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Preparation of Solid Lipid Nanoparticle-Containing Ovalbumin Based Reverse Micelle-Double Emulsion Technique

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ABSTRACT

Encapsulation of protein in the lipid-based nanoparticle is quite challenging. A reverse micelle-double emulsion method could be used for answering this challenge because reverse micelles able to protect the polar core in a nonpolar solvent. Thus, the protein solution can be kept in the interior of the reverse micelle particle and hosted in a lipid matrix of nanoparticles. Herein, we describe the preparation of solid lipid nanoparticles (SLNp) for the encapsulation of ovalbumin (Ova) with reverse micelle-double emulsion. Using several combinations of solid lipids and phospholipids, we prepared SLNp containing Ova-reverse micelle. The optimized formulation enabled the incorporation of Ova in the solid lipid matrix of SLNp with nano size and narrow particle size distribution (PdI), and high entrapment efficiency for Ova. The particle size and zeta potential were measured by a dynamic light scattering spectrophotometer 214.8±15.94 nm and −3.44±0.21 mV, respectively. The entrapment efficiency (EE%) was determined by Spectrofluorometer by determining the quantitative of Ova-FITC labeling was 98.97±0.89%.
Keywords: Solid lipid nanoparticle; Ovalbumin; Reverse micelle; Double emulsion

INTRODUCTION

Solid lipid nanoparticle (SLNp) is one type of lipid-based nanoparticle that is physiologically compatible and far from potential toxicity (Müller et al., 1996, Müller et al., 1997). SLNp is suitable for various types of drugs, including hydrophilic and lipophilic drugs because the lipid matrix of SLN is a property that can be modified by combining several types of solid lipids and phospholipids. However, a few researchers reported on the incorporation of protein into SLNp due to the lipophilic matrix of SLNp (Almeida et al., 1997).

Recently, protein-based therapy has become a concern in the field of immunotolerance therapy. Most of the proteins are administered via the subcutaneous route, which is inconvenient for patients. Consequently, alternative protein delivery is required, and oral administration might be the answer as it is the most convenient route. However, incorporating protein in a matrix of the carrier is required for oral administration to protect against proteolytic enzymes in the digestive tract.

To explore the functionality of SLNp as a carrier of protein, here we proposed a simple formulation of incorporating protein in SLNp. We chose ovalbumin (Ova) as the protein model because it is a common model for immunological research (Khadijah et al., 2018). Incorporated Ova in SLNp was prepared by reverse micelle-double emulsion method to achieve nano-size particles and increase the drug entrapment efficiency.

METHODS

Chemicals

1,2- Distearoyl- sn- glycerol- 3- phosphocholine (DSPC) was purchased from NOF Corporation (Tokyo, Japan). L-α Phosphatidylcholine (PC) from soybean, Soy lecithin, Ovalbumin (Ova), and Pluronic F-127) were purchased from Sigma-Aldrich. Glycerol monostearate (GMS) was purchased from Wako. FITC was purchased from Takara-Clontech. Glyceryl behenate (GB) was a gift from Gattefosse.

Preparation of Ova-SLNp

Incorporated Ova in SLNp was prepared by using the reverse micelle-double emulsion method. A mixture of cyclohexane-dichloromethane (1 mL, 1;1 v/v) containing several combinations of lipid and phospholipid (Table I) has been prepared. Then 30 µL Ova solution (1 µg/µL) was added to the organic solution. The two-phase solution was sonicated with a sonicator probe (Ultrasonic Disrupter UD 201, TOMY) for 2 min (10% power, 20 kHz, 50 W), reverse micelle emulsion (W/O) was

