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Characterisation of moisture uptake effects on the glass transitional behaviour of an amorphous drug using modulated temperature DSC

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Abstract

The purpose of this study was to investigate the depression of the glass transition temperature, T_g, of the protease inhibitor saquinavir in the first heating scan as a function of the quantity of sorbed water by the application of modulated temperature differential scanning calorimetry (MTDSC). Samples of amorphous saquinavir were pre-treated under various humidity conditions and the quantity of sorbed water measured by thermogravimetric analysis. MTDSC runs were performed using hermetically and non-hermetically sealed pans in order to determine the glass transition temperature. MTDSC allowed the separation of the glass transition from the enthalpic relaxation, thereby allowing clear visualisation of T_g for amorphous saquinavir in the first heating scan. The plasticizing effects of water were assessed, with the depression in T_g related to the mole fraction of water sorbed via the Gordon–Taylor relationship. An expression has been derived which allows estimation of the water content which lowers the T_g to the storage temperature, thereby considerably increasing the risk of recrystallisation. It is argued that this model may aid prediction of the optimal storage conditions for amorphous drugs.

Keywords: Amorphous; Glass transition; Modulated temperature DSC; Water uptake; Saquinavir

1. Introduction

Modulated temperature differential scanning calorimetry (MTDSC) is a recently introduced thermoanalytical technique which involves the superimposition of a modulation on the conventional linear temperature programme, allowing separation of the total heat flow signal into its heat capacity and kinetic components, these being known as the reversing and non-reversing heat flow respectively for the TA Instruments model (modulated DSC) (Reading et al., 1993). There is

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growing interest in the use of the technique as a means of characterising pharmaceutical systems, particularly in terms of measuring glass transitions (Hill et al., 1998a,b; Royall et al., 1998; van Winden et al., 1998).

One of the major advantages of MTDSC is that the technique allows direct measurement of the glass transition in the first heating scan when an associated enthalpic relaxation is also present, as demonstrated in a previous study involving the protease inhibitor saquinavir (Royall et al., 1998). The enthalpic relaxation may arise over the glass transition region either due to a difference in the cooling and heating rates used in the preparation and characterisation of an amorphous material or by stress relaxation on storage (Moynihan et al., 1996). The presence of this thermal feature is frequently problematic as it occurs over the same temperature range as the glass transition, thereby rendering visualisation of the latter difficult. Furthermore, the similarity in appearance between the relaxation endotherm and a melting response may result in confusion with regard to whether a melting or glass transitional event is taking place. Pharmaceutically, the conventional approach to characterisation of the T_g in the presence of a relaxation endotherm has been either to use one of a number of baseline extrapolations before and after the transitions, or alternatively to measure T_g not in the first heating scan but in a subsequent cycle which should remove the relaxation peak, providing equivalent cooling and subsequent heating rates are used. Both approaches have concomitant disadvantages: extrapolation of the baseline may lead to significant variability in the estimated T_g value (Royall et al., 1998), while temperature cycling results in the measured sample differing from the original material.

A particularly important consideration in the study of glassy drugs is the characterisation of the effects of sorbed water on the glass transition. It is well established (e.g. Levine and Slade, 1987) that the presence of water will plasticise the host material (lower the glass transition), leading to a greater probability of physical instability. The group of Zografi (Yoshioka et al., 1994; Hancock and Zografi, 1997; Andronis and Zografi, 1998) have addressed the issue of the relationship between the storage temperature and the crystallisation of the amorphous materials. These authors have emphasised that while there is a current understanding that storage below the T_g will reduce the probability of crystallisation over the lifetime of a product, recrystallisation may occur at temperatures up to 50°C (and possibly lower) below the T_g due to the system retaining sufficient molecular mobility to allow nucleation and crystal growth. Furthermore, Andronis and Zografi (1998) have demonstrated that the relaxation behaviour below T_g may be dependent not only on the storage temperature but also on the thermal history of the sample. The recrystallisation behaviour will inevitably be sample-dependent and it may not always be practically feasible (or even necessary over the shelf life of a product) to store an amorphous product at temperatures far below the T_g. There is, however, a clear need for a greater understanding of the relationship between the glass transition behaviour and the physical stability of the sample and it may be stated with reasonable certainty that storing a product above T_g may be regarded as inadvisable.

