



COLLAPSE TEMPERATURE MEASUREMENT BY FREEZE-DRY MICROSCOPY AND TRANSFERABILITY TO FREEZE DRYING PROCESSES: INFLUENCE OF SOLUTE CONCENTRATION ON COLLAPSE BEHAVIOR AND EFFECT ON CYCLE DESIGN

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OBJECTIVES

- To explain the dependency of a collapse temperature (T_c) on total solid content by evaluating physical properties (e.g. viscosity) of selected excipient solutions at 0°C. Thus, to gain a better understanding of collapse behavior and therefore the opportunity to further optimize formulations and freeze drying cycles.
- To evaluate the transferability of collapse temperatures measured by Freeze-Dry Microscopy (FDM) on freeze drying processes, and to establish a general relationship (guideline) between the onset of collapse as detected by microscopy and actual (micro)collapse of a structure in a vial during a freeze drying cycle. The degree of shrinkage was delineated by theoretical calculation and from SEM measurements.

MATERIAL & METHODS

Preparation of Solutions:

Trehalose, sucrose and polyvinylpyrrolidone (PVP) 10 kDa (Sigma-Aldrich, all of highest analytical grade) were used and prepared as aqueous solutions with total solid contents ranging from 0.01 to 0.3 g/g. Water was double distilled from an all-glass apparatus.

Freeze-Dry Microscopy (FDM):

The freeze-dry microscope consisted of a microscope (Axio Imager.A1, Zeiss) with a lambda plate plus analyser and a freeze-drying stage (FDGS 196, Linkam). Calibration was performed with 10% (w/w) solutions of KCl (-10.7°C), NaCl (-21.1°C) and MgCl₂ (-33.6°C). Self-made spacers (height 0.025 mm) were used to maintain the thickness of the sample layer constant. About 2 µL of solution were used during each experiment. Freezing rate was 10°C/min and heating rate 0.5 to 1.0°C/min with isothermal intervals if necessary. Pressure was measured using a calibrated Pirani gauge and was controlled below 0.03 mbar.

Measurement of Solution Density:

The density of each solution was measured at 0°C with a pycnometer (5 mL) and an ice-water bath. Obtained data were used for viscosity and surface tension measurements.

Viscosity Measurements:

The utilized VILASTIC Viscoelasticity Analyzer is a capillary viscometer with oscillatory flow principle. It was connected to a chiller unit (temperature between 0.00°C and 1.00°C). A frequency of 10 Hz was chosen for standard solutions and 5 Hz for PVP 10 kDa solutions with a high concentration.

Surface Tension Measurements:

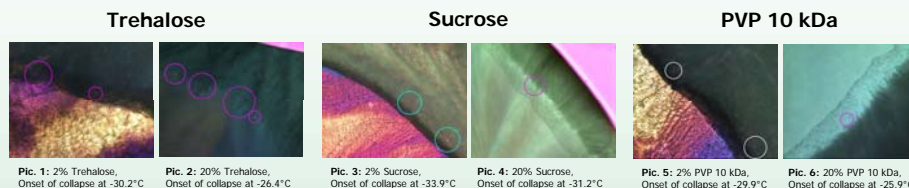
For the measurement of dynamic interfacial tension of sucrose and trehalose solutions a bubble pressure tensiometer (MPT 2, Lauda) served which was also connected to a chiller unit (temperature between 0.00°C and 1.00°C).

Freeze-Drying:

Sucrose solutions were freeze-dried in 20 mL vials containing 3 mL of solution (fill depth: 0.5 cm). Freeze drying runs were performed in a Lyostar II lab-scale freeze dryer with SMART™ Freeze Dryer software installed. According to the T_c evaluated by FDM the setting for the "critical temperature" was varied in the SMART software. Runs were performed with a T_c setting well above and below the T_c measured by FDM. The product temperature at the sublimation interface and bottom center was determined by Manometric Temperature Measurements (MTM).

Scanning Electron Microscopy (SEM):

Samples were fixed on Al stubs (Model G301, Plano) and then sputtered at 20 mA / 5 kV (Hummer JR Technics) for 1.5 min. Cake morphology was then examined using an Amray 1810 T Scanning Electron Microscope at 20 kV.



