FREEZE-DRY MICROSCOPY: CORRELATION BETWEEN PHYSICAL PROPERTIES AND COLLAPSE BEHAVIOR OF EXCIPIENTS FREQUENTLY USED FOR FREEZE-DRYING

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OBJECTIVES
To better understand collapse behavior of formulations observed by freeze-dry microscopy (FDM) and to predict collapse during a freeze-drying cycle.

MATERIALS & METHODS
Preparation of Solutions:
Aqueous solutions of sucrose, trehalose, glucose, polyvinylpyrrolidone (10 kDa), polyvinylpyrrolidone (40 kDa) and (2-hydroxpropyl)-ß-cyclodextrin (all of highest analytical grade from Sigma-Aldrich, Germany) were prepared with double distilled water from an all-glass apparatus. Total solid contents of the examined solutions were 0.1 g/g, 0.02 g/g, 0.03 g/g, 0.05 g/g, (0.07 g/g), 0.1 g/g, (0.12 g/g), 0.15 g/g, 0.2 g/g, (0.23 g/g), 0.25 g/g, (0.28 g/g) and 0.3 g/g (concentrations in brackets were not used in all studies).

Freeze-Dry Microscopy (FDM):
Collapse temperatures and equilibrium freezing points were determined using a freeze-dry microscopy setup consisting of a microscope (Axio Imager.A1, Zeiss, Germany) with a controlled layer thickness of the frozen solution. About 2 µL of solution was used during each experiment. Freezing rate was 1°C/min down to -40°C or -50°C for glucose. Heating rate applied during all experiments was 1°C/min. Pressure was measured using a calibrated Pirani gauge and was controlled between 0.03 and 0.1 mbar. 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 of the full structural collapse (Tfc) 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:
Solution densities were measured at 0°C by using an ice-water bath and at 20°C with a 5 mL pyknometer and sublimation temperature for viscosity measurements.

Viscosity Measurements:
Using a capillary viscometer with oscillatory flow principle (VLABS Viscocelastic Analyzer, Viscalog Scientific, USA) viscosity and elasticity of the solutions were measured at 0°C by using a connected chiller unit (temperature between 0°C and 1°C) and at a frequency of 10 Hz.

Differential Scanning Calorimetry (DSC):
The glass transition of the maximally frozen solute (Tg) was determined by using a Mettler Toledo DSC3+2e. About 35 µL of the sample were sealed in a 40 µL Al pan at room temperature. To determine the value of Tg, the solution was cooled down to -50°C (10°C/min), equilibrated (10 min) and then reheated to 10°C/min. The values for all transitions were analyzed as midpoints and points of inflection.

RESULTS & DISCUSSION
For all excipients used, a clear dependence of Tg on total solid content could be revealed. Each type of excipient showed a characteristic freezing and collapse behavior (data not shown). In the Tc over concentration curves, glucose, (2-hydroxypropyl)-ß-cyclodextrin and PVP 40 kDa show a plateau at higher concentrations (> 20% w/w) (Fig. 7, 2 and 8). Trehalose, sucrose and PVP 10 kDa slightly decrease for higher total solid contents which might be attributed to hygroscopcity of the dried matrix for these substances (Fig. 7 and 8). Due to the higher molecular weight the collapse temperatures for PVP 40 kDa are 6 to 15 K higher than for PVP 10 kDa and the dependence on total solid content is not that distinctive.

Variation in Tg may have a great impact on the robustness of Tc data due to the pore structure formed during this step. Tc cannot be controlled during an FDM measurement and Tg data are in the same order of magnitude relative to a product in an actual freeze-drying cycle.

Density and viscosity data may help to better understand collapse behavior and the interrelation of collapse and total solid content. Reporting Tc as the “collapse temperature” of a given product was found to be the most representative and reproducible way to describe the beginning of viscos flow in a dried matrix.

CONCLUSIONS

REFERENCES

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