Application Note

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Quantitation of Amorphous Content in Crystalline API via the Relative Heat Capacity at the Glass Transition Temperature

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Unintentional amorphization of crystalline active pharmaceutical ingredients (API) is a common phenomenon observed in the pharmaceutical manufacturing process. Amorphization could be induced by various manufacturing stresses experienced by the API, such as drying, milling, or wet/dry granulation. This application note outlines the development of a modulated differential scanning calorimetry (mDSC) technique to quantify the amorphous content in a crystalline API, compound X. While amorphous content quantitation via the melt endotherm relative to the fully crystalline API is typically used to quantify the percent crystallinity in the sample, this was not feasible due to the decomposition of compound X at the melt. Instead, the delta heat capacity at the glass transition temperature ($\Delta C_{p,Tg}$) relative to the neat amorphous API (referred to as "relative heat capacity") was used. Several samples with varying ratios of amorphous to crystalline material were prepared, and the respective $\Delta C_{p,Tg}$ were measured. The calculated amorphous content in each physical mixture obtained from the relative $\Delta C_{p,Tg}$ was in good agreement with the prepared amorphous ratio. The study demonstrated that the relative $\Delta C_{p,Tg}$, which only requires measurements of the $\Delta C_{p,Tg}$ of the neat amorphous material and the sample of interest, was suitable for amorphous quantitation in crystalline API without the need for a linearity curve.

Keywords: *Modulated Differential Scanning Calorimetry, Amorphous, Crystalline, Quantitation*

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Introduction

The crystalline form is generally the preferred solid form of an active pharmaceutical ingredient (API) from the early development to the final dosage form because of its desirable properties, such as excellent rejection of impurities, physical and chemical stability, and handleability [1-4]. However, API development involves many processes, such as drying, milling, and wet/dry granulation, that could induce stress upon the crystalline form and potentially generate an unintentional amorphous form. Since the amorphous form generally exhibits greater physical/chemical instability, enhanced dissolution rates, lower bulk density, and greater hygroscopicity, the potential amorphization must be monitored at each drug development stage to ensure consistent performance and product behavior. Therefore, this necessitates the development of analytical methods to detect and quantify the amorphous content in a crystalline API.

While various analytical techniques, such as X-ray/electron diffraction, spectroscopy, calorimetry, and physisorption, have been utilized in the literature to quantify the amorphous content in a crystalline material [5-10], the focus of this article is the use of differential scanning calorimetry (DSC). DSC offers several advantages compared to other techniques, such as high specificity between the amorphous and crystalline forms, a minimal amount of sample, straightforward sample preparation, and relatively simple data analysis (no chemometrics or elaborate processing/calculation needed). For a system containing a mixture of amorphous and crystalline materials, a typical DSC thermogram would exhibit the melt endotherm of the crystalline material, a re-crystallization exotherm from the amorphous form (if the crystallization kinetics are rapid relative to the DSC heating rate), and a reversible glass transition temperature (T_g) of the amorphous form [10-16]. All of these measurable, thermal events could potentially quantify the amorphous content in the sample.

In this study, a model proprietary compound (compound X), was used in the DSC method feasibility study to quantify the amorphous content in the sample. Crystalline and amorphous compound X were provided and analyzed to evaluate the most appropriate method for amorphous quantitation. Following the method development, the limit of detection (LoD) and limit of quantitation (LoQ) were estimated.

Instrument and Methods

Materials

Crystalline and amorphous compound X were provided by Gilead and used as-received.

Physical mixtures preparation

Physical mixtures were prepared by separately weighing crystalline and amorphous compound X, combining the materials in a mortar, and gently mixing using a pestle.

Differential Scanning Calorimetry (DSC)

DSC and modulated DSC (mDSC) analyses were performed using a TA Instruments DSC 2500 instrument. The instrument temperature calibration was performed using indium. Approximately 11 to 21 mg of the sample was weighed into an aluminum pan (a hermetically sealed pan was used in the initial evaluation of the crystalline material, but vented crimped pans were used in the remainder of the study) and loaded into the instrument. An empty pan of the same configuration was loaded into the reference position. The DSC cell was kept under a nitrogen purge of about 50 mL per minute during each analysis. In general, the mDSC was performed by equilibrating the sample at -50 °C for 10 minutes, followed by heating from -50 °C to 200 °C at 2 °C/min with a modulation amplitude of 1 °C and a period of 60 seconds. The instrument was controlled, and the data were analyzed using TA Instruments' TRIOS 5.7.2.101.

