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# An investigation into water interactions with amorphous and milled salbutamol sulphate: The development of predictive models for uptake and recrystallization

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## ABSTRACT

An investigation into the effect of water uptake on the glass transition of spray dried and milled salbutamol sulphate has been performed, with a particular view to exploring how the water uptake, $T_g$ value and recrystallization behaviour correlate. Samples of milled and spray dried drug were stored under controlled humidity conditions and the $T_g$ measured as a function of time. The $T_g$ was measured using modulated temperature differential scanning calorimetry (MTDSC) while the water content was measured using thermogravimetric analysis (TGA). A correlation was found between time of storage, water content and $T_g$ in that the samples showed time dependent equilibration with the storage environment (either gaining or losing water depending on the RH). The relationship between water content and stability, based on the concept of $T_g$ lowering, was modelled using the semi-empirical approach of Royall et al. (1999) as well as a derivation of the Kwei equation which allowed the interaction between the water and substrate to be accounted for. A method for predicting stability based on two simple DSC runs is proposed. In addition, we discuss the observation of a double glass transition for the spray dried samples.

## 1. Introduction

The importance and nature of the effect of water on amorphous materials has been extensively studied and is one of the key parameters that has to be considered when formulating amorphous products (Ahlneck and Zografi, 1990; Hancock and Zografi, 1994; Royall et al., 1999; Abdul-Fattah et al., 2007). In brief, water acts as a plasticizing agent, leading to a concentration-dependent reduction in $T_g$. The precise mechanism involved remains a matter of some debate, but the predominant theory involves consideration of the observation that the majority of (polymeric) amorphous systems have very similar viscosities at $T_g$ (circa $10^{12}$ Pa s), leading to development of the free volume theory which essentially states that the glass transition occurs when a material has a specific fractional free volume (Aharoni, 1998). On addition of a plasticizer, not only is a material with a lower $T_g$ being introduced but also the presence of the smaller molecule increases the free volume of the system, thereby both lowering the temperature at which that free volume equates to the critical value while also increasing the flexibility of the material as a result of the increased volume available for movement of the substrate molecules. It should be noted that alternative approaches to the interpretation of the glass transition (and hence by implication mechanism of plasticization) are available, particularly relating to the molecular interactions measured via cohesive energy density calculations which allow molecular flexibility to be assessed. More details of the theoretical background of plasticization may be found in Kern Sears and Darby (1982) and Marcilla and Beltran (2004).

As crystallization is greatly accelerated above $T_g$, this lowering may transform a stable product to one that is highly susceptible to physical (and quite possibly chemical) instability. From a pharmaceutical development perspective, therefore, water sorption from prolonged exposure to atmospheric water vapour poses a major formulation challenge (Ahlneck and Alderborn, 1989). As amorphous drugs and excipients are increasingly being considered as principal components of pharmaceutical formulations it follows that strategies to anticipate and understand the molecular events underlying their physical and chemical stability need to be developed.

Numerous studies have examined the relationship between water content and $T_g$, with the majority utilising the well known Gordon–Taylor equation as a means of modelling the relationship (Gordon and Taylor, 1952). The common assumption is that when the $T_g$ is lowered to the temperature of study, the increased molecular mobility afforded by the transition allows recrystallization to occur. This has been supported by numerous studies, although the relationship between $T_g$ lowering and the subsequent crystallization process do not appear to be a simple one. There may, for

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example, be significant delays between the $T_g$ being lowered to that of the experiment and the crystallization process commencing, or indeed, crystallization may commence prior to $T_g$ being reached (i.e. crystallization may commence below $T_g$) (Yoshioka et al., 1994; Surana et al., 2004). A further, associated complication lies in the use of recrystallization processes as a means of assessing the degree of crystallinity. Techniques such as gravimetric vapour sorption, microcalorimetry and some DSC-based approaches all rely on the plasticization/crystallization process for quantification of amorphous material, usually in comparison to a 100% amorphous standard. There is a significant question as to whether such crystallization, either for the sample or the standard, runs to completion or not, with several studies showing compelling evidence that it may not (Lefort et al., 2004; Qi et al., 2010; Grisedale et al., 2011). Overall, therefore, there is a common and well supported consensus that the lowering of $T_g$ may well lead to stability issues and hence should be avoided in most cases. However the precise nature of the relationship between water uptake, $T_g$ lowering and sample crystallization remains less well understood than may be ideally desirable.

In a previous study (Grisedale et al., 2011) we examined the effects of milling on the structure and behaviour of salbutamol sulphate, with particular emphasis on ascertaining the most appropriate means of quantifying the amorphous content and examining the use of spray dried material as a standard by which to compare the milled systems. In the current investigation, we examine the long term storage uptake of water into milled and spray dried material in terms of the lowering of $T_g$ and associated recrystallization behaviour. In particular we examine how the long term stability may be predicted from rudimentary short term experiments via the development of the model based on earlier work by Royall et al. (1999). As a supplementary issue, we also discuss the observation of a double glass transition for the spray dried material.

## 2. Materials and methods

### 2.1. Materials

Crystalline salbutamol sulphate was used as received (GSK; Stevenage, UK). The spray dried sample was prepared from a 10% aqueous crystalline salbutamol sulphate solution spray dried in a Büchi 290 Mini-Spray Dryer as described by Grisedale et al. (2011). The milled samples were prepared using a Planetary Micro Mill "Pulverisette 7" at a rotational speed of 800 rpm for 60 min. Previous studies (Grisedale et al., 2011) indicated that these samples had an amorphous content of circa 65–70%, although this communication compared numerous methods of making that assessment and found considerable variations in outcome between approaches.

### 2.2. Storage and water uptake studies

The storage studies were conducted at 20 °C. Airtight Kilner jars with enclosed drying agent (phosphorus pentoxide) and saturated salt solutions (magnesium chloride and sodium dichromate) were prepared as humidity chambers. Separate chambers were prepared for each testing point to prevent the disruption of equilibrium within the chamber owing to the continual opening and closing. All chambers were allowed 48 h to equilibrate before the samples were added. The relative humidity was measured using a hygrometer placed in the chamber, and the temperature checked daily. The nominal RH values were 0% (phosphorous pentoxide), 33% (magnesium chloride) and 54% (sodium dichromate); hygrometer readings were slightly higher than these values, possibly because of the slight temperature difference between the storage temperature (20 °C) and the temperature associated with the predicted RH environments (25 °C); the nominal values are used for simplicity. The samples were tested from 1 to 28 days; testing was ceased if a sample recrystallized before the end of the storage period. For studies whereby the water content and $T_g$ were modelled, the spray drying conditions were varied to allow production of material with varying initial water contents. More specifically, a range of combinations of inlet temperatures from 100 to 160 °C was used to generate material with water contents between 1 and 4% w/w.

