Development of Highly Stable Nifedipine Solid–Lipid Nanoparticles

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To improve the solubility of the drug nifedipine (NI), highly stabilized solid–lipid nanoparticles (SLNs) of nifedipine (NI-SLNs) were prepared by high pressure homogenization using two phospholipids, followed by lyophilization with individual sugar moieties (four monosaccharides and four disaccharides). The mean particle diameter, polydispersity index (PDI), zeta potential, drug loading, and the encapsulation efficiency of the NI-SLN suspension were determined to be 68.5 nm, 0.3, −62.1 mV, 2.7%, and 97.5%, respectively. In comparison with the NI-SLNs, the NI-SLNs lyophilized with trehalose (NI-SLN-Tre) showed a slight increase in the particle size from 68.5 to 107.7 nm, but the PDI decreased from 0.38 to 0.33, and no significant change in zeta potential was observed. Aqueous re-dispersibility study demonstrated that NI-SLNs lyophilized with trehalose had the maximum concentration (14.7 µg/mL) at 5 min, compared with lyophilized SLNs using other sugars; the use of other sugars also resulted in significant changes in the particle size, PDI, and zeta potential. A trehalose concentration of 2.5% w/v and a two-fold dilution of the SLN suspension were found to be the best conditions for lyophilization. Data from lyophilized SLNs using differential scanning calorimetry, powder X-ray diffraction, Fourier-transform infrared spectroscopy, and scanning electron microscopy indicated eventual transformation of SLN of NI-SLN-Tre from a crystalline to an amorphous state during the homogenization process. Finally, a stability study was performed with NI-SLN-Tre for up to 6 months at 30°C and 65% relative humidity, with no significant deterioration observed, suggesting that trehalose might be a useful cryoprotectant for NI-SLNs.

Key words nifedipine; solid–lipid nanoparticle; trehalose; cryoprotectant

Recently, about 40% of molecules being developed by the pharmaceutical industry have been reported to be poorly water soluble, which limits their absorption in the gastrointestinal tract and reduces the overall bioavailability.1,2 Most probably, many molecules have been rejected during the early stages of drug development because of this lack of water solubility. Therefore, the development of effective technologies and novel drug formulations for poorly water-soluble drugs is a mainstay of pharmaceutical research. Nanotechnology has revolutionized the field of drug delivery research. It offers some conventional delivery approaches, such as surface modification, complex formation, and use of colloidal lipid carriers for delivery to intestinal lymphatics.3,4 In addition, polymeric nanoparticles, self-emulsifying delivery systems, liposomes, microemulsions, micellar solutions, and solid–lipid nanoparticles (SLNs) have been exploited as possible drug carriers for oral intestinal lymphatic delivery. Since 1991, SLNs have been investigated comprehensively for use in drug delivery through various administration routes. SLN-based systems possess the characteristics of conventional carriers, as well as some additional characteristics that prevent the drawbacks and reported for conventional systems.3–7 SLNs have also been reported to be useful carriers for the successful delivery of peptides (e.g., insulin) and anticancer drugs (e.g., doxorubicin) through the oral intestinal route.8,9 Recently, Li et al. studied the pharmacokinetic aspects of quercetin-loaded SLNs after oral administration and found that SLNs improved the oral bioavailability.10

In particular, many formulations of SLN-like nanocarriers have been investigated with nifedipine (NI). NI is a highly potent calcium-channel blocker, which is poorly soluble in water (≈20 µg/mL). Previous attempts at formulations of NI nanocarriers have been reported, with mixed results. These approaches for the dissolution rate enhancement of NI include compaction with hydroxypropyl methyl cellulose (HPMC)11; co-grinding with HPMC,12 and bile salts13; and formation of co-precipitates or co-evaporates with mannitol,14 phosphatidylcholine esters,15 HPMC,16 chitosan derivatives,17 polyethylene glycols,18,19 and polyoxyethylene–polyoxypropylene copolymers.20 Recently, a high pressure homogenization technique has been successfully employed to prepare solid–lipid nanoparticles of NI using only phospholipids such as hydrogenated soybean phosphatidylcholine (HSPC) and dipalmitoylphosphatidyl glycerol (DPPG) as carriers to enhance the solubility of NI.21 This formulation has the advantage that no emulsifier and no toxic organic solvents were used. However, the limitation of this formulation was the reduced stability of the SLN suspension under ambient conditions. The suspension was only found to be stable, without suffering from any particle aggregation, for up to 4 months at 4°C.21 To overcome this limitation and to extend the storage life under ambient conditions, various sugars have been investigated as cryoprotectants. Sugars have proved to be very effective in preventing particle aggregation and inhibiting leakage of an active ingredient during the freeze drying of solid–lipid nanoparticles.22 Oshima et al. studied two monosaccharides (glucose and fructose) and two disaccharides (maltose and sucrose) as cryoprotectants, at arbitrary concentrations of 2% w/v, and found that disaccharides had higher cryoprotectant activity than monosaccharides.23 From these results, we speculated that sugars, specifically disaccharides, can be used to make SLNs physically stable. Therefore, there is a need for investigation

