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Pharmaceutical physical form characterisation with fast (>200 °C min^{-1}) DSC heating rates

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Abstract Differential scanning calorimetry (DSC) has many applications during preformulation screening of new drug candidates, but definitive assignment of peaks to specific events in the sample is difficult without supplementary data from other techniques. To some extent these problems can be overcome by running multiple experiments at different heating rates. Typically 2 and 20 °C min^{-1} are indicated. However, modern instruments are capable of achieving much faster heating rates (up to 750 °C commercially); with this extended range comes a new capacity for investigating the physical form of materials. Here, the use of fast DSC heating rates for materials characterisation is explored, focussing on determination of melting temperatures, glass formation and polymorph screening.

Keywords Fast-scan differential scanning calorimetry · Preformulation · Pharmaceutical · Polymorphism · Co-crystals

Introduction

Differential scanning calorimetry (DSC) is sensitive to the heat changes associated with thermally driven phase transitions and is thus particularly suited to physical form characterisation of pharmaceuticals; typical events that might indicate the physical state of a sample include glass transitions (T_g), loss of hydrate water, solid–solid conversions, crystallisation and melting. The proper interpretation of some, or all, of these transitions in a DSC thermogram can define whether the sample was amorphous or crystalline, whether any polymorphs exist and, if so, whether the sample exhibits monotropic or enantiotropic polymorphism. As such, a DSC screen of a new drug candidate early in preformulation can provide a lot of information on available physical forms that might direct future development of a product.

It is well known that interpretation of DSC data in isolation must be undertaken with caution, since the data do not contain any molecular information (in the way spectroscopic data do for instance); different phase transitions can give rise to very similar thermograms and hence assignment of exo- or endotherms to specific events is at best qualitative unless corroborative data are available. Typically thermogravimetric (TG) analysis or hot-stage microscopy (HSM) are employed in tandem with DSC analysis since all can record data with linear heating rates. However, much progress in interpretation of DSC data can be made simply by recording data with different instrumental parameters. One option is to use a modulated heating rate, since this allows separation of transitions into events that are 'reversible' and those that are 'irreversible'. This approach is particularly apt for isolation of glass transitions.

Another option is to use different heating rates. The rationale for this is that transitions that are purely thermodynamic (that is, require the sample only to attain a certain amount of energy before they occur) will occur at the same temperature independent of heating rate while those transitions that are kinetic (that is, the molecules in a sample require time to reorient following attainment of a certain amount of energy) will appear at increasing temperatures with increasing heating rate. Hence, kinetic events appear to 'move' with heating rate while thermodynamic events do not. Performing a DSC experiment at

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two heating rates thus allows a distinction to be drawn between kinetic (such as glass transitions, crystallisations or solid–solid transitions) and thermodynamic (such as melting) events. It is usually recommended that two heating rates an order of magnitude apart are used (typically 2 and 20 °C min^{-1}). However, the advent of instrumentation capable of achieving much higher heating rates (commercial instruments now operate up to 750 °C min^{-1}) mean that it is possible to explore the physical form of materials in new ways. In particular, where a kinetic event precedes a related thermodynamic event (such as crystallisation to a particular polymorph followed by melting of that polymorph) it is often possible to select a heating rate fast enough that the temperature at which the kinetic event occurs becomes higher than that of the associated thermodynamic event; when this is so, the kinetic event no longer happens and is said to have been 'inhibited'. The advantages of this approach to physical form characterisation of pharmaceuticals were first noted by Gabbott et al. [1] and used to study lactose [2] and carbamazepine [3].

Operating a DSC at fast heating rates requires specialised design; specific factors that must be considered include the overriding need to maintain control of temperature, the data capture rate and the effect of time constants. There are two current technologies with which fast heating rate measurements can be made. Either a conventional instrument can be modified to achieve fast heating and cooling rates [4] or solid-state 'chip' calorimeters can be employed [5]. The immediate benefit in the former case is one of familiarity and compatibility; with the instrument, software and sample handling apparatus. Drawbacks include rather modest heating rates, on the order of a few hundred K min^{-1} and, because of the relatively long time constant of a conventional DSC transducer (ca. 2–3 s), a reduced ability to resolve closely occurring events. Solid-state calorimeters can achieve extremely fast heating rates (up to 10^6 K s^{-1}) with very small time constants (on the order of 10^{-3} s). However, solid-state calorimeters are often very small and usually require samples to be deposited by film casting, frequently onto a thin protective membrane. This means it is difficult to weigh the sample and although they can be cleaned and re-used, solid-state calorimeters cannot at present be considered robust enough for general application outside of a specialised research laboratory.

