

# An equilibrium and kinetic sorption study of the interaction of amorphous trehalose with water vapour

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## Summary

Amorphous sugars and polyols are being increasingly used as stabilisers in lyophilised therapeutic protein products. However the relative thermodynamic instability of the amorphous state compared to the crystalline state necessitates a greater understanding of the amorphous to crystalline transition. The purpose of the work was to examine the use of a controlled moisture sorption analyser to provide a more detailed understanding of the kinetics and events that occur when an amorphous trehalose phase interacts with moisture. Two batches of spray-dried x-ray amorphous alpha-alpha-trehalose were exposed to a range of known controlled humidities at 25°C. Weight data was acquired at each humidity with the Avrami model being used to determine kinetic parameters and equilibrium uptake. The adsorption-desorption isotherms obtained featured a constant adsorption gradient between 5% and 55% RH, a loss of water accompanying the amorphous to crystalline phase transition to form the di-hydrate crystal form at 65% RH. Following this conversion the isotherm followed the classic reversible type III isotherm associated with the di-hydrate crystalline form. Notably, the kinetic features of the moisture interaction were not coincident although three regions were broadly assigned, which indicated a slowing down and then a speeding up of the diffusion of water through the amorphous structure which was attributed to a glass phase transition, and a third region of rapid sorption equilibrium with the crystalline phase. The approach presented here highlights the importance of evaluating kinetic data which is not routinely reported.

## Introduction

$\alpha$ -Trehalose is a non-reducing disaccharide comprised of two  $\alpha$ -D-glucosyl units connected by a 1,1-glycosidic linkage. The  $\alpha,\alpha$ -isomer is widespread in nature and is increasingly used in

pharmaceutical formulations, principally in the stabilisation of proteins [1] and the preservation of foodstuffs [2]. Since trehalose can be obtained in a variety of forms - anhydrous amorphous, anhydrous crystalline, crystalline dihydrate and glass, it is essential to have an understanding of the environmental and experimental conditions required to obtain and maintain these different forms. The purpose of the work was to examine the use of a controlled moisture sorption analyser to provide a more detailed understanding of the kinetics and events that occur when an amorphous trehalose phase interacts with moisture.

## Material and Methods

$\alpha,\alpha$ -Trehalose dihydrate was obtained from Quadrant Healthcare Ltd. A 10% w/v aqueous solution was spray-dried using a Buchi 190 mini-spray-drier. The powder obtained was X-ray amorphous (Siemens D5000 X-ray powder diffractometer) and was found to contain 2-3% w/w moisture by Karl Fischer titration analysis. The powder was then stored over phosphorous pentoxide at 20 °C for six months to provide Batch 1. A second batch (Batch 2) was freshly spray-dried following the same procedure as for Batch 1. Moisture sorption isotherms were obtained for the spray dried Trehalose samples using the IGAcorp Dynamic Vapour Sorption Analyser manufactured by Hiden Isochema Ltd. UK.

All studies were performed at 25°C. In turn each sample was loaded into the IGAcorp under atmospheric conditions and the initial weight measured. Sample sizes varied between 25 and 50mg. The environment around the sample was then set to 5%RH and the sample was allowed to equilibrate. A moisture adsorption desorption isotherm was performed on each sample which consisted of step like changes in RH between 5%RH and 95%RH for adsorption and step like changes in RH between 95%RH and 5%RH for desorption. At each set-point the equilibrium mass and kinetic rate constant were determined using the IGA Method described below. A second sample from Batch 2 of the spray dried Trehalose was studied under slightly different conditions. The sample was initially dried and the adsorption isotherm was studied in the range 0-95%RH and the desorption isotherm was studied in the range 95-0%RH.

