Inhibition of Mannitol Crystallization in Frozen Solutions by Sodium Phosphates and Citrates

Ken-ichi IZUTSU,* Chikako YOMOTA, and Nobuo AOYAGI

National Institute of Health Sciences; 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan.
Received November 10, 2006; accepted January 15, 2007; published online January 19, 2007

Effects of co-solutes on the physical property of mannitol and sorbitol in frozen solutions and freeze-dried solids were studied as a model of controlling component crystallinity in pharmaceutical formulations. A frozen mannitol solution (500 mM) showed a eutectic crystallization exotherm at −22.8 °C, whereas sorbitol remained amorphous in the freeze-concentrated fraction in the thermal scan. Various inorganic salts reduced the eutectic mannitol crystallization peak. Trisodium and tripotassium phosphates or citrates prevented the mannitol crystallization at much lower concentrations than other salts. They also raised transition temperatures of the frozen mannitol and sorbitol solutions (T_g': glass transition temperature of maximally freeze-concentrated amorphous phase). Crystallization of some salts (*e.g.*, NaCl) induced crystallization of mannitol at above certain salt concentration ratios. Thermal and near-infrared analyses of cooled-melt amorphous sorbitol solids indicated increased intermolecular hydrogen-bonding in the presence of trisodium phosphate. The sodium phosphates and citrates should prevent crystallization of mannitol in frozen solutions and freeze-dried solids by the intense hydrogen-bonding and reduced molecular mobility in the amorphous phase.

Key words amorphous; crystallization; formulation; freeze-drying; thermal analysis

Active ingredients and excipients in pharmaceutical solid formulations are in the amorphous or crystalline states. Application of amorphous solids is receiving increasing attention because of the unique physical and functional properties (*e.g.*, higher dissolution rate, stabilization of freeze-dried proteins).[1—4] Controlling the component crystallinity through optimizing the compositions and the manufacturing process (freeze-drying, quench cooling of hot-melt liquids) is a relevant method in formulation development because each ingredient and excipient possess different intrinsic propensity for crystallization.[5—7] Freeze-drying with a large amount of "inert" nonionic molecules (*e.g.*, disaccharides, soluble polymers) that are likely to form amorphous solid, is a popular way to obtain the intrinsically crystallizing ingredient in the non-crystalline dispersed state. The method, however, has some limitations in terms of the applicable excipients and the physical properties of the resulting solids.[8] Various inorganic salts also prevent crystallization of other solutes in frozen solutions and freeze-drying processes through mixing and/or complex formation.[9—11]

Mannitol is a popular excipient that tends to crystallize in frozen aqueous solutions.[12] The superior cake appearance and physical stability of the crystalline freeze-dried solids make mannitol a good bulking agent for many parenteral formulations of low-molecular-weight pharmaceutics. The crystallization process of mannitol in frozen solutions, the resulting crystal polymorphs, and the effect of co-solutes on the physical properties have been studied extensively as models to elucidate the component crystallization process in multi-solute systems.[13—19] Co-lyophilization with sucrose prevents crystallization of mannitol during freeze-drying at sucrose/mannitol weight concentration ratios above 2—3.[8,20,21] Some salts (*e.g.*, NaCl) that possess high melt miscibility with mannitol prevent its crystallization in frozen solutions at much lower concentrations than sucrose, whereas the low transition temperatures (T_g': glass transition temperature of the maximally freeze-concentrated amorphous phase) of the frozen solutions make it difficult to freeze-dry without physical collapse.[9,12,22]

Reduction of molecular mobility in the freeze-concentrated phase is another approach to obtain amorphous freeze-dried solids. Some salts (*e.g.*, sodium tetraborate, boric acid) that effectively raise T_g's of frozen polyol solutions (*e.g.*, saccharides, sugar alcohols) by complex formation would prevent spatial rearrangement of the mannitol molecules required for crystallization.[23—25] Recent studies showed that some pH-adjusting excipients (*e.g.*, sodium phosphate buffer) also raise glass transition temperature (T_g) of amorphous freeze-dried saccharides.[26,27] Some phosphate salts also prevent crystallization of mannitol during freeze-drying processes, suggesting contribution of the reduced molecular mobility in the amorphous mixture.[3,22,28] The purpose of this study was to elucidate the effect of phosphate and citrate salts on the physical properties of mannitol and sorbitol in the frozen aqueous solutions and in the dried solids. Different intrinsic tendency of mannitol and its isomer (sorbitol) for crystallization provided information on the mechanisms and requirements to obtain the stable amorphous solids. Controlling the component crystallinity by the widely used excipients should be of practical importance in developing formulations without particular safety concerns.

