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USE OF ISOTHERMAL MICROCALORIMETRY IN PHARMACEUTICAL PREFORMULATION STUDIES Part II. Amorphous phase quantification in a predominantly crystalline phase

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Abstract

Low amounts of amorphous phase present in predominantly crystalline powders were quantified by using various analytical techniques with an emphasis on the use of Isothermal Perfusion Microcalorimetry. The amorphous phase was plasticized using ethanol vapor and enthalpy of re-crystallization of amorphous phase was used for generation of a calibration curve. Amorphous content as low as 5% was quantified using this technique. Although baseline noise was very low, additional processes occurring during re-crystallization confounded quantification of lower amorphous fractions.

Keywords: amorphous phase, differential scanning calorimetry, isothermal microcalorimetry, powder X-ray diffraction, quantification, water vapor sorption

Introduction

Processing steps such as compression, milling, lyophilization and spray drying can result in partial or complete amorphization of a crystalline active pharmaceutical ingredient (API) [1]. Amorphous phases are thermodynamically less stable relative to the crystalline material. Under favorable environmental conditions, amorphous phases tend to crystallize to the more stable crystalline phase [1]. Presence of an amorphous phase and its subsequent crystallization to a crystalline phase in a marketed product may have a profound impact on performance of the product [2]. Moreover, variations in amorphous content in various batches, often leads to batch-to-batch variability in active pharmaceutical ingredient or the formulation [3]. Such variability may have an impact on the physical and chemical stability and in vivo performance of the product. Hence it is important to assess the impact of various processing steps on generation of amorphous phases as well as detect and quantify the amount of amorphous phase generated. Such an assessment early on during de-

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velopment process enables development of rational processing conditions to control the generation of an amorphous phase and its negative impact on product variability and stability.

Milling and compression during solid oral dosage form development are the two most common processing steps where the active ingredient is subjected to high stress conditions. Subjection of the active ingredients to these two processing conditions may result in amorphization of the crystalline phase [4]. The amount of amorphous phase generated is dependent on the processing conditions as well as the nature of material that is being processed. A small amount of amorphous phase generated on the surface of particles might have a significant effect on the performance of a dosage form. A number of analytical techniques are available for detection and quantification of amorphous phases. The detection and quantification limits by each technique are variable and the choice of analytical technique is dependent on the desired sensitivity [5].

The objectives of this study were two fold. The primary objective was to explore the use of isothermal microcalorimetry (IMC) to quantify low levels of amorphous phase. A number of studies have focused on the use of IMC for quantification of amorphous compounds [6–8]. IMC consists of a small closed reaction vessel in contact with a heat sink such that the reaction vessel is at a constant temperature. A heat flow sensor located between the sample cell and heat sink measures the heat generated due to a reaction such as crystallization of an amorphous phase [3]. The thermal power $P = \mathrm{d}Q/\mathrm{d}t$ due to the reaction is recorded and monitored as a function of time [9].

The second objective was to compare and contrast IMC results with results obtained from techniques such as differential scanning calorimetry (DSC), powder X-ray diffraction (XRD) and water vapor sorption. The model compound used for this purpose was a new chemical entity under development. The material was crystalline with a melting point of about 154–155°C and had low water solubility. The compound was considered ideal for the study due to its existence as a single polymorph and lack of thermal decomposition upon melting.

Methods

Preparation of amorphous phase

Amorphous phase of the material was prepared by melting approximately 2 g of the material at 160°C. The material was held above the melt temperature isothermally for 10 min to make sure all the crystalline nuclei were destroyed. An ice bath was used to rapidly cool the melt. The glassy material was milled in a liquid nitrogen cooled cryo-mill (Spex 6700 Freezer Mill). The milled material was stored in a glass vial in a dry box. The material was used as a 100% amorphous standard.

Preparation of crystalline phase

Crystalline material was size reduced using a Spex 6700 liquid nitrogen cooled cryo-mill. The material was milled for 10 s and milled material was stored over etha-

nol vapors to allow for crystallization of any amorphous phase. The material was used as a 100% crystalline standard.

Preparation of mixtures

Mixtures of amorphous and crystalline phases were prepared by mixing 100% amorphous with 100% crystalline phase in various proportions. Mixtures were prepared by geometric dilution method.

