Characterisation of the Glass Transition of an Amorphous Drug Using Modulated DSC[1]

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Purpose. The use of modulated differential scanning calorimetry (MDSC) as a novel means of characterising the glass transition of amorphous drugs has been investigated, using the protease inhibitor saquinavir as a model compound. In particular, the effects of measuring variables (temperature cycling, scanning period, heating mode) have been examined.

Methods. Saquinavir samples of known moisture content were examined using a TA Instruments 2920 MDSC at a heating rate of 2°C/min and an amplitude of ±0.159°C with a period of 30 seconds. These conditions were used to examine the effects of cycling between - 50°C and 150°C. A range of periods between 20 and 50 seconds were then studied. Isothermal measurements were carried out between 85°C and 120°C using an amplitude of ±0.159°C with a period of 30 seconds.

Results. MDSC showed the glass transition of saquinavir (0.98 ± 0.05%w/w moisture content) in isolation from the relaxation endotherm to give an apparent glass transition temperature of 107.0 °C ± 0.4°C. Subsequent temperature cycling gave reproducible glass transition temperatures of approximately 105°C for both cooling and heating cycles. The enthalpic relaxation peak observed in the initial heating cycle had an additional contribution from a Tg "shift" effect brought about by the difference in response to the glass transition of the total and reversing heat flow signals. Isothermal studies yield a glass transition at 105.9°C ± 0.1°C.

Conclusions. MDSC has been shown to be capable of separating the glass transition of saquinavir from the relaxation endotherm, thereby facilitating measurement of this parameter without the need for temperature cycling. However, the Tg "shift" effect and the number of modulations through the transition should be taken into account to avoid drawing erroneous conclusions from the experimental data. MDSC has been shown to be an effective method of characterising the glass transition of an amorphous drug, allowing the separate characterisation of the Tg and endothermic relaxation in the first heating cycle.

KEY WORDS: amorphous; glass transition; modulated differential scanning calorimetry; saquinavir.

INTRODUCTION

Modulated differential scanning calorimetry (MDSC) is a novel thermoanalytical technique which involves the application of a sinusoidal (modulated) heating signal to a sample, thereby allowing separation of the total heat flow response into the reversing and non-reversing components (1). The heat flow from a sample experiencing a linear heating (or cooling) programme will comprise a heat capacity (reversing) component and a contribution from any kinetically hindered (non-reversing) thermal events. The total heat flow is therefore given by;

$$\frac{dQ}{dt} = Cp\frac{dT}{dt} + f(t, T) \quad (1)$$

where dQ/dT is the total heat flow (Js^{-1} or W), C_p is the heat capacity (JK^{-1}) and $f(t, T)$ represents a function of temperature and time. The $C_p dT/dt$ term is always present in the signal and represents the reversing component which is dependent on the rate of change of temperature (heating rate, dT/dt) and the heat capacity. Chemical or physical events dependent on the absolute temperature achieved in the instrument and the rate of heat loss or gain for the process (or more correctly on the kinetics of the transition) will be seen as a contribution from the $f(t, T)$ component. The sample will not be in equilibrium with the temperature programme for the duration of the transition, hence the $f(t, T)$ component is considered to represent non-reversing kinetically controlled events and is a function of absolute temperature and time, whereas the heat capacity contribution is thermodynamically reversing and is a function of the heating rate.

Conventional DSC records the total heat flow at any temperature, hence both the reversing and non-reversing components are measured simultaneously. However, MDSC has the ability to separate the heat capacity and the kinetic components; this ability is a consequence of the different response of the two heat flow signals to the underlying and modulated temperature programmes (1). The signal separation is particularly useful when identifying and isolating glass transitions which, being changes in the heat capacity of the sample, are seen only in the reversing heat flow signal. More details of the principles of MDSC may be found in a number of texts (1,2,3,4).

This paper outlines the use of MDSC for the characterisation of an amorphous drug, saquinavir. The significance of the amorphous state in pharmaceutical systems has been widely discussed (e.g. 5,6,7). Furthermore, the determination of the glass transition temperature for amorphous solids is important for the determination of chemical and physical stability (e.g. 8,9). One of the most convenient techniques for measuring Tg is conventional DSC. However the predicted heat capacity step change for the glass transition is complicated by the presence of an enthalpic relaxation endotherm which is positioned directly over the glass transition response, often making proper identification of Tg extremely difficult. However, as the glass transition is a function of cooling rate whereas the endothermic relaxation is a function of absolute temperature and time (10), MDSC may separate these transitions into the reversing and non-reversing heat flow signals respectively.

