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Development of Fine Poly(D,L-Lactic-Co-Glycolic Acid) Particles for Hydrophilic Drug Using a Solid-in-Oil-in-Water Emulsion

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Abstract

Poly(D,L-Lactic-Co-Glycolic Acid) (PLGA) copolymers have been extensively used as controlled-release carriers for many hydrophilic drugs because they are non-toxic, biodegradable, bioavailable, and biocompatible. In general, PLGA particles have been produced by a solvent evaporation technique utilizing water-in-oil-in-water (W/O/W) emulsions. However, W/O/W emulsions are unstable, causing the outer and inner aqueous phases to easily fuse during particle preparation. Consequently, a sufficient amount of drug was not encapsulated inside the particles. In this study, we examined a new particle preparation method utilizing a solid-in-oil-in-water (S/O/W) emulsion technique. The advantages of S/O/W emulsions, wherein a surfactant-drug complex disperses into the oil phase, were as follows: 1) leakage of hydrophilic drugs from the emulsions was inhibited, and 2) facile control over the emulsion particle size. Thus, the PLGA particles prepared by this method showed high encapsulation efficiency of drugs and formation of fine particles of submicron size by membrane emulsification were achieved.

Keywords

PLGA, Hydrophilic drugs, S/O/W Emulsions, Solvent Evaporation

1. Introduction

Poly(D,L-Lactic-Co-Glycolic Acid) (PLGA) is a highly biocompatible and biodegradable synthetic polymer. In the body, this polymer is first hydrolytically

degraded into a non-toxic oligomer and finally converted to lactic acid and glycolic acid. PLGA particles have been widely studied as drug carriers. The monomer composition and molecular weight of PLGA are known to have an effect on the rate of drug release. Many preparation methods for PLGA particles have been reported in the literature [1] [2] [3]. PLGA particles loaded with lipophilic drugs, when compared with hydrophilic drug content, can be easily prepared. To load PLGA particles with lipophilic drugs, an O/W emulsion is prepared using an aqueous solution and a volatile organic solvent containing the drugs and PLGA. The volatile solvent is then evaporated to produce PLGA particles. This method is called the solvent evaporation method [4]. In contrast, the encapsulation of hydrophilic drugs requires more complex techniques. The simplest method is called the solvent diffusion method which is performed in water [5] [6] [7]. The drug is first dissolved in a water-miscible organic solvent and the resultant solution is then dispersed in an aqueous surfactant solution under mild agitation. The emulsion is slowly formed from the water and miscible organic solvent. The deposition of PLGA is initiated by the diffusion of the organic solvent in water. PLGA nanoparticles are prepared using this method. However, the entrapment efficiency of the drug is very low because the organic solution containing the drug diffuses out of PLGA. Other methods utilize a W/O emulsion that contains the hydrophilic drug in the aqueous phase and PLGA in the oil phase. One of these methods, the solvent evaporation method, uses a W/O/W emulsion [8] [9]. It is performed by evaporating the volatile organic solvent in the oil phase. The drug encapsulation efficiency is low because the water-soluble drugs leak from the internal to the external aqueous phase. Another method, called the phase separation method, is used in the oil phase [10]. PLGA-insoluble oil, such as silicone oil or liquid paraffin, is added into a W/O emulsion, inducing PLGA deposition. Some researchers also have reported the solvent evaporation method using Solid-in-Oil suspension containing amphiphilic compounds and the hydrophilic drugs in organic solvent [11] [12].

In this study, we examined a new method for the preparation of PLGA particles containing hydrophilic drugs using a solid-in-oil-in-water (S/O/W) emulsion. The hydrophilic drugs were converted into a lipophilic complex by coating with surfactant molecules. In this S/O/W emulsion technique, the surfactant-coated drugs formed a stable dispersion in the oil phase [13]. The S/O/W emulsion presented here has a few advantages: 1) high drug encapsulation efficiency in the particles, and 2) it is convenient for the formation of nano-sized particles. When preparing PLGA particles from W/O/W emulsions, the encapsulation of drugs in PLGA particles is low. The drugs contained in the internal aqueous phase of the W/O/W emulsion easily leak into the external aqueous phase. Two mechanisms for the leakage of drugs have been previously proposed by other researchers [14] [15]. One leakage mechanism is that the drugs pass through the thin lamellar layer of surfactants. This can partially form in the oil layer due to the fluctuation of its thickness. The other mechanism is diffusion across the oil layer by incorporation of drug into reverse micelles. These drug

leakage mechanisms are caused by the flow of water. We hypothesized that the S/O/W emulsion technique would reduce the leakage of drugs because this emulsion doesn't induce water flow. It is also difficult to form nano-sized particles using W/O/W emulsions because they contain internal aqueous droplets that hinder the formation of smaller particles. However, we found that S/O/W emulsions are able to form nanoparticles because the hydrophilic drug complexes and surfactants are dissolved in the oil phase. In this article, we examined the properties of PLGA particles prepared using S/O/W emulsions.

