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Research paper

The effects of cryoprotectants on the freeze-drying of ibuprofen-loaded solid lipid microparticles (SLM)

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Abstract

The effects of cryoprotectants on the diameter and the entrapment efficiency of ibuprofen-loaded solid lipid microparticles (SLM) during the freeze-drying process were investigated extensively. The SLM were prepared by the emulsion–congealing technique in which a glycerol behenate was used as the lipid matrix for the SLM and a soybean lecithin/bile salt used as the stabilizer. Also, trehalose, glucose, mannitol, and sucrose were chosen as the cryoprotectants. Trehalose and glucose proved to be the most effective in preventing particles aggregation and in inhibiting leakage from drug-loaded particles during the SLM freeze-drying process. The most suitable concentrations were proved to be 15% and 5% (wt), respectively.

Keywords: Solid lipid microparticles; Lyophilisation; Ibuprofen; Cryoprotectant

1. Introduction

During the last few decades, an increasing attention has been paid to the sustained release of various drugs [1–5]. Solid lipid microparticles (SLM) are micro- or nano-scale drug carrier systems composed of fatty acid, glyceride, fatty alcohol and solid wax. SLM are suitable as the carriers of lipophilic drugs [6–8]. SLM are characterized by their better bio-compatibility as compared to competing polymeric microparticles. Moreover, they are excellent in controlling and sustaining drug release efficiencies.

Ibuprofen is a well-known non-steroidal, anti-inflammatory drug which has been widely used for the treatments of inflammations, a variety of pains, and some rheumatism. However, it is limited by a short biological half-life time (1.5–2 h) [9] that leads to a short duration of action. In order to overcome the shortcoming, multiple intakes of ibuprofen are required to maintain the effective concentration in human bodies which potentially lead to the occurrences of some side effects [10]. A possible approach to resolve this problem is the application of SLM carrying ibuprofen [1].

In order to produce different pharmaceutical formulations which ensure the satisfaction of the specific needs related to storage, packing, and transportation, solid powder of SLM needs to be made by a drying process. Freeze-drying technology appears as one of the most suitable methods to stabilize and facilitate the handling of colloidal systems. It is widely used for drying pharmaceutical systems, e.g. liposomes [11–13], drug-loaded polymer microspheres [14,15], and solid lipid nanoparticles (SLN) [16]. At the same time, the freeze-drying of the drug-free and drug-loaded SLM was studied, and the diameters of both types of solid SLM were reported to increase as a result of drying [16].

Zimmermann et al. [17] investigated the protective efficiency of cryoprotectants, the freezing velocities, and the thermal treatments during the SLM freeze-drying process and optimized the freeze-drying process parameters. Schwarz and Mehnert [18] investigated the influence of differ-

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ent cryoprotectants and different redispersion methods on the diameters and poly-dispersities of SLM. These studies presented a comprehensive understanding of the influence of various parameters on the freeze-drying process and promoted the application of the lyophilisation technique in liposome drying.

In addition to above-mentioned contributions, we present a new approach for the development of strategies aiming at lowering of drug toxicity and increasing drug's storage time as well as reducing the costs of drug preservation. The study reported in this paper is focused on the development of a new SLM. The lyophilisation of ibuprofen SLM has been investigated. And in particular, the effects of freeze-drying on the particle size, the entrapment efficiency and the role of cryoprotectants were examined.

2. Materials and methods

2.1. Materials

Ibuprofen was provided by Shanghai Yuanji Chemicals Co. Bile salt was produced by Guangdong Huankai Biology Co. (China); glycerol behenate by Gattefosse (France); glucose, mannitol, and soybean lecithin by Shanghai Boao Biology Ltd. (China); trehalose with two coordinated water molecules by Guangxi Jiewoli Biology Ltd. (China), sucrose by Guangzhou Chemical Reagent Co. (China). Water was double distilled with quart still. All agents were of analytical grade.

2.2. The preparation of SLM

The emulsion–congealing technique was employed to prepare SLM. The SLM consist of 10% lipid, 3% stabilizer (wt), 10% ibuprofen (wt, relative to lipid only), and water as the rest. Soybean lecithin and bile salt were mixed together at the ratio of 2:1 as the stabilizer. The emulsion–congealing process is achieved as follows [7]: the lipid mixed with drug is first melted at 85 °C, and then emulsified into an aqueous phase in which the stabilizer is added. The emulsion is stirred at 300 rpm using a magnetic blender for 5 min, and subsequently treated with a high shear dispersing emulsifier (M200, Shanghai Fuluke Liquid Machinery Co., China) at 8000 rpm for 10 min. Finally, the emulsion is cooled down in a water bath at 5 °C and the lipid crystallizes.

2.3. Freeze-drying process

Trehalose, glucose, mannitol, and sucrose were selected, respectively, as the cryoprotectants for the freeze-drying process of ibuprofen-loaded SLM. Freeze-drying was performed in an ALPHA1-2 freeze-drier (CHRIST, Germany) equipped with the condenser operating at −50 °C and a chamber with cooled shelves. The freeze-drying process lasted for 24 h to allow for a complete solidification. Prior to drying, 50 ml liquid SLM with the cryoprotectants were frozen to −25 °C forming a 10 mm thick layer on a stainless steel tray in a refrigerator. The freeze-drying was conducted at a pressure of 5.0 Pa.

2.4. Freeze–thaw process and reconstruction of freeze-dried samples

After thawing been freeze-dried, the trays with SLM were placed into a desiccator at room temperature. Prior to use, purified water was added into the dried SLM to make the final volume to be 50 ml. Then an ultrasonic disperser was used for 5 min to ensure a complete dispersion of SLM. The rehydration was performed at room temperature.

