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Short communication

Evaluation of the amorphous content of lactose by solution calorimetry and Raman spectroscopy

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Abstract

Solution calorimetry can be used to determine the amorphous content of a compound when the solubility and dissolution rate of the compound in the chosen solvent are reasonably high. Sometimes, it can be difficult find a solvent in which a sample is freely soluble. The present study evaluated the use of solution calorimetry for the assessment of the amorphous content of a sample that is poorly soluble in a solvent. Physical mixtures of lactose and spray-dried lactose samples (the amorphous content varied from 0 to 100%) were analyzed by a solution calorimeter and the results were compared with Raman spectroscopy determinations. The heat of solvation of the samples was determined by solution calorimetry in organic solvents MeOH, EtOH, ACN, THF, acetone (400 mg sample/100 ml solvent). Lactose is virtually insoluble in ACN, THF and acetone and very slightly soluble in EtOH and MeOH. The amorphous content of the samples could not be determined by solution calorimetry in EtOH, ACN, THF or acetone. However, an excellent correlation was observed between the heat of solvation and the amorphous content of the samples in MeOH. Furthermore, the heat of solvation values of the samples in MeOH showed a linear correlation with the Raman quantifications. Therefore, our results demonstrate that solution calorimetry may represent a rapid and simple method for determining the amorphous content also in samples that are not freely soluble in the solvent.

Keywords: Amorphous content; Solution calorimetry; Quantification; Raman spectroscopy; Lactose

1. Introduction

The degree of crystallinity of drugs and excipients plays a very important role in drug formulations, e.g. affecting dissolution rate, physico-chemical stability, hygroscopicity and even the bioavailability of the drug [1]. Amorphous forms of drugs may be useful for increasing the dissolution rate of poorly soluble drugs, but the amorphous content may decrease both the physical and chemical stability of the drug [2–4]. For example, it has been shown that different physical forms of lactose can result in changes in drug deposition from dry powder inhalers [5,6] and changes in compaction due to variation in the mechanical properties of the powder [7].

Several methods have been developed to measure the amorphous content of a sample. For example, amorphicity can be determined by powder X-ray diffraction, isothermal microcalorimetry (IMC), thermal gravimetric analysis, differential scanning calorimetry (DSC) and spectroscopic methods. These methods differ greatly from each other in terms of their measurement principles, and in many previous publications their relative benefits and disadvantages have been discussed [8–11].

Two methods that have not been widely studied are solution calorimetry and Raman spectroscopy. A few earlier studies have revealed that solution calorimetry can be used for determining the amorphous content of drugs and excipients [12–14]. These methods typically are based on total and rapid dissolution of the sample in solvent during the measurement. Sometimes, it is not easy to find a solvent in which a sample is freely soluble. In this study, we show that total

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dissolution of the sample in a chosen solvent is not necessary, but the amorphous content of a very slightly soluble material can also be measured by solution calorimetry. Only a few previous reports have demonstrated the quantitative analysis of crystallinity using Raman spectroscopy [15,16]. Recently Niemelä et al. [17] reported a novel quantitative analysis of the amorphous content of lactose using CCD-Raman spectroscopy (CCD, charged coupled device). In their study, the amorphous content values obtained with Raman spectroscopy and isothermal microcalorimetry agreed well with each other. In the present study, this novel CCD-Raman spectrometer was utilized to determine the amorphous content of a sample, and the results were compared with the solution calorimetry determinations.

2. Experimental

2.1. Materials

Methanol (MeOH), acetonitrile (ACN), acetone and tetrahydrofuran (THF) were purchased from Rathburn (Walkerburn, Scotland), tris(hydroxymethyl)aminomethane from Sigma (St. Louis, USA) and hydrochloric acid (HCl) from Reagecon (Ireland). Ethanol (EtOH) was purchased from Primalco (Finland) and α-lactose monohydrate (mesh 325) from Tamro (Finland). All reagents were analytical grade and were used as received.

2.2. Sample preparation

Lactose was used as the test material, and physical mixtures and spray-dried samples were prepared.