### 2.3. Analytical methods

Thermogravimetric analysis (TGA; TA Instruments Hi-Res TGA2950, Delaware, US) was used to determine the changes in the water content of the samples during the storage study. Immediately on removal from the humidity chambers, approximately 3–4 mg of the sample was placed in open pan and heated at 10 °C min$^{-1}$ from 30 °C to 200 °C. The plateau region reached prior to decomposition (typically between 100 and 120 °C) was taken as the water loss. Towards the end of the study, Karl Fischer analysis became available and comparisons between the two methods indicated that the KF method yielded slightly higher results. However the TGA method used here was utilised in a consistent manner and hence the data used in the study is considered to be reliable.

Modulated temperature DSC (Q1000, TA Instruments, Delaware, US) experiments were used during the study to determine the temperature and the change in heat capacity of the glass transition of the samples. Hermetically sealed DSC pans and lids were used to encapsulate the samples as the water content of the samples was of interest. The samples were heated from 0 to 200 °C with an underlying heating rate of 2 °C min$^{-1}$ and modulation amplitude of ±0.212 °C every 40 s. Temperature calibration was performed using indium, benzoic acid and n-octadecane, while heat capacity calibration was performed using dried aluminium oxide.

ATR-FTIR spectrometry (Bruker Optics IFS66/S spectrometer (Coventry, UK) with a Golden Gate MkII Accessory from Specac Ltd. (Orpington, UK)) was used as a qualitative technique to confirm the recrystallization of the samples during the storage study. Spectra were collected from the region 4000 to 400 cm$^{-1}$ with 200 scans at a resolution of 8 cm$^{-1}$. Scanning electron microscopy (SEM; Jeol JSM5900LV SEM and a Quorum carbon coater) was also used at selected testing points during the study to observe changes in morphology of the samples on recrystallization.

## 3. Results

### 3.1. Storage studies

#### 3.1.1. Storage study at 0% relative humidity

Tables 1a and 1b summarise the data for the spray dried and milled samples respectively, including the water contents, the glass transition values determined from the MTDSC reversing heat flow signal and the baseline change in heat capacity ($\Delta C_p$) determined from the reversing heat capacity signal, while Fig. 1a and b shows the reversing heat flow for the fresh and stored spray dried and milled samples respectively. In both cases, an increase in $T_g$ is seen, indicating a loss of water on storage. It is also noted that, as previously reported (Grisedale et al., 2011), the spray dried samples showed clear separation of the $T_g$ and the non-isothermal recrystallization event (seen as a sharp discontinuity in the reversing heat flow profile) while the milled samples recrystallized immediately following the $T_g$. For the spray dried sample, two glass transitions were visible in the reversing heat flow signal throughout the storage study. The observation of double glass transitions in this sample has been previously reported (Grisedale et al., 2011) and is discussed in more detail in Appendix A; for simplicity, discussion of

**Table 1a**
Summary of the MTDSC and TGA results for spray dried salbutamol sulphate stored at 0% relative humidity.

| | Water content ± s.d. (%) | $T_g$ 1 ± s.d. (°C) | $T_g$ 2 ± s.d. (°C) | $\Delta C_p$ 1 ± s.d ($J\,g^{-1}\,°C^{-1}$) | $\Delta C_p$ 2 ± s.d ($J\,g^{-1}\,°C^{-1}$) |
|---|---|---|---|---|---|
| Day 0 | 2.26 ± 0.08 | 67.0 ± 1.7 | 82.7 ± 1.6 | 0.48 ± 0.08 | 0.18 ± 0.003 |
| Day 1 | 1.81 ± 0.26 | 80.5 ± 0.7 | 98.0 ± 0.3 | 0.39 ± 0.11 | 0.20 ± 0.01 |
| Day 2 | 1.51 ± 0.10 | 81.2 ± 2.3 | 101.3 ± 2.0 | 0.42 ± 0.02 | 0.18 ± ±0.02 |
| Day 4 | 1.57 ± 0.17 | 92.3 ± 7.1 | 106.0 ± 1.2 | 0.22 ± ±0.07 | 0.19 ± 0.04 |
| Day 8 | 1.10 ± 0.15 | 85.8 ± 2.8 | 103.5 ± 2.6 | 0.36 ± 0.07 | 0.10 ± 0.03 |
| Day 14 | 1.45 ± 0.08 | 84.2 ± 2.4 | Not seen clearly | 0.31 ± 0.08 | Not seen clearly |
| Day 28 | 0.96 ± 0.12 | 97.4 ± 4.6 | 113.9 ± 4 | 0.32 ± 0.04 | 0.08 ± 0.03 |

**Table 1b**
Summary of the MTDSC and TGA results for milled salbutamol sulphate samples stored at 0% relative humidity.

| | Water content ± s.d. (%) ($n$ = 4) | $T_{g1}$ ± s.d. (°C) ($n$ = 4) | $\Delta C_{p1}$ ± s.d. ($J\,g^{-1}\,°C^{-1}$) ($n$ = 4) |
|---|---|---|---|
| Day 0 | 1.71 ± 0.15 | 60.5 ± 0.8 | 0.26 ± 0.07 |
| Day 1 | 1.00 ± 0.04 | 82.7 ± 1.0 | 0.22 ± 0.03 |
| Day 2 | 1.39 ± 0.07 | 74.2 ± 4.0 | 0.26 ± 0.01 |
| Day 4 | 1.42 ± 0.12 | 79.7 ± 2.8 | 0.18 ± 0.02 |
| Day 8 | 1.05 ± 0.04 | 79.5 ± 2.9 | 0.24 ± 0.04 |
| Day 14 | 1.41 ± 0.22 | 74.3 ± 3.7 | 0.26 ± 0.02 |
| Day 28 | 0.76 ± 0.05 | 89.8 ± 3.2 | 0.24 ± 0.01 |

