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Impact of Solid Content on the Bulk Properties of Lyophilized Powders

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Abstract:	Interest in oral delivery of biological drug products, commonly prepared through lyophilization, is surging. Typically, low solid content solutions are employed for lyophilization to enhance mass transfer and minimize drying time. Yet, this approach often results in lyophilized powders with low bulk density and poor flowability, challenging downstream processing steps that are required for oral product development. Increasing solid content in a starting solution can, in theory, increase the density of lyophilized cakes and powders with higher bulk density post-milling. However, the effectiveness of improving powder density and flowability using a higher solid content has not been experimentally verified. In addition, the impact of using a higher solid content on other physicochemical properties of lyophilized materials remains uncertain. To address the knowledge gaps, we lyophilized three common bulk cryoprotectants at two different solid contents (5% and 10%) and systematically evaluated their solid-state properties, bulk density, flowability, compaction characteristics, and physical stability. We found that powders prepared at a higher solid content (10%) exhibited higher bulk density, but they still failed to meet the requirements for easy oral product development. A change in solid content also leads to different solid-state properties, compaction behaviors, and stability, highlighting the importance of thorough characterization of lyophilized materials when solid content is changed in the course of oral solid dosage formulation development.
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All changes in the manuscript text are highlighted in red for easy identification.

Reviewer #1:

Reviewer's comment: 1. Graphical abstract misleading and low-effort. Lyophilization AND milling result in the different bulk density properties, not lyophilization alone.

Author's response: The milling step was added to the GA.

Reviewer's comment: 2. Line 50-52 inaccurate. Solid content not primary way to influence supercooling/nucleation temperature. Also, larger pores enable faster mass transfer, not heat transfer.

Author's response: In the main text, we have revised this sentence. "For a specified formulation and lyophilization process, using a low solid content leads to larger pores, faster mass transfer, and shorter drying times."

Reviewer's comment: 3. Table 1 and much of the introduction on biologics and biologic products is not relevant to the content of the manuscript. Table 1 also includes several products that are not orally delivered as stated in table caption.

Author's response: Table 1 and the introductory section on oral biologics were removed from the manuscript.

Reviewer's comment: 4. Line 94 Need many more details on the milling of lyophilized powders. How did you mill? Was an automated system used or by hand? What determined when a powder was sufficiently milled?

Author's response: The milling process parameters were added.

Reviewer's comment: 5. Line 111-113 It is fine to only evaluate up to 10% solid content, but it is not accurate to say the lyophilization process is too resource intensive above that. You have already said this is a conservative FD cycle. It is very possible optimize for higher solid content mannitol within 75 hours.

Author's response: Agreed. It has been revised accordingly. "When the solid content of mannitol exceeds 10%, the primary drying process necessitates more than 75 hours following this drying process. Hence, a higher solid content solution was not evaluated. However, the drying time can be shortened by optimizing the primary drying process."

Reviewer's comment: 6. Figure 2 caption should include that moisture measured after 6 hours at 40% rh.

Author's response: Revised as suggested.

Reviewer's comment: 7. Line 246-247 Different polymorph compositions is interesting! There is no discussion of this later or theories given for why different polymorph composition was observed.

Author's response: We concur with the reviewer that the variations in mannitol's polymorphic compositions due to solid content are intriguing. However, literature has extensively documented how these compositions are influenced by the lyophilization process (*Pharm Res* **30**, 131–139 (2013); *J Pharm Sci*, **87**, 931-935 (1998); PDA J Pharm Sci Technol, 54, 13-22 (2000)). Although our current study reveals that polymorph formation is influenced by solid content, factors like cooling rate, annealing conditions, drying temperatures, and the presence of additives such as excipients and drug substances can change the outcomes. A more detailed discussion of is observation is outside the scope of our current study.

Reviewer's comment: 8. Line 251-253 Why does amorphous SUC/TRE explain higher moisture content? Source?

Author's response: The references have been added to the manuscript.

Reviewer's comment: 9. The interpretation and analysis of the experimental data in the results and discussion is excellent, but I am missing how all the details of the various solid state characterizations influence your conclusions. Spend more time on the characteristics that end up connecting to the desired powder properties like crystalline/amorphous in Fig. 9 / Table 4.

Author's response: In conclusion, solid content impacts the properties of lyophilized powders: mannitol shows varied polymorph compositions, sucrose exhibits complex thermal behaviors and water sorption properties, and trehalose displays distinct glass transition temperatures and stabilities, according to solid state characterization studies. Figure 9 and Table 4 illustrate the compaction properties of these powders compared to their crystalline counterparts. The enhanced tabletability of lyophilized sucrose and trehalose stems from their amorphous states. For mannitol, the improved tabletability is linked to particle morphology changes or polymorphic transformations. Considering the complexity of their compaction behaviors, we prefer not to correlate these observations further with solid state properties. Please add any suggestions or comments to expand on this discussion.

Reviewer's comment: 10. Line 467 Still not sure what biologics have to do with this manuscript.
Author's response: replaced the 'biologics' with 'lyophilized products'.

Reviewer #2:

Reviewer's comment: 1. The manuscript is very well written addressing the gaps in understanding the bulk properties and processing of solids obtained through lyophilization.
The manuscript in its current form is acceptable in my perspective.
Author's response: We thank the reviewer for the positive comments.

Reviewer #4:

Reviewer's comment: 1. Table 1: even though the table caption mentions orally delivered pharmaceutical peptides and other biological drugs, there are products that are delivered through the rectal and vaginal routes too. Suggest to either modify heading or remove those irrelevant examples

Author's response: Removed Table 1.

Reviewer's comment: 2. Line 90, "which ensured the product's temperature to reach the shelf temperature", please check for English

Author's response: Primary drying of the frozen solution occurred at -25 °C for 75 hours to ensure the product's temperature reached the shelf temperature.

Reviewer's comment: 3. Line 94, please elaborate on the milling process, including type of mill used, milling time, rpm etc.

Author's response: Added as suggested.

Reviewer's comment: 4. Fig 2, how many repeats were performed for each sample? Was n=1?

Author's response: Yes, N=1 for the presented data. We tested the lyophilized sucrose powders in triplicate and obtained consistent results.

Reviewer's comment: 5. Line 235, "lyophilized sample may indicates the presence of a higher fraction", please fix grammar

Author's response: Revised.

Reviewer's comment: 6. Line 268-280, (1) if the authors want to find the Tg during the 1st heating cycle but do not have modulated DSC, what can be done is to seal the pan

hermetically - that way, no moisture can escape and the Tg of the 1st cycle can be determined, but it would be the "wet" Tg. (2) I am very intrigued by the different Tgs obtained during the second heating cycle. Please explain how the pans were quenched to 15°C and if possible, provide the thermogram of the cooling cycle. Tg can be observed both during heating and cooling - what was the cooling Tgs for these 2 samples?

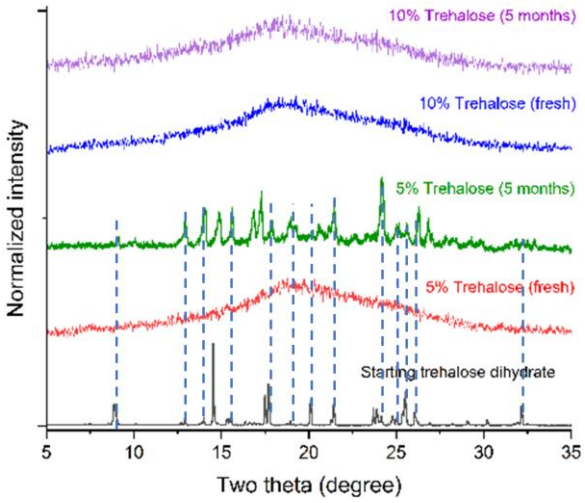
Author's response: In the first heating cycle, a pinhole was introduced to eliminate water, ensuring accurate measurements of the dry Tg. The samples cooled from 40 ℃ to target temperatures (0℃ or 15℃) at a rate of 10 ℃/min, but no distinct Tg was observed due to the baseline fluctuation.

Reviewer's comment: 7. Fig 6 and 13, the resolution of the scale bar needs to be improved.

Author's response: The scale bar has been added to Figure 6 and 13.

Reviewer's comment: 8. Fig 11, the PXRD patterns for trehalose dihydrate and the 5 month 5% sample do not exactly look very similar. Please explain how the conclusion that it's the dihydrate polymorph was made.

