약제학회지(2010), 제 40 권 제 1 호
J. Kor. Pharm. Sci., Vol. 40, No. 1, 39-43 (2010)

Preparation and Evaluation of Freeze-dried Solid Lipid Nanoparticles with Various Cryoprotectants

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(Received January 6, 2010 · Revised January 29, 2010 · Accepted February 5, 2010)

ABSTRACT – Solid lipid nanoparticles (SLNs) were freeze-dried to obtain a stable solid dosage form with the aid of various cryoprotectants such as trehalose, sucrose, glucose, fructose, and glycerol. Tricaprin(TC) and trilaurin(TL) were used as lipid matrices for SLNs and stabilizers were egg phosphatidylcholine and pegylated phospholipid. All cryoprotectants tested did not cause changes in mean particle size of SLNs when mixed with SLNs before freeze-drying. However, the mean particle sizes of reconstituted SLNs after freeze-drying were significantly different from those of the un-lyophilized original SLN dispersions depending on the types and concentration of cryoprotectants. Although the freeze-dried SLNs without any cryoprotectants were easily reconstituted by hand-shaking, the mean particle size drastically increased (>8 μm for TC SLNs and around 1 μm for TL SLNs) compared to that of un-lyophilized original dispersion (97 nm for TC SLNs and 164 nm for TL SLNs). Trehalose and sucrose were the most effective additives to protect the SLNs during lyophilization. The reconstituted SLNs were physically stable for 24 hours when lyophilized with 12.5% trehalose, sucrose, glucose, fructose or glycerol.

Key words – solid lipid nanoparticles, tricarprin, trilaurin, freeze-drying, cryoprotectants

Improvement of the aqueous solubility of poorly water-soluble drugs is a major challenge in pharmaceutical research. Nanotechnology has been extensively applied to formulate parenteral dosage forms or to enhance the dissolution and thus bioavailability of poorly water-soluble drugs.[1)] A reduction of particle size can not only improve the release rate of the incorporated drugs but also enhance the water solubility.[2)] Due to their superior physical stability and sustained release behavior resulting from the solid-state inner phase solid lipid nanoparticles represent an alternative to other nano-sized particulate carriers such as liposomes, polymeric nanoparticles and lipid emulsions.[3)] It has been shown that optimized SLNs are physically stable as an aqueous dispersion for 12 months[4,5)] and 24 hours.[6)] However, although some SLN formulations may be highly attractive in regard to toxicological considerations because of well-tolerated surfactants used, the physical instability may hamper the further development of SLNs as injectable dosage forms.[7)] For such cases, it is highly desirable to have a freeze-dried SLN formulation available. Freeze-drying is a commonly used technique and numerous studies have been done to improve the dispersability of nanoparticles.[8,9)]

In the present study, we evaluated the effects of cryoprotectants and their concentration on the particle size of reconstituted dispersion after freeze-drying of the nano-sized aqueous dispersion of solid lipids. First, optimized SLN formulations were prepared using tricaprin(TC) or trilaurin(TL) as solid-lipid core, which is a triglyceride lipid with monosaturated fatty acid chains (i.e. capric acid or lauric acid chains, respectively) and melts at around 31°C or 46°C, respectively. Phospholipid are well tolerated in clinically available fat emulsion where they are used as stabilizers.[10)] Thus we chose phospholipids to disperse the solid lipid nano-sized particles in the aqueous media.

Experimental

Materials

Tricaprin and trilaurin were purchased from Sigma Chemical Co, (St. Louis, MO, USA). Egg phosphatidylcholine (eggPC), distearoyl 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 SLN dispersions

TC or TL dispersions were manufactured by melt homogenization method as follows.[5)] TC (100 mg) or TL (100 mg), eggPC (30 mg), and $PEG_{2000}PE$ (3 mg) were weighed into glass tube followed by sonication for 1 hr at 65°C in bath type sonicator (Branson® ultrasonic cleaner, 3210R-DTH, Branson

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DOI : 10.4333/KPS.2010.40.1.039

Ultrasonics Corp., CT, USA). Preheated (65ºC) water for injection (1 mL) was added and sonicated for more than 3 hours until milky, homogeneous crude emulsions were obtained. These crude emulsions were homogenized for various cycles at 100 MPa using a high pressure homogenizer (Emulsiflex® EF-B3, Avestin Inc., Canada). The hot ultra fine emulsions obtained by high pressure homogenization were cooled by instantaneously freezing by dipping into liquid nitrogen followed by thawing in water bath at room temperature. The resulting dispersions were stored at 4ºC.