## The IGA Method

The IGAcorp is a fully automated and computer controlled instrument which continuously monitors the moisture content of the sample whilst accurately controlling both the temperature and relative humidity. A schematic of the IGAcorp is presented in figure 2. A sensitive microbalance (resolution of 0.1 $\mu$ g) is utilised to measure changes in the moisture content of the sample. The microbalance is mounted in a thermostated



Figure 1: The IGAcorp dynamic vapour sorption (DVS) analyser

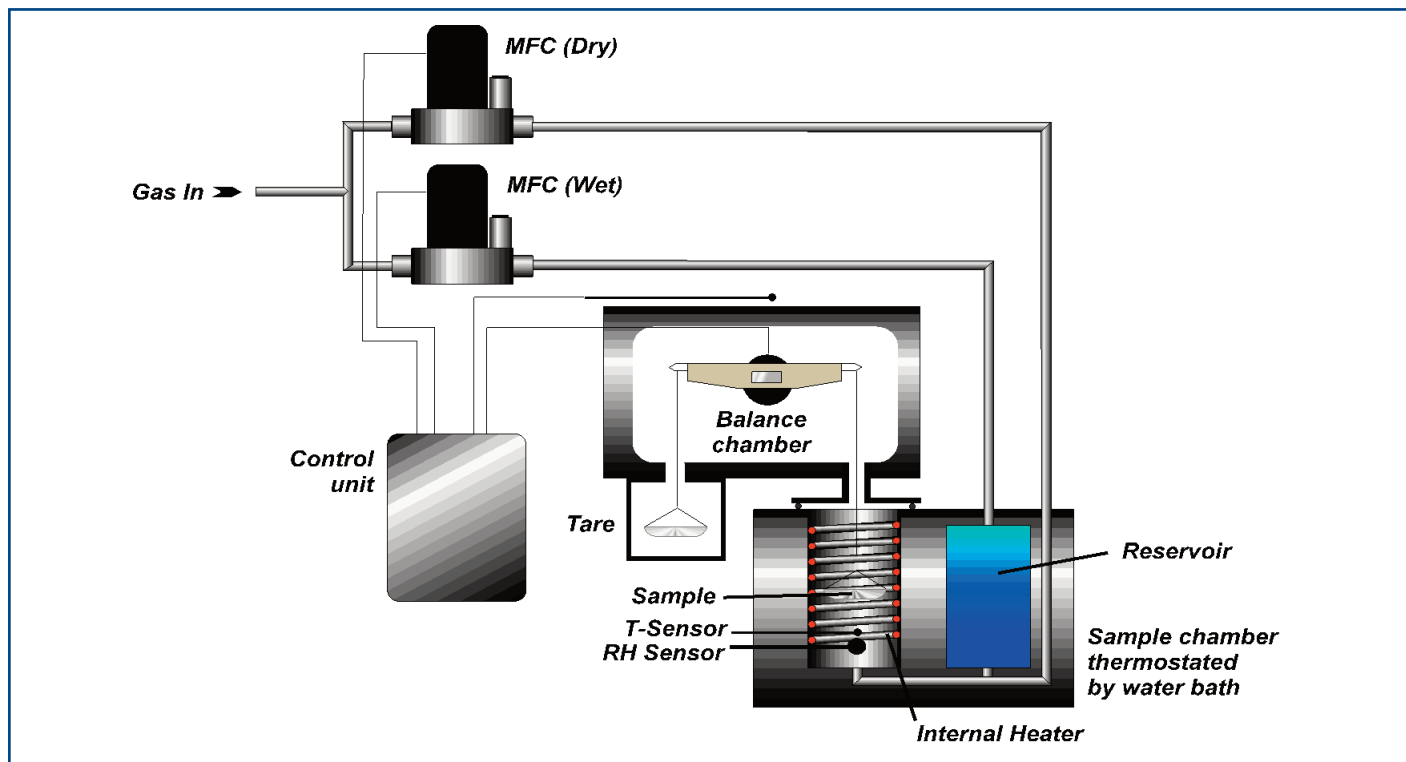


Figure 2: Schematic Representation of IGAsorp.