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into the use of sugar moieties as cryoprotectants that may be highly effective at inhibiting NI-nanoparticle aggregation.

To develop NI-loaded lyophilized SLN (NI-SLN) formulations with high physical stability, SLNs were prepared by high pressure homogenization and lyophilized with monosaccharides and disaccharides. The particle size and shape of the NI-SLNs were studied using dynamic light scattering (DLS) and scanning electron microscopy (SEM). X-Ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), and differential scanning calorimetric (DSC) analysis were performed to detect changes in the crystal structure and any chemical interactions between components. The results of these studies are discussed herein.

**Experimental**

**Materials** Hydrogenated soybean phosphatidylcholine [COATSOME® NC-21 (HSPC)] and dipalmitoylphosphatidylglycerol [COATSOME® MGLS-6060 (DPPG)] were purchased from Nippon Oil and Fats Co., Ltd. (Tokyo, Japan). Nifedipine (JPXIV; NI) was provided by Nippon Fine Chemical Co., Ltd. (Osaka, Japan). Glucose, fructose, galactose, xylose, lactose, trehalose, maltose, and sucrose were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The membrane filters (pore size: 0.20 and 0.45 µm) were purchased from Toyo Roshi Kaisha Ltd. (Tokyo, Japan). All reagents were of the highest grade commercially available and all solutions were prepared using de-ionized distilled water.

**Preparation of NI-Loaded Solid–Lipid Nanoparticle (NI-SLN) Suspension and Lyophilized NI-SLNs** To prepare the NI-SLN suspension, 40 mg of NI and 1000 mg of lipids (HSPC: DPPG, 5:1 molar ratio) were added to a mortar and physically mixed for 5 min. The mixture was then co-ground by a roll mill (R3-IR, Kodaira Seisakusho Co., Ltd.). The grinding part consisted of three rollers and the rotating velocity ratios for each roller were fixed as 1:2.5:5.8. Grinding was continued for 5 min. During grinding, most of the sample adhered to the rollers, but some partially fell down from the rollers. Therefore, the mill was stopped every 30 s to collect fallen material. The co-grinding cycle was repeated 10 times. The resultant roll mixture was dispersed in 200 mL of de-ionized distilled water and premixed using a Speed Stabilizer (10000 rpm, Kinematica Co.) at 9000 rpm for 10 min, followed by centrifugation at 12000 rpm, for 30 min using an Eppendorf® centrifuge (Germany). After this, the suspension was sonicated for 3 min, followed by centrifugation for 30 min using an Eppendorf® centrifuge (Germany). The membrane filter was placed in the filter device and centrifuged at 100 rpm for 10 min. The supernatant was filtered through a 0.2 µm syringe filter. The drug content was assayed by HPLC, as described above, using a calibration curve with at least three standard concentrations (10 µg/mL, 50 µg/mL, and 100 µg/mL) of NI.

EE and DL: The EE and DL were determined indirectly by calculating the amount of free drug (NI) concentration in the dispersions, according to the following equations.

**Total Drug Content (TDC), Encapsulation Efficiency (EE), and Drug Loading (DL) Determinations** TDC: NI-SLN suspensions (200 µL) were transferred to an eppendorf tube (1.5 mL). Exactly 800 µL of methanol was added and the suspension was sonicated for 3 min, followed by centrifugation at 7000 rpm for 10 min. The supernatant was filtered through a 0.2 µm syringe filter. The drug content was assayed by HPLC, as described above, using a calibration curve with at least three standard concentrations (10 µg/mL, 50 µg/mL, and 100 µg/mL) of NI.

