Optimization of Preparation Variables for Trimyristin Solid Lipid Nanoparticles

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ABSTRACT – Solid lipid nanoparticles (SLNs) have been regarded to behave similar to the vegetable oil emulsions because emulsions of lipid melts are formed before lipid droplets being solidified to turn into SLNs. Compared to lipid emulsion, however, it has been more difficult to obtain stable SLNs and needs more extensive considerations on stabilizer and manufacturing process. In the present study, we tried to prepare phosphatidylcholine-based trymyristin (TM) SLNs using high pressure homogenization method and optimize the manufacturing variables such as homogenization pressure, number of homogenization cycles, cooling temperature, co-stabilizer and freeze-drying with cryoprotectants. Nano-sized TM particles could be prepared using egg phosphatidylcholine and pegylated phospholipids (PEG$_{2000}$PE) as stabilizers. Based on the optimization study, the dispersion was manufactured by homogenization under the pressure of 100 MPa for more than 5 cycles, and solidifying the intermediately formed lipid melt droplets by dipping in liquid nitrogen followed by thawing at room temperature. In addition, TM SLNs could be freeze-dried and then redispersed easily without significant particle size changes after freeze drying with 10% and 12.5% sucrose or trehalose. The TM SLNs established in this study can be used as delivery system for drugs and cosmetics.

Key words – Solid lipid nanoparticles, Trimyristin, High pressure homogenization, Freeze drying

During the last decades much effort has been focused on the development of colloidal drug carriers, such as polymeric nanoparticles, liposomes, and lipid emulsions, for the parenteral delivery of poorly water soluble agents and also for targeting. One of the approaches investigated in recent years is the development of solid lipid nanoparticles (SLNs).[1] SLNs represent colloidal dispersions of non-polar lipids such as triglycerides. The idea behind the use of solid lipids is to combine the superiorities of colloidal lipid carriers with the advantages of the solid physical state of polymeric nanoparticles. Lipid carriers are biodegradable, biocompatable and can be easily manufactured. Polymeric nanoparticles have the advantages with respect to size stability, drug leakage and sustained drug release due to their solid state.[2] In the solid lipid matrix, the mobility of incorporated drugs is drastically reduced. A number of advantages can be theoretically deduced from the solid state of the dispersed lipid phase compared to the liquid state of lipid emulsions, and one of them we could expect is that SLNs could be freeze dried relatively easily compared to lipid emulsion and liposomes.[3] Owing to these potential advantages, SLNs have drawn attention as an alternative colloidal drug carrier system.

There have been several methods to prepare solid lipid nanoparticles and melt homogenization method have been widely used due to its high pressure enough to achieve nano-dispersion.[4,5] During the preparation of submicron solid lipid suspensions by melt homogenization, an emulsion of the lipid melt in the aqueous phase is intermediately created before the lipid droplets solidify to form SLNs. It is thus not surprising that emulsions of lipid melts behave physicochemically similar to the vegetable oil emulsions used in parenteral nutrition and drug delivery. However, it has been observed that the preparation of lecithin stabilized triglyceride suspensions with a composition similar to fat emulsions results in the formation of semisolid, ointment-like gels.[4,6] The gel formation can be avoided by the addition of co-surfactants. Many researchers have pointed out basic physicochemical differences between similarly composed lipid emulsions and lipid suspensions.[4] It is more difficult to obtain a stable SLNs than lipid emulsions and needs more extensive considerations on stabilizer, manufacturing process including shearing force and temperature, storage conditions and crystallinity of the solid lipids.

In the present study, we tried to prepare and optimize the variables for manufacturing nano-sized solid lipid particles using trimyristin (TM) as solid core. TM is a triglyceride lipid with monosaturated fatty acid chains (i.e. myristic acid chains) and melts at 56~57°C. At the beginning, we attempted to achieve nano-sized particle dispersion by high pressure homogenization using only egg phosphatidylcholine as primary stabilizer like in lipid emulsions and optimize manufacturing variables. Unlike lipid emulsions, additional manu-

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facturing variables such as cooling temperature and rate should be taken into considerations and it was difficult to obtain nano-sized particles without co-stabilizer, i.e. pegylated phospho-lipid in this study. In addition, we evaluated additives for freeze drying enabling dried SLNs to be redispersed to original particle size.

