



Using the DVS to Investigate Moisture-Induced Crystallization Kinetics

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Dynamic Vapour Sorption (DVS) allows the investigation of vapour-induced crystallization kinetics. Water sorption experiments were performed to study the crystallization behaviour of amorphous spray-dried lactose over a wide range of temperature and humidity conditions.

Introduction

Numerous low molecular weight amorphous materials will revert to their more thermodynamically stable, crystalline forms if exposed to conditions above the glass transition. Crystallization rates can be affected by temperature [1,2,3], relative humidity [4,5,6], and other neighbouring materials [7,8,9,10]. In particular, water vapour can have a dramatic effect on amorphous materials. Amorphous solids often absorb relatively large amounts of water vapour compared to their corresponding crystalline phases. Sorbed water can act as a plasticizing agent, thus significantly lowering the glass transition temperature below the storage temperature and cause phase transitions and lyophile collapse [11]. Additionally, moisture sorption can lead to particle agglomeration and powder caking.

Spray-dried lactose is commonly used in solid formulations and is likely to be the most commonly used (partially) amorphous material in the pharmaceutical industry [12]. Additionally, there is an abundance of information regarding its crystallization and polymorphic forms [13]. The degree of lactose crystallinity can affect tableting

properties [14,15], storage of micronised powders [16], texture [5], and flow properties [17].

Therefore, understanding the crystallization behaviour of amorphous lactose is vital for the successful development, processing, and storage of formulations containing amorphous lactose. This application note investigates the crystallization kinetics and mechanism over a range of temperatures and humidities. This aim of this paper is to understand the moisture-induced crystallization of lactose on a fundamental, mechanistic basis without assuming any previously derived crystallization models.

Theory

Above the glass transition, many low molecular weight amorphous materials will relax to their more stable, crystalline state. The amorphous material will typically have a greater water vapour sorption capacity than the crystalline material, due to increased void space, free energy, and/or surface area. This can be measured directly using gravimetric techniques and has been used previously to determine amorphous contents below one percent [18,19,20]. When the material undergoes an amorphous to crystalline transition, the water sorption capacity will typically decrease



drastically. This results in an overall mass loss as excess water is desorbed during crystallization. Therefore, this mass loss can be used to monitor an amorphous to crystalline transition.

Method

Gravimetric vapour sorption experiments have been carried out using the DVS instrument which measures the uptake and loss of vapour gravimetrically using a recording SMS UltraBalance with a mass resolution of $\pm 0.1 \mu\text{g}$. The vapour partial pressure around the sample is controlled by mixing saturated and dry carrier gas streams using electronic mass flow controllers. The desired temperature is maintained at $\pm 0.1 \text{ }^\circ\text{C}$. The samples ($\sim 25 \text{ mg}$) were placed into the DVS instrument at the desired temperature where they were initially dried in a 200-sccm stream of dry air ($< 0.1\%$ relative humidity) for several hours to establish a dry mass. The samples were then exposed to a step change in relative humidity (RH) and maintained at these conditions while monitoring the sample mass. The mass will initially increase as the sample sorbs water. The derivative of the mass versus time plots (dm/dt versus time) were performed and the onset time for crystallization was taken as the point where the derivative intercepts the x-axis (i.e. slope of mass versus time turns negative). The humidity was maintained at the desired level until crystallization was complete. Crystallinity of the end material was verified by exposing the sample to 95% RH. Absence of the mass loss feature at high relative humidity values was taken as an indication of complete crystallinity of the end material.

Amorphous lactose was prepared by GlaxoSmithKline (Collegeville, PA, USA) by dissolving crystalline lactose in water (10% w/w) and spray-drying at $190 \text{ }^\circ\text{C}$. No further processing, screening, or characterization was performed on the spray-dried lactose. The spray-dried lactose was stored over desiccant (anhydrous calcium sulphate) at $6 \text{ }^\circ\text{C}$, to limit any premature crystallization. The same batch of spray-dried lactose was used for these studies to

minimize any particle size, surface area, impurity, or similar batch-to-batch effects.