As it is well recognized that the presence of water lowers the T_g of amorphous systems, there are clear concomitant implications for product stability. The relationship between water content and T_g has been explored in a number of publications in the pharmaceutical literature (e.g. Hancock and Zografi, 1994). However, if one is attempting to quantitatively model the relationship between T_g and water content, the errors in T_g measurement arising due to the presence of the relaxation endotherm may be considerable. As mentioned above, it is possible to temperature cycle the sample in order to minimise the relaxation endotherm, although this may alter the sample in relation to the original system under study either due to changes in water distribution or to non-reversible alterations in structure such as collapse of freeze dried products. The former point has been demonstrated for HPMC films (McPhillips et al., 1999), whereby an endotherm corresponding to water evaporation was seen even when using hermetic pans due to evaporation of water into the pan headspace. There are therefore significant advantages to being able to measure

the T_g during the first temperature cycle, in isolation from the relaxation endotherm. In this investigation, we report on the use of MTDSC as a means of investigating the effects of water absorption on the T_g of the amorphous drug saquinavir in the first heating cycle.

2. Materials and methods

Amorphous saquinavir was obtained from Roche Pharmaceuticals and used as received. Samples of amorphous saquinavir were passed through a 125-micron sieve prior to experimentation. Sieved samples were stored over saturated salt solutions in desiccators stored at 25°C for approximately 1 week following the method of Nyqvist (1983). TGA and MTDSC experiments were run simultaneously so that the water content determined by the TGA experiments represented that of the samples run in the MTDSC.

Thermogravimetric analysis was conducted on a TA Instruments Hi-Res TGA 2950. Experiments were run with a 10°C/min scan rate from 29 to 200°C. The temperature had been previously calibrated using the melting of indium. Using the thermocouple above the sample pan on the TGA, it is possible to measure the rate of change of temperature during a temperature program. Upon melting of the indium sample a small peak is observed in this signal and is used to temperature calibrate the TGA. 'White spot' nitrogen was used as the purge gas through the furnace and balance, flowing at a rate of 60 and 40 cc/min respectively.

The MTDSC experiments were conducted using a DSC 2920 Modulated DSC (TA Instruments, Leatherhead, UK) with a Refrigerated Cooling System (RCS) unit attached. The instrument was calibrated using the melting of indium, cyclohexane and tin standards. Heat capacity calibration constants were derived as described in a previous study (Royall et al., 1998). 'White spot' nitrogen was used as the purge gas, flowing at a rate of 30 cc/min through the DSC cell, and at 150 cc/min through the RSC unit. TA Instruments aluminium hermetic pans were used throughout the study, except where stated when Perkin Elmer non-hermetic pans were used. The mass of each empty sample pan matched the mass of the empty reference to ± 0.1 mg. After loading with sample, the pans were sealed with a dry weld in a TA Instruments press.

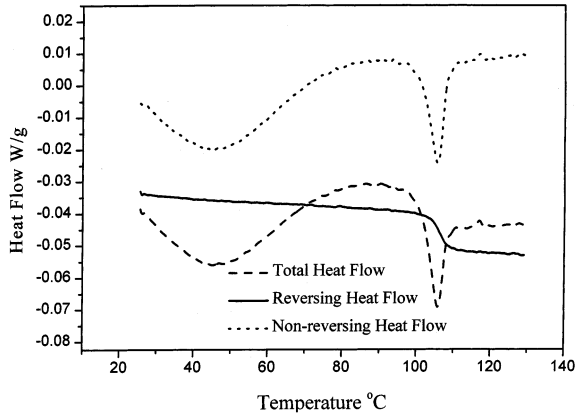

Fig. 1. MTDSC response of amorphous saquinavir exposed to 76% RH (2.4% w/w water) using non-hermetically sealed pans.

