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International Journal of Pharmaceutics 288 (2005) 101–108
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Effect of inorganic salts on crystallization of poly(ethylene glycol) in frozen solutions

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Received 19 July 2004; received in revised form 25 August 2004; accepted 21 September 2004
Available online 11 November 2004

Abstract

The effect of inorganic salts on eutectic crystallization of poly(ethylene glycol) (PEG) 1500–20,000 in frozen solution was studied to model the polymer and inorganic salt interaction in freeze-dried formulations. Thermal analysis of an aqueous PEG 3000 solution showed a eutectic PEG crystallization exotherm at approximately −47 °C and a subsequent PEG crystal melting endotherm at −14.9 °C. Addition of sodium chloride prevented the PEG crystallization in the freeze-concentrated solution surrounding ice crystals. Higher concentration NaCl was required to retain higher molecular weight PEG in the amorphous state. Various inorganic salts prevented the PEG crystallization to varying degrees depending mainly on the position of the anion in the Hofmeister's lyotropic series. Some salting-in and 'intermediate' salts (NaSCN, NaI, NaBr, NaCl, LiCl, KCl, and RbCl) inhibited the crystallization of PEG 7500 in frozen solutions. On the other hand, salting-out salts (NaH_2PO_4, Na_2HPO_4, Na_2SO_4, and NaF) did not show an apparent effect on the PEG crystallization. Some salting-out salts induced PEG crystallization in PEG and sucrose combination frozen solutions. The varying abilities of salts to prevent the PEG crystallization in frozen solutions strongly suggested that the solutes had different degrees of miscibility in the freeze-concentrates.

Keywords: Crystallization; Amorphous; Phase separation; DSC; Molecular interaction

1. Introduction

Freezing of an aqueous solution concentrates the solutes into a supercooled solution surrounding ice crystals. Various solutes crystallize or remain amorphous in the frozen solution depending on the nature of each solute, co-solute composition, and freezing methods (Chang and Randall, 1992; Franks, 1998; Pikal, 1999; Akers et al., 2002; Nail et al., 2002). A proper freeze-drying process without collapse or melt-back usually retains the solute crystallinity in the frozen solutions. The crystallinity of active ingredients and excipients is an important factor that affects the quality of pharmaceutical dosage forms (Pikal et al., 1977; Arakawa et al., 1993; Hancock and Zografi, 1997). Component crystallization in frozen solutions usually results in

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doi:10.1016/j.ijpharm.2004.09.015

good appearance of the freeze-dried cake, and better storage stability of the dried solids (Akers et al., 2002). Amorphous freeze-dried solids have the advantage of a faster dissolution rate of the ingredients, as well as other formulation properties (e.g., protein stabilization) that depend on the interaction between different molecules. In order to achieve the component crystallinity required for particular formulations, it is essential that composition and freeze-drying be optimized.

Co-lyophilization with a large amount of excipients that produce amorphous freeze-dried cake (e.g., disaccharides) is the most popular way to obtain a particular ingredient in the amorphous state (Nail et al., 2002; Telang et al., 2003). Although disaccharides can retain a wide range of chemicals in the amorphous freeze-dried solids, they often have insufficient physical stabilities, such as low collapse temperature during freeze-drying and low glass transition temperature of the dried solid at lower concentrations. Other categories of excipients (e.g., polymers, inorganic salts, and complexing agents) are receiving increasing attention due to their ability to retain certain solutes in the amorphous state (Martini et al., 1997; Izutsu et al., 2004). Some inorganic salts are effective at preventing the crystallization of other components (e.g., mannitol) in frozen solutions, although the mechanism of this effect remains unclear (Telang et al., 2003). Information on the effect of popular excipients (e.g., buffer salts) on the crystallinity of other solute components in frozen solutions should be valuable in pharmaceutical formulation design.