The modelling of the crystallization data was done using the routines found in NETZSCH Thermokinetics® software. This software allows for visual/manual manipulation of fit parameters and then performs the least squares optimization itself to generate the best fit parameters. Initial model selection is experience based as well as based on observation of the consequence of effects visually seen upon manual parameter manipulation.

Results

Gravimetric Data

A typical moisture-induced crystallization result is displayed in Figure 1 for amorphous lactose at $25.0 \text{ }^\circ\text{C}$. The solid line traces the percentage change in mass (referenced from the dry mass) on the left y-axis as a function of time. The dashed line follows the target relative humidity on the right y-axis as a function of time. During the drying period, the sample mass decreases by approximately 3 percent. As the humidity is rapidly increased to 55% RH, the sample mass increases dramatically due to water vapour sorption. After a period of time, the sample mass begins to steadily decrease. This characteristic mass loss feature has been well-documented and has been previously assigned to the crystallization of amorphous lactose [6,19,21]. The lag between the increase in humidity and the point where mass loss is observed is taken as the onset time for crystallization. This point was determined by plotting the derivative of the mass versus time and identifying the x-intercept (i.e. where the mass versus time slope becomes negative). After crystallization is complete, the mass loss slows and approaches an equilibrium value. As the humidity is then increased to 95% RH, the sample mass increases and rapidly approaches equilibrium. The absence of any mass loss during this step confirms the sample is completely crystalline. The slight mass increase observed during this step is due to surface water adsorption on the fully crystallized lactose.

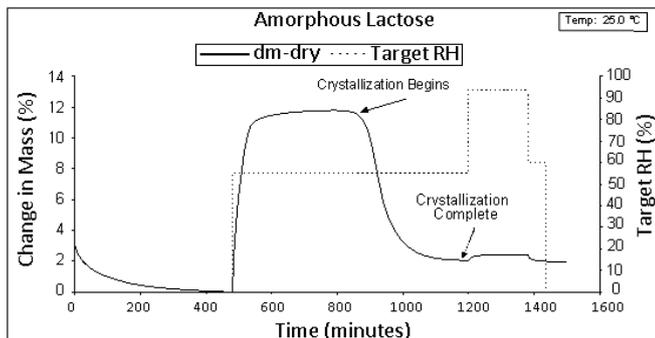


Figure 1. Amorphous lactose crystallization at 55% RH and 25 °C

In previous work [6], it was determined that water vapour induces a glass transition at 30% RH and a crystallization event at 58% RH for spray-dried lactose at 25 °C. Below 30% RH, no crystallization was observed over the time scale of the experiments (1 week) and above 58% RH crystallization was nearly instantaneous. Therefore, experiments have been performed on amorphous lactose at 25.0 °C over a range of relative humidities between the glass transition and crystallization humidity points. Over this regime, crystallization will be kinetically controlled. Figure 2 displays the moisture-induced crystallization results for amorphous lactose between 50% and 60% RH. The time has been normalized such that the onset point of crystallization has been shifted to a time of zero. The y-axis has been normalized to reflect the amorphous fraction. Before crystallization, the amorphous fraction is assumed to be one and after crystallization the amorphous fraction is set to zero. The gravimetric results in Figure 2 indicate a one-step crystallization process at 53%, 55%, 57%, and 60% RH. For these experiments, the amorphous fraction decreases precipitously in one step. However, at 51% and 50% RH, there is an initial decrease, followed by a 'levelling off' in the amorphous fraction. This is followed by a second decrease, resulting in an apparent two-step crystallization mechanism at these conditions. This is highlighted in Figure 3 where the derivative of the amorphous fraction is plotted versus time for the results at 60% RH (dashed line) and 50% RH (solid line). Clearly, a single peak is observed at 60% RH indicating a one step

mechanism, while two peaks are evident at 50% RH, illustrating a two step mechanism.

