Use of Heat of Adsorption to Quantify Amorphous Content in Milled Pharmaceutical Powders

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Abstract

Isothermal calorimetry operated in gas perfusion mode (IGPC) is often used to quantify the amorphous content of pharmaceutical powders. Typically, the calibration line is constructed using the heat of crystallisation as the sample is exposed to high levels of a plasticising vapour. However, since the physical form to which the amorphous fraction crystallises may be dependent on the presence of any crystalline seed, the calibration line is often seen to be non-linear, especially as the amorphous content of the sample approaches 100% w/w. Redesigning the experiment so that the calibration line is constructed with the heat of adsorption is an alternative approach that, because it is not dependent upon crystallisation to a physical form should ameliorate this problem. The two methods are compared for a model compound, salbutamol sulphate, which forms either a hydrate or an anhydrate depending on the amorphous content. The heat of adsorption method was linear between amorphous contents of 0-100% w/w and resulted in a detection limit of 0.3% w/w and a quantification limit of 0.92% w/w. The heat of crystallisation method was linear only between amorphous contents of 0-80% w/w and resulted in a detection limit of 1.7% w/w and a quantification limit of 5.28% w/w. Thus, the use of heat of adsorption is shown to be a better method for quantifying amorphous contents to better than 1% w/w.

Key words

Amorphous content quantification; isothermal microcalorimetry; gas perfusion; micronised pharmaceuticals; salbutamol sulphate

Introduction

Pharmaceutical powders are frequently milled to reduce their particle size distribution and improve their physical properties (for instance, to enhance blending, dissolution or pulmonary delivery). One unintended consequence of milling is that the high energies imparted to the sample can result in generation of crystal defects and amorphous regions, particularly on surfaces. This mechanical activation increases surface energy and means that milled powders are frequently highly cohesive/adhesive (Brodka-Pfeiffer et al, 2003). Additionally, their properties may change with time, temperature and/or relative humidity (RH) as any amorphous material present relaxes and/or crystallises with time. The amorphous fraction generated by milling may often be small (around 1% w/w) but occurring primarily on surfaces its impact on the properties of the bulk powder can be significant. This is especially true for dry-powder inhaler formulations, where aerosol performance is contingent upon forces of adhesion and cohesion (Sharma et al, 2013), but may also affect flow and blending of bulk powders. As a consequence it is important that methods to quantify small (<1% w/w) amorphous contents are available.

Of the many techniques that show potential for such an assay, isothermal gas perfusion calorimetry (IGPC) is particularly useful (Gaisford, 2012) because it requires a relatively small (typically 10-50 mg) mass of sample and can be applied to any pharmaceutical powder if a suitable plasticising vapour is available. The plasticiser (often water or ethanol) is perfused over the sample in an inert carrier gas (nitrogen); as the amorphous material absorbs the plasticiser, its glass transition temperature (Tg) is reduced, eventually resulting in crystallisation (and so an exothermic heat). The heat of crystallisation is quantitatively proportional to the mass of amorphous material; reference to a calibration line prepared by blending appropriate mass ratios of crystalline and amorphous material allows quantitative analysis. The quantification limit (QL) is often better than 1% w/w because the heat of crystallisation is usually substantial.

The method has some limitations however. Because the crystalline fraction of the sample will act as a seed, as the amorphous content of the sample increases, the number of crystalline seed particles reduces (and in the case of the 100% amorphous sample, there is no seed). One manifestation of this is that the calibration line is often seen to deviate from linearity as the amorphous content approaches 100% w/w (O'Neill and Gaisford, 2011). Another is that some samples may undergo solid-state conversion post-crystallisation (Gaisford et al, 2010), making determination of the heat of crystallisation difficult.

