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Preparation of Resveratrol-loaded Poly(ε-caprolactone) Nanoparticles by Oil-in-water Emulsion Solvent Evaporation Method

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Abstract Resveratrol-loaded poly(ε-caprolactone) (PCL) nanoparticles were prepared by oil in water (O/W) emulsion solvent evaporation method. The morphology of the nanoparticles was evaluated using atomic force microscope (AFM), in which well-shaped and rigid nanoparticles were prepared. The mean particle size of nanoparticles prepared using only dichloromethane (DCM) (523.5±36.7 nm) was larger than that prepared with a mixture of DCM and either ethanol (EtOH) (494.5±29.2 nm) or acetone (493.5±6.9 nm). The encapsulation efficiency of nanoparticles prepared only with DCM as dispersed phase (78.3±7.7%) was the highest of those prepared with solvent mixtures. An increase in the molecular weight of PCL led to an increase in encapsulation efficiency (from 78.3±7.7 to 91.4±3.2%). Pluronic F-127 produced the smallest mean size (523.5±36.7 nm) with the narrowest particle size distribution. These results show that dispersed phase, molecular weight of wall materials, emulsion stabilizer could be important factors to affect the properties of nanoparticles.

Keywords: resveratrol, nanoparticle, emulsion solvent evaporation, poly(ε-caprolactone), encapsulation efficiency

Introduction

Biodegradable colloidal nanoparticles have been used as delivery devices for various active agents (1-3). Biocompatible and biodegradable hydrophobic polymers such as poly(ε-caprolactone) (PCL), poly(DL-lactide-co-glycolides) (PLGA), and ammonio poly(methyl methacrylates) (Eudragits RL and RS) are widely used for the preparation of nanoparticles. Although the applications of nanoparticles in therapeutic systems have been well studied, they are relatively new in the food industry. Due to their subcellular size, nanoparticles offer promising means of improving the bioavailability of nutraceutical compounds, especially poorly soluble substances such as functional lipids (e.g., carotenoids, phytosterols, ω-3 fatty acids), natural antioxidants, and numerous other compounds that are widely used as active ingredients in various food products (4).

The emulsification of organic solutions of these polymers in an aqueous phase followed by the evaporation of organic solvents is a common method for the synthesis of nanoparticles (5-7). The crucial step of this method is the emulsification of the organic solutions of hydrophobic polymers containing additional bioactive substances as colloidal dispersions in an aqueous phase with the subsequent solidification of emulsion droplets by the evaporation of the organic solvent in a vacuum.

PCL is one of the biocompatible and biodegradable aliphatic polyester polymer that degrades slowly and does not generate acid environment unlike the polylactide (PLA) or polyglycolide (PLG) polymers (8). Although the permeability of macromolecules in PCL is low, such low permeability may be sufficient for the delivery of active agents (9,10). Other advantages of PCL include hydrophobicity, *in vitro* stability and low cost. Therefore, many investigations have focused on the application of PCL microspheres to drugs in recent years (6,9,11-15).

Resveratrol (*trans*-3,4,5-trihydroxystilbene), a phytoalexin belonging to the stilbene class of polyphenolic compounds, has been found in mulberries, peanuts, and grapes. It has been shown to be a potent antioxidant, anti-imflammatory, anticancer, and chemoprotective agent (16,17). However, the oral bioavailability and initial half life (8-14 min) are poor, leading to an irrelevant *in vivo* effect by oral administration compared to its powerful *in vitro* efficacy (18). The high hydrophobicity of resveratrol and its sensitivity to external agents such as air, light, and oxidative enzymes may constitute a serious problem for its bioavailability, formulation, and manipulation in the elaboration of functional foods.

However, there have been few studies about encapsulation of resveratrol to improve their stability and bioavailability. Therefore, the purpose of the present study is to use the emulsion solvent evaporation method in order to prepare resveratrol-loaded PCL nanoparticles, and to investigate the effect of the dispersed phase on the properties of nanoparticles.

Materials and Methods

Materials The following chemicals were obtained from commercial sources and used without further purification: resveratrol (Sigma-Aldrich, St. Louis, MO, USA); poly(ε-caprolactone) (PCL) with number-average molecular weight (Mn) of 10,000 and 65,000 Da (Sigma-Aldrich); dichloromethane (Junsei, Tokyo, Japan); acetone (Junsei); ethanol (Junsei); Pluronic F-127 (Sigma-Aldrich); polyvinyl alcohol (Sigma-Aldrich), Tween 80 (Sigma-Aldrich),

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Table 1. Processing conditions used in the study

Parameter		Processing condition
Dispersed phase	Core materials	Resveratrol 0.4 mg/mL
	Wall materials	Poly(ε-caprolactone) Molecular weight; 10,000, 65,000 Da
	Solvent	Dichloromethane Dichloromethane+Acetone Dichloromethane+Ethanol
Continuous phase	Emulsion stabilizer	PVA, Tween 80, Pluronic F 127 20 mg/mL
Dispersed:continuous phase		1:20 (v/v)
Homogenization		1 min at 15,000 rpm

acetonitrile (Sigma-Aldrich).