The ability of MDSC to separate reversing and non-reversing heat signals is therefore of clear potential significance for the study of pharmaceutical materials. However, the majority of work using MDSC has been associated with polymeric samples (1,2,4). We report here on the novel use of the technique to study the glass transitional behaviour of a model amorphous

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drug, the protease inhibitor saquinavir. As yet there is little information available regarding the use of the technique for such low molecular weight materials, nor is there literature available describing the optimal measuring conditions and available options, although a recent publication by Hill et al. (11) describes the effects of changing certain measuring parameters on the glass transitional behaviour of lactose. We therefore describe the effects of using a range of measuring conditions on the glass transitional behaviour of a model drug and outline some of the options available for the analysis of the data obtained.

MATERIALS AND METHODS

Materials

Amorphous saquinavir (molecular weight 671) was obtained from Roche Pharmaceuticals (Welwyn Garden City, UK) and used as received after passing through a 125 micron sieve. The amorphous nature of the sample had been previously confirmed by the manufacturing company using PXRD (data not shown). Thermogravimetric analysis (TA Instruments Hi-Res TGA 2950) was used to measure the moisture content of the sample, which was determined as 0.98 ± 0.05% weight of water (six replicates).

MDSC Studies

MDSC and conventional DSC experiments were conducted using a DSC 2920 Modulated DSC (TA Instruments), with a refrigerated cooling system (RCS) attached. "White spot" nitrogen was used as the purge gas, flowing at a rate of 40cc/min through the DSC cell, and at 150cc/min through the RCS unit. TA Instruments aluminium hermetic DSC pans were used throughout the study. The mass of each empty sample pan was matched with the mass of the empty reference pan to ± 0.1mg. Approximately 3mg of sample was used for the MDSC runs.

The instrument was calibrated for temperature using indium, cyclohexane and tin standards, using the same underlying heating rate and the same pan type as in the experiments described below. The heat flow and heat capacity signals were calibrated by comparing the response for a powdered sample of alumina, (3mg, 100 mesh) to the equivalent literature value for the heat capacity (12) over the experimental temperature range.

Standard DSC experiments were conducted at 2°C/min from −50°C to 150°C using the MDSC 2920 in its conventional mode in order to compare the response with that of the MDSC total heat flow signal. MDSC experiments were performed with a modulation amplitude of ± 0.159°C and a 30 second period with a 2°C/min underlying heating or cooling rate. The experimental methodology included an initial 20-minute isothermal period at −50°C to allow equilibration of the sample to the programmed temperature modulation, then heating to 150°C followed by subsequent cooling to −50°C. This was repeated for another 1.5 cycles producing a total of 3 heating scans and 2 cooling scans. The modulation period was then varied between 20 and 50 seconds with an underlying 2°C/min heating and cooling rate. The experimental method consisted of an initial 20 minute isothermal period at 70°C to allow equilibration of the sample to the programmed temperature modulation, then heating to 130°C followed by subsequent cooling to 70°C. The above experiments were repeated five times.

For quasi-isothermal studies, the basic MDSC parameters were a modulation amplitude of ± 0.159°C with a 30 second modulation period. The experimental method was initially identical to the one used for the temperature cycling experiments. However after the heating scan from −50°C to 150°C, with a heating rate of 2°C/min, the first cooling scan was stopped at 120°C. Below 120°C the temperature was reduced in 1°C isothermal steps until 85°C (with an approximate rate of 0.05°C/min). Each 1°C isothermal step lasted for 20 minutes, with data only recorded in the last 10 minutes of each step. The experiment was repeated three times.

RESULTS

Conventional and MDSC Responses

A typical result from a "conventional" DSC run is shown in Figure 1. The trace shows an endotherm just after 100°C, with a discontinuity in the baseline before and after the thermal event. Such step change discontinuities are indicative of glass transitions, although clearly there are problems associated with the verification that such a process is occurring and the identification of the exact temperature range over which the event is taking place due to the presence of the accompanying endothermic relaxation.