Experimental

Materials All the proteins and other chemicals employed in this study were of analytical grade and obtained from the following commercial sources: sucrose (Sigma-Aldrich, St. Louis, MO, U.S.A.); trisodium phosphate·12H_2O (Katayama Chemical, Osaka, Japan); disodium hydrogen citrate and sodium dihydrogen citrate (Kanto Chemical, Tokyo, Japan); D(+)-mannitol, D-sorbitol, and other chemicals (Wako Pure Chemical, Osaka).

Freeze-Drying and Preparation of Amorphous Solids Aqueous solutions (250 μl) containing 500 mM mannitol and various concentrations of co-solutes in flat-bottom glass vials (10 mm diameter) were lyophilized using a freeze-drier (Freezevac 1C; Tozai Tsusho, Tokyo). The solutions were frozen by immersion in liquid nitrogen, transferred to the shelf of the freeze-dryer, and lyophilized without a shelf temperature control for 12 h and at 35 °C for 4 h. The solids were applied for the thermal analysis within 48 h of the preparation. Mixed powders containing sorbitol (approx. 200 mg), salt, and

* To whom correspondence should be addressed. e-mail: Izutsu@nihs.go.jp

aliquot of water (50 μl) in crystal cuvettes or glass tubes were heated under vacuum at 160 °C for 20 min using a drying oven (DP23, Yamato Scientific Inc., Tokyo), then cooled at room temperature to prepare amorphous solids for thermal and spectroscopic analyses.

Thermal Analysis Thermal analyses of frozen solutions and freeze-dried solids were performed using a differential scanning calorimeter (DSC-Q10; TA Instruments, New Castle, DE, U.S.A.) with an electric refrigerating system and associated software (Universal Analysis). Indium and cyclohexane were used for the DSC calibration. Aliquots of solutions (10 μl) in aluminum cells were scanned from −70 °C at 5 °C/min after the system had been cooled at −10 °C/min to study the thermal profile of the frozen solutions. The effect of a heat treatment (annealing) on the thermal properties of the frozen solutions was studied after the initial heating scan was paused at −10 °C, and then the samples were maintained at the temperature for 30 min. Thermal data were acquired in the subsequent heating scan from −70 °C at 5 °C/min. The extent of mannitol crystallization exotherm was obtained from the peak area under the baseline of the heat flow. Peaks in the derivative thermograms were assigned to glass transition temperatures of the maximally freeze-concentrated amorphous phases (T'_gs). Thermal analyses of the freeze-dried solids (1.7—2.2 mg) were performed from −30 °C at 10 °C/min. The cooled-melt solids (approx. 5 mg) were scanned from −30 °C at 5 °C/min.

Near-Infrared Spectroscopy Near-infrared analysis was performed using a FT-NIR system (MPA, Bruker Optik GmbH, Germany) with OPUS software. Absorbance of the sample (1-mm light path length) at 4000 to 12000 cm^{-1} range was obtained at room temperature (25 °C) with a 2 cm^{-1} resolution in 128 scans.

Results

Figure 1 shows thermograms of frozen solutions containing an identical concentration (500 mM) of various solutes. Frozen citric acid and sodium citrate solutions showed thermograms typical for amorphous supercooled freeze-concentrated phase, with the baseline shifts (T'_g) at −53.4 °C (citric acid), −33.4 °C (sodium dihydrogen citrate), −38.8 °C (disodium hydrogen citrate), and −42.5 °C (trisodium citrate).[29] The transitions (T'_gs) of frozen sucrose and sorbitol solutions were observed at −33.8 °C and −44.2 °C, respectively. Some other frozen solutions (500 mM H_3PO_4, NaH_2PO_4, K_3PO_4, LiCl) showed a gradual shift of the thermogram baseline that suggested an amorphous freeze-concentrated phase with T'_g below −55 °C. Freezing of aqueous solutions containing di- and trisodium phosphate (Na_2HPO_4, Na_3PO_4) solutions showed endotherm peaks in the cooling process (data not shown) and flat thermograms in the heating scan, indicating that the salt crystallization was completed before the heating scan.[5,30] Some frozen salt solutions showed endotherm peaks that indicated eutectic crystal melting at −19.8 °C (NaCl), −8.9 °C (KCl), and −14.2 °C (RbCl) (data not shown). The peak temperatures were slightly (approx. 2 °C) higher than the values in some literature probably because of the higher scanning rate in this study.[5,6,31]