The most obvious benefit of fast heating rates is the concomitant reduction in the time required to run experiments, an important benefit in a world that demands ever-increasing throughput of samples. This benefit applies to all samples, but is of particular advantage to those materials whose properties may change upon prolonged exposure to increased temperatures, such as amorphous or metastable products, or compounds that degrade upon heating. The second benefit is that the increased heat flow signal allows investigation of events that occur with small thermal signals (alternatively, less sample is required to attain the same heat flow).

However, fast heating (and cooling) rates offer much wider potential for characterisation of physical form, including the ability of pharmaceuticals to form glasses, to convert between polymorphs and to determine 'true' (i.e. thermodynamic) melting temperatures. Thus, the specific aim of this work is to explore the use of fast DSC heating rates for physical form characterisation, allowing understanding to be gained of the range of experimental parameters in which DSC instrumentation can be used to aid quantitative data interpretation.

Materials and methods

D-Mannitol, carbamazepine (CBZ), nicatinamide (NCT) and sulfapyridine (all ALR grade) were purchased from Sigma-Aldrich (UK). Compound A was supplied by GlaxoSmithKline (UK). Ethyl acetate (HPLC grade) was purchased from Fisher Scientific (UK). All materials were used as received.

Preparation of CBZ–NCT Form I

Form I CBZ–NCT (CBZ–NCT(I)) co-crystals were prepared by the solvothermal methods described in [6]; a hot solution of NCT (350 mg) and CBZ (670 mg) in ethyl acetate (50 g) was cooled with agitation to room temperature to create a supersaturated solution. The co-crystals formed (1:1 molar ratio) were filtered under reduced pressure using a Whatman number 50 filter paper for 30 min to remove any remaining solvent and then stored over silica gel in a desiccator at 5 °C until use.

Differential scanning calorimetry

Measurements were performed with Q2000 and RHC DSC instruments (TA Instruments, LLC, USA). The instruments operate on similar principles, being heat-flux designs, but RHC has a very small furnace (<1 cm diameter, albeit of the same basic design as Q2000) and an array of infra-red lights is used to raise temperature in place of a conventional furnace. The result of this is that the instruments have different heating rate capabilities (Q2000 up to 200 °C min^{-1}; RHDSC up to 2000 °C min^{-1}) and sample sizes (Q2000 ca. 5 mg; RHC ca. 0.1 mg). Both instruments use aluminium pans and lids, but those of RHC are ca. 1.5 mm in diameter whereas those of Q2000 are ca. 10 mm in diameter. Q2000 uses an intercooler (−90 °C) for the cold-stage while RHC utilises liquid nitrogen (−196 °C).

This means that RHC can achieve faster cooling rates, although it is not possible to define them since the maximum rate of cooling will depend upon the temperature difference between the sample and the cold-stage and will asymptotically approach zero as the sample temperature approaches that of the cold-stage. Further details of the design and construction of RHC are available in the literature [7, 8]. Experiments were conducted in triplicate. The cell constant and enthalpy calibrations were performed with indium ($T_m = 156.6$, $\Delta_f H = 28.71$ J g^{-1}) in accordance with the manufacturer's instructions. Nitrogen (50 mL min^{-1}) was used as a purge gas.

Tzero non-hermetic aluminium pans and lids were used with both instruments. Data were analysed with Universal Analysis 2000 and all melting, crystallisation and glass transition temperatures are calculated as extrapolated onsets.

Glasses were prepared by quench-cooling either within the DSC or by immersion in liquid nitrogen. In all cases the sample (mannitol, sulfapyridine or CBZ–NCT(I)) was heated to ca. 10 °C above its melting point. For DSC experiments the equilibrate function (maximum cooling rate) was used to cool the sample. To prepare quench-cooled samples outside of the DSC, the sample was melted on a hot-plate over aluminium foil before being immersed in liquid nitrogen for 5 min.

In situ preparation of the metastable CBZ–NCT form II (CBZ–NCT(II)) was achieved by heating CBZ–NCT(I) to 170 °C, cooling to −30 °C to form a glass, heating at 100 °C min^{-1} to 100 °C, holding isothermally for 5 min and cooling to −30 °C.