enclosure to remove thermal coefficients of the weighing system and thus provide higher stability. The sample temperature is regulated to  $\pm 0.005^\circ\text{C}$  by a water bath which pumps water through a network of channels in the sample block. The temperature can be varied between 5 and  $85^\circ\text{C}$ . An additional internal heater is situated in the sample chamber for rapid drying, temperatures up to  $250^\circ\text{C}$  may be attained. The relative humidity is controlled in the sample chamber dynamically by combining different flow rates of wet and dry gas streams prior to injection. The reservoir in the sample block is utilised to humidify a gas stream and thus produces the wet stream. The RH sensor is used to measure and control the relative humidity in the sample chamber. The RH can be varied between 0 and 95%. Any sample will come to an equilibrium moisture uptake capacity at a defined humidity and temperature. If either of these conditions are altered the sample will either release or take up moisture to maintain energetic equilibrium. The IGAsorp software exploits the IGA method as shown in the Fig. 3. From an equilibrium state, the relative humidity is rapidly changed to a new set-point and then regulated at this new value. The sample will react by altering its adsorbed capacity. The trend of the data (mass versus time) is analysed in real-time as the sample capacity approaches the new equilibrium condition. This relaxation is modelled using the Avrami model: This is used to determine when the new equilibrium condition has been achieved. Once attained the equilibrium uptake values can be recorded and the system can proceed to measure the next isotherm point. Once the full adsorption has been measured the desorption can be similarly measured.

The method provides a consistent analysis point-to-point on the isotherm thus removing human subjectivity. The kinetic data usually discarded or not measured with other techniques

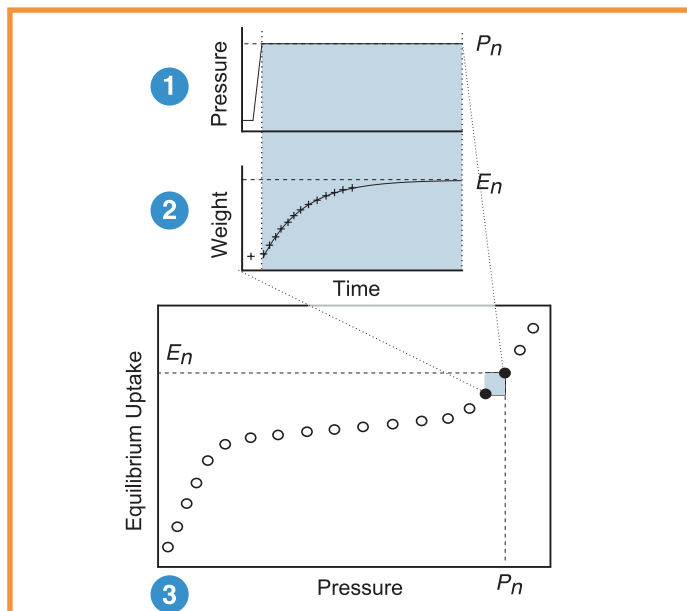


Figure 3: The IGA Method

- 1 The gas pressure or composition in the balance chamber is ramped to the setpoint,  $P_n$ , and then held constant through active control of the inlet gas flow
- 2 The weight change is analyzed in real time to determine kinetic parameters and simultaneously predict the equilibrium point,  $E_n$
- 3 Equilibrium points are then collected, corrected for buoyancy, and plotted as an isotherm

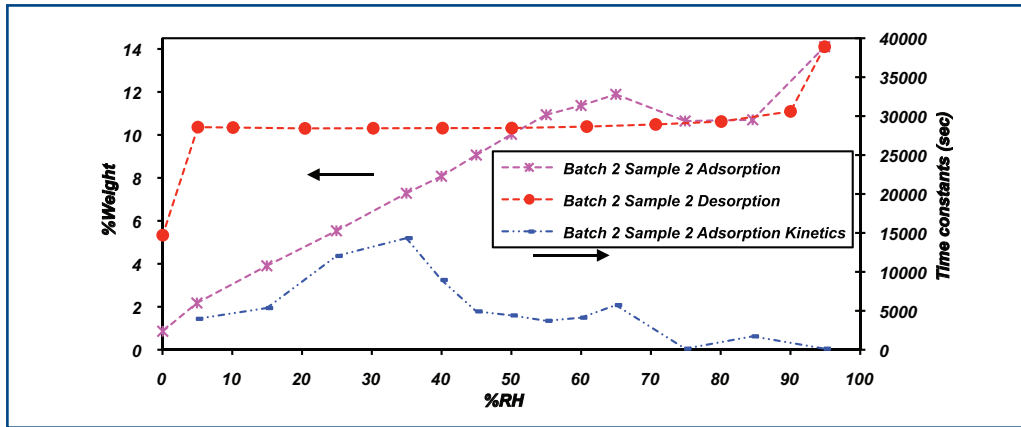


Figure 4: Moisture sorption isotherm (left axis) and kinetics (right axis) measured on spray dried Trehalose

