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Research Paper

Influence of Ethanol on Physical State of Freeze-Dried Mannitol

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Purpose. The purpose of this study is to characterize freeze-dried mannitol prepared from an ethanol-containing solution as a function of the ethanol ratio, mannitol concentration, and annealing in the freeze-drying cycle.
Methods. The characteristics of the freeze-dried mannitol were evaluated by X-ray diffractometry (XRD) and differential scanning calorimetry (DSC). The reconstitution time was measured for the freeze-dried solids as well as the residual moisture and ethanol by Karl–Fischer titration and gas chromatography, respectively.
Results. The XRD pattern of 5% (*w*/*v*) mannitol freeze-dried from aqueous solution with no annealing cycle showed all the five characteristic peaks at 13.6° and 17.2° 2θ for the α polymorph, at 14.6° and 23.4° 2θ for the β polymorph and at 9.7°2θ for the δ polymorph. The addition of ethanol to the initial solutions resulted in only a peak at 9.7° 2θ, indicating the presence of only the δ polymorph, regardless of the ethanol ratio in the initial solutions used [10, 20, 30, and 40% (*v*/*v*)]. However, annealing during freeze-drying influenced the XRD pattern; in particular, for the solid prepared from the 10% ethanol solution. Annealing of the 10% ethanol solution promoted the formation of the α polymorph and produced a different peak that might be attributable to another polymorph. In DSC thermograms, an endotherm and a subsequent exotherm were found in the temperature range of 150°C to 160°C, which corresponded to the transition of the δ form to α or β forms. The magnitude of this transition was smaller as the ethanol ratio increased for the solids from ethanol-containing solutions with an annealing cycle. In other words, annealing of the ethanol-containing solutions promoted δ polymorph formation in the lyophiles. In addition, the mannitol concentration affected the polymorphism in freeze-dried solids prepared from aqueous and 10% ethanol solutions. Addition of ethanol in the initial solution, in particular, at a lower ethanol level (10% *v*/*v*), and a higher concentration of mannitol could also promote the generation of lumps in freeze-dried solids during reconstitution, and result in longer reconstitution time. The residual moisture levels were less than 0.5%, and residual ethanol levels were less than 0.1%, irrespective of the formulation used.
Conclusions. The physical state and reconstitution time of the freeze-dried mannitol appears to be a complex function of the ethanol and mannitol concentrations in the initial solution before freeze-drying and of annealing during the freeze-drying process.

KEY WORDS: ethanol; freeze-dry; mannitol; polymorph; reconstitution.

INTRODUCTION

Freeze-drying is commonly used for manufacturing injectable pharmaceutical products. In general, freeze-dried formulations contain multiple components such as the active pharmaceutical ingredient, bulking agents, lyoprotectants, cryoprotectants, and buffers. The characteristics of freezing and freeze-drying, as well as the physical properties of the freeze-dried solid, have been studied extensively (1–4). Such investigations yield data that are useful in determining the most desirable formulations and freeze-drying conditions.

In freeze-dried formulations, mannitol is one of the substances most widely employed as a bulking agent. Mannitol has a strong tendency to crystallize during the freeze-drying process, which can result in relatively high allowable product temperature, thus achieving a faster drying rate and a more efficient freeze-drying cycle. However, after freeze-drying, mannitol hydrate and/or amorphous mannitol, which can potentially release water molecules due to its crystallization or dehydration during storage, can coexist with crystalline mannitol. This can affect the stability as well as other qualities of the freeze dried dosage form. The physical state and polymorphism of freeze-dried mannitol have also been studied by many scientists (5–17). Haikala *et al.* (6) investigated the effect of a surface-active agent, polysorbate 80, on the crystalline properties of freeze-dried mannitol and reported that polysorbate 80 promotes formation of the δ

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polymorph of anhydrous mannitol. Kim *et al.* (7) concluded that the physical form of mannitol was influenced by the mannitol concentration in the original aqueous solution, the freezing rate, and the presence of a noncrystallizing cosolute such as sucrose. In addition, Liao *et al.* (12) examined solid mannitol by XRD and DSC. They showed that the presence or absence of a protein and the processing conditions influence the physical form of mannitol in the final lyophile.