Results and discussion

Melting points

It is well known that the presence of trace levels of impurities in a sample can lower and broaden the melting endotherm (van't Hoff's law of freezing point depression), which is the basis for methods of purity determination by DSC [9]. However, even if a material is pure at the start of a DSC experiment, the reasonably long exposure to elevated temperatures during the measurement can cause chemical degradation of the sample in situ. Thus, by the time the sample reaches its melting temperature it is more impure than it was at the start of the experiment and the observed melting endotherm will appear at a lower temperature. The slower the heating rate the more the sample has time to degrade and the lower the melting point becomes. If this effect is not compensated for, then the melting temperature of the pure compound may not actually be determined. Riga [10] recently published a review of the use of fast-scan DSC to determine the stability through the melting point of a number of drugs classified in the United States Pharmacopoeia (USP) as stable, while Vanden Poel and Mathot [11] have discussed melting of calibration standards at fast heating rates.

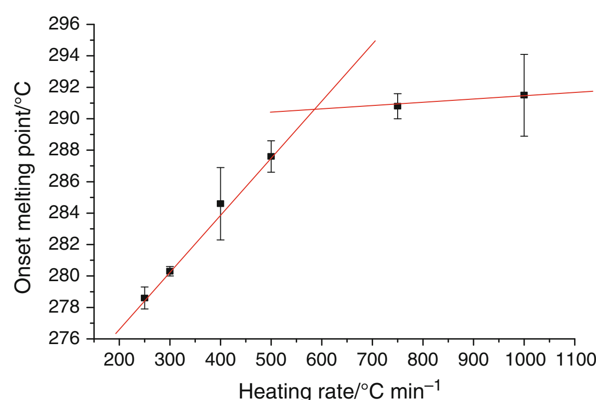

Fig. 1 Onset melting temperature as a function of heating rate for an experimental research compound (drug A)

It follows that one method to determine if the sample is degrading during the experiment is to conduct DSC experiments at two heating rates; if different melting temperatures are seen then the sample is likely degrading. If a compound is seen to degrade then it is also clear that degradation will be minimised with faster heating rates. The data in Fig. 1 show the (onset) melting temperatures as a function of heating rate for a research compound (drug A). At heating rates below 600 °C min^{-1} the melting temperature is seen to increase linearly with DSC heating rate. However, at heating rates above 600 °C min^{-1} the melting temperature becomes constant, at 291 °C. Thus, it can be assumed that the melting temperature of the pure compound is 291 °C.

Glass formation by quench-cooling

The starting point for many physical form studies is determining whether the sample can form a glass. Conventionally, drying techniques (such as spray- or freeze-drying) or quench-cooling by immersion in liquid nitrogen are used to prepare amorphous matrices. However, so long as the sample does not degrade upon melting DSC can be used to prepare glasses by melting the sample in the pan and then quench-cooling to a temperature below the T_g. Figure 2 shows the DSC curves for melted and then quench-cooled sulfapyridine in both Q2000 and RHC. It is apparent that in the Q2000 some proportion of the sample crystallises during the cooling phase, as evidenced by the crystallisation exotherm centred at 125 °C, while with RHC no crystallisation is seen and the sample forms a pure glass. The differences between the instruments are in

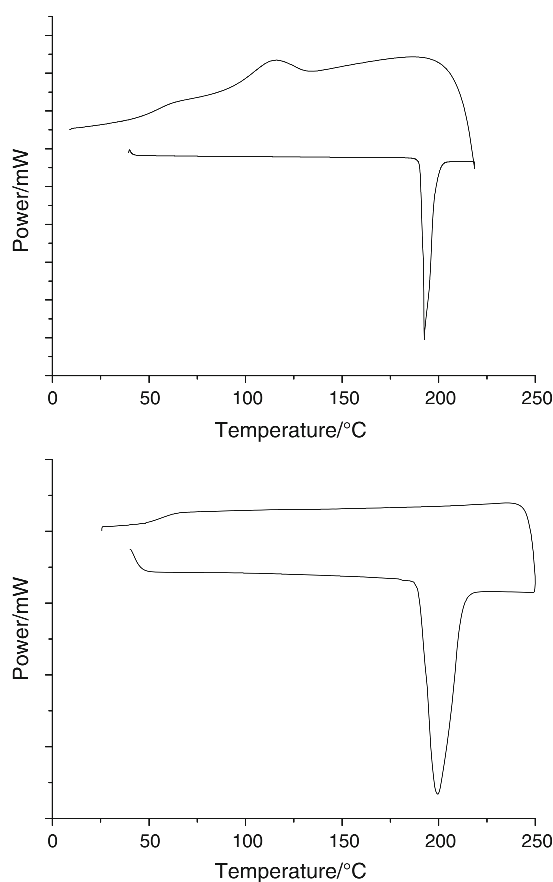

Fig. 2 DSC curves for melting and then quench-cooling sulfapyridine in Q2000 (*top*) and RHC (*bottom*)