2.5. Morphology and particle size analysis

The morphology of the particles was determined using an optical microscope (E200, Nikon, Japan). The volume based particle sizes of the formulations were analyzed using photon correlation spectroscopy (PCS) (Mastersize-2000, Malvern Instruments, UK). The PCS analysis yielded the mean diameter of the particles (Z-average). The particle size distribution was described by the monodispersity index δ, Eq. (1)

$$\delta = \frac{D_{90} - D_{10}}{D_{50}} \tag{1}$$

where D_{90}, D_{50}, and D_{10} refer to the particle sizes when cumulative total distributions were 90%, 50%, and 10%, respectively. The smaller the δ value is, the better the monodispersity is. The samples were diluted with distilled water to reach a suitable concentration. All measurements were repeated three times.

2.6. Determination of drug entrapment efficiency

The SLM were centrifuged for 20 min at 10,000 rpm (TGL-20M, Hunan Saitexiangyi Centrifuge Ltd., China). The drug contents in the supernatant after centrifugation were measured spectrophotometrically (751-GW spectrophotometer, HP Analytical Apparatus Ltd., Shanghai, China) at a wavelength of 222 nm. Prior to the measurement, the samples were filtered using a 0.22 μm pore size membrane. One milliliter of aliquot was withdrawn from the filtrate and added to a 25 ml volumetric flask. The flask was then filled with distilled water to make the final volume to be 25 ml. The drug entrapment efficiency in the microparticles was calculated using Eq. (2)

$$\text{Entrapment efficiency} = \left(\frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}}\right) \times 100\% \tag{2}$$

where $W_{\text{initial drug}}$ is the weight of drug added to the system, while $W_{\text{free drug}}$ is the analyzed weight of free drug in the aqueous phase.

2.7. Powder XRD analysis

An X-ray power diffractometer (XRD) was used to analyze the crystal structures of carriers and drug (D/max-IIIA, Rigaku, Japan). Before analysis, the freeze-dried SLM were ground to powder with an agate mortar. The angular range of XRD analysis ranged from 2°2θ to 50°2θ. Diffraction patterns were obtained under the continuous-scan mode and at a scanning rate of 12°2θ/min.

2.8. Differential scanning calorimetry (DSC) analysis

DSC analysis was carried out with STA4490 DSC (NETZSCH, Germany). Before analysis, the freeze-dried SLM were ground to powder with an agate mortar. Ten milligrams of each sample was sealed in a DSC aluminium pan using an empty pan as reference and submitted to calorimetric analysis at a scan rate of 5 °C/min under the temperatures ranging from 10 to 210 °C.

Microparticles may aggregate during the freeze-drying process. The XRD and DSC analyses samples need to be ground to separate the cluster of microparticles. It does not affect the crystallinity/amorphous content of the samples.

3. Results and discussion

3.1. Effects of freeze-drying on particle size

In order to choose the appropriate cryoprotectant, which effectively prevents particle aggregation, a screening of cryoprotectants at variable concentrations was performed. A range of weight concentrations of cryoprotectant to SLM ranging from 0% to 20% were tested during this screening. The effects of cryoprotectants on particle size and monodispersity δ are shown in Fig. 1. R refers to the SLM before freeze-drying.

Based on the comparisons of the experimental data from Figs. 1–3, several conclusions could be drawn. First, particle sizes of freeze-dried SLM increased obviously when there was no addition of cryoprotectants to the system. However, the particle sizes of SLM may be effectively preserved when a certain amount of cryoprotectants was added. Second, the cryoprotectants exhibited the best effect on the preservation of SLM particle size at different concentrations. For the case of 15% (wt) trehalose concentration, the size and monodispersity of freeze-dried microparticles show smallest change compared to that without cryoprotectants. And for glucose, mannitol and sucrose, the most suitable concentrations are 5%, 10%, and 10% (wt), respectively. Third, increasing cryoprotectant concentration to a certain level may eventually reach a limit of stabilization or even destabilize microparticles, especially in presence of sucrose. Fourth, every one of the cryoprotectants showed different preservation effects. The trehalose and glucose are proved to be more suitable than mannitol and sucrose for the ibuprofen-loaded SLM.

The particle size distribution and monodispersity of freeze-dried SLM are shown in Fig. 2 at suitable concentration, trehalose 15%, glucose 5%, mannitol 10%, and sucrose 10% (wt). It is also clear that trehalose and glucose are the most efficient cryoprotectants for ibuprofen-loaded SLM. We attribute the higher optimal concentration of trehalose than that of glucose to the trehalose's larger molecular weight. As for mannitol, its preservation effect is less visible, and the particle size distribution becomes wider. As for sucrose, the particle size, after the freeze-drying, is the largest and the distribution is the widest of the four cryoprotectants. Moreover, as shown in Fig. 2, the small peak at 120 μm in the particle size distribution of sucrose could be explained by the aggregation of microparticles.

The optical microscope pictures of SLM before and after the freeze-drying as well as the rehydration are shown in Fig. 3. Before the freeze-drying, the orientation of SLM is uniform and only a few aggregates were observed (Fig. 3a). However, after the freeze-drying, many SLM without the treatments of cryoprotectant agglomerated and seriously broke (Fig. 3b). With the pre-treatment using trehalose and glucose, the SLM are preserved well during the freeze-drying process (Fig. 3c and d). The freeze-dried SLM were well dispersed (this means that they did not agglomerate after the freeze-drying process), practically no significant changes of particle diameters were observed, and broken microparticles were not found. In contrast, after the treatment of mannitol, some aggregates and broken particles were observed in the SLM (Fig. 3e). Moreover, when treated with sucrose, the microparticles aggregated considerably and many of them broke (Fig. 3f). It is obvious that the aggregation of SLM caused the increases in particle sizes, and the broken particles resulted in the leakage of carried drug from the SLM. Thus, it can be concluded that trehalose and glucose are excellent cryoprotectants which preserve effectively the sizes and distribution of ibuprofen-loaded SLM after the SLM are freeze-dried.