Results and Discussion

Crystalline Compound X

Since thermal events at the T_g are generally small, a comparison of the melt endotherm of a given sample to that of a neat crystalline material is generally utilized to determine the percent crystallinity of the sample (and, thus, estimate the amorphous content). However, previous thermogravimetric analysis (TGA) and DSC thermograms of crystalline compound X (Figure 1) showed that the material decomposes nearly concurrently with the melt, as indicated by the appearance of an exotherm following the reported melt endotherm. An attempt to deconvolute the melt endotherm from the decomposition event was made using a slower heating rate of 2 °C/min (the heating rate was 10 °C/min in Figure 1) in a hermetically sealed pan (blue thermogram in Figure 2). This attempt was unsuccessful as the decomposition exotherm remained unresolved from the melt endotherm. An additional small endotherm at 166 °C (blue thermogram in Figure 2), which was not detectable in the initial thermogram (Figure 1), was observed in the

attempt with the hermetically sealed pan. This event was suspected to be caused by the DSC pan deformation that was visually observed after the analysis was completed. Further analysis with a vented crimped pan (green thermogram in Figure 2) did not show the small endotherm around 165 °C, indicating that this event was likely due to the DSC pan deformation (or at least this thermal event was irreproducible). In summary, it was concluded that amorphous quantitation could not be performed using the relative melt endotherm because of the subsequent decomposition event.

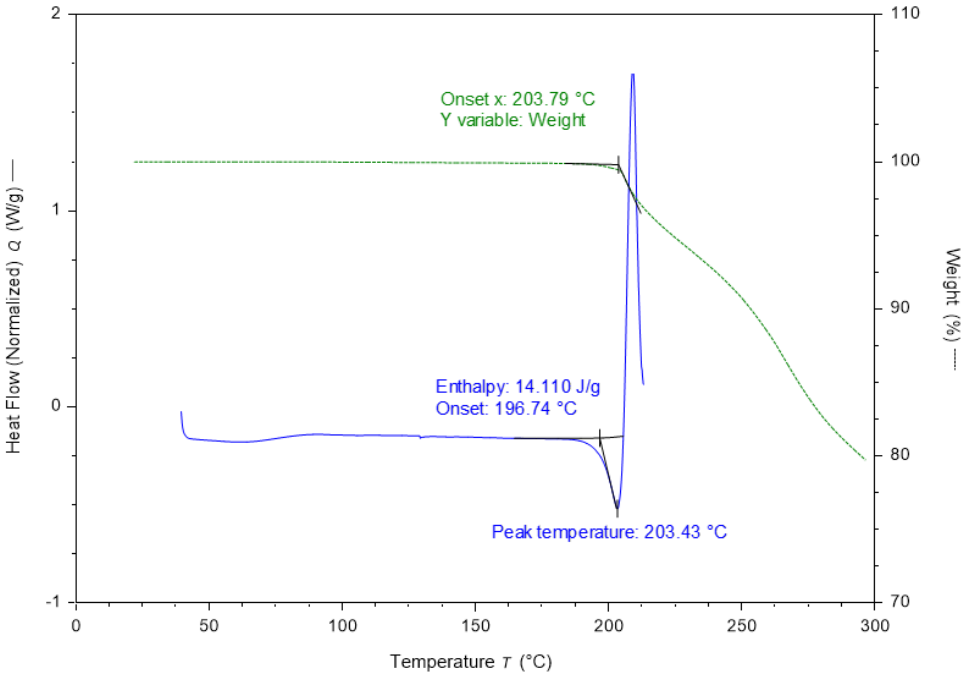

Figure 1. Historical TGA (blue) and DSC (green) thermograms of crystalline compound X.