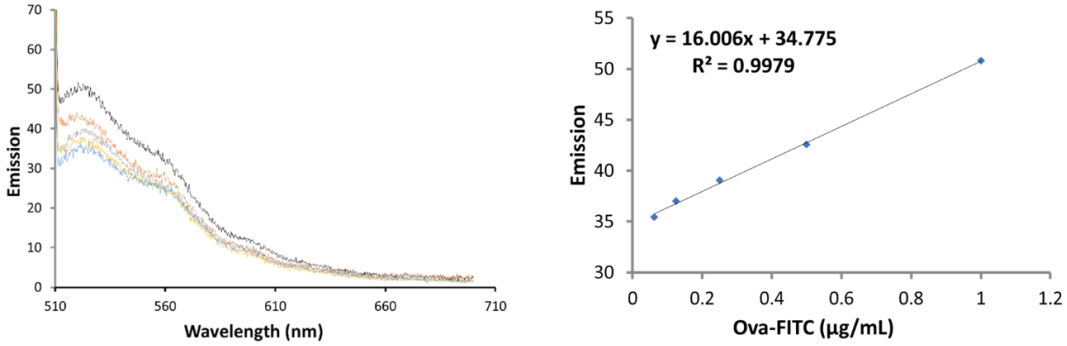

Figure 1. Calibration curve of Ova-FITC

formed. A reverse micelle-double emulsion was formed after mixing 24 mL of Pluronic F127 solution (1.25 mg/mL) with the previous W/O emulsion followed by sonication for 5 min (10% power, 20 kHz, 50 W). In the final step, the cyclohexane-dichloromethane was evaporated from the solution by overnight stirring.

Preparation of Ova-FITC labeling

2 mg/mL of Ova in 0.1 M Sodium carbonate buffer, pH 9 was prepared. Then, 1 mL Ova solution was added 50 µL of FITC solution in DMSO (1 mg/mL), followed by continuously stirring and incubation for 3h at room temperature. Free FITC was separated with Ova-FITC labeling by dialysis (10.000 Da). Dialysis was continuous until no detection of FITC-free in the medium of dialysis.

Particle size distribution and ζ-potential analysis

Physical properties of Ova-SLNp were measured by using a DLS (dynamic light scattering spectrophotometer, Malvern Instruments) at 25 °C.

Quantification of Ova-loaded SLNp

To determine the quantity of Ova-FITC labeling loaded into SLNp, the fluorescence method was utilized. The Ova-SLNp dispersion was placed into a filtration tube (100 kDa), and the dispersion was centrifuged for 10 min at 5000 rpm, 4oC with the purpose to separate the entrapped Ova from the free Ova. Afterward, the free Ova-FITC labeling in the supernatant was measured by using a spectrofluorometer. The emission spectrum was recorded between 510 and 700 nm by using Jasco Spectrofluorometer FP-8600. The free Ova-FITC in the aqueous phase was estimated by applying the calibration curve (Figure 1). The entrapment efficiency (EE) of Ova in SLNp was determined as follows:

$$\text{Entrapment efficiency (\%)} = \frac{(\text{Ova in feed} - \text{Ova in supernatant})}{\text{Ova in feed}} \times 100\%$$

FT-IR measurement

Confirmation of Ova in the SLNp matrixes, measurement of the Universal attenuated total reflectance (UATR) of Ova-SLNp were carried out by Spectrum Two FT-IR Spectrometer (PerkinElmer).

RESULTS AND DISCUSSION

Ova-SLNp preparation was optimized by the reverse micelle-double emulsion method. After several preliminary trials by following the combination of lipids and phospholipids as shown in Table I, the mixture of GMS and DSPC (2:1 molar ratio) was considered for this study because it produced high entrapment efficiency.

A summary of the characteristics of the Ova-SLNp prepared with the optimized GMS and DSPC combination is shown in Figure 2 and Table II.

Ova-SLNp was successfully prepared by using GMS as a solid lipid-based. GMS has a higher affinity to Ova than GB because the lipophilicity of GMS is lower than GB (log P value GMS: 6.73; log P value GB: 8.39). The type of phospholipid that can support the formation of reverse micelle-double emulsion for this study was DSPC. DSCP forms reverse micelles, which are stable in organic solvents (e.g., cyclohexane) and capable of encapsulating Ova in organic solvents (Vierros and Sammalkorpi, 2015). We failed to prepare Ova-SLNp using PC because PC requires an additive agent such as fatty acids to form stable spherical reversed micelles in the organic solvent (Shinji et al., 2001).

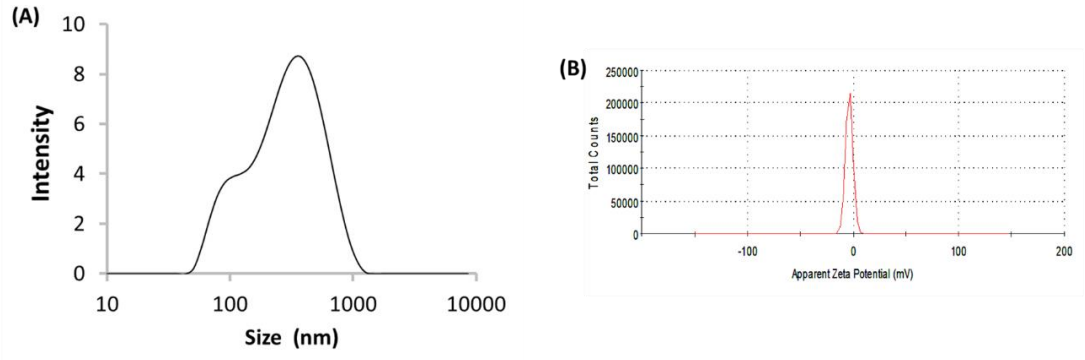

Figure 2. (A) Size distribution and (B) zeta potential of Ova-SLNp

Table I. Combination of two types of solid lipid with several types of phospholipids

