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International Journal of Pharmaceutics 294 (2005) 129–135

international journal of pharmaceutics

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# The use of inverse gas chromatography and gravimetric vapour sorption to study transitions in amorphous lactose

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Received 27 September 2004; received in revised form 13 January 2005; accepted 18 January 2005

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## Abstract

The aim of this study was to measure the glass transition of amorphous lactose under well-controlled temperature and humidity, using inverse gas chromatography (IGC) and to relate these data to gravimetric vapour sorption experiments. Amorphous lactose (spray-dried) was exposed to a stepwise increment in the relative humidity (%RH) under isothermal conditions in an IGC. At the end of each conditioning step a decane injection was made, and the retention volumes were calculated using the maximum peak height ($V_{\max}$) method. The pressure drop across the column was recorded using the pressure transducers. These measurements were performed at various temperatures from 25 to 40 °C. The extent of water sorption at identical humidity (%RH) and temperature conditions was determined gravimetrically using dynamic vapour sorption (DVS). At each $T$, it was possible to determine: (1) a transition at low RH relating to the onset of mobility; (2) changes in retention volume relating to the point, where $T_g = T$; (3) changes in pressure drop, which were related to the sample collapse. The rate and extent of water sorption was seen to alter at $T_g$ and also at a collapse point. Combinations of temperature and critical %RH (%cRH required to lower the dry glass transition temperature to the experimental temperature) obtained from IGC were comparable to those obtained from DVS. It was shown that at each $T$, the sample spontaneously crystallised, when $T_g$ was 32 °C below $T$. Inverse gas chromatograph can be used in this novel way to reveal the series of transitions that occur in amorphous materials.

*Keywords:* Amorphous; mobility; Glass transition; Collapse; Crystallisation; Inverse gas chromatography; Water sorption

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## 1. Introduction

Completely or partially amorphous materials (active pharmaceutical ingredients and excipients) are regularly produced, either during solidification (e.g. spray drying) or through subsequent processing (e.g. Briggner et al., 1994). The study of the amorphous state, the glass transition temperature ($T_g$) and the crystallisation from the amorphous state are all of importance as the material will change its physico-chemical properties with each transition, with likely impact on product performance.

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doi:10.1016/j.ijpharm.2005.01.034

Amorphous lactose has been the subject of many investigations, both as a model material and also as it is frequently used in pharmaceutical products in both the crystalline and amorphous forms. A number of approaches have been shown to be suitable for the study of the amorphous content of lactose, including isothermal microcalorimetry (Briggner et al., 1994; Ahmed et al., 1996) gravimetric water sorption (Buckton and Darcy, 1995), gravimetric sorption with near infrared spectroscopy (Buckton et al., 1999; Lane and Buckton, 2000), solution calorimetry (Hogan and Buckton, 2000), MDSC (Craig et al., 2000) and recently high-speed DSC (Saunders et al., 2004).

Recently, we (Buckton et al., 2004) have presented a new method for the study of the surface of amorphous materials. It was shown that the shape of the retention peak for an inverse gas chromatography (IGC) peak changed, when the surface of the particle reached conditions (relative humidity (RH) and temperature (*T*)) that resulted in the material passing through its glass transition temperature. The change in peak shape was monitored by following the changes in the peak maximum and the peak centre of mass for the retention of a probe (decane) at infinite dilution, on a model material (amorphous indomethacin) that did not readily absorb water. It was seen that the centre of mass of the peak deviated greatly from the peak height (in other words, the peak started to show greater tailing), at the point, where the surface was plasticized, due to the increased accessibility of the surface allowing absorption of the probe. An example of the deviation between the peak maximum (giving retention volume $V_{max}$) and the peak centre of mass (giving retention volume $V_{com}$) is shown in Fig. 1. This study (Buckton et al., 2004) was very valuable in showing the surface $T_g$ of a material in which water vapour did not readily equilibrate (i.e. it was not possible to know how much water remained at the surface and how much diffused into the bulk). The aim of the current work is to explore whether further information can be obtained on transitions in amorphous lactose as a function of temperature and RH in an IGC, with a view of understanding the data generated by other techniques.