Isothermal microcalorimetry

A thermal activity monitor (TAM) equipped with a perfusion cell, containing either ethanol or water in saturator chambers, was used. Dry nitrogen was used as perfusion gas. All experiments were performed at 25°C. Approximately 5 to 7 mg of the material was weighed into a perfusion cell, and lowered into the measurement position in four steps. The experimental program involved exposure of the material to a relative vapor pressure of 0.8 (p/p_0) for 180 min followed by exposure to dry gas for 180 min, with repetition of the cycle once.

Powder X-ray diffractometry

Powder diffractions patterns were obtained by using a Bruker D5000 X-ray powder diffractometer. Materials were exposed to CuK_α radiation (45 kV×40 mA) between an angular range of 3–40°2θ, with a step size of 0.02°2θ and counts were accumulated for 1 s at each step. For quantification integrated counts of the peak at 24.2°2θ were used.

Differential scanning calorimetry

Materials were analyzed using a Seiko robotic DSC (RDC 220). Sealed aluminum pans were used for analysis, with sample mass of approximately 5 mg. A dry nitrogen purge was used with materials scanned from 20 to 170°C at a rate of 5°C min^{-1}.

Water vapor sorption

A dynamic vapor sorption instrument DVS-1 (SMS) was used for this purpose. Approximately 25 mg of the sample was exposed initially to 0% RH for 180 min, after which the sample was exposed to 80% for 1500 min. The % mass change at 100, 500 and 1000 min was used for estimation of percent amorphous in the sample.

Results and discussion

The X-ray powder diffractometric patterns of 100% crystalline and 100% amorphous standard materials are shown in Fig. 1. No crystalline peaks were evident in the pattern of amorphous material and HPLC analysis confirmed no degradation during preparation of the amorphous phase. For quantification of the material by XRD anal-

ysis the peak at 24.4° 2θ was used for obtaining total integrated area counts. Water vapor sorption analysis revealed low affinity of the material to water due to its hydrophobicity. Exposure of 100% amorphous material to 80% RH resulted in a mass gain of about 1.4% whereas the crystalline standard adsorbed negligible (< 0.1%) amount of water. Samples containing amorphous fractions in between those limits presented intermediate levels of water uptake in a fairly linear fashion and were used to obtain a quantitative estimate of the amorphous phase (Fig. 2).

Differential scanning calorimetry (Fig. 3) of the amorphous phase revealed a glass transition temperature of approximately 60°C (onset). The glass transition event overlapped with an enthalpic relaxation endotherm, followed by re-crystallization of the amorphous phase at 73°C (onset). Analysis of the amorphous phase by XRD at various temperatures confirmed re-crystallization of amorphous phase (overlaid XRD patterns in Fig. 3). The re-crystallized phase was similar to the original crystalline phase (Fig. 3). The enthalpy of re-crystallization of amorphous phase was proportional to the amount present and hence was used for its quantification in mixtures with the crystalline material.

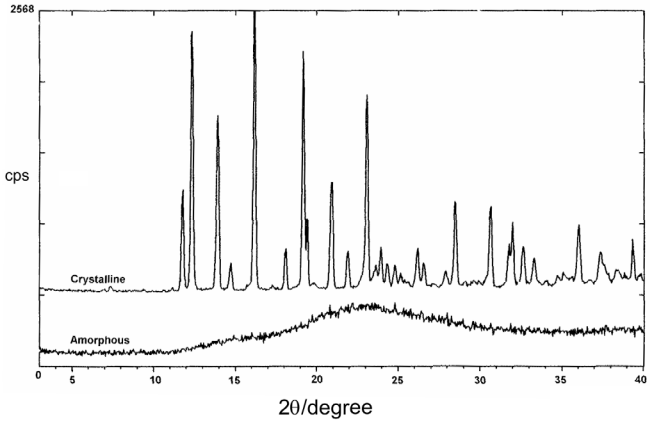

Fig. 1 Powder diffraction patterns of 100% crystalline and 100% amorphous forms

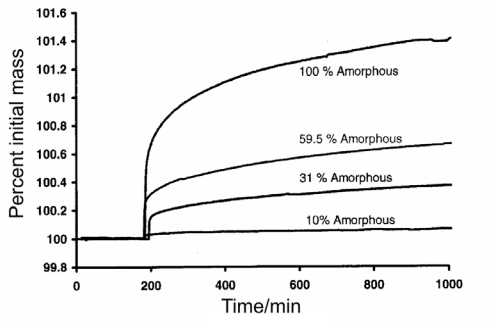

Fig. 2 Moisture uptake at 80% RH by samples containing varying fractions of amorphous material