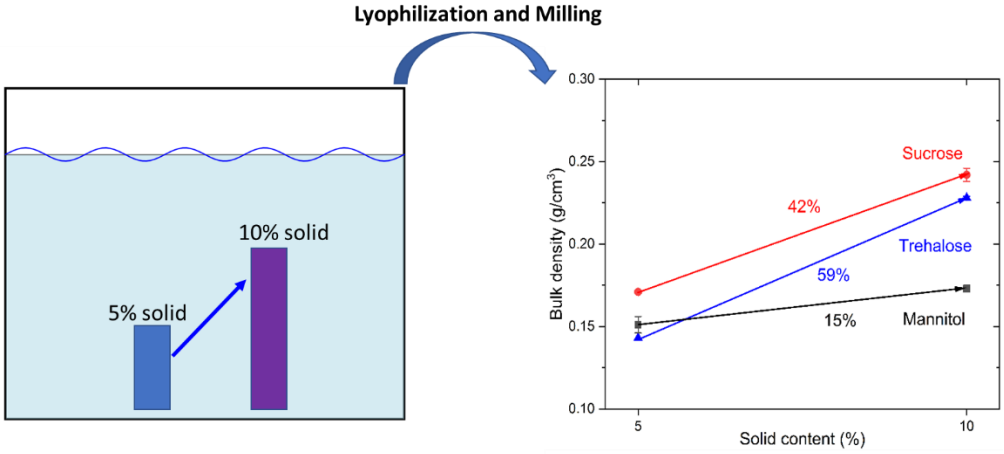
Author's response: The 5% trehalose sample showed significant crystallization, as evidenced by sharp XRD peaks similar to those of trehalose dihydrate, according to its PXRD pattern. Variations in peak intensity were due to preferred orientation, exacerbated by the large size of the initial trehalose dihydrate crystals.



Reviewer's comment: 9. For tableting studies, how many repeats were performed? Both Fig 9 and 10 don't have (visible) error bars.

Author's response: Each point in Figures 9 and 10 represents a single tablet.

Graphic abstract



Solid-state properties, compaction properties and physical stability are also changed!

Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

C Wang and H Zhang report a relationship with Evelo Biosciences Inc that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Impact of Solid Content on the Bulk Properties of Lyophilized Powders

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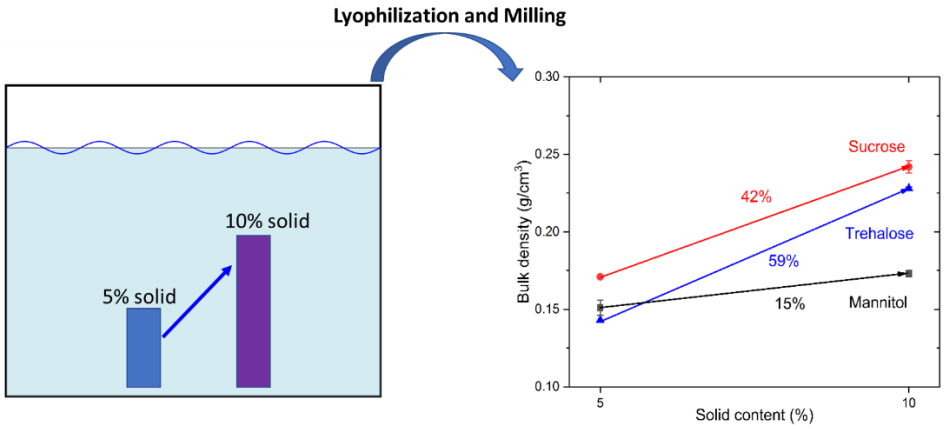
Abstract

Interest in oral delivery of biological drug products, commonly prepared through lyophilization, is surging. Typically, low solid content solutions are employed for lyophilization to enhance mass transfer and minimize drying time. Yet, this approach often results in lyophilized powders with low bulk density and poor flowability, challenging downstream processing steps that are required for oral product development. Increasing solid content in a starting solution can, in theory, increase the density of lyophilized cakes and powders with higher bulk density post-milling. However, the effectiveness of improving powder density and flowability using a higher solid content has not been experimentally verified. In addition, the impact of using a higher solid content on other physicochemical properties of lyophilized materials remains uncertain. To address the knowledge gaps, we lyophilized three common bulk cryoprotectants at two different solid contents (5% and 10%) and systematically evaluated their solid-state properties, bulk density, flowability, compaction characteristics, and physical stability. We found that powders prepared at a higher solid content (10%) exhibited higher bulk density, but they still failed to meet the requirements for easy oral product development. A change in solid content also leads to different solid-state properties, compaction behaviors, and stability, highlighting the importance of thorough characterization of lyophilized materials when solid content is changed in the course of oral solid dosage formulation development.

Key words: Lyophilization, solid content, density, flowability, compaction properties, physical stability, mannitol, sucrose, trehalose

Graphic abstract



Solid-state properties, compaction properties and physical stability are also changed!

1 Introduction

The lyophilization process is frequently used in solidifying delicate drug substances for improved stability, particularly in the production of biologicals (Allmendinger et al., 2023). The concentration of solids in a solution prior to lyophilization, termed solid content, significantly influences both the design of the lyophilization process and the performance of final lyophilized cakes (Tang and Pikal, 2004). For a specified formulation and lyophilization process, using a low solid content leads to larger pores, faster mass transfer, and shorter drying times. However, the resultant lyophilized cake of a drug substance is highly porous, which leads to a milled powder with sub-optimal bulk density and poor flowability. For example, most lyophilized powders incorporating soluble excipients exhibit a bulk density under 0.2 g/mL (Table S1).

Low bulk density and poor flowability of the lyophilized powders limit drug dose attainable by direct encapsulation and prevent successful commercial tablet manufacturing on a high-speed rotary press (Leane et al., 2015; Leane et al., 2018). This poses a challenge to the oral delivery of biological drug product development. Intuitively, a higher solid content in the starting solution is expected to form a proportionally denser cake and a milled powder with higher bulk density and better flowability. However, the effectiveness of this strategy for improving powder density and flowability has not been demonstrated. In addition, the structure and properties of a lyophilized product are impacted by size distribution and morphologies of ice crystal in the frozen solution, which are affected by solid contents. Therefore, the impact of increasing solid content on other physicochemical properties remains unknown. These knowledge gaps present a barrier for successful development of oral drug products (both capsules and tablets) for biologicals using lyophilized drug powders.

According to the Materials Science Tetrahedron (Sun, 2009), gaining a thorough understanding of the impact of solid content on various properties of lyophilized powders, e.g., density, flowability, compaction characteristics, and physical stability, is essential for their successful development into oral dosage forms. This work seeks to assess the potential impact of solid content on the properties of lyophilized powders, employing three widely used bulk cryoprotectants, i.e., mannitol, sucrose, and trehalose.

2 Materials and methods

2.1 Materials

Starting crystalline mannitol (Pearlitol® 50C, Roquette, Lestrem, France), sucrose (Sigma Aldrich, St. Louis, MO, USA), and trehalose dihydrate (Swanson, Fargo, ND, USA) were used as received.

2.2 Methods

2.2.1 Lyophilization and milling

One-liter solutions of mannitol, sucrose, and trehalose in distilled water with solid contents of either 5% or 10% were passed through a 0.2 µm filter before being poured into trays to form a layer with approximately 1 cm depth. Thermocouples were placed at the center bottom of each tray to monitor product temperature throughout the freeze-drying process. Initially, samples were equilibrated for half an hour at 4 ℃ on the freeze-dryer shelf. Then, they were cooled down to -40 ℃ at a rate of 10 ℃/h and held at -40 ℃ for 10 min to allow completion of the ice crystallization process (phase I). Primary drying of the frozen solution occurred at -25 ℃ for 75 hours to ensure the product's temperature reached the shelf temperature. The secondary drying process was carried out at 15 ℃ for the next 17 h (phase III), after which the temperature was increased to 25 ℃. Throughout the entire process, the vacuum pressure was maintained at 100 mTorr.

Lyophilized cakes were milled with a Quadro comil (model 197) equipped with a round bar impeller and a round hole cone mill screen (4L055R1008552B) at a speed of 2200 rpm. Before the density measurement, the powders were passed through a 35-mesh screen, and collected for further characterization. “5%” or “10%” are embedded in sample IDs of lyophilized powders to signify corresponding solid content used to prepare a sample.

A freeze-drying cycle for a 5% mannitol solution is shown in Figure 1a. In the primary drying phase, it is crucial to maintain the product temperature below a critical temperature to avoid cake collapsing. The collapse itself does not necessarily affect the stability of the biological formulation, but it suggests inadequate control of the manufacturing process and can lead to product rejections. Thus, a conservative cycle with a low temperature was used in this work to ensure the formation of elegant cakes (Figure 1b).

Drying time was estimated by the time taken for the shelf temperature to reach the target temperature, and the end of the drying occurs when the product temperature matches the shelf temperature (see Figure 1a**)**. A solution with a lower solid content forms larger, interconnected ice crystals, which exhibit lower resistance to water vapor transfer, leading to more efficient mass/heat exchange during drying. At a higher solid content, a longer drying time became necessary. For this reason, both primary and secondary drying times for the 10% solid content samples significantly exceeded those of corresponding 5% solid content samples (Table 1). For instance, the total drying time (primary plus secondary drying) for 5% mannitol was 18 h shorter than that for 10% mannitol. When the solid content of mannitol exceeds 10%, the primary drying process necessitates more than 75 hours following this conservative drying process. Hence, a higher solid content solution was not evaluated. However, the drying time can be shortened by optimizing the primary drying process.

