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Research paper

Mechanisms by which crystalline mannitol improves the reconstitution time of high concentration lyophilized protein formulations

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ABSTRACT

Lyophilized high concentration protein formulations often have long and variable reconstitution times. The aim is to understand the role of crystalline mannitol in lowering the reconstitution time of these formulations. Novel methods were developed for quantifying the effect of crystalline mannitol on cake attributes influencing reconstitution, specifically, cake wettability, liquid penetration into the cake and cake disintegration. Amorphous and partially crystalline cakes were obtained by varying the freeze-drying conditions, particularly, the freezing rate (slow vs. fast), annealing (annealed vs. unannealed), and primary drying (aggressive vs. conservative). Mannitol crystallinity was quantified using X-ray powder diffractometry. Phase separation of crystalline mannitol from the amorphous, protein rich matrix improved wettability of the cake solids and promoted penetration of the reconstitution fluid into the cake interior. The partially crystalline cakes offered less resistance to crushing in the dry state than the amorphous cakes. Crystalline mannitol provided "weak points" in the freeze-dried cakes, potentially enabling easier cake disintegration upon addition of the reconstitution fluid. There was no evident correlation between the degree of crystallinity and reconstitution time. While crystalline mannitol generally decreased reconstitution time by favorably affecting the cake attributes influencing reconstitution, it did not always reduce reconstitution time.

1. Introduction

Over the past decade, there has been a considerable rise in the number of biologics approved by the FDA [1]. Subcutaneous delivery is a common administration route for biological products and is often preferred over conventional intravenous delivery owing to convenient patient self-administration and reduced treatment costs [2,3]. The requirement for high doses, particularly for monoclonal antibodies (mAbs), coupled with the desire for subcutaneous delivery (injection volume limit < 1.5 ml) necessitates the development of certain biologics at protein concentrations higher than 100 mg/ml. Since the development of high concentration liquid protein products poses both in-process and storage stability challenges [3–5], lyophilization (freeze-drying) is commonly used to overcome these stability related issues [3,6,7]. Prior to patient administration, a lyophilized drug product is reconstituted into a solution by adding an appropriate fluid (usually Sterile Water for Injection). The endpoint of reconstitution as described by USP is the complete dissolution of solids after addition of the reconstitution fluid [8]. Rapid reconstitution is an important product quality attribute for lyophilized formulations. However, in the case of highly concentrated lyophilized proteins, reconstitution time is often long and variable. Reconstitution times of some FDA-approved high concentration lyophilized protein products range from < 5 min (Raptiva® [9], Nucala® [10]) to as long as 15–40 min (Ilaris® [11], Xolair® [12], Cosentyx® [13], and Cimzia® [14]). Long reconstitution times are undesirable in fast-paced clinical settings. Moreover, long and variable reconstitution of self-administered products can be dangerous, particularly if the patient attempts self-administration prior to complete reconstitution. As biosimilars and additional competing protein products come to market, reduced reconstitution time is expected to be a differentiating factor.

Research efforts have recently been directed towards understanding the causes of long reconstitution time and identifying ways to reduce it [15–19]. While evaluating multiple approaches to improve the

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Table 1
Prelyo solution compositions and solution characteristics.

Protein (mg/ml)	140[a]	127[b]	122[b]	117[b]	100[b]	70[b]
Trehalose (mg/ml)	40	36	35	33	29	30
Mannitol (mg/ml)	28	44	50	57	77	106
Sodium phosphate buffer (mg/ml)	1.3	1.2	1.1	1.0	0.9	0.6
Mannitol fraction (w/w relative to total solids)	0.13	0.21	0.24	0.27	0.37	0.51
Weight ratio (w:w) of mannitol to trehalose	0.7	1.2	1.4	1.7	2.7	3.5
Weight ratio (w:w) of protein to sugars	2.1	1.6	1.4	1.3	0.9	0.5
Total solids (% w/v)	20.9	20.8	20.8	20.8	20.7	20.7
Osmolality[c] (mOsmol/kg)	369 ± 1	414 ± 2	446 ± 0	524 ± 2	606 ± 1	979 ± 4

[a] As received from GSK.
[b] Prelyo solutions prepared by diluting the original 140 mg/ml solution as received from GSK with 20% w/v mannitol solution.
[c] Osmolality values were measured using a vapor pressure osmometer (Vapro®, Model 5520, Wescor Inc., Logan, UT, USA) and are reported as mean ± standard deviation (n = 3).

reconstitution of an Fc-fusion protein formulated with sucrose and mannitol, Cao et al. found that partially crystalline lyophilized cakes reconstituted in 3 min when compared to fully amorphous cakes which took as long as 13 min [15]. The authors attributed the significant reduction in reconstitution time to crystalline mannitol. They observed that during reconstitution, partially crystalline cakes "absorbed" the reconstitution fluid much faster than their amorphous counterparts. The authors surmised that partially crystalline cakes were more wettable, making the cake core more accessible to the reconstitution fluid. They also noted that sometimes the partially crystalline cakes disintegrated on exposure to the reconstitution fluid resulting in an increased surface area for faster dissolution. Although insightful, the inferences regarding the mechanisms by which crystalline mannitol lowered reconstitution time [15] were not supported by quantitative experimental data. Thus, underlying reasons for the improvement offered by crystalline mannitol are yet to be fully elucidated. Nonetheless, the work by Cao et al. pointed to a potential relationship between mannitol crystallinity and cake attributes such as cake wettability, penetration of reconstitution fluid into cakes and cake disintegration.