An alternative approach is to measure the heat of wetting of the sample before and after crystallisation. While the sample is partially amorphous, water will be both adsorbed and absorbed. Once crystalline, only water adsorption will be measured and so the difference gives the heat of absorption. This approach was first used by Mackin et al (2002) in an assay for amorphous content using dynamic vapour sorption, but has not been previously applied to IGPC. Hence, the specific aim of this work is to explore the feasibility of heat of absorption measurements as a method of quantifying small amorphous contents and to compare its use with (conventional) heat of crystallisation measurements. Salbutamol sulphate (SS) was selected as a model compound because it shows complex crystallisation.

Materials and Methods

Crystalline SS was supplied by Micron Technologies Ltd (UK) and was used as received. Amorphous SS was prepared by spray-drying an aqueous solution (10% w/v) using a B-290 mini spray-dryer (Buchi Labortechnik Ag). The following settings were used; inlet temperature 140 ^{o}C, outlet temperature 60 ^{o}C, aspirator 100%, pump 20%. The spray-dried sample was seen to be amorphous by X-ray powder diffraction (data not shown). SS hydrate was prepared by holding SS on a watch glass in a humidity chamber (100% RH) for 24h.

Experiments were performed with a gas perfusion accessory housed in a 2277 thermal activity monitor (TAM, TA Instruments Ltd, UK) operated at 25 ^{o}C. Samples (10 ± 0.1 mg) were weighed directly into the stainless steel ampoule (5 mL volume). Partially amorphous samples were prepared by dry-mixing appropriate mass ratios of crystalline and spray-dried SS.

The gas perfusion accessory splits the incoming gas into two streams; one is routed directly to the sample ampoule (the ‘dry’ line) and the other passes through two humidifying chambers, becoming saturated with (in this case) water, prior to entering the sample ampoule (the ‘wet’ line). Mass flow controllers adjust the proportional flow rates of the gas along the two lines in order to produce any desired RH in the sample ampoule. The total flow rate was 150 mL h^{-1}. A 9-step humidity program was used; 0-30-0-95-0-30-0-95-0% RH. The selection of 30 and 95% RH is arbitrary, but dynamic vapour sorption experiments (data not shown) showed that 30% RH was below cRH and 95% RH was above cRH (the critical relative humidity, above which crystallisation occurs). The time period of each step varied depending on the amorphous content of the sample, ranging from 1.5 to 10 h. The amplifier range was 3000 μW and data were recorded (1 point every 30 s) using the dedicated software

package Digitam 4.1. The TAM was calibrated prior to use with the electrical substitution method. Data were analysed with Origin 8.5 (Originlab Corp, USA). Note that while the TAM plots exothermic events as a positive power, by convention exothermic changes in heat are quoted as negative values.

Differential scanning calorimetry (DSC) measurements were made with a Q2000 DSC (TA Instruments Ltd, UK). Samples (5-10 mg) were weighed into hermetic Tzero aluminium pans. An empty pan, matched to the weight of the sample pan, was used as a reference. Samples were heated from 0 to 250 ^{o}C at 20 ^{o}C/min. The cell constant and enthalpy calibrations were performed with indium (Certified Reference Material LGC2601, Batch E1, LGC, London, T_m = 156.61^{o}C, $\Delta_f H$ = 28.70 J/g) in accordance with the manufacturer's instructions. The measured values were always in excellent agreement with those of the reference material (T_m ± 0.03^{o}C, $\Delta_f H$ ± 0.1 J/g). Nitrogen (50 mL min^{-1}) was used as a purge gas and data were analysed with Universal Analysis 2000.

All experiments were conducted in triplicate. Results are reported as mean ± standard deviation.

Results and discussion

Figure 1 shows the power time data for both crystalline and amorphous SS as a function of RH. A series of exothermic (positive) and endothermic (negative) powers are seen as the RH of the environment surrounding the sample is varied. Periods during which the sample is held under a dry gas are labeled $D_{1\text{-}4}$ (noting that the initial period D_1 represents drying of the sample following loading into the calorimeter) and periods during which the sample is exposed to raised humidity (either 30 or 95%) are labeled $W_{1\text{-}4}$. Although the data look complex, interpretation is relatively straightforward, remembering that the area under the curve for each period gives the net change in heat (in mJ).

