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QUANTITATION OF AMORPHICITY BY MICROCALORIMETRY

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Abstract

The amorphous state of solids is characterized by a higher chemical and physical reactivity and a hygroscopic behaviour. Furthermore processing of amorphous powders is often difficult, because of the instability. Fast crystallizations, precipitations and milling favour the formation of the amorphous state. Galenical processes like granulation, drying, lyophilization, mixing, may also induce amorphous regions in the drug products.

X-ray diffraction techniques can be used for the determination of the amorphicity of drug raw materials or drug products. Unfortunately, 10% is the detection limit, which in normal cases can be attained. Amorphous substances undergo an exothermic crystallization at temperatures above the glass transition point. Water which is a plasticizer decreases the temperature of the glass transition point, allowing the crystallization to occur at lower temperatures. The crystallization energy is measure of by microcalorimetry.

Examples show the influence of the choice of the experimental conditions, especially the influence of the amorphicity on the kinetic of the reaction. Critical steps are discussed for three different drug substances. Limits of detection in the magnitude of 1% are possible using microcalorimetry.

Keywords: microcalorimetry, quantitation of amorphicity

Introduction

Amorphous substances undergo an exothermic crystallization at temperatures above the glass transition point. Water which is a plasticizer decreases the temperature of the glass transition point, allowing the crystallization to occur at low temperatures.

The calorimetric method first described by K. Byström [1], is an application of this phenomenon. The amorphous sample is placed in a calorimetric cell in isothermal conditions under a high level of relative humidity. The measurement of the energy evolved by a complete crystallization is proportional to the amorphous content.

Sebhatu *et al.* [2] used the method for the analysis of amorphous lactose and found that 2% of amorphous lactose is easily detectable. Buckton *et al.* [3] studied the parameters for the measurement of amorphous salbutamol sulfate.

In order to develop a reliable analytical technique based on this principle; the different parameters were first studied for a purine derivative. Results with two other drug substances confirm the role of temperature, moisture and crystallization level for the setting of the analytical method. The critical steps and the limits of detection are discussed.

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Experimental and instrumentation

Amorphous samples are prepared either by lyophilization or by quick crystallization of diluted solutions under vacuum. The amorphicity of the samples are confirmed by X-ray diffraction. The samples are stored in tight containers in the refrigerator.

Micro DSC-II of Setaram with two stainless steel vessels was used. The instrument allows to work in isothermal mode as well as in scanning mode. Both modes were applied and the results compared for the purine derivative. Different levels of controlled humidity were obtained by using a small glass vial containing pure water or saturated solutions [4] according to the technique of Angerg *et al.* [5]. Different amounts of sample (5 to 130 mg) were investigated.

The reference vessel contains either undecane or crystalline drug substance. No relevant differences was found. Equilibration of 10–20 min are necessary before starting the measurement. The crystallinity of the samples after calorimetric mea-surements has been checked by X-ray diffraction and by DSC, for most of the experiments.

Results

Study of a purine derivative (MKS 492)

The X-ray diffractions and DSC curves of the amorphous samples and of the crystalline MKS 492 obtained after calorimetric measurement are very reproducible. At 92% r.h. the amorphous drug substance take up 3% moisture. Preliminary experiments reveal, that in isothermal mode 8 h are necessary at 30°C to transform the amorphous form into the crystalline state (36 J g^{-1}, number of determinations (n) $n=6$) [6]. The drug substance has several polymorphs. The crystalline form obtained in theses experiments is the thermodynamic stable form at room temperature. The obtention of the thermodynamic stable form has been also observed by Buckton *et al.* for Sabultamol [3].

The influence of the temperature on the duration of the experiment for a 100 mg amorphous sample is given in Table 1.

The influence of the sample mass on the duration of the crystallization and on the initial time before the starting of the crystallization (lag time) is given in Table 2.

This table shows also that the energy of the crystallization is very reproducible. Small samples are not suitable for practical reasons.