Preparation of resveratrol-loaded PCL nanoparticles by emulsion solvent evaporation methods The preparation of nanoparticles was based on O/W emulsion solvent evaporation method, which was adapted from the process described by Kim *et al.* (8). The procedures are as follows. Resveratrol and PCL were dissolved in solvent or solvent mixture, which were added to aqueous solution containing pluronic F-127 (emulsion stabilizer) and homogenized by a high speed homogenizer (Ultra-Turrax; Janke and Kunkel Ika-Labortechnik, Staufen, Germany) at 15,000 rpm to form O/W emulsion. After 1 min of homogenization, the emulsion was magnetically stirred at ambient temperature until the complete evaporation of organic solvent was accomplished (about 1 hr). Parameters for all the preparations are summarized in Table 1. All the formulations were made in triplicate.

Determination of resveratrol content by high performance liquid chromatography (HPLC) The resveratrol concentrations were obtained by direct injection of samples into a HPLC system (Jasco, Tokyo, Japan). A reversed phase column (μBondapak™ C18 125Å, 3.9×300 mm, 10-μm, Waters, Milford, MA, USA) was used for separation, while an acetonitrile-water (86:14) was used as the mobile phase. The flow rate and ultraviolet (UV) wavelength were 1.6 mL/min and 308 nm, respectively. The resveratrol concentrations were determined by measuring the peak areas and compared them with the peak areas of known standards.

Particle size and zeta (ζ)-potential analysis Mean particle size and ζ-potential of nanoprticles were measured using an electrophoretic light scattering (ELS) spectrophotometer (ELS-8000; Otsuka Electronics, Osaka, Japan).

Encapsulation efficiency The amount of encapsulated resveratrol was determined indirectly, i.e., by measuring the amount difference between the initial amount of resveratrol ($Resveratrol_{total}$) and amount of free resveratrol in the nanoparticle solution ($Resveratrol_{free}$). So the encapsulation efficiency was expressed as following equation (19).

$$\text{Encapsulation efficiency (\%)} = [(Resveratrol_{total} - Resveratrol_{free})/Resveratrol_{total}] \times 100$$

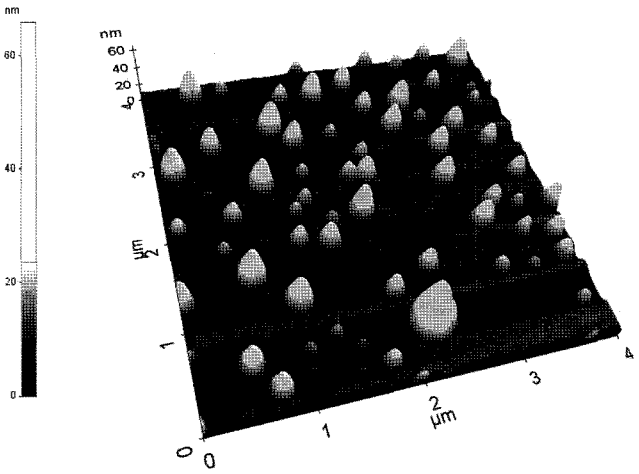

Fig. 1. AFM image of resveratrol-loaded poly(ε-caprolactone) (PCL) nanoparticles.

Morphology The morphological examination of nanoparticles was performed using an atomic force microscopy (AFM) (XE-150; PSIA, Seoul, Korea). Scanning was performed at a scan speed of 0.5 Hz with a resolution of 512×512 pixels. The tip loading force was minimized to avoid structural changes of the sample.

Results and Discussion

Preparation of resveratrol-loaded PCL nanoparticles One of the features of this process is the use of 2 solvents (termed as 'mixed solvent system' or MSS) (20,21) as dispersed medium. Components of the MSS can be selected from any of the commonly available organic solvents such as dichloromethane, ethyl acetate, acetone, acetonitrile, ethanol, etc (8,20-27).

Emulsion solvent evaporation method was successfully employed for the encapsulation of resveratrol with PCL, in which well-shaped and rigid nanoparticles were prepared (Fig. 1).