Starting with crystalline SS, the only process occurring should be adsorption (to a greater extent during W_2 and W_4) during periods $W_{1\text{-}4}$ and desorption during $D_{2\text{-}4}$ (the changes in heat should be equal and opposite to those of adsorption). Table 1 shows these data and these trends are seen.

Conversely, the amorphous sample shows a much larger power during initial exposure to 30% (W_1) and 95% RH (W_2). This is because several additional processes are occurring during these periods. During W_1 water is adsorbed onto all surfaces while the amorphous fraction absorbs water (but the Tg is not lowered sufficiently to cause crystallisation). During W_2 sufficient water is absorbed that the

sample crystallises, resulting in a complex series of exothermic events. Assignment of the processes that give rise to each exotherm is discussed below. It suffices here to note that the response of the amorphous sample is significantly different from that of the crystalline sample and that is the basis of the sensitivity of the method. After W_2 the sample, now crystalline, only adsorbs water during periods of exposure to raised humidity (W_3 and W_4) and consequently the heats are seen to reduce (Table 1). Indeed, the heats of adsorption are actually smaller than that of the crystalline reference sample, which is probably because the process of recrystallisation causes particle fusion and so a decrease in total surface area.

The complexity of the data increases for partially amorphous SS samples. Figure 2 compares the response of the amorphous reference sample and a 75% w/w amorphous blend during W_2. It is clear that while the same trends are seen during the exothermic region, the processes occur faster in the partially amorphous sample and are followed by a pronounced endotherm. The increase in rate is probably due to the presence of nucleation sites on the crystalline seed particles. The endotherm is seen for all partially amorphous samples but is never present in the amorphous reference sample. The implication is that the endothermic process is in some way associated with the presence of crystalline seed material.

To determine the physical form of the sample during exposure to 95% RH, samples were withdrawn from the TAM at various time intervals and analysed with DSC, Figure 3. Crystalline SS melts at 202 ^{o}C. SS hydrate shows more complex behaviour, with an endotherm at 111 ^{o}C corresponding to water loss followed by melt of the dehydrate at 214 ^{o}C. When removed from the TAM following initial exposure to 95% RH, the amorphous SS reference material shows the characteristic endotherms of the hydrate. Conversely, any partially amorphous SS sample, when removed from the TAM at the same point, shows behaviour characteristic of the anhydrate.

Thus, the exothermic events seen during W_2 may be ascribed to water adsorption, absorption and crystallisation to the hydrate. It is clear from the shape of the data that multiple stages occur during this crystallisation phase. One interpretation is that the outer layers of the sample absorb water and crystallise first. This results in expulsion of the absorbed water, some of which is pushed into the next layer of the sample causing crystallisation. This cascade effect is repeated until the sample is fully crystalline (Darcy and Buckton, 1998). The small, sharp exotherm signifies complete crystallisation, Figure 2. These events are seen in all SS samples with amorphous content. The additional endotherm seen when crystalline seed material is present can be ascribed to loss of water of hydration as the sample converts slowly to the anhydrate. As the higher melting physical form, the anhydrate is the

thermodynamically more stable form but presumably its formation in the humid environment of the TAM requires a seed.

Calibration lines for amorphous content may be prepared from the data in Table 1, using either the heat of adsorption (W_1-W_3), Figure 4, or the heat of crystallisation (W_2-W_4), Figure 5. Since water adsorption at 30% RH does not cause physical change in the sample, the calibration line is seen to be linear over the full range of amorphous content. The same is not true for the calibration line constructed using the heat of crystallisation because, as noted earlier, the sample crystallises either to the hydrate or the anhydrate, and so significant deviation from linearity is seen as the amorphous content approaches 100% w/w.