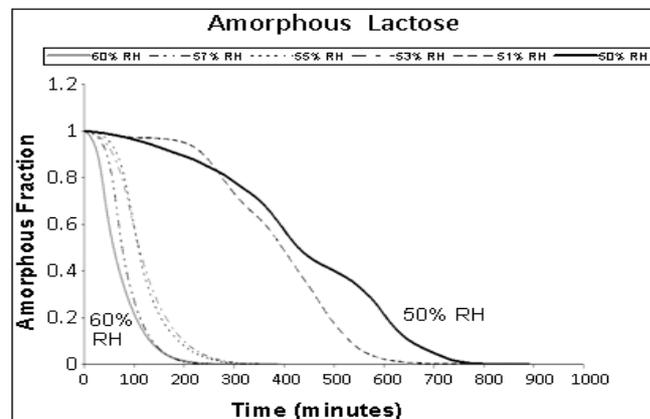


Figure 2. Amorphous fraction as a function of time between 50% and 60% RH at 25 °C.

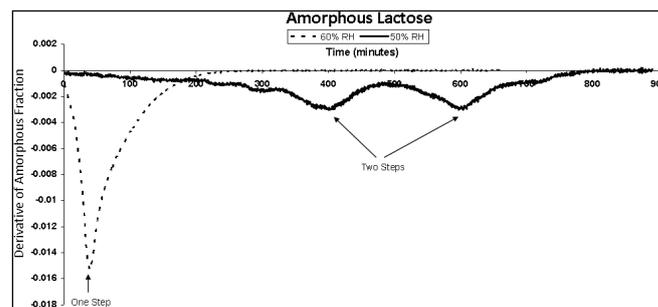


Figure 3. Derivative of amorphous fraction as a function of time at 50% and 60% RH at 25 °C.

The onset times for crystallization at 25 °C between 48% and 60% RH are displayed in Figure 4. These values were taken as the x-intercept from derivative of mass versus time plots (i.e. change in mass becomes negative). As the humidity is increased, the onset time for crystallization decreases. As the humidity is increased, the amorphous lactose sample will sorb more water, thus facilitating plasticization and lyophile collapse. Previous researchers have observed similar lactose crystallization trends with relative humidity [22]. An exponential fit was applied to the data as shown by the solid line in Figure 4.

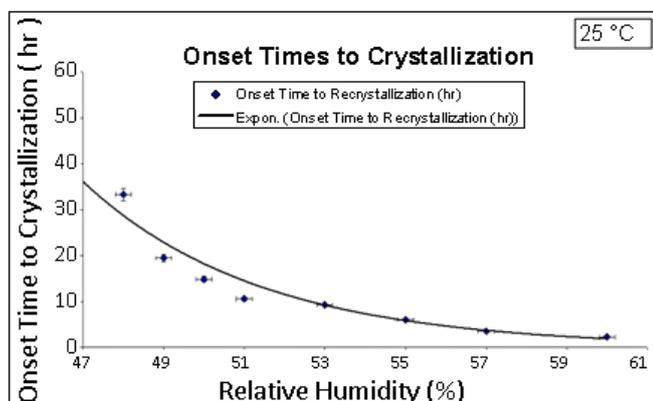


Figure 4. Onset times for crystallization as a function of relative humidity at 25 °C.

Additional experiments at a constant RH over a range of temperatures were performed to probe the effects of temperature on the crystallization behaviour of amorphous lactose. Figure 5 displays a series of experiments at 51% RH between 22 and 32 °C. Similar to Figure 2, these results show the amorphous fraction versus time. Again, the time scale is normalized such that the onset time for crystallization is taken as the zero point. For the experiments above 25 °C the amorphous fraction decreases sharply in one step, but at 25 °C and below the lactose appears to crystallize in two distinguishable steps. These results are similar to the results at 25 °C over a range of humidities (see Figure 2). At high humidities and high temperatures only one crystallization step is visible in the gravimetric data, but at low humidities and low temperatures evidence of two steps is visible. Derivative plots (data not shown) were performed for the data in Figure 4. As in Figure 3, there are two distinct peaks for the results at 25 °C and below (two step mechanism), while only one peak is observed above 25 °C (one step mechanism). It is quite possible that the same two reaction steps are present over all conditions studied, but only visible in the gravimetric data when the crystallization kinetics are significantly slowed down by low temperature or low humidity conditions.