A glass transition temperature of 102.7°C ± 0.5°C was determined by extrapolating the two baselines and judging the temperature for the mid-point of the transition by eye. In addition, a fictive glass transition temperature of 95.8°C ± 3°C was determined by taking the integral of the power/temperature plot and using the method developed by Richardson and Savill (10) to measure intercept of the extrapolated glass and liquid enthalpy lines. An estimation of the enthalpy for the relaxation endotherm was made by the construction of various types of baselines beneath the peak. A tangential baseline gave an enthalpy of 4.8 J/g, a stepped baseline 4.6 J/g and a sigmoidal baseline 4.1 J/g. This shows a 20% difference between the recognised methods for estimating the enthalpy of the endothermic relaxation.

Given the comparatively large breadth and magnitude of the thermal events shown in Figure 1, it was decided that

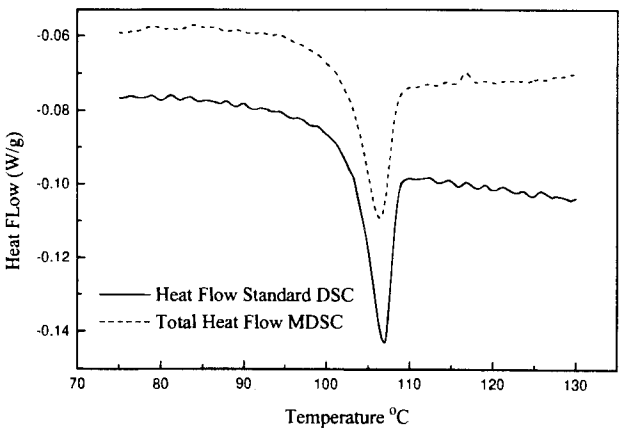

Fig. 1. Conventional DSC and total heat flow MDSC responses for amorphous saquinavir.

standard MDSC operating conditions of a modulation amplitude of ± 0.159°C, a 30 second period and a 2°C/min underlying heating rate would be used initially. It should be emphasised that one of the principle disadvantages of MDSC is that a comparatively slow underlying heating rate is required in order to allow a suitable number of modulations to appear through the thermal event; this will be discussed in more detail in a later section. Figure 1 also shows the average total heat flow response of the sample obtained using MDSC. This response is theoretically equivalent to that of conventional DSC run under equivalent conditions; comparison of the two curves supports this assertion, as has been shown in previous studies using lactose (11).

Figure 2 represents the response of amorphous saquinavir in an MDSC experiment, showing the total, reversing and non-reversing heat flow. The reversing signal shows a glass transition at 107.0°C ± 0.4°C, while the non-reversing signal shows a relaxation endotherm with an onset temperature of 102.5°C ± 0.3°C. The enthalpy for the relaxation endotherm in the non reversing heat flow signal was determined to be 6.7 ± 0.1 J/g for the MDSC experiment. Clearly, the use of MDSC allows separation of the two thermal events, although care is required in certain aspects of the interpretation which are outlined below.

Effects of Temperature Cycling

It is often useful when measuring glass transitions to temperature cycle, firstly to establish a defined thermal history for the sample, secondly to verify the existence of the glass transition which should be seen on cooling and re-heating and finally to remove the relaxation endotherm which may obscure the glass transition (as in the example shown here). Figures 3a & 3b show the effects of cycling the sample using the conditions outlined in the previous section. In the first heating scan, the total heat flow shows the large endothermic relaxation superimposed on the glass transition, which in the subsequent heating scans is reduced in size. The reappearance of the endothermic relaxation peak on reheating may be a result of annealing during the cycling process. The total heat flow signal in the cooling scans shows a broad step change in the baseline between 95°C and 105°C. Deconvolution of the total heat flow into the reversing and non-reversing components revealed that the glass

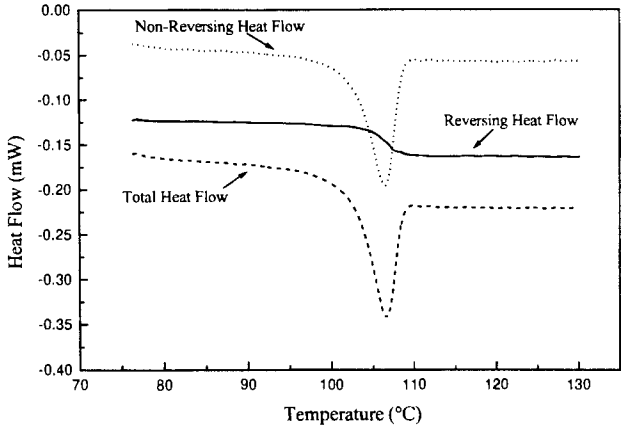

Fig. 2. Modulated DSC response of amorphous saquinavir, showing the reversing, non-reversing and total heat flow signals.