Type of lipid	Type of phospholipid	Ratio lipid: phospholipid	Step	Entrapment efficiency (%)
Glyceryl behenate	Soy PC	1:1	Preliminary study	0
	Soy lecithin			31.99
	DSPC			0
	DMPC			0
Glyceryl monostearate	Soy PC	1:1		0
	Soy lecithin			0
	DSPC			42.26
	DMPC			0
	DSPC	2:1	Optimize lipid and phospholipid ratio	98.97 ± 0.89
		1:2		12.62

Table II. Summarized characteristics of Ova-SLNp based GMS-DSPC (2:1 molar ratio)

Size	PdI	Zeta potential	Entrapment of Ova (%)
214.8 ± 15.94	0.29 ± 0.01	-3.44 ± 0.12	98.97 ± 0.89

The effect of the GMS/DSPC ratio on the entrapment efficiency of Ova in the SLNp matrix is shown in Table I. Initially, GMS/DSPC of 1:1 molar ratio had higher Ova affinity than GB/DSPC with the same ratio. However, the affinity of Ova to the GMS matrix becomes lower following increasing concentration DSPC concentration. The GMS/DSPC of 1:2 molar ratio was indicated as an optimum combination because it seemed to be the highest affinity for Ova to GMS as the solid matrix of SLNp and entrapment efficiency, and good size distribution of Ova-SLNp. Therefore, the Ova load pattern was supposed to be encapsulated Ova by reverse micelle of DSPC, then encapsulated Ova was dispersed in the solid matrix of SLNp (Figure 3).

The presence of Ova in the solid lipid matrix was also investigated by FT-IR analysis. As shown in Figure 4, the Ova shows characteristic amide I band at 1637 cm−1 and amide II bands at 1529.5 cm−1. We observed that the peak positions of amide I in the Ova-SLNp spectrum shifted from 1637 to 1641.5 cm−1. The shifted peak indicated the interaction of DSPC or GMS molecules with the O and N atoms of Ova (Ma et al., 2015, Krimm and Bandekar, 1986).

CONCLUSION

Here we optimized SLNp preparation loaded with ovalbumin. The obtained reverse micelle-double emulsion method successfully supported the encapsulation of protein in solid

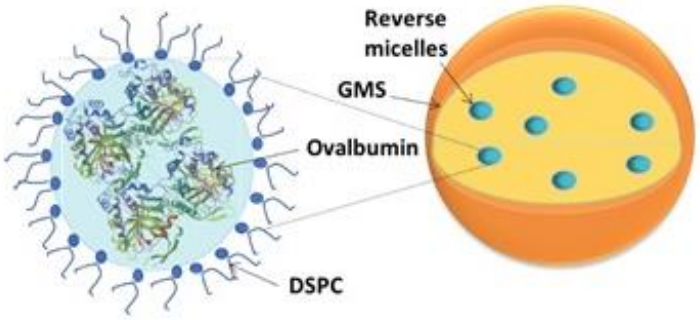

Figure 3. Schematic representation of Ova-SLNp structure

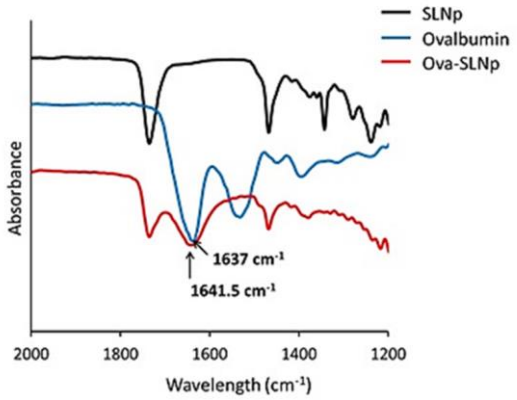

Figure 4. Representative FT-IR spectra of Ova, SLNp, and Ova-SLNp

lipid matrixes. The formulation is not limited to ovalbumin but can generally be applied to any protein.

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