2. Experimental

2.1. Materials

PLGA, polyvinyl alcohol (PVA), hexane, dichloromethane, cholic acid and theophylline were purchased from Wako Pure Chemicals Industries Ltd. (Japan). Albumin and insulin were obtained from Sigma-Aldrich (Japan). Sucrose palmitate and Sucrose laurate were provided by Mitsubishi-Kagaku Foods Co. (Japan).

2.2. Preparation of PLGA Particles

An aqueous solution (5.0 mL) of hydrophilic drug and a hexane solution (10.0 mL) containing sucrose palmitate (P-170) as a hydrophobic surfactant were mixed with a homogenizer (20,500 rpm) for 1 min. The resultant W/O emulsions were lyophilized for 1 day. Using this method, the drug was coated with surfactant. Next, the surfactant-coated drug was readily dispersed into methylene chloride (5.0 mL) and a S/O suspension was prepared. Then, PLGA (5 wt%) was added into the S/O suspension and stirred. This solution was used as the oil phase and an aqueous solution (20 mL) containing hydrophilic surfactant (3 wt%) was used as the aqueous phase. These two solutions were mixed and emulsified with a homogenizer (13,000 rpm) for 1 min. This formed the S/O/W emulsion. Next, methylene chloride in the emulsion was evaporated under vacuum. The formed PLGA particles were collected by centrifugation and washed with distilled water three times, followed by lyophilization for 1 day. For the preparation of PLGA particles using W/O/W emulsions, W/O emulsions were formed by mixing an aqueous drug solution (2.5 mL) and methylene chloride (5.0 mL) containing P-170 (instead of S/O suspension) at 20,500 rpm. In order to control the particle size, the emulsions were passed through a shirusu porous grass (SPG) membrane (pore size = 0.6 μm) prior to solvent evaporation. The preparation conditions and drug encapsulation efficiencies (EE) are shown in Table 1.

2.3. Examination of Particle Properties

The morphology of the prepared particles was observed with a scanning electron microscope (JSM-6060, JEOL, Japan). The particle size was measured using a laser diffraction particle size analyzer (LA-290, HORIBA, Japan). The encapsulation

Table 1. Preparation conditions for PLGA particles containing lipophilic assembly.

	Type	Lipophilic surfactant conc. [wt %][2]	Hydrophilic surfactant	Model drug	EE [%]
P1	W/O/W	5	PVA	Theophylline	19.7
P2	S/O/W	5	PVA	Theophylline	54.2
P3	S/O/W	3	PVA	Theophylline	22.1
P4	S/O/W	10	PVA	Theophylline	53.2
P5	S/O/W	5	P-1670	Theophylline	37.1
P6	S/O/W	5	Cholic acid	Theophylline	44.6
P7	S/O/W[1]	5	PVA	Theophylline	56.3
P8	S/O/W[1]	5	Cholic acid	Theophylline	45.0
P9	S/O/W[1]	5	Cholic acid	Insulin	72.4
P10	S/O/W[1]	5	Cholic acid	Albumin	87.1

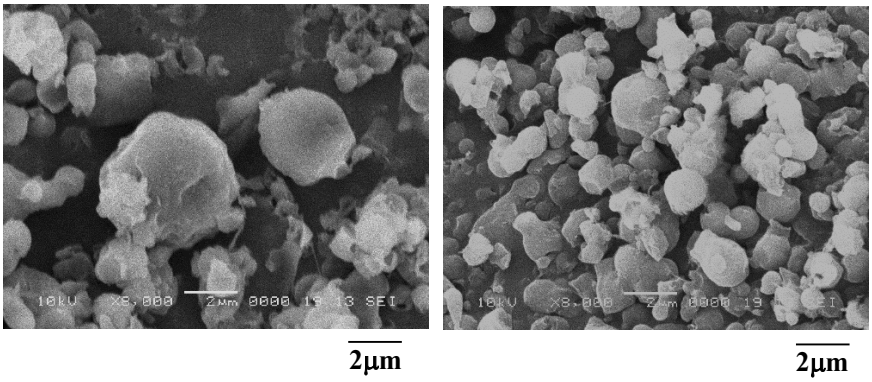
1: membrane emulsification, 2: Concentration of surfactant in oil phase used in the lipophilic complex preparation.