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**Aqueous Re-dispersibility Test** Aqueous re-dispersibility study of the lyophilized NI-SLNs was performed by mixing the freeze-dried nanoparticles equivalent to 290 µg of NI in 10 or 20 mL of water contained in a 50 mL beaker that was being continuously stirred magnetically at 100rpm at room temperature. The analyte samples (0.5 mL each) were withdrawn at intervals of 1, 5, 10 and 20 min, followed by the replacement of an equal volume of water. The solution was then filtered through a membrane filter (0.45 µm) to remove some aggregates of NI-SLNs. The amount of NI dispersed in the solution was monitored using the HPLC method as described above. The concentration of NI was plotted against time to derive the
re-dispersibility profile.

**Solid-State Characterization of Lyophilized NI-SLNs.** Characterization by DSC Thermograms of the different samples (NI-SLNs lyophilized with fructose, galactose, glucose, xylose, lactose, maltose, sucrose, and trehalose) were obtained from DSC (Exstar, SII DSC7020). Lyophilized SLN samples, the freeze-dried lipid mixture, and free NI (3–5 mg) were placed in sealed standard aluminium pans and heated from 0 to 300°C, at a scanning rate of 10°C/min, under nitrogen purge, with an empty aluminium pan as reference.

**Characterization by Powder X-Ray Diffraction System (PXRD)** An X-ray diffractometer (RAD-C, Rigaku Denki Co., Ltd.) was used for the diffraction studies. The samples were exposed to CuKα radiation (30kV, 50mA) and scanned from 2–40°, 2θ at a scanning rate of 5°/min. Samples used for XRD analysis were free NI, a physical mixture of the lipids alone, a ground mixture of NI and lipids, and the lyophilized products NI-SLNs-Fru, NI-SLNs-Gal, NI-SLNs-Glu, NI-SLNs-Xyl, NI-SLNs-Lac, NI-SLNs-Mal, NI-SLNs-Suc, and NI-SLNs-Tre.

**Characterization by SEM** A scanning electron microscope (SSX-500, Shimadzu, Japan) was used to obtain SEM micrographs of the lyophilized products and the respective sugars used for lyophilization, after coating with gold/palladium in a vacuum beforehand. An accelerating voltage of 15kV was used.

**Characterization by FT-IR** Lipid mixture alone (HSPC:DPPG, 5:1), the physical mixture of NI and lipid, NI-loaded ground mixture, and all the lyophilized products were analyzed by FT-IR. In addition, to find differences in the spectra, subtractions of the spectrum of the lipid mixture alone from that of the NI-SLNs physical mixture, and from that of the NI-SLNs roll mill mixture, were calculated. The samples were measured by the diffuse reflection method using an FT-IR spectrometer (IR-Prestige 21, Shimadzu Co.).

**Stability Studies** The stability of lyophilized NI-SLN-Tre was investigated by measuring the particle size, zeta potential, and dissolved concentration of NI in water at 0 (initial) months and after storage at 30°C and 65% RH for 6 months.

**Statistics** Statistical analyses were performed using the Student t-test. A probability value of p<0.05 was considered to indicate statistical significance.

**Results and Discussion**

**The Cryoprotectant Efficiency of Different Sugars towards Lyophilized NI-SLNs** The NI-SLN suspension had a particle size of 68.5 nm, and polydispersity index (PDI), ZP, DC, EE, and DL values of 0.3, –62.1 mV, 145.0 µg/mL, 97.5%, and 2.7%, respectively. Previously, electrostatic stabilization of SLNs was reported to require a ZP ≥−30 mV.26) Moreover, the re-dispersibility profiles indicated that the disaccharides had maximum cryoprotective activity with the highest solubility of NI. The lowest ZP values result in the highest efficiency of protection against particle aggregation. The four disaccharides, trehalose, maltose, lactose, and sucrose, exhibited almost identical maximum concentrations at 5 min of 14.7, 14.6, 14.1, and 13.9 µg/mL, respectively (Fig. 1). However, maltose, lactose, and sucrose containing NI-SLNs had relatively higher particle diameters, PDI, and ZP values (174.9 nm, 0.7, and 97.5%, respectively (Fig. 1). However, maltose, lactose, and sucrose containing NI-SLNs had relatively higher particle diameters, PDI, and ZP values (174.9 nm, 0.7, and 97.5%, respectively). Each sample was measured by the C6000 UV-Visible spectrophotometer (Shimadzu, Japan) and the absorbance was recorded at 260 nm. The absorbance values of the NI-SLNs were compared with those of free NI. The absorbance of free NI was 0.56 at 260 nm, and the absorbance of the NI-SLNs was 0.53 at 260 nm.

