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Determination of Amorphous Content of Lactose Samples by Solution Calorimetry

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ABSTRACT

Earlies studies suggest that solution calorimetry can be used to determine the extent of amorphous content of drug and excipient, when the solubility and dissolution rate of the compound in the chosen solvent are reasonably high. In the present study, the use of solution calorimetry for assessment of amorphous content of a sample that is not completely dissolved in a solvent was evaluated. Physical mixtures of lactose and spray-dried lactose samples were analysed. The amorphous content of the physical mixtures and the spray-dried samples varied from 0% to 100% determined by isothermal microcalorimetry. The enthalpy of solution ($\Delta_{sol}H$) was determined in water. The lactose samples were dissolved quickly in water. In addition, the enthalpy accompanied with an addition of a lactose sample in an over saturated aqueous solution ($\Delta_{sat}H$) (prepared from the corresponding lactose sample) was determined. The lactose sample did not completely dissolve in the over saturated aqueous solution. An excellent correlation was observed between $\Delta_{sol}H$ and the amorphous content of the samples. Interestingly, there was a linear correlation also between $\Delta_{sat}H$ and the amorphous content of the samples. Further, a linear relationship was observed between the $\Delta_{sat}H$ and the $\Delta_{sol}H$ of the samples. Therefore, solution calorimetry may represent a rapid and simple method for determining the amorphous content also in samples that are not completely dissolved in the solvent.

Key Words: Solution calorimetry; Lactose; Amorphous content.

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INTRODUCTION

Many pharmaceutical techniques, such as milling, spray drying, tablet compaction and lyophilization can induce amorphous regions within the crystal structure of the product. Evaluating the amount of amorphous material in a crystalline sample is very important, as amorphous regions may significantly alter the physical and chemical properties of the compound, e.g. amorphous regions can enhance the dissolution rate[1] and decrease the physical and chemical stability of a compound.[2]

The crystallinity of a compound can be determined by a variety of techniques, including powder X-ray diffraction, differential scanning calorimetry, thermal gravimetric analysis, isothermal microcalorimetry and solution calorimetry. The usefulness of these techniques has been reviewed in earlier reports.[3–8]

Few publications have utilized solution calorimetry to determine the extent of drug and excipient crystallinity.[3,6,8–10] In the earlier studies, the enthalpy of solution ($\Delta_{sol}H$) of a sample has been measured and dissolution rate of the sample has been high in the chosen solvent. Typically 100% crystalline and 100% amorphous materials have been physically mixed to prepare samples of varying percent crystallinities, and a linear relationship between the heat of solution and the weight percent crystalline fraction present in the resulting mixture has been demonstrated.[6,8] In addition, determination of the enthalpy of solution of the sample is helpful as a way to screen various batches to detect batch to batch differences and to guarantee stability.[9–14] Sometimes it is not easy to find a solvent in which a sample is freely soluble.

The aim of the present study was to evaluate whether solution calorimetry can be used for assessment of amorphous content of a sample that is not completely dissolved in a solvent. Therefore, the relationship between enthalpy accompanied with an addition of a lactose sample in the over saturated aqueous solution ($\Delta_{sat}H$) and the amorphous content of the physical mixtures and spray-dried lactose samples was determined.

MATERIALS AND METHODS

Preparation and Characterisation of the Spray-Dried Lactose

α-Lactose monohydrate (Pharmatose®, 325 Mesh, DMV, Holland) was used to prepare the spray-dried samples. Distilled water and ethanol (Aa, Primalco, Finland) were used to prepare the feed samples. The ratios of ethanol to water in the feed solution were 0:100; 20:80; 25:75; 30:70; 40:60 and 100:0. A 15% (w/w) lactose suspension or solution was spray dried with a Büchi Mini-Spray Drier 190 (Büchi Laboratorium-Technic AG, Switzerland) as described earlier.[15] After spray-drying, the samples were packed into tightly closed plastic bottles and stored in a silica desiccator for one week (at room temperature) prior to the studies.