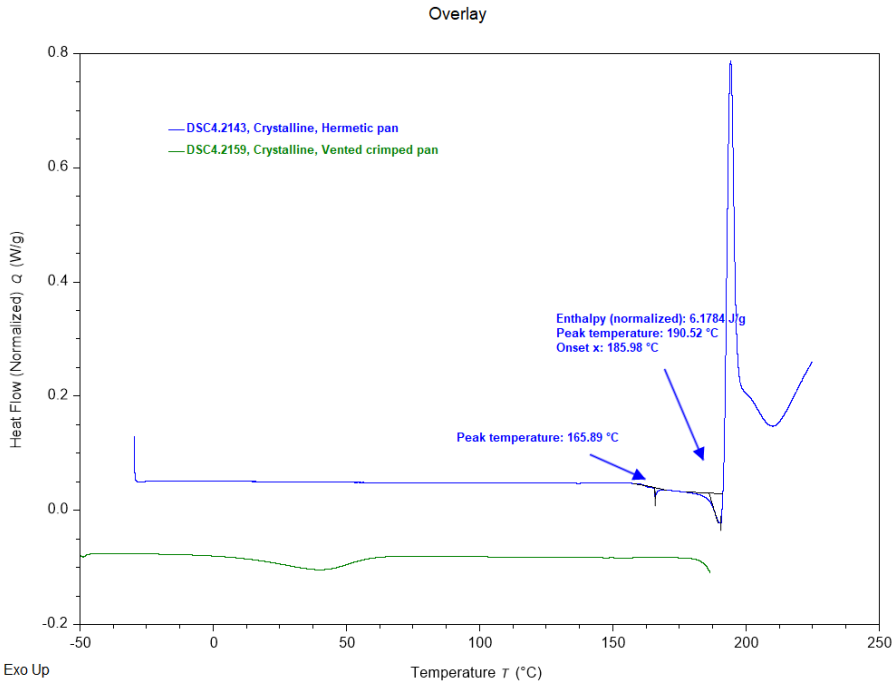

Figure 2. DSC thermograms of crystalline compound X using a hermetically sealed pan (blue) and a vented crimped pan (green).

Amorphous Compound X

Since amorphous materials are known to be hygroscopic (and the presence of water could plasticize and lower the material's T_g), an initial heat-cool-heat DSC analysis was performed to obtain a "dry" glass transition temperature (T_g) of the neat amorphous compound X. A broad endotherm from ~0 to 120 °C, which is characteristic of typical surface water evaporation, was observed in the first heating cycle (data not shown). Once the material was dried, the sample was cooled, and modulated DSC (mDSC) analysis was performed on the second heating cycle (blue thermogram in Figure 3). The result showed that the neat amorphous compound X has a T_g of 155 °C. Additional duplicate T_g analyses were conducted. Since the T_g occurs at a higher temperature than the endpoint of the surface water evaporation (~120 °C), the duplicate analyses were performed with only one heating cycle mDSC. The results (green and red thermograms in Figure 3) showed that a repeatable T_g of 154-155 °C (RSD of 0.36%) was obtained. In addition, an RSD of 6% (Table 1) was obtained for the $\Delta C_{p,Tg}$ calculated from the three analyses of

neat amorphous compound X. The results suggest that $\Delta C_{p,Tg}$ could be viable for quantifying the amorphous content in a given compound X.