Figure 2 shows DSC scans of frozen solutions containing 500 mM mannitol (approx. 91.1 mg/ml) and varied concentrations of sodium hydrogen phosphates (Na_2HPO_4, NaH_2PO_4, Na_3PO_4) in the first heating scan from −70 °C, and in the second heating scan after a heat treatment (annealing) at −10 °C for 30 min. The frozen mannitol solution showed a large eutectic crystallization exotherm peak at −22.8 °C in the first scan. Several smaller thermal events, including two possible T'_gs (T'_{g1}: −37.5 °C, T'_{g2}: −29.0 °C) and a small endotherm (−24.3 °C), were also observed prior to the crystallization exotherm.[17,21,28] A small exotherm peak that suggests partial crystallization was observed in the cooling process of some frozen mannitol solutions (data not shown). The second scan of the frozen mannitol solution after the

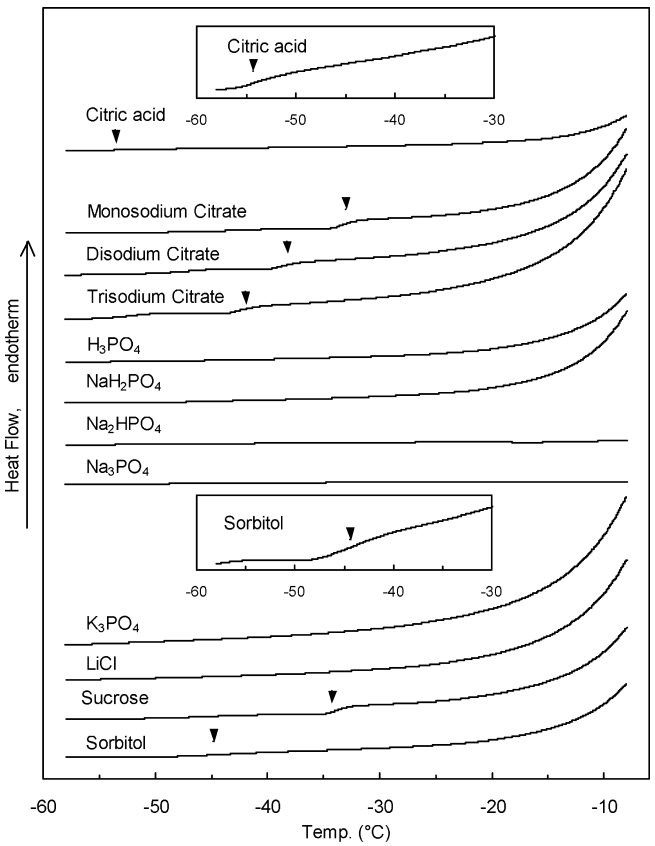

Fig. 1. Thermal Profiles of Frozen Single-Solute Aqueous Solutions (500 mM, 10 μl) Scanned from −70 °C at 5 °C/min

Glass transition temperatures of the maximally freeze-concentrated amorphous phases (T'_gs) are marked with reversed triangles (▼).

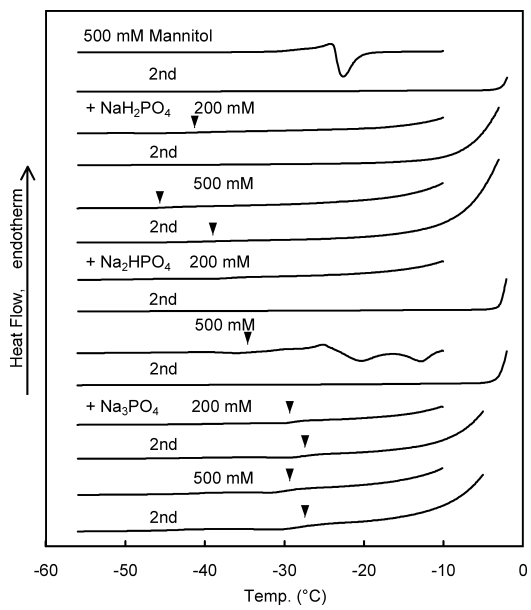

Fig. 2. Thermal Profiles of Frozen Solutions Containing Mannitol (500 mM) and Varied Concentrations of Sodium Phosphates in the First Scan from −70 °C at 5 °C/min (Upper) and the Second Scan after a Heat Treatment at −10 °C for 30 min

The glass transitions (T'_gs) are marked with reversed triangles (▼).

heat treatment resulted in a flat thermogram up to the ice-melting temperature, indicating the crystallized mannitol in the frozen solution.