Following earlier studies into the effects of experimental conditions on the MTDSC response (Royall et al., 1998), the following parameters were selected: a modulation amplitude of $\pm$ 0.159°C and a 30 s modulation period with a 2°C/min underlying heating rate. This particular set-up has the advantage that during the heating cycle no instantaneous cooling is applied within the modulation temperature programme. The experimental method consisted of an initial 20-min isothermal period at 25°C to allow equilibration of the sample to the programmed temperature modulation, then heating to 130°C. Analysis of the results was carried out by TA instruments Universal Analysis software. Fitting the data to the Gordon–Taylor equation and the derived treatment was conducted using Origin™.

3. Results and discussion

Figs. 1 and 2 show the total heat flow data and the reversing and non-reversing signals using hermetic and non-hermetic pans respectively for a sample stored at 76% RH for 7 days. The heat flow dQ/dt during a conventional DSC experiment is given by

$$dQ/dt = C_p dT/dt + f(t,T) \quad (1)$$

where C_p is the heat capacity, dT/dt is the heating rate, and $f(t,T)$ is a function of time and temperature representing kinetic events. For a modulated DSC experiment, involving the application of a sinusoidal signal with amplitude A and frequency ω, the heat flow is given by

$$dQ/dt = C_p(q + A\omega \cos(\omega t)) + f'(t,T) + C \sin(\omega t) \quad (2)$$

where q is the underlying heating rate (equivalent to dT/dt), hence $q + A\omega \cos(\omega t)$ is the derivative modulated temperature and C is the amplitude of the kinetic response to the modulation. The term $C \sin(\omega t)$ is usually negligible, due to the temperature oscillation being sufficiently small so as to render the kinetic response to the modulation linear with respect to the heating signal, hence the out-of-phase (sine) component may usually be ignored. It is, however, extremely advisable to check this assumption by examining the out-of-phase component of the response; this was performed in this case and the assumption of linearity was found to be valid. The data may be averaged using a discrete Fourier transform algorithm and deconvoluted into reversing and non-reversing signals which relate to changes in C_p and kinetic events respectively. Consequently, glass transitions may be seen in the reversing signal and kinetic events such as relaxation endotherms may be seen in the non-reversing signal. The total heat flow, given by Eq. (2), is generally taken to be equivalent to the conventional DSC response. Hill et al. (1998a) have compared the total heat flow of MTDSC to that using conventional DSC and have found this assumption to be valid, although small differences were seen between the two responses as a result of the former being essentially an average of an oscillating signal.

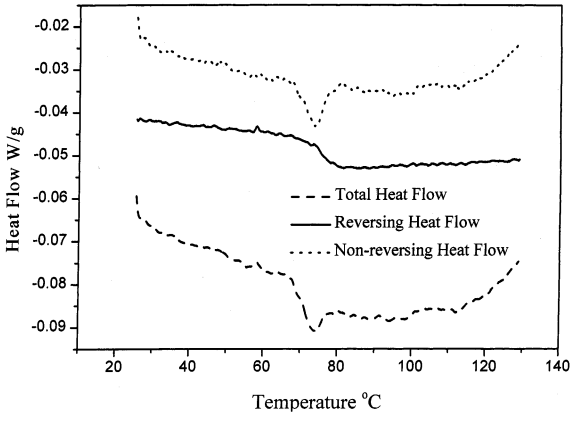

Fig. 2. MTDSC response of amorphous saquinavir exposed to 76% RH (2.4% w/w water) using hermetically sealed (crimped) pans.