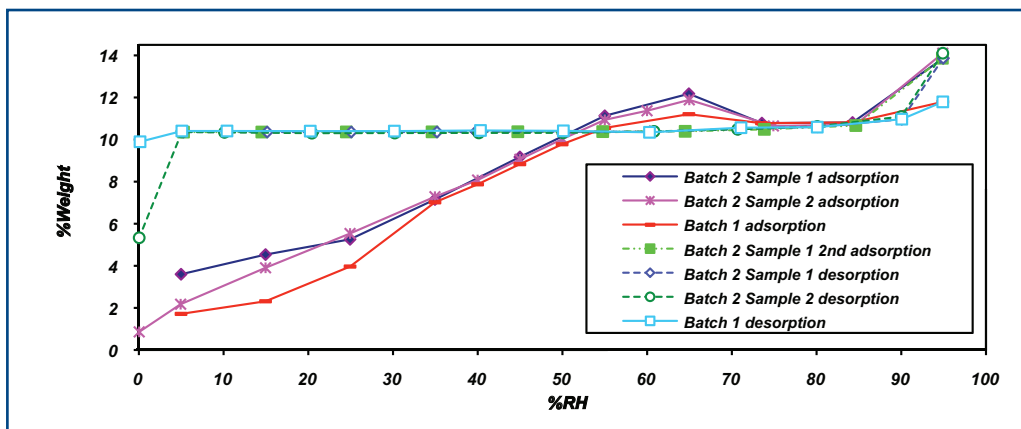


Figure 5: A comparison of moisture sorption isotherms measured on spray dried Trehalose samples

is accessible and can prove to be just as valuable as the equilibrium isotherm, as in the case of this study of Trehalose.

Each humidity step results in a determination of the equilibrium weight and a  $k$  and  $x$  value which describe the kinetics of the process. In the results section the kinetics are plotted as time constants which describe the time taken to reach 99% of the predicted equilibrium.

**Results**

The results of the equilibrium isothermal study are presented in Table 1 and results of the kinetic time constant study are shown in Table 2. Moisture contents were calculated from the reference point taken as the mass of the di-hydrate crystalline phase. This was the most acceptable reference point as any amorphous phase which may have been present would have been observable as irreversibility or uptake at lower RH's than those observed.

An adsorption-desorption isotherm measured on sample 2 of Batch 2 of the spray dried Trehalose, is displayed in Fig. 4. Features to note are the constant gradient between 5 and 55%RH, the classic loss of water accompanying the amorphous to crystalline phase transition at 65%RH after