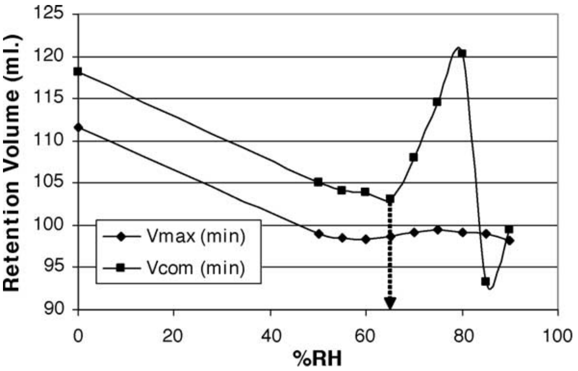

Fig. 1. Retention volume of decane on amorphous indomethacin, showing a major deviation in the $V_{com}$ and $V_{max}$ after 65% RH; column temperature, 30 °C (from Buckton et al., 2004).

## 2. Materials and methods

Amorphous lactose was prepared from α-lactose monohydrate (Borculo Whey Products, UK) by the spray-drying method described previously (Chidavaenzi et al., 1997). It was demonstrated to be amorphous by X-ray powder crystallography.

### 2.1. Inverse gas chromatography

The IGC was operated as described by Newell et al. (2001) using a commercial IGC (iGC, Surface Measurement Systems, London, UK).

### 2.2. Column preparation and conditioning

About 300–400 mg of amorphous lactose was packed in pre-silanated glass columns (i.d. 3 mm) using a standardized tapping method. The packed column was then placed in a column oven (temperature stable over ±0.2 °C of the set value) of the gas chromatograph and conditioned at 30 °C, using dry He (purity > 99.996%; moisture level < 6 ppm) (BOC, UK) as the carrier gas at a flow rate 20 sccm (standard $cm^3$/min) for 4 h at 0%RH to remove any adsorbed or absorbed water and other gasses from the packed sample. RH, which was initially set at 0%, was then sequentially increased in steps. Conditioning at each RH step was performed for 40 min before performing the injections of the probe gases. Moisture levels in the carrier gas were detected using a thermal conductivity detector (TCD). Control of RH was through mass flow controllers, which were electronically calibrated by Surface Measurement Systems and which are designed to give the same level of RH control that is obtained for the DVS experiment.

### 2.3. Elution method

Elutions using decane (Acros Organics; purity > 99%) injections with concentrations 0.08% p/p° were performed at a flow rate of 5 sccm of He at the required %RH, methane (BOC, UK) was used as internal standard. The solvent oven temperature was maintained at 50 °C (±0.2 °C). Elution times of decane and methane were measured using the flame ionisation detector. Retention volumes $V_{\max}$ and $V_{\mathrm{com}}$ were calculated using the IGC analysis software (Version 1.2 standard), from the time for maximum peak height and the centre of mass of the retention peak, respectively. The pressure drop across the column was monitored at each RH/T using the pressure transducers fitted to the IGC.

### 2.4. Water sorption

Gravimetric water sorption data were generated using a Dynamic Vapour Sorption apparatus (Surface Measurement Systems, London, UK), using steps in RH from 0 to 60% at temperatures of 25, 30, 35 and 40 °C; d$m$/d$t$ (<0.001 for 10 min) criteria were used while stepping up the RH conditions.

## 3. Results and discussion

It can be seen in Fig. 2 that unlike the amorphous indomethacin (Fig. 1), there is no place at which there is a break between the $V_{\mathrm{com}}$ and $V_{\max}$. Previously, the point at which $V_{\mathrm{com}}$ and $V_{\max}$ deviated (i.e. the point at which there was an increase in tailing of the retention peak on the IGC) was taken to be the point at which the surface had been plasticized such that $T_g = T$, (Buckton et al., 2004). The rationale for this was that the transition equated to a change in the ability for decane (the IGC probe vapour) to absorb into the sample. In the current study, $V_{\max}$ and $V_{\mathrm{com}}$ are similar and do not deviate, which would indicate that there is little tendency for decane to absorb into amorphous lactose. There is further work required to see if other probes can be selected, which would show changes in absorption and hence a deviation between $V_{\max}$ and $V_{\mathrm{com}}$ for lactose. Despite the fact that the $V_{\max}$ and $V_{\mathrm{com}}$ did not deviate from each other as RH changed, it is clear (Fig. 2) that the retention volume changes as a function of humidity. Also plotted on Fig. 2 is the pressure drop across the column, and it is clear that there are two regions in which this changes, one after 10% RH and another after 35% RH.