The phosphate salts showed varied effects on the thermal property of the frozen mannitol solution. The mannitol crystallization exotherm peak disappeared in the presence of NaH_2PO_4 (200, 500 mM), Na_2HPO_4 (200 mM) or Na_3PO_4 (200, 500 mM), presenting a thermal transition (T'_g) that indicated the amorphous freeze-concentrated phase surrounding ice crystals. The frozen solution containing 500 mM mannitol and 500 mM Na_2HPO_4 showed two exotherm peaks that suggest crystallization of mannitol (−20.3 °C) and Na_2HPO_4 (−12.8 °C). The second scan of frozen solutions containing mannitol and NaH_2PO_4 (500 mM) or Na_3PO_4 (200, 500 mM) also showed the T'_g transitions. A slight shift of the transition temperature (T'_g) in the second scan suggested retention of the amorphous freeze-concentrated phase and some re-ordering (*e.g.*, further ice crystal growth) of the frozen solution during the heat treatment.[17] The second scan of frozen solutions containing mannitol and Na_2HPO_4 (200, 500 mM) showed flat thermograms up to the ice-melting temperature, indicating the crystallized mannitol and the salt.[18]

Figures 3 and 4 show effects of various co-solutes (*e.g.*, phosphate, citrate, chloride salts) on the mannitol crystallization exotherm size and transition temperatures (T'_gs) of the frozen solutions. The crystallization exotherm of the frozen mannitol solution (500 mM) was 14.1±1.2 J/g (n=3). Most of the solutes, except H_3PO_4, apparently reduced the mannitol crystallization peak concomitantly with the upward shift of the peak temperature. Alkali-metal chloride salts with smaller cations (LiCl, NaCl) were more effective at reducing the mannitol crystallization peak than those with larger cations (KCl, RbCl). The mannitol crystallization peak also disappeared in the presence of 200 mM sucrose or 150 mM CH_3COONa, indicating the prominent effect of the salts, especially compared in their weight concentrations. Addition of some solutes slightly increased the exotherm at lower concentrations (<100 mM), probably because they alter thermal transitions prior to the crystallization peak. Sodium and potassium phosphates showed varied ability to reduce the mannitol crystallization peak depending on the ionic valencies. The mannitol crystallization peak disappeared at different phosphate salt concentrations (Na_3PO_4, K_3PO_4<Na_2HPO_4, K_2HPO_4<NaH_2PO_4<KH_2PO_4). Sodium phosphate buffers containing both NaH_2PO_4 and Na_2HPO_4 at different concentration ratios (3 : 1, 1 : 1, and 1 : 3) showed the crystallization-preventing effects intermediate between those of the individual salt solutions (data not shown). The mannitol crystallization peak also disappeared at different sodium citrate and citric acid concentrations (trisodium citrate<disodium citrate<monosodium citrate<citric acid). The pronounced effect of tri- and divalent salts suggests the significance of ion concentrations or alkaline pH on the ability of co-solutes to prevent the mannitol crystallization. The mannitol crystallization peak re-appeared upon further addition of some salts (300—500 mM Na_2HPO_4, 500 mM RbCl,

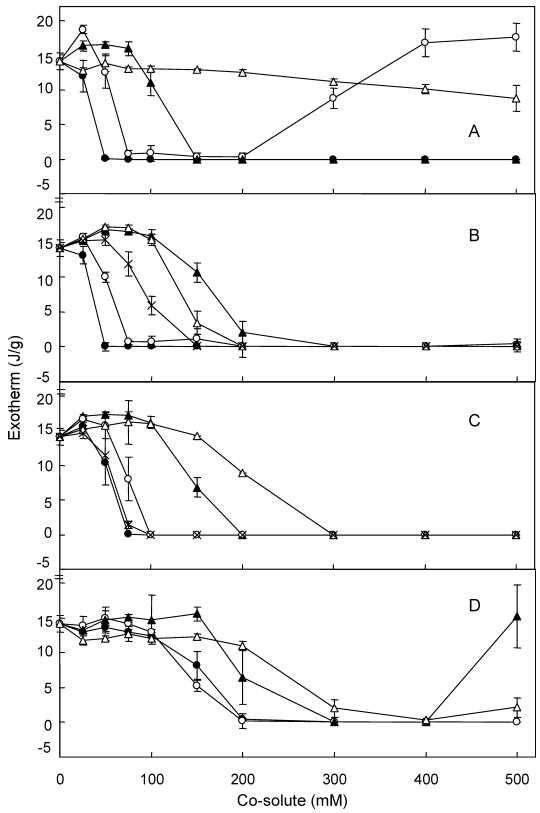

Fig. 3. Effects of Co-solutes on the Mannitol Crystallization Exotherm in Frozen Aqueous Solutions (10 μl) Scanned from −70 °C at 5 °C/min

The symbols denote frozen solutions containing (A) ●: Na_3PO_4, ○: Na_2HPO_4, ▲: NaH_2PO_4, △: H_3PO_4, (B) ●: K_3PO_4, ○: K_2HPO_4, ▲: KH_2PO_4, △: sucrose, ×: CH_3COONa, (C) ●: trisodium citrate, ○: disodium citrate, ▲: monosodium citrate, △: citric acid, ×: tripotassium citrate, (D) ●: LiCl, ○: NaCl, ▲: KCl, △: RbCl (mean value±S.D., n=3).

