

EVALUATION OF CALORIMETRIC AND GRAVIMETRIC METHODS TO QUANTIFY THE AMORPHOUS CONTENT OF DESFERAL

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Abstract

The amorphous content of different Desferal samples was quantified by recording its recrystallization using isothermal microcalorimetry in a static as well as in a flowing humid atmosphere. Furthermore water vapor sorption gravimetry was performed for the same purpose. These analytical methods result in a quantitative signal directly dependent on the content of the amorphous phase (recrystallization, water sorption equilibrium). Their sensitivity allows the detection of amorphous content below 1%. Methods are compared and advantages and disadvantages are discussed.

Keywords: amorphous phases, Desferal, gravimetry, isothermal microcalorimetry, recrystallization, relative humidity, water vapor sorption

Introduction

The presence of amorphous parts in crystalline pharmaceutical substances can have considerable impact on the bioavailability, the processability and stability of the final drug product. Typical amorphous solids have physical properties (heat capacity, vapor pressure, adsorption behavior e.g. enhanced water sorption, mechanical and rheological properties, solubility) quite different from those of their corresponding crystalline forms. Amorphous states are characterized by a higher internal energy and specific volume which enhances the dissolution behavior and bioavailability. The increased molecular mobility of the amorphous state above the glass transition temperature can decrease the chemical stability.

However, amorphous phases tend to recrystallize into the thermodynamic more stable crystalline state during processing or storage. Routes to induce amorphous phases are e.g. supercooling from melt, external mechanical stress due to milling, grinding or compaction and pharmaceutical processing as freeze or spray drying [1].

Most of the common techniques employed in the characterization of solid state properties of organic materials as X-ray powder diffraction, thermal analysis, gravimetry, IR-, Raman and dielectric spectroscopy, solid state NMR, microscopy

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and solubility, viscoelastic or density measurements are usually employed for characterization and quantification of the amorphous phase [2–21].

The lower quantification limit of the amorphous phase present in a mainly crystalline material achieved with common techniques is reported to be generally not better than 10%. However, if the amorphous phase exists predominantly at the particle surface, its level can be well below 1%. This is often observed in material which was subjected to high external forces encountered e.g. by micronisation of drug substances for use in inhalation products.

The amorphous state is characterized by an experimentally observable glass transition temperature (T_g). Below the glass transition the amorphous solid exists in a kinetically frozen glassy state which prevents it from recrystallization into a thermodynamic stable crystalline state usually in time scales sufficient for pharmaceutical relevant life times of years [1, 22]. Above the glass transition the molecules in the amorphous phase regain their mobility and behave like a highly viscous liquid. As the amorphous state is thermodynamically unstable in respect to the crystalline state, the likelihood for recrystallization is high and increases with temperature.

The use of isothermal microcalorimetric techniques has considerably improved the limits for quantification of the amorphous content [2–12]. The high sensitivity of modern microcalorimeters and the usually high enthalpy of recrystallization allows in ideal cases the quantification of amorphous contents below 1%.

In general water acts as a plastifier to the amorphous phase, thus, reducing the glass transition temperature. Therefore, the presence of water, e.g. due to increased relative humidity promotes the recrystallization of the amorphous phase. This effect is employed to initiate recrystallization.

The increased water sorption properties of the amorphous phase allows the use of gravimetric methods to determine the amorphous content by measuring the weight change due to the release of absorbed water during recrystallization of the amorphous phase [13–15].

In the present work we compared the quantification of the amorphous content of a Desferal methanesulfonate sample, an iron chelator agent, by isothermal microcalorimetry with static and dynamic humidity control as well as water vapor sorption gravimetry.

Experimental

Materials

Desferal methanesulfonate (Desferoxamine mesylate, $M=657 \text{ g mol}^{-1}$) was used as a lyophilized product. This material was predominately crystalline with varying amorphous contents ranging from 0 to approx. 30 wt%. Practically pure crystalline material was obtained by recrystallizing the amorphous parts at 40°C and 75% RH for at least 65 h. Its water content amounts to 6% according to thermogravimetric measurement.

