

Sugar and Its Role in Freeze-Drying Processes – Investigated by Means of DSC

Dr. Gabriele Kaiser



1 DSC 204 **F1** Nevio with automatic sample changer.

Introduction

Freeze-drying (lyophilization) is a technique widely used in pharmaceutical technologies to transform thermolabile substances such as proteins or liposomes – without thermal treatment – into usable and storable forms. The objective of lyophilization is the gentle removal of water from solutions to obtain a stable powder of defined residual moisture and porosity.

A product's composition has a decisive influence on the process parameters and thus also has an effect on the type, quality and stability of the resulting lyophilisate. Dynamic scanning calorimetry (DSC) provides important information for the selection of appropriate conditions.

The solutions to be lyophilized are usually complex multi-component systems consisting of active ingredients, additives and water. The auxiliaries include toning salts (for adjustment of isotonicity), buffer substances, cryoprotectors (for protection against damage during freezing) and builders

that give structure to the freeze-dried product. Sugars such as sucrose or trehalose have proven to be very effective in stabilizing proteins [5]. The following considerations are based on sucrose as a model substance. The solutions mentioned were produced from commercially available sucrose of pharmaceutical quality (Caesar & Loretz, Hilden) and double-distilled water.

The lyophilization process can generally be divided into 3 consecutive steps:

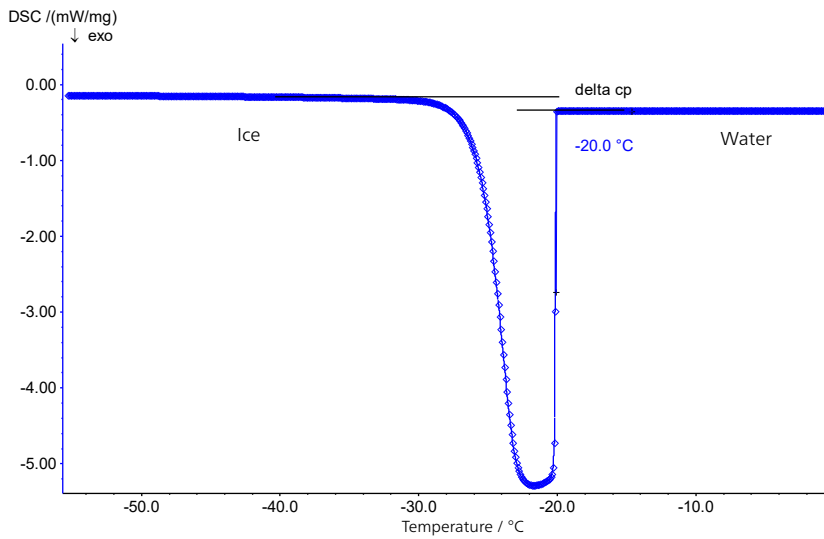
Deep-Freezing

Sugar solutions tend to oversaturate. Upon cooling, ice and an increasingly viscous sucrose solution are formed. The increasing viscosity complicates diffusion processes, which would be necessary for crystallization. As a result, the system does not crystallize but solidifies as an undercooled liquid without complete phase separation (glass). The glass transition temperature of the maximally concentrated solution is designated T_g' and is substance-specific [3].

During cooling, supercooling can often be observed. Pharmaceutical solutions for parenteral application (administration avoiding the gastrointestinal tract), which must be particle-free, represent an extreme case. They have virtually no heterogeneous impurities that could act as crystallization nuclei. Therefore, crystal nucleation in such solutions is often only likely as the temperature approaches -40°C .

The freezing behavior of a 10% sucrose solution is shown in figure 2. The sample was cooled with the NETZSCH DSC 204 **F1** (see figure 1) in a closed aluminum crucible at a controlled cooling rate of 5 K/min. The supercooled solution solidifies extremely quickly at -20°C (extrapolated onset temperature).

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2 Exothermal solidification of a 10% sucrose solution; cooling rate: 5 K/min

Rough spots on the inside of the crucible or traces of contamination caused by preparation can serve as seed crystals. For this reason, solidification temperatures determined this way can generally not be correlated with the concentration of the sugar solutions used.