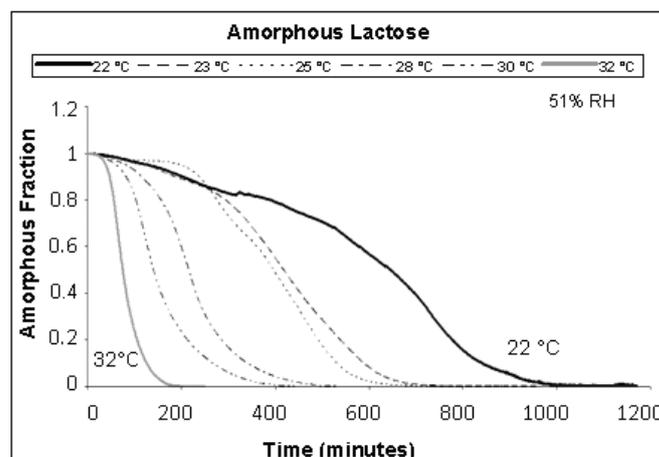


Figure 5. Amorphous fraction as a function of time between 22 and 32 °C at 51% RH.

The onset times for crystallization at 51% RH over a range of temperatures are displayed graphically in Figure 6. Again, these onset times are obtained from the x-intercept of mass derivative versus time plots, as discussed previously. As the temperature is increased, the induction time to crystallization decreases rapidly. Similar results have been obtained previously using DSC, PLV, and gravimetric methods [8,11,23]. These results are expected since viscosity decreases considerably as temperature increases. The increased molecular motion facilitates sample crystallization. In this study there appears to be a strong relationship between the crystallization induction time and temperature at 51% RH. An exponential equation has been fit to the data (solid line in Figure 6) indicating a general agreement with the data. An exponential relationship between crystallization induction times and temperature was observed for lactose crystallization using DSC and PLV [23].

The data in Figure 6 was fit to an Arrhenius plot in order to obtain an apparent activation energy. The reciprocal of the induction time was taken as the rate; therefore the activation energy obtained would only be for the initial reaction step. Using the data in Figure 6, an apparent initial activation energy of 146 kJ/mol was obtained. Activation energies of 112 and 104 kJ/mol were obtained using similar gravimetric techniques at 57.5% RH between 18 and 32 °C [12]. The value obtained in this report at 51% RH is higher than the values

obtained by other researchers at 57.5% RH. As Figures 2 and 4 clearly show, crystallization occurs faster at higher relative humidities when measured at the same temperature. Therefore, higher apparent activation energies would be expected at lower humidity conditions, which is consistent with the current results.

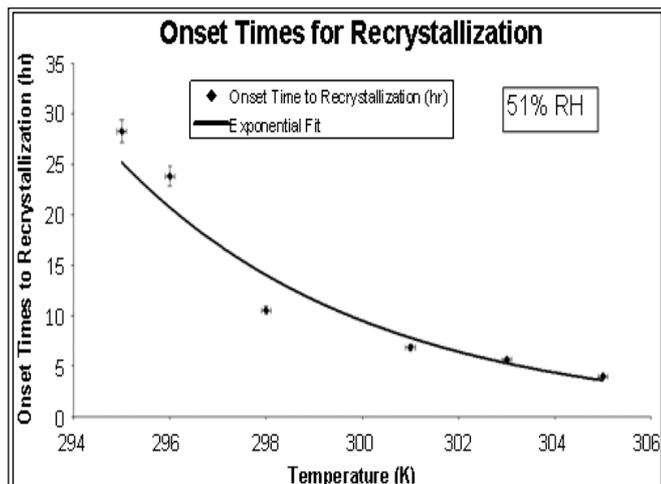


Figure 6. Onset times for crystallization as a function of temperature at 51% RH.

