Investigation of PEG Crystallization in Frozen PEG–Sucrose–Water Solutions. I. Characterization of the Nonequilibrium Behavior during Freeze-Thawing

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ABSTRACT: Our objective was to characterize the nonequilibrium thermal behavior of frozen aqueous solutions containing PEG and sucrose. Aqueous solutions of (i) sucrose (10%, w/v) with different concentrations of PEG (1–20%, w/v), and (ii) PEG (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 differential scanning calorimeter. Annealing was performed at temperatures ranging from −50 to −20°C for 2 or 6 h. Similar experiments were also performed in the low-temperature stage of a powder X-ray diffractometer. A limited number of additional DSC experiments were performed wherein the samples were cooled to −100°C. In unannealed systems with a fixed sucrose concentration (10%, w/v), the T'_g decreased from −35 to −48°C when PEG concentration was increased from 1% to 20% (w/v). On annealing at −25°C, PEG crystallized. This was evident from the increase in T'_g and the appearance of a secondary melting endotherm in the DSC. Low-temperature XRD provided direct evidence of PEG crystallization. Annealing at temperatures ≤−40°C did not result in crystallization and a devitrification event was observed above the T'_g. In unannealed systems with a fixed PEG concentration (10%, w/v), the T'_g increased from −50 to −40°C when sucrose concentration was increased from 5% to 50%, w/v. As the annealing time increased (at −25°C), the T'_g approached that of a sucrose–water system, reflecting progressive PEG crystallization. A second glass transition at ∼ −65°C was evident in unannealed systems [10%, w/v sucrose and 10 (or 20%), w/v PEG] cooled to −100°C. Investigation of the nonequilibrium behavior of frozen PEG–sucrose–water ternary system revealed phase separation in the freeze-concentrate. Annealing facilitated PEG crystallization. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:2609–2619, 2010

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INTRODUCTION

Freeze-dried protein formulations are complex multicomponent systems containing crystallizable and noncrystallizable components. The phase behavior of these components is dependent on formulation (solute concentration) and process variables (cooling rate, annealing temperature, and time). Ice crystallization ("primary" crystallization)[1] during the freezing stage of lyophilization causes concentration of solutes, which can eventually lead to solute crystallization ("secondary" crystallization). Crystallization of bulking agents such as glycine and mannitol provides a crystalline matrix, thereby enabling primary drying at elevated temperatures.[2–4] On the other hand, selective crystallization of buffer salts (e.g., sodium dihydrogen phosphate dodecahydrate) or cryoprotectant can lead to product instability.[5,6]

Poly(ethylene glycol) (PEG), a water soluble polymer, is widely used in the manufacture of oral, parenteral, and topical dosage forms.[7] PEG is employed as a binder, co-solvent, and plasticizer in solid oral dosage forms, and as a cryoprotectant in

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lyophilized formulations.[8–11] In addition, chemical modification of peptides and proteins with PEG (i.e., PEGylation) can increase *in vivo* half-life and reduce immunogenicity.[12] A number of PEGylated protein formulations have been approved for therapeutic use by the FDA and several are currently under development.[13] One potential limitation is that PEGs can crystallize during freeze-drying and storage, leading to protein degradation.[10,14]

The phase behavior of PEG–water systems has been investigated by differential scanning calorimetry,[15–23] dielectric spectroscopy,[24–26] light microscopy,[27] low-temperature X-ray diffractometry,[28] nuclear magnetic resonance spectroscopy,[29] and Raman spectroscopy.[30–33] The eutectic composition and hydration number has been determined for PEG of average molecular weights ranging from 400 to 1.8×10^6.[15–17,19–22,34,35] The crystallization of PEG during freeze-thawing or freeze-drying from multicomponent systems containing polymers (PVP, dextran)[36–38] or salts has been reported.[14–17,22,28,39–41] However, the thermal behavior of frozen PEG–sugar–water ternary systems has not been widely investigated.[14,37,39–41] Izutsu et al.[37] investigated the effect of sugars and polymers on PEG crystallization in frozen solutions. The inclusion of sugars prevented crystallization of PEG 3000 in frozen solutions. The effectiveness of sugars in preventing PEG crystallization was rank ordered as: sucrose > palatinose > maltose > cellobiose > trehalose > lactose > melibiose.

In a recent study, crystallization of PEG was inhibited in frozen solutions containing PEG (2%, w/v) and sucrose (5%, w/v).[41] However, the inclusion of disodium phosphate (10–100 mM) enabled PEG crystallization. Thus the solute crystallization can be influenced by the other formulation components, thereby presenting a potentially challenging issue in the development of formulations containing multiple components.

PEG crystallization during freeze-drying could pose serious concerns, both from formulation and product performance perspectives. On the other hand, PEG crystallization presents a potential processing advantage, in that a formulation may no longer need a bulking agent. A comprehensive understanding of PEG crystallization is particularly relevant in the development of lyophilized PEGylated protein formulations.

There are at least ten PEGylated products in the market and many more are under development.[42–44] A significant fraction of these products are manufactured by freeze-drying. In spite of the potentially important role of PEG in freeze-dried formulations, little is known about its low-temperature phase behavior. A comprehensive understanding of its phase behavior will require characterization of the equilibrium (PEG—ice melting, also known as "eutectic melting" in the pharmaceutical literature) and nonequilibrium (crystallization and glass transition of the freeze-concentrate) behavior in frozen aqueous systems. Since lyophilized formulations often contain a sugar, it will be instructive to perform similar characterization studies of PEG–sucrose–water systems. The phase behavior of frozen PEG–sugar solutions are unlikely to reflect that of PEGylated protein–sugar systems. However, in order to develop a comprehensive understanding of the PEGylated protein formulations, characterization of PEG–sugar systems is the necessary first step. Additionally, systematic studies of PEG–sucrose–water systems have not been reported in the literature. The effects of solution composition and annealing on the T'_g in PEG–sucrose–water ternary systems are discussed in this paper. The equilibrium behavior of these systems, and the impact of freeze-drying on the phase behavior of PEGylated API, will be the subject of future manuscripts.

MATERIALS AND METHODS

Materials

Sucrose (Lot # 063617) was obtained from Fisher Scientific (Pittsburgh, PA). Poly(ethylene glycol) (a proprietary PEG, average molecular weight ~40,000) was obtained from Nektar (Huntsville, AL). Distilled and deionized water was filtered (0.45 μm) and used for solution preparation. 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. The other solutions were prepared at room temperature.

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. A Universal Analysis Program (Version 4.1D, TA Instruments) was used for data processing. 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 an organic solvent (cleaning in methanol followed by sonication in acetone for 15 min) and air-dried, prior to use in the DSC experiments. Approximately, 10 mg of the solution was weighed in an aluminum pan, and sealed hermetically.

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