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Effect of polyethylene glycol on preparation of rifampicin-loaded PLGA microspheres with membrane emulsification technique

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ABSTRACT

Monodisperse poly(lactide-*co*-glycolide) (PLGA) microspheres containing rifampicin (RFP), anti-tubercle drug, as hydrophobic model drug were prepared by solvent evaporation method with a membrane emulsification technique using Shirasu Porous Glass (SPG) membranes. Five kinds of rifampicin-loaded PLGA (RFP/PLGA) microspheres with different sizes were prepared by changing pore size of the membranes. Effect of polyethylene glycol (PEG) added to polyvinyl alcohol (PVA) solution (continuous phase) upon the monodispersity of microspheres was studied. PEG was used as a stabilizer for microspheres dispersing in PVA solution. The most suitable molecular weight of PEG as a stabilizer was 20,000. RFP/PLGA microspheres prepared with PEG20000 were apparently more uniform than those prepared without PEG. The yield of RFP/PLGA microspheres was 100%. The initial burst observed in the release of RFP from RFP/PLGA microspheres was suppressed by the addition of PEG.

1. Introduction

Biodegradable microspheres containing bioactive materials with a uniform size have a great potential as carriers in drug delivery system (DDS), since the release rate and loading efficiency of drug can be constant in each microsphere [1]. Poly(lactide-*co*-glycolide) is a widely used biodegradable polymer in DDS. Usually, PLGA microspheres containing drug are prepared by a solvent evaporation technique [2–7]. The polymer solution dissolved in organic solvent, (oil phase), is dispersed in aqueous phase (continuous phase) containing surfactants by mixing under mechanical agitation. By evaporating organic solvent from this emulsion, polydisperse PLGA microspheres are generated. The monodisperse microspheres with various sizes can be prepared by membrane emulsification technique using Shirasu Porous Glass (SPG) membrane [8,9]. We have reported that PLGA microspheres containing rifampicin, RFP, with various sizes between 1.0 and 9.0 μm are prepared by changing the pore size of membranes, and the release rate of RFP from the microspheres was studied [10,11]. PLGA microspheres containing RFP with molecular weight of 10,000 were relatively monodisperse and the values of the coefficient of variation (CV) for the size distributions of the microspheres were in the range between 7.0 and 16.0%. From RFP/PLGA microspheres with average diameters of 1.3 and 2.2 μm, almost 60% of RFP loaded in the microspheres was released in the initial day and the release was terminated almost within 10 days. On the other hand, from those with average diameters of 5.2, and 9.0 μm, the release of RFP was observed even 20 days after the release started [11]. Further examination to progress properties of PLGA microspheres containing drug was studied. In this research article, we will show the effects of PEG on monodispersity of PLGA microspheres containing RFP with molecular weight of 5000. Also, the effects of PEG on loading efficiency and release rate of RFP will be discussed.

2. Experimental

2.1. Materials

PLGA7505 (poly(lactide-*co*-glycolide), 75:25, Mw 5000) purchased from Wako Pure Chemical Industry, Japan was stored at

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Table 1
Preparation of RFP/PLGA microspheres without PEG

	Sample #				
	1	2	3	4	5
SPG pore size (μm)	1.00	1.50	1.95	2.60	3.63
PLGA7505 con. % (w/v)	5.00	5.00	5.00	5.00	5.00
RFP con. % (w/v)	0.50	0.50	0.50	0.50	0.50
DCM (v) (ml)	10.0	10.0	10.0	10.0	10.0
PVA con. % (w/v)	2.00	2.00	2.00	2.00	2.00
Distilled water (ml)	190	190	190	190	190
Total volume (ml)	200	200	200	200	200
Particle size (μm)	3.11	4.31	4.50	5.82	8.79
CV (%)	16.4	17.4	17.1	10.6	18.0
Yield (%)	32.0	36.0	46.2	45.6	52.5
Loading efficiency of RFP (%)	51.6	62.6	55.1	60.6	61.9
Critical pressure (kgf/cm^2)	0.29–0.33	0.12–0.15	0.09–0.11	0.06–0.08	0.02–0.03

−80 °C prior to use. Anti-tuberculosis drug, rifampicin (RFP) purchased from SIGMA was used as hydrophobic model drug. Polyvinyl alcohol (PVA) with the degree of polymerization of 500 and the saponification of 86–90 mol% purchased from Wako Pure Chemical Industry, Japan was used as a stabilizer of microspheres. Polyethylene glycol (PEG) (Wako Chemical Industry, Mw 200, 1000, 6000, 20,000) was used as a stabilizer. Other chemicals were those of the highest grade commercially available.