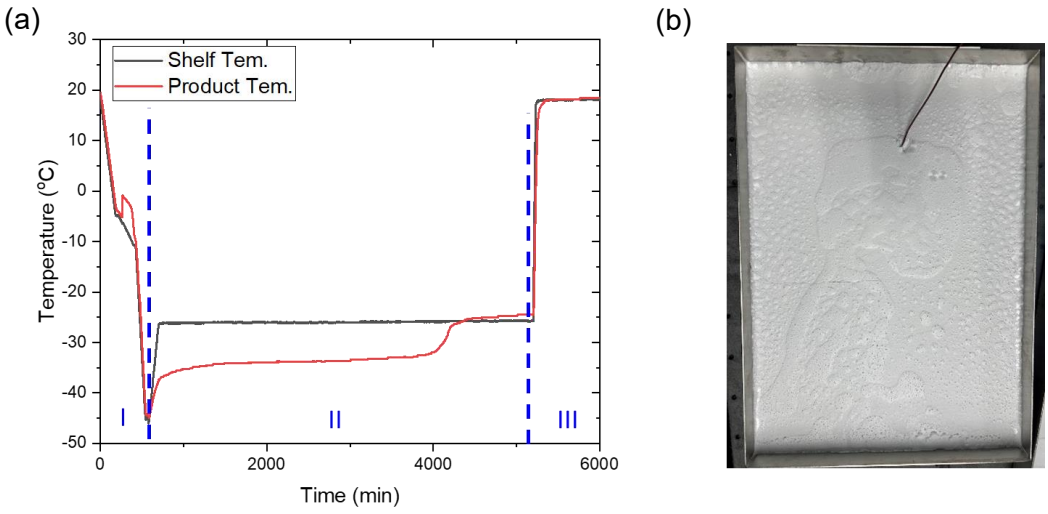


Figure 1. a) Freeze-drying cycle profile featuring three phases for a 5% mannitol solution (Phase I – freezing; Phase II – primary drying, Phase III – secondary drying). Shelf temperature profile is shown as black line and product temperature is shown as red line, b) resulting cake.

Table 1. Primary and secondary drying times of lyophilized samples.

	5% mannitol	10% mannitol	5% sucrose	10% sucrose	5% trehalose	10% trehalose

Primary drying time (h)	59	72.5	51.8	57.6	61.3	71.0
Secondary drying time (h)	1.5	6.4	3.3	4.8	4.1	6.4

2.2.2 Powder X-ray diffractometry (PXRD)

PXRD patterns of all powders were obtained using an X-ray diffractometer (X'pert Pro; PANalytical, Westborough, MA, USA) with Cu Kα radiation (λ = 1.540598 Å). Samples were scanned between 5°–35° 2θ angles with step size 0.016° and a dwell time of 1 s. Tube voltage and amperage were set as 45 kV and 40 mA, respectively.

2.2.3 Differential scanning calorimetry (DSC)

Approximately 5 mg of each powder was loaded into a Tzero hermetically sealed aluminum pan with a pinhole for analysis using a differential scanning calorimeter (Q1000; TA Instruments, New Castle, DE, USA) at a heating rate of 10 °C/min under continuous nitrogen purge at a flow rate of 25 mL/min. Different ranges of experimental temperature were chosen for the three materials based on their crystallization and melting temperatures, i.e., – 50 °C to 180 °C for lyophilized mannitol powders; 0 °C to 200 °C for lyophilized sucrose powders, and 15 °C to 220 °C lyophilized trehalose powders (two heating cycles).

2.2.4 Polarized light microscopy (PLM)

All powder samples were dispersed in silicone oil between a glass slide and a cover glass and observed under a polarized light microscope (Olympus BX51, Japan). Images were captured with a digital camera (AmScope, USA) at 10X and 40X magnifications.

2.2.5 Scanning electron microscopy (SEM)

Particle size and shape were assessed using a Phenom XL desktop scanning electron microscope (Thermal Fisher Scientific, Waltham, MA, USA) operating at an excitation voltage of 10 kV under low vacuum mode. Specimens were affixed to a copper stage and observed without coating.

2.2.6 Water content and dynamic vapor sorption (DVS)

Water content and moisture sorption of all lyophilized powders were analyzed using an automated vapor sorption analyzer (Intrinsic, Surface Measurement Systems Ltd., Allentown, PA, USA) at 25 °C. Nitrogen flow rate was 50 mL/min. Approximately 10 mg of each powder was first

equilibrated at 40% RH (mimicking ambient humidity) for 6 h. Then, the sample was exposed to 0% RH over a period of 2 h. Water content was calculated from the change in sample weight from 40% RH to 0% RH. Next, the sample was then exposed to a series of RHs from 0% to 90% with a step size of 10% RH. At each specific RH, the equilibration criterion was dm/dt ≤0.002% with a minimum equilibration time of 0.5 h and a maximum equilibration time of 6 h. The RH was changed to the next target value when one of the criteria was met.

2.2.7 Densities

Bulk and tapped densities of all lyophilized powders were measured (n = 3) using a TD1 Tap Density Tester (SOTAX, Hopkinton, MA, USA), following method 1 in USP <616> (USP, 2024). True density (ρ_t) of all samples was measured using a helium pycnometer (Quantachrome Instruments, Ultrapycnometer 1000e, Byonton Beach, FL, USA). An accurately weighed sample was placed into the sample cell, occupying approximately half to three-quarters of the cell volume. The measurement was concluded once the standard deviation of five successive measurements was less than 0.005% and the mean of the last five measurements was taken as the sample's true density.

2.2.8 Flow properties

Carr's index and Hausner ratio were calculated from bulk density (ρ_{bulk}) and tapped density (ρ_{tap}) using Eq. (1) and (2), respectively.

$$Carr's\ index\ (\%) = \frac{\rho_{tap} - \rho_{bulk}}{\rho_t} \times 100\% \quad (1)$$

$$Hausner\ ratio = \frac{\rho_{tap}}{\rho_{bulk}} \quad (2)$$

A ring shear cell tester (RST-XS, Dietmar Schulze, Wolfenbüttel, Germany), with a 30 mL cell, was also used to conduct powder flow testing (n = 3) at a pre-shear normal stress of 3 kPa, following a standard 230 method (Wang et al., 2022). The shear cell was over filled with a powder under investigation and excess powder was gently scraped off using a spatula to obtain a surface flush with the upper edge of the shear cell. Attention was paid to prevent compression or agitation of the powder bed when loading powder and removing excess powder.

2.2.9 Compaction properties

Starting crystalline sucrose was milled into smaller particles using a mortar and pestle. All powders were passed a 125 μm sieve (mesh 120) to minimize possible effects of particle size on compaction properties. A series of tablets with approximately 150 mg of each powder were compressed at various pressures (10 MPa to 350 MPa) on a compaction simulator (Styl'One, Medelpharm, Beynost, France), simulating a Korsch XL100 press. Forces exerted on the upper and lower punches were recorded using load cells, and the punch displacement was tracked using incremental sensors. Round flat-faced punches (8mm diameter) were used for tablet compaction, which was carried out at both low speed (dwell time of 98 ms or 21 rpm) and high speed (dwell time of 20 ms or 101 rpm). MgSt spray (Styl'One Mist) was applied to punch tips and die wall as an external lubricant before each compaction. Tablet dimensions were measured using a digital caliper to calculate tablet envelope density. Tablets were broken diametrically using a texture analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY, USA) at a speed of 0.002 mm/s with a 5 g trigger force. Tablet tensile strength (σ), was calculated using Eq. (3) from the breaking force (F), tablet diameter (d), and tablet thickness (h), following a standard procedure (Fell and Newton, 1970).

$$\sigma = \frac{2F}{\pi d h} \tag{3}$$

In-die elastic recovery (% IER) of tablet was determined from the minimum tablet thickness under compression (h_1) and tablet thickness at the end of the decompression (h_2) using Eq. (4).

$$IER\ (\%) = \frac{h_2 - h_1}{h_1} \times 100\% \tag{4}$$

Tablet porosity (ε) was calculated from tablet envelope density (ρ) and true density (ρ_t) of powder using Eq. (5).

$$\varepsilon = 1 - \frac{\rho}{\rho_t} \tag{5}$$

In-die Heckel analysis was conducted following a standard procedure (Vreeman and Sun, 2021), where the linear portion of the in-die tablet porosity, ε, vs. P plot was analyzed based on the Heckel Eq. (6), to obtain in-die mean yield pressure ($P_{y,i}$) (Heckel, 1961a, b).

$$-\ln(\varepsilon) = \frac{1}{P_{y,i}} P + A \quad (6)$$

Strain rate sensitivity (SRS) was calculated from $P_{y,i}$ values obtained at low speed ($P_{y,l}$) and obtained at high speed ($P_{y,h}$) using Eq. (7) (Roberts and Rowe, 1985).

$$SRS\ (\%) = \frac{P_{y,l} - P_{y,h}}{P_{y,l}} \times 100\% \quad (7)$$

2.2.10 Physical stability under storage

All lyophilized powders were stored under ambient conditions for 5 months, during which environmental humidity varied from 10% RH to 40% RH. To assess sample stability, PXRD patterns and PLM and SEM images of fresh samples and samples after storage were compared for signs of instability. Additionally, changes in bulk powder appearance after storage were captured using a digital camera (Canon EOS R50, Japan).

3 Results and discussions

3.1 Solid state characterization

The freshly lyophilized powders have a moisture content of less than 2%. However, upon reaching equilibrium at RH 40% for 6 hours, the moisture content of lyophilized powders followed a descending order of trehalose > sucrose >> mannitol (Figure 2). 5% lyophilized sucrose and trehalose exhibited slightly higher water content than their corresponding 10% lyophilized samples. This difference may be attributed to the fact that a lyophilized powder from a higher solid content has fewer or smaller pores and smaller specific surface area, which is translated into a lower tendency to adsorb moisture.

