration (% (w/v))	0.83	0.94	1.07	1.25	1.50
DCM (ml)	9.00	8.00	7.00	6.00	5.00
Outer water phase					
PVA concentration (% (w/v))	1.00	1.00	1.00	1.00	1.00
Distilled water (ml)	190	190	190	190	190
Primary emulsion					
Stirring rate (rpm)	10,000	10,000	10,000	10,000	10,000
Stirring time (s)	90	90	90	90	90
Total (ml)	200	200	200	200	200
Particle size (μm)	6.51	7.20	7.73	8.31	11.8
Span	2.55	2.04	1.99	1.54	0.90
Loading efficiency of blue dextran (%)	12.2	10.7	11.1	11.3	11.8
Viscosity of w_1/o emulsion (mPa s)	0.94	1.93	4.24	8.96	13.7

[a] Sample 12 is the same as sample 9 in Table 2.

sion increased as the molecular weight of PLGA increased. Span values also increased with increasing polymer molecular weight. The increase in the viscosity of w_1/o emulsion made the particle size and the span value larger, which is caused by the coagulation between primary emulsions during preparation of the secondary emulsion. The loading efficiency of blue dextran was affected by molecular weight of PLGA, as observed when hydrophobic drug, rifampicin, was loaded in PLGA microspheres [6]. The drug loading efficiency of microspheres prepared from PLGA having 50% lactide was lower than that from PLGA having 75% lactide. In addition, when PLGA having 75% lactide was used, higher loading efficiency was obtained in PLGA microspheres composed of PLGA having higher molecular weight. On the other hand, the dependence of drug loading efficiency on molec-

Table 4
Effects of the composition and molecular weight of PLGA on the properties of blue dextran-loaded PLGA microspheres

	Sample					
	16[a]	17	18	19	20	21
Inner water phase						
Blue dextran concentration (% (w/v))	5.00	5.00	5.00	5.00	5.00	5.00
Distilled water (ml)	2.00	2.00	2.00	2.00	2.00	2.00
Oil phase						
PLGA concentration (% (w/v))	6.25	6.25	6.25	6.25	6.25	6.25
PLGA	7505	7510	7520	5005	5010	5015
Sunsoft concentration (% (w/v))	0.94	0.94	0.94	0.94	0.94	0.94
DCM (ml)	8.00	8.00	8.00	8.00	8.00	8.00
Outer water phase						
PVA concentration (% (w/v))	1.00	1.00	1.00	1.00	1.00	1.00
Distilled water (ml)	190	190	190	190	190	190
Primary emulsion						
Stirring rate (rpm)	10,000	10,000	10,000	10,000	10,000	10,000
Stirring time (s)	90	90	90	90	90	90
Total volume (ml)	200	200	200	200	200	200
Particle size (μm)	6.20	6.94	7.86	6.35	6.71	8.44
Span	1.15	1.43	2.25	1.16	1.42	2.22
Loading efficiency of blue dextran (%)	21.7	34.6	44.6	18.0	16.2	12.8
Viscosity of w_1/o emulsion (mPa s)	1.54	3.21	4.21	1.32	3.40	4.40

[a] Sample 16 is the same as sample 2 in Table 1 and sample 7 in Table 2.

Table 5
Effects of PLGA amount in primary emulsion on the properties of blue dextran-loaded PLGA microspheres

	Sample				
	22	23[a]	24	25	26
Inner water phase					
Blue dextran concentration (% (w/v))	5.00	5.00	5.00	5.00	5.00
Distilled water (ml)	2.00	2.00	2.00	2.00	2.00
Oil phase					
PLGA 7505% (w/v)	3.13	6.25	9.38	12.5	15.6
Sunsoft concentration (% (w/v))	0.94	0.94	0.94	0.94	0.94
DCM (ml)	8.00	8.00	8.00	8.00	8.00
Outer water phase					
PVA concentration (% (w/v))	1.00	1.00	1.00	1.00	1.00
Distilled water (ml)	190	190	190	190	190
Primary emulsion					
Stirring rate (rpm)	10,000	10,000	10,000	10,000	10,000
Stirring time (s)	90	90	90	90	90
Total volume (ml)	200	200	200	200	200
Particle size (μm)	5.38	6.20	9.40	9.81	11.0
Span	1.50	1.15	0.93	1.04	0.94
Loading efficiency of blue dextran (%)	13.9	21.7	21.3	21.3	18.2
Viscosity of w_1/o emulsion (mPa s)	1.07	1.54	3.43	5.18	7.53

[a] Sample 23 is the same as sample 2 in Table 1, sample 7 in Table 2, and sample 16 in Table 4.

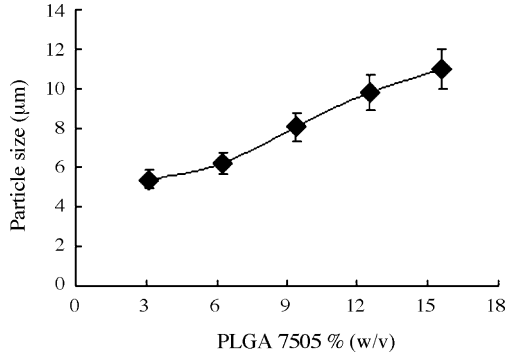

Fig. 3. Effects of PLGA concentration on the particle sizes of blue dextran-loaded PLGA microspheres.

ular weight of PLGA showed the opposite tendency when PLGA having 50% lactide was used.

The effects of PLGA concentration in an oil phase on the properties of blue dextran-loaded PLGA microspheres were examined as shown in Table 5. PLGA 7505 in the range between 0.25 and 1.25 g was dissolved in 8.00 ml of DCM. As shown in Fig. 3, particle size of microspheres increased as PLGA concentration increased as same as reported in a previous paper [3]. As shown in Table 5, the viscosity of w_1/o emulsion increased as concentration of PLGA increased. The thicker polymer layers were formulated at the interface between oil and w_2 phase, then the particle size of microspheres increased. From these observations, it is clear that the viscosity of w_1/o emulsion on PLGA solution affects the particle size, size distribution of water-soluble drug-loaded PLGA microspheres.

Acknowledgements

This work was supported by "High-Tech Research Center" Project for Private Universities: matching fund subsidy from Ministry of Education, Culture, Sports, Science and Technology (MEXT), 2004–2008.

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