The particle size distribution of the spray-dried lactose samples was determined in 2-propanol by laser diffraction, using a Mastersizer 2000 (ultrasonication time was 1 min) (Malvern Instruments, Malvern, UK). The particle size distribution of the spray dried lactose samples is shown in Table 1. The amorphous content of the spray-dried lactose samples is shown in Table 2. When the ratio of ethanol to water was decreased in the feed solution, the degree of disorder increased in the product as discussed earlier.[15] As a result, the lactose spray-dried from pure ethanol was 100% crystalline, whereas the lactose spray-dried from pure water was 100% amorphous.

Table 1. Particle size (μm) (n=10) distribution of the spray dried lactose samples.

	Particle size distribution		
Amorphous content % (w/w)	10% undersize	50% undersize	90% undersize
100	1.42 (±0.01)	16.35 (±0.02)	32.72 (±0.05)
51	2.63 (±0.07)	47.79 (±0.16)	93.16 (±0.31)
44	5.43 (±0.01)	34.30 (±0.15)	82.99 (±0.24)
34	15.11 (±0.15)	45.95 (±0.12)	84.53 (±0.25)
25	12.26 (±0.10)	48.07 (±0.08)	87.81 (±0.18)
0	19.52 (±0.24)	54.08 (±0.13)	95.50 (±0.29)

Mean values ± SD are shown.

Table 2. Amorphous content (% w/w) of the spray dried lactose samples determined by isothermal microcalorimetry and by solution calorimetry.

Ratio of ethanol to water % (w/w) in the feed solution	Amorphous content (% w/w)		
	Isothermal microcalorimetry	Solution calorimetry	
		Water	Over saturated aqueous solution
0:100	100 (±0.4)	100 (±2.6)	96 (±4.8)
20:80	51 (±4.6)	49 (±1.4)	56 (±5.1)
25:75	44 (±2.6)	45 (±2.1)	47 (±1.6)
30:70	34 (±2.5)	37 (±1.1)	37 (±3.9)
40:60	25 (±2.2)	21 (±2.9)	21 (±2.4)
100:0	0 (±0.0)	1 (±0.6)	−4 (±1.0)[a,b]

[a]Data significantly different if compared to amorphous content determined by isothermal microcalorimetry.
[b]Data significantly different if compared to amorphous content determined by using water as a solvent.
Mean values ± SD are shown (n=4–8).

Preparation of Physical Mixtures of Lactose

Quantities of 100% amorphous lactose and 100% crystalline lactose were accurately weighed and combined to produce physical mixtures with an amorphous content of 0–100% w/w (Table 3). The 100% amorphous lactose was prepared by spray drying of lactose from 100% water. Isothermal microcalorimetry measurements showed that an untreated commercial lactose (Pharmatose®, 325 Mesh, DMV, Holland) was 100% crystalline. 100% amorphous lactose and 100% crystalline lactose were mixed with a mortar and pestle for 10 min. The amorphous content of the physical mixtures was confirmed by isothermal microcalorimetry. The samples were packed and stored as described above.

Isothermal Microcalorimetry Measurements

The amorphous content of the lactose samples was measured by an isothermal heat-conduction microcalorimeter TAM 2277 (Thermometric AB, Sweden). The miniature humidity chamber technique[16,17] was employed to detect the thermal response for the recrystallization of amorphous lactose. The extent of heat evolution was directly related to the degree of amorphicity. During the measurement, the sample was recrystallized by moisture absorbed from the saturated

Table 3. Amorphous content (% w/w) of the physical mixtures determined by isothermal microcalorimetry (n=2–4) and by solution calorimetry (n=4–8).