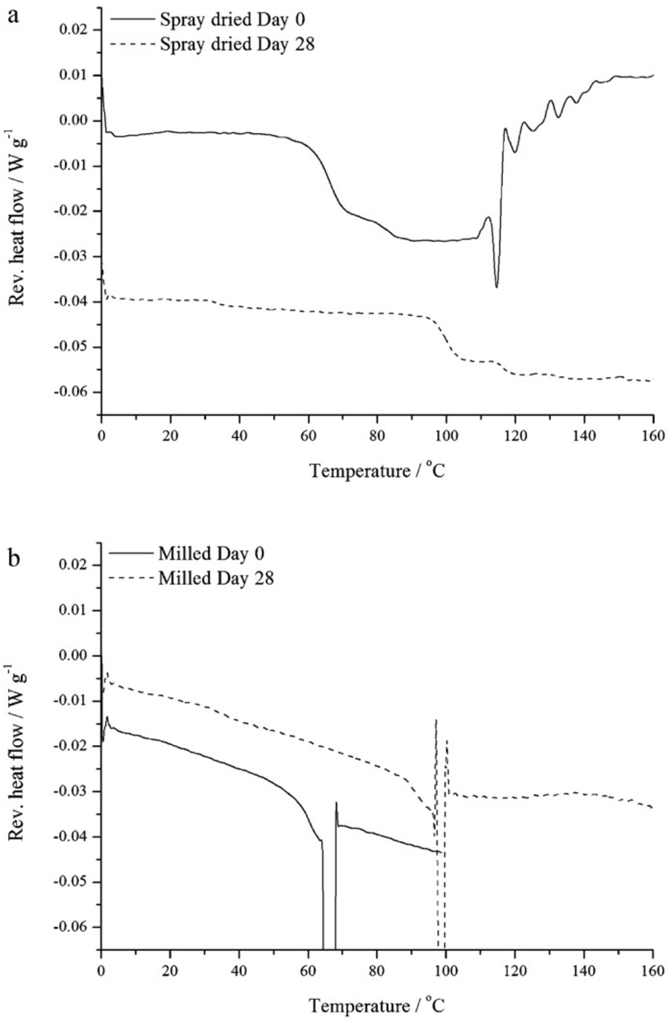

**Fig. 1.** (a) MTDSC reversing heat flow signals for the spray dried samples stored at 0% relative humidity on day 0 and day 28. Hermetically sealed pans used. Signals offset for clarity. (b) MTDSC reversing heat flow signals for the milled salbutamol sulphate samples stored at 0% relative humidity on day 0 and day 28. Hermetically sealed pans used. Signals offset for clarity.

the glass transition of the spray dried sample in this chapter will only refer to the first and larger of the two transitions.

The data in Tables 1a and 1b show an initial steady decrease in water content from day 0 to day 2 after which the water content of the samples largely equilibrated with the surrounding environment (although some fluctuation is noted, almost certainly associated with the inevitable noise associated with such measurements at low water contents). Coinciding with the decrease in water content was a corresponding increase in the glass transition temperature for both samples before a similar plateau was reached. The change in heat capacity ($\Delta C_p$) provided an indication of the magnitude of the glass transition. The (total) heat capacity change remained largely constant, indicating no evidence of a change in the degree of crystallinity of the samples. The use of $\Delta C_p$ as a means of quantifying crystallinity has been discussed (and indeed criticised) in our previous study (Grisedale et al., 2011); however despite its inherent weaknesses it does provide a simple means of assessing whether change is occurring to the samples. ATR-FTIR also indicated no evidence for recrystallization.

### 3.1.2. *Storage study at 33% relative humidity*

The results for the 33% RH study are given in Fig. 2a and b. These figures show an opposite trend to that seen for storage at 0% relative humidity in that the samples stored at 33% RH sorbed water from the surrounding environment, leading to a decrease in $T_g$. It is clear from both figures that the relationship between $T_g$ and water content with time inversely mirrored each other, as expected from basic plasticization theory but nevertheless of interest to see demonstrated so clearly. The precise relationship between water content and $T_g$ is discussed in more detail in a subsequent section. A further observation is that the spray dried sample sorbed over 1% more water than the milled sample as a result of the greater quantity of amorphous material present. No physical signs of recrystallization were seen in the samples during the testing period, confirmed by ATR-FTIR (Fig. 2c). As discussed previously (Grisedale et al., 2011), it is possible to use this technique to both identify and quantify amorphous or crystalline content. In the current context, we use the technique merely as a means of identifying crystallization, hence simple observation of the 2500–3500 $cm^{-1}$ allows one to ascertain changes relating to physical structure; more sophisticated analysis is possible by scrutinizing the lower wave number regions but was not considered necessary for the current study. It can therefore be inferred from the results that the water content of both samples reached a stable equilibrium with their

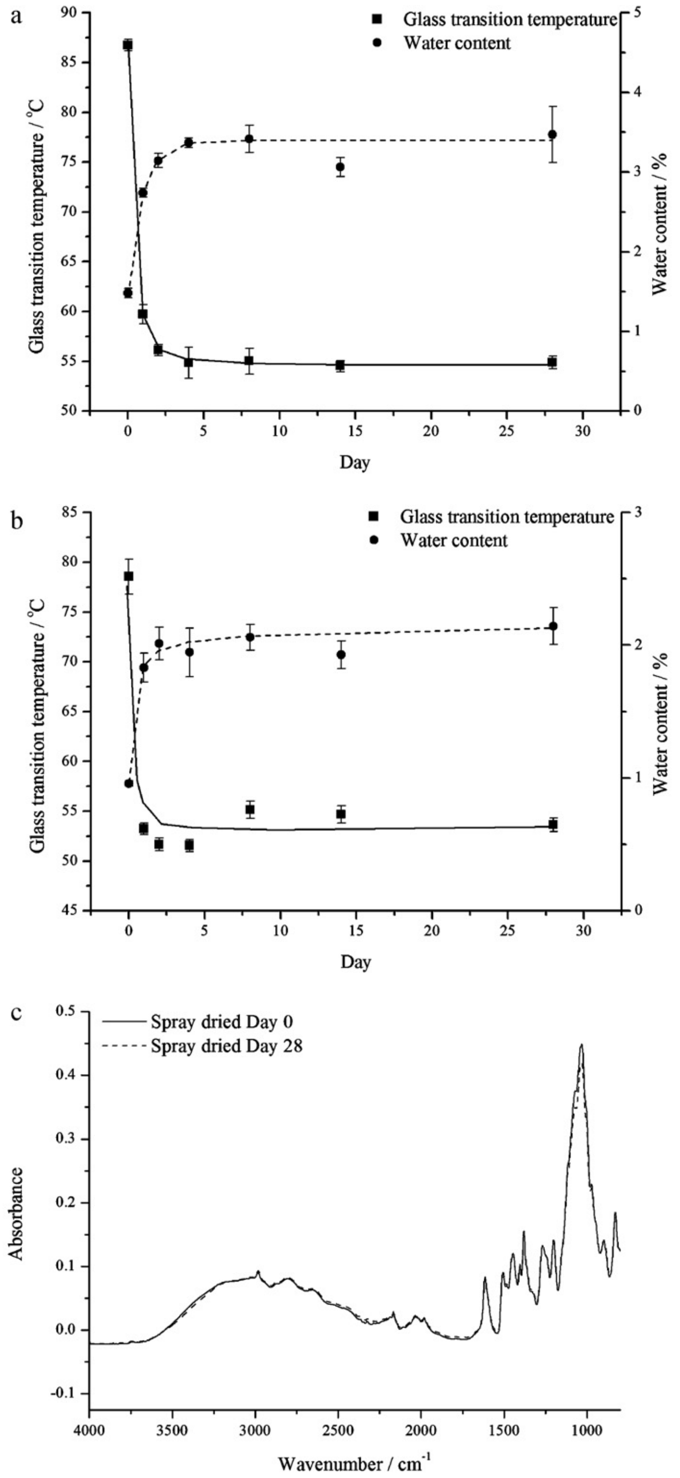

**Fig. 2.** (a) Glass transition temperature and percentage water content for the spray dried salbutamol sulphate samples stored at 33% relative humidity. (b) Glass transition temperature and percentage water content for the milled salbutamol sulphate samples stored at 33% relative humidity. (c) ATR-FTIR spectra for the spray dried salbutamol sulphate samples stored at 33% relative humidity on day 0 and day 28.

environment without adversely affecting the physical stability of the samples. It was also noted that the preponderance of the double $T_g$ decreased with storage time, with the smaller, higher temperature event becoming less visible. No changes in morphology for either the 33% or 0% RH samples were apparent using SEM.