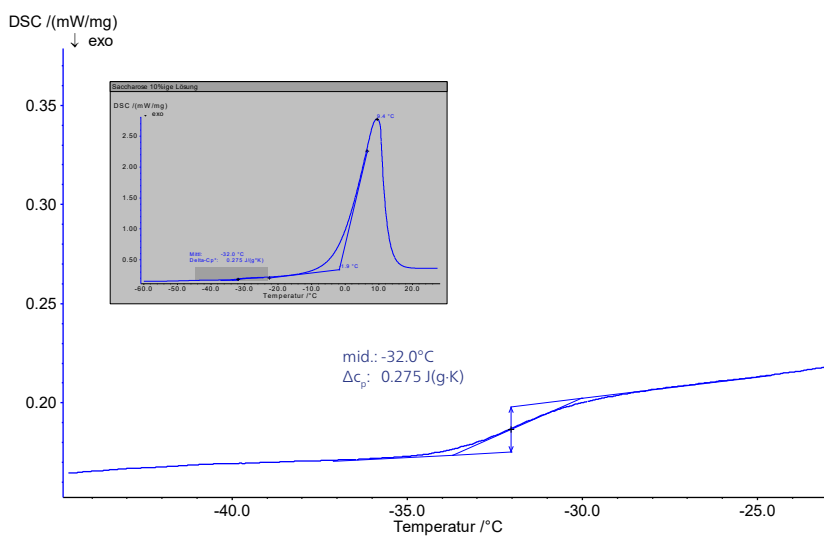
During the water-to-ice transition, a change in specific heat from 4.18 J/g·K (water) to 2.1 J/g·K (ice, just below the freezing point) occurs, which is primarily responsible for the clear baseline shift before and after the solidification/melting peak (fig. 2: water-to-ice transition – and figure 3: ice-to-water transition).

In the subsequent heating at a heating rate of 5 K/min (figure 3), the glass transition of the maximally concentrated

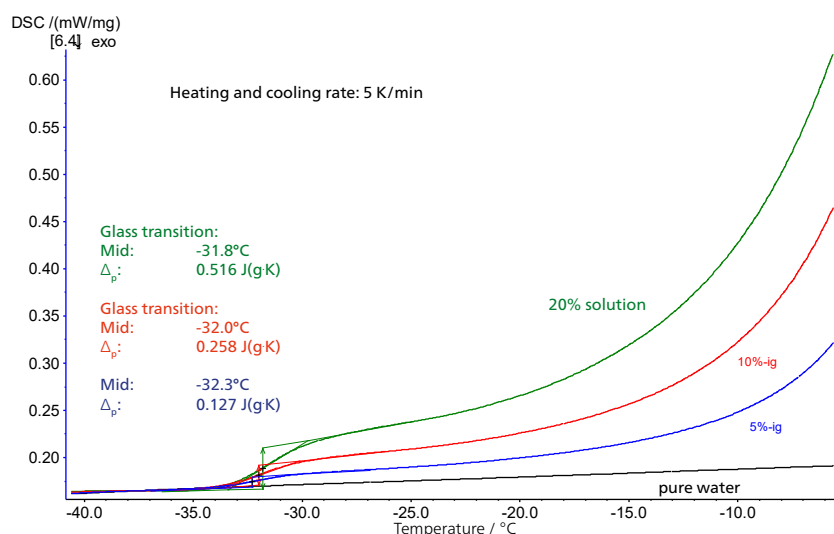
solution appears at -32°C (midpoint). This value is in good agreement with literature data assuming -32°C and -33°C [2], [4].

The glass transition is followed by an endothermic peak during heating (inset in figure 3), the extrapolated onset temperature of which, T_m' , describes the start of melting of the ice. According to Roos [1], the maximum “freeze concentration” can only be observed at freezing temperatures between T_g' and T_m' .

The area below the melting peak corresponds to the free water portion. The reference point here is the heat of fusion of the ice of 333.7 J/g.



3 Glass transition of the maximally concentrated solution; heating rate: 5 K/min



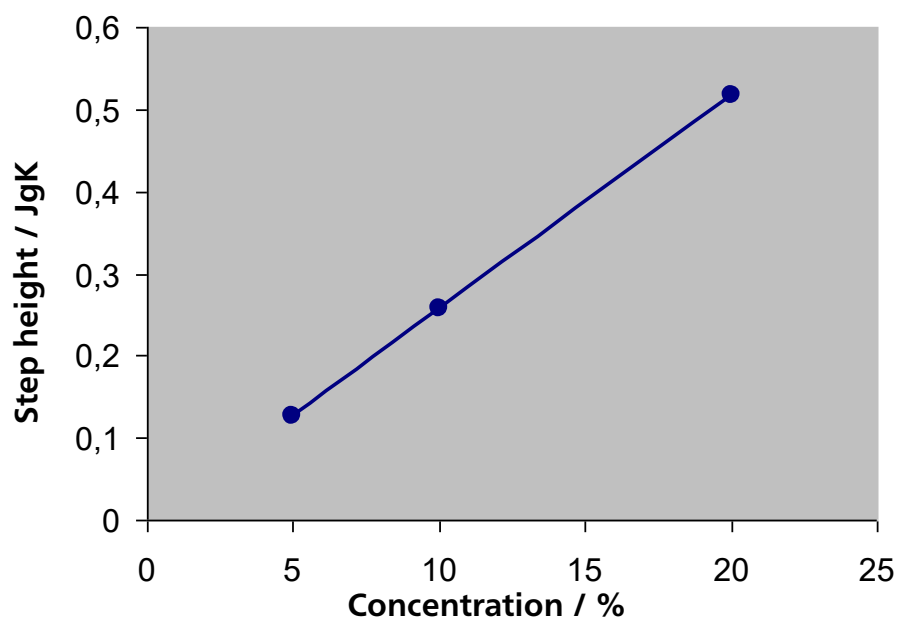
4 Step height of the glass transition as a function of the concentration of the sugar solution