As expected from previous studies (e.g. Hill et al., 1998a), the choice of pan has a profound effect on the measured glass transition, with a lower T_g/relaxation endotherm measured for the samples in hermetic pans. This may be ascribed to the water remaining within the sample pan during the heating run, leading to plasticization of the material. In contrast, the water within the non-hermetic pans escapes, hence at the T_g the sample is in a dehydrated state, a hypothesis supported by the observation of a broad endotherm (between 20 and 80°C), for the non-hermetic pans which corresponds reasonably to the water loss range noted using TGA. Clearly, if one is conducting studies specifically to examine the effects of water uptake on T_g then it is generally assumed that hermetic pans will be used. The difficulty lies with studies whereby the intention is to measure the T_g of a drug as an 'absolute' value. If one wishes to obtain a figure for the non-plasticized drug, it is arguably preferable to use non-hermetic pans such as pin-holed pans in order to drive off the moisture prior to the glass transition (this will obviously depend on the value of T_g and the water binding properties of the drug, as well as the experimental conditions used); it is in any case essential to state which pans have been used. There are two important additional considerations in this respect. Firstly, there is no 'absolute' T_g value for a drug as the value will inevitably depend on the thermal history of the sample. Secondly, consideration must also be given to the integrity of the seal of the hermetic pan. Our own experience has been that, depending on the pan type used and the water content of the sample, the integrity of the seal becomes an issue for consideration above approximately 150°C. In the present

study, a lack of the characteristic endothermic peak below the T_g/relaxation endotherm indicates that no water was lost from the hermetic pan, with the dry weld seal remaining intact throughout the temperature program. This was confirmed by the mass of the hermetic pan remaining constant before and after the MTDSC run. For the crimped pan, the mass loss during the heating run was equivalent to that measured in the TGA experiments run in open pans.

Figs. 1 and 2 clearly demonstrate the ability of MTDSC to separate the associated endothermic relaxation from the glass transition of amorphous saquinavir. The water loss peak, observed in Fig. 1 for the crimped pan, appears in the non-reversing heat flow together with the endothermic relaxation peak as it is a kinetically controlled event. Sorbed water significantly lowered the glass transition temperature of amorphous saquinavir, as can be seen in Fig. 2, where the T_g for saquinavir stored at 76% RH, run in hermetic pans, was determined to be $74 \pm < 1$°C. This compares to a measured value of $105 \pm < 1$°C obtained when using the crimped pans. Overall, therefore, the data indicate that MTDSC is capable of separating the glass transition from the accompanying relaxation endotherm, thereby facilitating quantification of T_g in the first heating cycle for systems containing a range of moisture contents.

The extent of the depression of T_g is clearly related to the weight fraction of sorbed water (Fig. 3). Previous authors (Hancock and Zografi, 1994) have indicated that the relationship between moisture uptake and T_g may be described in terms of the Gordon–Taylor relationship (Gordon and Taylor, 1952). Assuming perfect volume additivity with no specific interaction between the components, the glass transition of the mixture, $T_{g_{mix}}$ is given by:

$$T_{g_{mix}} = \phi_1 T_{g_1} + \phi_2 T_{g_2} \quad (3)$$

where ϕ is the volume fraction and the subscripts represent the two components. Re-defining Eq. (1) in terms of weight fraction gives

$$T_{g_{mix}} = \frac{(w_1 T_{g_1}) + (K w_2 T_{g_2})}{w_1 + (K w_2)} \quad (4)$$

where w_1 and w_2 are the weight fractions of water and drug respectively and K can be considered to be the ratio of the free volumes of the two components. It can be seen from Fig. 3 that the Gordon–Taylor equation fits the data reasonably well. A K value of 0.198 ± 0.005 was determined, implying that the free volume of amorphous saquinavir is approximately five times that of absorbed water. More specifically, by applying the Simha–Boyer rule (Simha and Boyer, 1962) it is possible to define K in terms of the densities of the two components:

$$K = \frac{(\rho_1 T_{g_1})}{(\rho_2 T_{g_2})} \quad (5)$$

Using Eq. (5), a value of 1.79 ± 0.05 g cm^{-3} was determined for the density of amorphous saquinavir assuming the density of the absorbed water to be 1 g cm^{-3}. The T_g of water was estimated by linear regression in Origin™ using Eq. (4) to be 135.9 ± 6.1 K with a K value of 0.198. This compares reasonably well with the published value of 135 K (Sugisaki et al., 1968). A value of 1.35 ± 0.07 g cm^{-3} for the density of saquinavir was determined by air pycnometry (using seven replicates) which is in reasonable agreement with the density value stated above.