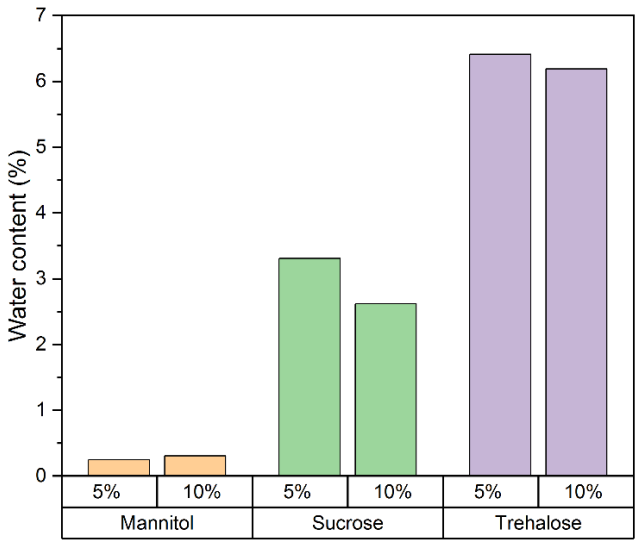


Figure 2. Moisture contents of mannitol, sucrose, and trehalose powders lyophilized from 5% and 10% solid solutions and stored at 40% RH after 6 hours (n=1).

Anhydrous crystalline mannitol can exist in three known polymorphs (α, β, and δ forms), with the β form being the most thermodynamically stable at room temperature (Pitkänen et al., 1993; Smith et al., 2017). The 2θ positions of characteristic peaks for mannitol polymorphs are identified as 13.6° and 17.3° for the α form (Fronczek et al., 2003), 14.6°, 16.8°, and 23.4° for the β form (Berman et al., 1968), and 22.3° for the δ form (Fronczek et al., 2003). During the freezing stage, the only exothermic event at -4 °C is attributed to crystallization of ice (Figure 1a). The starting crystalline mannitol used in this work is a mixture of α and β forms (Figure 3a). The lyophilized mannitol powders remain crystalline as shown by sharp PXRD peaks (Figure 3a), which show that the 5% lyophilized mannitol is a mixture of α, β, and δ forms, while the 10% lyophilized mannitol only contains the α and δ forms. The relatively higher intensity of α characteristic peaks in the 5% lyophilized sample suggests a higher content α form. In the DSC thermograms, both lyophilized mannitol samples showed a small endothermic peak immediately followed by an exothermic peak in the temperature range of 150 °C - 157 °C (Figure 4a), which is consistent with a previous study (Yoshinari et al., 2002). These events are attributed to the events of melting of the δ form, followed by recrystallizing to the β form. The larger value of heat of fusion of the δ form for the 5% lyophilized mannitol (5.613 J/g) than the 10% lyophilized mannitol (1.676 J/g) suggests a larger proportion of the δ form in the 5% lyophilized mannitol powder. Both the 5% and 10% lyophilized mannitol samples exhibited prominent endothermic peaks at ~167 °C, corresponding to melting of α form, β form of mannitol, or both (Figure 4a). Melting events for

the α and β forms could not be discerned solely from DSC profiles because of the close proximity of their melting points (α: 166 °C; β: 167 °C) (Paul et al., 2015; Pitkänen et al., 1993). The different polymorph compositions in the two lyophilized mannitol samples are expected to contribute to disparities in their bulk properties.

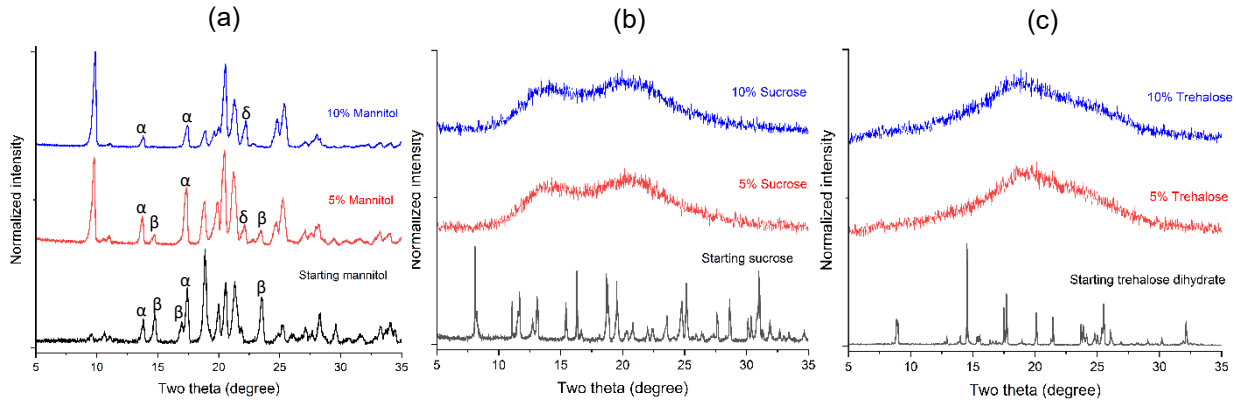


Figure 3. PXRD patterns of freshly prepared lyophilized powders and their corresponding crystalline form, a) mannitol; b) sucrose; c) trehalose.

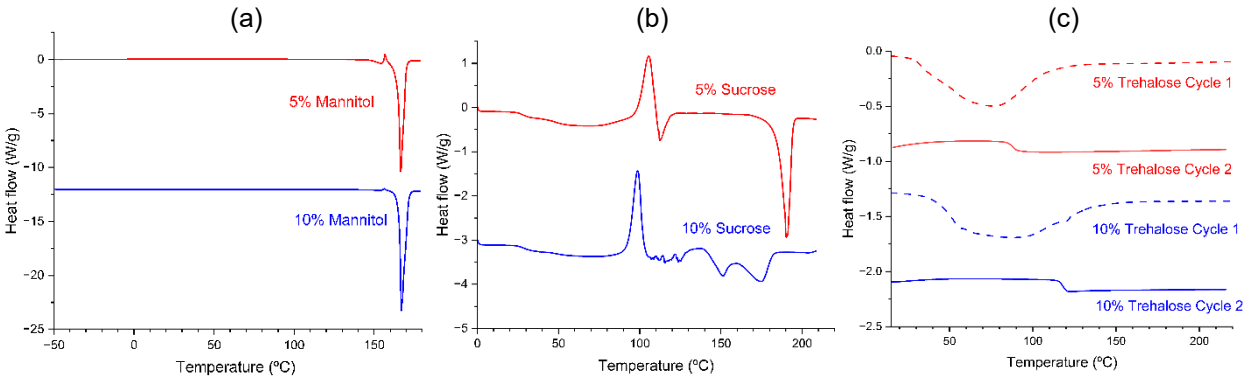


Figure 4. DSC heating profiles of lyophilized powders, a) mannitol; b) sucrose; c) trehalose.

The PXRD patterns of all lyophilized sucrose and trehalose samples exhibited broad halos without sharp peaks (Figure 3b, 3c), indicating an absence of detectable crystalline phases. The amorphous nature of the sucrose and trehalose sample explains their relatively much higher moisture contents (Iglesias et al., 1997; Yu et al., 2008) than the crystalline lyophilized mannitol powders (Figure 2). The thermal properties of sucrose samples prepared using different solid contents were distinct, as revealed by their DSC thermograms (Figure 4b). The lower T_g values of

sucrose powders than the literature values (T_g > 50 °C) (Hancock and Zografi, 1994; Imamura et al., 2010) are attributed to the higher moisture contents within the samples in this work (Figure 2). Moisture loss above T_g leads to a broad endotherm (or downward baseline shift) before the crystallization event at ~ 90 °C, as evidenced by an exothermic peak. In the case of 5% sucrose, the endothermic event at 190 ℃ following the crystallization event is assigned as melting since it falls in the reported melting point range for sucrose, 160 °C - 192 °C (Beckett et al., 2006; Hurtta et al., 2004; Okuno et al., 2003; Roos, 1993). The 10% sucrose sample exhibited complex thermal behaviors after the crystallization event, featured as multiple thermal events in the temperature range 105 °C - 180 °C. It was reported that multiple endothermic peaks of crystalline sucrose could be induced by decomposition of sucrose before melting, where the decomposition products may serve as a solvent to dissolve sucrose crystals, leading to an endothermic peak (Beckett et al., 2006; Lee et al., 2011; Roos, 1993; Schmidt et al., 2012).

Both trehalose samples exhibited a broad endothermic peak in the 30 - 120 °C temperature range (Figure 4c), due to the evaporation of residual water in the samples, which masked the potential T_g of lyophilized trehalose powders. Since no crystallization or melting event was observed up to the known melting point of 203 °C for crystalline trehalose (Sussich et al., 1998), both dehydrated trehalose samples remained amorphous. This offers an opportunity to measure the T_g value by performing a second thermal scan of a sample dried *in situ*. Indeed, distinct T_gs were observed in both samples (91 °C for the 5% trehalose sample and 118 °C for the 10% trehalose sample) by quenching the cell to 15 °C after the first thermal scan and then heating at 10 ºC/min (Figure 4c). The different T_g values suggest two possible amorphous states of trehalose since different extents of plasticization by moisture can be excluded as a reason for different T_g*s*. However, both values fall in the reported range of T_g (75 to 120 °C) for amorphous trehalose (Roe and Labuza, 2005; Sussich and Cesàro, 2008). Again, no crystallization or melting events were observed after T_g for these samples when heated to 220 °C (above the melting point of trehalose).