Experimental

Materials

Trimyristin were purchased from Sigma Chemical Co, (St. Louis, MO, USA). Egg phosphatidylcholine (eggPC), dis-tearoyl phosphatidylethanolamine-N-poly (ethylene glycol)$_{2000}$ (PEG$_{2000}$PE) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). All other chemicals were reagent grade and used without further purification.

Preparation of TM dispersions

TM dispersions were manufactured by melt homogenization method.[6] TM, eggPC, and PEG$_{2000}$PE were weighed into pear-shaped 10-mL glass tube followed by sonication for 1 hr at 65°C in bath type sonicator (Branson ultrasonic cleaner, 3210R-DTH, Branson Ultrasonics Corp., CT, USA). Preheated (65°C) water for injection was added and sonicated for more than 3 hours until milky, homogeneous crude emulsions were obtained. These crude emulsions were homogenized for var-ious cycles at 50, 100 or 150 MPa using a high pressure homogenizer (Emulsiflex EF-B3, Avestin Inc., Canada) wired with heating tape (Thermolyne). The hot fine emulsions obtained by high pressure homogenization were cooled by three different methods; incubation at room temperature, 4°C, or by instantaneously freezing by dipping into liquid nitrogen followed by thawing in water bath at room temperature. The resulting dispersions were stored at 4°C.

Freeze drying of TM SLNs

The optimized composition of SLNs consisted of TM: eggPC:PEG$_{2000}$PE (100:50:5, weight ratio) were used for freeze drying study. The SLN dispersions were diluted (1:1) with the cryoprotectant solution before freezing to give the concentration of 2.5, 5, 10 and 12.5% of cryoprotectant. The cryoprotectants tested were fructose, glucose, glycerol, sucrose, and trehalose. SLNs were freeze dried in Ultra 35EL freezer dryer (Virtis, USA) as follows: frozen to −60°C in 3 hr and maintained for 12 hr; primary drying at −40°C for 10 hr; and then stepwise elevation of temperature to 20°C; secondary drying at 20°C for 25 hr. After the freeze dried SLN was recon-stituted with water for injection at room temperature, mean

particle size was determined by Submicron Particle Sizer.

Measurement of particle size

The mean particle size of the TM dispersions was deter-mined by dynamic light scattering method using Submicron Particle Sizer (Nicomp 370, Particle Sizing Systems, Inc., CA, USA). TM dispersions were diluted with water for injection to give an intensity of 300 Hz as recommended by manufacturer. For optimization study, the number of particles larger than 1 μm was counted with Optical Particle Sizer (Accusizer™ 770, Particle Sizing Systems, Inc., CA, USA).