efficiency of hydrophilic drugs into the particles was estimated by detection of the drugs that leaked into the external aqueous phase during the particle preparation process. Theophylline was measured using a spectrophotometer (λ_{abs} = 270 nm, S-3100, SCINCO, Korea). Insulin and albumin used in the modified fluorescent dye, FITC, were measured using fluorometer (λ_{Ex} = 490 nm, λ_{Em} = 520 nm, Versa Fluor, Bio-rad, Japan).

3. Results and Discussion

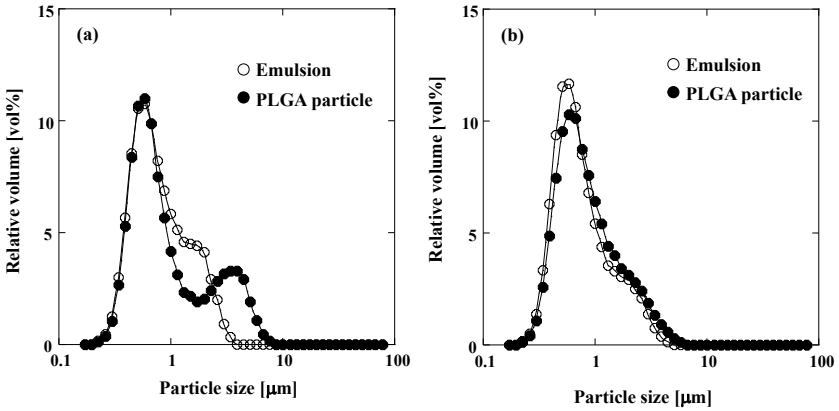
3.1. PLGA Particles Prepared from S/O/W and W/O/W Emulsions

The properties of the PLGA particles prepared using the S/O/W emulsions were compared with those prepared using the W/O/W emulsions. It is known that when W/O/W emulsions are used to encapsulate hydrophilic drugs in PLGA particles, the entrapment efficiency is low. In this study, we focused on PLGA particles prepared using a S/O/W emulsion technique. As shown in **Table 1**, the theophylline entrapment efficiency of the particles (P2) prepared using the S/O/W emulsion was clearly higher those (P1) prepared using the W/O/W emulsion. This result shows the effect of coating the drug with surfactant. SEM images of each particle are shown in **Figure 1** and it was found that each particle is spherical. It was confirmed that the PLGA particles can be prepared using the S/O/W emulsion and the particles obtained have the desired entrapment effect on the lipophilic drug. The size distributions of PLGA particles and emulsions are shown in **Figure 2**. From this figure, it was found that the size of the PLGA particles reflects the oil droplet size of the emulsion used during the particle preparation. In other words, the PLGA particle size can be controlled by controlling the emulsion droplet size. From these results, it is confirmed that S/O/W emulsions are adequate for the preparation of PLGA particles as hydrophilic drug carriers.



(a) (b)

Figure 1. SEM images of the particles prepared using (a) W/O/W emulsions and (b) S/O/W emulsions.



(a) (b)

Figure 2. Size distribution of emulsions before the solvent evaporation process and prepared PLGA particles. Emulsion type used in particle preparation: (a) W/O/W emulsion; (b) S/O/W emulsion.

3.2. Effect of Preparation Conditions on the Preparation of PLGA Particles

The preparation conditions for particle formation using the S/O/W emulsions were examined. We examined the effect of lipophilic surfactant concentration. As shown in **Table 1**, when the surfactant concentration in S/O/W emulsion was above 5% (P2, P4), the entrapment efficiency of theophylline inside PLGA particles was nearly constant. However, when the concentration was 3% (P3), the encapsulation efficiency inside of the particles was lower than the others. When we observed SEM images of these particles, it was found that the particles containing 10% surfactant were bigger than the others. **Figure 3** shows SEM images of particles prepared using 3% and 10% surfactant (P3, P4). From this figure, it is clear that the particle prepared using 10% surfactant is larger than that prepared using 5% surfactant. We concluded that using high concentrations of lipophilic surfactant makes unstable S/O/W emulsions. Accordingly, we decided to use 5% lipophilic surfactant for future experiments.