The PLM results showed that mannitol particles exhibited birefringence (Figure 5a), which is a characteristic of crystalline phases. On the contrary, no birefringence was observed in sucrose and trehalose samples (Figure 5b, c), which is consistent with their amorphous nature shown by their PXRD patterns (Figure 3b, c). SEM images revealed that all lyophilized samples consisted of thin-flakes, which is distinct from the block-shaped starting crystalline particles (Figure 6). Notably, the rough surface of lyophilized mannitol particles (Figure 6a) is reminiscent of

crystallization, whereas the smooth particle surfaces of lyophilized sucrose and trehalose particles (Figure 6b, c) are consistent with an absence of crystallization.

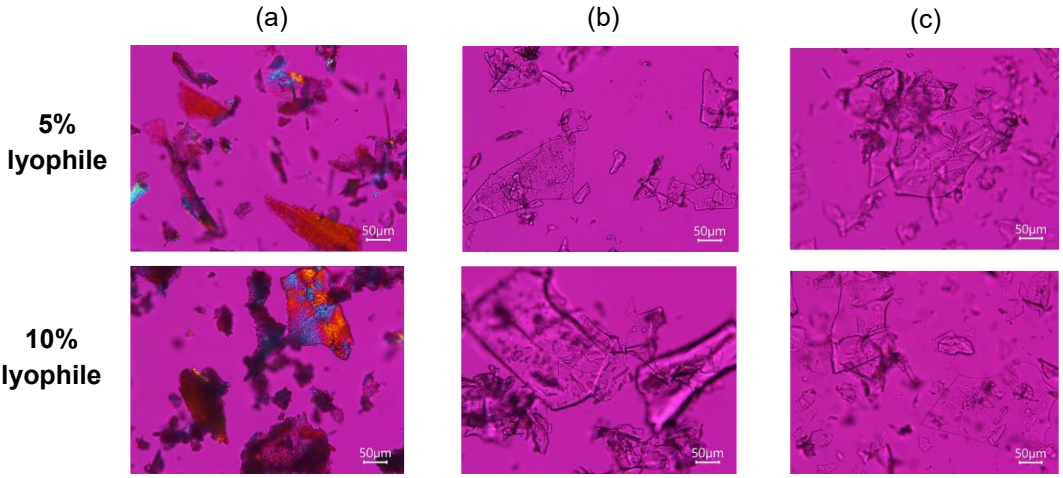


Figure 5. PLM images of fresh lyophilized particles, a) mannitol; b) sucrose; c) trehalose

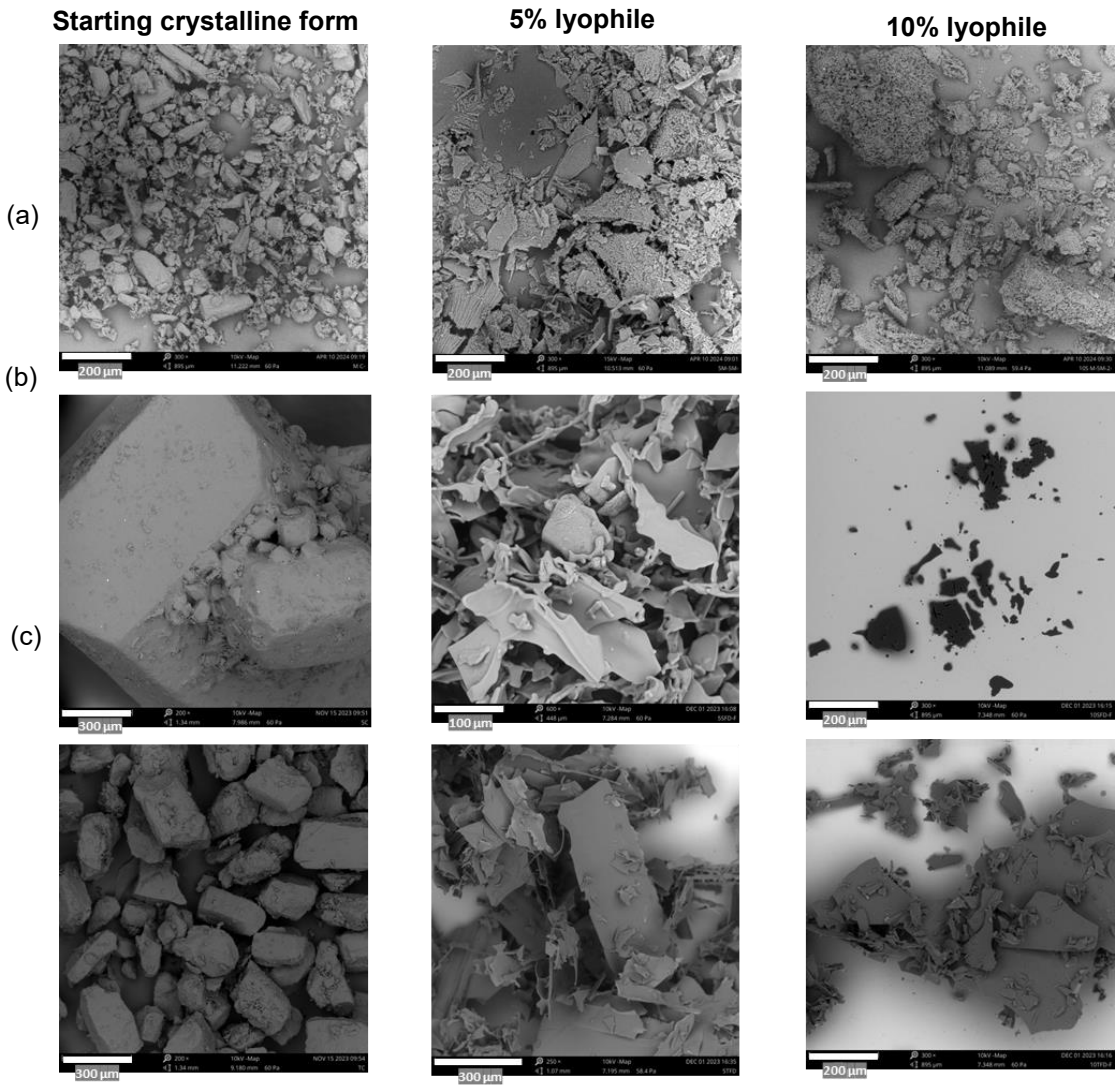


Figure 6. SEM images of starting crystalline and lyophilized powders from solutions of different solid contents for a) mannitol, b) sucrose and c) trehalose.

Both lyophilized mannitol samples gradually gained ~2% weight when RH increased from 0% to 90%, indicating limited surface adsorption of moisture (Figure 7a). Two lyophilized sucrose powders absorbed a negligible amount (~0.1%) of water up to 80% RH but gained a substantial amount of moisture (22.0% for 5% solid content sample and 28.5% for 10% solid content sample) at 90% RH (Figure 7b). The low moisture sorption observed at RH < 80% is unexpected for hydrophilic amorphous materials. However, this observation is in line with the widely observed shape of adsorption isotherms for both amorphous and crystalline sugars (Mathlouthi and Rogé, 2003).

The 5% lyophilized trehalose sample absorbed water rapidly with increasing RH and converted into trehalose dihydrate at 50% RH, corresponding to an approximately 10% weight gain (Figure 7c). The 10% trehalose sample absorbed less water than the 5% trehalose sample at RHs <90%. The amount of water adsorbed by this sample also increased rapidly up to 50% RH but dropped at 60% RH and then maintained relatively constant up to 80% RH. A drop in absorbed water at a higher RH indicates crystallization of amorphous materials. Since the plateau value of ~6% water content is close to that in a monohydrate (4.75%), the crystalline phase is likely a monohydrate (Figure 7c). The sharp increase in weight at 90% RH indicates possible deliquescence or conversion into the dihydrate form. Although interesting, no further efforts were made to elucidate the phase nature and phase transformation of sucrose and trehalose samples since these aspects were outside the scope of this project.

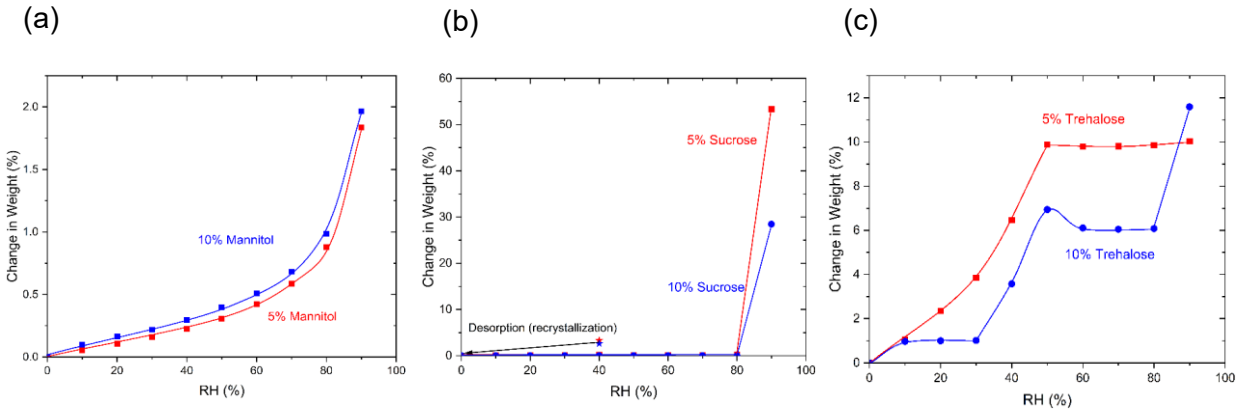


Figure 7. DVS plots of lyophilized a) mannitol, b) sucrose, and c) trehalose.