Ratio of 100% amorphous lactose to 100% crystalline lactose	Amorphous content (% w/w)		
	Isothermal microcalorimetry	Solution calorimetry	
		Water	Over saturated aqueous solution
100:0	100 (±0.4)	98 (±2.5)	102 (±4.6)
93:7	90 (±1.5)	94 (±1.1)	92 (±2.4)
45:55	47 (±0.4)	45 (±1.6)	45 (±4.3)
38:62	39 (±5.4)	37 (±3.9)	33 (±6.0)
27:73	23 (±10.0)	25 (±1.2)	22 (±1.5)
0:100	0 (±0.0)	1 (±0.8)	5 (±1.2)[a,b]

[a]Data significantly different if compared to amorphous content determined by Isothermal microcalorimetry.
[b]Data significantly different if compared to amorphous content determined by using water as a solvent.
Mean ± SD are shown.

salt solution ($Na_2Cr_2O_7$: 54% RH), which was included in the hermetically sealed 3 ml glass ampoule as a desiccant, together with the sample. The samples stored in a silica desiccator at room temperature were accurately weighed just prior to the measurements. After preparation, the samples and identical reference ampoules containing only the saturated salt solution were immediately placed in the equilibrium position of the TAM. The samples were lowered into the measuring position after 15 minutes of equilibration. All of the spray-dried batches were measured in quadruple and physical mixed lactose batchs were assayed in duplicate. The lactose sample that was spray dried from pure water was considered to be totally amorphous (100%) because x-ray diffraction studies (Philips PW 1820, The Netherlands) showed only diffuse scattering with no characteristic reflections of crystallinity in the diffractogram (Fig. 1). The corresponding heat (48.54 J/g) for the recrystallization process was taken as the reference value in the calculations of amorphous content of the other samples.

Solution Calorimetry Measurements

The enthalpy of solution ($\Delta_{sol}H$) and the enthalpy accompanied with an addition of a lactose sample in a over saturated aqueous solution ($\Delta_{sat}H$) were determined at room temperature using a Parr 1455 Solution Calorimeter (Parr Instrument Company, Moline Illinois, USA) equipped with a chart recorder. The solution calorimeter was calibrated using 0.500 g tris(hydroxymethyl)aminomethane (Tris® Parr Instruments) and 100.00 g 0.1 N hydrochloride acid. The thermograms were recorded at a sensitivity of 10 mV or 0.1°C full scale and a chart speed of 3 cm/min (total measurement time was about 5 min). The samples were prestored in a silica desiccator at room temperature, and accurately weighed (about 400 mg) just before the measurements. The $\Delta_{sol}H$ was determined in distilled water (100.00 g). The $\Delta_{sat}H$ was determined in a saturated aqueous solution (100.00 g), prepared from the corresponding lactose. A negative value for the $\Delta_{sat}H$ and $\Delta_{sol}H$ indicated an evolution of heat (exothermic process), and a positive value indicated an absorption of heat (endothermic process). The values of $\Delta_{sol}H$ (Fig. 2A and 2B) or $\Delta_{sat}H$ (Fig. 3A and 3B) were plotted against the amorphous content of the samples (determined by isothermal microcalorimetry). These regression lines were utilized when the amorphous content of the samples was calculated by using the values of the $\Delta_{sol}H$ and $\Delta_{sat}H$ (Tables 2, 3).

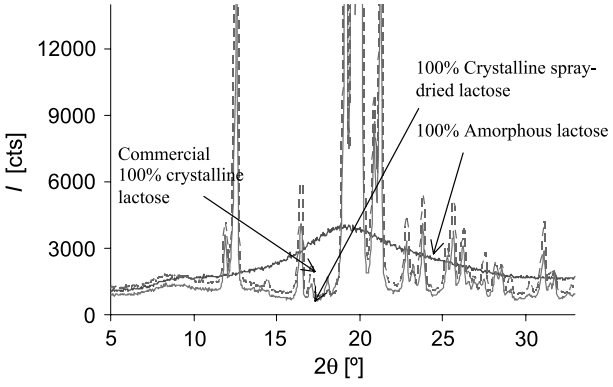

Figure 1. X-Ray diffraction pattern of the commercial 100% crystalline lactose (the broken line), the spray-dried 100% crystalline lactose (the solid line) and 100% amorphous lactose (the dark line).