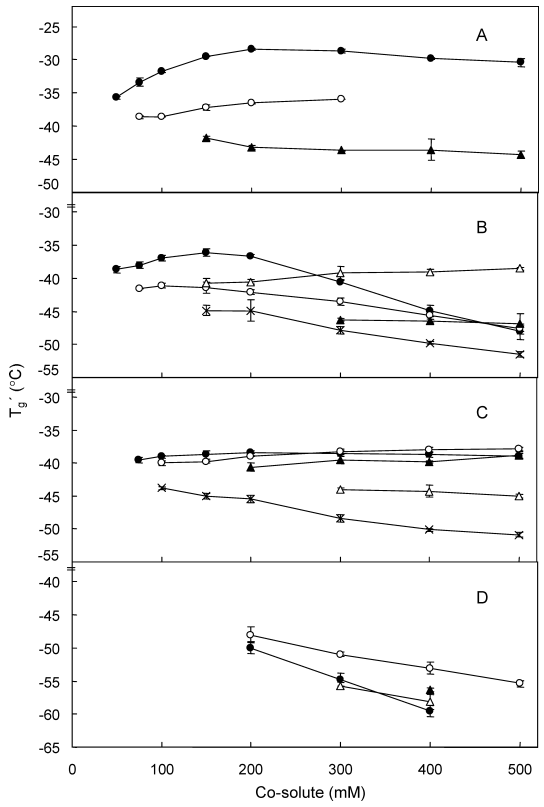

Fig. 4. Thermal Transition Temperatures (T'_g: Glass Transition of the Maximally Freeze-Concentrated Amorphous Phases) of the Frozen Solutions (10 μl) Containing Mannitol (500 mM) and Various Concentrations of Co-solutes Scanned from −70 °C at 5 °C/min

Symbols denote the co-solutes shown in Fig. 3 (mean value±S.D., n=3).

500 mM KCl). Crystallization of these salts in the single-solute frozen solutions (Fig. 1) suggested that the salt crystallization at high salt/mannitol ratio induced the mannitol crystallization in the remaining phase.

Frozen solutions containing the amorphous state mannitol and co-solute combinations showed apparent T'_g transition (Fig. 4). Extrapolating the transition temperatures of these mixture solutions suggested that T'_g of the frozen single-solute mannitol solution occurred at approximately −40 °C, which should correspond to the reported lower-temperature transition (T'_{g1}).[21,28] Addition of some salts (*e.g.*, NaH_2PO_4, CH_3COONa, LiCl, NaCl, KCl, RbCl) lowered the transition temperature linearly depending on their concentrations. Sucrose slightly shifted the transition toward its intrinsic T'_g (−33.8 °C, Fig. 1).[21] These findings suggest simple mixing of the solutes in the freeze-concentrated phase, which transition temperature depends on the T'_gs of the individual components and their concentration ratios. In contrast, some phosphate and citrate salts non-linearly raised the transition temperature. Frozen solutions containing mannitol and trisodium or tripotassium salts (Na_3PO_4, K_3PO_4, trisodium citrate) showed the highest transition temperatures at certain (150—300 mM) co-solute concentrations. The transition temperatures above those of the individual solutes (*e.g.*, trisodium citrate: −42.5 °C) suggested reduced molecular mobility in the freeze-concentrated phase.

Sorbitol remained amorphous in the single-solute (500 mM) frozen solution, presenting a T'_g transition at −44.2 °C (Fig. 1). Effect of the salts on the transition temperature of the frozen sorbitol solution (T'_g, Fig. 5) showed similar trends with the mannitol-salt systems. The non-linear upward shift of T'_gs suggested some interaction between the Na_3PO_4 and the sugar alcohols, or altered environment in the freeze-concentrated phase. A salt crystallization exotherm peak was observed in the solutions containing 500 mM sorbitol and 300—500 mM Na_2HPO_4. Addition of 500 mM Na_2HPO_4 resulted in low T'_g (−45.0 °C) that suggested transition of phase-separated sorbitol fraction.