It may be seen from Fig. 3 that the presence of relatively small quantities (up to 5% w/w) sorbed water may lower the T_g by up to 50°C for this

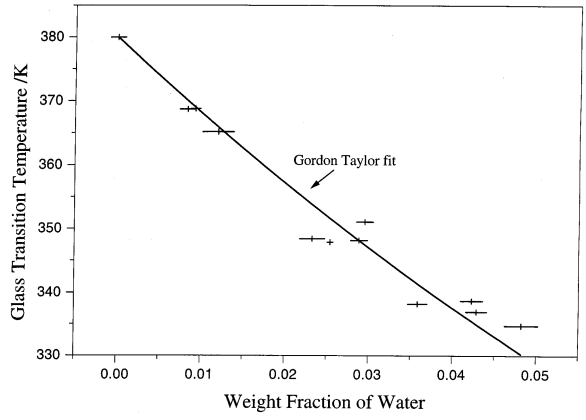

Fig. 3. Fitting of the depression of the glass transition temperature for amorphous saquinavir to the Gordon–Taylor equation.

material. This has clear implications for the storage stability of products containing this drug and leads to the question, what is the water content that will result in the lowering of T_g to below the storage temperature, thereby considerably increasing the risk of recrystallisation? An estimate of this figure at any storage temperature may be easily obtained simply by considering the Simha–Boyer rule in the case of water uptake, whereby T_{g_1} and ρ_1 are known, hence

$$K = \frac{1}{\rho_2} \times \frac{135}{T_{g_2}} \tag{6}$$

Inserting Eq. (6) into the Gordon–Taylor equation and setting $w_2 = 1 - w_1$ gives

$$T_{g_{mix}} = \frac{135 T_{g_2}(w_1\rho_2 - w_1 + 1)}{T_{g_2} w_1 \rho_2 + 135(1 - w_1)} \tag{7}$$

Rearranging to make w_1 the subject of the equation

$$w_1 = \frac{135(T_{g2} - T_{g_{mix}})}{T_{g2\rho\,2}(T_{g_{mix}} - 135) + 135(T_{g_2} - T_{g_{mix}})} \tag{8}$$

$$w_1 = \left[1 + \frac{T_{g_2}\rho_2[T_{g_{mix}} - 135]}{135[T_{g_2} - T_{g_{mix}}]}\right]^{-1} \tag{9}$$

If one now considers a critical water content w_c at which the T_g is lowered to the storage temperature T_{ST}, then at w_c the value of $T_{g_{mix}}$ becomes T_{ST}. Eq. (9) then becomes

$$w_c = \left[1 + \frac{T_{g_2}\rho_2[T_{ST} - 135]}{135[T_{g_2} - T_{ST}]}\right]^{-1} \tag{10}$$

Alternatively, one can derive an expression for the moisture content (w'_c) which would result in the value of T_g falling to a value 50 K above the storage temperature, thereby giving a much greater margin of safety with regard to the possibility of recrystallisation.

$$w'_c = \left[1 + \frac{T_{g_2}\rho_2[T_{ST} - 85]}{135[T_{g_2} - T_{ST} - 50]}\right]^{-1} \tag{11}$$

A demonstration of the use of this expression is given in Fig. 4. The curves indicate the critical water content (i.e. the water content that will lower the T_g to the storage temperature) in relation to the glass transition of the dry material, assuming a density of 1.2 g/cm^3 (which is a reasonable estimate of the density of a typical pharmaceutical solid). The curves refer to three storage temperatures. For example, if a material with a 'dry' glass transition of 360 K is stored at 303 K, then the material may take up approximately 9% w/w water without the glass transition being lowered to below the storage temperature (although as mentioned previously this does not guarantee stability).