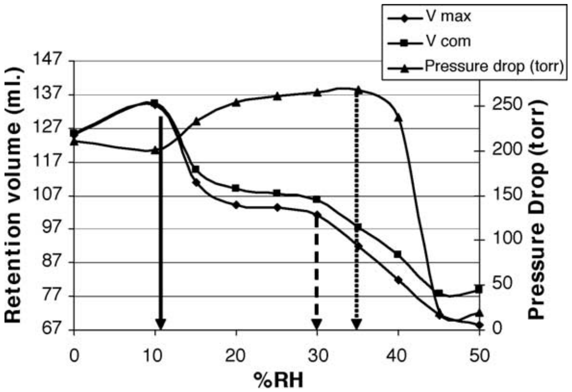

Fig. 2. Retention volume calculated as $V_{\mathrm{com}}$ and $V_{\max}$ and pressure drop across the IGC column for amorphous lactose at 30 °C initially 0%RH and sequentially increasing RH. Arrows indicating the transitions in the amorphous lactose: (—) first transition; (···) glass transition; (- - -) collapse.

### 3.1. The low RH transition region (ca. 10% RH)

The low RH region was explored further by studying the retention behaviour of decane as a function of T and RH. It can be seen (Fig. 3) that the retention

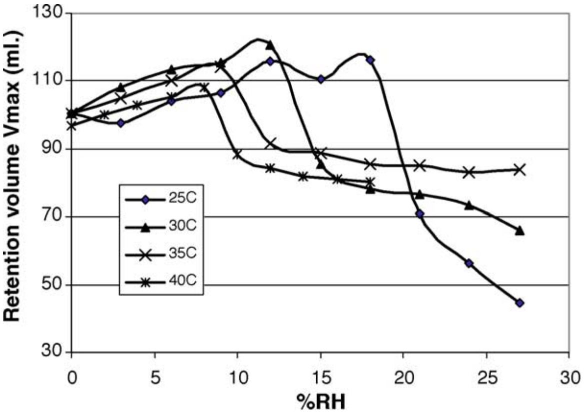

Fig. 3. The retention volume ($V_{\max}$) of decane as a function of RH at different $T$, showing the first transition in amorphous lactose.

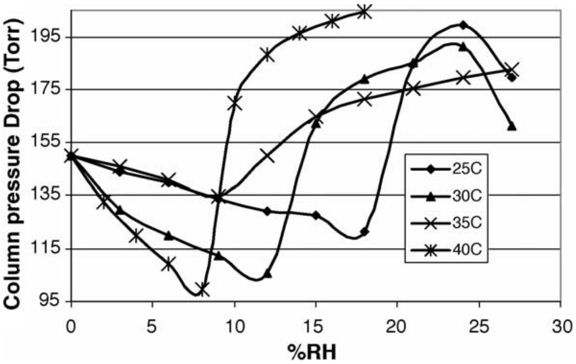

Fig. 4. The column pressure drop for as a function of RH at different $T$, showing the first transition in amorphous lactose.

behaviour showed a clear break at the following conditions: 25 °C/18% RH; 30 °C/12%RH; 35 °C/9% RH and 40 °C/8% RH. These transitions exactly match the change in pressure drop in the column (Fig. 4), demonstrating that the sample is altering its geometry at a defined point, which alters the retention volume of the decane.

The gravimetric data for mass uptake at each temperature are shown in Fig. 5. It can be seen that from 8 to 18% RH, the mass change starts to deviate between each isotherm, but the range of mass change at all temperatures is 2–3%. By use of the Gordon–Taylor equation

$$T_{g_{\text{mix}}} = \frac{w_1 T_{g_1} + w_2 k T_g}{w_1 + k w_2}$$

(where $w_1$ and $w_2$, are the masses and $T_{g_1}$and $T_{g_2}$ glass transition temperatures for dry amorphous lactose and water, respectively, $T_g$ for water is −135 °C and for lactose 110 °C, $k$ was taken as 6.56 (LRoos, 1993)) it is possible to calculate the theoretical $T_g$ for any selected water content. For 2% water content, the calculated $T_g$ is 81 °C, and for 3%, it is 67 °C. This equates to $T_g$ being approximately 40 °C above the experimental temperature at the point of this transition, this is in keeping with the proximity to $T_g$ below which there is no, and above which, there is significant molecular mobility (Hancock and Zografi, 1997). It is likely, therefore, that the IGC method is revealing a transition that equates to the onset of significant molecular mobility in the amorphous material.