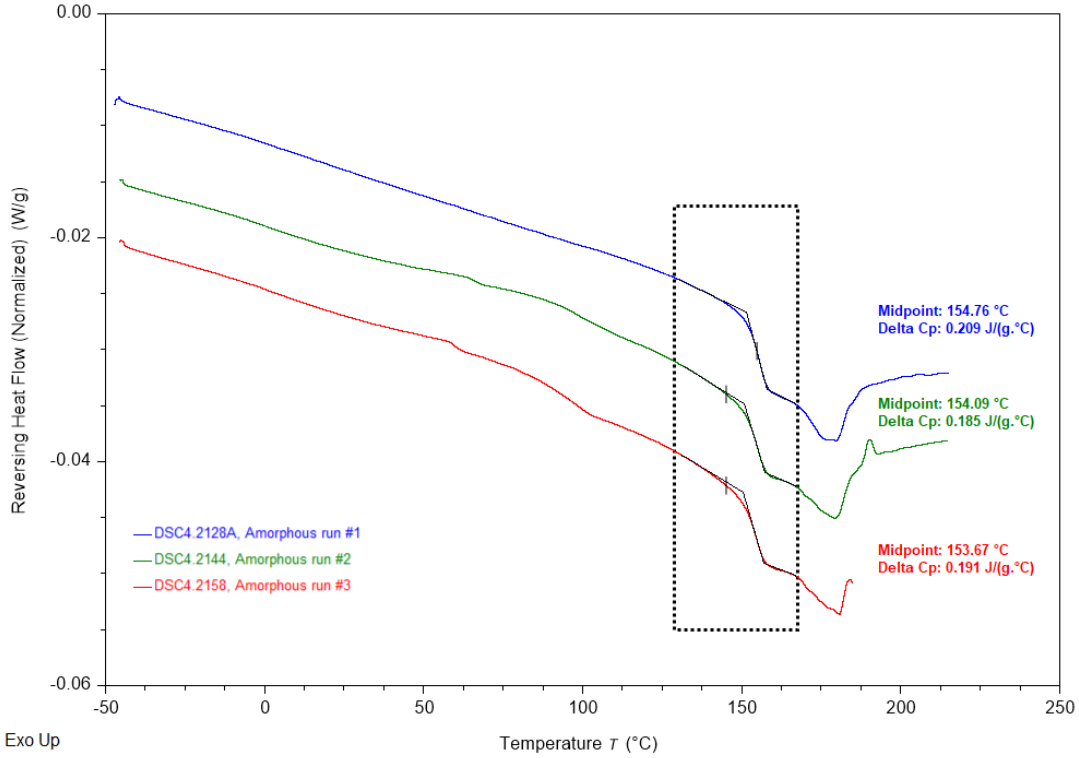

Figure 3. Reversing heat flow from the mDSC thermograms of amorphous compound X. The area in the dotted box represents the region in which T_g was measured.

Table 1. Repeatability of T_g data of neat amorphous compound X.

File	$\Delta C_{p,Tg}$ (J/(g·°C)	Mid-point T_g (°C)
DSC4.2128	0.209	154.76
DSC4.2144	0.185	154.09
DSC4.2158	0.191	153.67
Average	0.195	154.17
SD	0.012	0.55
RSD	6.41%	0.36%

Amorphous Content in Amorphous/Crystalline Compound X Mixtures

To verify the suitability of the relative $\Delta C_{p,Tg}$ (i.e., ratio of found $\Delta C_{p,Tg}$ to that of neat amorphous) for amorphous quantitation, several physical mixtures containing varying amounts, i.e., between 5 and 40% of amorphous in crystalline compound X, were prepared and analyzed. Figure 4 shows the T_g and $\Delta C_{p,Tg}$ for neat crystalline, amorphous/crystalline physical mixtures, and neat amorphous compound X. As expected, the observed T_g event amplitude progressively increased with increasing amorphous content. The ratio of the $\Delta C_{p,Tg}$ for each physical mixture to the average $\Delta C_{p,Tg}$ of the neat amorphous (i.e., 0.195 J/(g·°C) obtained in the previous section) was then calculated to estimate the amorphous content in these physical mixtures (Table 2). Overall, an agreement between the prepared and calculated amorphous content within 2% was achieved. An 8% difference was observed for the 20% w/w amorphous physical mixture. The high error in the 20% w/w amorphous sample could have been due to additional amorphization during the mixing/grinding in preparing the physical mixtures (though hand-grinding was performed as gently as possible). Another potential source of error could be sampling heterogeneity since not all of the prepared physical mixture amount was analyzed. Furthermore, because the DSC domain size detection limit is 20-30 nm [17], partial amorphization in the crystalline material (or conversely partial crystallization in the amorphous material) that is lower than this domain size would not be observed and potentially contribute additional errors to the calculation.