Table 3 summarizes the experimental results in scanning mode (0.1°C min^{-1} 10 to 60°C). The calculation of linearity gives a factor R of 0.996. For theoretical values, the crystallization energy of 36 J g^{-1} is taken into account.

Table 1 MKS 492: Influence of the temperature of the duration of the crystallization for 100 mg amorphous substance at a 100% r.h.

Temperature/°C	Energy/J g^{-1}	Total duration/h
20	33.4	30
25	34.5	17
30	35.0	10

Table 2 MKS 492: Influence of the samples mass. Temperature 30°C, 100% r.h.

Sample mass/mg	Initial time/h	Total duration of transition/h	Energy/J g^{-1}
100	2.00	11.48	35
50	1.54	10.52	38
25	0.52	6.40	36
15	0.35	5.53	38
10	0.33	4.00	29
5	0.32	3.35	27

Table 3 MKS 492: Scanning mode (10 to 60°C, 0.1°C/min) with 100% r.h. Influence of the duration of the crystallization

Theoretical % amorphous	Total duration of transition/h	Energy/J g^{-1}	Micro-DSC II % amorphous found	Difference/%
100	7.57	40.1	111.4	+11.4
75	7.41	27.4	76.1	+1.1
50	6.42	20.0	55.5	+5.5
25	6.11	7.2	20.0	–5.0
15	4.47	5.8	16.1	+1.1
10	4.45	3.0	8.3	–1.7
5	4.26	1.9	5.3	+0.3
1	3.55	0.3	0.8	–0.2

Table 4 summarizes the experimental results in the isothermal mode at 20°C and 100% r.h. between 1 and 10% amorphous MKS. 2% is the limit of quantitation, 1% the limit of detection. The linearity is also good: $R=0.990$. Figure 1 shows the microcalorimetric curve for a 2% amorphous sample.

The comparison of the isothermal mode and of the scanning mode shows that they have both advantages and disadvantages. The scanning mode allows a faster optimization of the determination and a quicker analysis. Only one method can be used for low and high levels of crystallinity. The peaks are higher, therefore a lower limit of detection may be expected.

Disadvantages are the difficulty to have a proper integration of the start of the peak, and the decrease of the base line after 50°C. Furthermore several process may overlap. The isothermal mode allows a faster stabilization, and an easier integration of the peaks. The optimizations of the temperature and the humidity level take more time. Different temperatures for analysis in low range of crystallinity and in high range of crystallinity are necessary in order to achieve analysis in reasonable time.

Since better integration is achieved in isothermal mode, we prefered this technique for the accurate determination of low amounts of amorphous phase of MKS 492.

Table 4 MKS 492: Mixtures of crystalline form with amorphous form in isothermal mode at 20°C and in 100% of r.h.: Determination of amorphous content (2–10%)

Theoretical % amorphous	Micro-DSC II % amorphous found	Difference/%	Experimental energy /J g^{-1}
10.6	9.1	–1.5	3.3
10.6	8.7	–1.9	3.1
10.1	8.9	–1.2	3.2
10.6	8.1	–2.5	2.9
5.2	4.8	–0.4	1.7
4.9	3.5	–1.4	1.3
5.1	4.7	–0.4	1.7
3.0	2.7	–0.3	1.0
3.1	2.4	–0.7	0.9
3.1	2.3	–0.8	0.8
2.0	1.5	–0.5	0.5
2.1	1.2	–0.9	0.4
2.4	1.2	–1.2	0.4
2.4	1.9	–0.5	0.7
2.3	1.7	–0.6	0.6
1.0	1.1	+0.1	0.4
1.1	0.6	–0.5	0.2