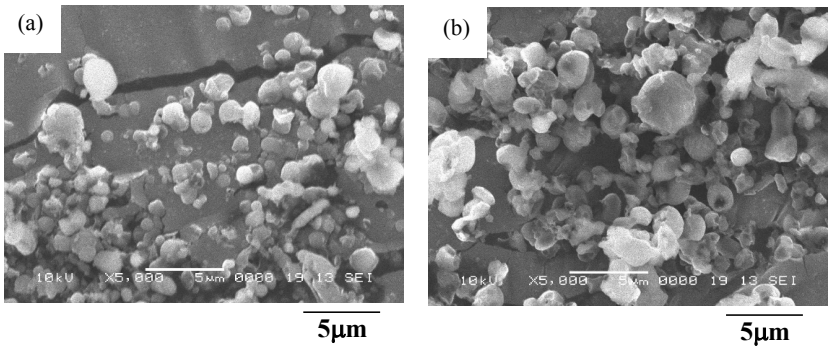


Figure 3. SEM images of the particles with the different content of lipophilic surfactant. (a) 3 wt%, (b) 10 wt%.

Next, the effect of hydrophilic surfactants used for emulsion formation was examined. When PLGA particles were prepared by the solvent evaporation method for O/W emulsions, PVA is commonly used as surfactant because PVA has a strong interaction with PLGA as well as good redispersibility in aqueous solution. Moreover, PVA improves the stability of emulsion by increasing the viscosity of the aqueous solution. Here, we compared PVA (P2) with other surfactants such as sucrose palmitic ester (P-1670) (P5) and cholic acid (P6). The theophylline encapsulation efficiency of the particles prepared using PVA was the highest (**Table 1**). In comparison, the particles prepared using P-1670 leaked large amounts of theophylline. Also, we found that the encapsulation efficiency of the particles prepared using cholic acid was higher than P-1670 and that the particle size was relatively small (**Figure 4**).

We also attempted to control the size of PLGA particles. The carrier particles used for drug delivery applications require strict size control to be administered. Thus, we sought to control the oil droplet size of the S/O/W emulsion by the membrane emulsification method. This is a method used to control the size of oil droplets by passing through an inorganic porous membrane. By careful tuning of the preparation conditions, it is known that the average diameter of the emulsion droplets becomes nearly equal to the pore size of the membrane used. The selection of a hydrophilic surfactant suitable for membrane emulsification is critical to obtain proper size control of PLGA particles. First, we used PVA as a surfactant because it is suitable to encapsulate hydrophilic drugs in PLGA particles. **Figure 5(a)** shows the size distribution of PLGA particles (P7) prepared using an aqueous PVA solution. As shown in this figure, membrane emulsification using an aqueous PVA solution did not allow control over PLGA particle size. Alternatively, cholic acid was found to have a miniaturizing effect on the particle size in S/O/W emulsions when the membrane emulsification method was used. **Figure 5(b)** shows the size distribution of PLGA particles (P8) prepared using that solution. The size distribution of the particles prepared showed little variance and the average particle size was nearly equal to the pore size of the membrane. This was also confirmed from the SEM images of the prepared particles (**Figure 6**). From these results, it was confirmed that submicron size