Organic solvents are occasionally used to dissolve and/or stabilize active pharmaceutical ingredients. In most cases, cosolvent systems, which consist of water and an organic solvent in an appropriate ratio, are chosen for use in parenteral formulations (18,19). Telang *et al.* (20) studied the crystallization of cephalothin sodium, which does not crystallize easily in frozen aqueous solutions, using organic solvents. Tertiary butyl alcohol (TBA), ethanol, and isopropyl alcohol (IPA) were studied using DSC and XRD experiments, and the TBA-water cosolvent system was found to be a suitable medium with respect to crystallization of cephalothin sodium, the amount of residual solvent in the lyophilized powder, and the appearance of the freeze-dried cake. Wittaya-Areekul *et al.* (21) investigated residual TBA in the lyophile of sucrose and glycine as a function of the physical state of the solute, initial TBA concentration, freezing rate, thickness of the freeze-dried cake, and secondary drying conditions, and concluded that the crystallization of the solute was the most important factor in minimizing the residual TBA. Ethanol is a typical organic solvent that is easily miscible with water. In the author's experience, ethanol enhanced the solubility and reduced the rate of hydrolysis in aqueous solutions for a few compounds (unpublished data). In addition, ethanol has a reasonably low safety risk (22) even though a small amount remains in the freeze-dried powder as a residual solvent, as well as relatively low cost for commercial manufacture. Therefore, the inclusion of ethanol as a solubilizing and/or stabilizing agent in the formulations is worthwhile for some pharmaceutical products. However, little information has been published regarding the use of ethanol as a co-solvent for parenteral freeze-dried formulations. We have been studying the formulation and freeze-drying process in which mannitol is added as a bulking agent and ethanol for the enhancement of API solubility and stability. In this study, the characteristics of the freeze-dried mannitol prepared from ethanol-containing solutions are reported.

MATERIALS AND METHODS

Materials

D-Mannitol was obtained from Kyowa Hakko Kogyo Co., Ltd. (Japan) and was used as received. The mannitol used in this study met Japanese Pharmacopoeial (JP) requirements and the purity was 99.5% in the vendor's analysis. The anhydrous ethanol used in this study was USP grade (Aaper Alcohol and Chemical Co., Shelbyville, KY). Water was purified by ion exchange, followed by distillation. For freeze-drying, 25-mL glass vials, 30 mm diameter (provided by Namicos, Japan), and rubber stoppers (provided by Daikyo, Japan) were used. All the vials and stoppers were in compliance with JP. Vials and stoppers were also washed with purified water and dried under ambient conditions prior to use.

Methods

Preparation

Mannitol was dissolved in water and ethanol was added until the desired ethanol concentration [% (*v*/*v*)] was obtained. The total volume was adjusted by adding water, yielding the final mannitol concentration. Unless otherwise stated, the concentration of mannitol in this study was 5% (*w*/*v*). All of the solutions were filtered with polyvinylidene fluoride (PVDF) membranes (Millex®-GV, 0.22 μm, Durapore membranes from Millipore Corporation, Bedford, MA) prior to being filled into the vials and freeze-dried.

Freeze-Drying

Freeze-drying was carried out using a laboratory freeze-dryer (Lyostar Model MNL-055-A, SP Industries, Stone Ridge, NY). Five mL of solution was filled into each vial, resulting in a freeze-dried powder approximately one cm deep. The filled vials were partially stoppered and placed on the shelf of the freeze-dryer. The shelf was cooled from ambient temperature to −50°C at a rate of 0.5°C/min and maintained at −50°C for 30 min. For cycles with an annealing step, 60 min of annealing was inserted into the freezing process at −20°C for aqueous solutions and at −30°C for ethanol-containing solutions, referring to our previous study (23). In addition, two methods of primary drying were carried out, one for the aqueous solutions and one for the ethanol-containing solutions. For the aqueous solutions, the shelf temperature was maintained at −10°C for 35 h during the primary drying process. The primary drying process for the ethanol-containing solutions, however, involved two steps: in the first step, the shelf temperature was maintained at −50°C for 10 h to evaporate the unfrozen ethanol. The low shelf temperature was used to prevent sudden boiling of ethanol. In the second step, the shelf temperature was raised to −10°C and maintained at −10°C for 20 h. The chamber pressure was maintained at 10 Pa throughout primary drying in all the experiments. Finally, the secondary drying process was performed at 30°C for 10 h.

Differential Scanning Calorimetry (DSC)

The DSC measurements were carried out using a calorimeter from TA Instruments (model 2920, Newcastle, DE). The calibration was carried out using the melting point (156.6°C) and the enthalpy of indium (28.71 J/g). Nitrogen gas was used for purging at a rate of 25 mL/min. Five to 10 mg of freeze-dried mannitol was placed in an aluminum sample pan, which was then covered by a lid with a pin hole. The powder samples were heated from room temperature to 200°C at 10°C/min. The samples were stored under ambient condition prior to the DSC measurements.

X-Ray Diffractometry (XRD)

LabX XRD-6000 (Shimadzu, Japan) was used to record the X-ray diffraction patterns of the freeze-dried powders. The CuKα radiation source was operated at a voltage of 40 kV and a current of 40 mA. The freeze-dried cake was

gently ground in a mortar and placed in an aluminum sample holder. The samples were scanned from 6° to 50° 2θ at a rate of 4°/min. The samples were stored under ambient condition prior to the XRD measurements.

Thermal Gravimetric Analysis (TGA)

TGA was carried out with a TGA2050 thermogravimetric analyzer (TA Instruments, Newcastle, DE). Nitrogen was used for purging at a rate of 60 mL/min. Five to ten mg of the freeze-dried powder was placed in an aluminum pan, which was then covered by a lid with a pinhole. The powder samples were heated from room temperature to 200°C at 5°C/min, and the reduction in the weight of the powder was monitored.