**Table 1. Effect of Different Sugars on the Particle Size, Polydispersity Index (PDI), and Zeta Potential (ZP) of Nifedipine Loaded Solid–Lipid Nanoparticles**

<table>
<thead>
<tr>
<th>Sample</th>
<th>PDI</th>
<th>Particle size, Dp (nm)</th>
<th>ZP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI-SLN (Control)</td>
<td>0.4</td>
<td>9.1×10^{9}</td>
<td>−29.0</td>
</tr>
<tr>
<td>NI-SLN-Glu</td>
<td>1.0</td>
<td>156.45</td>
<td>−38.0</td>
</tr>
<tr>
<td>NI-SLN-Fru</td>
<td>1.0</td>
<td>2.3×10^{4}</td>
<td>−38.5</td>
</tr>
<tr>
<td>NI-SLN-Gal</td>
<td>1.0</td>
<td>227.59</td>
<td>−34.5</td>
</tr>
<tr>
<td>NI-SLN-Xyl</td>
<td>0.6</td>
<td>2.1×10^{1}</td>
<td>−38.9</td>
</tr>
<tr>
<td>NI-SLN-Lac</td>
<td>0.5</td>
<td>109.3</td>
<td>−51.1</td>
</tr>
<tr>
<td>NI-SLN-Mal</td>
<td>0.7</td>
<td>174.9</td>
<td>−56.4</td>
</tr>
<tr>
<td>NI-SLN-Tre</td>
<td>0.3</td>
<td>107.7</td>
<td>−58.6</td>
</tr>
<tr>
<td>NI-SLN-Suc</td>
<td>0.4</td>
<td>162.9</td>
<td>−43.7</td>
</tr>
</tbody>
</table>

**Fig. 1. Aqueous Re-dispersibility Profile of Solid Lipid Nanoparticles Using Various Sugars as Cryoprotectants**

NI-SLN (control): Freeze-dried SLNs with NI only; NI-SLN-Glu: freeze-dried NI-SLN with 2.0% glucose; NI-SLN-Fru: freeze-dried NI-SLN with 2.0% fructose; NI-SLN-Gal: freeze-dried NI-SLN with 2.0% galactose; NI-SLN-Xyl: freeze-dried NI-SLN with 2.0% xyllose; NI-SLN-Lac: freeze-dried NI-SLN with 2.0% lactose; NI-SLN-Mal: freeze-dried NI-SLN with 2.0% maltose; NI-SLN-Tre: freeze-dried NI-SLN with 2.0% trehalose; NI-SLN-Suc: freeze-dried NI-SLN with 2.0% sucrose. The study was performed using 20mL of water at room temperature. Since the NI in the SLN suspension is completely dissolved, the concentration should theoretically be 14.5 µg/mL. This value was adopted for 100% dissolved NI. Each value represents mean±S.D. (n=3).
drying was determined by a freeze thawing test using 2% optimum concentration of the NI-SLN suspension for freeze
Water for Freeze Drying (Freeze Thawing Test) to be the best cryoprotectant for use in further studies.