Completely amorphous material was obtained by rapid supercooling the sample from the melt. The amorphous material shows at room temperature a viscous and sticky behavior which did not allow to mix it homogeneously with crystalline mate-

rial. Therefore we used a partially amorphous sample (referred as sample X) as starting material for the further study.

DSC

DSC measurements were performed using a low temperature DSC-7 system (Perkin Elmer, USA). Samples of typically 3–10 mg were sealed in closed aluminum pans and were measured using a heating rate of 10 K min⁻¹.

Isothermal microcalorimetry

Isothermal microcalorimetric measurements were performed using the Thermal Activity Monitor TAM 2277 (Thermometric AB, Sweden). Measurements were carried out at 40°C. Samples between 5 and 350 mg were placed in 3 ml glass vials and closed with a rubber sealing and aluminum caps. The atmosphere in the vial was adjusted to 75% RH by adding a small glass tube containing a saturated NaCl-solution. The glass vials, sealings and caps as well as the salt solution were preconditioned at the measuring temperature for at least 24 h. Measurements were performed for 65.0 h. The reaction enthalpy was calculated by integrating the whole obtained curve of the heat flow as function of time.

Sorption gravimetry (dynamic vapor sorption; DVS)

Water vapor sorption measurements were performed using the sorption balance DVS-1 (Surface Measurements Systems, UK). Relative humidities are programme-controlled adjustable between 0 and 95% at variable temperatures between 5 and 80°C.

The change of the sample mass by the loss and uptake of water due to recrystallization and sorption processes in a humidity of 75% RH at 40°C was determined. The equilibrium value was related to the dry mass of the sample obtained in a dry nitrogen flow at the end of each experiment.

Results

Differential scanning calorimetry

A typical DSC curve of a lyophilized sample of Desferal shows a glass transition at around 0°C followed by a melting endotherm at ca. 110°C, a recrystallization and a second melting peak at 145°C (Fig. 1). When cooling the sample from the melt, only a glass transition is observable in a second heating run. The DSC results show that Desferal can exist in at least two crystalline modifications (Figs 1 and 2).

Isothermal microcalorimetry

The amorphous content of the starting material (sample X) was measured using isothermal microcalorimetry. Figure 3 shows the microcalorimetric measured heat flow of Desferal samples with different amorphous contents. The exothermic peak is

mainly caused by recrystallization of the amorphous phase. The measured enthalpy of recrystallization at 40°C of pure amorphous Desferal is $76 \pm 2 \text{ J g}^{-1}$ related to the waterfree sample. This result is lower than the melting enthalpy of the crystalline material of 84 J g^{-1} at the fusion temperature of 97°C (waterfree). The microcalorimetric measured enthalpy of the partially amorphous material, sample X, was found to be $23.2 \pm 2.0 \text{ J g}^{-1}$. Assuming a linear dependence between recrystallization enthalpy and amorphous content over the whole range, this results in an amorphous content of sample X of $29 \pm 3\%$ (Table 1).

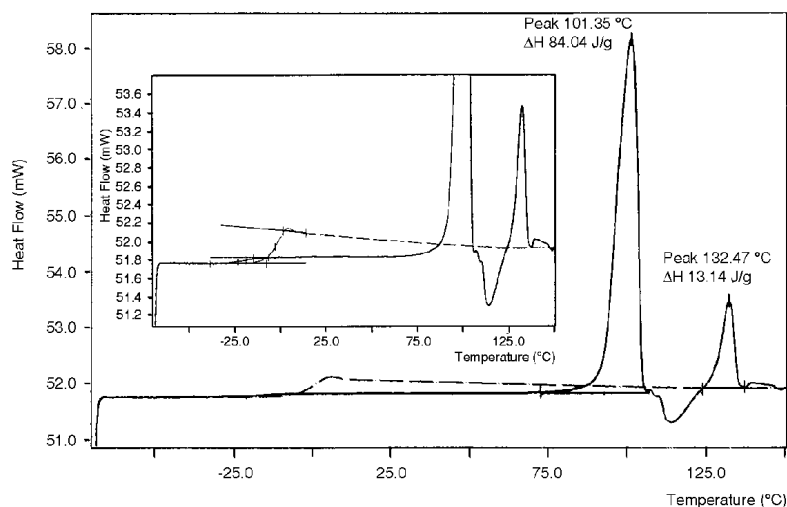


Fig. 1 DSC curve of partially (1. Scan) and completely (2. Scan) amorphous Desferal (heating rate 10 K min^{-1} ; sample X)

