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DVS Application Note 33

Impact of Protein Concentration on the Moisture-Induced Phase Transitions of Protein-Sugar Formulations

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In the development of solid-state dosage forms of biopharmaceuticals the effect of moisture on the stability and structure have become increasingly important. This paper investigates the affects of bovine serum albumin (BSA) loading on the moisture sorption behaviour with co-lyophilised sugars. For maltose and sucrose, increasing BSA content increased the moisture-induced crystallization humidity. For mannitol, the water sorption results indicate a higher mannitol amorphous content is possible with higher BSA loadings.

Introduction

As the number of solid-state protein-based therapeutics increase, the role of water on the long-term stability, activity, structure, and drug-carrier interactions becomes increasingly critical to the successful development of biopharmaceuticals. There has been a recent increase in the number of papers addressing the effects of water on a range of potential biopharmaceuticals [1- 10]. Water may have a range of affects on proteins including: hydrolysis, oxidation, deamidation, and folding. Each of these interactions affect the bioactivity and stability of the formulation. An area of particular concern is water-induced protein aggregation [1,2,5,10-12]. Protein aggregation causes drastically decreased biological activity, increased immunogenicity, and poor release due to diffusional and solubility restrictions [1,2]. Further, when hydrated above a critical level, protein reactivity is accelerated due to its ability to enhance conformational flexibility, participate in additional degradation pathways, and mobilize reactants [13]. If the protein is dried too much it may lead to protein instability [14].

To increase the stability of solid-state proteins, they are often embedded in an amorphous sugar matrix [15]. This can be accomplished by co-lyophilising, co-spray drying, or through solid dispersions of the protein and sugar. Although these sugar matrices provide some stabilization, environmental conditions such as temperature and humidity have a strong impact on product performance and storage. 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 [4]. Also, if the hydration level of the sugar is too high, the excess water may be drawn into the protein, thus affecting the ultimate stability [13]. Further, water is a key determinant for protein-matrix integrity and protein-sugar interactions [3].

For the above reasons knowledge of physico-chemical properties such as glass transition and water sorption behaviour is important for protein-sugar formulations. As a result, the interaction of water with the amorphous sugar/protein matrix becomes essential in the overall formulation development. Ultimately, the interactions between the incorporated sugars and the protein

determine the formulation stability. The formulation's glass transition (T_g) is commonly used to evaluate the physical stability of amorphous materials. Additionally, crystallization rates of the amorphous sugar will affect the stability of the final formulation. The T_g and crystallization rates can be greatly affected by the moisture content; therefore investigation of the water sorption behaviour for these protein-sugar formulations is paramount in determining the ultimate product stability.

In the current study, we investigate the water sorption properties of various lyophilized protein-sugar mixtures. Bovine serum albumin (BSA) was chosen as a model protein while mannitol, sucrose, and maltose were used as model pharmaceutical sugars. The water sorption properties of the co-lyophilized mixtures are compared to the pure BSA and sugar components. At the same time, samples with varying BSA content were studied to investigate the role of protein concentration in the water sorption properties of co-lyophilized protein-sugar mixtures.

Method

Mannitol, sucrose, and maltose samples were obtained by freezing 3mL volumes of formulation within 10 mL vials to a temperature of -50 °C. Freeze drying was conducted with a shelf temperature of -30 °C for a period of 24 hours with a constant 200 μbar vacuum before secondary heating at 20 °C and 50 μbar for 9 hours. The following ratios in the dried material were studied for each sugar: pure sugar, 11% BSA, 20% BSA, 33% BSA, as well as pure BSA.

Water sorption experiments have been carried out by Dynamic Vapour Sorption (DVS) at 25 C. DVS is a well-established method for the gravimetric determination of vapour sorption isotherms using a SMS UltraBalance. The vapour partial pressure around the sample was controlled by mixing saturated and dry carrier gas streams using electronic mass flow controllers.

Experiments were carried out in two manners: step and ramp. For the ramping experiments, each sample was initially dried at 0% relative humidity (RH) at the desired temperature. Then, the sample was exposed to a linearly increasing RH profile up to 95% RH while monitoring the change in mass. For the sugar containing species, the sample mass would initially increase due to surface adsorption. If the material passes through a glass transition, the vapour uptake will increase dramatically as bulk absorption dominates. The transition between surface adsorption and bulk absorption regimes can be taken as the glass transition RH (RH_g) [16]. If the temperature and humidity were great enough to induce a crystallization event, there would be a measurable mass loss as the sample crystallizes. The crystalline phase typically has a lower surface area and affinity, resulting in a lower capacity for water vapour and the decrease in mass. This transition can be taken as the crystallization RH (RH_c) [16].