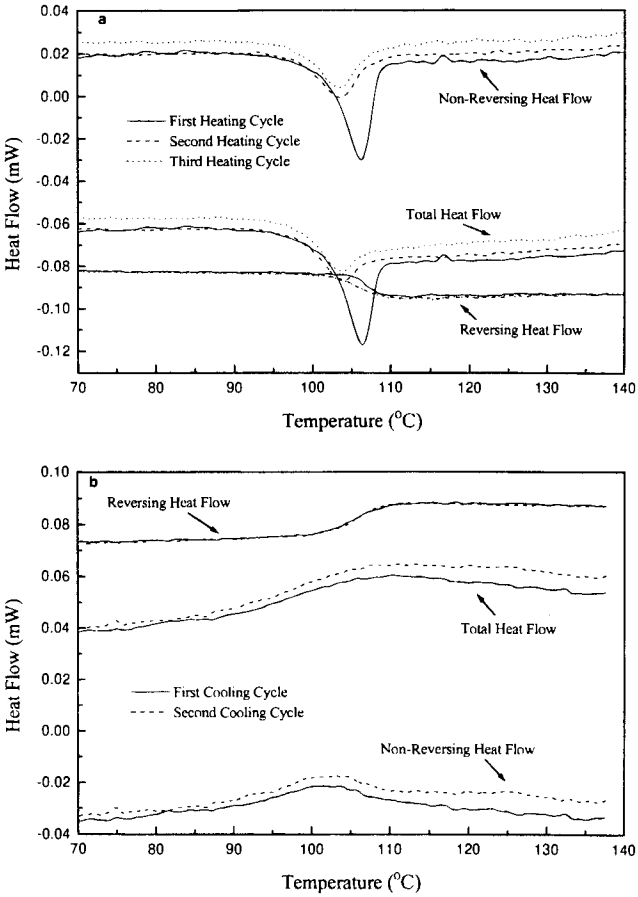

Fig. 3. Modulated DSC response of temperature cycled amorphous saquinavir (a) heating scans (b) cooling scans.

transition, observed in the reversing heat flow signal, was reproducible within experimental error between the heating and cooling runs after the initial heating cycle; the three heating cycles gave Tg values of 107.0 ± 0.4°C, 105.2 ± 0.7°C and 104.7 ± 0.9°C, while the cooling cycles gave values of 105.6 ± 0.5°C and 105.3 ± 0.3°C. The difference between the Tg values observed in the first heating cycle and subsequent cycles was relatively small (≈2°C). In the cooling scans a very broad exothermic peak is seen in the non-reversing signal over the glass transition region. An explanation of this effect is given in the discussion section of this paper.

Effects of Modulation Period

The effect of modulation period on the glass transitional behaviour of saquinavir is illustrated in Figures 4a and b. Figure 4a shows the calibrated complex heat capacity signal for the heating scan at various periods between 20 and 50 seconds at a constant underlying heating rate and modulation amplitude of 2°C/min and ± 0.159°C respectively. It can be seen that for the present system the range of modulation periods used has little effect on the value for the observed Tg in either heating and cooling modes. The difference in heat capacity through the glass transition is approximately the same for each period with the changes in the underlying heat capacities likely due to the slight discrepancies in the average mass of the samples pans