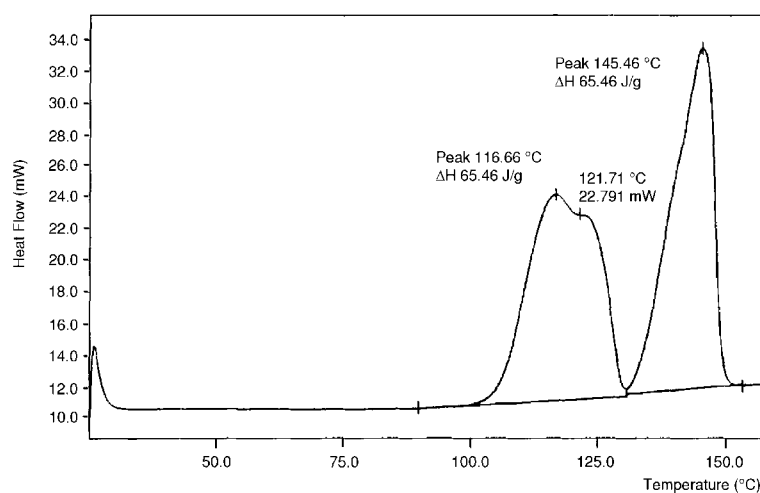


Fig. 2 DSC curve of Desferal polymorphs. Sample recrystallized by TAM at 40°C and 75% RH (sample dried in waterfree nitrogen, heating rate 10 K min^{-1})

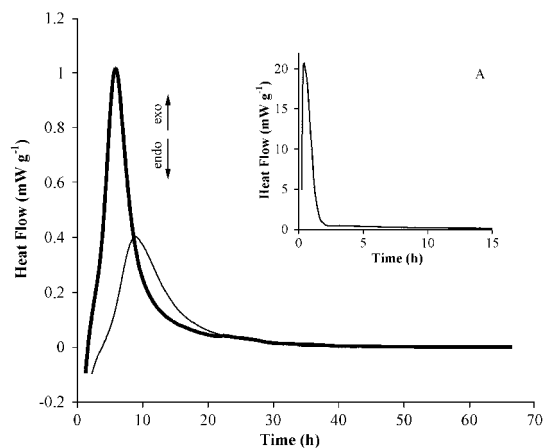


Fig. 3 Heat flow of recrystallization of Desferal samples with different amorphous contents recorded by isothermal microcalorimetry at 40°C and 75% RH. **—** sample X; **—** 50% sample X+50% crystalline; **—** crystalline material. A – Heat flow of recrystallization of completely amorphous Desferal

Table 1 Microcalorimetric results of the amorphous content of Desferal

Sample	$\Delta H_{\text{recryst}}/\text{J g}^{-1}$	Amorphous content/wt%	Repetitions
Crystalline	2.0 ± 0.8	0	6
X	23 ± 2	29 ± 3	3
Amorphous	76 ± 2	100	4

To obtain samples with varying amorphous contents (30–1%), mixtures of sample X with crystalline material were prepared and investigated. As shown in Fig. 4, the linear relationship between the exothermic heat of recrystallization and the amorphous content calculated using the above result for sample X could be confirmed within the given error limits. The regression line intersects approximately the ordinate at the origin, as expected.

During most experiments a strong exothermic effect during the first hour was observed, which is believed to be related to sorption and equilibration processes. This first peak (not shown in Fig. 3) was excluded from the calculation of the reaction heat. It was also found that the kinetics of the processes strongly depend on the quantity of the sample. As shown in Fig. 5 high quantities of material reduce the reaction rate because of water-diffusion controlled sorption and recrystallization processes through the sample bulk which occur in several successive phases.