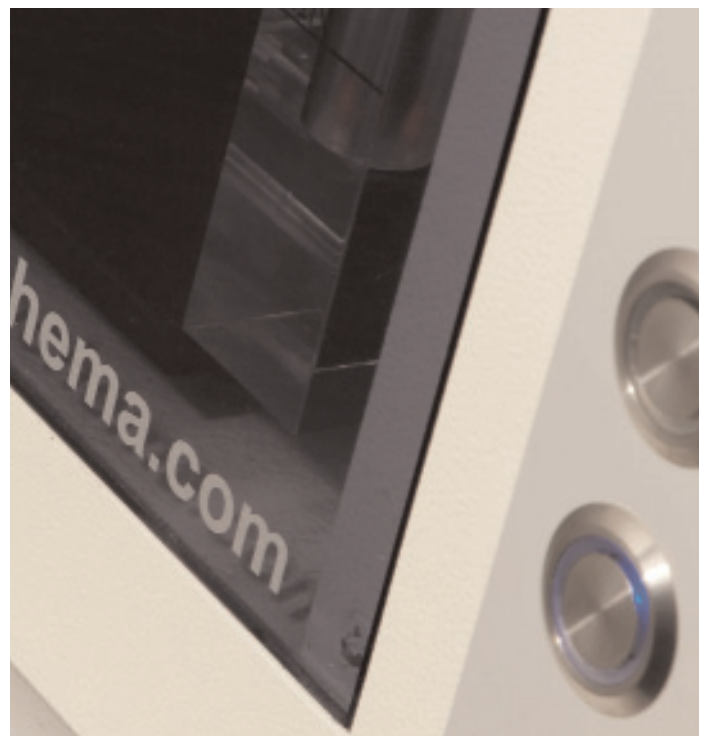


Table 1: Equilibrium % weights obtained at 25 °C for Spray Dried Trehalose

%-RH	Batch 1	Batch 2 - Sample 1 1st cycle	Batch 2 - Sample 1 2nd cycle	Batch 2 - Sample 2
0				0.9
5	1.7	3.6	10.4	2.2
15	2.3	4.5	10.4	3.9
25	4.0	5.3	10.4	5.5
35	7.0	7.2	10.4	7.3
40	7.9			8.1
45	8.8	9.2	10.4	9.1
50	9.8			10.0
55	10.6	11.1	10.4	10.9
60				11.4
65	11.2	12.2	10.4	11.9
75	10.8	10.8	10.5	10.7
85	10.8	10.9	10.7	10.7
95	11.8	13.9	13.9	14.1
95	11.8	13.9		14.1
90	11.0	11.0		11.1
80	10.6	10.6		10.6
70	10.6	10.5		10.5
60	10.3	10.4		10.4
50	10.4	10.4		10.3
40	10.4	10.4		10.3
30	10.4	10.4		10.3
20	10.4	10.4		10.3
10	10.4	10.4		10.3
5	10.4	10.4		10.4
0	9.9			5.3

which the isotherm becomes a classic reversible type III isotherm associated with the 100% di-hydrate crystalline form. The 0%RH point on the desorption isotherm shows the water of hydration being lost from the crystal structure. Interesting observations can be made from comparing the kinetic time constants with the equilibrium isotherm.

Firstly the kinetics show a clear peak which indicates a slowing down and then a speeding up of the diffusion of water through the structure. Water is known to function as a plasticiser and lower the glass transition temperature,  $T_g$ , of amorphous polymers. It is also known that the viscous-elastic properties

the moisture content that will reduce  $T_g$  to the temperature of solids are altered significantly above  $T_g$  (rubbery state) to below  $T_g$  (glass state). Thus, the distinct change in the water diffusion properties, that we observe from the kinetic data, is likely to be attributable to a glass phase transition. Applying this interpretation then the three plateaus can be associated with the initial amorphous glass phase (5-15%RH), the post glass transition amorphous phase (45-55%RH) and the crystalline phase after the amorphous to crystalline phase transition (75-95%RH). The glass and rubbery state amorphous phase kinetics are more similar in value when compared with the more rapid kinetics associated with the crystalline phase. Using equilibrium data, Oksanen and Zografis [3] have found that the moisture content at which the moisture sorption isotherm increases significantly correlates well with the isotherm. Our preliminary findings indicate that kinetic data may be equally valuable in identifying glass state transitions. Since compaction, gelatin capsule and freeze-dried product properties can vary above or below glass state transition, a kinetic analysis may find much wider application.

A comparison of equilibrium isotherms measured on three samples: sample 1 and sample 2 of Batch 2 of the spray dried Trehalose and one sample from Batch 1 of the spray dried Trehalose (Table 1) shown in Figure 5, reveals that isotherms are comparable above 35%RH with the comparability region