2.2. Preparation of RFP/PLGA microspheres

Five hundreds milligrams of PLGA and 50 mg of RFP were dissolved in 10.0 ml of dichloromethane (DCM). After mixing with vortex mixer, they (oil phase) were injected to the oil tank of an apparatus of SPG (Shirasu Porous Glass) membrane emulsification. This apparatus soaked into 190 ml of 2.0 wt% PVA solution (continuous phase), and the SPG membrane was stored in the solution under a gentle agitation with a magnetic stirrer for 1 h. The nitrogen gas was flowed into the tank forces the dispersion droplets through stainless steel module with a SPG membrane to a continuous phase containing dispersion stabilizer. SPG membranes with membrane pore sizes of 1.00, 1.50, 1.95, 2.60, and 3.63 μm were used. After the membrane emulsification, the emulsion was poured into 100 ml of 2.0 wt% (w/v) PVA solution. The solvent evaporation at room temperature was carried out at 250 rpm for 4 h. After passing through a 22 μm sieve, monodisperse polymer microspheres were collected by centrifuged and washed with distilled water for three times.

2.3. Measurement of yield of prepared monodisperse microspheres

The microspheres which do not pass through a 22 μm sieve were collected and the weight was measured after drying. The yield of monodisperse microspheres was calculated from the following equation:

$$\text{Yield} = \frac{w_\text{i} - w_\text{e}}{w_\text{i}} \times 100, \quad (1)$$

where w_i is an initial weight of microspheres, w_e is the weight of microspheres which do not pass the sieve.

2.4. SEM observation of microspheres

A droplet of the suspension of the prepared microspheres was placed on the aluminum sample stage, and was dried for 1 day under reduced pressure. The gold sputtering with an ion sputtering device (JFC-1100, JEOL Ltd.) was performed. The microscopic observation of RFP/PLGA microspheres was carried out with a scanning electron microscope (SEM, JSM-T20, JEOL Ltd.).

2.5. Measurements of microsphere size

An average-diameter and the value of coefficient of variation (CV) for prepared microspheres were calculated from 200 microspheres on the taken SEM photograph. The value of CV was calculated from the following equation:

$$\text{CV} = \frac{\sigma}{D_\text{p}} \times 100 \quad (2)$$

where σ is a standard deviation, and D_p is an average microsphere diameter obtained by SEM observation. The lower value of CV means the higher monodispersity of the microspheres.

2.6. Measurements of ζ-potential of prepared microspheres

A droplet of suspension of the prepared microspheres was diluted to 5000 times in 10 ml test tube, and a droplet of this diluted suspension was dispersed in PBS (phosphate buffer solution) of 1.54×10^{-2} M. The ζ-potentials of the microspheres at 25 °C were measured with ζ-potential measurement apparatus (ELS-800, Otsuka Electro. Co., Ltd.).

2.7. Measurements of loading efficiency of RFP in PLGA microspheres

The prepared RFP/PLGA microspheres were dissolved in chloroform of 5.0 ml. The RFP concentration in the solution was spectrophotometrically measured at 475 nm. The measurements were carried out three times.

2.8. Release study

Ten milligrams of the prepared RFP/PLGA microspheres were dispersed in 5.0 ml PBS (phosphate buffer solution) of pH 7.4 with the ionic strength of 0.154 M. The suspension was shaken with 60 times/h at 37 °C. The suspension was centrifuged 1 h or 20 min later, and RFP concentration in the supernatant was spectrophotometrically measured at 475 nm. The PBS was changed with fresh one every hour or 20 min.

3. Result and discussion

3.1. Preparation of RFP/PLGA microspheres

Effects of the pore sizes of SPG membrane on microspheres size, CV value, loading efficiency has been studied, as shown in Table 1. As reported in previous papers [11,12], the value of critical pressure to pass the oil phase through SPG membrane was dependent