Many solutes show different miscibility in the freeze-concentrate depending on the component composition and freezing method (Her et al., 1995; Heller et al., 1996, 1997; Izutsu et al., 1996, 1998; Randolph, 1997). Various combinations of polymers or a polymer and a low molecular weight solute separate into multiple freeze-concentrated phases rich in one of the components because of the increasing concentration and thermodynamically unfavorable interactions. The solute miscibility in the multi-component frozen solution is one of the significant factors that determine the crystallinity of solutes with an intrinsic propensity for crystallization (Heller et al., 1996; Izutsu et al., 1996). Mixing of solutes in the freeze-concentrate often prevents solute crystallization by altering the molecular interactions and reducing the molecular mobility. For example, different saccharides have been shown to have different abilities to prevent the crystallization of poly(ethylene glycol) (PEG) in frozen solutions depending on their miscibility (Izutsu et al., 1996).

In this report, we studied the effect of various inorganic salts on the crystallization of PEG in frozen solutions to model the component interaction in freeze-dried formulations. PEG forms a helical eutectic crystal that possesses two or three hydration water molecules per oxyethylene unit in the single-solute frozen solution, with the eutectic crystallization and melting transitions apparent in thermal analysis (Hager and Macrury, 1980; Bogdanov and Mihailov, 1985; Vringer et al., 1986; Graham et al., 1989). PEG and inorganic salts show varied miscibility in aqueous solutions and dehydrated states. Solutions containing sufficiently high levels of PEG and some salts (e.g., sodium phosphate and sodium sulfate) separate to form aqueous two-layer systems (Albertsson, 1958; Bailey and Callard, 1959; Ataman and Boucher, 1982; Florin et al., 1984; Ananthapadmanabhan and Goddard, 1987; Zaslavsky, 1995). Mixing of some salts and a poly(ethylene oxide) (PEO), which is usually referred to as a large PEG molecule, has been studied extensively for possible use in the production of solid electrolytes (Wright, 1989; Bruce and Vincent, 1993). The results obtained in frozen solutions were compared with published information on the component miscibility in aqueous solutions and dried states.

2. Materials and methods

Sucrose was obtained from Sigma Chemical Co. (St. Louis, MO). PEGs of various molecular weights (PEG 1500, 3000, 7500, and 20,000) and other chemicals were of reagent grade purchased from Wako Pure Chemical Co. (Osaka, Japan).

Thermal analysis of frozen solutions was performed using a differential scanning calorimeter (DSC-Q10; TA Instruments, New Castle, DE) with a refrigerating cooling unit. Aliquots (10 μl) of solutions in hermetic aluminum cells were cooled from room temperature to −70 °C at approximately 20 °C/min, and kept at −70 °C for 1 min. Thermal data were obtained in the scans at 5 °C/min. The relative PEG melting endotherm (%) in frozen solutions was obtained from the ratio of the PEG melting enthalpy to that of a solution

containing only PEG. All the solutions employed in this study were in single phase at room temperature.

The mobility of solute and water molecules in frozen solution was studied as described previously (Izutsu et al., 1996) using a modified version of the method of Fung and McGaughy (1974) with a broad-line pulsed NMR spectrometer with Larmor frequency of 25 MHz (JNM-MU25; JEOL, Tokyo, Japan) and a temperature-controlling unit (DVT-2; JEOL). Free induction decay (FID) signals of frozen aqueous solutions (in H_2O or D_2O, 1 ml, frozen by immersion in liquid nitrogen) in glass tubes (10 mm in diameter) were sampled every 0.2 μs after 90° pulses. Measurements were made every 2 °C from −70 °C by warming at a rate of 0.5 °C/min. The results were resolved into two components indicating long and short spin–spin relaxation times (T_2), which were considered to represent protons of liquid- and solid-state molecules. The proportions of long T_2 protons in the sample were determined by extrapolating the portions of FID curve between 40 and 120 μs back to time zero. In experiments using D_2O as the solvent, samples were stored overnight at room temperature prior to the NMR analysis. Relative amounts of long T_2 proton were expressed as the proportion (%) in pure H_2O at 25 °C, using a calibration curve obtained from various concentrations of H_2O/D_2O solutions.