In the food industry, the important steps for reconstitution of milk, cocoa and whey protein powders as well as infant formulas have been identified as (a) powder wetting, (b) liquid penetration into the porous powder by capillary action leading to sinking of the powder into the reconstituting liquid, (c) disintegration of powder agglomerates into primary particles, and (d) dissolution of the primary particles [20–22]. Analogously, reconstitution of high concentration lyophilized protein formulations can be considered to depend on cake attributes such as (a) cake wettability, (b) liquid penetration to the cake interior, (c) cake dispersibility or disintegration upon fluid addition, and (d) dissolution of the disintegrated particles.

The primary goal of the present work was to better understand the role of crystalline mannitol in decreasing reconstitution time by comparing the cake attributes (e.g., wettability, penetration of reconstitution fluid into the cake, and disintegration) of both amorphous and partially crystalline lyophilized formulations. A second goal was to develop methods to quantitatively assess wettability of cake solids, liquid penetration through the cake and cake dispersibility or disintegration. Sane et al. introduced methods to quantify the wettability and disintegration rates of lyophilized cakes [18]. However, their methods relied on either formulations lyophilized in glass tubes (rather than in vials) or on samples that were crushed and removed from the vial for analyses. Hence, the objective behind developing newer methods was to reduce sample handling by making measurements on intact cakes without disrupting the cake structure during measurement. In doing so, the measured cake attributes would be more representative of the cake in the vial. This is especially relevant while studying reconstitution because cake structure has been pointed out as an important factor influencing reconstitution of high concentration proteins in amorphous cakes [16,17].

Mannitol crystallization in multicomponent systems comprising of a protein, lyoprotectant and mannitol is affected by both formulation composition [15,23,24] and processing conditions [23,25,26]. Hence, a range of formulations and freeze-drying cycles were used to obtain amorphous and partially crystalline cakes with the end goal of understanding the effect of crystalline mannitol on cake attributes influencing reconstitution. The percent solids in the cake has been proposed as a major factor governing reconstitution time [4,15,19,27]. In general, cakes with higher density have longer reconstitution times [4,27]. To eliminate the influence of cake density on reconstitution time, formulations were designed with the same total solids content (21% w/v) but with different compositions.

2. Materials and methods

2.1. Materials

β-D-Mannitol (catalog # M4125; Sigma-Aldrich, St. Louis, MO, USA) was used to prepare a 20% w/v solution, which was filtered through a 0.22 μm syringe filter (Millex®, MilliporeSigma, Burlington, MA, USA). The pre-lyophilization (prelyo) solution was provided by GlaxoSmithKline and consisted of 140 mg/ml recombinant protein in 40 mg/ml mannitol, 28 mg/ml trehalose and 10 mM sodium phosphate buffer (pH 7.0) with total solid content of 20.9% w/v. Portions of the 140 mg/ml prelyo solution were diluted with the filtered 20% w/v mannitol to obtain protein concentrations at 127, 122, 117, 100 and 70 mg/ml. The resulting prelyo solution compositions are listed in Table 1. All the solutions had approximately the same total solids at 21% w/v. The prelyo solution provided by GSK also contained 0.01% w/v polysorbate 80. Upon dilution with 20% w/v mannitol solution, the surfactant concentration was < 0.01%. Polysorbate 80, at concentrations up to 1%, has been shown to have no observable effect on reconstitution time [15]. Hence, the surfactant concentration in the formulations was considered negligible from a reconstitution point of view and has not been reported in Table 1.

2.2. Methods

2.2.1. Pre-lyophilization solution characterization

2.2.1.1. Determination of glass transition temperature of the freeze concentrates (T_g'). Aliquots of each solution (10–15 mg) were hermetically sealed in aluminum pans. Each solution was cooled to -50 °C at 1 °C/min, held for 30 min and then scanned at 10 °C/min to room temperature in a differential scanning calorimeter (DSC; Model Q1000, TA Instruments, New Castle, DE, USA). To study the effect of annealing on T_g', the solutions were cooled to −50 °C at 1 °C/min, heated to −20 °C at 1 °C/min, held at −20 °C for 12 h, then cooled back to −50 °C before scanning at 10 °C/min to room temperature. The midpoint of the glass transition was reported as T_g' (Table 2).