At times the ramping experiments may not pick up phase transitions, due to kinetic limitations. Therefore, for some protein-sugar samples isotherm experiments were completed. The isotherm experiments might be more sensitive in picking up subtle or severely kinetically limited transitions; the humidity accuracy is limited by the RH step size. For the isotherm experiments, the sample was dried at 0% RH to establish the dry mass. Then the humidity was increased to 95% RH in 5% RH increments. At each stage, the sample mass was allowed to reach equilibrium before the relative humidity was increased to the level. As with the ramping experiments, a crystallization event can be observed by a net mass loss at a particular humidity step. Again, the water acts as a plasticizing agent, thus inducing a crystallization event. This phenomenon is well characterized for amorphous lactose and can be used to detect a water vapour-induced crystallization event [17].

Results

A. Maltose-BSA Mixtures

Figure 1 shows the humidity ramping experiments for a series of maltose-BSA mixtures, including pure maltose and pure BSA. In this experiment the humidity is linearly ramped from 0 to 95% RH at 5% RH per hour. A glass transition is observed for all samples containing maltose around 26% RH. The glass transition of pure maltose has been reported previously at 25 °C and 30% RH [18], which agrees well with the current data. The glass transition RH (RH_g) does not change significantly with increasing BSA content. Protein concentration had little affect on the T_g of other co-lyophilised protein-sugar mixtures, as determined by differential scanning calorimetry [13]. A crystallization event is observed above 40% RH for all maltose containing samples. Crystallization shifts to higher humidities as the BSA loading is increased (see Figure 2), indicating BSA acts as a stabilizer. Clearly there are significant BSA-maltose interactions such that crystallization is inhibited. The protein-sugar interactions may delay sugar crystal nucleation. Previous researchers have hypothesized the sugar is stabilized via hydrogen bonding between the sugar and protein [13, 19, 20]. The strong interactions between BSA and maltose most likely stabilize both materials. Obviously, the ultimate goal in solid protein therapeutics is to use the sugars to stabilize the protein over a wide range of humidity and temperature conditions. The stabilization of the amorphous sugar by the BSA may be a side effect of a mutual stabilization process.

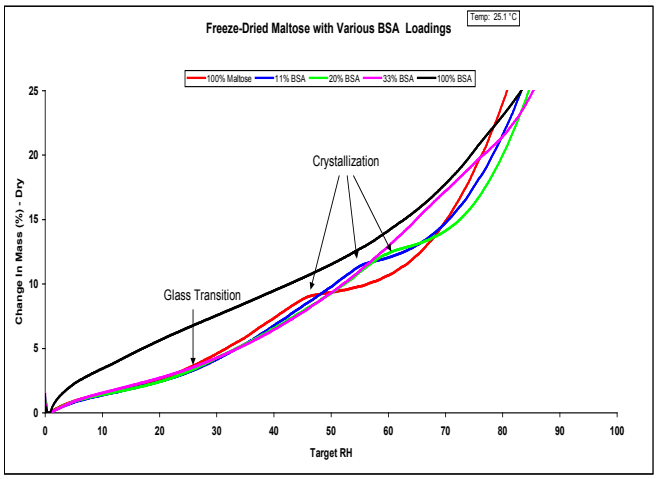

Figure 1. 5% RH/hour ramping experiments for pure maltose (red), 11% BSA (blue), 20% BSA (green), 33% BSA (pink), and pure BSA (black) lyophilized samples at 25.0 °C.

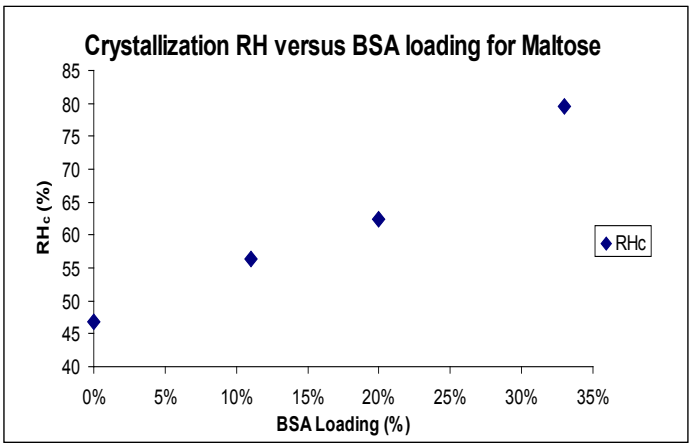

Figure 2. RHc versus BSA loading for maltose at 25.0 °C.