3. Results

Fig. 1 shows the thermograms of frozen aqueous solutions containing 0–20 mg/ml PEG 3000 and 0–200 mM NaCl. A single-solute frozen PEG 3000 solution showed a broad eutectic PEG crystallization exotherm (−47.3 °C) and its melting endotherm (−14.9 °C) below the ice-melting temperature (Vringer et al., 1986; Izutsu et al., 1996). The fact that the PEG crystallization exotherm was smaller than its melting endotherm suggested that partial PEG crystallization occurred in the cooling process of the thermal analysis. A frozen 100 mM NaCl solution showed a sharp endotherm of eutectic NaCl melting at −21.2 °C (DeLuca and Lachman, 1965; Jochem and Körber, 1987). Addition of 10 or 20 mM NaCl reduced the eutectic PEG crystallization and crystal melting peaks, and shifted the peaks to higher (crystallization) and lower (melting) temperatures, respectively. The thermograms of frozen solutions containing 20 mg/ml PEG 3000 and 40–100 mM NaCl did not have the PEG crystallization and melting peaks before the ice-melting endotherm, indicating the solutes remain amorphous in the freeze-concentrate. The eutectic PEG melting endotherm disappeared at a NaCl/ethylene oxide unit concentration ratio of approximately 0.1 in two initial PEG 3000 concentration solutions (20 and 100 mg/ml, data not shown). Excess NaCl above the concentration ratio crystallized in the frozen solution.

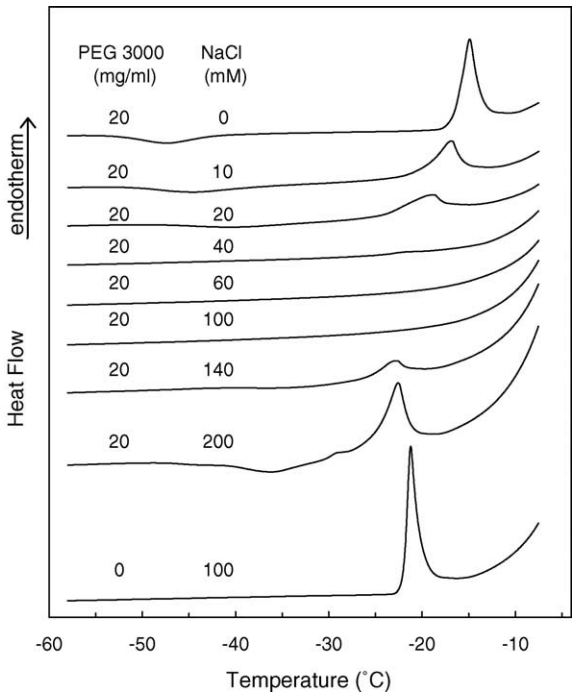

Fig. 1. Thermograms of frozen solutions containing PEG 3000 and NaCl. Aqueous solutions (10 μl) in hermetic aluminum cells were scanned from −70 °C at 5 °C/min.

The physical properties of the PEG and NaCl combination frozen solutions were further studied by using pulsed NMR. Fig. 2 shows the temperature dependence of the proton ratio with liquid-like relatively long spin–spin relaxation time (T_2) in aqueous (H_2O or D_2O) frozen solutions. The long T_2 proton ratio in D_2O solutions shows the proportion of solute molecules with liquid-like translational motion. The H_2O solution data indicate the proportions of mobile solute and water molecules. The increase in the long T_2 protons suggests increasing molecular mobility at crystal melting and/or thermal transition of the amorphous phase (e.g., glass

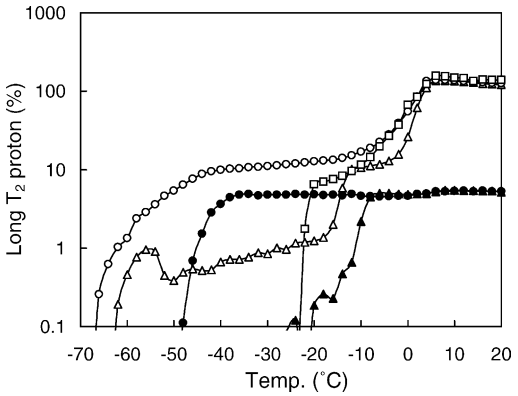

Fig. 2. Proportion of long-T_2 proton components in frozen solutions. (▲) 20 mg/ml PEG 3000 in D_2O; (●) 20 mg/ml PEG 3000 and 100 mM NaCl in D_2O; (△) 20 mg/ml PEG 3000 in H_2O; (○) 20 mg/ml PEG 3000 and 100 mM NaCl in H_2O; (□) 100 mM NaCl in H_2O. The relative amount of long T_2 proton was obtained from the ratio using H_2O (25 °C) as a standard.

