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The Use of Inverse Phase Gas Chromatography to Study the Glass Transition Temperature of a Powder Surface

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Purpose. To measure the glass transition temperature (Tg) at the surface of a hydrophobic particle at different temperatures and humidities, on the hypothesis that the surface may be plasticized to a different extent to the bulk due to slow water sorption giving a concentration gradient of water through the particles.

Methods. Amorphous indomethacin was exposed to a range of relative humidities (RH) and temperatures in an inverse gas chromatograph (IGC). The retention volumes of decane were calculated at all conditions using center of mass (Vcom) and peak height (Vmax) methods. The extent of water sorption was determined gravimetrically.

Results. The Vcom retention volumes were found to deviate from Vmax results at certain critical humdities at each temperature. This was taken as a novel method for determining the Tg of the sample surface at different experimental conditions. Extrapolating the critical RH to lower the Tg to experimental temperature to 0% RH yeilded a Tg similar to literature values. Water sorption data provided valuable information on changes in mobility of the amorphous form as a function of temperature and RH.

Conclusions. It is possible to use IGC to determine the Tg of the surface of particles at defined conditions. This overcomes the problems of conventional methods of assessing Tg, relating to disruption of water sorption on heating. This helps in the understanding of the physical form of the surface of hydrophobic particles and how and when the surface will start to crystallize.

KEY WORDS: amorphous; glass transition; inverse gas chromatography; water sorption.

INTRODUCTION

There are major differences in physico-chemical properties between the amorphous and crystalline states of materials. Many crystalline materials become partially amorphous during processing, and it is the surface of the sample that is most affected (1). Consequently, processed materials can have a surface (amorphous) with very different properties to the bulk (crystalline) of the material. Newell *et al.* (1) have demonstrated that the surface energy of an amorphous material changes and assimilates to the surface energy of the crystalline material, prior to crystallization. Hogan and Buckton (2) have shown that amorphous particles develop the external shape of crystals prior to crystallization. Consequently, it is clear that relaxation processes within the amorphous state will result in changes in the physico-chemical properties of the amorphous form prior to any crystallization event taking place.

One factor that has a major influence of many amorphous materials is the absorption of water. Water is well-known to plasticize amorphous materials, resulting in spontaneous crystallization as the glass transition temperature (Tg) is depressed below room temperature. This induction of crystallization is very rapid when water absorbs freely into the sample, however when the material is more hydrophobic the reduction in Tg results in an increased rate of crystallization than is seen for dry samples, but this can still be slow and occur over many months. For example, Andronis *et al.* (3) have reported the effect of relative humidity (RH) on the crystallization of indomethacin, and have shown the amorphous sample to take more than 2 months to crystallize completely at 75% RH.

For hydrophobic materials that show absence of spontaneous crystallization, but an increase in rate of crystallization, it is clear that there is limited access for water absorption and hence the suppression of Tg is also limited. The issue that remains to be addressed is about water distribution within the amorphous material. Hydrophilic amorphous materials rapidly absorb very substantial amounts of water [upwards of 10% w/w, e.g., lactose, (4)], whereas hydrophobic samples sorb much less [perhaps 1–3% e.g., indomethacin (3)]. One possibility is that water distributes equally throughout the sample, but to a limited extent. This would mean that the Tg would be depressed to a limited extent throughout the sample. Alternatively, there could be a concentration gradient of water throughout the sample, where the surface has higher water content than the middle of the particle and thus the effective Tg is lower at the surface than in the bulk, this second option would allow the surface to be sufficiently plasticized to allow crystallization while the bulk would remain amorphous. The second type of behavior would provide an explanation for the surface crystallization described for amorphous indomethacin (3). Once the surface crystallizes, there would be a packing of certain regions to a higher density (crystallization) and this may well provide routes for water to access more of the bulk.

The measurement of Tg as a function of RH is difficult as methods for the study of Tg involve changes in temperature which will alter the water sorption, as well as water distribution within a sample. Consequently it is difficult to be clear about what effect water is having at the powder surface under conventional storage conditions.