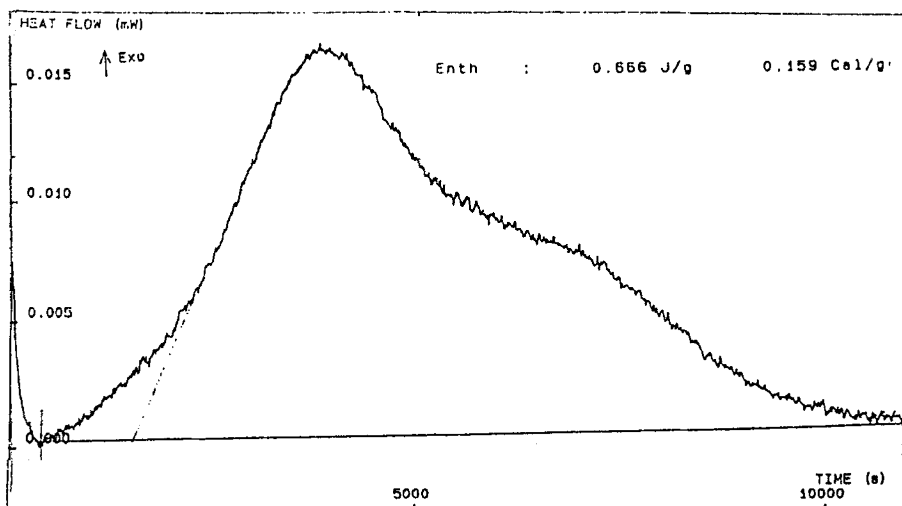

Fig. 1 MKS 492. Example of microcalorimetric heat flow for a mixture containing 2% amorphous form at 20°C, 100% r.h.

Study of an isoquinoline derivative (as hydrochloride)

The X-ray diffractions and the DSC curves of the crystalline drug substance and of the amorphous form were carried out. The crystalline substance obtained after the calorimetric experiments is the original crystalline form.

The highest sample mass which can be put in the vessel (100 mg) was chosen in order to attain the lowest limit of detection.

Table 5 shows that the same results are obtained at 30°C, 75% r.h. or 30°C, 100% r.h. for a 100% amorphous sample or for a mixture containing only 5% amorphous form. The same energy is obtained.

Table 6 shows that the amount of sample does not play an important role for a sample 100% amorphous (linearity see Table 7).

The durations of the analysis at 25, 30, 40°C and 75% r.h. and 100% r.h. were compared. The same energy was obtained. Since the recrystallization is very fast, a longer lag time is necessary for precise determination of the calorimetric output. Therefore the isothermal conditions at 75% r.h. were chosen. A new amorphous sample was prepared for the measurement of the linearity of the energy *vs.* amorphous content.

Table 5 Isoquinoline derivative. Influence of humidity on the energy of recrystallization at 30°C

Sample	r.h./%	Energy of the sample/J g^{-1}	Difference/%	Energy/J g^{-1} 100% amorphous
5% amorphous form in the mixture	100	1.85	2.1	36.7
	75	1.81		
100% amorphous	100	36.4	2.2	36.9
	75	37.3		

Table 6 Isoquinoline derivative. Influence of the sample mass at 100% r.h. and 30°C

Mass of the 100% amorphous/mg	Energy/J g^{-1}
130	36.4
30	35.3

Table 7 Linearity of the calorimeter output at 100% r.h., 30°C

Amorphous form/%	Energy/J g^{-1}	Theoretical energy/J g–1
3	1.08	1.09
4	1.43	1.46
5	1.85	1.82
7	2.45	2.55
10	3.58	3.6
100	36.4	–

Figure 2 shows the heat flow curve for a mixture containing 6% amorphous form. The linearity is given in Fig. 3. A limit of detection of 1% was obtained.

