Investigation of PEG Crystallization in Frozen PEG–Sucrose–Water Solutions: II. Characterization of the Equilibrium Behavior During Freeze-Thawing

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ABSTRACT: Our objective was to characterize, by DSC and XRD, the equilibrium thermal behavior of frozen aqueous solutions containing polyethylene glycol (PEG) and sucrose. Aqueous solutions of (i) PEG (2.5–50% w/w), (ii) sucrose (10% w/v) with different concentrations of PEG (1–20% w/v), and (iii) PEG (2% or 10% w/v) with different concentrations of sucrose (2–20% w/v), were cooled to −70°C at 5°C/min and heated to 25°C at 2°C/min in a DSC. Annealing was performed for 2 or 6 h at temperatures, ranging from −50 to −20°C. Experiments under similar conditions, on select compositions, were also performed in a powder X-ray diffractometer. Two endotherms, observed during heating of a frozen PEG solution (10% w/v), were attributed to PEG–ice eutectic melting and ice melting, and were confirmed by XRD. At higher PEG concentrations (≥37.5% w/w), only the endotherm attributed to the PEG–ice eutectic melting was observed. Inclusion of sucrose decreased both PEG–ice melting and ice melting temperatures. In unannealed systems with a fixed sucrose concentration (10% w/v), the PEG–ice melting event was not observed at PEG concentration ≤5% w/v. Annealing for 2–6 h facilitated PEG crystallization. In unannealed systems with a fixed PEG concentration (10% w/v), an increase in the sucrose concentration increased the devitrification but decreased the PEG–ice melting temperature. The PEG–ice melting temperatures obtained by DSC and XRD were in good agreement. In ternary systems at a fixed PEG to sucrose ratio, the T'_g as well as the PEG–ice melting temperature were unaffected by the total solute concentration. XRD confirmed the absence of a PEG–sucrose–ice ternary eutectic. When the PEG to sucrose ratio was systematically varied, the PEG–ice and ice melting temperatures decreased with an increase in the sucrose concentration. However, at a fixed PEG to sucrose ratio, the PEG–ice melting temperature, was unaffected by the total solute concentration. J Pharm Sci 99:4510–4524, 2010

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INTRODUCTION

Polyethylene glycol (PEG), a water-soluble synthetic polymer, is employed as an additive in pharmaceutical formulations intended for oral and parenteral delivery.[1] In addition to chemical modification (i.e., PEGylation of proteins),[2] it has been utilized to facilitate protein crystallization.[3–5] Several PEGylated proteins are formulated as freeze-dried powders where sucrose or other sugars are also included as lyoprotectants.[6] An understanding of the phase behavior of PEGylated protein–sucrose systems is critical for the optimization of the formulation as well as the freeze-drying process.

The low temperature phase behavior (i.e., crystallization during freezing, thawing, and drying) of several excipients including sugars, sugar alcohols, salts, and polymers has been extensively studied.[7–12] While mannitol and glycine are examples of solutes which crystallize during freeze-drying, other solutes such as sucrose, trehalose, and PVP remain

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amorphous.[13] Solutes which crystallize as hydrates (e.g., disodium hydrogen phosphate crystallizes as a dodecahydrate) in the frozen state can undergo simultaneous dehydration and crystalline → amorphous transition during drying and exist in the noncrystalline state in the freeze-dried cake.[14] PEGs have been shown to crystallize during freeze-drying and are retained crystalline in the final freeze-dried cake.[15–17] The extent of PEG crystallization is dependent on the concentration of the other formulation components such as sugars and polymers.[8] While the phase transitions during freeze-thawing of PEG–water systems have also been documented, the phase behavior in frozen PEG–sugar–water systems has not been adequately addressed.[7,17–25] A detailed characterization of the PEG–sugar–water systems is the first step towards understanding the phase behavior of the more complex PEGylated protein–sugar–water systems.

During freeze-thawing of aqueous PEG solutions, both "nonequilibrium" (glass transition of the freeze concentrate and PEG–ice eutectic crystallization) and "equilibrium" (PEG–ice eutectic melting and ice melting) transitions were observed.[12,17] Glass transition occurred at temperatures (−67°C for PEG 8000) much lower than the processing temperatures (−40 to 45°C) typically employed during lyophilization. PEG crystallization in frozen aqueous solutions containing a polymer (dextran, PEG, PVP, or Ficoll),[8] salt (sodium phosphate, sodium sulfate, or an alkali halide),[26] or a sugar (mono-, di-, or tri-saccharides) was evaluated by differential scanning calorimetry. The compatibility between PEG and the additive appeared to govern the ability of the additive to inhibit crystallization. While PEG was retained amorphous in frozen solutions containing PEG and sucrose, the inclusion of disodium phosphate promoted PEG crystallization.[26] This conclusion, based on an endotherm observed in the DSC heating curve of the frozen solution, was attributed to PEG melting. However, the crystalline phase was not identified by a spectroscopic or a diffractometric technique.

Even though it is known that solutes such as sucrose inhibit PEG crystallization, the nonequilibrium and equilibrium behavior of such ternary systems has not received adequate attention in the literature. In a recent report, we documented the nonequilibrium behavior in frozen PEG–sucrose–water solutions using DSC and low temperature XRD.[12] Specifically, the impact of solute composition and annealing on the T'_g and PEG–ice crystallization was determined. At high solute concentrations, two glass transition events were observed, suggesting phase separation in the freeze-concentrate. In the present manuscript, we have extended the investigation to the equilibrium behavior of these systems with the goal of developing a comprehensive understanding of the phase behavior in frozen aqueous PEG–sucrose systems. Understanding these "simpler" ternary systems will facilitate the study of more "complex" systems containing PEGylated proteins and sucrose. This will be addressed in a future manuscript on the investigation of PEG crystallization during freeze-thawing and freeze-drying of PEGylated protein–sucrose solutions.

MATERIALS AND METHODS

Materials

Sucrose (lot # 063617, Fisher Scientific, Pittsburgh, PA) and poly(ethylene glycol) (a proprietary PEG, average molecular weight ~40000, Nektar, Huntsville, AL) were used as received. Filtered, distilled, and deionized water was employed for preparation of the solutions. While most solutions were prepared at room temperature, solutions containing high PEG concentrations (>20% w/v) were prepared by heating PEG–water mixtures in sealed weighing bottles in a water bath at 65°C for 5 min. The solutions were cooled to room temperature prior to use in the DSC experiments.

METHODS

Differential Scanning Calorimetry

A modulated differential scanning calorimeter (model 2920, TA Instruments, New Castle, DE) equipped with a refrigerated cooling system (TA Instruments) was employed. The data analyses were performed using a Universal Analysis Program (Version 4.1D, TA Instruments). The cell constant was determined using indium and temperature and enthalpy calibrations were performed using indium, tin, and water as standards. The aluminum sample pans (TA Instruments) were sonicated in methanol and acetone separately for 15 min and air dried, prior to use in the DSC experiments. Approximately, 10 mg of the solution was sealed hermetically in an aluminum pan.

Frozen PEG–Water Solutions

The transitions in frozen aqueous PEG solutions (2–50% w/w) were investigated for constructing the PEG–water phase diagram. The solution was cooled to −70°C, at 5°C/min, where it was held for 10 min followed by heating to 25°C at 5 heating rates ranging from 0.25 to 2.0°C/min. The PEG–ice eutectic melting and ice melting temperatures noted as $T_{eutectic}$ and T_{ice}, respectively were determined from the endotherm peak temperatures obtained over the range of the employed heating rates and extrapolated