Because of the closed system the effect of exothermic condensation of water at the solid surface is compensated by the corresponding endothermic evaporation enthalpy of water from the micro-vessel. Only wetting and adsorption processes at the surface could give an enthalpic effect which is expected as being neglectable related

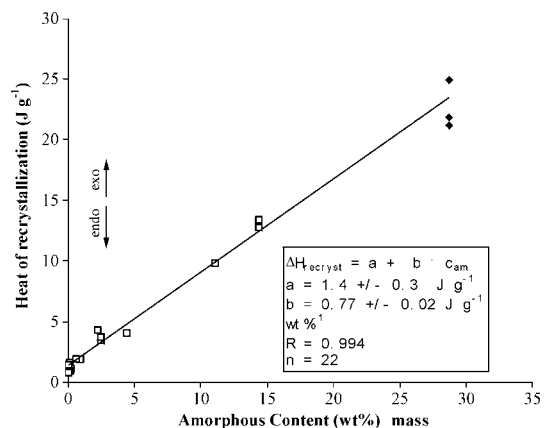


Fig. 4 Linear relation between heat of recrystallization and amorphous content of Desferal measured at 40°C and 75% RH by isothermal microcalorimetry (batch). ♦ sample X. Completely amorphous Desferal: $76 \pm 2 \text{ J g}^{-1}$

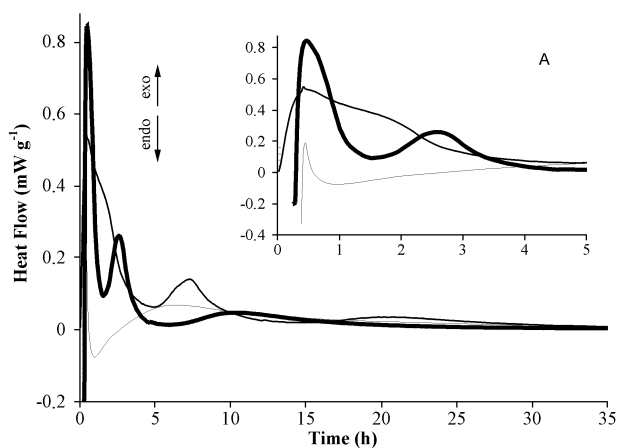


Fig. 5 Influence of sample mass on the course of recrystallization by isothermal microcalorimetry (batch) (amorphous content 2–3%). — 242 mg; — 193 mg; — 71 mg

to the experimental error. The recrystallization proceeds in parallel with the release of water as it was observed in the gravimetric measurements.

Isothermal microcalorimetry (perfusion cell)

In a further series of measurements a 4 ml perfusion steel cell was used that was flowed through by a continuous nitrogen stream under humidity control. The initial samples were dried in waterfree nitrogen and after that subjected for 24 h to 65% RH (Fig. 6). The heat effect was assumed as being caused by wetting and water sorption processes at the solid surface. Drying the sample again for 24 h gave the desorption enthalpy which was subtracted.

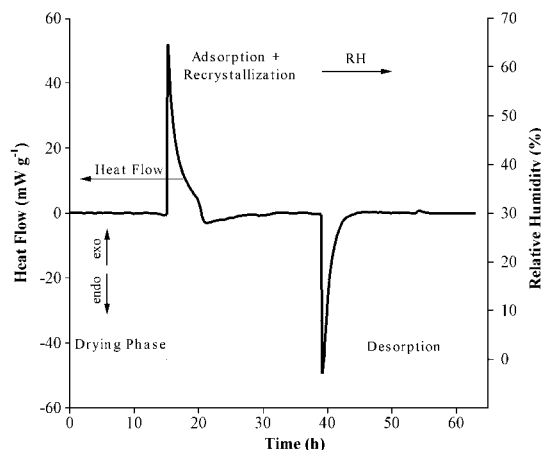


Fig. 6 Heat flow of sample X using the perfusion cell. — relative humidity; — sample X