Karl–Fischer Titration

Residual moisture content in freeze-dried mannitol was measured by volumetric Karl–Fischer titration, using Moisturemeter CA-100 (Mitsubishi Chemical Corporation, Japan). A mixture of Aquamicron Solvent GEX and Aquamicron Solvent FM (50/50% *v*/*v*) and Aquamicron Titrant (SS-Z 3 mg) were used for the titration. Both the solvent and the titrant were provided by Mitsubishi Chemical Corporation (Japan). The Karl–Fischer system was calibrated with three injections of purified water (10 μL for each) before sample analysis. Amounts of 100–120 mg of freeze-dried mannitol were accurately weighed and transferred to the titration vessel for measurement.

Gas Chromatography (GC)

Residual ethanol content in freeze-dried mannitol was measured by GC, using GC-2010 (Shimadzu, Japan) with flame ionization detection. The column used in this study was a DB-WAX Fused Silica Capillary Column, 30 m, 0.25 mm ID, 0.5-μm film (lot. no. US3398443H, Agilent Technologies, Santa Clara, CA). The following temperature program was used in all injections: initial temperature of 40°C, hold for 3 min., 10°C/min ramp to 200°C and hold for 10 min. Quantities of 180–200 mg of the freeze-dried solids were accurately weighed and dissolved in 5 mL of purified water. The sample solutions were placed on the heating module, TurboMatrix 40, (Perkin Elmer Japan) and heated at 100°C for 60 min. to obtain vapor for injection. The injected volumes were controlled by time (0.08 min.). The injector and detector temperatures were maintained at 170°C and 250°C, respectively. The detector make-up flow of helium gas was maintained at 40 mL/min, the hydrogen flow at 40 mL/min., and the air flow at 400 mL/min. Helium was also used as a carrier gas and flowed at 3 mL/min. Data acquisition was conducted by Shimadzu GC solution. Standard solutions of ethanol in purified water were used for calibration.

Reconstitution Time

Five mL of water was added along the inner wall of the vial including freeze-dried powder, and the vial was gently swirled. The time from the start of the agitation to the complete dissolution of the freeze-dried powder was measured and recorded as the reconstitution time.

RESULTS AND DISCUSSION

Freeze Dry

The freeze dry process was monitored by product temperature and chamber pressure. Fig. 1 shows the trends of the product temperature and the chamber pressure in the freeze drying of 5% (*w*/*v*) mannitol/10% (*v*/*v*) ethanol solution as a representative freeze-drying chart for ethanol-containing solutions. The first 10 h in the primary drying was a process aimed for evaporation of ethanol. In this step, the chamber pressure was below the desired pressure (10 Pa) and decreased as the drying progressed. This observation suggested that evaporation of ethanol proceeded gradually and little sublimation of ice occurred due to the low temperature. Then as the shelf temperature was increased to −10°C, the ice sublimation started and the chamber pressure went up to the desired value. In addition, the product temperature increased and the chamber pressure decreased at 20–25 h. Both responses indicated the end of primary drying and the transition to secondary drying. Other ratios of ethanol solutions had similar trends of the product temperature and the chamber pressure during the freeze-drying process regardless of whether an annealing step was used.

The appearance of the freeze-dried solids prepared from 5% mannitol solution is shown in Fig. 2. Regardless of annealing, the solids prepared from aqueous solutions were a cake, while the solids from ethanol-containing solutions were particles. In addition, the particle size of the powders obtained from the ethanol-containing solutions decreased as the ethanol ratio in the initial solution increased. Especially, the particle size of 10% ethanol sample was larger than other samples as indicated by the observation of powder volumes (Fig. 2).

Effect of Ethanol on Freeze-Dried Mannitol

The characteristics of the freeze-dried mannitol prepared from aqueous solution and from ethanol-containing solutions were investigated by XRD and DSC. Fig. 3 shows the powder XRD patterns of the freeze-dried mannitol prepared from a 5% solution with a non-annealing lyophilization cycle. XRD analysis of mannitol from the ethanol-containing solutions

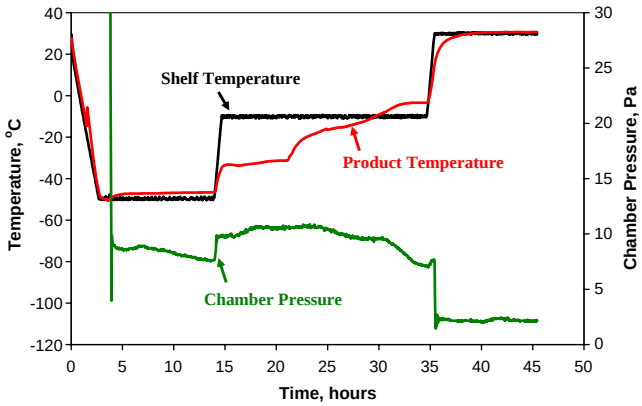

Fig. 1. Freeze-drying chart of 5% (*w*/*v*) mannitol/10% (*v*/*v*) ethanol solution with no annealing cycle.