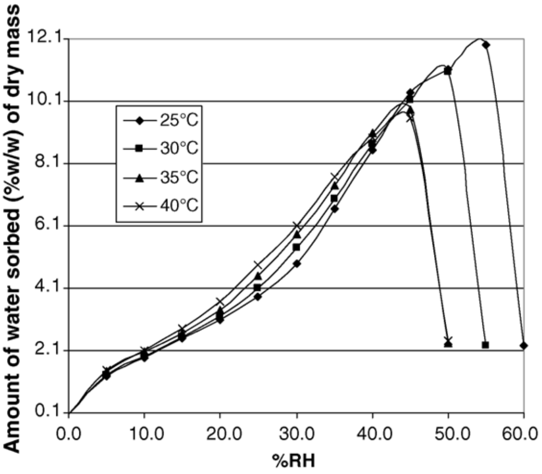

Fig. 5. Sorption isotherms for amorphous lactose at different temperatures showing changes in the onset of spontaneous crystallisation and differences in the sorption between the $T_g$ region and the point of crystallisation.

The deviations seen in the sorption isotherms in this 8–18% RH region are also in keeping with a change from apparent monolayer to multilayer sorption. The use of adsorption isotherms to fit absorption data for amorphous material is clearly not correct, but is commonly undertaken (Lechuga-Ballesteros et al., 2003). As a comparison, the water sorption data were fitted to a BET isotherm using the water uptake at RHs from 0 to 30%RH at 25 and 40 °C. The initial linear part was used to calculate the water volume ($V_m$) for monolayer coverage of the sample (Fig. 6). Values of $V_m$ and the corresponding relative humidity (%RH$_m$) (Table 1)

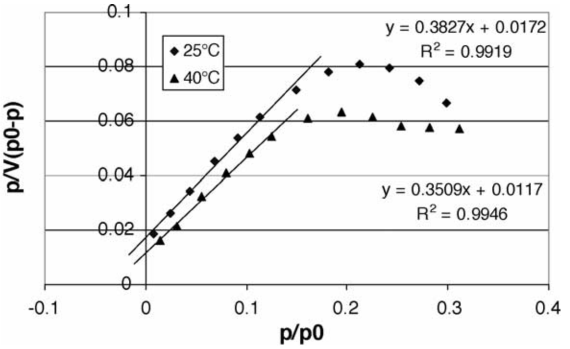

Fig. 6. BET plot for water sorption of amorphous lactose to calculate the monolayer volume coverage ($V_m$) and the corresponding vapour pressure at 25 and 40 °C using DVS; values obtained are shown in Table 1.

Table 1
$V_m$ values calculated from BET equation for water sorption on amorphous lactose at 25 °C and 40 °C and corresponding vapour pressure (RH) for monolayer coverage

| Temperature (°C) | $V_m$ (cc/g) | %$RH_m$ |
|---|---|---|
| 25 | 25.00 | 17.2 |
| 40 | 27.58 | 15.2 |
| Reported value at 25 °C[a] | | 16 |

[a] Lechuga-Ballesteros et al. (2003).

at 25 and 40 °C are close to the point, where the first transition is seen in the IGC.

### 3.2. The second transition region (30–40% RH)

As described above, and shown in Fig. 2, a further transition occurs in the sample in the region between 30 and 40% RH.

A typical water sorption profile for amorphous lactose (25 °C) is shown in Fig. 7. The sample readily equilibrates with water vapour at 5, 10, 15, 20 and 25% RH, where plateau values are seen. At 30 and 35% RH, the sample does not reach a plateau water uptake, despite longer equilibration times. At 40, 45, 50 and 55% RH, there is substantial water sorption and in each case, a plateau is reached, without the need for long equilibration times. At 60%RH, the sample spontaneously crystallises. These water sorption data show that after 30% RH, there is a change in behaviour, with this value being in the region shown to be the point, where the IGC pressure drop and retention changed (Fig. 2). Collapse of the lactose column is evident from the fall in the pressure drop values with the original column offering more resistance to the flow of carrier gas than the collapsed column (35–40%, Fig. 2). Retention of the decane probe also changed (30–35%) just before the onset of collapse as seen from the break in the retention volume versus %RH plot. It is reasonable to argue that the changes in retention volume and pressure drop (Fig. 2) are, respectively, the glass transition and consequential collapse in the amorphous lactose as a function of change in RH.