3.2. The effects of the freeze-drying on the drug entrapment efficiency

The drug leakage from the SLM is a very serious problem during the freeze-drying process. The pre-treatment of SLM with certain cryoprotectants not only preserves the particle sizes efficiently, but also eliminates the chance of drug leakage from the SLM. The effects of four cryoprotectants on the ibuprofen entrapment efficiencies are shown in Table 1.

The entrapment efficiency of freeze-dried SLM without the pre-treatment of cryoprotectants dropped from 91.26% to 75.56%. It is worth mentioning that the entrapment efficiency can be promoted by adding some cryoprotectants to the SLM prior to the freeze-drying process. There exists a non-linear increase in the entrapment efficiency with the increase of the cryoprotectant concentration. Trehalose is the most effective one, while sucrose is

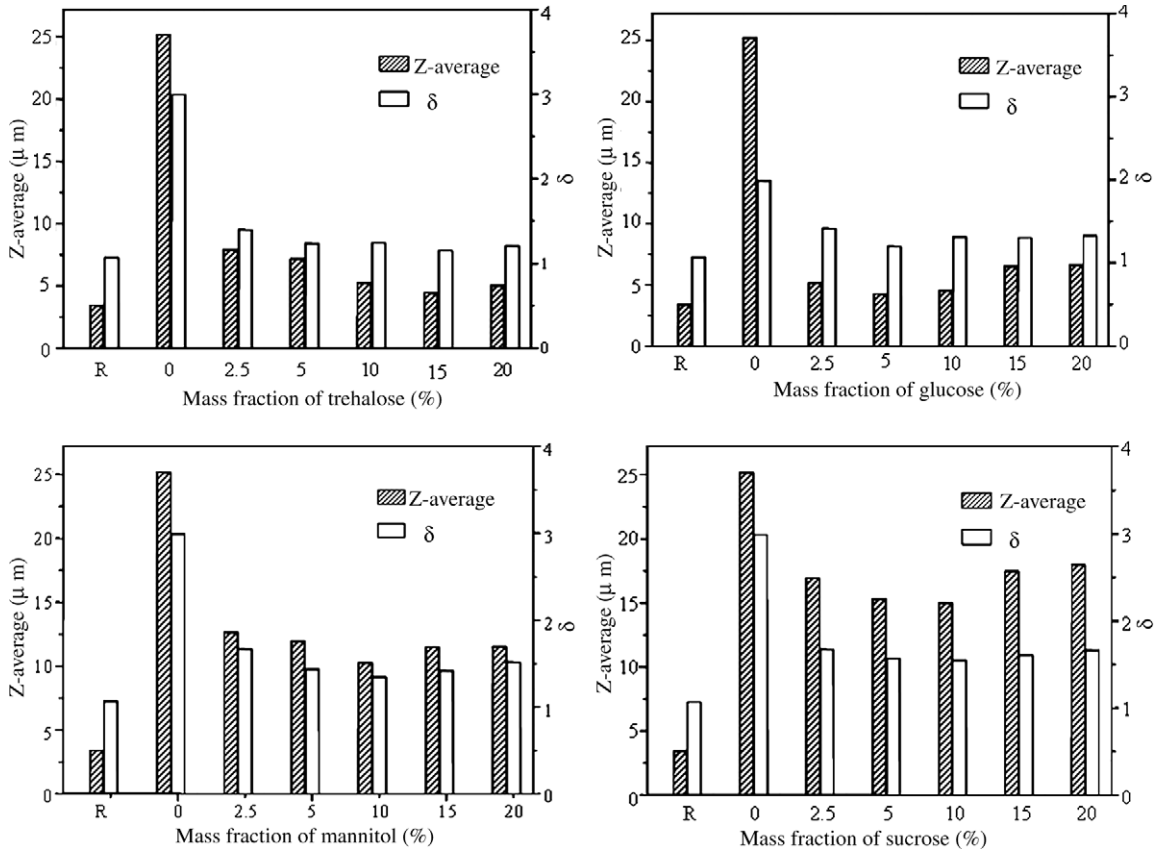

Fig. 1. *Z*-average and monodispersity δ after thawing of the frozen SLM in the presence of different cryoprotectants.

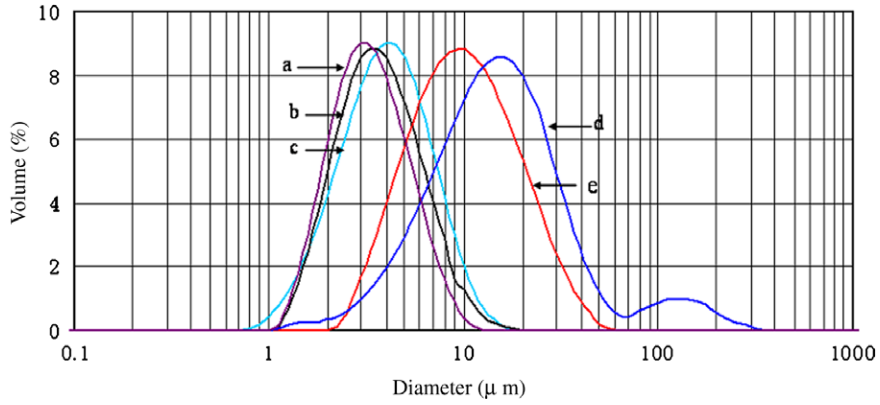

Fig. 2. The particle size distribution of SLM before freeze-drying (a) and freeze-dried SLM after thawing in the presence of different cryoprotectants with suitable contents (wt): 15% trehalose (b), 5% glucose (c), 10% sucrose (d), and 10% mannitol (e).

the worst cryoprotectant in the ibuprofen-loaded SLM. The entrapment efficiency of drug in SLM in presence of trehalose at the concentration of 15% (wt) reached values above 90%, which is practically the same as the efficiency before the freeze-drying of the SLM. It shows that the drug almost does not leak from the SLM after the freeze-drying.