Non-annealing Cycle

(A) (B) (C) (D) (E)

Annealing Cycle

(F) (G) (H) (I) (J)

Fig. 2. Freeze-dried solids prepared from 5% (*w/v*) mannitol in aqueous and ethanol-containing solutions with (*upper*) and without (*lower*) annealing cycles: *A* Aqueous, *B* 10% EtOH, *C* 20% EtOH, *D* 30% EtOH, *E* 40% EtOH by non-annealing cycle, and *F* Aqueous, *G* 10% EtOH, *H* 20% EtOH, *I* 30% EtOH, *J* 40% EtOH by annealing cycle.

resulted in XRD patterns that were different than those prepared from the aqueous solution. Irrespective of differences in the ethanol ratios in the co-solvents, the powders prepared from ethanol-containing solutions all yielded essentially the same XRD patterns which had a characteristic peak at 9.7° 2θ and no peak in the range of 10 to 18° 2θ.

Previous investigations of mannitol polymorphs (7–9, 24–27) were referred to in order to identify these specific peaks. Kett *et al.* (24) summarized the investigations in their report, and concluded that the α, β and δ forms were present as polymorphs of anhydrous mannitol. The reference X-ray diffractgrams of α, β, and δ mannitol are illustrated in Fig. 4 which has been published by Kim *et al.* (7). Cannon and Trappler (9) also used the International Center for Diffraction Data (ICDD) standards prescribed for the α, β, and δ forms to identify the polymorphs of the freeze-dried mannitol in their study. The mannitol polymorphs obtained in the experiments reported here were identified using the same representative peaks which Cannon and Trappler used (shown in Fig. 3 as three kinds of the symbol). The α form was identified using the peaks at 13.6° and 17.2° 2θ. The peaks at 14.6° and 23.4° 2θ were used to identify the β form. The δ form was identified using a peak at 9.7° 2θ. As reported by Yu *et al.* (8), one of the characteristic peaks for mannitol hydrate is present at 9.6° 2θ. This peak is very close to the peak for the δ form identification at 9.7° 2θ, and these two peaks are not easily distinguishable. Yu *et al.* confirmed that

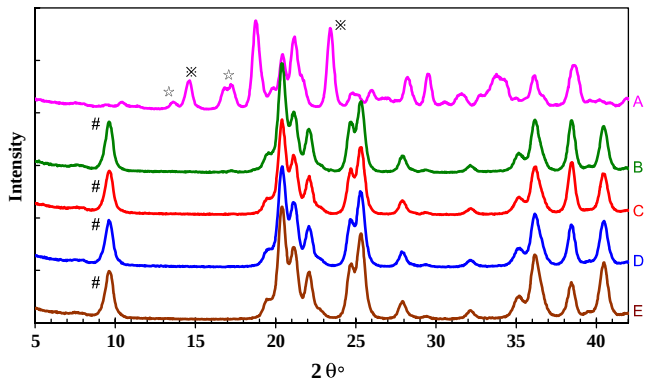

Fig. 3. Powder XRD patterns of freeze-dried mannitol prepared with no annealing cycle from ethanol-containing solutions of 5% (*w/v*) mannitol: *A* 0% (aqueous), *B* 10%, *C* 20%, *D* 30%, and *E* 40% EtOH. The symbols, ☆, ※ and #, are characteristic peaks of α, β and δ mannitol, respectively.

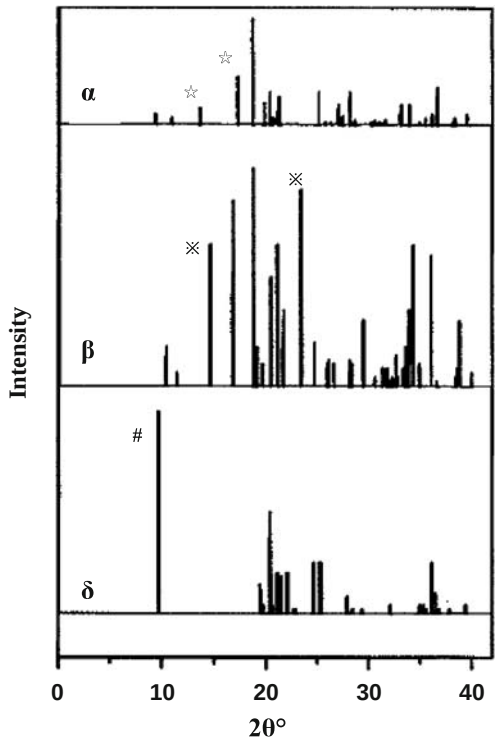

Fig. 4. Reference X-ray diffractograms of mannitol. The symbols, ☆, ※ and #, are characteristic peaks of α, β and δ mannitol, respectively, which were used for identification in this study. These diffractograms has been published by Kim *et al.* (7).

water molecules were present in the freeze-dried mannitol on the basis of differential thermal analysis (DTA), TGA, and DSC experiments, and they concluded that the peak at 9.6° 2θ could be assigned to mannitol hydrate. A 6% weight loss was indicated at around 50°C in the TGA experiment conducted by Yu *et al*. However, the TGA measurements in this study resulted in no appreciable weight loss (less than 0.2%) across the temperature range of ambient to 200°C shown in Table I. Accordingly, the peak observed at 9.7° 2θ in our study was interpreted as indicative of the δ form. The long primary and secondary drying processes and the higher shelf temperature in our study would have led to a drier powder, and it is probable that little mannitol hydrate was present in the solids.