The depression of melting point is determined in proportion to the curvature, 1/r, of a spherical nanoparticle, according to the Gibbs–Thomson equation. Figure 2 gives an overview of the melting process of intact NI, a freeze-dried mixture of lipids, and the lyophilized NI-SLNs. The endothermic (melting point) peak of the pure drug NI occurs at 176.9°C, whereas the DSC thermal curve for the lipid mixture shows endothermic peaks at 75.0, 86.4, and 131.4°C. The thermogram for lyophilized NI-SLNs without any sugar (control) showed an endothermic peak of drug melt at 76.5°C, which corresponds to the peaks of the lipids, probably because of the low concentration of NI compared with the lipids. After the use of fructose (mp 103.0°C), galactose (mp 167.0°C), glucose (mp 146.0°C), xylo-lose (mp 144.0°C), lactose (mp 202.0°C), and sucrose (mp 186.0°C) as cryoprotectants for lyophilization, the NI-SLNs showed melting points for the SLNs at 133.1, 171.1, 135.5, 159.5, 152.0, and 190.0°C, respectively, that do not correspond with the melting points of the respective sugars. Whereas, NI-SLNs lyophilized with maltose and trehalose showed no peaks near to the melting point of the corresponding sugars (102 and 203°C, respectively), only peaks attributed to the lipid (at 57.9 and 60.0°C, respectively) were observed, indicating the conversion of crystalline SLNs to an amorphous state.

As shown in Fig. 2, almost all the lyophilized SLNs showed melting peaks due to the lipids with more or less intensity in the range 51.1–135.5°C, except for NI-SLN-Gal with a high intensity peak at 171.1°C, corresponding to that of NI (176.9°C). This peak may be due to the transformation of NI polymorphs from I to II. However, no monosaccharide demonstrated efficiency in altering the crystallinity of the SLNs. Among the disaccharide-containing SLNs, NI-SLN-Lac and NI-SLN-Suc showed sharp melting peaks at 152 and 190°C, with a lower

### Table 2. Effect of the Concentration of the NI-SLN Suspension on Freeze Drying (Freeze Thawing Test)

<table>
<thead>
<tr>
<th>Dilution ratio (NI-SLN suspension : water)</th>
<th>Particle size (nm) after freeze thawing, ( P_1 )</th>
<th>Particle diameter ratio, ( P_1/P_0 )</th>
<th>PDI after freeze thawing, ( D_s )</th>
<th>PDI ratio, ( D_s/D_b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:0</td>
<td>107.7</td>
<td>3.1</td>
<td>0.5</td>
<td>1.6</td>
</tr>
<tr>
<td>5:1</td>
<td>125.5</td>
<td>2.3</td>
<td>0.6</td>
<td>2.0</td>
</tr>
<tr>
<td>2:1</td>
<td>103.8</td>
<td>1.9</td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>1:1</td>
<td>79.6</td>
<td>1.5</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>1:2</td>
<td>85.9</td>
<td>1.6</td>
<td>0.4</td>
<td>1.3</td>
</tr>
<tr>
<td>1:5</td>
<td>53.8</td>
<td>1.0</td>
<td>0.7</td>
<td>2.3</td>
</tr>
</tbody>
</table>

PDI: Polydispersity index, \( P_1 \): particle diameter before freeze thawing, \( D_s \): PDI before freeze thawing.

### Table 3. Effect of the Concentration of Trehalose on Freeze Drying

<table>
<thead>
<tr>
<th>Trehalose concentration (%w/v)</th>
<th>PDI</th>
<th>Particle size (nm)</th>
<th>ZP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.43±0.29</td>
<td>7.32±1.26×10^4</td>
<td>−40.2±2.4</td>
</tr>
<tr>
<td>0.5</td>
<td>0.72±0.17</td>
<td>5.8±1.25×10^4</td>
<td>−46.6±1.0</td>
</tr>
<tr>
<td>1.0</td>
<td>0.47±0.26</td>
<td>134.8±22.1**</td>
<td>−45.7±2.3</td>
</tr>
<tr>
<td>1.5</td>
<td>0.66±0.11</td>
<td>102.5±29.8**</td>
<td>−49.0±0.9</td>
</tr>
<tr>
<td>2.0</td>
<td>0.52±0.09</td>
<td>131.9±44.1**</td>
<td>−48.6±4.6*</td>
</tr>
<tr>
<td>2.5</td>
<td>0.39±0.04</td>
<td>111.7±9.6**</td>
<td>−59.1±3.9**</td>
</tr>
<tr>
<td>3.0</td>
<td>0.38±0.04</td>
<td>112.5±18.2**</td>
<td>−45.9±2.9*</td>
</tr>
</tbody>
</table>

PDI: Polydispersity index. *p<0.05, **p<0.01 versus each 0% value.