Assuming a linear dependence between the results of the crystalline and amorphous material, sample X contains $28 \pm 4\%$ amorphous part, which agrees with the result of the batch method. Because of adjustable humidity, the beginning of recrystallization can very well be controlled and observed. The enthalpic effect due to changing the humidity was neglectable. However, the method gave a low reproducibility. The results are extremely sensitive to the fixing of the baselines which is influenced e.g. by changes in heat capacity and noise. Errors in the baseline however, causes a twofold effect in one direction. Besides this, the totally crystalline samples show a reaction enthalpy of -14 J g^{-1} as calculated from the regression line of samples with crystallinity between 0 and 100%. Explanations could be different polymorphic forms (Fig. 2) or changing surface conditions during the perfusion cell cycle leading to differences in the sorption enthalpy.

Gravimetry (dynamic vapor sorption; DVS)

The observed change in mass of the samples (Δm) can be explained by the recrystallization of the amorphous part which leads to a decrease in mass due to the lower water affinity of the crystalline compared to the amorphous or partially amorphous material. Recrystallization is partially overlapped by water sorption of the whole sample. This process is assumed as being completed during maximal one hour for sufficiently small samples dependent on the kinetic behavior of the crystalline material.

The recrystallization branch could be described very well by an exponential function, which was extrapolated to time equal to zero (Fig. 7). The intersection with the y-axis gives a_1 which is the water uptake of a sample with a certain amorphous content at 75% RH. a_0 is the amount of adsorbed water at the crystalline substance.

$$\Delta m(\%) = \frac{100\%}{\text{dry mass}} a_1 - 100\% \quad \text{with } a_1 = a_0 \text{ for totally crystalline material}$$

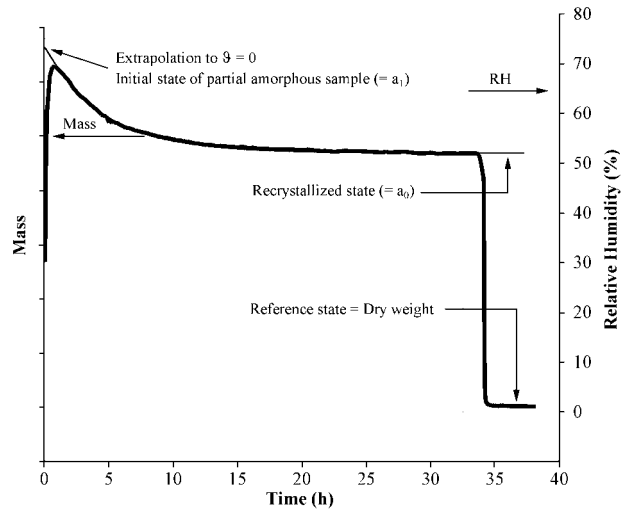


Fig. 7 Example of change in mass of partial amorphous Desferal by DVS at 40°C and 75% RH. — relative humidity; — mass; — extrapolated curve

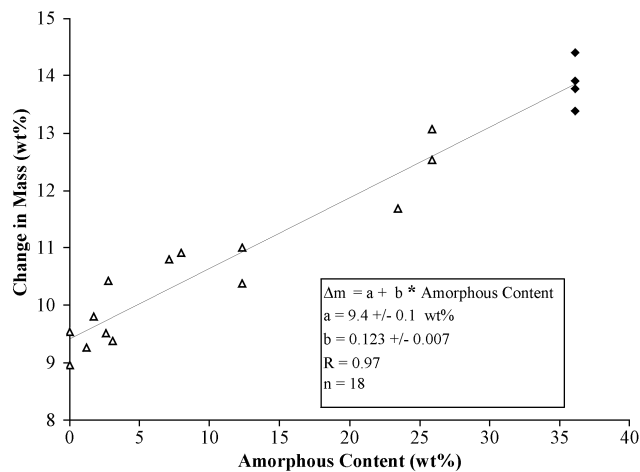


Fig. 8 Linear relation between change in mass and amorphous content of Desferal measured at 40°C and 75% RH by DVS. ◆ sample X. Completely amorphous Desferal: 22.1 ± 0.7 wt%

The change in mass (Δm) should show a linear dependence on the amorphous parts present in the sample. The investigation of a series of mixtures of sample X and crystalline material confirmed the linear dependence between change in mass and amorphous content (Fig. 8). The amorphous content of sample X was found to be 36 ± 3 wt% (Table 2).