The XRD patterns in Fig. 3 reveal that only one of the five representative peaks (that peak observed at 9.7° 2θ) was present in the freeze-dried solid prepared from the ethanol-containing solutions, while the peaks at 13.6°, 14.6°, 17.2°, and 23.4° 2θ are more pronounced for the powder prepared from the aqueous solution. Therefore, three polymorphs (α, β and, δ) of mannitol were formed (predominantly the α and β forms) when the aqueous solution was lyophilized, while only the δ form was formed by the freeze-drying of the ethanol-containing solutions.

Furthermore, the physical state of freeze-dried mannitol prepared from 5% (*w*/*v*) solutions by the no annealing lyophilization cycle was characterized by DSC. The DSC thermograms were obtained in the temperature range from ambient to 200°C. The upper panel in Fig. 5 illustrates the DSC thermogram of the freeze-dried powder prepared from 10% ethanol solution. A large endotherm was observed at approximately 168°C and small thermal events appeared from 152°C to 158°C in the thermogram. There were no thermal events below 150°C or above 180°C. The smaller thermal events in the range of 152°C to 158°C are not clear in Fig. 5. Therefore, the expanded thermograms around 155°C is shown in Fig. 6. The thermograms of the powders prepared from ethanol-containing solutions appear to consist of an endotherm followed by an exotherm. Table II shows the peak temperatures and the enthalpies of the endotherms and exotherms. The enthalpy of the endotherm and exotherm has no clear trend depending on the ethanol ratio in the original solution. In addition, the identification of the thermal events seen in Figs. 5 and 6 can be discussed referring to the report by Burger *et al.* (27) These investigators obtained similar DSC thermograms to those reported in this study and investigated their characteristics including the melting temperatures. The melting points of α, β and δ mannitol (Burger

Table I. Effect of Ethanol Ratio and Annealing on Polymorphic Forms, Weight Loss in TGA, Residual Moisture and Ethanol and Reconstitution Time for 5% (*w*/*v*) Mannitol

EtOH (%, *v*/*v*)	Annealing	Polymorphs	Weight loss (TGA; %, *w*/*w*)	Water content (%, *w*/*w*)	Residual EtOH (ppm)	Reconstitutitime (s)
0	No	α, β (major) δ (minor)	0.15	0.47	Not measured	12.5
	Annealing	α, β (major) δ (minor)	0.15	0.48	Not measured	14.0
10	No	δ	0.05	0.35	574	8.8
	Annealing	δ (major) α (minor) unknown	<0.01	0.35	752	34.8
20	No	δ	0.05	0.37	692	8.8
	Annealing	δ	<0.01	0.35	858	7.2
30	No	δ	0.03	0.36	619	11.5
	Annealing	δ	<0.01	0.37	830	10.3
40	No	δ	0.07	0.30	358	15.5
	Annealing	δ	0.05	0.30	601	11.0

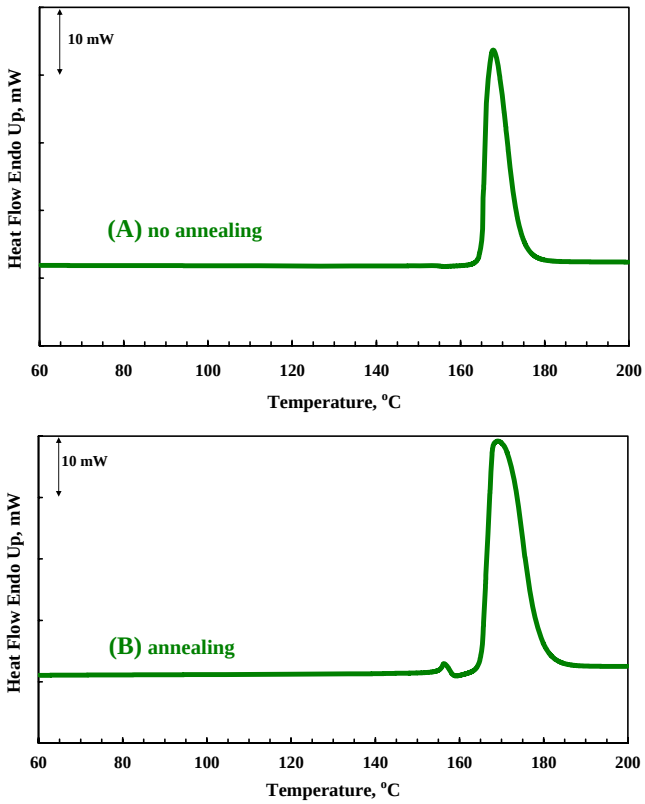

Fig. 5. DSC thermograms of freeze-dried mannitol prepared from 10% (*v*/*v*) ethanol-containing solution of 5% (*w*/*v*) mannitol with: **A** no annealing cycle, **B** annealing cycle (for 60 min at −30°C).

