

INTRODUCTION

Proteins are commonly freeze-dried in combination with stabilizers such as amorphous saccharides. However, environmental conditions such as temperature and humidity also have a profound impact on product performance and storage properties. Freeze-drying conditions can significantly affect the ultimate lyophile stability, its dryness, morphology, porosity and surface area. An increase in moisture content or temperature may cause a glass transition and crystallization of the sugar resulting in a loss of thermal stability of the protein [1]. For this reason knowledge of physico-chemical properties such as glass transition and water sorption behavior is important.

METHOD

Dynamic Vapor Sorption (DVS) is a well-established gravimetric method for the determination of vapor sorption isotherms. The DVS-HT instrument allows the measurement of up to 10 samples simultaneously. In the current study, water sorption and glass transition behavior of bovine serum albumin (BSA), co-lyophilized with a range of saccharides at a series of ratios and using two different freeze-drying cycles, were investigated at 25 °C. The difference between freeze-drying conditions in the two lyo-cycles studied here was the addition of an annealing (warming) step prior to sublimation (primary drying), in order to determine the effect of annealing on the resulting lyophile.

Mannitol, sucrose, and maltose samples were obtained with varying amounts of BSA: pure sugar, 11% BSA, 20% BSA, 33% BSA, as well as pure BSA. The samples were exposed to a 2% RH/hour humidity ramp. The sample mass would initially increase gradually due to surface adsorption. If the material passes through a glass transition, the vapor uptake will increase dramatically as bulk absorption dominates. If the conditions were great enough to induce a crystallization event, there would be a measurable mass loss. The crystalline phase typically has a lower surface area and affinity, resulting in a lower capacity for water vapor and the decrease in mass [2].

RESULTS/DISCUSSION

A. Sucrose-BSA Mixtures

Figure 1 shows the humidity ramping experiments for a series of sucrose-BSA mixtures, including pure maltose and pure BSA. The samples that included an annealing step are represented by the solid lines and those without an annealing step are in dashed lines. The glass transition point is not greatly affected by protein loading or annealing step. The crystallization humidity increases with increasing BSA content. All sucrose containing samples deliquesce above 87% RH. The addition of the annealing step delays the onset of crystallization to higher humidities. This indicates a stronger BSA-sucrose interactions, thus stabilizing the formulation.

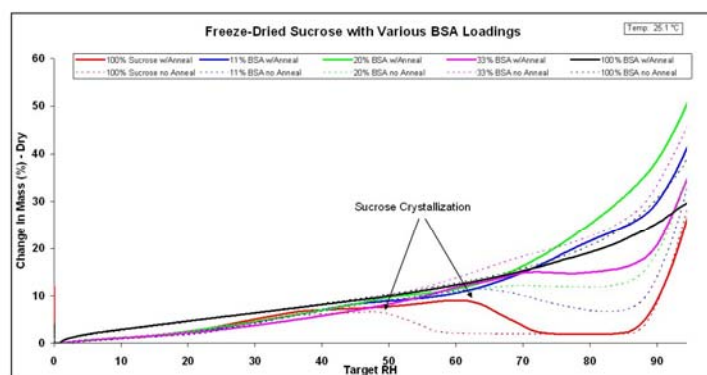


Fig. 1 Humidity ramping experiments (2% RH/hour) for various Sucrose-BSA mixtures at 25 °C. Samples with annealing step in solid lines, without in dashed lines.

B. Maltose-BSA Mixtures

Figure 2 shows the humidity ramping experiments (2% RH/hour) for a series of maltose-BSA mixtures, including pure maltose and pure BSA. Again, the freeze-drying cycle with an annealing step is in solid lines and the cycle without the annealing step is in dashed lines. Again, the glass transition point is not greatly affected by addition of annealing step or BSA content. Maltose deliquesces at high humidities. Unlike sucrose samples, the addition of the annealing step during the freeze-drying process does not greatly affect any sample crystallization. Therefore, annealing does not increase interactions between maltose and BSA.

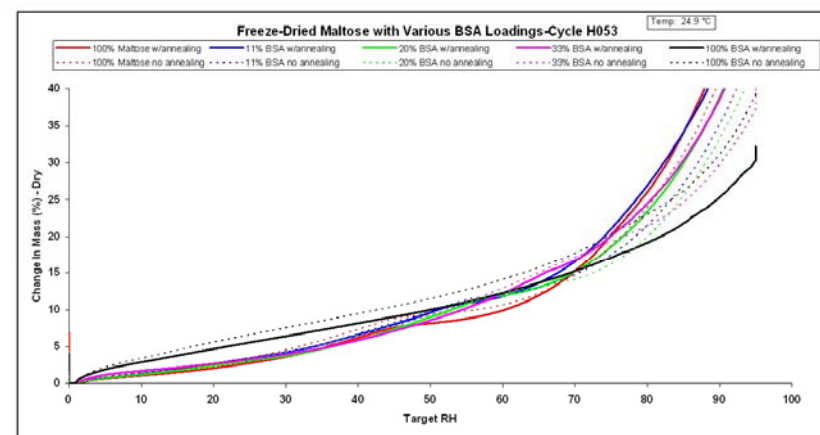


Fig. 2 Humidity ramping experiments (2% RH/hour) for various Maltose-BSA mixtures at 25 °C. Samples with annealing step in solid lines, without in dashed lines.