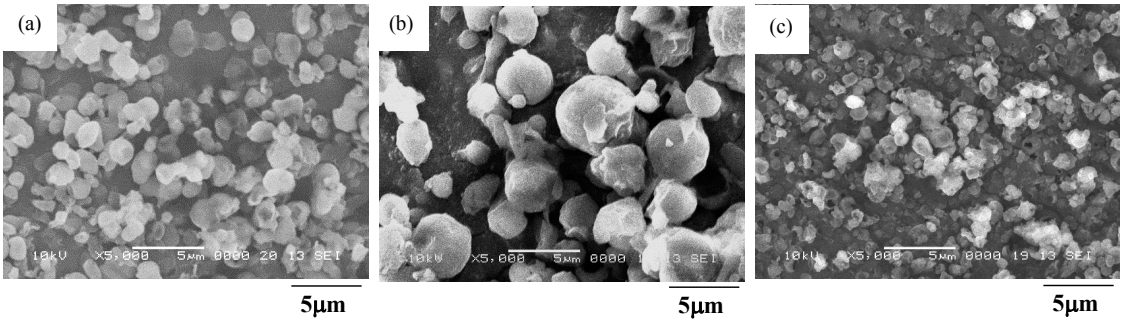
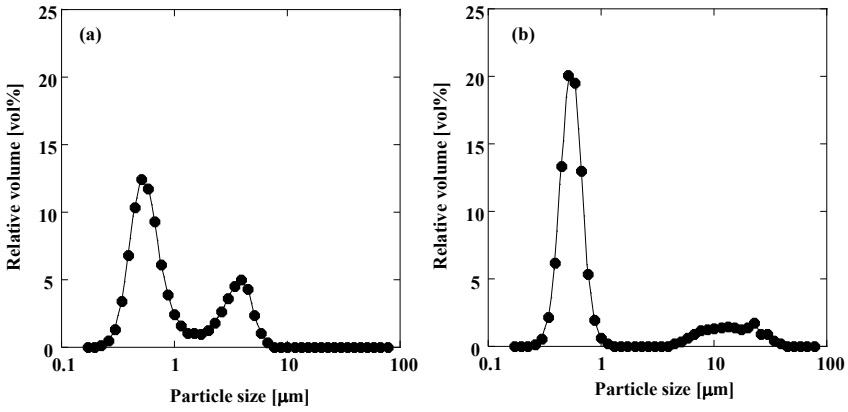


Figure 4. SEM images of the particles prepared with the emulsions containing different hydrophilic surfactants. Surfactant: (a) PVA; (b) L-1695; (c) Cholic acid.



(a) (b)

Figure 5. Size distribution of the particles controlled by membrane emulsification. Hydrophilic surfactant: (a) PVA; (b) Cholic acid.

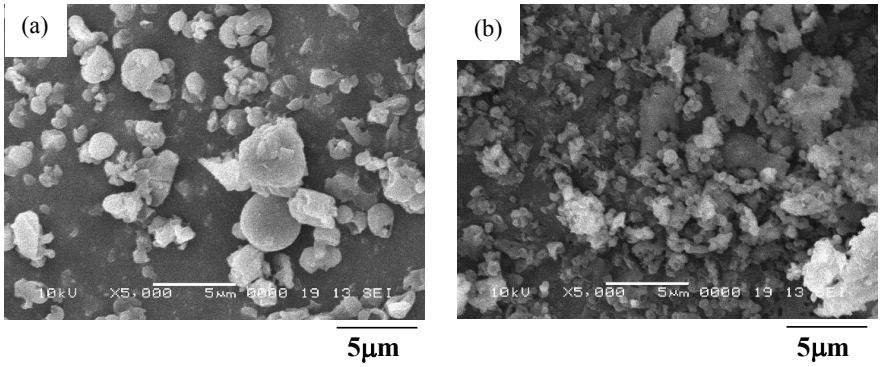


Figure 6. SEM images of the particles controlled by membrane emulsification: Surfactant: (a) PVA; (b) Cholic acid.

control of PLGA particles, prepared using S/O/W emulsions, by the membrane emulsification method is possible.

Finally, the effect to the encapsulation efficiency of molecular weight of hydrophilic drug was also studied. Three model drugs: theophylline (Mw: 180), insulin (Mw: 5800) and albumin (Mw: 66,000) were encapsulated into PLGA particles. The encapsulation efficiency for each drug is shown in **Table 1** (P8, P9

and P10). Higher encapsulation efficiency was observed for larger molecular weight drugs. From this result, we hypothesize that polymer drugs, such as peptides or proteins, can inhibit the dissociation from the complex with lipophilic surfactant. In contrast to this, we found that the PLGA particles prepared using S/O/W emulsions are sufficient at the encapsulation and can act as the carrier of protein drugs.

4. Conclusion

PLGA particles containing hydrophilic drugs were prepared by a solvent evaporation method utilizing S/O/W emulsions. The encapsulation efficiency of drugs into the particles was dramatically improved when compared with those formed with commonly used W/O/W emulsions. Moreover, we confirmed that size control of the PLGA particles can be easily performed by the preparation of S/O/W emulsions using the membrane emulsification method. We expect that this method can be used to encapsulate hydrophilic drugs, especially biopolymers such as peptides or proteins, into PLGA nanoparticles.

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