et al. noted them modification II, I and III, respectively) were 166°C, 166.5°C and 155°C, respectively. Therefore, the small endotherm and exotherm around 155°C can be interpreted as transformation of mannitol polymorph from δ form to α or β form, and the large endotherm at 168°C corresponds to the melting of α and/or β mannitol. Similar thermograms were obtained for the powders prepared from the other ratios of ethanol-containing solutions. On the other hand, the thermogram of the freeze-dried powder prepared from aqueous solution had only the large endotherm at 168°C and no other thermal events even in the range of 152°C to 158°C. These DSC results are consistent with XRD results shown above that α and β forms were predominant in the powder from aqueous solution, while δ form was predominant in the powder from ethanol-containing solutions.

Effect of Annealing on Mannitol Polymorphs

The effect of annealing on the solid state of mannitol in the freeze-dried powder was also evaluated by XRD and DSC. Fig. 7 illustrates the XRD patterns of the powder obtained from 5% mannitol solution prepared from ethanol-containing solutions with annealing. The XRD patterns of freeze-dried mannitol prepared from 0%, 20%, 30%, and 40% ethanol solutions appear to be almost identical to those of the corresponding XRD patterns prepared without annealing (Fig. 3). However, for the freeze-dried mannitol prepared from the 10% ethanol solution, the XRD pattern showed several different peaks depending on the existence of annealing in the freeze-drying cycle. Two peaks at 13.6° and 17.2° 2θ corresponded to the α form, but one peak at 15.9° 2θ is not assignable to any peaks seen in the standards for the α, β, and δ forms. Moreover, it could not be found in any published papers (6–14,16,17,27) yet. However, it was evident that annealing promoted the slight change of polymorphs only for the 10% ethanol sample.

The DSC thermogram of freeze-dried powder with annealing of 5% (*w*/*v*) mannitol in 10% (*v*/*v*) ethanol-containing solution is shown in the lower panel in Fig. 5. A large endotherm, which is attributed to the melting of α or β mannitol, was observed at approximately 168°C and another endotherm followed by an exotherm, which was characterized by transformation of the δ form to α and/or β, are seen in the range of 155°C to 160°C, while no thermal events occurred below 150 or above 180°C. Such observations are similar to the DSC traces for the powder prepared with no annealing (the upper panel in Fig. 5), but the thermal events at temperatures between 155°C and 160°C are more pronounced relative to the no-annealing sample. Fig. 8 illustrates the expanded DSC thermograms of mannitol freeze-dried with annealing for the powders prepared from 0%, 10%, 20%, 30% and 40% ethanol-containing solutions. The endotherms and the following exotherms became broader as the ethanol ratio in the original solutions was decreased. The enthalpy values are indicated in Table I. From these results, it was concluded that the formation of the δ form was promoted during freeze-drying with annealing, and that the lower the ethanol ratio in the original solution, the more pronounced the effect.

Effect of Mannitol Concentration on Polymorph Distribution

The effect of mannitol concentration on the polymorphs in the freeze-dried powder was also investigated. When 1%, 5%, and 10% mannitol aqueous solutions were freeze-dried without annealing, the XRD patterns for all the freeze-dried solids were almost identical, indicating that the three polymorphs (α, β, and δ) were present (data not shown).

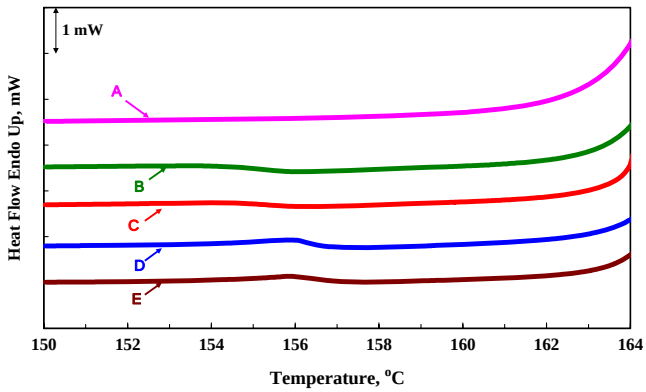

Fig. 6. Expanded DSC thermograms of freeze-dried mannitol prepared with no annealing cycle from ethanol-containing solutions of 5% (*w*/*v*) mannitol: *A* 0% (aqueous), *B* 10%, *C* 20%, *D* 30%, and *E* 40% EtOH.