As shown in Table 1, the entrapment efficiency of drug in SLM increases as increase of cryoprotectant amounts. However, increase of cryoprotectant concentration to a certain level may eventually cause the increase of sizes and monodispersity of the freeze-dried SLM. The drug entrapment efficiency of SLM/15% trehalose is almost the same as that of SLM/20% trehalose, whereas sizes and monodispersity of the particles with 15% trehalose content are smaller than those with 20% trehalose content, as shown in Figs. 1 and 2, respectively. Therefore, 15% trehalose content was proved to be the most suitable concentration for ibuprofen-loaded SLM. For glucose, the particle size and monodispersity increases significantly when the content of glucose is increased to 10%. The particle accu-

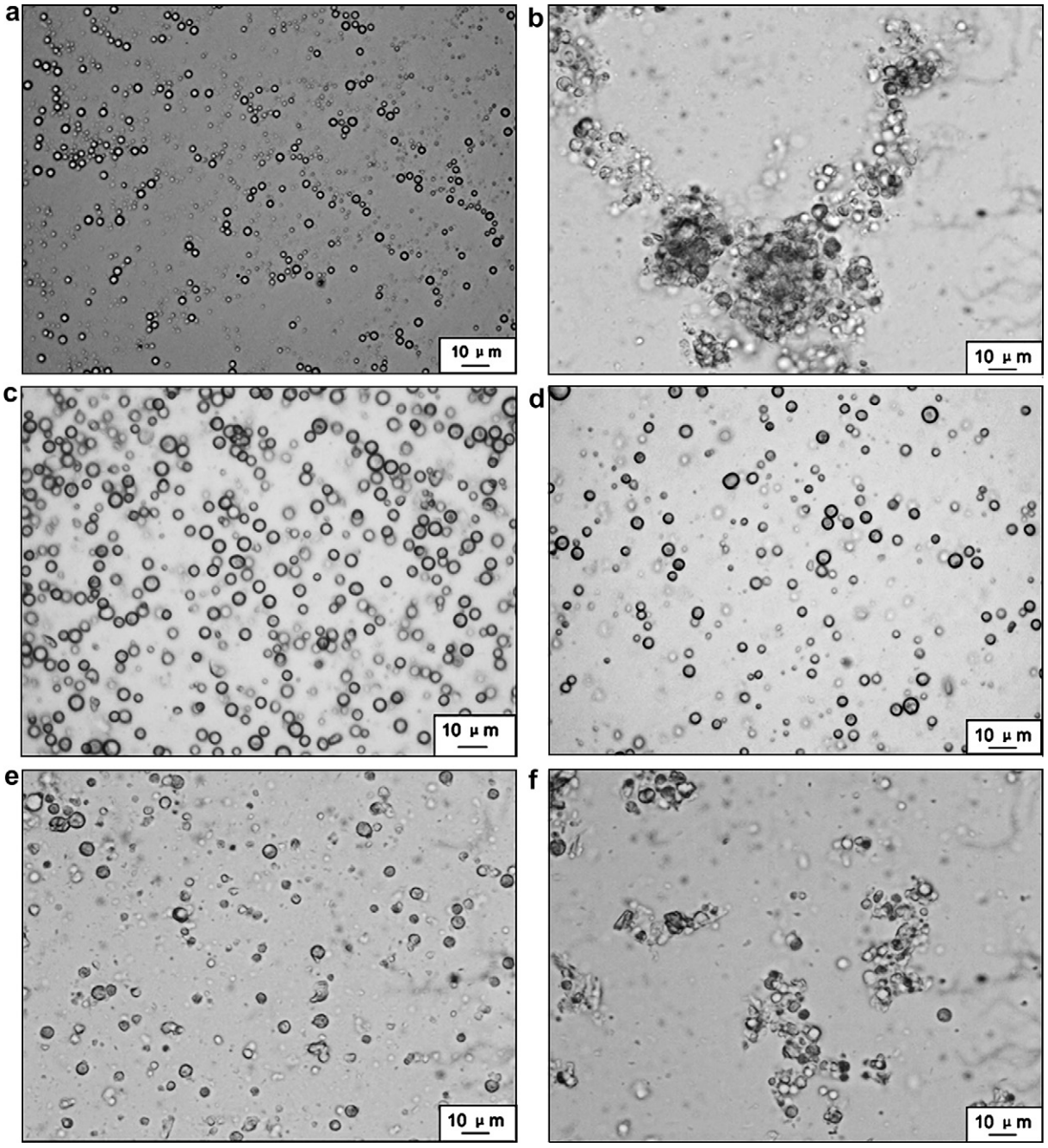

Fig. 3. The optical microscope pictures of SLM before freeze-drying and after thawing of the frozen SLM in the presence of different cryoprotectants with optimal concentration (ensuring a constant particle diameter and eliminating the drug leakage); (a) before freeze-drying, (b) in the absence of cryoprotectant (wt), (c) trehalose 15%, (d) glucose 5%, (e) mannitol 10%, and (f) sucrose 10%.