Effects of sodium phosphates and citrates on the physical properties of freeze-dried mannitol was studied (Fig. 6). Freeze-drying of mannitol resulted in α or β polymorph crystal that showed a large crystal melting endotherm at approximately 170 °C.[18,32] Co-lyophilization with the phosphate and citrate salts resulted in the small crystal melting peaks that suggested lower crystallinity or different polymorph (*e.g.*, δ-form) in the solid.[9,18,32] Some solids showed glass transitions (35—55 °C), exotherm peaks of putative mannitol crystallization (60—80 °C), and small mannitol crystal melting peaks (150—170 °C). Transition temperatures (T_gs) of these co-lyophilized solids were higher than the reported T_g of amorphous mannitol (approx. 13 °C).[33] Freeze-dried solids containing mannitol and trisodium phosphate or citrate showed T_gs (52.9, 43.7 °C) higher than those of the corresponding disodium salts (44.5, 40.0 °C). Co-lyophilization with a higher concentration (200 mM<) of the co-solutes further reduced the mannitol crystallization during the process (data not shown). The upper shift of the glass transition temperature (T_g) suggested altered interaction (*e.g.*, hydrogen-bonding) between mannitol molecules, as was reported in co-lyophilization of disaccharides with the phosphate salts.[26]

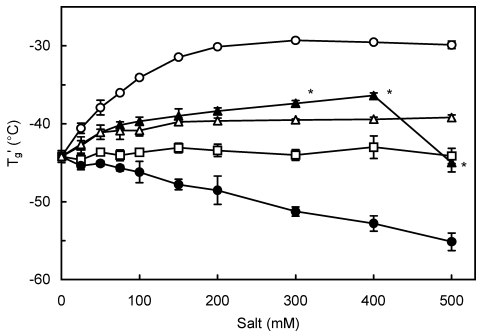

Fig. 5. Effect of Co-solutes on T'_g of Frozen Sorbitol (500 mM) Solutions

An aliquot of solution (10 μl) in aluminum cell was scanned from −70 °C at 5 °C/min. Symbols represent measured midpoint values±S.D. (n=3, ○: Na_3PO_4, ▲: Na_2HPO_4, △: trisodium citrate, □: NaH_2PO_4, ●: NaCl).

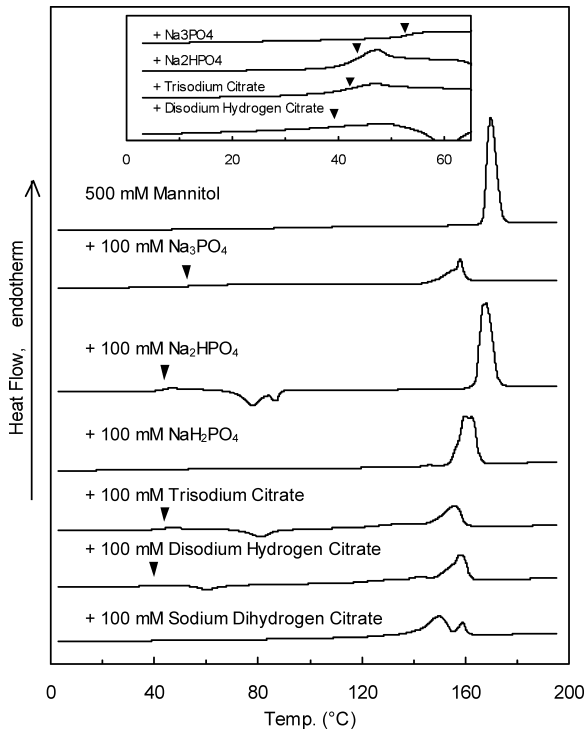

Fig. 6. Thermal Profiles of Freeze-Dried Solids Obtained from Initial Solutions Containing Mannitol (500 mM) and Various Co-solutes (100 mM)

Some thermograms were magnified to indicate the glass transition temperatures of the solids (▼).

Thermal and near-infrared analyses of cooled-melt amorphous sorbitol solids were performed to study the possible salt-induced changes in molecular interactions. A thermogram of cooled-melt sorbitol solid, and effects of the salts on the glass transition temperature (T_g: −1.1 °C) are shown in Fig. 7. The amorphous sorbitol solid showed only a glass transition in the thermal scan up to 125 °C. Trisodium phosphate and citrate exhibited much larger effect to raise the T_g of the amorphous sorbitol solid than Na_2HPO_4 and NaCl, suggesting significant reduction in the molecular mobility. Near-infrared spectra of the cooled-melt amorphous sorbitol solid showed several broad bands that indicate random molecular configurations (Fig. 8). The bands in the 6000 to 7000 cm^{-1} range have been assigned as O–H stretch first overtones of hydroxyl groups with intermolecular (around 6350 cm^{-1}) and intramolecular (around 6800 cm^{-1}) hydro-

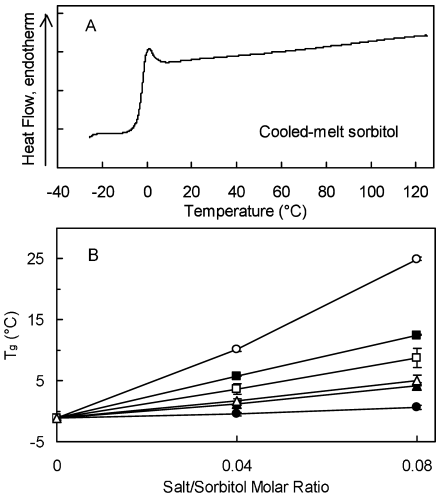

Fig. 7. Thermal Profile (A) and Glass Transition Temperatures (B) of Cooled-Melt Sorbitol–Salt Mixture Solids

Symbols represent measured midpoint T_g values±S.D. (n=3, ○: Na_3PO_4, ▲: Na_2HPO_4, ■: trisodium citrate, □: disodium citrate, △: monosodium citrate, ●: NaCl).