Table II. Thermal Events in DSC Thermograms for Powders Prepared from 5% (*w*/*v*) Mannitol Solutions

		Smaller endotherm		Exotherm		Larger endotherm	
Annealing	EtOH (%, *v*/*v*)	Peak temp. (°C)	Δ*H* (J/g)	Peak temp. (°C)	Δ*H* (J/g)	Peak temp. (°C)	Δ*H* (J/g)
No	0	n.d.	n.d.	n.d.	n.d.	168.5	301.0
	10	153.6	0.302	156.1	0.305	167.8	300.9
	20	154.2	0.389	156.4	0.029	168.4	299.2
	30	155.9	0.343	157.5	0.027	170.2	295.3
	40	155.9	0.136	157.6	0.136	168.8	302.8
Annealing	0	n.d.	n.d.	n.d.	n.d.	168.7	290.5
	10	156.3	2.631	159.3	1.285	169.1	298.0
	20	156.1	0.950	158.8	0.809	170.0	269.4
	30	155.5	0.662	158.1	0.242	168.0	312.4
	40	155.5	0.277	157.9	0.019	169.6	301.3

However, the XRD peaks at 14.6° and 23.4° 2θ, ascribed to the β form, were higher in intensity for the powders prepared from the 5 and 10% mannitol solutions than for that from the 1% mannitol solution. Kim *et al.* (7) reported that low concentrations of mannitol in solution favored the formation of the δ form, while high concentrations favored the formation of the β form, which is consistent with the results reported here Also, annealing resulted in a different distribution of polymorphs depending on the mannitol concentration, as summarized in Tables I and III. Although the XRD patterns for the powders prepared from the 1 and 5% mannitol aqueous solutions were almost the same with or without annealing, for the powder prepared from the 10% mannitol aqueous solution, the intensity of the peak at 9.7° 2θ, which corresponds to the δ polymorph, increased after annealing, and the peaks indicative of the α and β forms became almost nonexistent. Secondly, for the freeze-dried powders prepared from ethanol-containing solutions (10%, 20%, 30%, and 40%), the XRD patterns were evaluated as a function of the mannitol concentration. The XRD patterns of powders prepared without annealing were distinctive in that only the δ form existed, irrespective of the mannitol concentration used. When an annealing step was introduced into the lyophilization cycle, there was no change in the XRD patterns for the freeze-dried mannitol prepared from the 20%, 30%, and 40% ethanol solutions, regardless of the mannitol concentration used. However, the physical states of mannitol in the powder prepared from 10% ethanol solution with annealing differed depending on mannitol concentration. A concentration of 10% mannitol promoted the formation of the β polymorph, and 5% mannitol yielded the α form as well as an unknown form described earlier; however, the XRD patterns of the powder prepared from the 1% mannitol solution remained unchanged. These results support the conclusion that annealing and mannitol concentration affect which mannitol polymorphs are present in a freeze-dried powder.

Reconstitution Time

The reconstitution time of freeze-dried mannitol was evaluated. The averages of three to five determinations of the reconstitution times are shown in Tables I and III. The difference of the reconstitution times between 1% and 5% mannitol was not significant, but it was evident that 10% mannitol reconstituted more slowly. This tendency was independent of the ethanol ratio in the initial solutions and

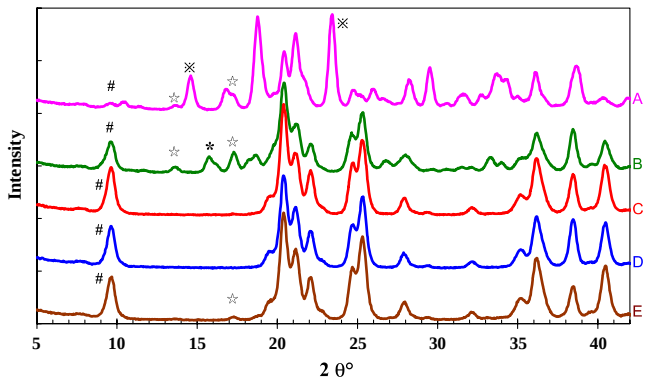

Fig. 7. Powder XRD patterns of freeze-dried mannitol prepared with annealing cycle (for 60 min at −30°C) from ethanol-containing solutions of 5% (*w*/*v*) mannitol: *A* 0% (aqueous), *B* 10%, *C* 20%, *D* 30%, and *E* 40% EtOH. The symbols, ☆, ※ and #, are characteristic peaks of α, β and δ mannitol, respectively. The symbol *asterisk* is a non-identified peak.

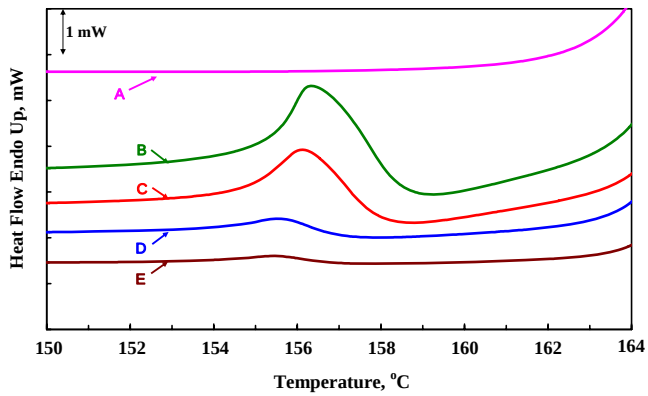

Fig. 8. DSC thermograms of freeze-dried mannitol prepared with annealing cycle (for 60 min at −30°C) from ethanol-containing solutions of 5% (*w*/*v*) mannitol: *A* 0% (aqueous), *B* 10%, *C* 20%, *D* 30%, and *E* 40% EtOH.