3.2 Densities and flowability

Bulk density is an important powder property that plays a critical role in the development of pharmaceutical solid products. A low powder bulk density can adversely affect drug loading, content uniformity, manufacturing efficiency, and flow properties (Leane et al., 2015). Poor flow properties are detrimental to downstream processing, such as blending, granulation, compression, and encapsulation (Guerin et al., 1999). Thus, a high bulk density of APIs is typically preferred when processing pharmaceutical powders. For the three 5% solid content lyophilized powders, bulk densities were all less than 0.171 g/cm^3 (Table 2), which is common for lyophilized powders (Table S1). With increasing solid content, the bulk densities of three materials were increased to different extents, e.g., mannitol (14.6%), sucrose (41.5%), and trehalose (59.4%). However, even

the highest bulk density of 0.242 g/cm^3 (10% Sucrose) is still likely too low for efficient downstream processing considering the role of particle density on flow. In a prior study involving a range of mannitol solutions with variable solid contents from 1% to 15%, a linear relationship between solid contents and bulk densities was observed (Kaialy et al., 2016). Nevertheless, the maximum bulk density for 15% lyophilized mannitol was 0.11 g/cm^3, which is notably lower than the 5% lyophilized mannitol powder in this work. This discrepancy is likely caused by the different freezing steps in the two research endeavors.

Powder flow initiates when the cohesive forces between particles are overcome by external forces, such as gravitational force. Due to the low density of lyophilized powders, the cohesive forces, consisting of *van der* Waals forces, electrostatic forces, and hydrogen bonding, become stronger than the gravitational force. Consequently, particles tend to form agglomerates and resist flowing. Additionally, the flake-shaped lyophilized particles tend to interlock with each other during packing, further hindering powder flow. This speculation was examined by measuring established flow parameters of these powders.

Carr's index and Hausner ratio were used to characterize powder flowability (Table 2), where a higher Carr's index or Hausner ratio value suggests poorer flowability (Tan et al., 2015; Tharanon et al., 2024). All lyophilized powders in this work, despite different bulk densities, belong to the category of "approximately no flow" by the measure of both Carr's index (> 38%) and Hausner ratio (> 1.60). The poor flowability of these lyophilized powders is also confirmed by shear cell data (Figure 8), which put them into the class of either "very cohesive" (flowability index: 1 – 2) or "cohesive" (flowability index: 2 – 4) powders. The flowability of all these powder was also much poorer than Avicel PH102 (flowability index: ~ 8 at 3 KPa), which is considered as a reference material exhibiting minimum flowability required for high speed tableting (Sun, 2010). Only lyophilized trehalose powder showed a slight increase in flowability index with the 10% solid content sample. No significant difference in flowability index between two solid contents was observed for lyophilized mannitol and sucrose powders.

Table 2. Densities and flowability parameters of the materials studied in this work.

Material	Solid content	ρ_{bulk} (g/cm^3)	ρ_{tap} (g/cm^3)	Carr's index (%)	Hausner ratio
Mannitol	5%	0.151 (0.005)	0.258 (0.003)	41.5	1.7

Sucrose	10%	0.173 (0.002)	0.303 (0.004)	43.0	1.8
	5%	0.171 (0.001)	0.341 (0.001)	49.9	2.0
	10%	0.242 (0.004)	0.443 (0.012)	45.4	1.8
Trehalose	5%	0.143 (0.000)	0.278 (0.002)	48.6	1.9
	10%	0.228 (0.001)	0.419 (0.002)	45.6	1.8

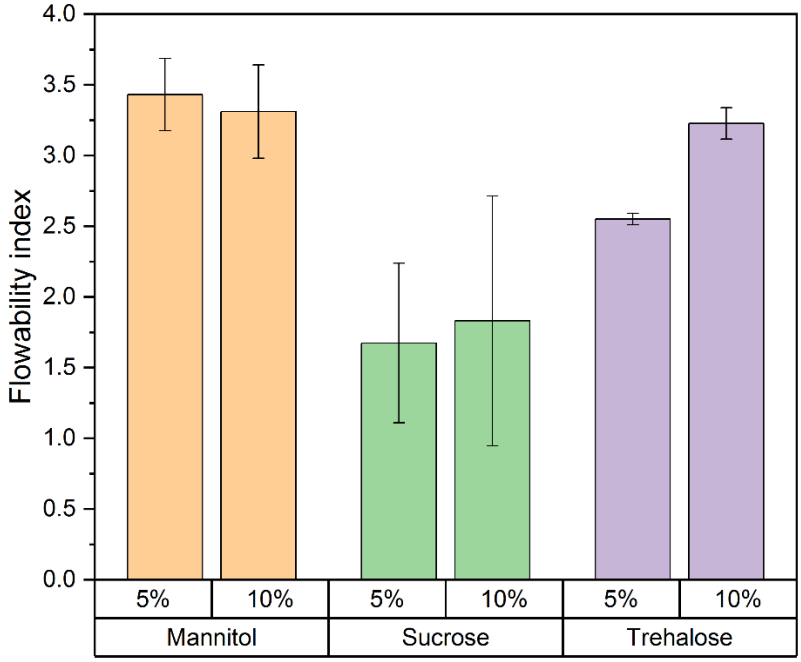


Figure 8. Flowability index of all lyophilized powders (n=3).

3.3 Compaction properties

All lyophilized powders were able to form tablets at both slow and fast speeds with tensile strength higher than 2 MPa (Figure 9, Table S2). Thus, they exhibit adequate tabletability for making sufficiently strong tablets that can withstand stresses during transportation and handling (Sun et al., 2009).

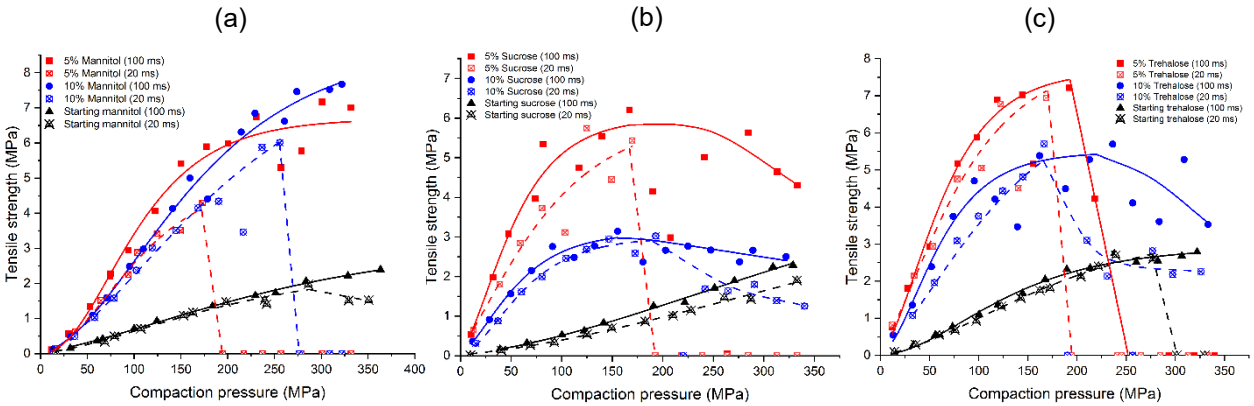


Figure 9. Tabletability profiles of lyophilized powders compressed at two tableting speeds, a) mannitol; b) sucrose; c) trehalose (n=1). Crystalline starting materials are included for comparison.

It was suggested that disordered molecular arrangement in amorphous solids allows for greater molecular mobility and better plasticity (Rozanski and Galeski, 2013). If so, amorphous particles can exhibit better tabletability than their crystalline counterparts since they can likely form larger bonding area while maintaining similar bonding strengths (Sun, 2011). All amorphous sucrose and trehalose samples in this work were indeed more plastic than their crystalline counterparts, as indicated by their lower P_y values (Table 3) and lower β values (Table S2). This is consistent with the better tabletability of lyophilized powders at both tableting speeds (Figure 9). Although both lyophilized mannitol samples are crystalline, they still exhibit significantly better tabletability than the starting crystalline form. This can be, in part, explained by the flake-shaped particles, surface roughness, and polymorph composition, which contribute to stronger bonding between particles.

At the slow tableting speed, 5% sucrose and trehalose exhibit higher plasticity and better tabletability than corresponding 10% samples (Figure 9b-c, Table 4). The more porous structures of 5% lyophilized samples result in greater compressibility, which contributes to better plasticity and tabletability than the less porous 10% lyophilized samples. However, there was no significant difference in plasticity and tabletability between the two crystalline mannitol lyophiles (Figure 9a, Table 3).