Freeze drying of SLNs

The optimized composition of SLNs consisted of TC (or TL):eggPC:PEG_{2000}PE (100:30:3, 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 diluted dispersions were subjected to the particle size measurements before freeze-drying. 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 -60ºC in 3 hr and maintained for 12 hr; primary drying at -40ºC for 10 hr; and then stepwise elevation of temperature to 20ºC; secondary drying at 20ºC for 25 hr. After the freeze dried SLNs were reconstituted 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 aqueous SLN dispersions was determined via dynamic light scattering method using a Submicron Particle Size Analyzer (Nicomp 370, Particle Sizing Systems, Inc., CA, USA). SLN dispersions were diluted with water for analysis to give an intensity of 300 Hz as recommended by manufacturer. The medium for size measurement was water for injection.

Results and Discussion

Effects of cryoprotectants on the particle size before freeze-drying

SLNs consisting of lipid (TC or TL), egg PC and PEG $_{2000}$PE (weight ratio of 100:30:3) were used for the further freeze drying study. The SLN dispersions were diluted (1:1) with the cryoprotectant solution before freeze-drying, resulting in a cryoprotectant concentration of 2.5, 5, 10 and 12.5%. Before freeze-drying, the diluted dispersions were subjected to the size measurement to evaluate the effect of the cryoprotectants on the mean particle size of the dispersions. As shown in Figure 1, there were no significant increases of mean particle size of TC SLNs when mixed with cryoprotectants compared to the control dispersion which was diluted with water only, which led us to expect that cryoprotectants would not cause detrimental effect to the dispersions. Similar phenomena was observed in TL SLNs as well as shown in Figure 2 although the mean particle sizes of TL SLNs were larger than those of TC SLNs. Based on this results, we expected cry-

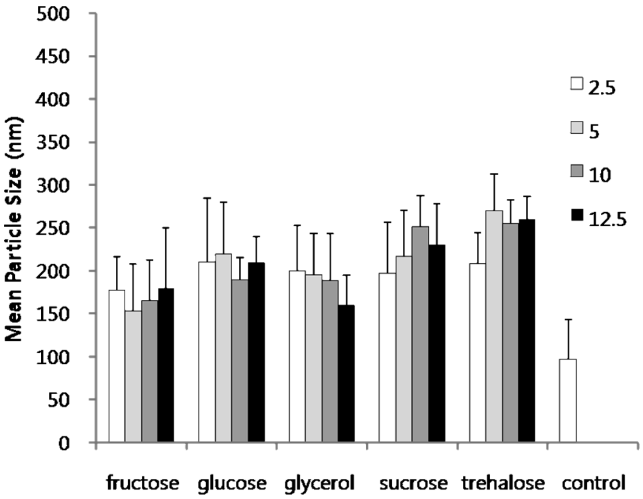

Figure 1–Effect of dilution with cryoprotectant solution on the mean particle size of TC SLNs. SLN dispersions were mixed with equal volume of cryoprotectant solution to make final concentration of 2.5, 5, 10, and 12.5% as cryoprotectant and their mean particle size was measured within 1 hr after mixing. Control was SLNs diluted with water.

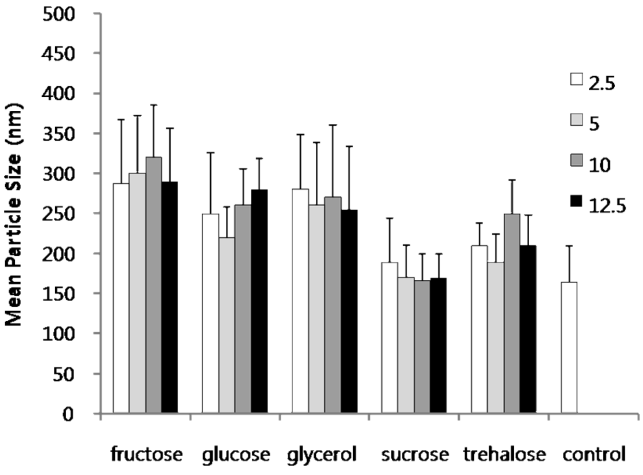

Figure 2–Effect of dilution with cryoprotectant solution on the mean particle size of TL SLNs. SLN dispersions were mixed with equal volume of cryoprotectant solution to make final concentration of 2.5, 5, 10, and 12.5% as cryoprotectant and their mean particle size was measured within 1 hr after mixing. Control was SLNs diluted with water.

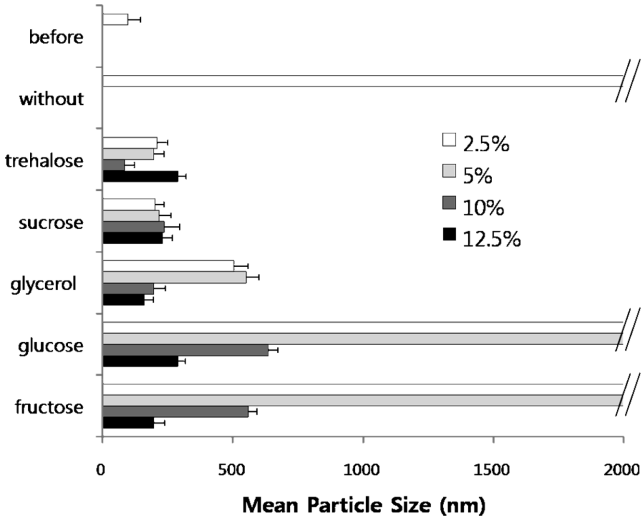

Figure 3–The effect of type and concentration of various cryoprotectants on the mean particle size of reconstituted TC-SLN dispersions after freeze-drying. The breaks represent that the mean particle size exceeded 2 μm.