The aim of this study was to investigate the surface of an amorphous hydrophobic powder as a function of temperature and humidity and to attempt to identify the critical RH that caused the surface to have the Tg lowered below the experimental temperature (T). The major tool used in this work was inverse phase gas chromatography, which provides a means of probing the surface of a material. More specifically, the retention of a single probe has been studied. If, as should be the case, the chromatographic retention peak is symmetrical, then using the time to peak height (tmax) and the time to reach the calculated center of mass of the peak (tcom) (5) would yield identical retention volumes (Vmax and Vcom, respectively,

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which would be identical). If however, the peak deviates from Gaussian, due to an altered interaction of the probe with the sample, for example absorption, then the peak will be distorted (tailing) and consequently tmax and tcom will differ (and so will calculated Vmax and Vcom). Hence a novel aspect of this work will be to consider differences in Vmax and Vcom as indicators of a change in retention mechanism for the probe, which in turn reflects a change in the nature of the sample in the column.

MATERIALS AND METHODS

Indomethacin was obtained from Becpharm (Essex, England).

Amorphous indomethacin was prepared by quenching using the method reported earlier (4). Briefly, crystalline indomethacin (γ form) was melted in a crucible and the melt was poured into a flask containing liquid nitrogen. Liquid nitrogen was evaporated and the sample was dried in an evacuated dessicator containing phosphorous pentoxide for 1–2 h. Lumps of amorphous indomethacin were then ground gently and passed through a 355 μm sieve. The sieved sample was then dried over phosphorous pentoxide and stored at −70°C to avoid any temperature induced crystallization. The material was tested using high speed DSC (Perkin Elmer Pyris 1, hermetic aluminium pans, 300°C/min, scanning to 250°C) to demonstrate that it was amorphous, which showed absence of any melt response.

The IGC was operated as described previously (1), using a commercial IGC (iGC, Surface Measurement Systems, London, UK).

Column Preparation and Conditioning

About 300–400 mg of amorphous indomethacin was packed in pre-silanated glass column (Internal diameter 3 mm) using a standardized tapping method. The packed column was then placed in a column oven of the gas chromatograph and conditioned at 30°C, using He as the carrier gas at a flow rate 20 sccm for 4 h at 0% RH.

Elution Method

Elutions using decane injections (concentrations 0.08% p/p^0) were performed (methane was used as an internal standard) at a flow rate of 5 sccm of He at the required %RH. The solvent oven temperature was maintained at 50°C. Elution times of decane and methane were measured using the FID detector and retention volumes were calculated, using both the maximum height and the center of mass of the retention peak (Vmax and Vcom, respectively). These measurements were performed at different RH conditions in the steps of 5%RH under isothermal conditions at 27, 30, 33, and 35°C. Once the transition region of interest was identified these measurements were performed at 3% RH steps to get the % critical RH at each temperature (%RH which, lowers the Tg to experimental temperature). These experiments were repeated twice to get the average % critical RH at each temperature (three injections were measured on each of two columns). Conditioning at each RH was for a period of 40 min and resulted in a flat response in the thermal conductivity meter, indicative of a pseudo equilibrium in water uptake (in keeping with gravimetric data, but not a full equilibration of the sample due to very slow diffusion of water into the bulk of the hydrophobic solid).

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 90% at temperatures of 25, 30, 35, and 40°C.

Differential scanning calorimetry (DSC) was undertaken using a Perkin Elmer Pyris 1, on samples after exposure to different conditions of RH and T in the DVS and IGC. The samples (ca. 2 mg) were loaded into hermetically sealed aluminium pans and scanned at 300°C/min under a nitrogen atmosphere.

RESULTS AND DISCUSSION

Typical retention volumes (Vmax and Vcom) determined for crystalline indomethacin as a function of RH at 30°C are shown in Fig. 1. It can be seen that there is a difference between the retention volumes calculated by peak maximum and center of mass calculations, which indicates that the peaks are not perfectly Gaussian (although they appear to be so when viewed by eye). However, the Vmax and Vcom data mirror each other as RH is changed, with a drop in retention volume at high RH values, presumably reflecting a lower affinity of decane for the surface when some water adsorption has built up.