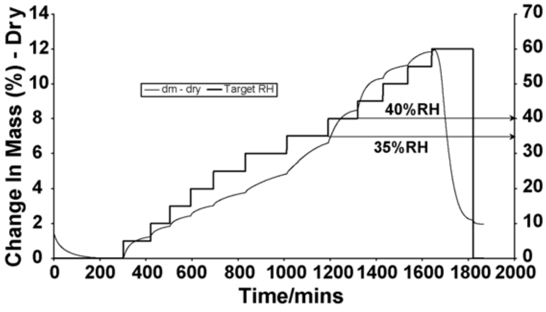

Fig. 7. The sorption of water into amorphous lactose, as a function of RH, at 25 °C.

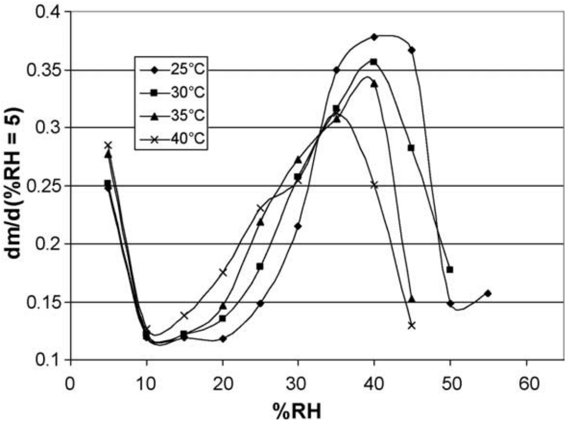

Fig. 8. The mass change differential with each 5% increase in RH at each $T$ for amorphous lactose (all data up to the RH immediately before the one which resulted in spontaneous crystallisation).

In Fig. 8, the differential change in mass for each step increase in RH is plotted for different temperatures. Differential weight change was calculated by measuring the additional water sorbed by the amorphous lactose during each 5% step rise in the RH. It can be seen from Fig. 8 that between 10 and 35/40%RH, each step change in RH results in sample sorbing more water than the previous step and a maximum is reached (35–45%RH at 25 °C, 35–40%RH at 30 and 35 °C, 30–35%RH at 40 °C). This critical region in water sorption (30–40% RH) is in keeping with the region, where major changes are seen in the IGC data. Using the Gordon–Taylor equation (as described above), it is possible to calculate the mass of water that will result in $T_g$ being reduced to $T$. For $T = 25$ °C, this is 7.5%, 30 °C, it is 6.9%, 35 °C, it is 6.3% and 40 °C, it is 5.7%. By looking at the water sorption data in Fig. 5, it is clear that these mass changes cover the region between approximately 30–35% RH. The very clear deviation in retention volume (Fig. 2) is in keeping with the point, where the rate and extent of water sorption are seen to change (Figs. 7 and 8) and also with the calculated

Table 2
Combination of temperature and %RH for glass transition and collapse of amorphous lactose obtained from IGC

| Temperature (°C) | Glass transition (%RH) | Collapse (%RH) |
|---|---|---|
| 25 | 34–38 | 38–42 |
| 30 | 26–30 | 30–34 |
| 35 | 18–21 | 21–24 |
| 40 | 18–21 | 21–24 |

Table 3
The extent of water sorbed at the point, where spontaneous crystallisation is detected (see Fig. 7) and the calculated $T_g$ using the Gordan–Taylor equation

| Temperature $T$ (°C) | Weight of water sorbed before onset of crystallisation (%) | $T_g$ mix (GT equation) (°C) | $T - T_g$ mix |
|---|---|---|---|
| 25 | 11.9 | −6.8 | 31.8 |
| 30 | 11.2 | −1.9 | 31.9 |
| 35 | 10.3 | 3.7 | 31.3 |
| 40 | 9.6 | 7.9 | 32.1 |

The final column shows the difference between the isotherm temperature $T$ and the calculated $T_g$.

point at which $T_g$ should equal $T$. Thus, this break point on IGC retention volume is a very clear indication of when the sample passes through $T_g$.