Detection and quantification limits can be calculated from these data in several ways. Here the definitions in ICH guideline Q2 (Validation of Analytical Procedures) were used;

$$\text{Detection limit (DL)} = \frac{3.3\sigma}{S} \qquad \text{Equation 1}$$

$$\text{Quantification Limit (QL)} = \frac{10\sigma}{S} \qquad \text{Equation 2}$$

Where σ is the standard deviation of the blank (i.e. heat of the crystalline sample) and *S* is the slope of the calibration line. For the heat of adsorption method this gives; DL = 3.3 x 0.8 / 8.68 = 0.3% w/w, QL = 10 x 0.8 / 8.68 = 0.92% w/w and for the heat of crystallisation method; DL = 3.3 x 4.5 / 8.52 = 1.7% w/w, QL = 10 x 4.5 / 8.52 = 5.28% w/w (calculated over the region 0-80% w/w amorphous content). The improved DL and QL values for measurements based on heats of adsorption arise almost entirely because of the reduced error of the blank measurement. Although not expressly explored here, any RH below cRH could be used. 30% RH was an arbitrary selection which matched the previous DVS method (Mackin et al, 2002); an increase to a higher value should result in a proportionately large heat of adsorption and so a further improvement in DL and QL. Furthermore, the calibration line constructed from heat of crystallization data can only be used up to 80% w/w amorphous content.

Conclusion

IGPC is an excellent technique for quantifying amorphous contents in pharmaceutical powders but the calibration line may be seen to be non-linear in the case that the sample crystallises to different physical forms depending on the presence or absence of seed material. Reconfiguring the experiment so that the heat of adsorption is measured produces a linear response over the full range of amorphous content. In addition, because only one process contributes to the power, the standard deviation of the measurement is reduced, improving the DL and QL values.

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Period	Crystalline SS AUC (mJ)	Amorphous SS AUC (mJ)
W_1	-63.6 ± 1.4	-989.0 ± 30.3
D_2	64.0 ± 1.9	916.3 ± 10.2
W_2	-270.4 ± 6.7	-3806.9 ± 20.4
D_3	250.2 ± 5.9	232.2 ± 20.5
W_3	-49.9 ± 0.8	-28.1 ± 1.1
D_4	47.5 ± 0.8	29.5 ± 1.1
W_4	-212.4 ± 2.8	-163.9 ± 17.3

Table 1. Area under curve (AUC) data for the various dry and elevated RH periods for the crystalline and amorphous SS data shown in Figure 1