Crystallization Mechanism Modelling

The results at 51% RH between 22 and 32 °C were used to elucidate the lactose crystallization mechanism. These five data sets were subjected to a Friedman Analysis. The resulting Friedman plot in Figure 7 shows the activation energy (left y-axis; E) and pre-exponential factor (right y-axis; A) versus the fractional mass loss during crystallization. The activation energy is changing significantly throughout the course of the reaction, indicating a multi-step process. If there was a single reaction step, then the activation energy would be constant throughout the entire reaction.

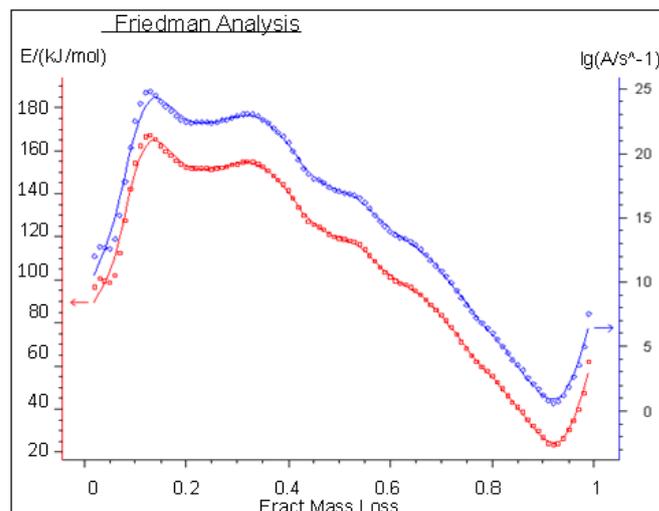


Figure 7. Friedman plot using the gravimetric crystallization results measured between 22 and 32 °C at 51% RH.

The Netzsch Thermokinetics® software was used to elucidate the reaction mechanism. The best fit was obtained when using only the data above 25 °C. This coincided with the results where only one step was visible in the gravimetric data. The resulting mechanism and fit is displayed in Figure 8 for the data at 32.0, 30.0, and 28.1 °C. The correlation coefficient for this mechanism is 0.9997. The mechanism in Figure 7 indicates two competing, independent reaction sequences. For both reaction sequences, the first step is auto-catalyzed by B and E, respectively. The second step in each reaction sequence is three-dimensionally diffusion limited. The first step in each reaction sequence is most likely crystal nucleation from the amorphous phase, as nucleation would be auto-catalyzed. The initial crystal seeds would propagate, thus creating more crystals. The second step is most likely water diffusing from the bulk of the sample. Amorphous lactose has the ability to absorb water into the bulk structure, whereas water sorption would be limited to surface adsorption for crystalline lactose. Therefore, as the amorphous material crystallizes it must desorb water, which would be diffusion limited.

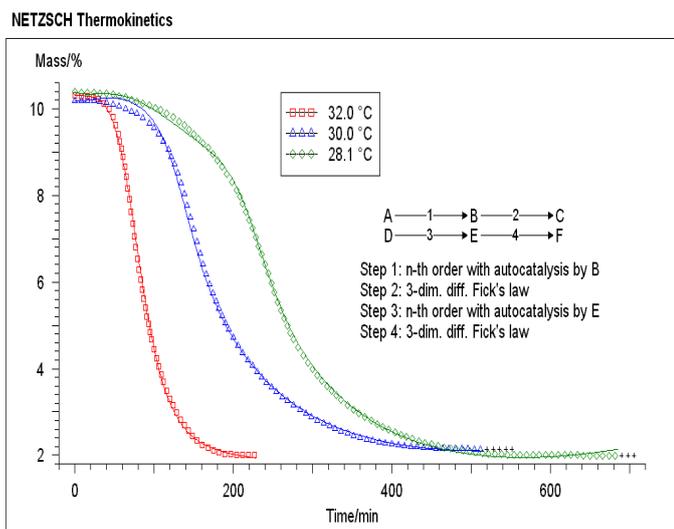


Figure 8. Gravimetric data above 25 °C at 51% RH with best-fit mechanism.