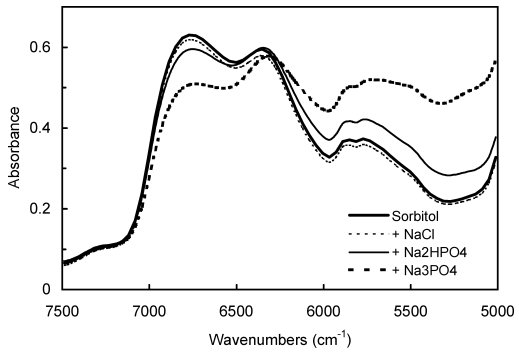

Fig. 8. Transmission Near-Infrared Spectra of Cooled-Melt Solids Containing Sorbitol and Salts (Na_3PO_4, Na_2HPO_4, NaCl) in the Molar Ratio of 12.5 : 1

gen-bonding.[34,35] Addition of Na_3PO_4 reduced intensity of the band at 6800 cm^{-1} with concomitant increase in the bands below 6350 cm^{-1}, suggesting increased intermolecular hydrogen-bonding in the solid. The intense molecular interaction should result in the higher transition temperatures. Absorbance by the phosphate salts should also contribute to the increased intensity in the lower wavenumber region (5500—6000 cm^{-1}).[34,35] Partial crystallization of cooled-melt mannitol made the solid inappropriate for the NIR study.

Discussions

Various co-solutes, including popular excipients in pharmaceutical formulations, kinetically and/or thermodynamically prevented the crystallization of mannitol in frozen solutions at certain concentrations. Trisodium and tripotassium phosphates or citrates showed larger effect to prevent the mannitol crystallization in the frozen solutions and freeze-dried solids. The sorbitol and salt combinations indicated reduced mobility of molecules by intense hydrogen-bonding in the freeze-concentrated phase, which should contribute to retain mannitol in the amorphous state.

Miscibility of mannitol and co-solutes in the concentrated fraction should be the first requisite to prevent the mannitol crystallization in frozen solutions. Mannitol and the various co-solutes were freeze-concentrated into the same phase at certain concentration ratios, and under this condition mixing should kinetically perturb the spatial rearrangement of mannitol molecules required for crystallization. Reported significant melt miscibility of mannitol and some salts (*e.g.*, NaCl) at elevated temperatures suggested their thermodynamic compatibility in the concentrated mixture phase in frozen solutions.[9] On the other hand, thermodynamic solute immiscibility and/or co-solute crystallization (*e.g.*, Na_2HPO_4) at above their critical mixing co-solute/mannitol concentration ratios should separate the solutes to different phases in a frozen solution.[36] The co-solute crystallization should induce crystallization of the remaining mannitol molecules. Various factors, including the structure, ionized state and concentrations of co-solutes, as well as the thermal process, should determine the miscibility with mannitol in the frozen solutions.

Reduction of the molecular mobility of mannitol should be another significant requirement to prevent its crystallization in frozen solutions. The upward shift of the transition temperatures (T'_gs) in the presence of some phosphate and citrate salts should indicate the reduced molecular mobility in the freeze-concentrated phases. Some of the combinations showed their highest T'_gs at certain salt concentrations, which suggested direct interaction between mannitol and co-solute ions and/or altered molecular interactions between mannitol molecules rather than simple mixing.[23,24] It is plausible that the salt-induced intense intermolecular hydrogen-bonding observed in the cooled-melt sorbitol system also contributes to prevent mannitol crystallization in frozen solution and freeze-dried solids. The different effects of phosphate and citrate salts suggested that ionic valence and local pH are the significant factors involved in altering the molecular interactions. The effect of pH on the molecular interaction between polyols are subject to debate, and will require further study.[26,37]