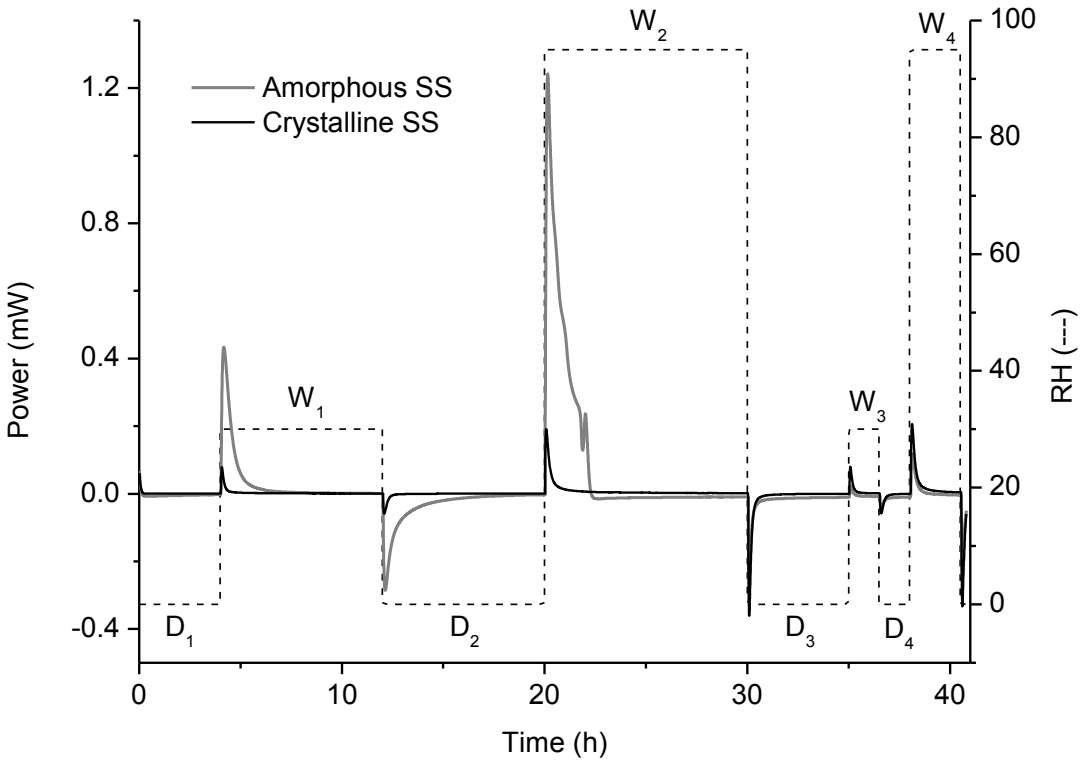
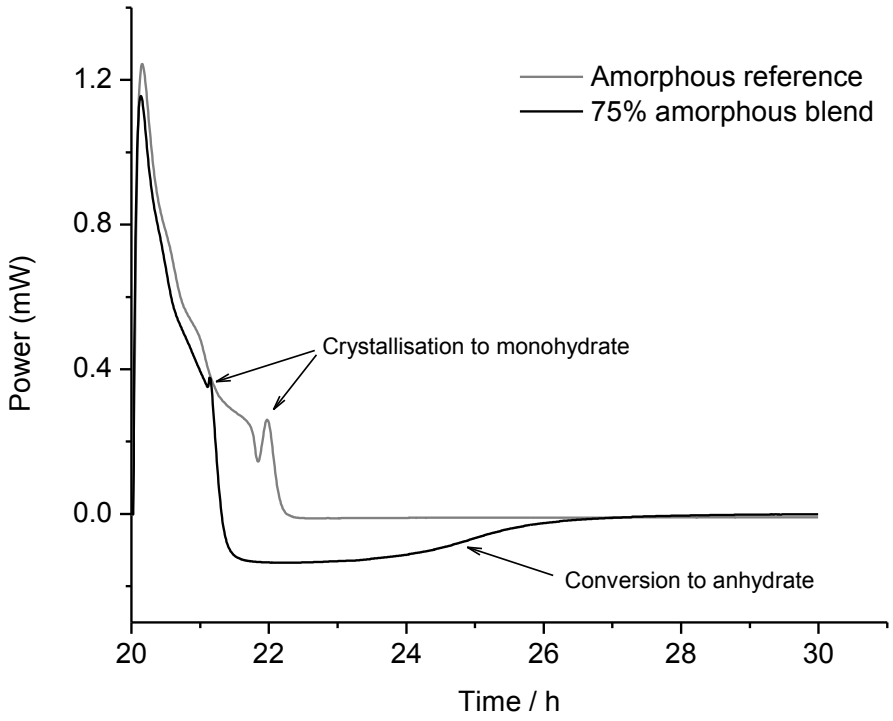


Figure 1. Power-time data for amorphous (grey line) and crystalline (black line) SS as a function of RH