B. Mannitol-BSA Mixtures

Figure 3 shows the humidity ramping experiments (2% RH/hour) for a series of mannitol-BSA mixtures, including pure mannitol and pure BSA. There is no clear glass transition humidity observed in these experiments. The transition may be very subtle due to the mannitol being highly crystalline. Freeze-dried and spray-dried mannitol has been reported to be highly crystalline [21, 22]. The Tg of mannitol has been estimated to be 11 °C by using sorbitol as an impurity and extrapolating to sorbitol concentration of zero [23]. Therefore, the RH_g may not be detectible for these experiments. However, there is clearly some amorphous

material present as a small, but measurable crystallization event is observed at higher humidities. This is more readily observed in the isotherm experiments shown in Figure 4. The RH_c is taken as the humidity step where a net mass loss is observed. Assignment of this point as the RH_c has been previously discussed in the experimental section. The onset humidity for crystallization decreases with increasing BSA content: pure lyophilized mannitol does not crystallize until the 95% RH step, while the addition of 33% BSA decreases the RH_c to 75% RH. This trend in crystallization humidity is clearly shown in Figure 5. As mentioned above, XRD evidence indicates spray-dried mannitol is highly crystalline. However, when spray-dried with various proteins the amorphous fraction increased significantly [20,22]. In fact, when spray-dried with bovine panacreatic ribonulease A, no crystalline mannitol was observed in the XRD pattern [22]. Additionally, when spray-dried with salmon calcitonin, mannitol was 100% amorphous with a salmon calcitonin weight fraction above 0.5 [20]. Therefore, the protein is able to stabilize the amorphous mannitol. In this study, we detect a lower crystallization humidity as the BSA content increases. This is most likely due to an increased mannitol amorphous content. As the amorphous content increases it is more readily available to the water vapour, thus decreasing the crystallization humidity. Therefore, the decrease in crystallization humidity as the BSA content increases is due to kinetic effects and not due to BSA destabilizing the mannitol.

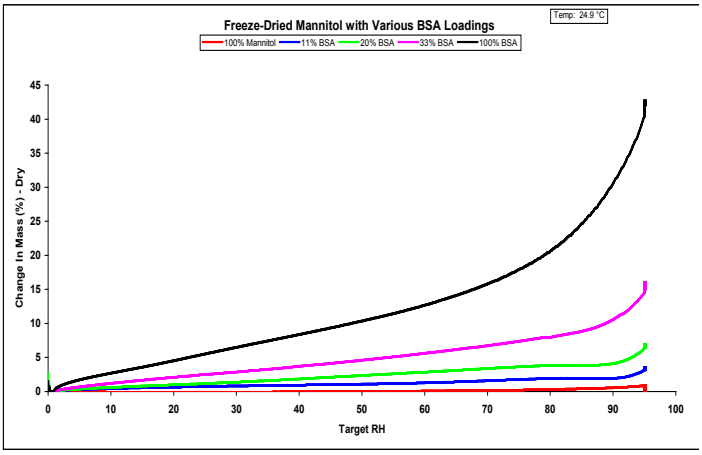

Figure 3. 2% RH/hour ramping experiments for pure mannitol (red), 11% BSA (blue), 20% BSA (green), 33% BSA (pink), and pure BSA (black) lyophilized samples at 25.0 °C.

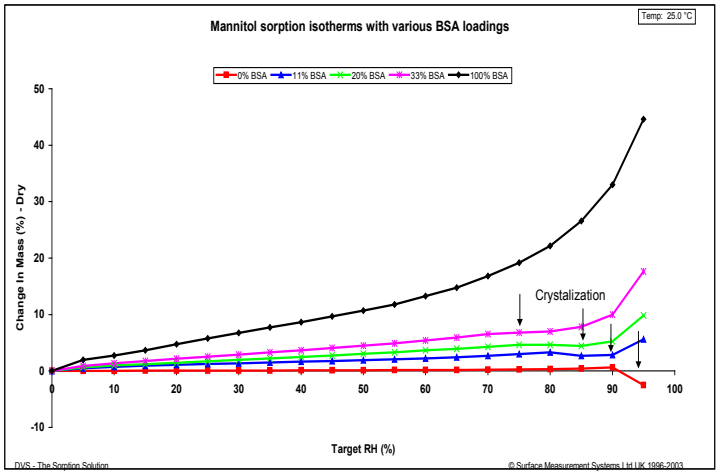

Figure 4. Water sorption isotherms for pure mannitol (red), 11% BSA (blue), 20% BSA (green), 33% BSA (pink), and pure BSA (black) lyophilized samples at 25.0 °C.