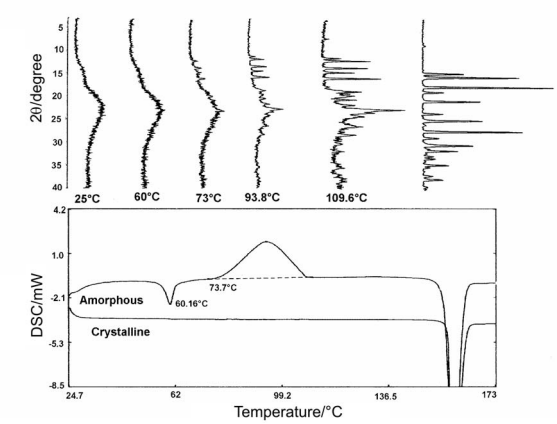

Fig. 3 DSC curve of 100% amorphous phase overlaid with XRD patterns of the amorphous phase obtained at temperatures corresponding to thermal events in the DSC curve

Similarly for quantification by IMC, the enthalpy of crystallization of the amorphous phase was utilized. However, the isothermal nature of IMC experiments did not allow for ramping up of temperature to re-crystallization temperatures. Alternately, increasing molecular mobility by exposing the amorphous phase to water or solvent vapor results in its re-crystallization. Absorbed water is a good plasticizing agent lowering the glass transition temperature of an amorphous phase leading to its crystallization at temperatures lower than crystallization of dry material [8]. However, due to hydrophobic nature of the compound, absorbed water even at high relative humidity conditions did not sufficiently plasticize the amorphous phase to result in its re-crystallization at IMC experimental temperatures. Therefore it was necessary to use a solvent vapor, which plasticizes the amorphous phase sufficiently to result in its re-crystallization. The model compound was soluble in ethanol and for that reason ethanol was chosen as a plasticizing agent of the amorphous phase [10]. The amorphous phase was exposed to ethanol vapors in a glass chamber and the material was analyzed by XRD. The diffraction patterns after 20 min and 12 h exposure are shown in Fig. 4. It was apparent that crystallization of the amorphous phase had occurred rapidly within 20 min of exposure to ethanol vapors with essentially no change after 12 h of exposure. The rapid crystallization of amorphous phase, on exposure to ethanol vapors, made ethanol an ideal choice for perfusion IMC experiments.

The power-time plot for approximately 32% amorphous material in a mixture with the crystalline phase in a perfusion IMC is shown in Fig. 5. The heat flow curve can be essentially divided into three different sections. The initial 0–2 h section consisted of an exotherm, which was apparent soon after exposure to 0.8 p/p_0 of ethanol. The exotherm was attributed to heats associated with sorption, crystallization and expulsion of the sorbed ethanol from the re-crystallized phase. The phase (2–4 h) consisted of an endothermic peak, which occurred after switching to 100% dry nitrogen gas. The endotherm was due to desorption of ethanol associated with the completely

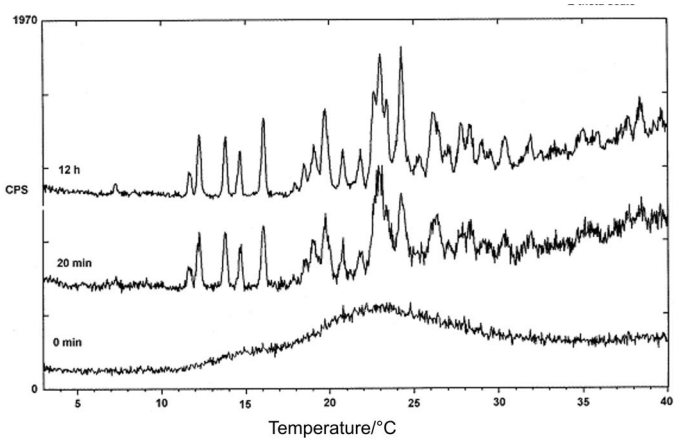

Fig. 4 Diffraction patterns of amorphous phase after exposure to ethanol vapors for 20 min and 12 h