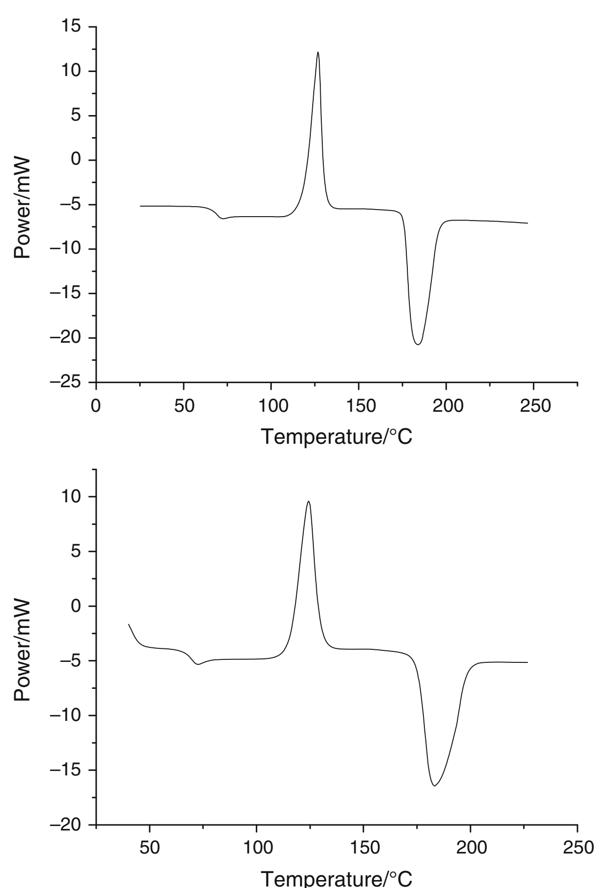

Fig. 3 Comparison of the DSC curves of sulfapyridine quench-cooled in RHC (*top*) and in liquid nitrogen (*bottom*)

sample mass and cold-stage temperature. The RHC instrument is able to cool at a faster rate and hence is able to force the sample into a totally glassy phase. Figure 3 shows that the DSC thermograms for sulfapyridine glasses formed in RHC and by immersion in liquid nitrogen are identical.

The use of RHC to study poor glass formers is shown by the data for D-mannitol in Fig. 4. D-Mannitol crystallises very rapidly and it is clear from the data in Fig. 4 that even during quench-cooling in RHC some of the sample crystallises. However, a proportion of the sample is forced into an amorphous form, as shown by the glass transition at ca. 10 °C; this value is comparable with that recorded by Ye and Byron [12], also using fast heating DSC, and by Kim et al. [13] who blended D-mannitol with a non-crystallising co-solvent.

Polymorph screening

Understanding the available polymorphic forms of a new drug candidate is an essential part of preformulation characterisation. Usually, the less thermodynamically stable the polymorphic form, the faster its dissolution rate (leading, as a consequence, to higher non-equilibrium solubility and better bioavailability). However, metastable polymorphs will convert (with time, temperature and/or humidity) to a more stable form, often with a consequential reduction in bioavailability. There is thus a compromise to be made between stability and bioavailability of the polymorphic form selected for development; making this decision depends upon knowing the polymorphic forms available.

McGregor et al. [3] used fast-scan DSC to characterise the polymorphs of carbamazepine. They noted that the enthalpy of fusion of form III had never been determined because of its rapid conversion to form I during heating. By heating the sample at 500 K min^{-1}, they were able to inhibit this change in form and hence determine a value for the enthalpy of fusion of form III. Further, they showed it was possible to quantify small proportions (ca. 1% w/w) of form III of the drug in a sample that was predominantly form I. Further work by the same author [14] resulted in determination of the enthalpies of fusion of two polymorphs of a Merck development compound, using scan rates greater than 400 K/min.