The retention volumes for amorphous samples showed different behavior to that seen in Fig. 1 for the crystalline material. At each temperature, there was a point where the result for the Vcom deviated from that seen for Vmax. An example of this is seen in Fig. 2 and this is typical of the results at all temperatures, the difference being that the point at which the Vmax and Vcom data deviated was different at each temperature. The point at which the Vmax and Vcom deviate in Fig. 2 is the point where the chromatographic retention peak shows a substantial deviation in shape, relating to a tailing of the peak (deviation from near Gaussian to a skewed distribution). This tailing clearly is a reflection of a change in interaction between the decane probe and the column. It follows that the structure of the particles (hence the column) has altered, but not in a way that causes a major disruption to the peak position. The onset of the rise of re-

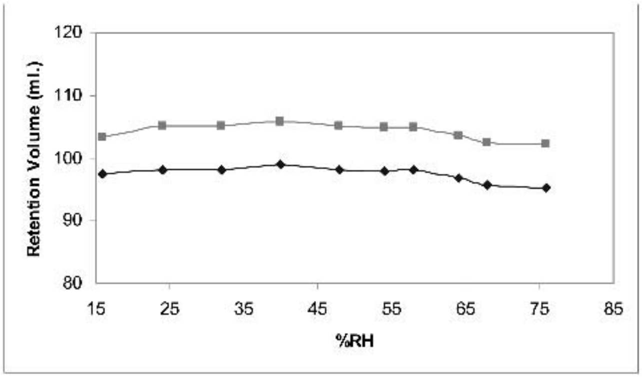

Fig. 1. Retention volumes for decane on crystalline indomethacin as a function of relative humidity at 30°C. (■) Vcom (ml) and (♦) Vmax (ml).

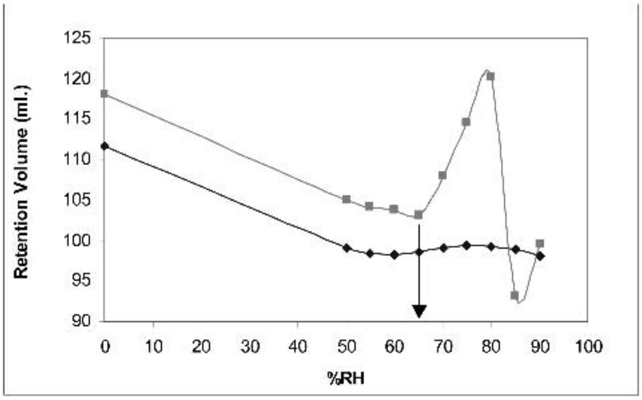

Fig. 2. Retention volumes for decane on amorphous indomethcain as a function of relative humidity at 30°C. (■) Vcom (ml) and (◆) Vmax (ml). Arrow shows the designated critical RH

tention volume (measured by Vcom) implies that some of the probe is being retained, and as decane will not favorably interact with water, this retention is likely to be by a change in absorption into the amorphous material. It is therefore a reasonable assumption that the onset of the change in retention peak shape (deviation between the Vcom and Vmax plot) correlates to the point at which the surface is plasticized such that Tg drops below T. In other words this defines the critical humidity required to plasticize the surface of the particles such that Tg is below T.

In Fig. 3, the critical RH (i.e. the RH after which the Vcom plot deviated) has been plotted as a function of 1/T (T = temperature of the experiment in K). This yielded a straight line relationship. If this relationship holds over a wide range of temperature and humidity, then extrapolation to 0% RH should correspond to the dry Tg for amorphous indomethacin. Carrying out such an extrapolation gives a figure of 48.6°C, which compares favorably to literature values of glass transition temperature of amorphous indomethacin shown in Table I (6–11). Thus the extrapolated value is in excellent agreement with the dry Tg for indomethacin. We believe that this is the first time Tg has been reported as a function of RH and T by use of the change in the retention peak shape. It is of course not possible to measure the true Tg of a sample in the presence of water vapor by use of techniques that involve heating (such as DSC) as the sample will inevitably change during heating, even if hermetically sealed pans are used.

Table I. Reported Glass Transition Values (Onset Temperature) Showing Variation of Tg from 42 to 50°C

Tg (°C)	Heating rate (°C/min)	Reference
42	10	(6)
44.7	20	(7)
~50	20	(8)
50	20	(9)
47	20	(10)
44.7 ± 1.3	20	(11)
44.1	1 (MDSC)	