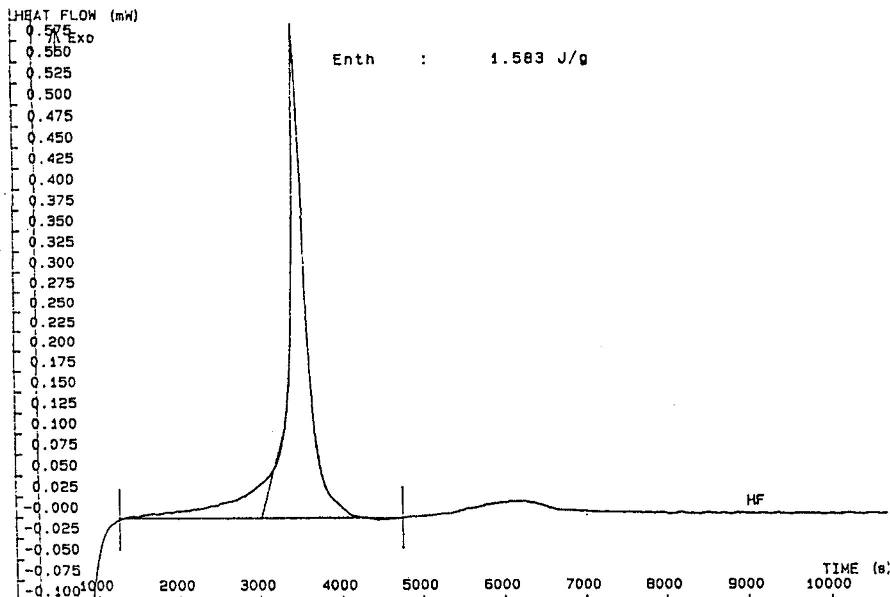

Fig. 2 Isoquinoline derivative. Example of microcalorimetric heat flow for a mixture containing 6% amorphous form at 75% r.h. and 30°C

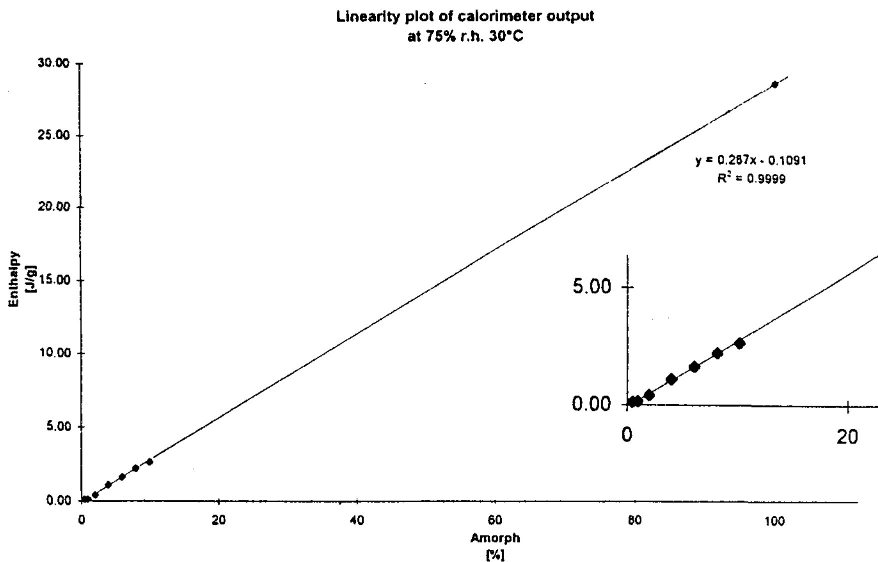

Fig. 3 Linearity of the heat flow *vs.* content of amorphous form of the isoquinoline derivative at 75% r.h. and 30°C

All the results were obtained with samples directly introduced in the measuring vessel. Comparison with well mixed crystalline and amorphous forms shows that the heat flow is not affected, but the lag time is considerably reduced if amorphous and crystalline are well mixed.

Study of a peptide

The peptide under investigation was known to have two hydrates and an amorphous form. This amorphous form could be stored unchanged even in tropical climate. Therefore it was interesting to investigate the microcalorimetric method by using different temperatures.