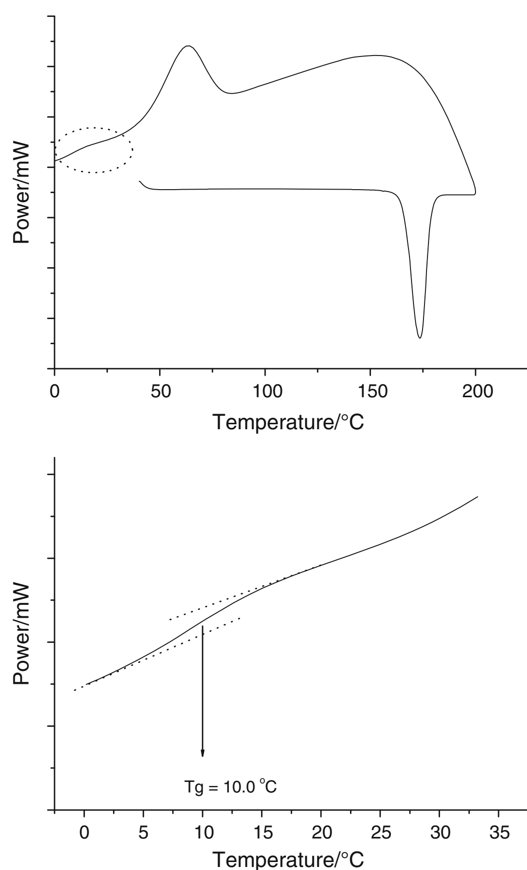

Fig. 4 DSC curve for melting and then quench-cooling mannitol in RHC with the glass transition *circled* (*top*) and an expanded graph showing the glass transition (*bottom*)

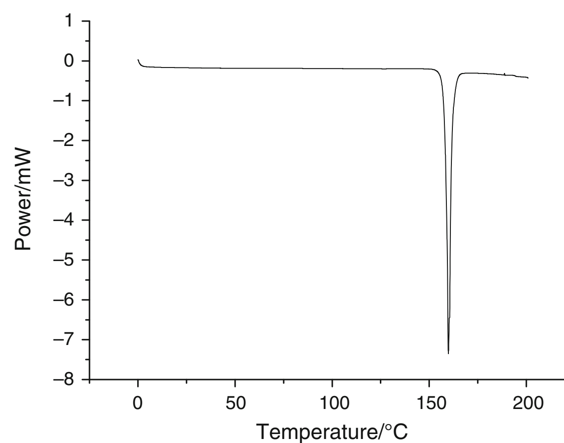

Fig. 5 DSC curve of CBZ–NCT(I) (heating rate 10 °C/min)

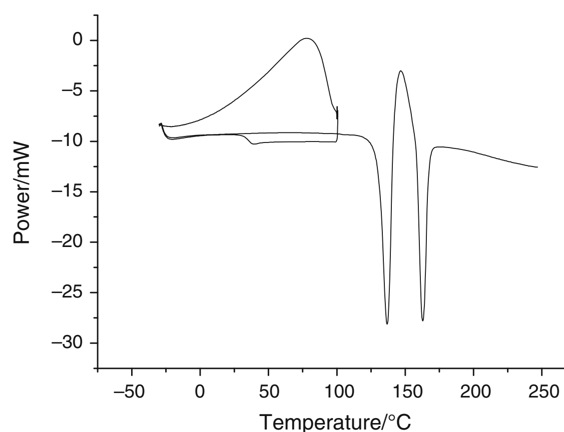

Fig. 6 DSC curve for the heat–cool–heat cycle for CBZ–NCT(II), showing a glass transition upon initial heating and the melt of form II, crystallisation to form I and melt of form I on the second heat

Fast-scan DSC also offers the potential for screening polymorphic forms if the compound can form a glass. The underlying experimental principle for formation of metastable forms is based on Ostwald's rule of isolation in stages, which supposes that when a material crystallizes from a non-equilibrium, high-energy state (such as a glass) it will do so via progression through any available lower energy states; the physical manifestation of this is that the sample will crystallize in a sequence, progressing through any metastable polymorphs to the stable crystalline form. Thus, a sample can be quench-cooled in the DSC to form a glass, then held at an elevated temperature to allow crystallisation to the (least stable) polymorphic form. When this metastable form is heated an endothermic melt should be seen, along with crystallisation to, and melting of, any other physical forms available.