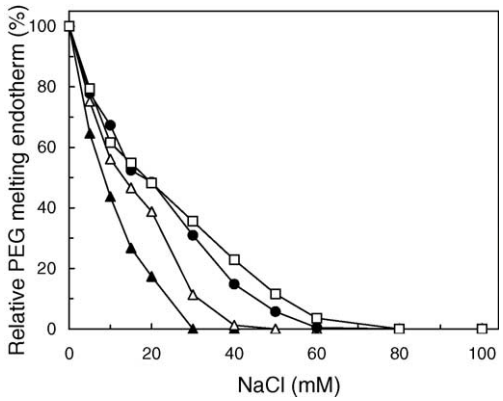

Fig. 3. Effect of NaCl on the relative eutectic melting endotherm (%) of PEG molecules of various molecular weights in frozen solutions. The endotherm enthalpies of respective PEGs were used as a standard. (▲) PEG 1500; (△) PEG 3000; (●) PEG 7500; (□) PEG 20,000 ($n = 2$).

transition). The PEG 3000 molecules in D_2O solution showed the translational motion at the temperature of the eutectic crystal melting (−15 to −10 °C) (Izutsu et al., 1996). Water molecules around PEG moved from a much lower temperature (approximately −60 °C), and showed reduced mobility in the eutectic crystallization temperature range (−50 to −15 °C). Melting of eutectic NaCl crystals (approximately −22 °C) conferred the translational motion to surrounding water molecules. The solute and water molecules of the frozen solutions containing both PEG and NaCl showed the translational motion from much lower temperatures than in the corresponding single-solute solutions. The moving PEG molecules in the PEG and NaCl combination frozen solution (in D_2O) appeared at −48 °C, then reached to a plateau at −38 °C. The long T_2 protons of the combination solution in H_2O appeared at −64 °C, and gradually increased in number as the temperature was raised. The results indicate that the solute and solvent molecules are in the amorphous freeze-concentrated mixture with substantial molecular mobility with low T_g' (glass transitions of maximally freeze-concentrated solute).

Fig. 3 shows the effect of NaCl on the relative melting endotherm (%) of PEGs of various molecular weights in frozen solutions. The frozen PEG 1500–20,000 solutions (20 mg/ml) showed the eutectic crystal melting endotherm at different temperatures (PEG 1500: −16.5 °C; PEG 7500: −12.4 °C; PEG 20,000: −10.6 °C) with similar enthalpy changes (standard deviations within 5%) in the absence of NaCl (Hager and Macrury, 1980; Vringer et al., 1986; Graham et al., 1989). Eutectic crystallization of larger PEG molecules was prevented at higher NaCl concentration, which was consistent with the effect of some saccharides reported previously (Izutsu et al., 1996).

Figs. 4–6 show the effect of inorganic salts on the crystallization of 20 mg/ml PEG 7500 in frozen solutions. The degree to which the various sodium salts altered the PEG crystallinity was mainly dependent on the position of anions in the Hofmeister's lyotropic series (Fig. 4) (Hofmeister, 1888). Some salting-in sodium salts (water-structure breaking salts: NaSCN, NaI, and NaBr) prevented the PEG 7500 crystallization at lower concentrations than those of NaCl. On the other hand, salting-out salts (water-structure enhancing salts: NaF, NaH_2PO_4, Na_2HPO_4, and Na_2SO_4) did not show apparent effects on the PEG crystallization even at 100 mM. For example, addition of NaF (40–100 mM) induced the eutectic NaF crystal melting endotherm without changes in the PEG 7500 melting peak (Fig. 5). Various potassium salts other than potassium fluoride showed the same trend as the sodium salts in their ability to prevent the PEG crystallization depending on the position of anion in the Hofmeister series (data not shown). Although NaF showed little effect on the PEG crystallization in the