Table 1
Entrapment efficiency of ibuprofen in SLM in presence of different cryoprotectants

Cryoprotectants	Before freezing	Content of cryoprotectants (%)					
		0	2.5	5	10	15	20
Trehalose	91.26 ± 1.18	75.56 ± 1.21	84.97 ± 1.15	87.89 ± 1.08	89.54 ± 1.12	90.56 ± 1.11	90.82 ± 1.16
Glucose			80.16 ± 1.06	84.52 ± 1.15	85.36 ± 1.16	87.15 ± 1.13	87.89 ± 1.20
Mannitol			79.16 ± 1.19	81.25 ± 1.21	82.51 ± 1.25	82.94 ± 1.20	83.12 ± 1.17
Sucrose			76.99 ± 1.23	77.15 ± 1.21	79.42 ± 1.16	79.89 ± 1.15	80.19 ± 1.24

mulation even happened at a content of 15% glucose, even though the entrapment efficiency of the drug improved. Thus the suitable glucose content is 5%. For mannitol and sucrose, 10% of the contents are reasonable, in consideration of the entrapment and particle size and monodispersity.

3.3. The protective mechanism of cryoprotectants

3.3.1. Protective mechanism

The protective mechanism of the cryoprotectants may be explained by several factors.

(1) The Cryoprotectants are multi-hydroxy compounds that may form a eutectic in the presence of water that lead to the formation of amorphous or imperfect ice crystalloids. With development of the ice crystalloid, the extrusion and mechanical destruction of the microparticles in the freezing process is inhibited [19].

(2) With the addition of cryoprotectants, the viscosity of solution usually rises due to the interaction between the hydroxyl of cryoprotectants and H_2O molecules. The rise of viscosity of the suspension can suppress the ice crystallization and limit the mechanical damage of the microparticles.

(3) The multi-hydroxy compounds can maintain the spatial orientation and distance among the SLM when the ice sublimes during the freeze-drying process. In consequence, the SLM are prevented from forming the aggregates.

(4) The multi-hydroxy compounds can inhibit the amalgamation of the lecithin layer. The SLM were prepared using the glycerol behenate as the carrier and lecithin/bile salt as the stabilizer. The bile salt is a catalyst promoter inserted in the lecithin layer to increase the curvature of the lecithin layer [7]. The schematic structure diagrams of lecithin molecule and SLM are shown in Fig. 4. The molecule is comprised of a polar head and two nonpolar hydrocarbon chain tails in the lecithin molecule. Lecithin molecules can be adsorbed to the SLM surface and form a lecithin layer (monolayer or multilayer) via the two nonpolar hydrocarbon chain tails. And water molecule can form a hydrated layer on the lecithin by forming the H-bonding with the polar head of lecithin molecule. As the two lecithin layers come close enough, the hydrated layer will produce a repulsive force to prevent the amalgamation of the lecithin layers. Therefore, the microparticles aggregation is prevented. During the freeze-drying process without any treatment of cryoprotectants, the hydrated layer will gradually disappear, resulting in the amalgamation of lecithin layers, thus the particle sizes increase. On the contrary, during the freeze-drying process with the treatment of cryoprotectants, the positions of water molecules are replaced by the cryoprotectant molecules that form a new protective layer. This new protective cryoprotectant layer now will protect the lecithin from amalgamating. Therefore, the amalgamation process of the lecithin layer is stopped, the particle sizes will increase and the drug's leakage will be inhibited [20].

(5) Theoretically, if the water content in the lecithin layer is below 20% (molar percentage), the lecithin molecules will arrange closer, and the lecithin is in the gel state. However, once the water content in the lecithin layer increases to more than 20% (molar percentage), the lecithin molecules take a larger space, and the distances between two lecithin layers shrink. Thus, the lecithin is in the liquid crystalline state [21]. During the freeze-drying process, the lecithin will transit from liquid phase to the gel phase inevitably while water content is diminishing. And during the rehydrating process, the lecithin will transform from the gel phase to the liquid crystalline phase. In the transition, the resulting increase in permeability of the lecithin layer

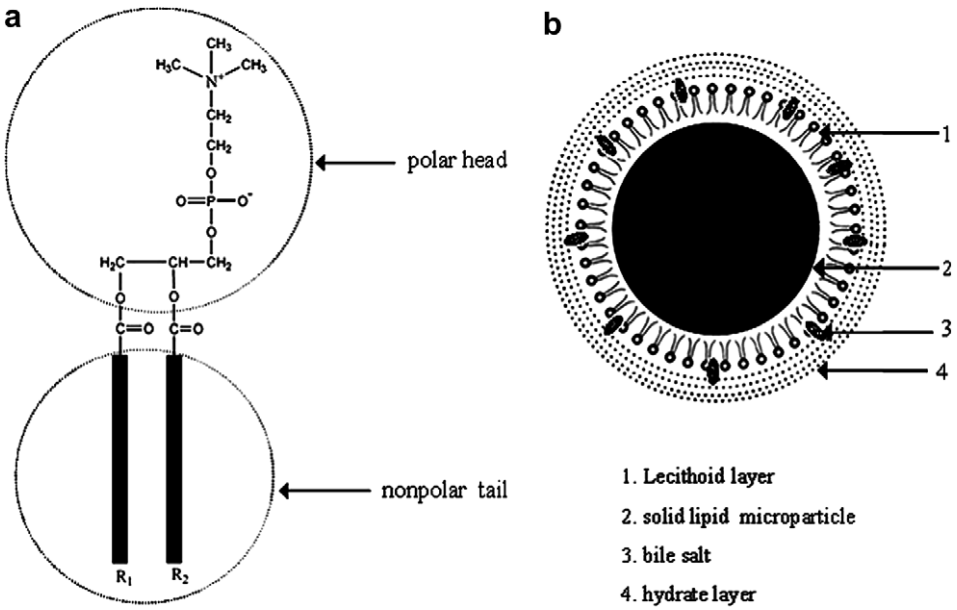

Fig. 4. Schematic structures of lecithin molecule (a) and SLM (b).

for the lateral phase separation causes the leakage of carried drug from the SLM. However, the utilization of these multi-hydroxyl compounds as cryoprotectants successfully compensates for the spacing between lecithin molecules arising from the sublimation of water. This resolves the leakage issue by replacing the hydrate layer with the cryoprotectant while water content is diminishing. Therefore, the moving resistance of lecithin molecule is enhanced, and the carried drug is effectively protected. In general, the more the cryoprotectant is used, the thicker the cryoprotectant layer will become, and finally a higher entrapment efficiency will be obtained.