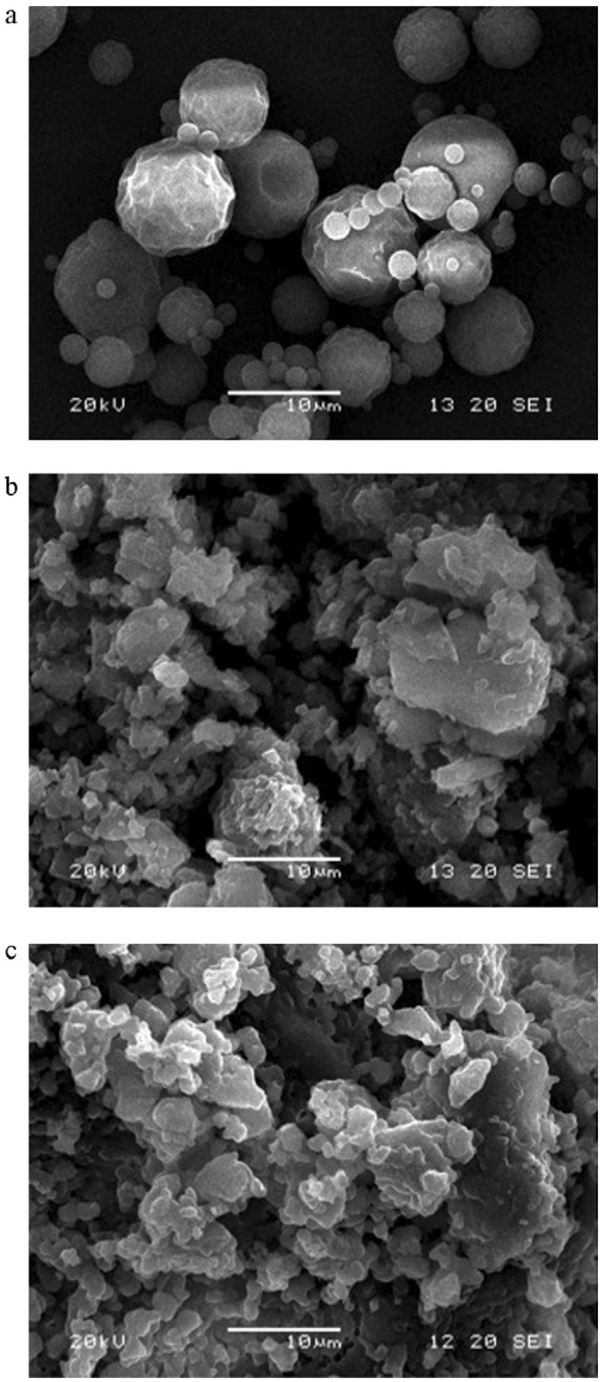

**Fig. 3.** SEM images for the spray dried salbutamol sulphate samples stored at 54% relative humidity on day 0 (a) and day 1 (b) and for milled salbutamol sulphate on day 1 (c).

### 3.1.3. *Storage study at 54% relative humidity*

Physical inspection of the samples after 24 h of storage revealed that both the spray dried and milled samples had compacted and fused, as shown in Fig. 3a–c. Indeed, while the milled material appeared to maintain its basic morphology but with clear fusion apparent (Fig. 3c), the spray dried material had undergone a radical change and bore closer resemblance to the milled material than the original spherical particles (Fig. 3a and b). This implied that the samples had recrystallized, confirmed using ATR-FTIR and MTDSC. In the case of the former, new absorption peaks were clearly seen for the spray dried material in the fingerprint 2500–3500 cm$^{-1}$ region (Fig. 4), while MTDSC showed no evidence of a glass transition or

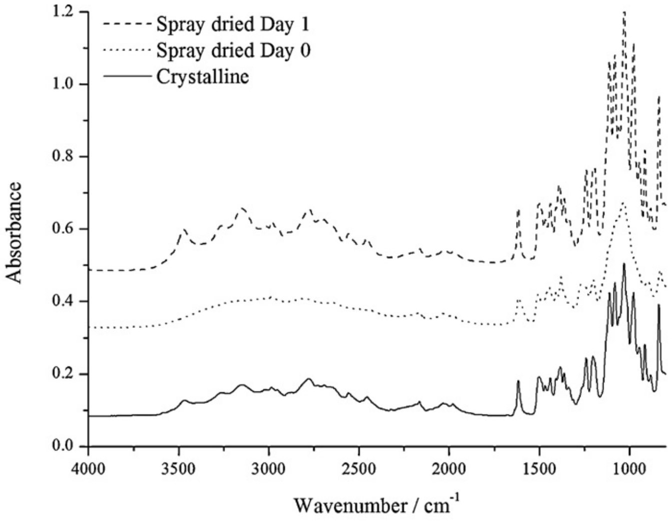

**Fig. 4.** ATR-FTIR spectra for spray dried salbutamol sulphate samples stored at 54% relative humidity on day 0 and day 1. Spectra offset for clarity.

recrystallization event for either sample (data not shown). Overall, therefore, while water content changes were in evidence for all three storage conditions (albeit by implication for the 54% samples), only the higher RH (54%) storage study showed evidence of physical changes to the materials other than water content and $T_g$. This in turn implies that, over the 30 day period of study, the recrystallization presents as an ‘all or nothing’ process, rather than a gradual one. This in turn implies that if the trigger for crystallization can be identified and predicted, it should be possible to predict storage conditions that will prevent this trigger from occurring.

### 3.2. *Predicting recrystallization from water uptake and $T_g$ measurements*

#### 3.2.1. *Fitting $T_g$ data as a function of water content*

As outlined above, a fundamental issue regarding amorphous materials is their stability, especially during storage. Hancock et al. (1995) suggested that to ensure physical stability a maximum storage temperature for an amorphous material should be no greater than $T_g$ – 50 °C, corresponding to the estimated Kauzmann temperature, below which the translational molecular mobility is considered to be negligible. However, atmospheric water vapour can plasticize the glass transition temperature of an amorphous material, leading to the effective $T_g$ reaching temperatures below or close to the temperature of storage. On this basis the effect of water uptake on $T_g$ has been studied in more detail and a model developed to predict the recrystallization profile as a function of water content. The raw data of the $T_g$ as a function of water content of spray dried material (prepared using a variety of spraying conditions) is shown in Table 2. The $T_g$ of the dry sample was taken to be 121.7 ± 0.7 °C (averaging the pinholed pan values, as outlined by Grisedale et al. (2011)).