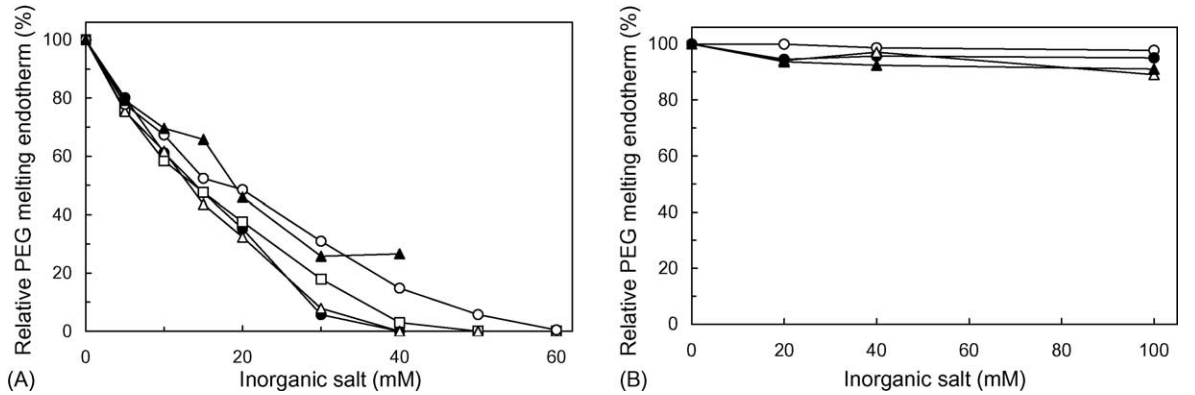

Fig. 4. Effect of various salts on the relative PEG 7500 melting endotherm (%) in frozen solutions. The endotherm enthalpy of frozen PEG 7500 solution without inorganic salt was used as a standard. (A) (●) NaSCN; (△) NaI; (□) NaBr; (◯) NaCl; (▲) KF. (B) (▲) NaF; (◯) Na_2SO_4; (●) Na_2HPO_4; (△) NaH_2PO_4 ($n = 2$).

frozen solutions, addition of 10–40 mM KF reduced the crystallization of PEG 7500 (20 mg/ml) in the frozen solutions (Fig. 4A). A eutectic KF melting endotherm appears at around −22 °C above the KF concentration. The F^- ion may have less favorable interaction with PEG molecules compared to larger halogen ions (I^-, Br^- and Cl^-), which may vary the crystallization-preventing effect between the sodium and potassium salts. Other salts with different cations showed smaller difference in their ability to prevent the eutectic PEG crystallization. Some alkali chlorides (LiCl, NaCl, KCl, and RbCl) indicated that salts with larger cation maintain PEG in the amorphous state from lower concentrations (Fig. 6). Alkali chlorides interact differently with anhydrous PEO molecules depending on the cations (Ohno et al., 1994). The salts employed in this study showed a different tendency to crystallize in the single-solute frozen solutions. Some frozen salting-in (e.g., KCl, NaCl, and NaBr) and salting-out (e.g., KH_2PO_4) salt solutions showed eutectic crystal melting endotherm in the thermal scan (data not shown). No apparent relationship was observed between the propensity of salts for crystallization and the ability of

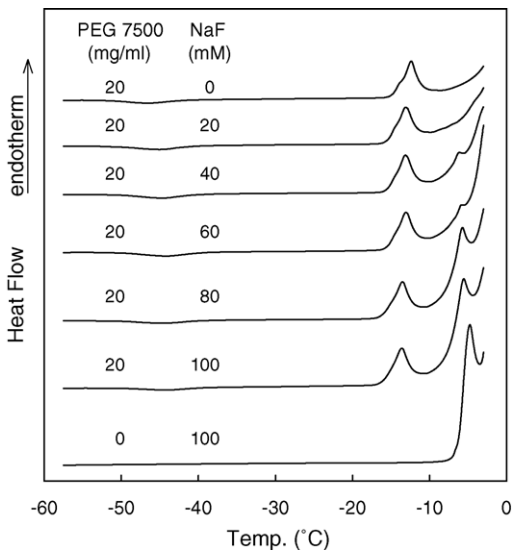

Fig. 5. Thermograms of frozen solutions containing PEG 7500 and NaF. Aqueous solutions (10 μl) in hermetic aluminum cells were scanned from −70 °C at 5 °C/min.