Dilution Effect of the NI-Nanoparticle Suspension in Water for Freeze Drying (Freeze Thawing Test) The optimum concentration of the NI-SLN suspension for freeze drying was determined by a freeze thawing test using 2% trehalose as the cryoprotectant. Therefore, trehalose appeared to be the best cryoprotectant for use in further studies.

### Effect of the Concentration of Trehalose on Freeze Drying

-56.4mV; 109.3nm, 0.5, and −51.1mV; and 162.9nm, 0.4, and −43.7mV, respectively) than those observed for trehalose (107.7nm, 0.3, and −58.6mV). Therefore, trehalose appeared to be the best cryoprotectant for use in further studies.

#### Dilution Effect of the NI-Nanoparticle Suspension in Water for Freeze Drying (Freeze Thawing Test)

The optimum concentration of the NI-SLN suspension for freeze drying was determined by a freeze thawing test using 2% trehalose as the cryoprotectant. The particle size (\( P_1 \)) and PDI (\( D_s \)) of the nanoparticle suspension before freeze thawing were 53.2nm and 0.3, respectively. The lowest particle diameter ratio, \( P_1/P_0 \) (1.0) but highest PDI ratio \( D_s/D_b \) (2.3) were observed for a dilution ratio of 1:5 (suspension:water) (Table 2). In spite of having a moderately high value for \( P_1/P_0 \) (1.4), the sample with a 1:1 dilution ratio showed the least PDI ratio \( D_s/D_b \) (1.0). Guan et al. reported that a two-fold dilution of nanoliposomes, using trehalose as a cryoprotectant, shifted the \( P_1/P_0 \) value from 1.9 to 1.2 and a value smaller than 2.0 is preferable for freeze drying purposes. The smaller the \( P_1/P_0 \) and \( D_s/D_b \) values, the better will be the freeze drying effect. In our study also, a dilution ratio of 1:1 gave satisfactory results.

#### Effect of the Concentration of Trehalose on Freeze Drying

- The effectiveness of various concentrations of trehalose on the freeze drying of NI-SLNs was examined (Table 3). A mean particle size around 100nm was observed for lyophilized nanoparticles containing 1.0%, 1.5%, 2.0%, 2.5%, and 3.0% trehalose. The mean PDI of 0.39 and 0.38 were observed for concentrations of 2.5% and 3.0% w/v trehalose, respectively, suggesting that these might be effective concentrations of trehalose for cryoprotection during lyophilization. The lowest ZP (−59.1mV) was observed for 2.5% trehalose, indicating that this concentration should have more efficacy in preventing particle aggregation than 3.0% trehalose (ZP=−45.9mV).

#### Solid-State Characterization

- The thermal stability of the SLNs and the compatibility of the constituents in the formulation were analyzed by simultaneous DSC. DSC also gives insight into the melting and re-crystalline behavior of crystalline materials like SLNs. Therefore, DSC experiments are useful to understand solid dispersions, such as solid solutions, simple eutectic mixtures, and, as in this case, the effect of lipid mixtures and cryoprotectant sugars on crystal ordering.

- Table 3 gives the melting points due to the lipids with more or less intensity in the range 51.1–135.5°C, except for NI-SLN-Gal with a high intensity peak at 171.1°C, corresponding to that of NI (176.9°C) peak of the pure drug NI occurs at 176.9°C, whereas the DSC thermal curve for the lipid mixture shows endothermic peaks at 75.0, 86.4, and 131.4°C. The thermogram for lyophilized NI-SLNs without any sugar (control) showed an endothermic peak of drug melt at 76.5°C, which corresponds to the peaks of the lipids, probably because of the low concentration of NI compared with the lipids. After the use of fructose (mp 103.0°C), galactose (mp 167.0°C), glucose (mp 146.0°C), xylose (mp 144.0°C), lactose (mp 202.0°C), and sucrose (mp 186.0°C) as cryoprotectants for lyophilization, the NI-SLNs showed melting points for the SLNs at 133.1, 171.1, 135.5, 159.5, 152.0, and 190.0°C, respectively, that do not correspond with the melting points of the respective sugars. Whereas, NI-SLNs lyophilized with maltose and trehalose showed no peaks near to the melting point of the corresponding sugars (102 and 203°C, respectively), only peaks attributed to the lipid (at 57.9 and 60.0°C, respectively) were observed, indicating the conversion of crystalline SLNs to an amorphous state.