**Table 2**
$T_g$ values for amorphous (spray dried) salbutamol sulphate prepared using a range of spraying protocols as a function of water content.

| Water content (%) | $T_g$ (°C) (hermetic pans) |
|---|---|
| 1.29 ± 0.07 | 83.9 ± 2.7 |
| 1.61 ± 0.08 | 77.5 ± 0.5 |
| 2.20 ± 0.09 | 67.5 ± 1.4 |
| 2.26 ± 0.08 | 67.0 ± 1.7 |
| 2.73 ± 0.19 | 60.8 ± 1.3 |
| 3.29 ± 0.17 | 50.1 ± 1.1 |

There are several means of modelling this data, the most widely used being the Gordon–Taylor equation:

$$T_{\mathrm{gmix}} = \frac{w_1 T_{g1} + K w_2 T_{g2}}{w_1 + K w_2} \tag{1}$$

whereby $T_{g1}$, $T_{g2}$ and $T_{\mathrm{gmix}}$ are the glass transition temperatures for amorphous water (literature value 135 K; Sugisaki et al., 1968), the dry amorphous drug and the hydrated amorphous drug respectively, while $w_1$ and $w_2$ are the weight fractions of water and drug. $K$ is a sample-specific parameter which can be considered to be the ratio of the free volumes of the two components (water and drug in this case). Knowledge of the $K$ value is fundamental to predict $T_{\mathrm{gmix}}$ as all other values in the Gordon–Taylor equation can be derived experimentally. There are several approaches to obtaining $K$. One method is to use the Couchman–Karasz equation (Couchman and Karasz, 1978) which calculates $K$ on the basis of the ratio of the heat capacity changes through $T_g$ for the two components. However we are not aware of a reliable value of $\Delta Cp$ for water through the glass transition and hence have not pursued this approach further. More usually, $K$ is estimated using the Simha–Boyer rule or by data fitting to the Gordon–Taylor equation. The Simha–Boyer rule (Simha and Boyer, 1962) defines $K$ in terms of density of the two components:

$$K = \frac{\rho_1 T_{g1}}{\rho_2 T_{g2}} \tag{2}$$

whereby $\rho_1$ and $\rho_2$ are the densities of water and the drug, respectively. The density of spray dried salbutamol sulphate, as determined by helium pycnometry, was 1.29 g cm$^{-3}$. Water is assumed to have a density of 1 g cm$^{-3}$. $K$ for amorphous salbutamol sulphate was calculated to be approximately 0.265 using these values according to the Simha–Boyer rule. Otherwise if the density of the pharmaceutical material being studied is unknown it is common to assume all low molecular weight organic compounds exhibit a true density of approximately 1.2 g cm$^{-3}$. Using this value, $K$ for amorphous salbutamol sulphate was calculated to be approximately 0.285. Alternatively the $K$ value can be derived by fitting experimental data (i.e. $T_g$ at a range of water contents) to the Gordon–Taylor equation; this yielded a $K$ value of 0.088. Other approaches include the Fox (1956) equation, which does not use the K value at all but instead assumes simple additivity and equivalence of free volume of the materials involved.

$$\frac{1}{T_{\mathrm{gmix}}} = \left(\frac{w_1}{T_{g1}}\right) + \left(\frac{w_2}{T_{g2}}\right) \tag{3}$$

This approach was not considered appropriate in the present case due to the inherent assumptions outlined above. Instead the experimental and Simha–Boyer estimates of $K$ were examined in the first instance.

Fig. 5 shows the difference between the predicted glass transitions temperatures, calculated via the Gordon–Taylor equation, depending on the $K$ value used. The results of this study showed that $K$ estimated by the Simha–Boyer did not model the data correctly. Almost inevitably, the experimentally derived value fitted the data well. Interestingly, however, the $K$ value found by fitting all experimental data and that estimated using single hermetic/pinholed pan runs (Grisedale et al., 2011) were very similar, with the previous estimate being calculated as 0.085. In brief, this method involves heating a sample of known water content in hermetically sealed and pinholed DSC pans. The former gives the $T_g$ of the plasticized material, while the latter gives the ‘dry’ $T_g$ as the water escapes on heating. By fitting these two sets of values into the Gordon–Taylor equation, the value of $K$ can be easily and quickly obtained. The close agreement between this rapid evaluation of $K$ (0.085) and that obtained from a much more extensive data set of equilibrated samples (0.088) does indicate that the former yields reliable values. As a practical point, it is also worth noting that exchanging

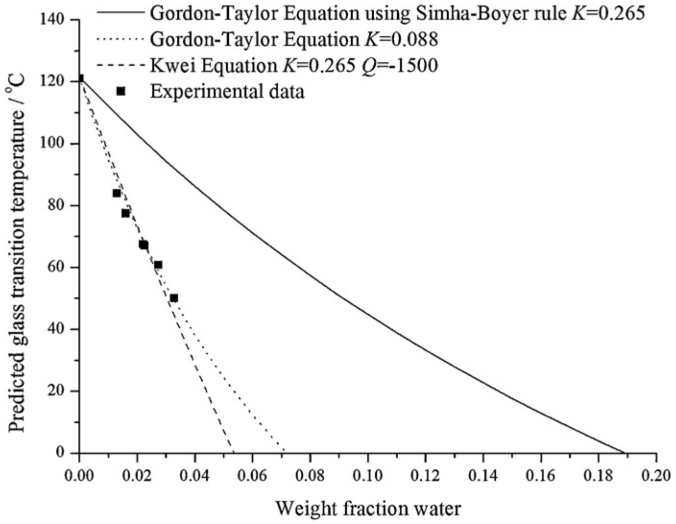

**Fig. 5.** Prediction of the glass transition of amorphous salbutamol sulphate with varying weight fraction of water according to the Gordon–Taylor equation using the fitted value of *K*, the Gordon–Taylor equation using *K* derived from the Simha–Boyer rule and the Kwei equation using *K* derived from the Simha–Boyer rule. Experimental data is shown for comparison.

the assignments 1 and 2 for the drug and water will result in the mathematical inversion of the calculated value of *K*.