Based on the Gordon Taylor equation (12) the water content needed to lower Tg of indomethacin to 35°C is 2.44% w/w (using 48.6°C as Tg of amorphous indomethacin from IGC) and 1.8% w/w (using 44.7°C as Tg of amorphous Indomethacin from literature) (7,11). The IGC data show that the critical RH at 35°C is 50%. Water sorption data (Fig. 4), show that the mass gain for amorphous indomethacin at 35°C was just below 1% w/w. Assuming that the mass of water sorbed in the IGC experiment is similar to that sorbed in the DVS (Note: This is a perfectly reasonable assumption as the rate of sorption is clearly limited by the solid itself and not by the rate of supply of water vapor. The rate of supply of water vapor is rapid in both the DVS and IGC and both the DVS and IGC experiments show pseudo equilibration, that is, levelling of mass gain, and the IGC shows a flat response with the thermal conductivity meter.), then the amount of water actually sorbed to lower the Tg to of the surface of the powder to 35°C (1%), is lower than that calculated using the Gordon Taylor equation (2.44 and 1.8% w/w). It follows that the concentration of sorbed water at the surface is higher than in the bulk (i.e. that at the surface is high enough to lower Tg to the experimental temperature (T), even though the sorbed mass in the entire sample is too low to reduce the Tg of the entire sample mass to T). These data would be consistent with a concentration gradient of water through the hydrophobic mass, with the surface being plasticized to a greater extent than the bulk of the sample.

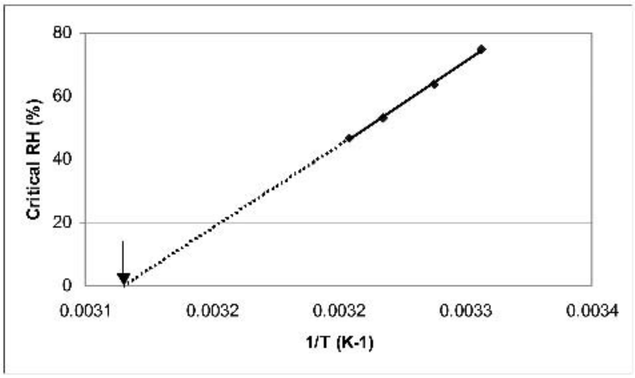

Fig. 3. Plot of critical RH (that after which there was a deviation between Vcom and Vmax) as a function of 1/T (K^{-1}) showing straight line relationship with best fit line equation $y = 338238 - 1015.8$ and $R^2 = 0.998$; value of extrapolated dry Tg = 48.6°C. Extrapolation to 0% RH shows dry Tg.

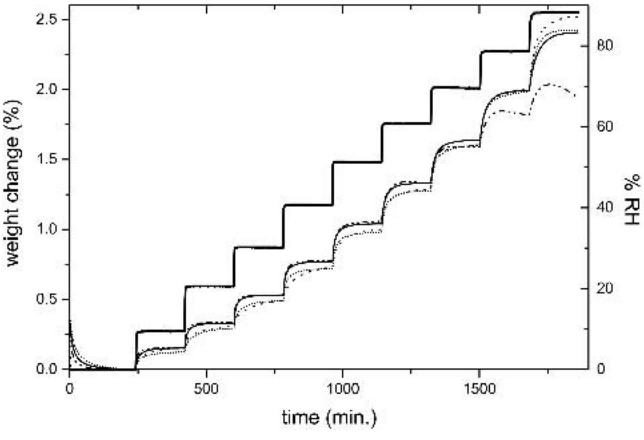

Fig. 4. Moisture sorption data for amorphous indomethacin at 25 (------), 30 (······), 35 (——————), and 40°C (—··—··—··—), and %RH (————).

The water sorption traces (Fig. 4) also provide interesting information; firstly the total mass change (2–2.5%) is higher than expected for adsorption, but much lower than expected for absorption into hydrophilic samples. It can be seen that for these samples the results up to 70% RH show the data at 35 and 40°C to be essentially identical to each other and the results at 25 and 30°C to be different to those obtained at higher temperature, but similar to each other. The results at the lowest temperature show the slowest rate of sorption and at least up until 60% RH the lowest extent of sorption (perhaps full equilibration has not been achieved at the lowest temperature). Obviously the norm of physical sorption processes is that the extent of absorption is less as temperature is increased. The fact that the results do not reflect this show that water can absorb slightly more readily as temperature is increased, probably due to increasing mobility of the amorphous form as the temperature approaches Tg. At high RH values it is clear that desorption of water is occurring to some extent for the 40°C sample, in keeping with the onset of some surface crystallization. At 80% RH the mass gain for the samples at 25, 30, and 35°C is identical (with 40°C being lower due to signs of crystallization). By 90% RH the mass gain is highest at 25°C, then 30, 35, and 40°C have the lowest net mass gain, showing that the samples are all changing and that the change toward crystallization is clearly related to the temperature. More prolonged storage at these conditions would see all of these samples crystallize, although the complete crystallization would take a very long time (3). The water sorption data reveal much about the structure, and changes in structure, as a consequence of changes in temperature and humidity, but do not provide as clear an indication of the point where Tg reaches T as is obtained when using IGC.