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intensity than that observed with the monosaccharides, which also demonstrates some crystalline nature for the SLNs. However, NI-SLN-Mal and NI-SLN-Tre do not possess such peaks that indicate crystallinity, only very low intensity peaks corresponding to lipids at 57.9 and 60.0°C, respectively.

The DSC thermograms of the four disaccharides show moderately less intensity peaks indicative of crystallinity than those of the four monosaccharides, indicating the higher efficiency of the disaccharides for protecting the SLNs from crystal formation. Among the disaccharides, only trehalose and maltose could prevent crystal formation of the SLNs, as proved by the maximum dissolved concentrations (14.7 and 14.6 µg/mL, respectively) (Fig. 1).

The PXRD pattern of NI (intact) exhibited sharp peaks with high intensity at 2θ scattered angles 8.1, 11.8, 16.2, 19.6, and 24.4° indicating the highly crystalline nature of NI (Fig. 3). Moderately intense peaks were observed for the lipid mixture and the ground mixture of NI and lipids. Among the NI-SLNs containing monosaccharides, high intensity peaks indicating crystallinity were observed for NI-SLN-Gal (18.9, 22.1, 25.5, 28.6, and 36.7°), NI-SLN-Glu (19.9, 22.9, and 28.7°) and NI-SLN-Xyl (17.6 and 35.0°). The presence of these peaks gives further proof of the crystalline nature of NI-SLN-Gal, NI-SLN-Glu, and NI-SLN-Xyl. However, no significant peaks were observed for NI-SLN-Fru indicating crystallinity of SLN, only peaks attributed to lipids (Fig. 2).

No peaks of significant intensity attributable to NI were found in NI-SLNs containing lactose, maltose, sucrose, and trehalose. Thus, the PXRD pattern along with the re-dispersibility profile and DSC thermograms provide further evidence that disaccharides might have the best cryoprotectant properties for NI-SLNs.

The FT-IR spectra (Fig. 4) of the physical mixture of lipids and the ground mixture of NI and lipids correspond with each other, but differ in intensities, because the spectrum of the lipids is strong due to its weight ratio being about 25 times higher than that of NI. The spectrum of the ground mixture was apparently different from that of NI in the range 2800–3000 cm$^{-1}$. The appearance of peaks outside the regions of C=O and N–H stretching vibrations suggests that partial structural changes or new interactions have occurred. It has been reported that roll mixing of poorly water-soluble drugs with carriers alters the drug from crystalline to the amorphous state and induces intermolecular interactions.31)

In the lyophilized NI-SLNs (without sugar), an interaction was observed in the range 3200–3400 cm$^{-1}$ (Fig. 4). Subtraction of the spectrum of the freeze-dried sugar from that of the respective lyophilized NI-SLNs showed a common difference can be observed in the range 2800–3000 cm$^{-1}$, suggesting that an intermolecular interaction between the NI-SLN and the sugar has occurred, as indicated by O–H stretching. This stretching is due to the formation of a hydrogen bond between the O–H groups of the sugar moieties with the polar head group of the lipids, resulting in the cryoprotectant activity of the sugars in different order.
Figure 5 shows the SEM images for the ground mixture of NI and lipids, lyophilized NI-SLNs (without sugar), the free sugars, and the lyophilized NI-SLNs with those sugars. NI-SLN-Mal (l) and NI-SLN-Tre (p) have a morphology that is discrete and regular in shape. The smooth surface ensured the absence of drug on it; rather the drug is entrapped within the lipid matrix. Lyophilized NI-loaded SLNs containing trehalose (p) also appear to have a network-like structure that facilitates the penetration of water within the structure to allow quick re-dispersibility.