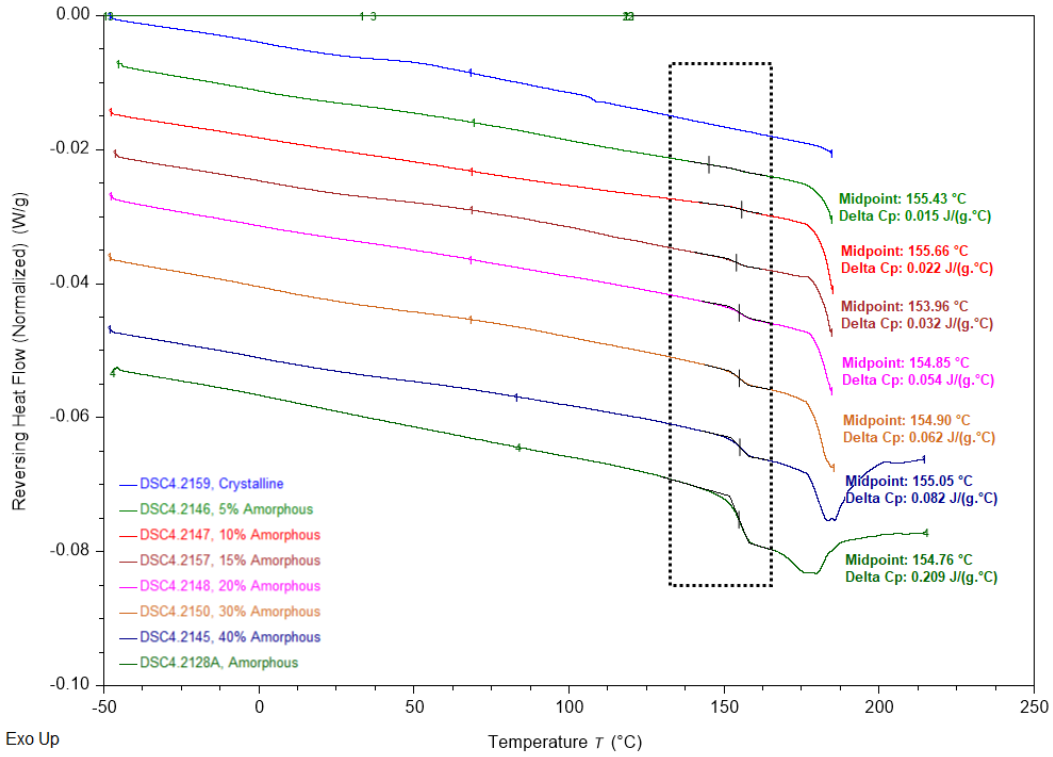

Figure 4. Reversing heat flow from the mDSC thermograms of neat crystalline, amorphous/crystalline mixtures, and neat amorphous compound X. The area in the dotted box represents the region in which T_g was measured.

Table 2. Sample information and results of amorphous/crystalline compound X physical mixtures.

Sample Name	File	Amorphous mass (mg)	Crystaline mass (mg)	Prepared Amorphous Content (% w/w)	$\Delta C_{p,Tg}$ (J/(g·°C)	Calculated % Amorphous from DSC
5% amorphous	DSC4.2146	3.0615	57.0505	5.0930	0.015	7.692
10% amorphous	DSC4.2147	6.0625	54.0775	10.081	0.022	11.282
15% amorphous	DSC4.2157	8.9805	50.9370	14.988	0.032	16.410
20% amorphous	DSC4.2148	11.9735	48.1380	19.9188	0.054	27.692
30% amorphous	DSC4.2150	18.1005	41.9355	30.1494	0.062	31.795
40% amorphous	DSC4.2145	24.0190	35.9060	40.0817	0.082	42.051

Furthermore, the LoD and LoQ were estimated by calculating the standard error of the response ($\Delta C_{p,Tg}$) from the slope [18], assuming the data generated herein could be used as a calibration curve. Figure 5 shows the plot of $\Delta C_{p,Tg}$ as a function of the prepared percent amorphous in the physical mixture. The estimated LoD and LoQ, as shown in Table 3, were approximately 8% and 25% w/w amorphous, respectively.