3.3.2. Effects of the cryoprotectant transition from crystal forms to amorphous states

Trehalose, glucose, mannitol and sucrose are very similar in the chemical characters. However, as it has been discussed above, their efficiencies in protecting SLM during the freeze-drying process are varied. This is largely attributed to the crystallization behavior of the cryoprotectants during the freeze-drying process. The amorphous cryoprotectants in the final freeze-dried products are more effective than crystal ones in protecting SLM [22,23].

On the contrary, these multi-hydroxyl compounds will separate from the system if they crystallize during the freeze-drying process. As a result, such compounds are unable to combine with the lecithin layer to form the compound layer. Therefore, such multi-hydroxyl compounds cannot protect the SLM effectively during the freeze-drying process. Moreover, the crystallization and separation of multi-hydroxyl compounds from the system can lead to the development of crystalloid, which can cause mechanical damage to the microparticles.

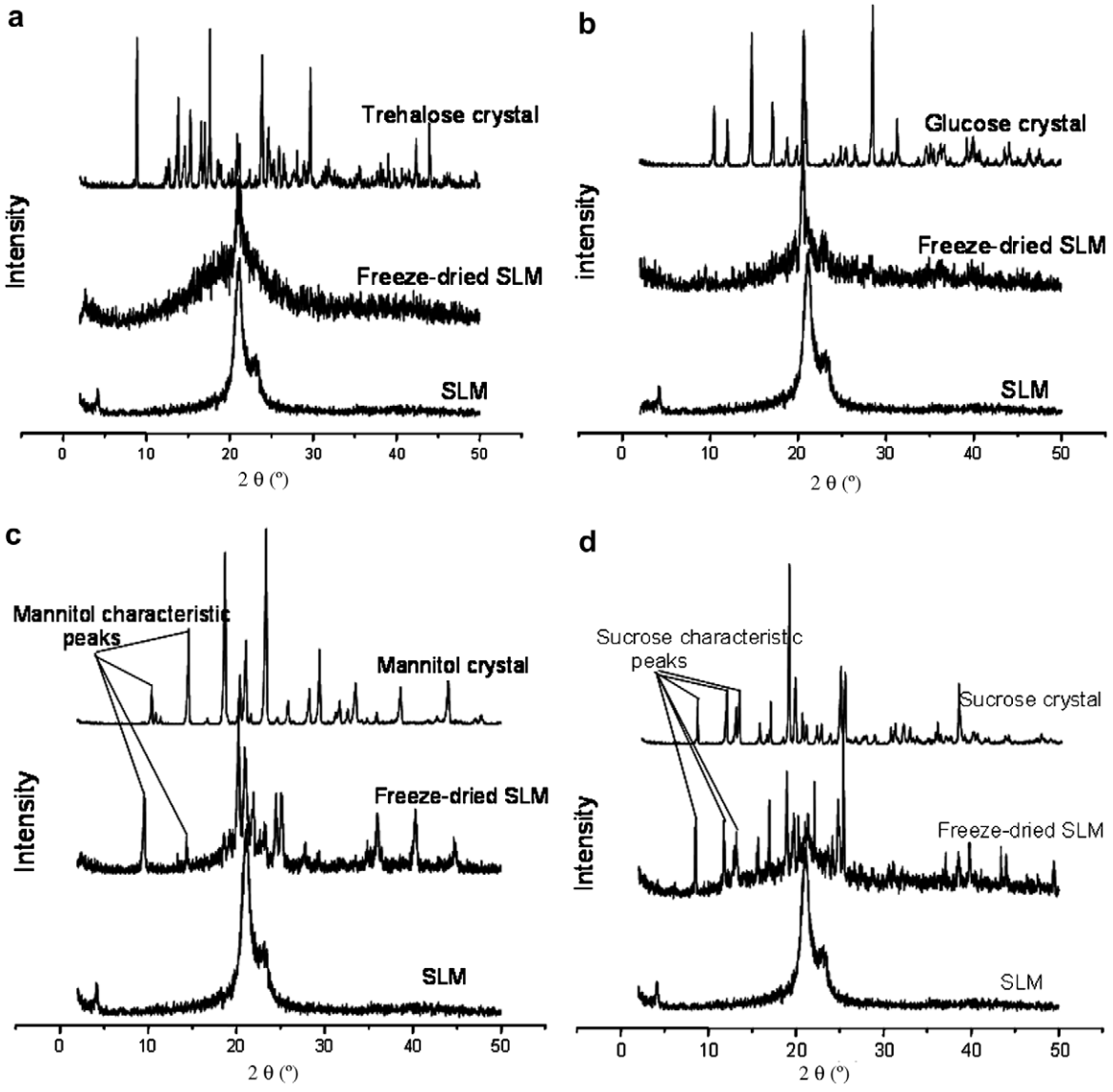

Fig. 5. Powder X-ray diffraction patterns of undried and freeze-dried ibuprofen-loaded SLM with different cryoprotectants (wt); (a) trehalose 15%, (b) glucose 5%, (c) mannitol 10%, and (d) sucrose 10%.