Previous researchers have observed evidence of multiple reaction products during lactose crystallization. For instance, Kedward et al. observed evidence of a shoulder during the crystallization peak in DSC experiments [2,5]. They hypothesized this shoulder was due to crystallization into different forms of lactose. For instance, the lactose may crystallize into the α -monohydrate form and other anhydrous polymorphs. This hypothesis is given further support by XRD data of amorphous lactose crystallized at different humidity conditions [4,22]. Amorphous lactose was found to crystallize into the α -lactose monohydrate, anhydrous β -lactose, and the anhydrous forms of α - and β - lactose in molar ratios of 5:3 and 4:1. α -Lactose monohydrate was the predominant species at high humidities, with the anhydrous forms becoming more prevalent at lower humidity conditions [4]. In fact, anhydrous β -lactose was only observed at 44.4% RH, the lowest humidity investigated in the above study. In all, the XRD and DSC results by the above researchers support the multi-reaction sequence obtained by the modelling studies in this report.

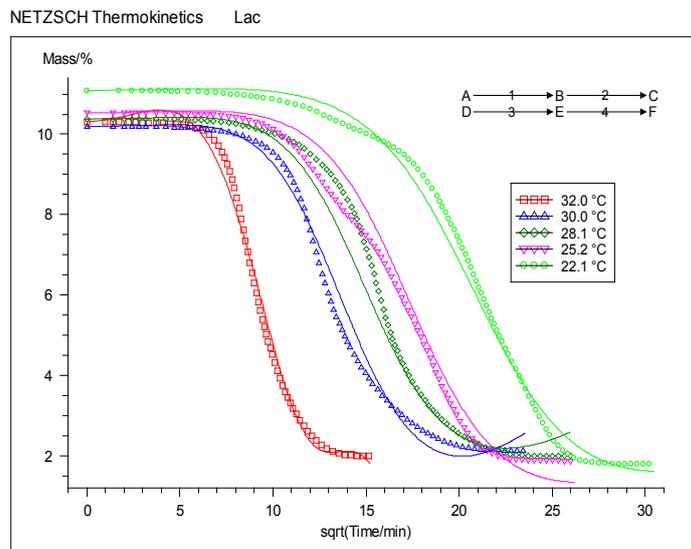


Figure 9. Gravimetric data between 22 and 32 °C at 51% RH with best-fit mechanism.

When the data at 25 °C and below is considered, the quality of the reaction mechanism fit diminishes. Figure 9 displays the same reaction mechanism obtained in Figure 8 applied to all temperatures. When all temperature data are included the correlation coefficient decreases to 0.977. These results are not fully understood at the present time. The lower temperature data exhibit a distinctive shoulder, which is absent in the other higher temperature experiments. There is also a 'shoulder' in the lower humidity data at 25 °C. This 'shoulder' may be due to two reaction sequences with induction times that are affected differently by temperature and humidity. For instance, the first step in one reaction sequence may more dependent on temperature and/or humidity than the other reaction sequence. Then, there may be humidity and temperature regimes where the reactions will be concurrent and other regimes where the reaction sequences are independent. Alternatively, a branched reaction may only occur at low temperature and/or humidity conditions. This hypothesis may be supported by the XRD data by Roos et al. [11] where anhydrous β -lactose was only observed at the lower humidities. Additional studies over a broader temperature and humidity matrix may help elucidate the nature of this not fully understood feature in the gravimetric data.



Conclusion

Amorphous lactose was crystallized over a range of humidity and temperature conditions using a gravimetric sorption apparatus. The induction times for crystallization indicated a strong relationship with both temperature and humidity. Gravimetric experiments and mechanistic modelling indicated a multi-step crystallization process. Crystallization data above 25 °C at 51% RH indicated a mechanism consisting of two competing reaction sequences. The first step of each reaction is auto-catalyzed, while the second step was three-dimensionally diffusion limited. The auto-catalyzed first step is probably due to crystal nucleation, while the second step is most likely water diffusing out of the lactose. The data at low temperature and humidity conditions is not fully understood, but may be due to the formation of multiple conformers of crystalline lactose or reaction sequences with differing induction periods.

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