Effect of dispersed phase Figure 2 and 3 show the influence of the dispersed phase on the properties of nanoparticles prepared by the O/W emulsion solvent evaporation method, in which the following solvents were investigated as dispersed media: dichloromethane (DCM), mixture of dichloromethane with either ethanol (DCM/

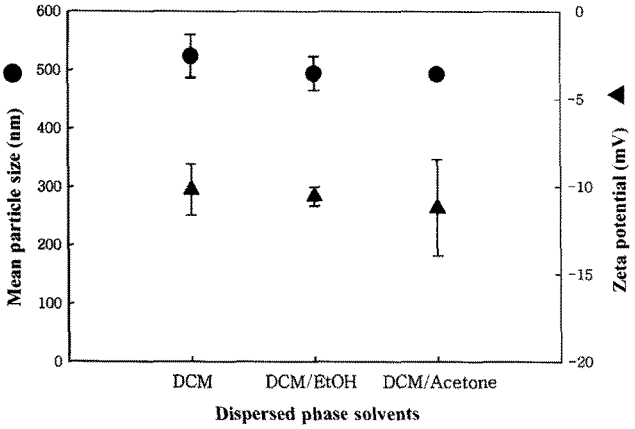

Fig. 2. Changes in mean particle size and ζ-potential of resveratrol-loaded poly(ε-caprolactone) (PCL) nanoparticles with different dispersed phase (mean±SD, *n*=3). DCM, dichloromethane.

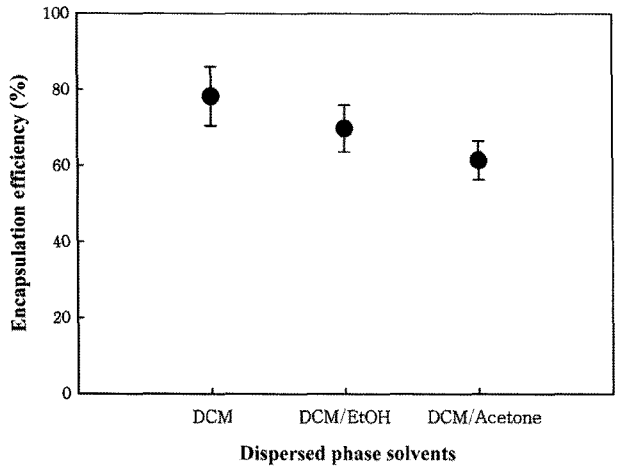

Fig. 3. Changes in encapsulation efficiencies of resveratrol-loaded poly(ε-caprolactone) (PCL) nanoparticles with different dispersed phase (mean±SD, *n*=3). DCM, dichloromethane.

EtOH; 1:1, v/v) or acetone (DCM/Acetone; 1:1,v/v).

The mean particle size of nanoparticles prepared using only DCM (523.5±36.7 nm) was larger than that prepared with a mixture of DCM and either EtOH (494.5±29.2 nm) or acetone (493.5±6.9 nm). Evaporation through the aqueous phase depends on the solubility of the solvents in water (8). Practically, water solubility of acetone or EtOH is higher than that of DCM. Therefore the rate of precipitation is in the same range. Since the higher the water solubility, the higher the diffusion rate before nanoparticle hardening. This is the major reason of smaller mean size of nanoparticles (Fig. 2). Sah (28) also showed that, although boiling point of the DCM is a little higher than that of ethyl formate, the high evaporating rate is contributed to the difference in water solubility.

The differences in the evaporation rate of solvents may have influence on the encapsulation efficiency of the nanoparticles. As shown in Fig. 3, the encapsulation efficiency of nanoparticles prepared only with DCM as dispersed phase (78.3±7.7%) was the highest of those prepared with solvent mixtures. These are also related to

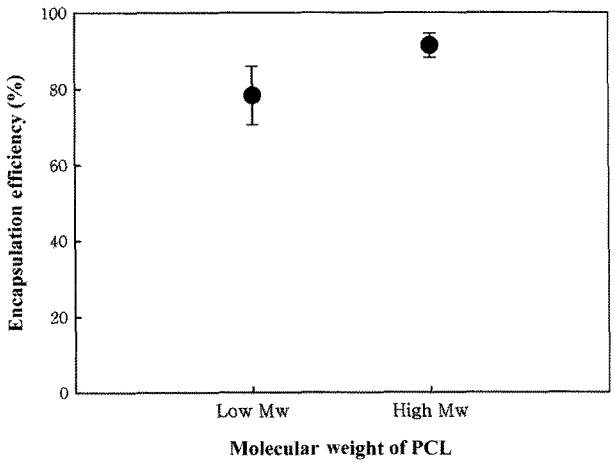

Fig. 4. Changes in encapsulation efficiencies of resveratrol-loaded poly(ε-caprolactone) (PCL) nanoparticles with molecular weight of PCL (mean±SD, n=3). DCM, dichloromethane.

the water solubility of the solvents. Generally, core partitioning into the aqueous phase can occur during the initial stages of nanoparticle formation prior to polymer precipitation. During evaporation of the solvent from the aqueous phase, the core materials can be migrated with solvent into aqueous phase. So, the higher the evaporation rate, the lower the encapsulation efficiency of the nanoparticles, which is the similar results of our previous study (8). However, in morphological analysis, no particular differences could be observed (data not shown).