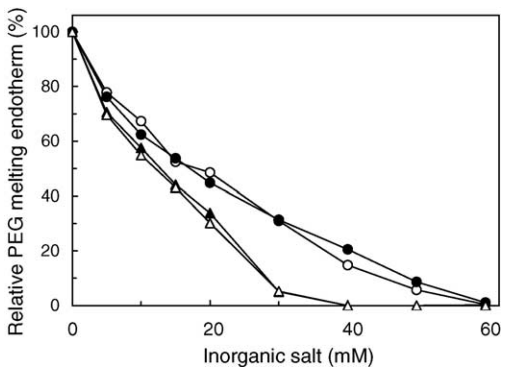

Fig. 6. Effect of various alkali chlorides on the relative melting endotherm (%) of PEG 7500 in frozen solutions. The endotherm enthalpy of frozen PEG 7500 solution without inorganic salt was used as a standard. (◯) NaCl; (●) LiCl; (▲) KCl; (△) RbCl ($n = 2$).

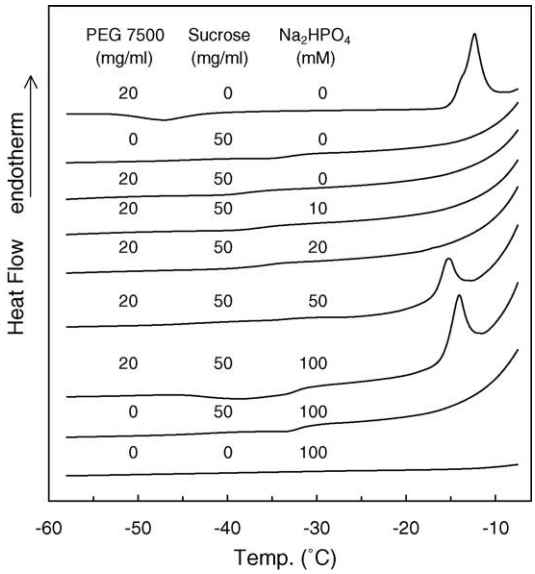

Fig. 7. Thermograms of frozen solutions containing PEG 7500, sucrose, and disodium phosphate. Aqueous solutions (10 μl) in hermetic aluminum cells were scanned from −70 °C at 5 °C/min.

the salts to prevent PEG crystallization in the frozen solutions.

Some mono- or disaccharides prevent crystallization of PEG in frozen solutions (Izutsu et al., 1996). We therefore studied the effects of sucrose and a salting-out salt (disodium phosphate) on the crystallization of PEG 7500 in frozen solutions (Fig. 7). The PEG 7500 (20 mg/ml) remained amorphous in a frozen solution with sucrose (50 mg/ml), showing an apparent T_g' at −37.8 °C. Addition of more than 50 mM disodium phosphate to the PEG and sucrose combination frozen solution induced the eutectic PEG crystal melting peak. Some other salting-out salts (e.g., NaH_2PO_4, Na_2SO_4, KH_2PO_4, and K_2SO_4) also induced the PEG crystallization in the PEG and sucrose combination frozen solutions (data not shown). The results suggest that the crystallinity of a solute is determined by complex interplay among the co-solutes in the frozen solution.

4. Discussion

The effect of various salts on the eutectic PEG crystallization in frozen solutions was primarily dependent on the position of anions in the Hofmeister's lyotropic series. The different effect of inorganic salts is attributed to their different miscibility with PEG in the freeze-concentrate. PEG and various salting-in or 'intermediate' salts in the lyotropic series are miscible in aqueous solutions in ultrafiltration and immobilized polymer analysis (Fujita et al., 1980; Florin et al., 1984; Sartori et al., 1990). Some salting-in salts bind to the surface of PEG. Some of these salts (e.g., KI, NaI, and NaSCN) are also miscible with dehydrated PEO (larger PEG) melt, and reduce the polymer crystallinity in the solid states (Lundberg et al., 1966; Wright, 1989; Bruce and Vincent, 1993). Ice formation should concentrate the PEG and salting-in salts in an aqueous solution into a highly viscose amorphous mixture, where the solute and surrounding water molecules have a substantial degree of mobility from low temperatures.