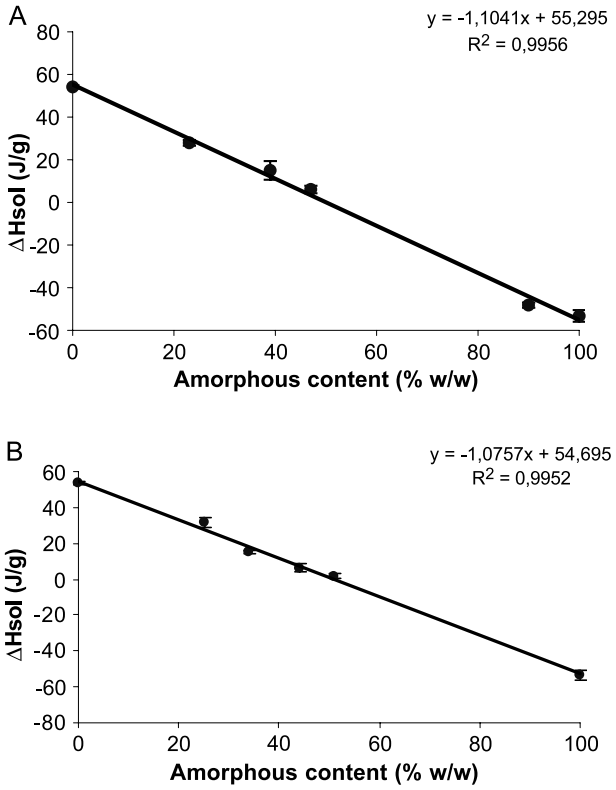

Figure 2. (A) Relationship between the $\Delta_{sol}H$ and the amorphous content of the physical mixtures. Mean values ± SD are shown (n=4). (B) Relationship between the $\Delta_{sol}H$ and the amorphous content of the spray dried samples. Mean values ± SD are shown (n=4).

Statistical Analysis

A non-parametric Kruskal-Wallis test was used to test the differences between multiple groups; significance in the differences in the means was tested using

the Games-Howell's multiple range test. The level of significance was taken as $p<0.05$.

RESULTS AND DISCUSSION

The $\Delta_{sol}H$ of the 100% amorphous lactose was -53.3 ± 2.7 J/g, (n=4), indicating an exothermic process. The untreated commercial 100% crystalline lactose revealed the value of the $\Delta_{sol}H$ to be 54.2 ± 0.9 J/g, (n=4). These results can be explained by the fact that an amorphous material has a different energy state than a crystalline material. The amorphous material always possesses better solubility because the amorphous material has higher energy, entropy and free energy value than the corresponding crystal form.[18] Our results are in line with an earlier report from Hogan and Buckton.[8] The present and earlier[8] results indicate that the interactions within the crystalline lactose are stronger than the hydration process and thus, the dissolution of the crystalline lactose is an endothermic process. A linear correlation between the $\Delta_{sol}H$ and the amorphous content of the physical mixtures was obtained ($R^2=0.9956$) (Fig. 2A). In addition, a linear correlation between the $\Delta_{sol}H$ and the amorphous content of the spray-dried sample was obtained ($R^2=0.9952$) (Fig. 2B).

In addition to the $\Delta_{sol}H$, $\Delta_{sat}H$ was determined in order to clarify the effect of solubility of a sample on solution calorimetry measurements. Measurements of $\Delta_{sat}H$ were performed in the over saturated aqueous solutions, prepared from the corresponding lactose. When compared to the measurements in water, the solubility and dissolution rate of the lactose samples were lower in the over saturated aqueous solutions. In fact, lactose was not completely dissolved in the over saturated aqueous solutions. $\Delta_{sat}H$ may include several processes, such as wetting, crystallisation, the breakage of bonds, liquid penetration, hydration and possibly rearrangement.