One may also use Eqs. (10) and (11) for a single material (in this case saquinavir) in order to ascertain the relationship between the storage temperature and the glass transition, with a particular view to identifying the combinations of water content and temperature which will result in a greater or lesser danger of recrystallisation. This is exemplified in Fig. 5 for saquinavir whereby above the solid line ($T_{ST} > T_g$) the storage temperature is above that of the glass transition, hence there is considerable risk of recrystallisation. In the region between the solid and dashed curves, the storage temperature is below the glass transition temperature, hence stability is more likely but is not guaranteed. In the region below the dashed curve, the glass transition is at least 50 K greater than the storage temperature, hence molecular mobility may be expected to be extremely limited. This therefore represents a 'safety zone' for the formulator in that recrystallisation is extremely unlikely to occur under these conditions.

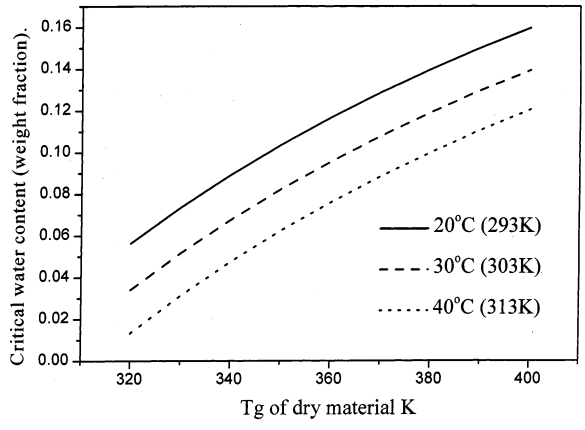

Fig. 4. Relationship between the critical water content (water content at which T_g is lowered to the storage temperature) as a function of 'dry' T_g for three storage temperatures (assuming a density of 1.2 g/cm^3), calculated using Eq. (10).

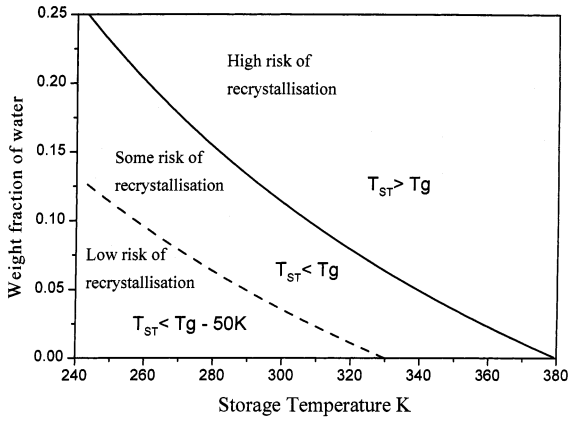

Fig. 5. Relationship between the storage temperature and the water contents which will lower the T_g of saquinavir to that storage temperature (solid line) and a temperature 50 K below the glass transition temperature (dashed line), calculated using Eqs. (10) and (11).

Clearly, the analysis described above yields only an approximation. However, the method may be of use for initial formulation, as only the 'dry' glass transition and the density are required. The T_g value may be obtained using crimped pans if the value is above that required to drive off the sorbed water, while the density may in the first instance be estimated or measured using a small quantity of material. Consequently, the above analysis may be of use during the early stages of formulation when only very limited quantities of material are available.

4. Conclusions

The study has demonstrated the ability of MTDSC to separate the glass transition from the accompanying relaxation endotherm and has also examined the effects of experimental conditions on the measurement of T_g for hydrated systems. Furthermore, an analysis has been proposed whereby the relationship between the moisture content and the storage stability may be predicted, albeit approximately. Nevertheless, the ability to assess whether water uptake is likely to lead to stability problems using comparatively simple measurements may be of use to the formulator at the early stages of drug development.

Acknowledgements

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