Results and Discussion

Homogenization pressure

The effect of homogenization pressure on the number of par-ticles larger than 1 μm in TM dispersions consisted of 10% TM and 1.2% eggPC were determined (Figure 1). The TM dis-persions were prepared by cooling at room temperature of the hot emulsion obtained after 1 hr sonication at 65°C and high pressure homogenization. At 50 and 100 MPa, the number of particles larger than 1 μm was not significantly changed as homogenization cycles increased. But at 150 MPa the number of particles larger than 1 μm was dramatically increased after 5 cycles. Previously, the kinetic energy has been shown to be sufficient to overcome the stabilizing energy barrier of elec-trostatic repulsion.[6] In the present study, it was possible that the droplet coalescence due to its high kinetic energy intro-duced to the system by homogenization at cycle 5 under the pressure of 150 MPa. Therefore, dispersion and coalescence
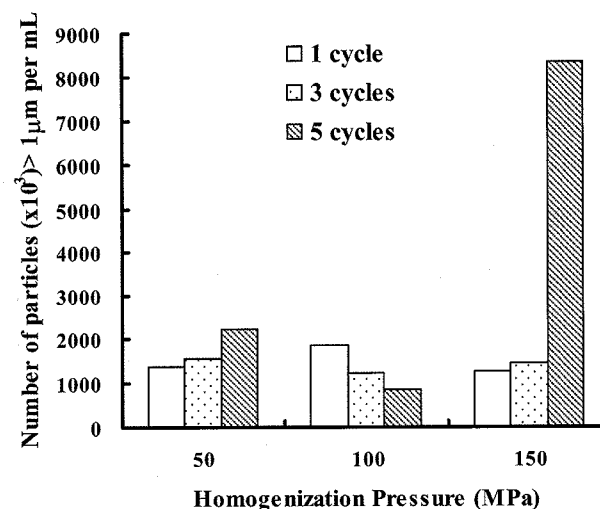

Figure 1−The effect of homogenization pressure on the number of particles larger than 1 μm in TM dispersion consisted of 10% TM and 1.2% eggPC.
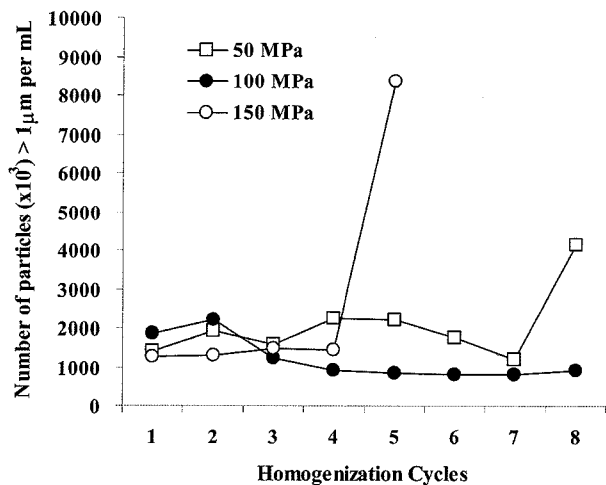
Figure 2—The effect of number of homogenization cycles on the number of particles larger than 1 μm in TM dispersion consisted of 10% TM and 1.2% eggPC.

Figure 3—The effect of cooling temperature on the number of particles larger than 1 μm in TM dispersion consisted of 10% TM and 1.2% eggPC.

occurring simultaneously within one cycle might have caused the higher number of particles.

The number of homogenization cycles

The effect of homogenization cycles was also evaluated with TM/eggPC (10% and 1.2%) dispersion. As shown in Figure 2, the number of particles larger than 1 μm markedly increased at 8 cycles under the pressure of 50 MPa and at 5 cycles of 150 MPa. These increases would be due to the imbalance between segregation and aggregation caused by kinetic energy introduced. In contrast, at 100 MPa, the number of particles showed the tendency to decrease with increasing cycles and was maintained constant after 4 cycles. Based on the results of homogenization pressure and cycles, it was concluded that the optimum homogenization conditions were at 100 MPa with more than 4 cycles.