Figure 3A and 3B demonstrate the clear correlation between the $\Delta_{sat}H$ and the amorphous content for the physical mixtures ($R^2=0.9895$) and for the spray-dried lactose ($R^2=0.9852$). The process evolved more heat, the higher the amorphous content of the sample

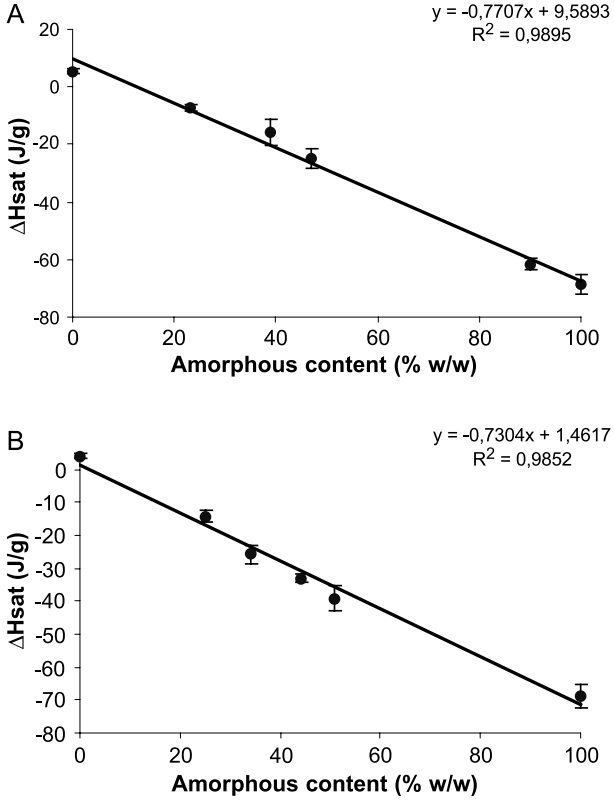

Figure 3. (A) Relationship between the $\Delta_{sat}H$ and the amorphous content of the physical mixtures. Mean values ± SD are shown (n=4–8). (B) Relationship between the $\Delta_{sat}H$ and the amorphous content of the spray dried samples. Mean values ± SD are shown (n=4–8).

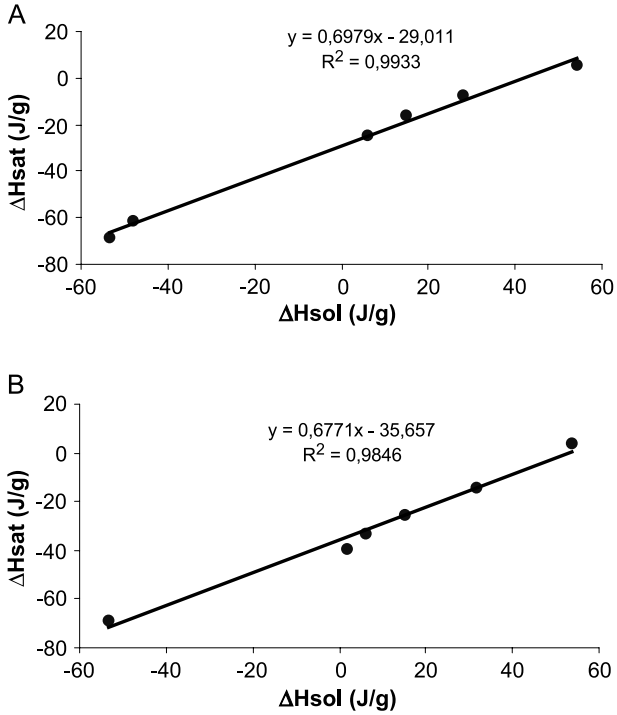

Figure 4. (A) The relationship between the $\Delta_{sat}H$ and the $\Delta_{sol}H$ of the lactose physical mixtures (amorphous content 0–100%). (B) The relationship between the $\Delta_{sat}H$ and the $\Delta_{sol}H$ of the spray dried lactose samples (amorphous content 0–100%).