Pic. 1: 2% Trehalose, Onset of collapse at -30.2°C
Pic. 2: 20% Trehalose, Onset of collapse at -26.4°C

Pic. 3: 2% Sucrose, Onset of collapse at -33.9°C
Pic. 4: 20% Sucrose, Onset of collapse at -31.2°C

Pic. 5: 2% PVP 10 kDa, Onset of collapse at -29.9°C
Pic. 6: 20% PVP 10 kDa, Onset of collapse at -25.9°C

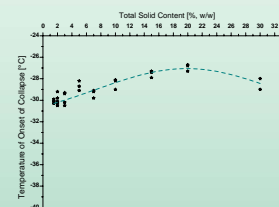


Fig. 1: Trehalose solutions: Temperature of onset of collapse as a function of total solid content

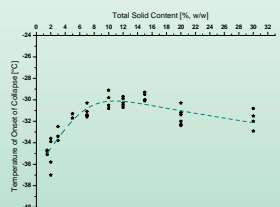


Fig. 2: Sucrose solutions: Temperature of onset of collapse as a function of total solid content

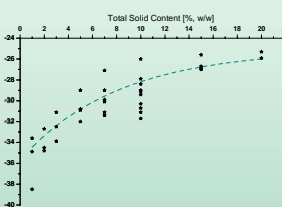


Fig. 3: PVP 10 kDa solutions: Temperature of onset of collapse as a function of total solid content

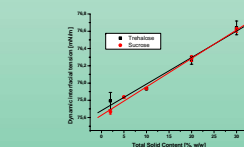


Fig. 4: Surface tension as a function of total solid content (measured at 0°C)

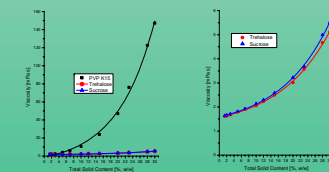
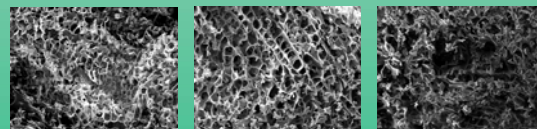


Fig. 5 and 6: Viscosity as a function of total solid content (measured at 0°C)

| Total solid content of solution | T_c (FDM) | T_c (TC) | T_c -max (MTM) | T_c -max (MTM) | Shrinkage | SEM |
|---------------------------------|-------------|------------|------------------|------------------|-----------|---------|
| 2.5% (w/w) -26°C (SMART) | -33.6 °C | -32.9 °C | -33.5 °C | -34.4 °C | - | Pic. 10 |
| 2.5% (w/w) -28°C (SMART) | -33.6 °C | -33.8 °C | -33.7 °C | -34.5 °C | - | Pic. 11 |
| 2.5% (w/w) -32°C (SMART) | -33.6 °C | -35.2 °C | -35.2 °C | -35.8 °C | 19 - 20% | Pic. 12 |
| 2.5% (w/w) -34°C (SMART) | -33.6 °C | -35.0 °C | -35.5 °C | -36.0 °C | 16 - 18% | Pic. 13 |
| 2.5% (w/w) -36°C (SMART) | -33.6 °C | -37.0 °C | -37.6 °C | -37.9 °C | - | Pic. 14 |
| 10% (w/w) -28°C (SMART) | -30.2 °C | -29.9 °C | -31.8 °C | -32.4 °C | - | Pic. 7 |
| 10% (w/w) -32°C (SMART) | -30.2 °C | -33.7 °C | -34.5 °C | -34.8 °C | - | Pic. 8 |
| 10% (w/w) -34°C (SMART) | -30.2 °C | -35.1 °C | -35.3 °C | -35.6 °C | 0% | Pic. 9 |

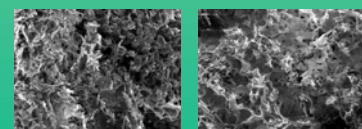
Tab. 1: Data of SMART runs for sucrose solutions