Cooling temperature

For manufacturing SLNs, cooling was a very critical step to solidify the core lipid without gel formation. The various cooling methods were tested for TM/eggPC SLNs prepared by 1-hr sonication and homogenization for 3 cycles at 65°C as follows: (1) instantaneous freezing by dipping into liquid nitrogen; (2) relatively slow freezing in −20°C freezer; (3) cooling in 4°C chamber; (4) slowly cooling at room temperature (RT). At all of the pressures tested, freezing in liquid nitrogen or at −20°C followed by thawing in room temperature exhibited satisfactory results with respect to the number of particles larger than 1 μm and appearance after thawing (Figure 3). At 100 MPa, although freezing and cooling in 4°C chamber showed similar number of particles, it was thought that dipping into
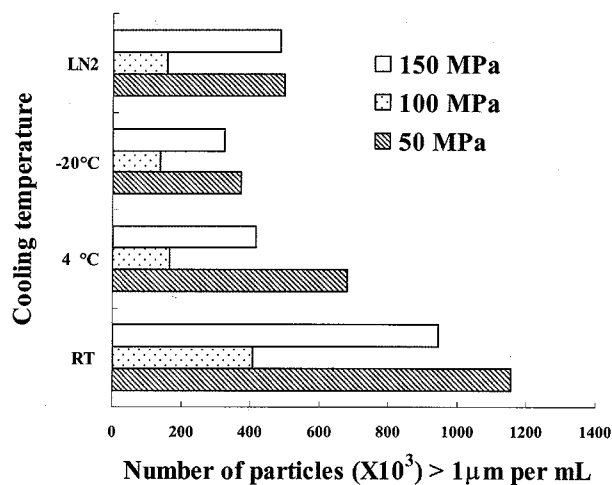
liquid nitrogen could assure the complete solidification of core lipid. Therefore, dipping in liquid nitrogen was chosen for preparation of SLNs.

The effect of co-stabilizer, $PEG_{2000}PE$ on the dispersion and particle size

SLNs were initially considered as similar system to lipid emulsion except solid lipid used as inner phase instead of oil. Thus, we tried to make similar composition of SLNs to commercial lipid emulsion except using solid lipid, TM. Commercial lipid emulsion consists of 10~30% vegetable oil, 0.6~1.5% lecithin and glycerol or xylitol for isotonicity.[7] Based on these compositions, only eggPC was initially applied to make solid lipid nanoparticles in our study. As shown in the above optimization study, the particles were large with eggPC only, and thus the number of particles larger than 1 μm was used as evaluation parameter for optimization of manufacturing process. Only with eggPC, nano-sized SLNs were not obtained even forming gel in some cases. In addition to lecithin, more hydrophilic co-stabilizer seemed to be required to achieve stable nano-sized particles. In this study, we used $PEG_{2000}PE$ as hydrophilic co-stabilizer because it has been widely used in many lipid-based nanoparticles such as liposomes, emulsions and micelles and it has shown excellent compatibility with phosphatidylcholine. As shown in Table I, with 1.2% eggPC without $PEG_{2000}PE$, the dispersion showed phase separation and aggregation of lipids on the wall of glass tube. Although homogenous dispersion was obtained by increasing eggPC up to 5%, the mean particle size was 714.1 nm and lipids aggregation was formed after 1 day at 4°C. Insufficient eggPC and too much of $PEG_{2000}PE$ to eggPC

Table I—*The Effect of eggPC and PEG$_{2000}$PE on TM Dispersion*

| Composition	Particle size[a] (nm)	Appearance
In 1 mL TM:eggPC:PEG$_{2000}$PE (mg weight ratio)		
100:12:0	ND[b]	Phase separation Lipid aggregation on the wall
100:20:0	ND[b]	Phase separation Lipid aggregation on the wall
100:20:2	ND[b]	Lipid aggregation on the wall
100:20:4	ND[b]	Lipid aggregation on the wall
100:20:10	ND[b]	Lipid aggregation on the wall
100:50:0	714.1 ± 120.5	Homogenous (Day 0) Lipid aggregation (Day 1)
100:50:2	792.2 ± 92.6	Homogenous, No aggregation
100:50:4	487.8 ± 70.5	Homogenous, No aggregation
100:50:5	123.1 ± 38.7	Homogenous, No aggregation

[a]Mean and standard deviation (S.D.) of 3 batches.
[b]Not detected due to lipid aggregation.

resulted in phase separation and precipitation as seen in the compositions of 100:20:2, 100:20:4, and 100:20:10 of TM:eggPC :PEG$_{2000}$PE. In contrast, the mean particle size decreased in a dose-dependent manner by adding PEG$_{2000}$PE with sufficient eggPC (5% eggPC) as in 100:50:2, 100:50:4, and 100:50:5 of TM:eggPC:PEG$_{2000}$PE.