In low-concentration solutions, the sucrose proportion can be determined from the height of the respective glass transition. In figure 4, the step heights (Δc_p values) for solutions of 5%, 10% and 20% – with results of 0.127 J/g·K, 0.258 J/g·K and 0.516 J/g·K – are in very good agreement with a scaling of the concentration by a factor of 2, while the glass transition temperatures remain largely constant. There is a linear relationship between the step height and the concentration (fig. 5).

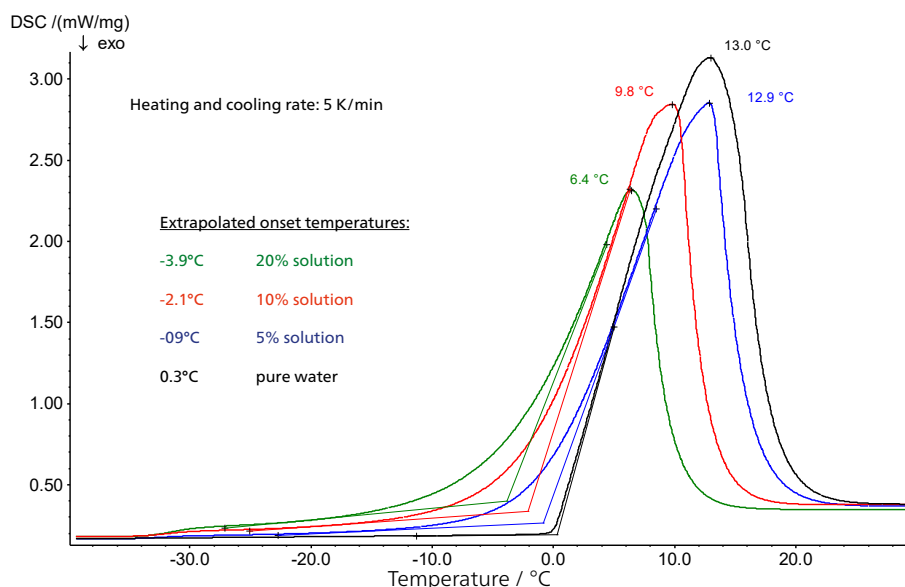
Additionally, as the concentration of the sucrose solutions increases, the start of the melting of ice (extrapolated onset temperature) is shifted to lower values in figure 6. At higher

concentrations, this results in a lower interval between the glass transition of the maximally concentrated solution and the start of melting of the free water.

Some amorphous substances again crystallize upon heating above the glass temperature. This effect, called devitrification or cold crystallization, can be used to change the porosity and residual moisture of the lyophilisate [2] by tempering of the material above the recrystallization temperature (extrapolated onset). Due to recrystallization, a phase separation occurs and the “unfrozen” water released turns to ice. As shown in figure 3, however, no post-crystallization occurs in the case of sucrose.



5 Linear relationship between the step heights of the glass transitions and the corresponding concentrations of the sucrose solutions



6 Start of melting of ice as a function of the concentration of the sugar solution

Primary Drying

In this step, the frozen ice is removed in a vacuum by sublimation (transition from the solid to the gaseous aggregate state).

During this process – in which heat is supplied from the outside – the temperature in the product should not rise above the temperature of the glass transition since this leads to softening of the framework structure and collapse of the system [5]. The destruction of the framework structure during the drying phase is called collapse.

Although collapse temperatures are reported that are an average of 1 to 5 K higher than the corresponding glass transition temperatures [6], the glass transitions of the maximally concentrated solutions, T_g' , which can be determined by means of DSC, are good reference points for their position.

Secondary Drying

In this step, the product is dried to the desired final moisture level by desorbing the water contained in the matrix via a slow increase in temperature.

In amorphous lyophilisates, the water must diffuse from the glassy phase to the surface. This rather slow process is the reason that the post-drying step is often the one which determines the speed of freeze drying for amorphous lyophilisates [2].

Due to the softener effect of water, the glass transition temperature of the amorphous phase is directly related to the entrapped water content. As dewatering progresses, the T_g (the glass transition of sucrose as a solid) increases; its position can also be determined quickly and precisely by means of DSC.

Conclusion

Essential characteristics for designing the primary drying process are the glass transition temperature of the maximally concentrated solution (T_g') and the collapse temperature at which the material softens so that it can no longer support its own structure and begins to flow. Using DSC (sometimes as TM-DSC*), the T_g' can easily be determined.

The collapse temperature is slightly higher than the T_g' ; the exact interval between the T_g' and the collapse temperature is formulation-dependent.

*Temperature-modulated DSC

Literature

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