The salting-in salts and 'intermediate salts' may prevent the PEG crystallization by two possible mechanisms. An increase in viscosity due to the salt adsorption could retard the PEG nucleation and crystal growth in the non-ice phase (Sartori et al., 1990). Another possibility is that direct interaction with the salting-in salts could prevent the structural re-ordering of PEG molecules required for the eutectic crystallization. Both of the crystallization-preventing mechanisms would require that the PEG and salts be in the same freeze-concentrated phase at certain mass ratios. Higher-concentration NaCl was required to prevent the crystallization of higher molecular weight PEGs because these PEGs showed a larger tendency to undergo intramolecular structure reordering.

The fact that the salting-out salts had only a marginal effect on the eutectic PEG crystallization was attributed to the phase separation in the freeze-concentrated solution. The mutual exclusion between PEG and some salting-out salts (e.g., Na_2SO_4, K_2SO_4, and Na_3PO_4) separates the high concentration solutions into two layers that are relatively rich in one of the solute components (Albertsson, 1958; Bailey and Callard, 1959; Ananthapadmanabhan and Goddard, 1987). Salting-out salts also lower the PEG cloud point (lower critical solution temperature (LCST)) (Albertsson, 1958; Ataman and Boucher, 1982; Florin et al., 1984; Karlström, 1985). Ions of the salting-out salts are 'preferentially' excluded from the immediate surface of the polymer to form a salt-deficient zone even in homogenous solutions (Florin et al., 1984;

Ananthapadmanabhan and Goddard, 1987). Various salting-out salts also show poor miscibility with PEG (PEO) melts (Bruce and Vincent, 1993). Freeze-concentration separates some solute combinations with repulsive interaction into multiple phases (Her et al., 1995; Heller et al., 1996, 1997; Izutsu et al., 1996, 1998; Randolph, 1997). Separation of PEG and salting-out salts in a frozen solution would results in the PEG crystallization in the PEG-dominant phases as occurs in single-solute frozen solutions. Some salting-out salts (e.g., sodium phosphates) show apparent effect to prevent crystallization of small molecules (e.g., mannitol) in the miscible freeze-concentrated phase (Izutsu and Kojima, 2002). The miscibility-dependent ability of salts to prevent the eutectic PEG crystallization resembles that of saccharides, despite the possible differences between their mechanisms for maintaining the amorphous freeze-concentrated phase (Izutsu et al., 1996). Saccharides may largely dilute PEG molecules in the amorphous freeze-concentrate. Further studies on the physical properties of multi-solute frozen solutions and their dried solids will be needed for pharmaceutical formulation design.

The different effects of inorganic salts on the eutectic crystallization made PEG a good model for studying the solute miscibility in frozen solutions. Many other polymers remain amorphous in the frozen solutions, whereas the solute miscibility in the amorphous freeze-concentrate is often difficult to determine experimentally (Her et al., 1995). The occurrence of phase separation in various high concentration polymer and salt combination solutions (Zaslavsky, 1995; Tolstoguzov, 2000), and the two amorphous freeze-concentrated phases observed in some polymer and inorganic salt combinations (e.g., PVP and sodium phosphate) (Her et al., 1995), suggest that the polymer and salt combinations had different degrees of miscibility in the freeze-concentrate. The different polymer and salt miscibilities in frozen solutions could affect the quality of freeze-dried formulations by altering the component crystallinity, glass transition temperature, and pH. It is also plausible that the different miscibilities with inorganic salts affect conformation of biological polymers (e.g., protein) in the freeze-dried solid. Proper choice of salts, including buffers, based on the component miscibility information would be important in the design of freeze-dried formulations.

5. Conclusion

PEG remained amorphous in frozen aqueous solutions containing salting-in or ‘intermediate’ salts that are miscible in solution. On the other hand, some salting-out salts showed little or smaller effect on the PEG crystallization. The varied effects of inorganic salts on the eutectic PEG crystallization were attributed to the different miscibilities of PEG and each salt in the freeze-concentrate.

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