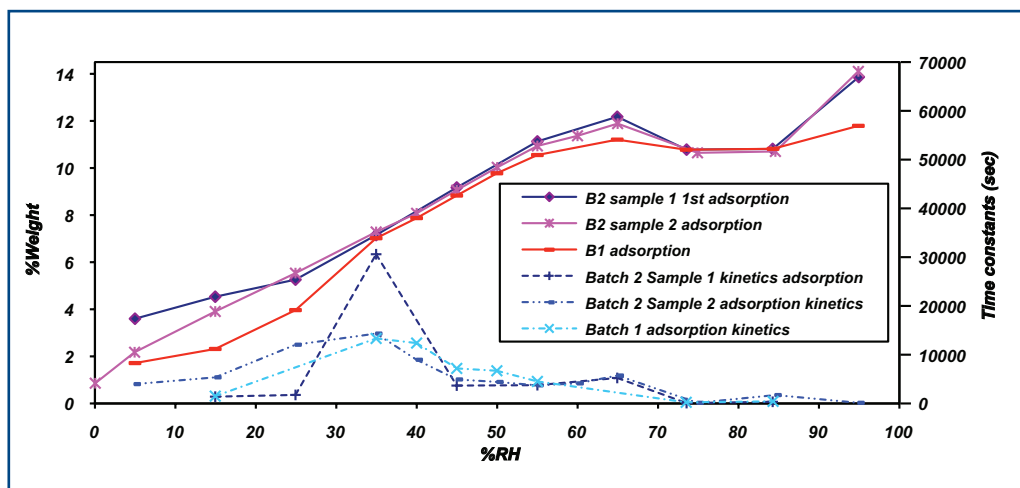


Figure 6: Comparison of moisture sorption isotherms and kinetics measured on spray dried Trehalose samples

extending to 25%RH for the two samples from Batch 2. Though the isotherms are comparable in this region the sample from Batch 1 consistently shows a slightly lower uptake even at 95%RH in the crystalline phase. The differences observed below these points indicate that there is a history effect in the moisture isotherms i.e. some kind of hysteresis occurs. Further investigation in this region is required to ascertain the true equilibrium paths. All samples showed a transformation from amorphous phase to crystalline phase at 65%RH. Desorption data (Table 1) on Batch 2 measured after the crystalline phase transition show the type III isotherm to be fully reversible.

Inspection of kinetic data (Table 2 and Figure 6) reveals that samples show similar features in their time constant curves to those presented in Figure 4. A peak is observed showing the slowing down and then speeding up of the moisture diffusion within the structure as the glass phase transition takes place. The three plateaus associated with: the amorphous phase before the glass transition, the glass phase and the crystalline

phase can also be observed. The kinetics associated with the points before the glass transition on the Batch 1 sample are noticeably slower than those observed on the Batch 2 samples. After the glass transition has taken place the kinetics of the batches are comparable. The most interesting feature is the coincidence of the glass transition indicated by the peak in the kinetics with the convergence of the equilibrium isotherms. This suggests that the differences in the isotherms at RH's below this point are due to differences in the initial amorphous phase and are probably due to the hysteresis, but the data indicates that the variations in the initial phase have no apparent effect on the moisture sorption initiated transitions and the subsequent moisture sorption on the phases formed. Further work is required to enable fitting of the kinetics of the process associated with the glass to crystalline phase transition. This is complicated by several processes occurring concurrently these include moisture uptake into any amorphous phase present, moisture loss from the crystalline phase and the molecular motion of the sugar molecules to form the crystalline phase.

Table 2: Time-constants of absorption (sec) obtained at 25 °C on Spray Dried Trehalose

%-RH	Batch 1	Batch 2 - Sample 1 1st cycle	Batch 2 - Sample 2
5	1502.99	1397.6	3970.88
15		1752.1	5355.34
25	13327.45	30597.56	12044.18
35	12385.24		14332.48
40	7186.71	3669.49	8960.04
45	6705.71		4926.51
50	4516.68	3750.23	4411.87
55			3717.14
60		5230.63	4118.06
65	239.79	79.03	5733.59
75	427.63	342.95	137.13
85			1723.22
95			138.16

In conclusion, the study has demonstrated that water acting as a plasticiser can allow the molecules of the sugar to move and re-order into more energetically favourable structures and reveal the regions over which each phase is present. Furthermore, the data provided some evidence to show that aging of spray dried amorphous Trehalose may occur. (Lower uptakes observed slower kinetics in the initial amorphous phase). However correlation with crystallinity data is required to fully interpret this data. However, despite any changes in sample crystallinity that may have occurred on storage, the aging only has a slight effect after the sample has been transformed into the rubbery phase.

#### References

- [1] C. A. L. S. Colaco, C. J. S. Smith, S. Sen, D. H. Roser, Y. Newman, S. Ring and B. J. Roser. ACS Symposium Series. 567 (1994) 222-240
- [2] B. Roser, BioPharm 4(8) (1991) 47
- [3] C. A. Oksanen and G. Zografi, Pharm. Res. 7(6) (1990) 654-657