References

1) Hancock B. C., Zografi G., *J. Pharm. Sci.*, **86**, 1—12 (1997).
2) Craig D. Q., Royall P. G., Kett V. L., Hopton M. L., *Int. J. Pharm.*, **179**, 179—207 (1999).
3) Izutsu K., Yoshioka S., Terao T., *Chem. Pharm. Bull.*, **42**, 5—8 (1994).
4) Pikal M. J., Dellerman K. M., Roy M. L., Riggin R. M., *Pharm. Res.*, **8**, 427—436 (1991).
5) Chang B. S., Randall C., *Cryobiology*, **29**, 632—656 (1992).
6) MacKenzie A. P., *Phil. Trans. R. Soc. Lond. B.*, **278**, 167—189 (1971).
7) Suzuki T., Franks F., *J. Chem. Soc., Faraday Trans.*, **89**, 3283—3288 (1993).
8) Kim A. I., Akers M. J., Nail S. L., *J. Pharm. Sci.*, **87**, 931—935 (1998).
9) Telang C., Suryanarayanan R., Yu L., *Pharm. Res.*, **20**, 1939—1945 (2003).
10) Tong P., Taylor L. S., Zografi G., *Pharm. Res.*, **19**, 649—654 (2002).
11) Izutsu K., Fujimaki Y., Kuwabara A., Aoyagi N., *Int. J. Pharm.*, **301**, 161—169 (2005).
12) Nail S. L., Jiang S., Chongprasert S., Knopp S. A., *Pharm. Biotechnol.*, **14**, 281—360 (2002).
13) Cannon A. J., Trappler E. H., *PDA J. Pharm. Sci. Technol.*, **54**, 13—22 (2000).
14) Yu L., Milton N., Groleau E. G., Mishra D. S., Vansickle R. E., *J. Pharm. Sci.*, **88**, 196—198 (1999).
15) Lu Q., Zografi G., *Pharm. Res.*, **15**, 1202—1206 (1998).
16) Williams N. A., Guglielmo J., *J. Parenter. Sci. Technol.*, **47**, 119—123 (1993).
17) Pyne A., Surana R., Suryanarayanan R., *Pharm. Res.*, **19**, 901—908

(2002).
18) Kett V. L., Fitzpatrick S., Cooper B., Craig D. Q., *J. Pharm. Sci.*, **92**, 1919—1929 (2003).
19) Martini A., Kume S., Crivellente M., Artico R., *PDA J. Pharm. Sci. Technol.*, **51**, 62—67 (1997).
20) Johnson R. E., Kirchhoff C. F., Gaud H. T., *J. Pharm. Sci.*, **91**, 914—922 (2002).
21) Lueckel B., Bodmer D., Helk B., Leuenberger H., *Pharm. Dev. Technol.*, **3**, 325—336 (1998).
22) Telang C., Yu L., Suryanarayanan R., *Pharm. Res.*, **20**, 660—667 (2003).
23) Miller D. P., Anderson R. E., de Pablo J. J., *Pharm. Res.*, **15**, 1215—1221 (1998).
24) Izutsu K., Ocheda S. O., Aoyagi N., Kojima S., *Int. J. Pharm.*, **273**, 85—93 (2004).
25) Yoshinari T., Forbes R. T., York P., Kawashima Y., *Int. J. Pharm.*, **258**, 109—120 (2003).
26) Ohtake S., Schebor C., Palecek S. P., de Pablo J. J., *Pharm Res.*, **21**, 1615—1621 (2004).
27) Kets E. P. W., IJpelaar P. J., Hoekstra F. A., Vromans H., *Cryobiology*, **48**, 46—54 (2004).
28) Cavatur R. K., Vemuri N. M., Pyne A., Chrzan Z., Toledo-Velasquez D., Suryanarayanan R., *Pharm. Res.*, **19**, 894—900 (2002).
29) Lu Q., Zografi G., *J. Pharm. Sci.*, **86**, 1374—1378 (1997).
30) Murase N., Franks F., *Biophys. Chem.*, **34**, 293—300 (1989).
31) Deluca P., Lachman L., *J. Pharm. Sci.*, **54**, 617—624 (1965).
32) Burger A., Henck J. O., Hetz S., Rollinger J. M., Weissnicht A. A., Stottner H., *J. Pharm. Sci.*, **89**, 457—469 (2000).
33) Yu L., Mishra D. S., Rigsbee D. R., *J. Pharm. Sci.*, **87**, 774—777 (1998).
34) Ozaki Y., Kawata S., "Near-Infrared Spectroscopy," Japan Scientific Societies Press, Tokyo, 1996.
35) Shenk J. S., Workman J. J., Jr., Westerhaus M. O., "Handbook of Near-Infrared Analysis," ed. by Burns D. A., Ciurczak W. W., Taylor & Francis, New York, 2001, pp. 419—474.
36) Randolph T. W., *J. Pharm. Sci.*, **86**, 1198—1203 (1997).
37) Eriksson J. H., Hinrichs W. L., de Jong G. J., Somsen G. W., Frijlink H. W., *Pharm. Res.*, **20**, 1437—1443 (2003).