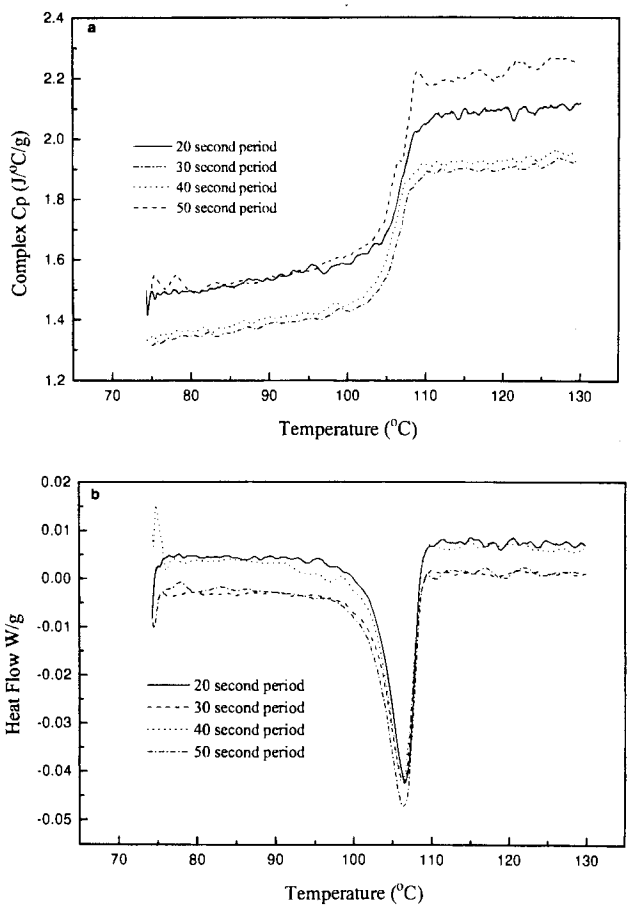

Fig. 4. Effect of period on (a) the complex heat capacity and (b) the endothermic relaxation of amorphous saquinavir on heating.

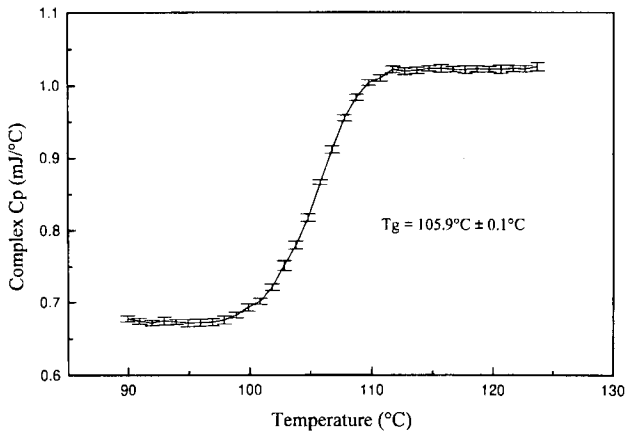

Fig. 5. Quasi-isothermal MDSC studies for amorphous saquinavir.

used in the different experiments. Calibrated non-reversing heat flow signals for this set of experiments are shown in Figure 4b for the heating scans. Again no clear relationship between response and modulation period can be observed outside the experimental error.

Quasi-Isothermal Studies

Figure 5 illustrates the ability of MDSC to operate in a quasi-isothermal mode, which effectively consists of holding the sample at a particular temperature and imposing a sinusoidal modulating heating and cooling programme around this point. The raw signals obtained permit the determination of a complex heat capacity. To allow for equilibration, the heat capacity was recorded only in the last 10 minutes of the isothermal interval, over 400 data points were recorded at each temperature, averaged and then plotted against temperature to reveal the glass transition. The glass transition was determined by taking the first derivative to estimate the mid point and had a value of 105.9°C.

DISCUSSION

The data presented here demonstrate the strengths of the MDSC technique compared to conventional analysis but also serve to highlight some of the disadvantages involved. Figure 1 exemplifies some of the difficulties associated with measuring glass transitions using conventional DSC, notably with respect to the presence of the endothermic relaxation peak. The endothermic relaxation arises as a result of two phenomena; a kinetic effect due to the difference in the rate of formation and detection and an "annealing" effect due to the relaxation of the sample upon storage (13); both are related to the thermal history of the sample. It is advantageous to determine the glass transition during an initial heating cycle, because this will be representative of the sample as received and not that imposed by the analytical technique when the Tg is measured in the second cooling run. Therefore the ability of MDSC to measure an apparent glass transition temperature in the first heating cycle allows a more non-invasive assessment of the nature of the amorphous material; this may be particularly important in multicomponent systems (i.e. formulated products) whereby cycling may result in permanent alteration of the product.