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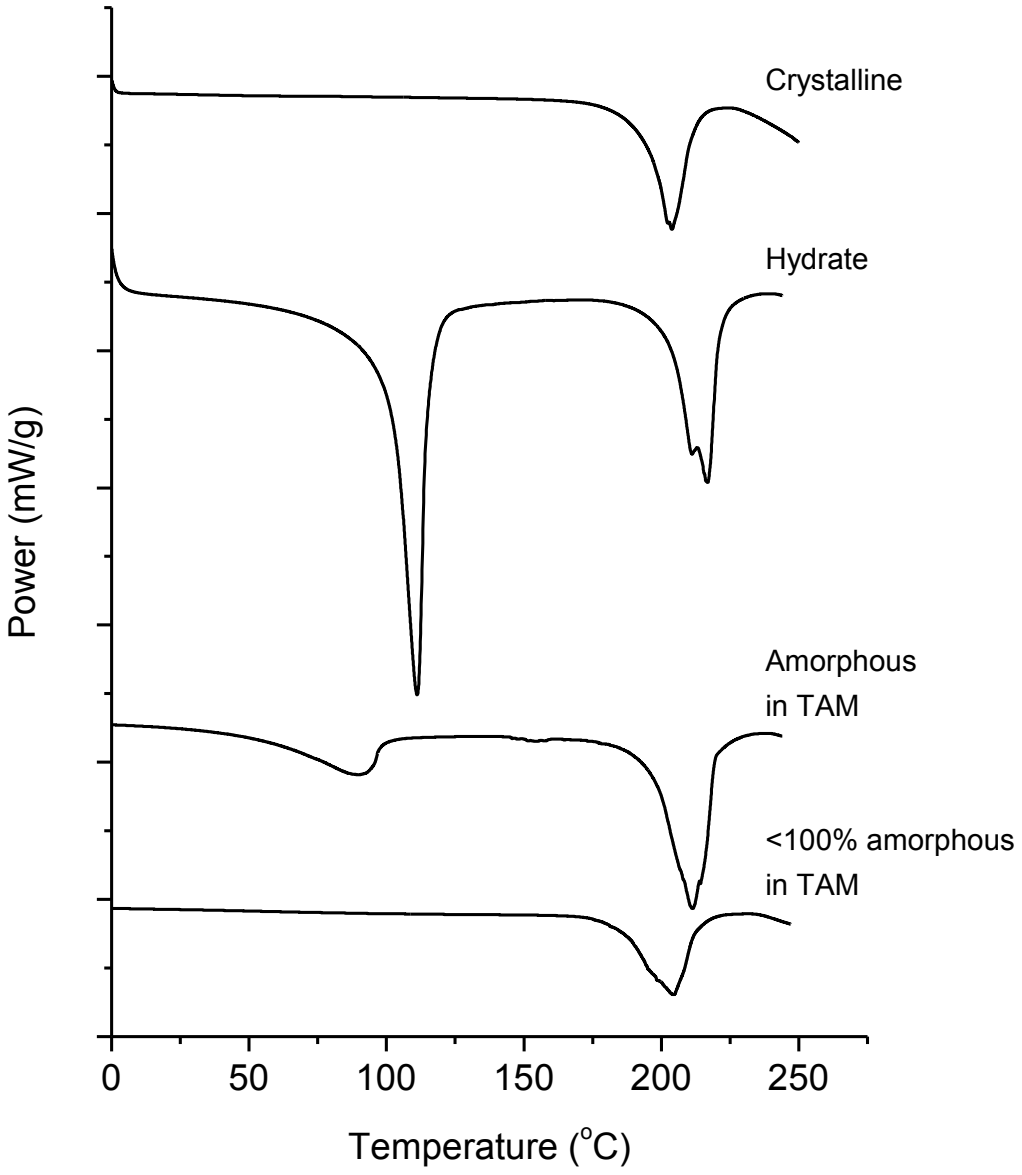
270 **Figure 2. Power-time data for amorphous (grey line) and partially amorphous**

271 **(75% w/w, black line) SS upon initial exposure to 95% RH**



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273 **Figure 3. DSC data for crystalline SS, SS hydrate and two samples removed**