Table 2 Dynamic vapor sorption results of the amorphous content of Desferal

Sample	$\Delta m/\text{wt}\%$	Amorphous content/wt%	Repetitions
Crystalline	8.8*	0	2
X	13.9 \pm 0.4	36 \pm 3	4
Amorphous	22.1 \pm 0.7	100	3

*Only 2 measurements (8.6, 9.0 wt%)

Discussion

The amorphous part of Desferal recrystallizes spontaneously when subjected to a humid environment. This effect was used for its quantification. The high sensitivity of the used isothermal microcalorimeter allowed the detection of amorphous contents as low as 1%. The limit of detection is mainly given by the kinetics of the recrystallization process. The microcalorimetric technique requires a sufficient equilibration phase before an undisturbed signal can be measured.

Best results were obtained with small amounts of sample and by minimizing the equilibration time to 10–15 min, using an already well equilibrated salt solution. Optimizing the measuring conditions (e.g. lower temperature and humidity, larger sample amounts in 25 ml vials to reduce the sample height) should enhance the resolution of sorption and recrystallization processes as well as the reproducibility of the method. A difference between the recrystallization enthalpy of 72 J g⁻¹ (TAM) and the melting enthalpy of 84 J g⁻¹ (DSC) was observed which can be explained by the two different ways to obtain crystalline material: crystallization from solution (Fig. 1) or recrystallization of the amorphous phase by a solid state reaction in a humid atmosphere (Fig. 2). Furthermore, these enthalpies were determined at unequal temperatures and differences between heat capacity would have to be taken into account for a detailed evaluation.

The main problems of the static microcalorimetric method are the necessity of a thermal equilibration phase of the sample, in particular the salt solution, during which the recrystallization already starts and furthermore the disturbance of the system due to insertion of the vials. Effects of wetting, sorption and possible recrystallization of unstable polymorphic forms have to be taken into account, too.

An interesting alternative seems to be the use of the perfusion cell. The adjustable humidity offers the possibility of controlled measurements. However, further work should be done for optimizing and better understanding of the results.

The gravimetric method allows a sensitive detection of amorphous phases in Desferal. The humidity is controlled over the whole experiment and the measuring time is very short. Small sample amounts and a high gas stream minimizes the diffusion processes and enhance the kinetics. An important disadvantage of the method is the necessity of a model assumption (extrapolation of the curve to the initial state).

The high standard deviation of the results and the difference to the calorimetric data is attributed to the strong dependence of sorption on the surface conditions which are expected as being different in the lyophilized product and the quenched melt: these effects are poorly reproducible.

Conclusions

The amorphous content of Desferal was quantified by isothermal microcalorimetry using the heat of recrystallization as measure for the amorphous content.

The limit of detection was found to be as low as 1%. The precision of quantification of the amorphous content amounted to about 10% relatively. The detectability of amorphous parts by microcalorimetry is limited by the equilibration time of the instrument and the sample during which the beginning of the recrystallization process is overlapped and so not recordable. When large amounts of sample are used, obviously water diffusion controls the recrystallization which results in the observed multiple recrystallization processes.

The use of a perfusion cell instead of closed vials seems to be an interesting alternative because of the controllable humidity and thus, of the measured reaction. However, the open and dynamic system is more sensitive to disturbance and the measurements are overlapped by the sorption processes, which complicates the evaluation and lowers the reproducibility. Furthermore, the amorphous phase was quantified by the different sorption behavior of the crystalline and amorphous surface using water vapor sorption gravimetry. Under the chosen measuring conditions sorption and recrystallization overlap in that way, that the sorption behavior of a given sample has to be calculated by a model assumption.

All measurements are influenced by the fact that amorphous phases were generated by different processes e.g. from melt or by lyophilization. Furthermore, crystalline material obtained by different ways can differ with regard to surface area and activity or the existence of polymorphic forms.

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