The use of fast-scan DSC for isolation and characterisation of paracetamol form III has recently been discussed [15]. In this work, its use for investigation of co-crystals is discussed. Pharmaceutical co-crystals are defined as unit cells comprising at least two compounds both of which interact by hydrogen bonding and/or any other non-covalent bonds and which are solids at room temperature and pressure. The DSC curve of the stable, form I co-crystal of carbamazepine and nicatinamide (CBZ–NCT(I)) is given in Fig. 5, where a sharp melt at 159 °C is seen. Quench-cooling the sample post-melting resulted in a glass. The glass was heated to 100 °C and held isothermally for 5 min, resulting in crystallisation to the metastable CBZ–NCT(II), Fig. 6. Heating CBZ–NCT(II) resulted in a melting endotherm at 130 °C followed immediately by crystallisation to CBZ–NCT(I) and then melting of the stable form at 159 °C, Fig. 6.

As noted earlier, crystallisation, requiring movement of molecules to occur, is a kinetic event while melting, requiring the sample only to have a certain minimum amount of energy, is termed a thermodynamic event. If the heating rate is fast enough, such that the temperature at which crystallisation occurs becomes higher than the melting temperature of the next available polymorphic form, crystallisation will effectively be 'inhibited'. Thus,

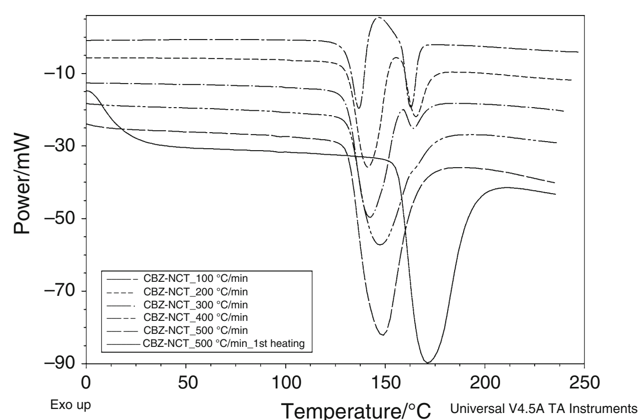

Fig. 7 DSC curves for CBZ–NCT(II) at heating rates from 100 to 500 °C min^{-1}. Also shown is the DSC curve for CBZ–NCT(I) for reference (*solid line*)

application of fast heating rates to CBZ–NCT(II) should enable isolation of its melt. DSC data for heating rates up to 500 °C min^{-1} are shown in Fig. 7.

At heating rates up to 400 °C min^{-1} the melt of CBZ–NCT(II) at 130 °C is always observed. Since the sample should consist entirely of the metastable form, if the heating rate used is sufficiently fast to inhibit crystallisation, no melt for the stable CBZ–NCT(I) should be seen (it is difficult to use the exothermic crystallisation peak as an indicator of crystallisation because the extent of crystallisation will fall with increasing heating rates and thus while it is still present it becomes masked by the melting endotherms). At a heating rate of 500 °C min^{-1}, however, no CBZ–NCT(I) melt was detectable, implying that this is the minimum heating rate required to inhibit conversion between polymorphs. This is consistent with two earlier studies of metastable polymorphs [3, 14] where heating rates of 400–500 °C min^{-1} were required to prevent conversion between forms. The single endotherm observed at a heating rate of 500 °C min^{-1} for CBZ–NCT(II) occurred with an onset temperature of 131.8 ± 0.3 °C and $\Delta_f H = 125.1 \pm 2.4$ J g^{-1}. Also shown in Fig. 7 is the melting endotherm for CBZ–NCT(I) at a heating rate of 500 °C min^{-1} (onset temperature 157.2 ± 0.3 °C, $\Delta_f H = 157.6 \pm 4.5$ J g^{-1}).

Summary

Fast DSC heating rates offer a lot of benefits; shorter run times (hence faster throughput), greater sensitivity, less potential for change in the sample during heating and the ability to inhibit kinetic phase transitions. The latter two benefits in particular increase the application of the technique to preformulation. Fast heating rates allow the investigation of samples that change during heating by reducing the amount of change that can occur during measurement. Here, it has been shown how fast heating rates allow determination of 'true' melting temperatures and characterisation of metastable polymorphic forms.

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