Pic. 7: 10% Sucrose, -28°C (SMART), 100x

Pic. 8: 10% Sucrose, -32°C (SMART), 100x

Pic. 9: 10% Sucrose, -34°C (SMART), 100x



Pic. 10: 2.5% Sucrose, -26°C (SMART), 100x

Pic. 11: 2.5% Sucrose, -28°C (SMART), 100x

Pic. 12: 2.5% Sucrose, -32°C (SMART), 100x

Pic. 13: 2.5% Sucrose, -34°C (SMART), 150x

Pic. 14: 2.5% Sucrose, -36°C (SMART), 100x

RESULTS & DISCUSSION

Dependence of T_c on Total Solid Content:

For all three excipient solutions T_c as measured by FDM showed a great dependence on total solid content of the solution (Fig. 1-3). At a low total solid content measured T_c was low with an increase at higher concentrations. The overall difference between the minimum and the maximum of detected T_c is 5 K for sucrose, 3 K for trehalose and 8 K for PVP 10 kDa. At very high concentrations hygroscopic effects of the dried matrix lead to a decrease in T_c for the two sugars. The highest T_c for sucrose was evaluated as -30.1°C at a total solid content of 0.11 g/g and for trehalose as -27.0°C at 0.2 g/g. These alterations are not reflected in measurements of dynamic surface tension (Fig. 4). Values for all concentrations were detected in the same magnitude with a linear dependence of surface tension on total solid content. Viscosity data show differences for sucrose and trehalose with slightly higher values for sucrose. This may serve to explain the unequal collapse behavior. For PVP 10 kDa the molecules overlap for solutions with a high total solid content [1] so that the exponential increase is much stronger compared to that of the sugars.

Transferability of T_c (FDM) on Freeze Drying Processes:

As illustrated in Pic. 1-6, the "onset" of collapse was determined in this study when the first fissures or holes in the structure appeared as bright spots in the image. This measurement methodology is found consistent for very low (e.g. 2%) and high (e.g. 20%) total solids. T_c for a 2.5% sucrose solution was determined to be -33.6°C by FDM. For all low concentrated sucrose solutions, microcollapse in the structure was found after the freeze drying cycle, even when freeze dried under very conservative conditions (T_c setting: -36°C, T_p (max): -37.9°C, Tab. 1) [2]. In contrast, no shrinkage was found for a 10% sucrose solution and more severe process conditions. Here, the T_c was measured more than 3 K higher by FDM compared to the lower concentrated solute. As illustrated from the SEM pictures, the 10% cake structure is much more rigid (Pic. 7-9) although freeze dried at much higher product interface temperature (-32.4°C). For a 2.5% sucrose concentration the system tolerated both a T_p -max (MTM): -35.8°C (T_c setting: -32°C) and -34.4°C (T_c setting: -26°C) without a full structural loss (Pic. 12). However, it is important to note that SEM pictures of 2.5% sucrose and the T_c setting of -28°C showed much more droplet formation in the structure (indicating viscous flow) compared to a T_c setting of -26°C. T_b (MTM) and T_p (MTM) values indicate a temperature gradient within the product, where the temperature at the bottom never exceeded the collapse temperature of the 10% sucrose solution, but did exceed T_c (FDM) at the low concentrated solute and a T_c setting of -26°C/-28°C. Note when comparing the temperatures in Tab. 1 that the bias between the T_c setpoint and the real temperature data arises from a "safety margin" which is build into the software.

CONCLUSIONS

- The dependence of T_c on total solid content (observed for trehalose, sucrose and PVP 10 kDa solutions) is reflected in viscosity values of the solutions at 0°C, but further investigation is necessary to explain unequal collapse behavior.
- A case study for different concentrated sucrose solutions during vial freeze drying clearly indicates that higher concentrated solutes may tolerate higher product temperatures which is consistent with collapse temperature data obtained by FDM.

REFERENCES

- Elias H-G. *An Introduction to Polymer Science*. VCH Verlagsg. mbH (1997), Weinheim, p. 259.
- Rambhata S. et al. *Cake Shrinkage during freeze-drying: a combined experimental and theoretical study*. Pharmaceutical Development and Technology (2005), 10 (1), pp. 33-40.