ζ-Potentials of these nanoparticles showed negative values with few differences between dispersed phase (−10.2 ±1.5, −10.6±0.5, and −11.2±2.8 mV for DCM, DCM/EtOH mixture, and DCM/Acetone mixture, respectively).

Effect of molecular weight of PCL on encapsulation efficiency In this study, 2 different molecular weight PCLs were used as wall materials, i.e., 10,000 and 65,000 Da. An increase in the molecular weight of PCL led to an increase in encapsulation efficiency (Fig. 4) (from 78.3±7.7 to 91.4±3.2%). These results show that increasing the molecular weight of the PCL led to an increase in viscosity of organic phase, which reduced resveratrol diffusion in the external aqueous phase before nanoparticle hardening. Similar results were reported for PCL microspheres with an oily core (15).

These can be described by another ways. Crystallinity of the polymer is very important for their barrier properties. Since the crystalline phase of the polymer is essentially impermeable to water, encapsulation is likely to occur in the amorphous region of the polymer, the higher the crystalline region, the lower the encapsulation efficiency, and *vice versa* (15). In our previous study, the percentage of crystallinity was estimated using differential scanning calorimetry (DSC), in which crystallinities of PCL with 10,000 and 65,000 Da were 93.5 and 85.7%, respectively (8). In this study, encapsulation efficiency was also in inversion proportion to crystallinity (Fig. 4). However, mean size of nanoparticles was not affected by molecular weight; there were few differences in ζ-potentials with molecular weight of PCL (from −10.2±1.5 to −12.0±2.2 mV).

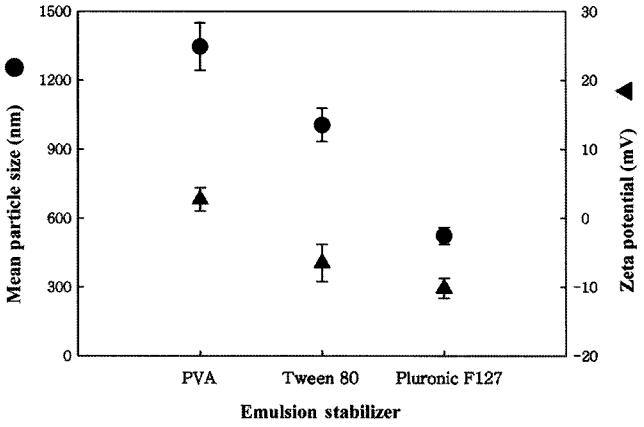

Fig. 5. Changes in mean particle size and ζ-potential of resveratrol-loaded poly(ε-caprolactone) (PCL) nanoparticles with different emulsion stabilizers (mean±SD, n=3). PVA, polyvinyl alcohol.

Effect of emulsion stabilizer Figure 5 shows the effect of emulsion stabilizers on mean size and ζ-potential of PCL nanoparticles prepared by different emulsifiers, i.e., polyvinyl alcohol (PVA), Tween 80, and pluronic F-127, which are commonly used emulsion stabilizers.

Pluronic F-127 produced the smallest mean size (523.5 ±36.7 nm) with the narrowest particle size distribution. However, Tween 80 and PVA showed higher value of mean size (1005.3±72.2 and 1347.1±103.4 nm, respectively). This is due to the changes in ζ-potentials. Generally, PCL lead to negative ζ-potentials due to the presence of terminal carboxylic groups (5,29). However, when PVA was used as emulsion stabilizer, ζ-potential was changed to positive one (2.7±1.7 mV) in contrast to that of pluronic F-127 (−10.2±1.5 mV) indicating the poor stability. These results show that emulsion stabilizer could be one of the important factors to maintain the ζ-potentials of systems.

In this work, encapsulation efficiencies of all preparations were all over 61.5±5.1% and the mean particle size was between 493.5±6.9 and 1347.1±103.4 nm. Encapsulation efficiency and mean particle size of nanoparticles were affected by changing the experimental parameters, such as type of dispersed phase, emulsifier and molecular weight of PCL, indicating it is very important to control the experimental parameters to improve the properties of nanoparticles.

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