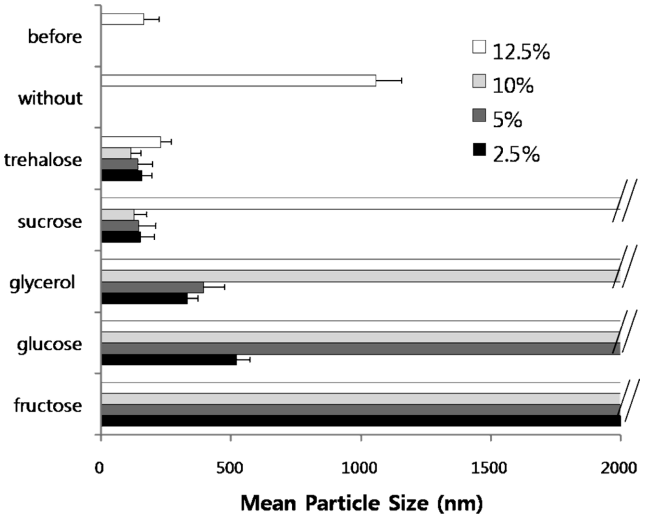

Figure 4–The effect of type and concentration of various cryoprotectants on the mean particle size of reconstituted TL-SLN dispersions after freeze-drying. The breaks represent that the mean particle size exceeded 2 μm.

oprotectants would protect the dispersions from the particle size changes and thus measurement of particle size of SLNs mixed with cryoprotectants would act as a useful tool to predict the behavior of SLNs under lyophilization. Unfortunately, this preliminary size measurement did not provide useful information consistent to the changes of particle sizes after freeze-drying as shown in particle size changes after freeze-drying (Figure 3 and 4).

Effects of cryoprotectants on the particle size of the reconstituted dispersions

The dried cakes of all the SLNs were easily reconstituted in water by hand-shaking. Lyophilization of TC SLNs without cryoprotectants showed dramatic increase of particle size (>10 μm) compared to 97.3 nm prior to lyophilization. Some cryoprotectants, including trehalose and sucrose, enabled lyophilization without changes in particle size of TC SLN (Figure 3). The freeze-dried cakes of TC SLN with trehalose, at all contents tested (2.5%, 5%, 10%, 12.5%), maintained the original particle size and were easily reconstituted in water for injection. Although the mean particle sizes of the reconstituted SLNs after lyophilization with trehalose were increased, those were below 300 nm. With 10% trehalose, the mean diameter of the reconstituted SLNs (84.5 nm) was not changed compared to that before drying (97.3 nm). Sucrose was also effective excipient showing the particle size of 201.1 nm-230.3 nm. In the case of glycerol, the shape of cakes changed to wax-like state. TC SLNs with glycerol could be reconstituted by vortex mixing. The mean particle sizes at higher glycerol concentrations, 10% and 12.5%, were within intravenously injectable range with submicron range. Fructose and glucose, except for 12.5% glucose, were not effective as cryoprotectants of TC SLNs leading to poor cakes and large mean particle size (>10 μm).

TL SLNs presented similar pattern to TC SLNs in lyophilization with trehalose and sucrose (Figure 4). Even at 2.5% trehalose, mean particle size of the reconstituted TL SLN was slightly increased (from 164.1 nm to 229.1 nm after drying). Thus trehalose was very effective cryoprotectant also in TL SLNs leading to no changes in particle size. Sucrose also gave good results at concentrations of 5% and more. As in the case with TC SLN, lyophilization with glycerol resulted in wax-like cakes in TL SLN. In TL SLNs, glycerol, fructose and glucose were not effective in freeze-drying. TL-SLNs were stable during freezing and thawing cycle and gave small particle size (data not shown). 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 all SLN preparations. It has been reported that trehalose is very effective cryoprotectant for liposomes.[11,12,13] Sucrose was also effective cryoprotectant for SLNs. Sucrose has been used as excipient in parenteral formulations.[14] Therefore, sucrose would be preferred to trehalose because of its safety. Apart from the nature and concentration of the cryoprotectants, freez-

ing velocity and pressure for drying are also of importance. It may be possible to improve the quality of the products by optimization of freezing parameters.[7)]