Table 3. Values of in-die P_y and SRS of materials studied in this work.

Material	Solids	$P_{y,l}$ (MPa)	$P_{y,h}$ (MPa)	SRS (%)
Mannitol	5%	107.7 (2.0)	109.9 (3.6)	4.0
	10%	108.1 (1.7)	113.8 (2.5)	5.0
	Starting	120.0 (2.3)	126.6 (1.0)	5.5
Sucrose	5%	82.8 (1.8)	86.6 (1.6)	4.4
	10%	89.4 (1.5)	100.4 (1.1)	10.9
	Starting	168.8 (3.0)	171.1 (2.4)	1.4
Trehalose	5%	57.1 (0.6)	74.0 (2.2)	22.8
	10%	89.7 (1.8)	98.8 (1.0)	9.2
	Starting	106.1 (1.3)	109.0 (1.7)	2.7

Tableting speed only has a marginal impact on the tabletability of starting crystalline powders. However, all lyophilized powders exhibited decreased tabletability with increasing tableting speed, though the extent varied (Figure 9). Above certain pressures, tablet tensile strength of lyophilized samples also decreased with increasing pressure, and tablet lamination was observed in some cases. This overcompression phenomenon can be attributed to air entrapment due to the porous structure of lyophilized samples that leads to more initial air in the sample, as indicated by their low bulk densities and difficulty for air to escape, especially during high-speed compression. This mechanism explains the earlier onset of the overcompression problem for each of the six lyophilized powders than their crystalline counterpart and the more severe overcompression at a higher tableting speed (Figure 9). For starting crystalline materials, the overcompression problem is significantly less severe, which is consistent with their higher bulk densities and easier escape by air from their powder beds due to the more regular particle shapes (Figure 6). Expansion of entrapped air during decompression can break bonding between particles in a compact, which weakens tablet and even causes tablet laminations (Hiestand et al., 1977; Mazel et al., 2015; Vreeman and Sun, 2022). One parameter for assessing the extent of air expansion during decompression is the in-die elastic recovery (IER) (Vreeman and Sun, 2024). As expected, IERs were higher at a higher speed for all lyophilized powders (Figure 10). A jump in the IER profile signifies the onset of significant air entrapment during compression, where pores are sealed due to extensive plastic deformation at a sufficiently high pressure (Vreeman and Sun, 2022). Moreover, compared to the 5% lyophilized samples, the less pronounced overcompression phenomenon in

the 10% lyophilized samples is consistent with their lower IERs, resulting from their less porous structures and higher bulk densities.

Distinct speed sensitivities in plasticity were observed among samples studied (Table 4). The plasticity of mannitol powders is not sensitive to tableting speed, as indicated by the comparable SRS values across the three types of mannitol powders. However, the SRS values of starting crystalline sucrose and trehalose are much lower than those of their corresponding lyophilized samples, i.e., the plasticity of the lyophilized powders is more sensitive to tableting speed. The high SRS of 5% trehalose, 22.8% (Table 3) is consistent with a previous work (Hsein et al., 2023).

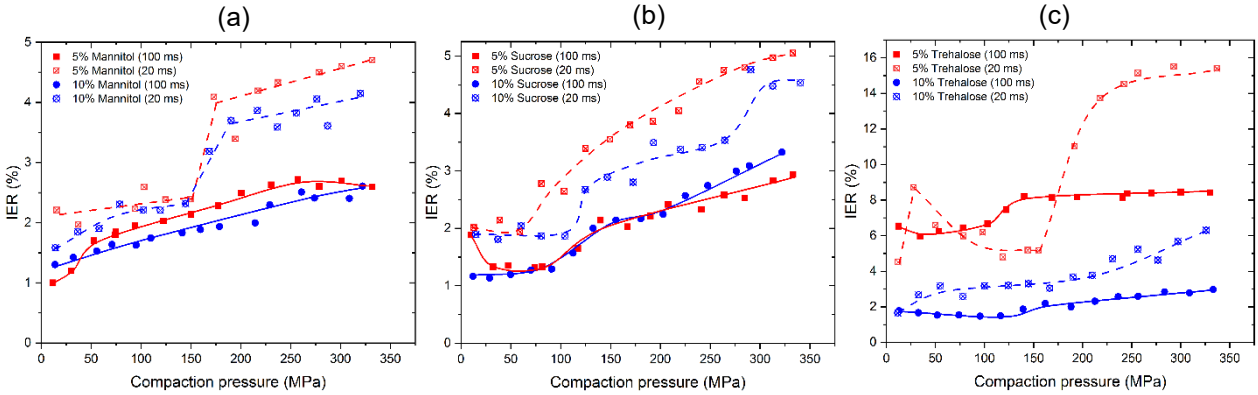


Figure 10. IER of lyophilized a) mannitol, b) sucrose, and c) trehalose tablets at two tableting speeds.

3.4 Physical stability

Stability of all lyophilized powders under ambient conditions over a period of five months was assessed, during which ambient humidity shifted from 40% to 10%. No evidence of form transformation during storage was observed in both lyophilized mannitol samples since their PXRD patterns remained essentially unchanged (Figure 11a) and no discernible change was observed in their PLM images (Figure 12a).

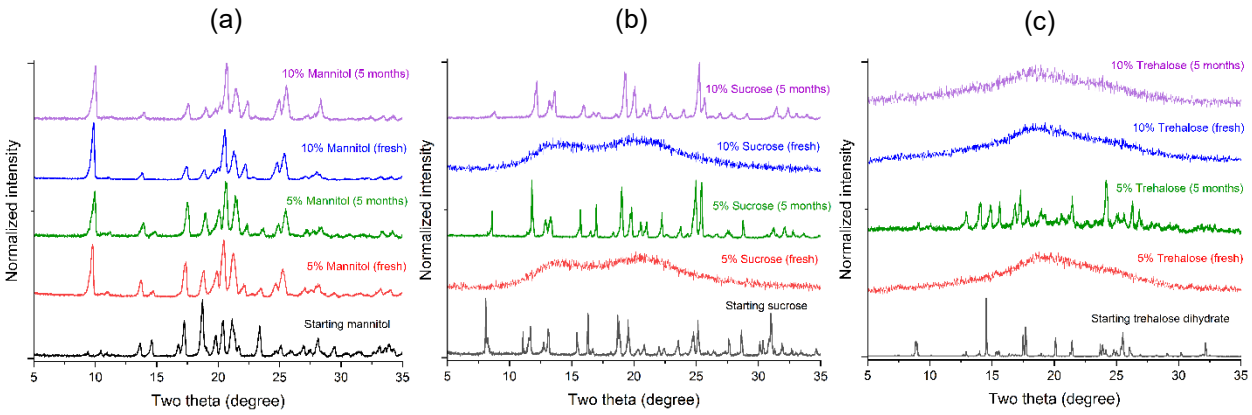


Figure 11. Comparison of PXRD patterns between fresh samples and lyophilized samples stored for 5 months for a) mannitol; b) sucrose; c) trehalose.

Both lyophilized amorphous sucrose samples crystallized over time, as indicated by the appearance of shape XRD peaks matching those of the starting crystalline sucrose (Figure 11b) and the observation of birefringence in their PLM images (Figure 12b). Crystallization was further evidenced by changes in particle morphology, where the initially flake-shaped particles with poorly defined edges in both lyophilized sucrose samples transformed into block-shaped particles with well-defined edges, similar to starting crystalline sucrose particles (Figures 6b, 13a).

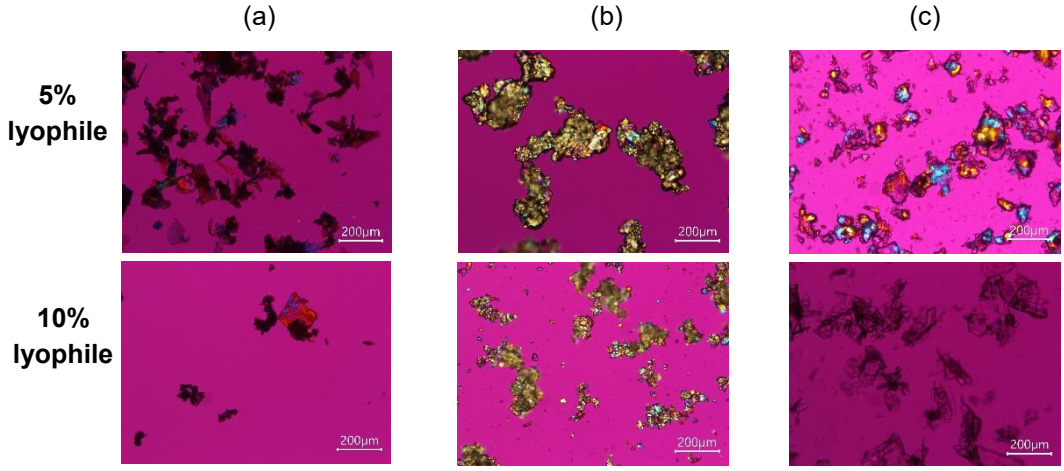


Figure 12. PLM images of lyophilized a) mannitol, b) sucrose, and c) trehalose particles stored for 5 months.