274 **from the calorimeter following exposure to 95% RH**

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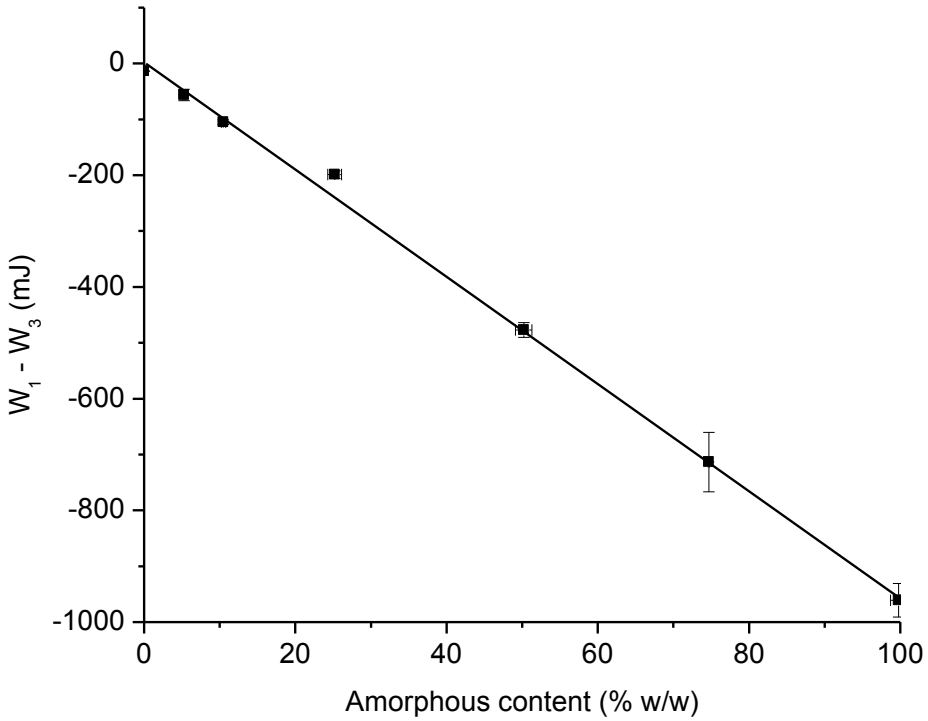

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278 **Figure 4. Calibration line for amorphous content determined from the heat of**
279 **adsorption**
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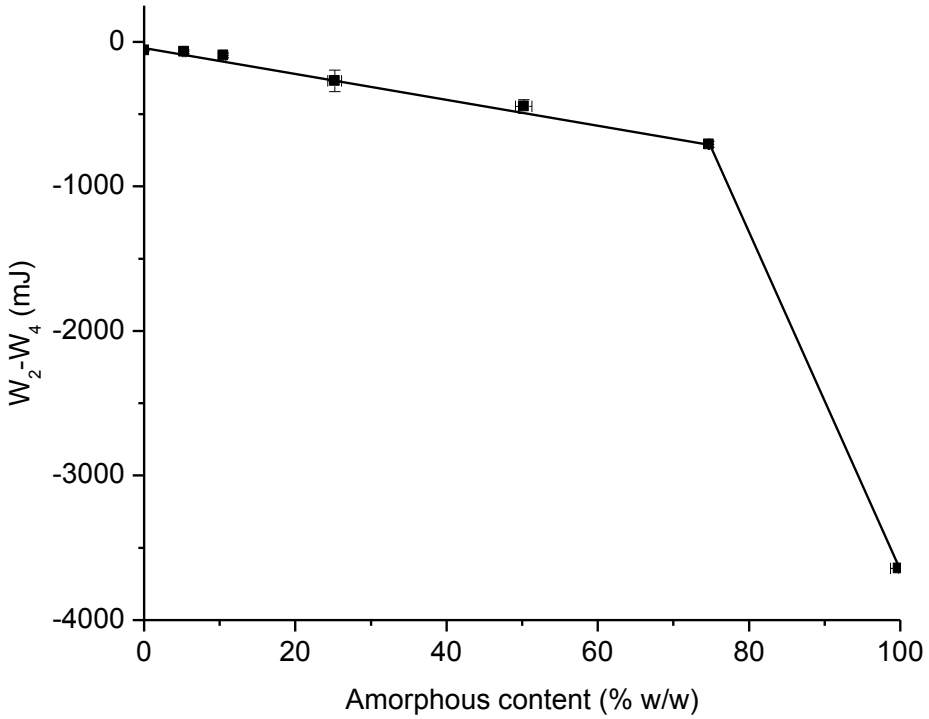

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282 **Figure 5. Calibration line for amorphous content determined from the heat of**
283 **crystallisation**

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