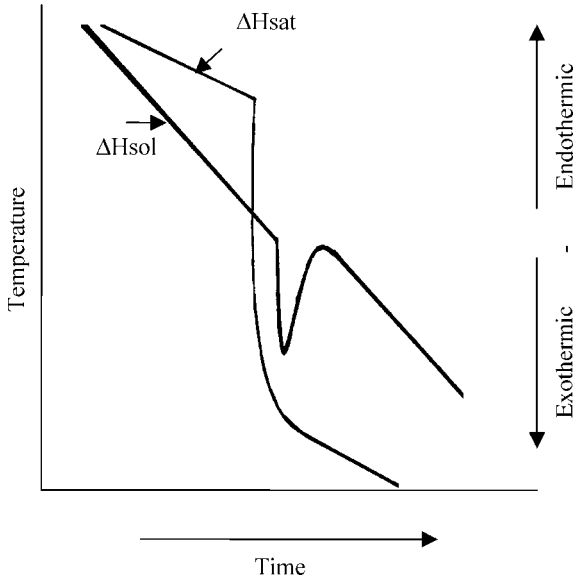

Figure 5. Thermograms ($\Delta_{sol}H$, $\Delta_{sat}H$) of 44% amorphous lactose sample determined by solution calorimetry.

(Fig. 3A and 3B). The $\Delta_{sat}H$ of the 100% amorphous lactose was -68.7 ± 3.5 J/g (n=8), indicating an exothermic process. The value of the $\Delta_{sat}H$ was 5.45 ± 0.9 J/g (n=4), for the commercial 100% crystalline lactose and 4.1 ± 0.7 J/g (n=4), for the 100% crystalline spray-dried lactose, indicating endothermic processes.

Figure 4 clearly shows the linear relation between the $\Delta_{sol}H$ and the $\Delta_{sat}H$ for lactose samples ($R^2=0.9933$ for the physical mixtures and $R^2=0.9846$ for the spray-dried samples). One might expect that the $\Delta_{sat}H$ would be more of a surface-composition sensitive quantity whereas the $\Delta_{sol}H$ would be a bulk-composition sensitive quantity and thus, unless a sample was fully homogeneous with respect to its amorphous character, the correlation shown in Fig. 4 could not exist.

When the $\Delta_{sat}H$ of the 0–100% amorphous lactose was determined, we observed only either a clear exothermic or a clear endothermic response. When the $\Delta_{sol}H$ of 25–51% amorphous lactose samples were determined, an initial exothermic response was observed, followed by an endothermic response. The overall result for the dissolution process of the 25–51% amorphous lactose samples was endothermic. The initial exothermic process is thought to be attributable to moisture sorption, recrystallization and dissolution of the amorphous lactose all of which evolve heat during the dissolution process. The subsequent endothermic process could be due to dissolution of the crystalline lactose in the sample, as suggested earlier by Hogan and Buckton.[8] As an example, the thermograms of 44% amorphous lactose sample are shown in Fig. 5.

The regression lines shown in Figs. 2 and 3 were utilized when the amorphous content of the samples was calculated by using the values of $\Delta_{sol}H$ or the values of $\Delta_{sat}H$ (Tables 2 and 3). Tables 2 and 3 show that when compared to isothermal microcalorimetry, the value for the amorphous content of a sample was comparable when determined by solution calorimetry. Either of the values for the $\Delta_{sol}H$ or the $\Delta_{sat}H$, could be utilized if the amorphous content was to be determined by solution calorimetry. The theoretical limits of detection (LOD) and quantification (LOQ) were determined from the data (IUPAC). LOD and LOQ values were determined from the residual standard deviation (SD_0) in the y-intercepts from the linearity data and slopes (b) using the equations $LOD=3*SD_0/b$ and $LOQ=10*SD_0/b$. In cases of the $\Delta_{sol}H$ measurements, LODs of 2.3% and 1.8% (amorphous content) were obtained for the physical mixtures and the spray-dried samples, respectively. In cases of the $\Delta_{sat}H$ measurements, LODs of 3.5% and 3.0% were obtained for the physical mixtures and the spray-dried samples, respectively. The LOQ for the determination of amorphous content of a sample using the $\Delta_{sol}H$ values was established at 7.8% and 6.0% for the physical mixtures and the spray-dried samples, respectively. The LOQ value was 11.6% and 10.0% for the physical mixtures and the spray-dried samples, respectively, when the $\Delta_{sat}H$ was measured.

CONCLUSION

The present results demonstrate the correlation between the $\Delta_{sat}H$ and the amorphous content of the lactose samples. This study indicates that solution calorimetry may be a rapid and simple method for determining the amorphous content also in samples that are not completely dissolved in the solvent.

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