The point in Fig. 8 at which the differential water sorption starts to decline follows just after $T_g$ and is indicative of structural collapse. This is due to water having less ready access to the collapsed mass. This change in water sorption is mirrored by the pressure drop change in the IGC, which is a very good and clear indication of collapse.

The RH regions in which there is a deviation of retention volume (glass transition) and pressure drop (collapse), measured using IGC, are shown in Table 2 for each experimental temperature. The RH values required to lower the $T_g$ of the amorphous lactose to the experimental temperature (25 °C) determined by IGC are close to the ones calculated using the Gordon–Taylor equation with DVS data. A direct comparison is performed in Fig. 9, the RH required to lower the $T_g$ to 25 °C calculated from DVS data was 37%, whereas the RH measured by IGC using the break in the retention volume line for decane was 34–38%. In the same RH range, the maximum in the differential water uptake was observed. Collapse, as observed from IGC (fall in the pressure drop values) was initiated at 42% and continued till 54%RH, whereas the same phenomena observed by fall in the water uptake was initiated at 45%RH continuing till 50%RH.

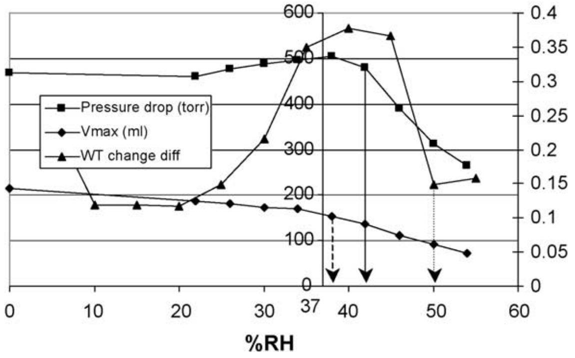

Fig. 9. Comparison of the glass transition and collapse seen using DVS and IGC at 25 °C for amorphous lactose: (- - -) glass transition from change in retention volume; (—) collapse from pressure drop; (· · ·) collapse from reduced water uptake by DVS, value predicted using Gordon–Taylor equation 37%RH.

### 3.3. Crystallisation

The onset of crystallisation is very clear from the data in Fig. 5. This occurs above 45% RH and is well above the region in which $T_g$ has fallen to $T$. It is often said that spontaneous crystallisation occurs, when $T_g = T$, but this is in fact not the case. Crystallisation can occur, when $T_g$ is above $T$ and will occur more rapidly, when $T_g$ is at, and much more rapidly as $T_g$ falls below $T$.

The significance of the crystallisation point (Fig. 5) for each RH can be demonstrated by again considering the Gordon–Taylor equation. By using the amount of water sorbed at the onset of crystallisation, the extent of $T_g$ depression was calculated and compared to the experimental temperature (Table 3). In each case, the difference was the same at 32 °C.

## 4. Conclusion

A combination of IGC and gravimetric sorption data has allowed a thorough understanding of transitions that occur during the water sorption to amorphous lactose.

Firstly, there is a transition in the material (by IGC) approximately at the point of the first inflection of the sorption data. At this point, $T$ is ca. 40 °C below $T_g$, and this can be regarded as the point, where mobility of the amorphous form becomes significant.

It has been shown that IGC can identify the $T_g$ of amorphous materials by following retention of a single probe and that this is seen to correlate to the region, where the rate and extent of water sorption to the amorphous material changes. These changes in gravimetric data are not obvious from the sorption isotherm, but are clear, when the raw data of mass change as a function of time are considered.

Just after the point, where the glass transition is detected, the pressure drop across the IGC column shows substantial deviation due to the collapse of the amorphous structure.

For each temperature studied the point, where spontaneous crystallisation is seen is the combination of $T$ and RH that causes $T_g$ to be 32 °C below $T$.

IGC, in combination with moisture sorption data, has proved powerful in showing these four significant features (initial mobility, $T_g$, collapse, crystallisation), and this is seen as a valuable advance in the study of amorphous samples.

## Acknowledgement

AA is grateful for financial support from Glaxo SmithKline.

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