In this study the Simha–Boyer rule was shown to have underestimated the plasticizing effect of water on the amorphous samples and therefore overestimated the predicted glass transition temperatures. This may be related to the fact that the Gordon–Taylor equation, and indeed Simha–Boyer rule, assumes no specific interaction between the two components. Indeed, the parameter *K* has a theoretical physical basis which relates simply to the relative free volumes of the components. Therefore, using an experimentally derived value of *K*, while perhaps producing a better fit, represents a deviation from the original assumptions inherent in developing the model. Kwei (1984), Kwei et al. (1987) suggested the addition of a quadratic parameter to the Gordon–Taylor to account for any such interaction that may occur between the two components of a mixed system:

$$T_{\text{gmix}} = \frac{w_1 T_{g1} + K w_2 T_{g2}}{w_1 + K w_2} + q w_1 w_2 \tag{4}$$

where $q$ is a constant and the term $qw_1w_2$ represents the presence of interactions such as hydrogen bonding. The previous section showed the Gordon–Taylor equation where the *K* value was determined from the Simha–Boyer rule did not model the data correctly, yet the density values were experimentally determined and hence should be considered to be reliable. This discrepancy may therefore reflect interaction between the water and substrate that are effectively hidden by the experimentally derived value of *K*, i.e. one may postulate that the Simha–Boyer value is a better representative of the 'true' value of *K*. The additional $qw_1w_2$ expression in the Kwei equation may account for this missing interaction parameter. Using the *K* value of 0.265 (according to the Simha–Boyer rule) a $q$ value of −1500 was determined by data fitting. Fig. 5 shows the predicted glass transitions for amorphous salbutamol sulphate fitted according to the Kwei equations using the above values. The predicted glass transitions according to the Kwei equation modelled the experimental data reasonably well, although one must bear in mind that the $q$ value is empirically derived.

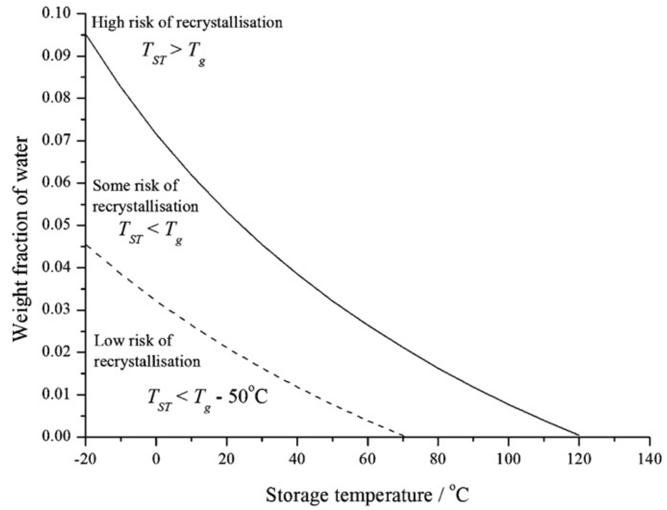

**Fig. 6.** Calculated phase diagram displaying the relationship between storage temperature and the water contents that may lower the glass transition of amorphous salbutamol sulphate to that of (or 50 °C below) the storage temperature.

### 3.3. Extending the Gordon–Taylor model to predict recrystallization

Previously, Royall et al. (1999) suggested a model based on the Gordon–Taylor equation, whereby both the effects of storage temperature and water content on the glass transition could be predicted and displayed as a phase diagram. The approach they used was based on the recommendation from Hancock et al. (1995) that an amorphous material should be stored at 50 °C below its glass transition at any given water content to prevent recrystallization. The critical water content ($w_c$) for a given storage temperature ($T_{\text{ST}}$) can be calculated from Eq. (5) and the critical water content ($w_{c'}$) arising from the glass transition 50 °C below its storage temperature from Eq. (6) (Abdul-Fattah et al., 2007). These equations were based on the Gordon–Taylor equation (Eq. (1)) where $T_{\text{ST}}$ and $T_{\text{ST}}$-50 replaces $T_{\text{mix}}$ at water content $w_c$ and $w_{c'}$ respectively, and by considering *K* determined from the Simha–Boyer rule.

$$w_c = \left[1 + \frac{T_{g2}\rho_2(T_{\text{ST}} - 135)}{135(T_{g2} - T_{\text{ST}})}\right]^{-1} \tag{5}$$

$$w_{c'} = \left[1 + \frac{T_{g2}\rho_2(T_{\text{ST}} - 185)}{135(T_{g2} - T_{\text{ST}} - 50)}\right]^{-1} \tag{6}$$

Regarding amorphous salbutamol sulphate, the previous discussion has shown that the Gordon–Taylor equation with the Simha–Boyer rule did not correctly model the experimental data. Thus, the explicit form of the equations proposed by Royall et al. (1999) would not accurately model the instability of amorphous salbutamol sulphate. Nevertheless, we can use the model without reference to this approximation. The corresponding phase diagram (Fig. 6) maps the approximate relationship between storage temperature and water content required to lower the glass transition of amorphous salbutamol sulphate by 50 °C, given a *K* value was 0.088. This provided an indication of the storage safety margins required to prevent the recrystallization of amorphous salbutamol sulphate. Samples stored at temperatures above the predicted glass transition temperature (solid line) may be considered to be at a higher risk of recrystallization than those stored below the predicted glass transition temperature. Between the predicted glass transition temperature (solid line) and predicted glass transition temperature −50 °C (dashed line), the stability of the sample is not guaranteed and recrystallization may occur. Below the predicted glass transition temperature minus 50 °C (dashed line), the samples maybe considered to be at lower risk from recrystallization compared to above these temperature values.

The question then arises as to the extent to which the model is able to actually predict crystallization behaviour. We know from the current study that the sample is unstable at 54% RH, did not recrystallize within 28 days at 33% RH (and equilibrated at a water content of circa 3.5% (w/w) and also did not recrystallize at 0% RH (with a water content in the region of 1% w/w). Examination of the gravimetric vapour sorption data from Grisedale et al. (2011) indicates that the water uptake at 54% in the short term was circa 4% (w/w), although this figure was obtained from a dynamic experiment and one would expect significantly higher values on equilibration. Examination of Fig. 6 indicates that these data are reasonable in the context of the predicted behaviour; the 0% RH falls within the safety area (low risk), the 33% RH system will be below $T_g$, but not by a wide margin, while the 54% system would almost certainly fall within the high risk region on equilibration. Obviously, to validate the model one would need real time studies over a much longer time period, but even at this stage one can use the model as a simple warning system. Indeed, by combining the model (using the pinholed/hermetic method of obtaining $K$) with gravimetric water sorption studies one could, in the course of a matter of one or two days of experimental work, derive a profile that matched RH to water uptake to likelihood of crystallization, thereby presenting the formulator with an early assessment of optimal storage conditions.

## 4. Discussion

The study has focused on two aspects of water sorption in relation to amorphous behaviour, namely the uptake of water on storage under different RH conditions and secondly the modelling of that uptake in terms of predicting recrystallization. The water uptake profile for the milled and spray dried material followed the theoretical expectations quite well, the surprising result being the rapidity of the recrystallization of both samples under ambient (54% RH) conditions. Overall, however, the two sets of samples did not show any evidence of behaving significantly differently from each other, with the same crystallization pattern seen for the stored samples as the fresh material (Grisedale et al., 2011), in that for the milled material, crystallization followed $T_g$ immediately while a delay was seen for the spray dried material, at least on heating. It is not possible to say whether this delay manifested on storage due to the rapidity of crystallization for both sets of samples. However it is reasonable to conclude that, in the context of storage over days, any such lag for the spray dried material did not result in any practically significant difference in behaviour. This effectively means that the milled and spray dried material behaved essentially similarly in real time, leading to the conclusion that the key issue is the sample $T_g$ rather than issues that are pertinent to non-isothermal recrystallization such as nucleation and processing history.