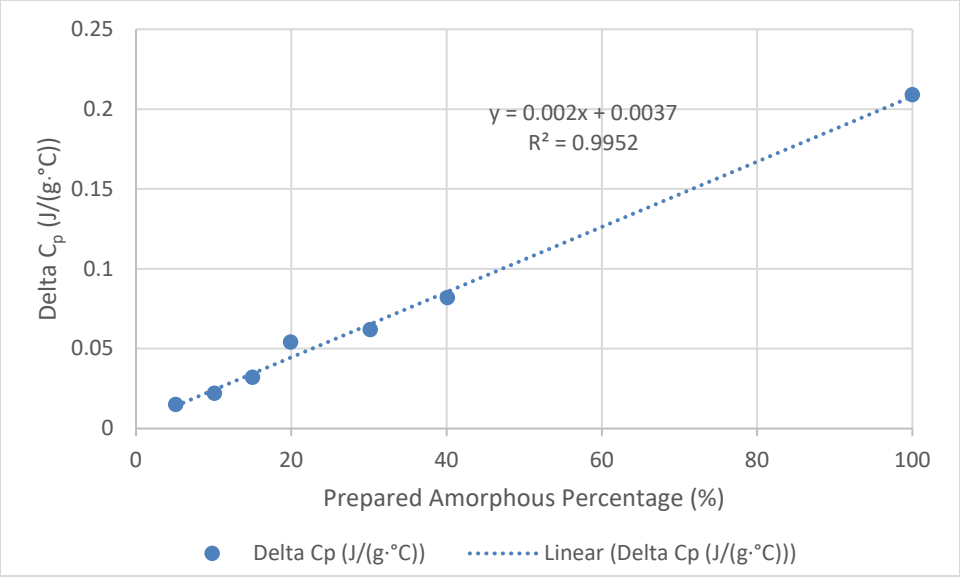

Figure 5. Delta Cp (at T_g) as a function of the prepared amorphous content.

Table 3. LoD and LoQ estimates for quantitation of amorphous compound X.

Performance Criteria	Values
Sigma	0.0051
Slope	0.0020
LoD	8.2% w/w amorphous
LoQ	24.8% w/w amorphous

Further Considerations

While DSC has been historically used as a technique to quantify amorphous content in crystalline materials, relative $\Delta C_{p,Tg}$ (i.e., $\Delta C_{p,Tg}$ of the sample relative to that of the neat amorphous) has not been widely utilized. To the authors' knowledge, while Gunaseelan et al. have demonstrated the linear relationship between $\Delta C_{p,Tg}$ and amorphous content (between 1-10% w/w amorphous) in a given matrix [15], the use of the relative $\Delta C_{p,Tg}$ for amorphous quantitation has not been published to date. The study conducted herein underscores the utility of the method, which requires only two $\Delta C_{p,Tg}$ measurements (for

the sample of interest and neat amorphous) to estimate the amorphous content in a given sample, without the need for a linearity curve or other elaborate data processing. In cases where neat amorphous material is unavailable or too unstable to prepare ex situ, it can be generated in situ in the DSC pan via a quench-cool of the melt. Furthermore, while 8% LoD is relatively high, inclusion of additional physical mixtures at lower amorphous contents in the calibration standard would likely provide a much lower LoD. Indeed, an LoD as low as 0.5-1% has been reported via DSC [11, 13, 16].

Conclusion

A DSC method was developed to demonstrate the feasibility of quantifying amorphous content in a crystalline material. Compound X was used as a model compound. The DSC results of the crystalline material showed that decomposition occurred nearly simultaneously with the melt; therefore, the melt endotherm could not be used for quantifying the amorphous content. On the other hand, a consistent T_g was observed in several analyses of the amorphous material, and upon further evaluation, it was determined that a simple ratio of the $\Delta C_{p,Tg}$ from a given sample to that of the neat amorphous material ("relative $\Delta C_{p,Tg}$") could be used to estimate the amorphous content in the sample. This study demonstrates that the relative $\Delta C_{p,Tg}$, which only requires the measurements of the $\Delta C_{p,Tg}$ of the neat amorphous material and the sample of interest, could be used for rapid quantitation of amorphous content in a given sample without the need for a linearity curve or elaborate data processing.

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