After five months of storage, the 5% trehalose sample crystallized significantly, exhibiting sharp XRD peaks that closely resembled those of crystalline trehalose dihydrate, as confirmed by its PXRD pattern, with variations in peak intensity due to preferred orientation. (Figure 11c).

However, the 10% trehalose sample remained amorphous. The disparity in solid-state stability between the two samples was also apparent in their PLM and SEM images. Birefringence was observed in the aged 5% trehalose sample but not in the aged 10% trehalose sample (Figure 12c). Additionally, the morphology changed for 5% trehalose particles after 5-month storage but remained unchanged for the 10% trehalose particles (Figures 6c, 13b), indicating an absence of crystallization. The better physical stability of the 10% trehalose sample may be attributed to its significantly higher T_g than 5% trehalose (Figure 4c).

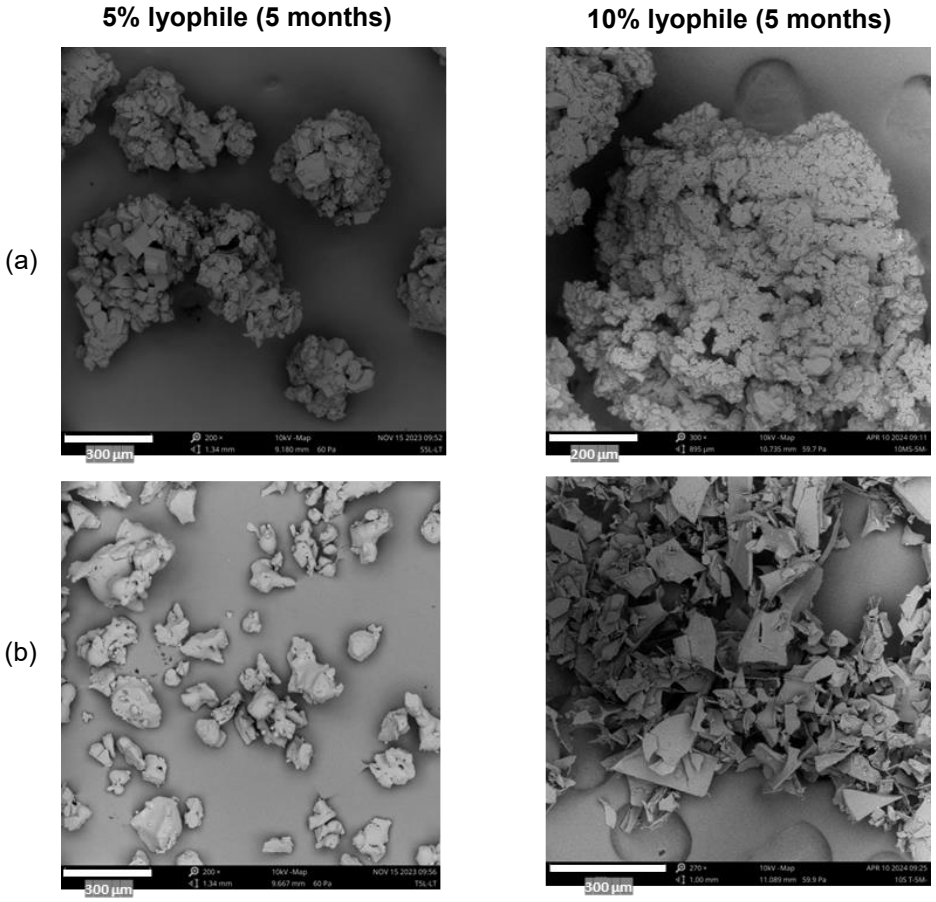


Figure 13. SEM images of lyophilized powders stored for 5 months, a) sucrose; b) trehalose.

The bulk powders of stored lyophilized samples exhibited varied appearances (Figure 14). Initially, all freshly milled freeze-dried samples were loose powders without any agglomeration. For samples that retained their initial solid form, such as 5% and 10% mannitol and 10% trehalose, the appearance of powders remained visually unchanged. Although a few loose agglomerates formed in lyophilized mannitol bulk powders, they could be easily broken by applying a gentle

external force. However, lyophilized powders that underwent solid form transformation during storage, i.e., 5% and 10% sucrose and 5% trehalose, formed strong and dense cakes, an indication of solid bridge formation. One mechanism for the formation of solid bridges is a three-step process: 1) form liquid brideges at the contact point between particles due to initially high mositure uptake, 2) dissolution of sugar into liquid water, and 3) crystallization of sugar upon evaporation of water with decreasing humidity (Dupas-Langlet et al., 2015; Leaper et al., 2012). Another possible mechanism for the formation of solid bridges is solid-state transformation (Hartmann and Palzer, 2011), where the recrystallization of amorphous powders leads to formation of solid bridges. Hence the 10% trehalose powder, initially contained high water content, did not form a large lump after storage (Figure 14c), solid-state transformation is likely the principal mechanism for powder caking observed in this study.

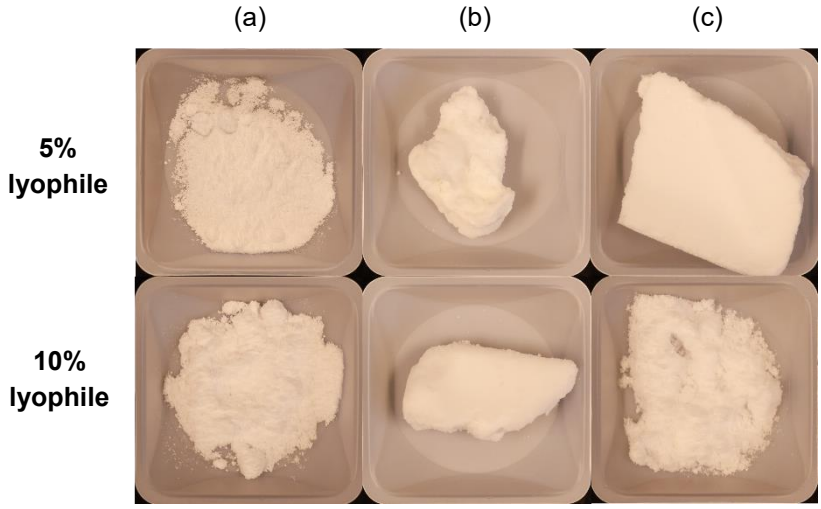


Figure 14. Powder appearance of lyophilized powders stored for 5 months, a) mannitol; b) sucrose; c) trehalose.

4 Conclusions

Increasing the solid content during lyophilization can increase cake density and bulk density of milled powders. However, even with a doubled solid content (from 5% to 10%), lyophilized powders still demonstrate inadequate bulk density and flowability required for direct encapsulation or tableting. Importantly, altering the solid content can yield lyophilized powders with distinct bulk properties, which require careful characterization and assessment on their impact on stability in

addition to processability. Impact of solid content on properties of lyophilized powders also varied, resulting in different polymorph compositions for mannitol, different and complex thermal behaviors and water sorption properties for sucrose, and different glass transition temperatures and stabilities for trehalose. For all three sugars, samples prepared with a lower solid content tend to possess more porous structures, contributing to better compressibility and higher plasticity. However, these porous structures may result in tablet defects or lamination at high tableting speeds and pressures due to air entrapment. This comprehensive investigation into the impact of solid content on lyophilized powders of three common cryoprotectants provides valuable baseline knowledge for the preparation and formulation development of future lyophilized products.

CRediT Authorship Contribution Statement

Zijian Wang: data curation, formal analysis, writing – original draft, writing – review & editing, visualization. **Sichen Song**: data curation, review and editing. **Hongwei Zhang**: data curation, review and editing. **Xiaohong Liu**: data curation, review and editing. **Ronald A. Siegel**: writing – review & editing. **Changquan Calvin Sun**: Conceptualization, supervision, writing – review & editing. **Chenguang Wang**: conceptualization, data curation, supervision, formal analysis, writing – original draft, writing – review & editing.

Declaration of Competing Interest

The work was initiated while C.W. and H.Z. were employed at Evelo Biosciences and completed at the Department of Pharmaceutics, University of Minnesota. The authors state that this research was carried out without any commercial or financial interests that might be interpreted as a potential conflict of interest. Parts of the data were presented at the 6th David Grant Symposium held in June 2023 at the University of Minnesota – Twin Cities.

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Dear Dr. Zhengrong Cui,

Thank you for arranging the review process regarding our manuscript titled **Impact of Solid Content on the Bulk Properties of Lyophilized Powders**. We deeply appreciate the time and effort the reviewers invested in evaluating our manuscript. We have carefully considered their feedback and have revised the manuscript accordingly.

We are pleased to submit the revised version of our manuscript for further consideration by *IJP*. The revision has addressed each point raised by the reviewers, and we believe that these changes have significantly improved the manuscript.

We have attached a detailed response letter listing all the reviewers' comments and specifying the changes we made to the manuscript. We believe that these revisions have improved our manuscript and hope that the changes meet with the approval of the reviewers. We appreciate the opportunity to resubmit our work and look forward to your response.

Thank you once again for the opportunity to refine our manuscript. Please do not hesitate to contact me should you require any further information.

Sincerely,

Chenguang Wang
Evelo Bioscience