This then leads to the conclusion that, in the context of long term stability, if one is able to understand the fundamental glassy behaviour one should be able to predict recrystallization as a function of water uptake. To this effect we have re-examined the model of Royall et al. (1999) and have examined modifications to account for the assumptions inherent in the original approach, namely the use of the Simha–Boyer approximation for $K$ or, alternatively, the use of an empirical $K$ with no basis in a physical model for the system. While we were able to introduce alternative expressions (particularly involving the Kwei approach) that were arguably more satisfactory from a theoretical viewpoint, comparison with experimentally derived recrystallization data indicated that the original Royall et al. (1999) model demonstrated reasonably predictive behaviour and has the advantage of comparative simplicity. This in turn means that one may identify the critical water contents that induce varying risks of recrystallization from a minimal number of data points, as all that is required is a knowledge of the $T_g$/water content relationship. It is also interesting to note that if one knows the water uptake profile, one could in theory extend the model to present stability in the context of storage RH rather than water uptake. These findings also have implications for the use of accelerated storage stability conditions (particularly the use of 40 °C/75%RH) as clearly this will be applying dual stability stresses to the material that will augment each other to render such assessment extremely harsh.

## 5. Conclusions

A study has been conducted which has examined the water uptake profile of amorphous (spray dried) or partially amorphous (milled) salbutamol sulphate in relation to subsequent recrystallization behaviour. It was noted that by storing under different humidity conditions, water was taken up or lost to an equilibrium value, with a concomitant change in $T_g$, although at higher RH conditions (54%) both sets of samples recrystallized within 24 h. We examine models to predict recrystallization as a function of water uptake and conclude that, despite its theoretical limitations, the model proposed by Royall et al. (1999) provides a predictive means of identifying critical water uptake parameters as a function of storage temperature. We also, as a supplementary issue, discuss the appearance of double glass transitions for the spray dried material and suggest that water is present in two regions within such samples.

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## Appendix A. The appearance of a double $T_g$ in spray dried salbutamol sulphate

In the published literature there is little information concerning the occurrence of multiple glass transitions within a ‘single’ amorphous system. Such transitions have been discussed with regards to glass forming aqueous solutions and are accounted for as either liquid–liquid phase separation on cooling or phase separation associated with ice nucleation (Mehl, 1996). Indeed, multiple transitions for phase separated materials are well recognized and have been described for polymer-solvent systems in terms of the presence of nanodistinct cooperative regions of differing molecular mobility (Lipson and Milner, 2006). In the present case, initial characterisation of spray dried salbutamol sulphate showed the presence of two step changes in the reversing heat flow signal of the MTDSC experiments, despite this system being, at least in theory, a simple plasticized material. While this was initially dismissed by us as an anomaly, repeated MTDSC experimentation confirmed that the double glass transition was a real phenomenon as evidenced by its consistent recurrence. Since this work was conducted, we have observed the same phenomena for several other systems, hence have reason to believe that it may be a more common occurrence than is appreciated. On this basis we outline here a possible explanation, or set of explanations, for this thermal event.

The double glass transition was first noticed when modulated temperature DSC using hermetically sealed pans were used to characterise the amorphous salbutamol sulphate produced by spray drying. Fig. 7 shows the MTDSC total, reversing and derivate reversing heat flow signals for a typical freshly prepared sample of spray

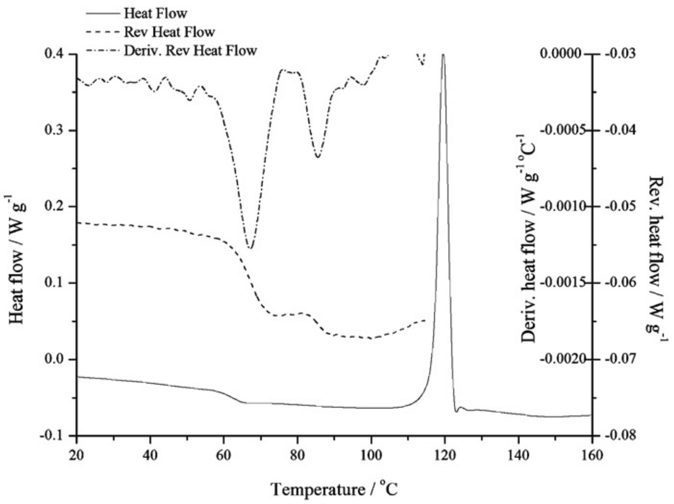

**Fig. 7.** Typical MTDSC total, reversing and derivate reversing heat flow signals for spray dried salbutamol sulphate, runs in hermetic pans.

dried salbutamol sulphate. While the total heat flow signal shows a single glass transition, the reversing heat flow signal on the other hand shows two step changes in the baseline. This second step change was consistently the smaller of the two. Likewise, the derivative of the reversing heat flow signal shows two distinct peaks indicating two separate processes had occurred. These events were reproducible on two separate TA Instruments Q1000 DSC instruments. Only one glass transition was seen in the reversing heat flow signal when pin holed pans were used and likewise for the milled sample using either pan type. Therefore, this phenomenon was assumed to be specific to the spray dried sample and to the use of hermetically sealed pans.

Several hypotheses were developed to explain the double glass transition; each one will be discussed in turn. The first hypothesis was that water had moved through the sample and into the headspace of the pan during the applied heat program, resulting in two amorphous phases of different plasticized states being generated during the heating run itself. To test this hypothesis a series of cycling modulated temperature DSC experiments were performed. The samples were modulated through both glass transitions to 100 °C, then cooled down to 80 °C, before the first glass transition and reheated through to 200 °C. By cycling through the glass transition region it was expected that the glass transition temperature recorded would shift to the higher of the two glass transition values thus confirming that water had evolved up through the sample leaving a dryer sample in contact with the base of the pan. The results of the cycling experiment demonstrated that the sample had only one glass transition event, as predicted. However, this event occurred at an almost identical temperature to the lower of the two glass transition temperatures, which would correspond to the higher water content state; this is incompatible with the idea of the water leaving the solid sample. The result of this experiment implied that the double glass transition was therefore not caused by water movement during the heating run.

The second hypothesis was that the spray dried particles redistributed themselves within the sample pan as a result of heating (such movement has been previously reported (Passerini and Craig, 2001) and also noted here during decomposition in our preliminary studies), hence improving their surface contact with the pan and generating a further apparent thermal event. The third, related hypothesis was the possibility that the morphology of the spherical spray dried particles changed and became flatter, again improving surface contact between them and the pan base. The spray dried salbutamol sulphate sample was a "fluffy" and static powder thus it was more than reasonable to imagine a poor initial surface contact with base of the sample pan. However, if the issue was one of contact then changes in signal to noise ratio would have been expected; the signal to noise ratio during these MTDSC experiments had never been an issue or changed during the cycling experiments. Furthermore, the use of pin holed pans would be expected to yield identical results if sample redistribution was indeed of relevance. These two explanations were therefore considered to be unlikely.