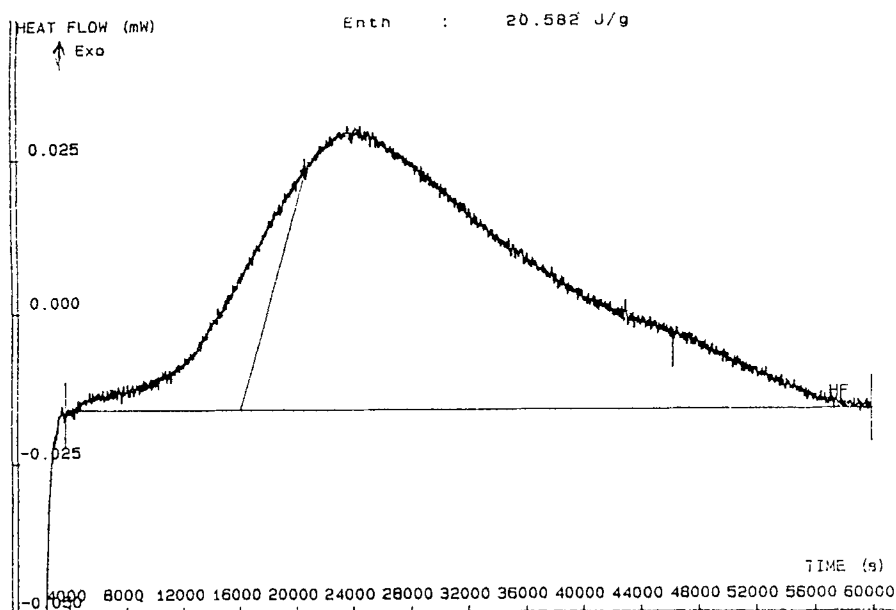

Fig. 4 Peptide. Microcalorimetric heat flow for a 100% amorphous sample at 80°C and 100% r.h.

X-ray diffractions of the crystalline form obtained at 80°C/100% r.h. showed that the amorphous form converted to the monohydrate. The corresponding calorimetric output is shown in Fig. 4. For the crystallization of 50 mg, 13 h are necessary in these drastic conditions. The same experiment at 50°C/100% r.h. shows a considerable long lag time. For this peptide an analytical method can be considered only by using very drastic conditions: high temperature and high relative humidity.

Discussion and conclusion

The experiments performed have brought forward two major points of discussion. The first point is the number of peaks detected. Depending of the amount of amorphous substance, the humidity level and the drug substance, we observed one,

two or three peaks. According to Sebhatu *et al.* [2] and Buckton *et al.* [3], three energetic processes should be taken into consideration: adsorption of water on the amorphous powder, recrystallization and expulsion of the water. Furthermore the liberation of the water should accelerate the crystallization [7]. If the substance is very soluble in water, a solution can also be obtained in very high humidity levels.

Therefore we preferred the experimental conditions which give only one peak, easy to be integrated (or in the worst case all the peaks are to be integrated). The resulting calorimetric output should be always proportionnal to the amount of amorphous substance.

The second point is the influence of the glass transition temperature on the parameters temperature, humidity and samples. The lowering effect of water on this transition has been described [8]. Therefore the knowledge of this temperature should guide the experimental conditions.

The best conditions for the analytical methods are very different for the three substances described: 20°C and 100% r.h. for MKS 492, 30°C and 75% r.h. for the isoquinoline derivative and 80°C and 100% r.h. for the peptide.

In conclusion, the quantitative determination of low levels of amorphous content in drugs is possible by the calorimetric technique and is specially attractive for those levels where the X-ray diffraction fail.

A proper development of an analytical method for the determination of amorphous content need several steps. The first step is the obtention of the amorphous form. It is very important to obtain a 100% amorphous sample in order to determine properly the energy of crystallization. The scanning mode is very convenient for optimization.

The isothermal mode is preferred for quantitative determinations in cases of quick crystallizations. Since the equilibration time of the instrument is 10–20 min, the temperature and humidity have to be chosen in order to permit a correct integration of the beginning of the crystallization. Once the linearity demonstrated, the calculations of the amorphous content for analysis samples should be calculated by using the value of the heat of crystallization of the 100% amorphous sample. In these studies 1% was easily detected, but not quantified properly. More sensitive instruments would permit to attain a lower limit of detection.

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