Material was studied after exposure to humidity and temperature variation in both the DVS and IGC, in all cases it was possible to see a glass transition indicating that amorphous sample remained. Depending upon the conditions to which the sample had been exposed varying levels of crystallization and melt were seen in the DSC experiment. These data are not presented here due to uncertainties about transformation during the DSC experiment itself, but are mentioned to show that the samples do not show substantial crystallization during the DVS and IGC experiments.

CONCLUSION

IGC has been used in a different way in order to determine the Tg at the surface of the particle whilst maintaining temperature and relative humidity. This is very important as it allows a better understanding of real systems, without the need to disturb the system greatly in order to make a measurement. The measured surface Tg values extrapolate to the dry Tg for indomethacin, however the water sorption to achieve the surface Tg is lower than that which would be expected when using the Gordon Taylor equation, indicating that there is a concentration gradient of water in the sample and the surface is plasticized to a greater extent than the bulk.

The water sorption data show interesting differences in mass uptake as temperature and humidity are altered, revealing how absorption and crystallization change under different conditions.

These results show that it is possible to better understand the slow and complex changes that occur in hydrophobic amorphous materials as a function of temperature and relative humidity.

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REFERENCES

1. H. E. Newell, G. Buckton, D. A. Butler, F. Thielmann, and D. R. Williams. The use of inverse phase gas chromatography to study the change of surface energy of amorphous lactose as a function of relative humidity and the process of collapse and crystallization. *Int. J. Pharm.* **217**:45–56 (2001).
2. S. E. Hogan and G. Buckton. Water sorption/desorption–near IR and calorimetric study of crystalline and amorphous raffinose. *Int. J. Pharm.* **227**:57–69 (2001).
3. V. Andronis, M. Yoshioka, and G. Zografi. Effect of sorbed water on the crystallization of indomethacin from the amorphous state. *J. Pharm. Sci.* **86**:346–351 (1997).
4. G. Buckton and P. Darcy. Water mobility in amorphous lactose below and close to the glass transition temperature. *Int. J. Pharm.* **135**:141–146 (1996).
5. F. Thielmann. iGC analysis software manual, Version 1.2 (Standard), Surface measurement systems. *London.* Page 12. June (2002).
6. T. Mastumato and G. Zografi. Physical properties of solid molecular dispersions of indomethacin with poly(vinylpyrrolidone) and poly(vinylpyrrolidone-co-vinyl-acetate) in relation to indomethacin crystallization. *Pharm. Res.* **16**:1722–1728 (1999).
7. P. Tong and G. Zografi. Solid state characteristics of amorphous sodium indomethacin relative to its free acid. *Pharm. Res.* **16**: 1186–1192 (1999).
8. M. Yoshioka, B. C. Hancock, and G. Zografi. Crystallization of Indomethacin from the amorphous state below and above its glass transition temperature. *J. Pharm. Sci.* **83**:1700–1705 (1994).
9. M. Yoshioka, B. C. Hancock, and G. Zografi. Inhibition of indomethacin crystallization in poly(vinylpyrrolidone) coprecipitate. *J. Pharm. Sci.* **84**:983–986 (1995).
10. B. C. Hancock, S. Shamblin, and G. Zografi. Molecular mobility of amorphous pharmaceutical solids below their glass transition temperature. *Pharm. Res.* **12**:799–806 (1995).
11. P. Tong and G. Zografi. A study of amorphous molecular dispersions of Indomethacin and its Sodium salts. *J. Pharm. Sci.* **90**:1991–2004 (2001).
12. M. Gordon and J. S. Taylor. Ideal co-polymers and the second order transitions of synthetic rubbers. 1. Non-crystalline copolymers. *J. Appl. Chem.* **2**:493–500 (1952).