Table III. Effect of Ethanol Ratio and Annealing on Polymorphic Forms and Reconstitution Time for 1% and 10% (*w*/*v*) Mannitol

EtOH (%, *v*/*v*)	Mannitol (%, *w*/*v*)	Polymorphs		Reconstitution time (s)	
		No annealing	Annealing	No annealing	Annealing
0	1	α, β (major) δ (minor)	α, β (major) δ (minor)	16.3	13.0
	10	α, β (major) δ (minor)	α, β, δ	28.0	19.0
10	1	δ	δ	12.0	10.5
	10	δ	δ (major) β (minor)	35.8	96.3
20	1	δ	δ	9.3	10.0
	10	δ	δ	32.3	36.4
30	1	δ	δ	10.7	6.0
40	1	δ	δ	11.0	8.0

the use of annealing during the freezing process. An exception was the sample obtained from 10% ethanol with annealing. For this sample, the 5% and 10% mannitol dissolved in 35 and 96 s, respectively. In fact, most of the powders reconstituted quickly, but a few lumps remained for an extended time. Other samples for which reconstitution required more than 30 s were 10% mannitol from 20% ethanol with or without annealing and 10% ethanol with no annealing.

The residual moisture and ethanol content in the freeze-dried solids of 5% mannitol was measured and is summarized in Table I. All the samples contained slight amounts of moisture and ethanol although the moisture levels were a little higher for aqueous samples and the residual ethanol levels were higher for annealing samples. Therefore, these experiments suggested that there was little effect of the residual solvents on the reconstitution time. In addition, Kim *et al.* (7) suggested that the different reconstitution times could be attributed to differences in the specific surface areas of the freeze-dried solids. However, considering the fact that the longer reconstitution time resulted from a small part of the remaining powders, the difference in reconstitution time in this study may not be associated with the difference of surface area of the powders. On the other hand, this study indicated that addition of ethanol in initial solution, in particular at lower level (e.g. 10% *v*/*v*), and higher concentration of mannitol could promote the generation of lumps, and thus result in a longer reconstitution time.

Implications for Pharmaceutical Products

Finally, we discuss the application of ethanol-containing mannitol formulations to pharmaceutical products. As was found in this study, mannitol can exist in various polymorphic forms depending on the ethanol ratio in the original solution, the mannitol concentration, and annealing. Other conditions, including freezing rate, primary and secondary drying parameters, and other components of the formulation may play important roles in determining the physical state of mannitol in the freeze-dried solid. The polymorphs of mannitol might influence the stability of coexisting drug substances and the reconstitution of the dried powder although the extent of the influence, if any, is unclear at present. Furthermore, ethanol-containing solution can cause powder blow-out from the vials during freeze-drying. Such a phenomenon was observed in this study. The powder blow-out can become a significant issue in the commercial manufacturing of pharmaceutical products because it may cause contamination as well as distribute powder containing drug products onto containers that are shipped to medical facilities. If the extents of the powder blow-out are significant in freeze dry of commercial products, the content of the active pharmaceutical ingredient in a vial can reduce and it results in out of specification of final products. We believe that the freezing and freeze-drying characteristics of the ingredients in the formulation need to be understood well and that an appropriate freeze-drying cycle should be established on the basis of the studies to prevent powder blow-out during the freeze-drying.

CONCLUSIONS

The effect of ethanol ratio, mannitol concentration, and annealing on the physical state of freeze-dried mannitol prepared from ethanol-containing solutions was evaluated in this study. In the absence of ethanol, the α, β, and δ polymorphs of mannitol coexisted in the freeze-dried solid, predominantly the α and β forms. Annealing promoted the formation of the δ form from the 10% mannitol solution, while it reduced formation of the δ form from the 5% mannitol solution. In the presence of 20%, 30% and 40% ethanol, both with and without annealing, only the δ form was produced. In the presence of 10% ethanol, annealing resulted in the formation of the α form and an unknown polymorph for powder from the 5% mannitol solution, and promoted the formation of the β polymorph for powder from the 10% mannitol solution. Furthermore, addition of small amounts of ethanol in initial solution (e.g. 10% *v*/*v*) and higher concentration of mannitol promoted the generation of lumps in the freeze-dried solids, and they result in longer reconstitution time, but residual moisture and ethanol level did not affect reconstitution time.

Thus, the physical state and reconstitution of freeze-dried mannitol were influenced by the ethanol and mannitol concentrations in the solutions before freeze-drying, and by the inclusion of annealing in the freeze-drying process. In other words, the environment of the mannitol molecules, for

example, the density of mannitol molecules and the presence of ethanol, may determine which polymorphs are present in the dried solid.

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