Fig. 5 shows the powder XRD patterns of the final freeze-dried SLM products treated with different cryoprotectants of trehalose, glucose, mannitol, and sucrose. In the XRD patterns of freeze-dried SLM treated with trehalose and glucose, no typical and distinct peaks for trehalose and glucose were observed (Fig. 5a and b). It is suggested that the trehalose and glucose molecules combine so closely with lecithin molecules that the crystallization process is inhibited. Consequently, the ''vitreous state'' is created. Thus, the effective preservation of SLM by trehalose and glucose during the freeze-drying process is achieved. However, several new peaks were observed in the XRD patterns of freeze-dried SLM treated with mannitol and sucrose. Based on the positions of the peaks, the crystallized substance is identified as mannitol and sucrose, respectively (Fig. 5c and d). Interestingly, compared with the peaks of pure mannitol, several peaks positions were shifted. The shifts might be due to the lecithin influences on mannitol and finally on the crystallization process. Especially, the peaks attributed to the sucrose are very intense in the XRD pattern of freeze-dried SLM. It is expected that the crystallization degree of sucrose is so high that the sucrose is well separated from the lecithin layer during the freeze-drying process. Hence, the sucrose is the least efficient cryoprotectant for the SLM.

DSC is a tool to investigate the recrystallization and melting behaviors of crystalline materials like SLM. The DSC curves of the cryprotectants and SLM before and after the freeze-drying are shown in Fig. 6. The melting point, 70.7 °C, of lipid matrix before and after the freeze-drying remained consistent in all cases. But for trehalose, the melting point of trehalose bulk material was 97 °C. However, there were no peaks observed at the original position for freeze-dried SLM (Fig. 6a). For glucose, which is very similar to trehalose, the peak attributed to glucose disappeared after the freeze-drying (Fig. 6b). Figs. 5 and 6 show that trehalose and glucose are amorphous in the SLM after freeze-drying. It is assumed that trehalose and glucose molecules combine closely with the polar head of lecithin molecules by hydrogen bonds, and this interaction is stronger than that between trehalose (or glucose) molecules. So the crystallization process of trehalose and glucose is inhibited [24]. At the same time, trehalose and glucose molecules can form a stable layer on the surface of SLM to prevent SLM aggregation. DSC curve of SLM/trehalose shows an endothermic peak at about 115 °C, and for SLM/glucose, two endothermic peaks at about 100 °C and 113 °C, respectively. It is suggested that these endothermic peaks are independent of the glass transition temperatures (T_g) of trehalose and glucose.

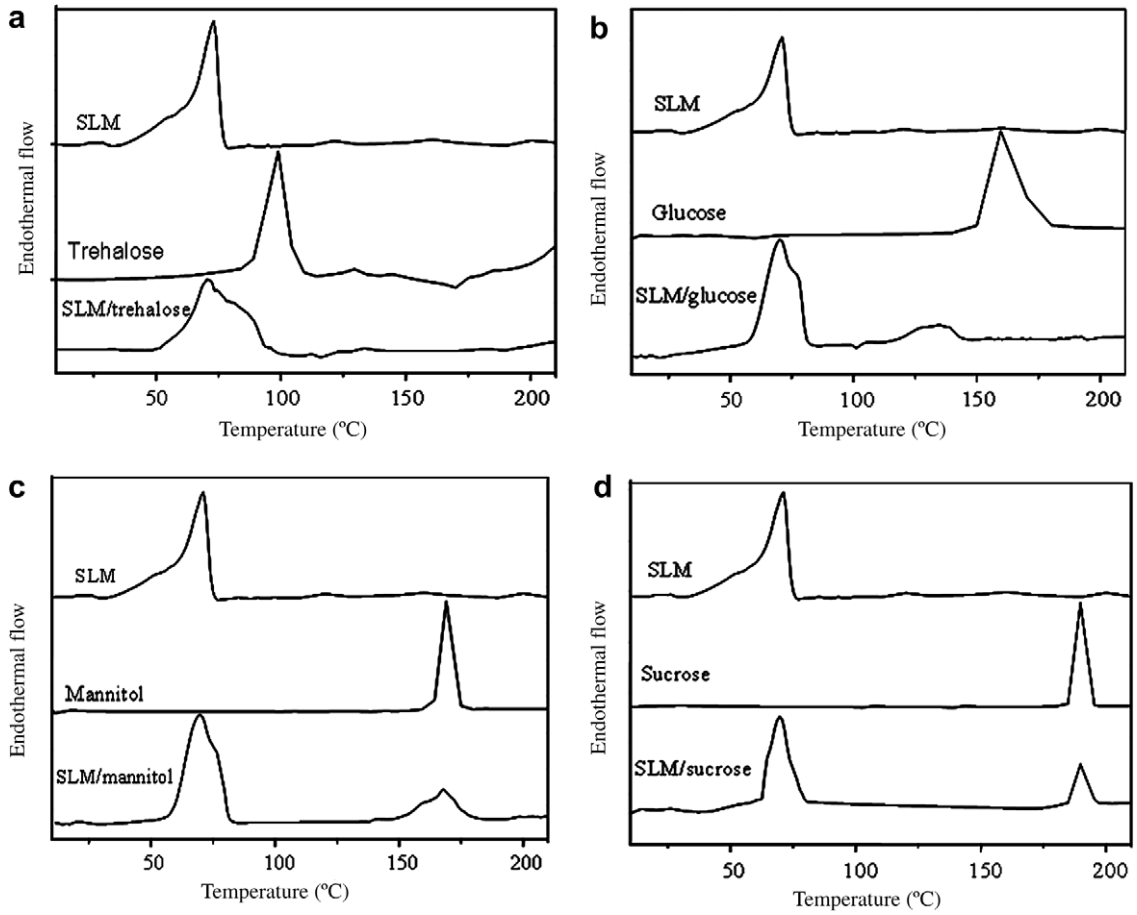

Fig. 6. DSC curves before and after freeze-drying of ibuprofen-loaded SLM with different cryoprotectants (wt); (a) trehalose 15%, (b) glucose 5%, (c) mannitol 10%, and (d) sucrose 10%.