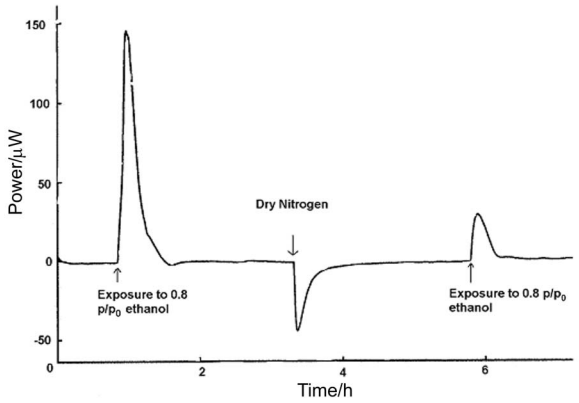

Fig. 5 Microcalorimetric response of 32.6% amorphous phase in an amorphous crystalline phases mixture up on exposure to ethanol vapors in a perfusion cell

crystalline phase. The third phase (4–6 h) consisted of an exothermic response, which occurred soon after exposure to 0.8 p/p_0 due to adsorption of ethanol by the crystalline phase [8]. The heat of desorption obtained from the endotherm was approximately equal to the heat of adsorption obtained from the third exotherm [11].

The first exotherm is a sum of heats associated with sorption of ethanol by the crystalline and amorphous phases and re-crystallization of the amorphous phase. The heat obtained from the endotherm (phase II) was subtracted from the first exotherm to obtain the value associated with the re-crystallization of the amorphous phase. The heat of crystallization obtained was proportional to the amount of amorphous phase and was used for quantification purposes. The calibration curve obtained by exposure of mixtures of amorphous and crystalline phases in various proportions is shown in Fig. 6. The calibra-

tion curve was linear across the range studied (5–100% amorphous) and can be used for quantification of amorphous phase in unknown samples. Perfusion IMC was used successfully to quantify amorphous content as low as ~5% in unknown samples. It was not possible to get a good measurement below this level because the crystallization exotherm is confounded with other events such as absorption and heat generated by expulsion of ethanol from the re-crystallized phase. If the crystallization is delayed and separated from sorption, as observed with compounds like lactose, much lower levels of amorphous content may be quantified by this method. Although this method was considered adequate for the intended development project, it could be possible to further refine it to achieve much lower limits of quantification.

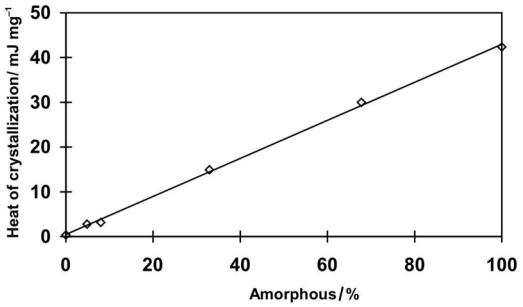

Fig. 6 Calibration curve for quantification of amorphous phase by isothermal microcalorimetry

The quantification sensitivity of amorphous phase was compared with the sensitivity obtained with other techniques such as water vapor sorption, DSC, and XRD. DSC analysis resulted in a linear calibration curve from 5 to 100% amorphous phase. Water sorption analysis provided similar results but the experimental procedure was much longer and tedious. X-ray diffraction methods were sensitive enough to quantify samples with an amorphous content greater than 10%.

Conclusions

Crystallization induced by exposure to ethanol vapor using a perfusion cell of an isothermal microcalorimeter was used to quantify low amounts of amorphous phase present in a predominantly crystalline hydrophobic material. Water has often been used as a plasticizer in such experiments and this case study presents the possibility of using non-aqueous plasticizer for quantification of amorphous phase. By varying experimental conditions, it is possible to develop methods with very low limits of detection and quantification of amorphous phase using non-aqueous solvent vapor as plasticizer.

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