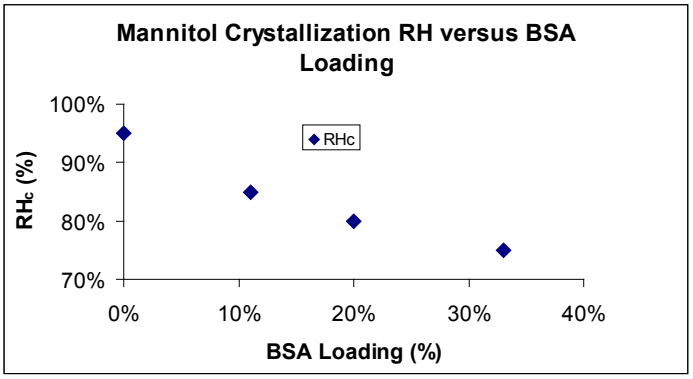

Figure 5. RHc versus BSA loading for mannitol at 25.0 °C.

C. Sucrose-BSA Mixtures

Figure 6 displays the humidity ramping experiments (2% RH/hour) for various freeze dried sucrose-BSA samples. A glass transition RH is observed around 23% RH for all sucrose samples at 25 °C. This is in excellent agreement to DSC results which revealed a T_g of 27.9 ± 2.4 °C at 23% RH [24]. The DVS experiments are performed under precisely controlled humidity conditions. The DSC experiments are performed by storing a sample at a particular RH, sealing a pan, and then heating the sample. As the temperature changes, the sample RH changes

correspondingly. Therefore, the DVS experiments are expected to give more realistic humidity effects on the glass transition.

Similar to the maltose-BSA samples there is little change in the glass transition RH, but a drastic change in the crystallization RH with BSA content. As the BSA loading increases, the crystallization RH increases dramatically, indicating BSA has a stabilizing affect on the freeze-dried sucrose. This is clearly shown in Figure 7 where the RH_c is plotted versus the BSA content. This is similar to the results obtained for the maltose-BSA samples, but in contrast to the trend observed for the freeze-dried mannitol-BSA samples.

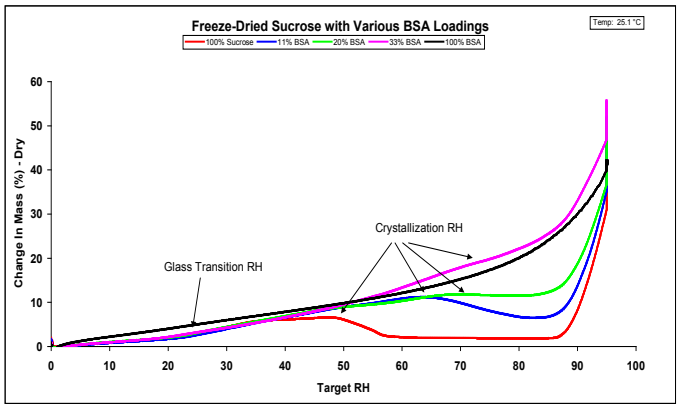

Figure 6. 2% RH/hour ramping experiments for pure sucrose (red), 11% BSA (blue), 20% BSA (green), 33% BSA (pink), and pure BSA (black) lyophilized samples at 25.0 °C.

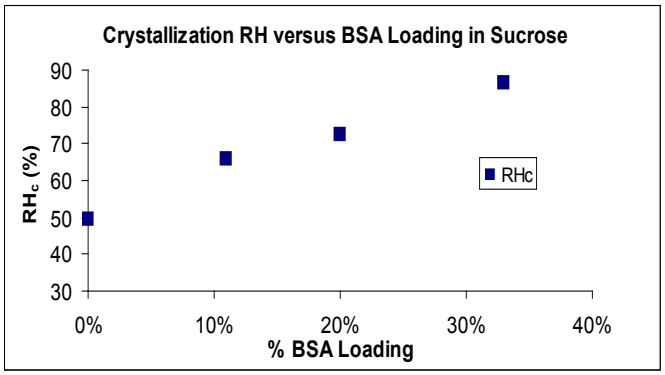

Figure 7. RHc versus BSA loading for sucrose at 25.0 °C.

Conclusion

Humidity experiments were performed on a series of BSA-sugar samples. For maltose-BSA samples, increasing BSA content had a stabilizing effect on the amorphous maltose. Increasing the BSA content in mannitol-BSA samples increased the mannitol amorphous content. Finally, the BSA had a stabilizing effect on spray-dried sucrose and increased the crystallization RH. Clearly, the type of the sugar matrix has a strong effect on the nature of protein-sugar interactions. DVS proves to be an extremely sensitive technique for the investigation of protein-sugar interactions and the determination of moisture-induced phase transitions.

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