The effect of freeze-drying additives

We attempted to make dried form of nanoparticles because powder or dried cake form can give several advantages such as improved stability and convenience of storage and delivery. Due to their solid property of inner phase, SLNs has been expected to be easier to be dried without changing particle shapes than emulsions having liquid oily phase inside. In the present study, the SLN dispersions were lyophilized without or with various cryoprotectants. The SLN dispersions were diluted (1:1) with the cryoprotectant solution before freezing, yielding cryoprotectant concentrations of 2.5%, 5%, 10% and 12.5%. The dried cakes were easily reconstituted in water for injection by hand-shaking except for glycerol. The particle size of the reconstituted SLN was determined using submicron particle sizer.

Only higher concentrations (10% and 12.5%) of cryoprotectants, trehalose and sucrose produced the submicron particle size (<500 nm) (Figure 4). The TM SLNs consisted of TM: eggPC:PEG$_{2000}$PE (100:50:5, weight ratio) without any cryoprotectants were stable during freezing and thawing cycle and

(A)
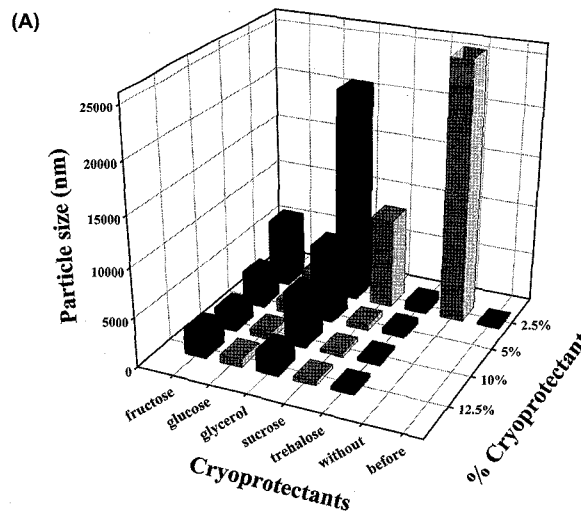
(B) Particle size (nm) before and after freeze drying

Before	202.9 nm	
After	10%	12.5%
Trehalose	413.7 nm	392.8 nm
Sucrose	379.2 nm	408.7 nm

Figure 4—The particle sizes of reconstituted TM dispersions TM:ePC:PEG$_{2000}$PE 100:50:5) after freeze drying with 2.5%, 5%, 10% and 12.5% various cryoprotectants (A). The dried particles were reconstituted in water for injection. The dispersions with 10% and 12.5% of trehalose and sucrose showed mean diameter smaller than 500 nm when reconstituted after freeze drying (B).

gave small particle size. But drying caused the increase in particle size and poor reconstitution behavior. Looking at different concentrations of the cryoprotectants used, in general, high concentrations proved to be more effective. Trehalose was found to be the most effective for SLN preparations. It has been reported that trehalose is very effective cryoprotectant for liposomes.[8,9,10] Sucrose was also effective cryoprotectant for SLNs. Sucrose has been used as excipient in parenteral formulations.[11] Therefore, sucrose would be preferred to trehalose because of its safety. Apart from the nature and concentration of the cryoprotectants, freezing velocity and pressure for drying are also of importance. It may be possible to improve the quality of the products, TM SLNs, by optimization of freezing parameters.

Conclusion

Nano-sized solid lipid dispersion using trimyristin as solid core was prepared by optimization of preparation variables such as homogenization pressure, number of homogenization cycles, cooling temperature and ratio of stabilizers. In addition,

SLN dispersions were freeze-dried without significant changing particle sizes with the aid of cryoprotectants and the dried SLNs were easily re-dispersed to the original state. The nano-sized TM dispersion will be able to be used as delivery system for drugs and cosmetics.

Acknowledgements

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