Figure 2 shows the ability of MDSC to separate the overlapping glass transition and endothermic relaxation. This is due to the glass transition being a change in heat capacity rather than a phase change, hence this response will appear in the reversing heat flow signal. Likewise the endothermic relaxation is a non-reversing transition which is dependent on the temperature and time scale of the measurement rather than the heating rate and will therefore appear in the non-reversing heat flow signal. This separation not only allows clearer visualisation of the glass transition but also allows verification of the presence of such thermal events for samples whereby differentiation between a glass transition/relaxation endotherm and a melting response is not a trivial problem. However, caution is required in terms of quantitatively determining the size of the relaxation endotherm from MDSC data. Examination of Figure 3 indicates that in the total and non-reversing signals, peaks are seen after the first heating scan which, according to the argument outlined above, should not be observed. Current thinking (14) suggests that these peaks may be caused by differences in the response to the glass transition in the total and reversing heat flow signals. The glass transition seen in the reversing signals will differ from that seen in the total heat flow as Tg is heating rate dependent and will therefore be marginally higher in the reversing signal, effectively because glass transitions are higher at rapid rates of cooling (10) and the reversing signal represents the response to the oscillation rather than the underlying heating rate, which will be slower. Reference to Eq. 1 shows that the

de-convolution process involves the subtraction of the reversing from the total heat flow signal, hence the difference outlined above (the Tg "shift" effect) will result in an apparent non-reversing response being observed. As the underlying heating and cooling rates are equal the peak observed in the non reversing heat flow for the cooling scans is solely attributed to this "shift" effect, and has an area of 2.7 ± 0.1 J/g. This was then used to adjust the relaxation enthalpy observed for the first heating scan to its true value by subtracting the area of the "shift" peak in the cooling scans away from the observed relaxation peak. Such a procedure gave a value for the enthalpic relaxation of 4.0 ± 0.2 J/g which is close to the value estimated in the standard DSC experiments. The high variance in the estimations for the enthalpic relaxation in the conventional DSC experiments is due to the difficulty in assessing where the glass transition lies under the peak; this problem is at least partially resolved using MDSC.

It is possible to use the frequency dependency of the glass transition to produce information concerning the kinetics of that transition (15,16). However, in the study outlined here the frequency dependence was found to be too small over the range of periods measurable. It is essential (and one of the limitations of the technique) to have at least four modulations through the thermal event under study in order to allow reliable de-convolution of the data (14), hence the data obtained using a period of 50 seconds starts to approach this limit. Theoretically, one can simply shorten the period. However, if periods of approximately 20 seconds are used one approaches the frequency limit of the MDSC cell, whereby the cell itself has difficulty maintaining the modulation temperature programme due to its inherent thermal lag. In practice, a compromise is reached whereby slow underlying heating rates are used and periods between the approximate range of 20 to 50 seconds are employed. However, the limitation outlined above is particularly pertinent to low molecular weight pharmaceutical samples, as, unlike the studies on Tg of polymeric systems which form the bulk of the literature, drugs and excipients tend to have far narrower glass transition ranges, hence careful consideration of the modulation period and underlying heating rate is required for such samples. It should be emphasised that if the above limitations are not appreciated it is very possible to produce artefacts which in turn may lead to misinterpretation of the data.

A very interesting approach described by Boller et al. (4) and used in this study is to employ a quasi-isothermal method to measure the change in Cp at specific points through the glass transition. Obviously, as the glass transition is a response to a temperature signal then there is no such event as an isothermal Tg, however, the method outlined here allows the Tg at effectively infinitely slow heating rates to be obtained, thereby providing a rate-independent value. This method also overcomes some of the problems associated with the width of the glass transition for low molecular weight pharmaceuticals, as it may allow measurement to be made over a narrow temperature increment range.

CONCLUSIONS

The study has demonstrated the potential benefits and limitations of using MDSC for the characterisation of glass transitions of low molecular weight drugs substances. In particular, the ability to de-convolute the glass transition from the relaxation endotherm has highly important implications in terms of verification and quantification of glass transition phenomena; such knowledge is of considerable practical benefit in terms of understanding the behaviour not only of amorphous drugs but also spray and freeze dried systems and polymeric drug delivery vehicles. However, the study has also highlighted the issues over which caution is required, particularly the choice of experimental parameters and the quantification of the relaxation endotherm. In general, the choice of underlying heating rate, period and amplitude must be made with full appreciation of how these parameters may alter the response, while interpretation of the reversing and non-reversing responses must be made with as full an understanding as possible of the underlying mechanisms responsible for the generation of these signals.

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