Taken together, the FTIR, DSC, PXRD, and SEM data indicate that both maltose and trehalose are highly efficient cryoprotectants for the lyophilization of NI-SLN suspension. However, trehalose has a mean particle diameter, and PDI and ZP values that are more conducive to cryoprotectant ability compared with maltose. Probably for this reason, Patist and Zoerb reported that trehalose is a more effective cryoprotectant than other disaccharides. In addition, trehalose has a very high \( T_g \) (glass transition temperature) and has the ability to form a dihydrate to maintain an elevated \( T_g \) in the sample. Moreover, trehalose has a membrane-protecting effect, not only because of the formation of hydrogen bonds with the polar head groups of lipids, but also because trehalose can disrupt the tetrahedral hydrogen bond network of water and reduce the amount of freezable water. Thus, trehalose can be reported as one of the best cryoprotectant of all the disaccharides investigated for NI-SLNs and was used for further studies.

Stability Study Upon storage at 30°C and 65% RH for 6 months, NI-SLN-Tre was found to be stable in terms of mean particle diameter, PDI and ZP (Table 4) and re-dispersibility rate (Fig. 6). No significant increase in particle size, PDI, or ZP was observed. The concentration profile of NI-SLN-Tre at
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6 months showed drug release almost identical to the initial (0 month) values. The physical appearance of NI-SLN-Tre in aqueous medium was also found to be transparent as similar to the original. In addition, although DSC, PXRD, and FT-IR analyses were performed to determine the physicochemical changes of SLNs after long-term storage, any significant changes were not observed (data not shown). Taken together, this study revealed that NI-SLN-Tre was more stable under ambient conditions than the NI-SLN suspension was at 4°C.

**Conclusion**

This study demonstrates that lyophilization of solid–lipid nanoparticles of water insoluble or poorly water-soluble drugs (such as nifedipine) with disaccharides can enhance the solubility of the drug. Nifedipine was chosen as a model drug in this study because this drug is one of extensively studied class II group drugs in accordance with biopharmaceutics classifi-

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**Table 4.** Stability Data [Particle Diameter, PDI, and ZP] for Lyophilized NI-SLNs with 2.5% Trehalose

<table>
<thead>
<tr>
<th>Time (month)</th>
<th>Particle size (nm)</th>
<th>PDI</th>
<th>ZP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>111.7±9.6</td>
<td>0.39±0.04</td>
<td>−59.1±3.9</td>
</tr>
<tr>
<td>4</td>
<td>109.4±9.9</td>
<td>0.38±0.04</td>
<td>−59.9±3.5</td>
</tr>
<tr>
<td>6</td>
<td>104.5</td>
<td>0.3</td>
<td>−63.0</td>
</tr>
</tbody>
</table>
cation system. As for an aqueous re-dispersibility test, maximum concentration of nifedipine was obtained with solid–lipid nanoparticles lyophilized with trehalose (14.7 µg/mL) at 5 min that was more than three times higher than that of without trehalose (4.6 µg/mL). The particle size and ZP can be controlled at around 100 nm and −60 mV, respectively. This is because nifedipine, during high pressure homogenization at 175 MPa with up to 100 pass cycles, is transformed from a crystalline to an amorphous state. Trehalose had a strong intermolecular interaction with the phospholipids, which resulted in trehalose performing as the best cryoprotectant to prevent the agglomeration of solid–lipid nanoparticles of nifedipine. Moreover, storage limitations could be overcome by using trehalose as the cryoprotectant for nifedipine solid–lipid nanoparticles. Previously, nifedipine solid–lipid nanoparticles in suspension could be stored at 4°C for up to 4 months. In our study, lyophilization with trehalose at a concentration of 2.5% w/v could successfully keep the nanoparticles physico-chemically constant for up to 6 months at 30°C and 65% RH. This also indicates that, under ambient conditions, the shelf life of nifedipine loaded solid–lipid nanoparticles may be increased by more than 6 months, although further study is needed. In addition, our recent study demonstrated that nifedipine solid–lipid nanoparticles just after preparation showed improved oral absorption rate in comparison to nifedipine itself[30]; further in vivo pharmacokinetic study using lyophilized nifedipine solid–lipid nanoparticles with trehalose should also be conducted. In conclusion, nifedipine solid–lipid nanoparticles lyophilized with trehalose might provide effective drug delivery of nifedipine for the treatment of hypertension and angina pectoris.

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References