C. Mannitol-BSA Mixtures

Figure 3 displays the humidity ramping experiments (2% RH/hour) for various freeze dried mannitol-BSA samples. When there was no annealing step, mannitol was purely crystalline. However, when an annealing step was added amorphous mannitol was created. Further, the addition of BSA was able to increase the amorphous content of the mannitol for both freeze-drying cycles due to interactions between the mannitol and BSA. The annealing step was able to stabilize the BSA-mannitol samples and delay or eliminate any moisture-induced crystallization. The results could be further complicated by the formation of different polymorphs during lyophilization.

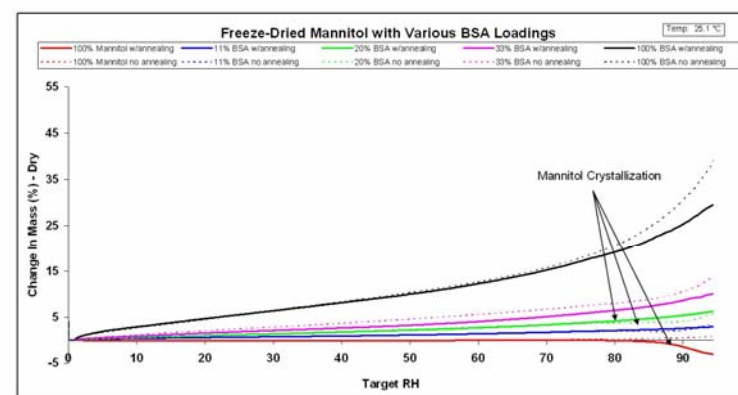


Fig. 3 Humidity ramping experiments (2% RH/hour) for various Mannitol-BSA mixtures at 25 °C. Samples with annealing step in solid lines, without in dashed lines.

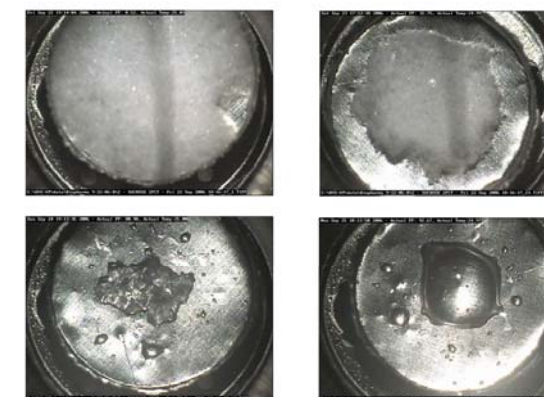


Fig. 4 In-situ images collected on freeze-dried pure sucrose at 0%, 32.7%, 80.9%, and 92.6% RH.

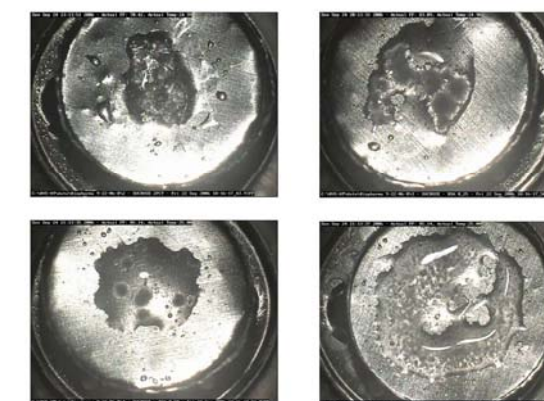


Fig. 5 In-situ images of crystallization transition points for pure sucrose (63.0% RH), 11% BSA (71.2% RH), 20% BSA (75.3% RH), and 33% BSA (85.1% RH).

D. In-situ video images

Figure 4 displays video images collected during the humidity ramping experiments for the pure sucrose sample with the annealing step. The images clearly show the different phase transitions observed in the mass data. By 32.7% RH, the sample undergoes a glass transition (sample cake shrinks). By 80.9% RH the sample crystallizes, and at 92.6% RH the sucrose deliquesces. Figure 5 compares the crystallization transition for the sucrose sample with different BSA loadings. The video images confirm the delay in crystallization onset with increasing BSA content. The pure sucrose crystallizes at 63% RH, while the 33% BSA sample doesn't crystallize until 85.1% RH. These video images confirm the stronger interaction between BSA and sucrose.

CONCLUSIONS

Humidity experiments were performed on a series of BSA-saccharine samples. The addition of an annealing step increased protein-sugar interactions, resulting in a more stable formulation. In-situ video images confirm the physical changes in the sample observed in the gravimetric data.

REFERENCES

- [1] Immara, K. et. al, *J. Chem Eng. Jpn.* 33 (2000), 657.
- [2] Burnett, D. and Thielmann, F., *Intern. J. Pharm.* 287 (2004), 123.