Actually, T_g values of trehalose and glucose as aqueous solution change with the solution concentration. Saez et al. [14] reported that T_g values of trehalose and glucose aqueous solution at the concentration of 30% (w/w) are −29.8 °C and −43.6 °C, respectively. When trehalose and glucose were added to PCL and PLGA nanoparticle formulations, their T_g values were almost the same as the corresponding aqueous solutions. It may suggest that these endothermic peaks observed in thermograms of SLM/trehalose and SLM/glucose were attributed to the formation of a complex between trehalose (or glucose) and lecithin [25]. Although their case is similar to ours, formation of the complex has not been observed in our experiment. Our further related study is on going. Further results will be reported in another paper.

The melting peaks in the DSC curves of SLM/mannitol and SLM/sucrose were clearly observed at 167.9 °C and 189.8 °C, respectively (Fig. 6c and d). This could be attributed to the presence of bulk materials in the freeze-dried SLM. Also, Fig. 5 clearly indicates that mannitol and sucrose crystallized and separated from the system during the freeze-drying process.

The protective properties of trehalose and glucose are better than those of mannitol and sucrose. The protective efficiencies of mannitol and sucrose were impaired due to their crystallization during the freeze-drying process, which correlates well with the findings obtained using XRD. However, some literatures [14,26] reported that the sucrose was an effective cryoprotectant which is contrary to our work. This contradiction may be due to the different compositions and particles' structures of the SLM systems. In the ibuprofen-loaded SLM system, ibuprofen is an acrylic compound. The most ibuprofen molecules were distributed on the surface of the particles [8]. The hydroxyl groups of sucrose might not combine with the lecithin layer, thus causing the separation of the sucrose from the lecithin layer. Moreover the sizes of the particles were at a micron scale that was much bigger than those reported in above-mentioned literatures. This may also have caused the separation of sucrose from the lecithin layer. These unbonded sucrose molecules crystallized more easily during freeze-drying process and caused the worse protective efficiency.

3.3.3. The effects of molecular structures of cryoprotectants

The differences of efficiencies of protecting SLM using different cryoprotectants are controlled by the molecular structure of the cryoprotectants. Fig. 7 shows the molecular structure of the four cryoprotectants: trehalose, glucose, mannitol, and sucrose.

The structures of trehalose and glucose molecule are annular and that of mannitol is catenulate. The spatial resistance becomes larger when the annular molecule combines with the polar lipid of SLM to form the protective layer that is relatively more compact. Therefore, trehalose and glucose are efficient cryoprotectants. However, the catenulate molecules have more of a linear structure and the formed protective layer has less spatial resistance [27].

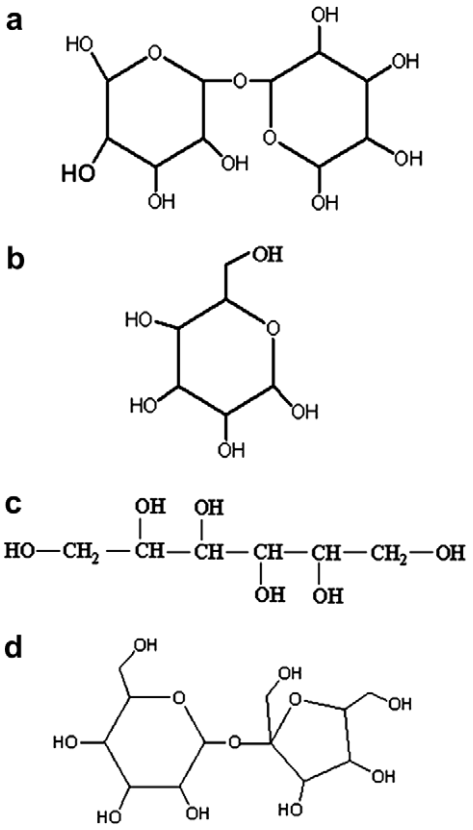

Fig. 7. The molecular structures of cryoprotectants, (a) trehalose, (b) glucose, (c) mannitol, and (d) sucrose.

Thus, their preservation efficiency is lower than that of trehalose or glucose. Although the sucrose molecules have also an annular shape similar to those of trehalose and glucose, they crystallize easily as proved by the XRD and DSC data. As a result of that, sucrose was separated from the lecithin layer during the freeze-drying process. Consequently, it is hard for sucrose molecules to form a stable protective layer, thus the protective efficiency is greatly abated [28].

4. Conclusion

The study shows that cryoprotectants such as trehalose, glucose, mannitol, and sucrose are capable of maintaining the particle sizes and drug entrapment efficiencies of ibuprofen-loaded SLM during the freeze-drying process. The optimal concentrations of the cryoprotectants ensuring the best preservation of the particle shape and best inhibition of drug leakage are 15%, 5%, 10%, and 10% (wt) for trehalose, glucose, mannitol, and sucrose, respectively. The cryoprotectants can replace water molecules with a newly formed strong layer that combines closely with SLM molecules during the sublimation process of the freeze-drying process. The newly formed layer effectively prevents a size expansion of the SLM due to the chemical and structural properties of the cryoprotectants. Also, due to the compact interaction with H-bonding between

the SLM surface and the cryoprotectant molecules, the drug leakage is effectively inhibited. We conclude that trehalose and glucose are effective cryoprotectants and mannitol and sucrose are not very good cryoprotectants for the ibuprofen-loaded SLM as they crystallize during the freeze-drying process.

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