FREEZE-DRY MICROSCOPY: DRYING AND COLLAPSE BEHAVIOR OF HUMAN SERUM ALBUMIN AND BOVINE SERUM ALBUMIN BASED FORMULATIONS

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OBJECTIVES

To optimize the freeze-drying cycle for high concentrated protein formulations by understanding their drying and collapse behavior and using human serum albumin (HSA) and bovine serum albumin (BSA) as model substances. Furthermore:

- to evaluate if a collapse temperature (Tc) is detectable by FDM for a pure protein (i.e., HSA and BSA),
- to understand and explain the drying behavior of a protein in combination with excipients, in particular sugars,
- to compare the individual drying and collapse behavior of HSA and BSA.

MATERIAL & METHODS

Preparation of Solutions:

BSA, HSA, sucrose and trehalose, all of highest analytical grade (Sigma-Aldrich, Germany) were prepared as aqueous solutions. Water was double distilled from an all-glass apparatus. The individual composition of each formulation is listed in Table 1.

Freeze-Dry Microscopy (FDM):

Drying and collapse behavior of the formulations were analyzed by using a freeze-dry microscopy system consisting of a microscope (Axio Imager.A1, Zeiss, Germany) with a calibrated freeze-drying stage (FDICS 106, Linkam, UK) and an analyzer/polarizer system. During all experiments custom-made spacers with a height of 25 μm were used to ensure a constant layer thickness of the freeze-drying. About 2 μL of solution was used during each experiment. Freezing rate was 10°C/min to -40°C. Heating rates of 0.5 to 2.0°C/min were applied to imitate typical cycle conditions. Pressure was measured using a calibrated Pirani gauge and was controlled at about 0.1 mbar. Images of the sample were recorded with a digital camera (1.3 Mpix) and analyzed by using the commercial Linksys software (Linkam, UK).

Freezing temperatures (Tf) were determined by observing the nucleation of each solution. The onset of collapse (Tc) was defined as the very first fissures and holes which appear close to the sublimation front. The temperature at which the full structural collapse (Tf) was recorded at the temperature when the product forms no coherent, product layer adjacent to the sublimation interface right after the sublimation process.

Measurement of Solution Density:

HSA formulations were cooled to 0°C using an ice-water bath and density of the solutions was measured with a pyknometer (volume 5 mL). Data were used for viscosity and elasticity measurements.

Viscosity and Elasticity Measurements:

A capillary viscometer with oscillatory flow principle (VILASTIC Viscelasticity Analyzer, Vilastic Scientific, USA) was used to evaluate the viscosity and elasticity of HSA solutions. Experiments were performed at 0°C, utilizing a connected chiller unit and a frequency of 10Hz.

RESULTS & DISCUSSION

Freezing Phase:

Nucleation temperatures (Tf) for both the pure protein solutions and the binary mixtures with sucrose and trehalose were observed in a temperature range from approximately -9°C to -20°C (Fig. 1 and 2). Performing replicate experiments under the same experimental conditions revealed that Tc is not constant (except for a few cases) reproducible for the same sample solution. The nucleation temperature is, however, in the same order of magnitude as it would be expected in a laboratory scale freeze-drying experiment, independent of the applied freezing rate.

Drying and Collapse Behavior:

BSA and HSA show both as pure solutions as well as in mixtures with sucrose or trehalose a similar drying and collapse behavior (Tab. 3 and 4). The pure solutions of BSA and HSA with a total solid content of 3% and 5% (w/w) respectively dry with fissures and disruptions even when keeping the temperature far below the collapse temperature (see Pic. 1). Their onset of collapse was detected in the temperature range where melting of ice started. This collapse could be described as "towing gum-like" behavior. Compared to the formulations which contained one of the used sugars, this observation may be explained by the higher viscosity and elasticity of the pure BSA/HSA solutions measured at 0°C (Tab. 2). Viscosity and elasticity values for HSA + sucrose and HSA + trehalose formulations were in the same order of magnitude for the different total solid contents. Drying and collapse behavior of the binary mixtures with sucrose or trehalose is predominantly influenced by the sugars, especially at higher total solid contents (Fig. 3, 4 and Tab. 3). Sucrose was found to decrease the collapse temperature more than trehalose in the mixture with HSA/BSA, which correlates well to the prediction for glass transitions by the Fox equation [2]. Data presented in Tab. 3 and 4 indicate that Tc for pure sucrose solutions is lower than for pure trehalose solutions (based on calculated values from recent experiments [1]). Note that both sugars have a significant impact on Tc for total solid contents of 2.5% and 4% (w/w). For those formulations collapse characteristics are very similar to those of pure sugar solutions (Pic. 3, 4, 5 and 6) with a sharp and easily recognizable onset of collapse. For all formulations used, the difference between the onset of collapse and full collapse is quite high (up to 80°C) which was found typical for BSA/HSA and their collapse characteristics. For solutions of pure sucrose or trehalose, the bias between Tc and Tbc was revealed less significant. (data not shown).

CONCLUSIONS

Our study shows that it is feasible to determine the collapse temperature of a pure protein (HSA and BSA) by FDM. The drying behavior of HSA and BSA in a binary mixture with either sucrose or trehalose is very similar. Nevertheless, the drying characteristics of this mixture is predominantly influenced by the sugar component, in particular at higher sugar/ protein ratio.

Drying and collapse behavior of the pure proteins showed a very similar pattern. This may be typical for the entire class of these materials.

REFERENCES