Physical stability of reconstituted SLN dispersions

The changes in mean particle size of reconstituted SLN dispersions were monitored for 48 hours. The mean particle sizes of reconstituted TC-SLN dispersions with 12.5% trehalose or sucrose remained smaller than 300 nm for 48 hours suggesting the reconstituted TC SLNs were physically stable in this period (Figure 5). However, in other cryoprotectants such as glycerol, glucose and fructose, reconstituted TC SLNs significantly changed in size after 48 hours ($p<0.05$). Reconstituted TL SLNs were also stable for 24 hours when freeze-dried with 12.5% trehalose or sucrose ($p<0.05$). Although the mean particle size of TL SLNs with 12.5% glycerol or glucose remained unchanged for 24 hours, the size increased significantly exceeding 1 μm when measured after 48 hours after reconstitution. Overall, freeze-dried SLNs were physically stable for 24 hours after reconstitution when lyophilized with 12.5% cryoprotectants such as trehalose, sucrose, glucose, fructose and glycerol. Recently, other authors also reported that sugars such as glucose and trehalose proved to be very effective in preventing particle aggregation during freeze-drying process of SLNs.[15,16,17)] Sugars have been also used as cryoprotectants for liposomes.[18)] The precise protective effects were different depending on the type of nano-carriers. In the present study, pattern of particle size change of SLNs after lyophilization were different between TC and TL SLNs with the same cryoprotectants. It appeared that SLNs made of solid lipid with higher melting point (i.e. TL) were easier to freeze-dry than those with low melting solid lipid (i.e. TC). It may be important to choose optimum cryoprotectant considering the physicochemical properties of inner core lipid of SLNs. It would be worth studying further the effect of inner core lipids and composition of stabilizers of SLNs on the freeze-drying behavior.

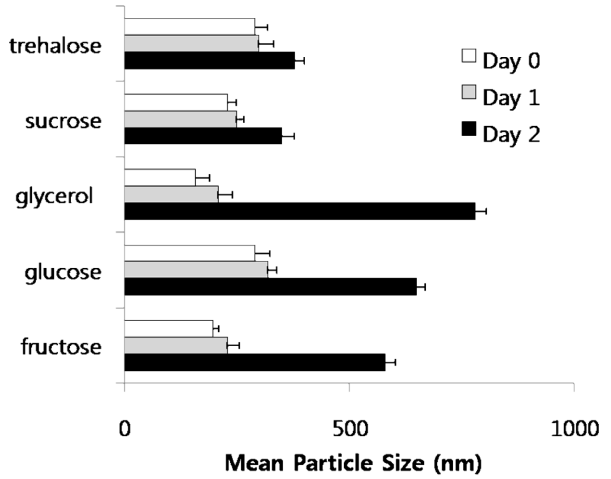

Figure 5–Mean particle size change of reconstituted TC SLN dispersion with time. The particle size was measured on the day of reconstitution (within 1 hr after reconstitution; Day 0), after 24 hr (Day 1) and 48 hr (Day 2) after reconstitution.

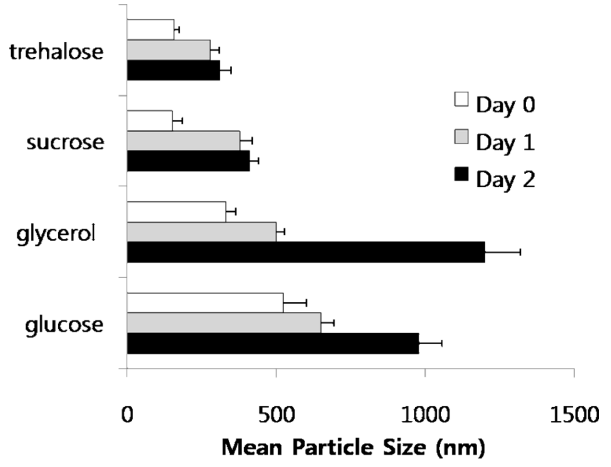

Figure 6–Mean particle size change of reconstituted TL SLN dispersion with time. The particle size was measured on the day of reconstitution (within 1 hr after reconstitution; Day 0), after 24 hr (Day 1) and 48 hr (Day 2) after reconstitution.

Conclusion

SLNs were freeze-dried with various cryoprotectants to minimize the change in particle size. Trehalose and sucrose were effective additives for the lyophilization of SLNs, showing no particle size changes between before and after lyophilization. Furthermore SLNs lyophilized with trehalose and sucrose as cryoprotectants were physically stable for at least 24 hours after reconstitution. These results could lead to a stable, solid dosage form of SLNs for drug delivery.

Acknowledgements

This research was supported by the Grant of the Korean Ministry of Education, Science and Technology (The Regional Core Research Program/ Center for Healthcare Technology Development) and also in part by Woosuk University.

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