The fourth hypothesis was the possible presence of a rigid amorphous fraction within the sample. A rigid amorphous fraction is part of the amorphous structure which is restrained by a crystalline phase and as such the sample is unable to fully relax as it passes through its glass transition (Wunderlich, 2003). The first glass transition recorded may have corresponded to the relaxation of the mobile amorphous phase and the second glass transition to that of the rigid amorphous phase. However, this was discounted as a similar event would have been expected when pin holed DSC pans were used. Additionally, this theory would require three phases, namely a mobile amorphous phase, a rigid amorphous phase and a crystalline phase. As there was no crystalline phase present in this case the explanation was again considered to be unlikely.

Nevertheless the principle behind a rigid amorphous fraction may be developed to a final fifth explanation by considering that within the sample there are in fact two distinct regions of localised structural relaxation, the simplest explanation being that the two regions contain varying quantities of water. This is distinct from the first explanation in that the two regions are not created by the heating run but are there as an intrinsic component of the particle structure, possibly reflecting a dry surface and a more hydrated bulk. Therefore, the first and larger of the two glass transitions may well have resulted from a plasticized region created within the bulk of the particle, the second smaller glass transition resulting from the less plasticized amorphous region created on the surface of the particle. The results of the storage study also support this hypothesis, as the results showed a decrease in the occurrence of the second glass transition with increasing water sorption and equilibration time. This indicates that the passing of time had allowed the spray dried particles to reach equilibrium with the humidity of the surrounding environment and had resulted in a homogenised particle exhibiting a signal glass transition. For pinholed pans, the ease of water escape negates this effect and a single 'dry' value is seen.

## References

Abdul-Fattah, A.M., Truong-Le, V., Yee, L., Nguyen, L., Kalonia, D.S., Cicerone, M.T., Pikal, M.J., 2007. Drying-induced variations in physico-chemical properties of amorphous pharmaceuticals and their impact on stability (I): stability of a monoclonal antibody. J. Pharm. Sci. 96, 1983–2008.

Aharoni, S.M., 1998. Increased glass transition temperature in motionally constrained semicrystalline polymers. Polym. Adv. Technol. 9, 169–201.

Ahlneck, C., Alderborn, G., 1989. Moisture adsorption and tabletting 1. Effect on volume reduction properties and tablet strength for some crystalline materials. Int. J. Pharm. 54, 131–141.

Ahlneck, C., Zografi, G., 1990. The molecular basis of moisture effects on the physical and chemical stability of drugs in the solid state. Int. J. Pharm. 62, 87–95.

Couchman, P.R., Karasz, F.E., 1978. A classical thermodynamic discussion of the effect of composition on glass-transition temperatures. Macromolecules 11, 117–119.

Fox, T.G., 1956. The influence of diluent and of copolymer composition on the glass temperature of a polymer system. Bull. Am. Phys. Soc. 1, 123.

Gordon, M., Taylor, J.S., 1952. Ideal copolymers and the second-order transitions of synthetic rubber. I. Non-crystalline copolymers. J. Appl. Chem. 2, 493–500.

Grisedale, L., Jamieson, M., Belton, P.S., Barker, S.A., Craig, D.Q.M., 2011. The effect of milling on the physical structure and properties of salbutamol sulphate: a thermal, spectroscopic and water vapour sorption study. J. Pharm. Sci. 100, 3114–3129.

Hancock, B.C., Zografi, G., 1994. The relationship between the glass-transition temperature and the water content of amorphous pharmaceutical solids. Pharm. Res. 11, 471–477.

Hancock, B.C., Shamblin, S.L., Zografi, G., 1995. Molecular mobility of amorphous pharmaceutical solids below their glass-transition temperatures. Pharm. Res. 12, 799–806.

Kern Sears, J., Darby, J.R., 1982. The Technology of Plasticizers. John Wiley and Sons, New York.

Kwei, T.K., 1984. The effect of hydrogen bonding on the glass transition temperatures of polymer mixtures. J. Polym. Sci. 22, 307–313.

Kwei, T.K., Pearce, E.M., Pennacchia, J.R., Charton, M., 1987. Correlation between the glass transition temperatures of polymer mixtures and intermolecular force parameters. Macromolecules 189, 1174–1176.

Lefort, R., De Gusseme, A., Willart, J.E., Danede, F., Descamps, M., 2004. Solid state NMR and DSC methods for quantifying the amorphous content in solid dosage forms: an application to ball-milling of trehalose. Int. J. Pharm. 280, 209–219.

Lipson, J.E.G., Milner, S.T., 2006. Multiple glass transitions and local composition effects on polymer solvent mixtures. J. Polym. Sci. Part B: Polym. Phys. 44, 3528–3545.

Marcilla, A., Beltran, M., 2004. Handbook of Plasticizers. Chem. Tech. Publishing, Toronto, Canada.

Mehl, P.M., 1996. Observations of multiple glass transitions in the system water/1,3-butanediol. Effect on ice crystallisation. Thermochim. Acta 284, 191–212.

Passerini, N., Craig, D.Q.M., 2001. An investigation into the effects of residual water on the glass transition temperature of polylactide microspheres using modulated temperature DSC. J. Control. Rel. 73, 111–115.

Qi, S., Weuts, I., De Cort, S., Stokbroekx, S., Leemans, R., Reading, M., Belton, P., Craig, D.Q.M., 2010. An investigation into the crystallisation behaviour of an amorphous cryomilled pharmaceutical material above and below the glass transition temperature. J. Pharm. Sci. 99, 196–208.

Royall, P.G., Craig, D.Q.M., Doherty, C., 1999. Characterisation of moisture uptake effects on the glass transitional behaviour of an amorphous drug using modulated temperature DSC. Int. J. Pharm. 192, 39–46.

Simha, R., Boyer, R.F., 1962. On a general relation involving the glass temperature and coefficients of expansion of polymers. J. Appl. Phys. 37, 1003–1007.

Sugisaki, M., Suga, H., Seki, S., 1968. Calorimetric study of the glassy state. IV. Heat capacities of glass water and cubic ice. Bull. Chem. Soc. Jpn. 41, 2591–2599.

Surana, R., Pyne, A., Suryanarayanan, R., 2004. Effect of preparation method on physical properties of amorphous trehalose. Pharm. Res. 21, 1167–1176.

Wunderlich, B., 2003. Reversible crystallization and the rigid-amorphous phase in semicrystalline macromolecules. Prog. Polym. Sci. 28, 383–450.

Yoshioka, M., Hancock, B.C., Zografi, G., 1994. Crystallization of indomethacin from the amorphous state below and above its glass-transition temperature. J. Pharm. Sci. 83, 1700–1705.