Lactose samples with various degrees of amorphicity (0–100%) were prepared by spray- drying α-lactose monohydrate as described earlier [18]. A 15% (w/w) lactose suspension or solution was spray-dried using a Büchi Minispray Dryer 190 (Büchi Laboratorium-Technic AG, Switzerland). The ratio of ethanol to water in the feed solution varied between 0:100 and 100:0. The amorphous content of spray-dried lactose increased in conjugation with an increase in the amount of water in the feed solution [18]. Totally amorphous lactose was prepared by spray-drying using water as the feed solution and 100% crystalline lactose was prepared by spray-drying using ethanol as the feed solution. Lactose was mixed in an ethanol–water solution for 5 min at room temperature before spray-drying. The spray-drying variables were kept constant (Table 1). The only exception was totally amorphous lactose, which was spray-dried from pure distilled water at an outlet temperature of 112 °C and at an inlet temperature of 143 °C. After preparation, the samples were immediately stored in a dry atmosphere produced by silica gel.

Physical mixtures with an amorphous content of 0–100% (w/w) were prepared immediately before reaction by mixing accurately weighed quantities of 100% amorphous lactose and 100% crystalline lactose.

Table 1
Spray-drying parameters used to prepare lactose samples (nozzle, 0.7 mm)

Parameter	Value
Air flow rate (dial setting)	15
Feed rate (ml/min)	5.1
Atomizer air flow rate (norm l/h)	500
Inlet temperature (°C)	115–120
Outlet temparature (°C)	78–82
Heating rate (dial setting)	8

2.3. Solution calorimetry measurements

Solution calorimetry measurements were made at room temperature with a Parr Solution Calorimeter 1455 (Parr Instrument Company, USA) equipped with a Parr 1672 precision thermometer and a chart recorder. The thermograms were recorded at a sensitivity of 10 mV and 0.1–0.5 °C full scale and a chart speed of 3 cm/min (total measurement time was about 5 min). The measurements were performed under vigorous stirring (450 rpm). Heat calibration was conducted before the measurements, and the energy equivalence obtained was used for the corresponding heat calculations. For calibration, the mass of tris(hydroxymethyl)aminomethane was 500 mg and the mass of 0.1 M HCl was 100.0 g. The lactose samples were prestored in the dry atmosphere produced by silica gel at room temperature, and accurately weighted (about 400 mg with precision of 0.1 mg) just before the measurements. MeOH, ACN, EtOH, THF and acetone were used as solvents (100 ml). The temperature change during the reaction was detected and the reaction enthalpies were calculated. A negative value for the reaction enthalpy indicated heat evolution (exothermic reaction), and a positive value indicated heat absorption (endothermic reaction).

2.4. Raman spectra

The Raman spectra of lactose samples were measured with a CDD-Raman spectrometer (RAMSTAS) developed by VTT Electronics (Finland) [17,19]. In the present study, acquisition time for each spectrum was 1 min, and the power of the 830 nm laser at the sample was 100 mW. To average out the sample inhomogeneities, the spectra were measured at 10 different points for each sample. The diameter of the measurement spot was 0.5 mm. Two spectral bands were chosen for the determination, one centred at 440 cm^{-1} for amorphous lactose and another centred at 470 cm^{-1} for crystalline lactose. The spectra were baseline corrected, and band areas were integrated between spectral regions 490–410 and 490–450 cm^{-1}. The ratio of those areas was 0.328 for the totally amorphous sample and 0.729 for the lactose monohydrate.

3. Results and discussion

The present study evaluated the effect of solubility of a sample on solution calorimetry measurements. Earlier

Table 2
Total reaction enthalpies (J/g) of physical mixtures of lactose in different organic solvents measured by solution calorimetry

Amorphous content (%)	MeOH (J/g)	EtOH (J/g)	ACN (J/g)	THF (J/g)	Acetone (J/g)
0	115.1 ± 0.6	0.0 ± 0.2	20.5 ± 0.4	23.8 ± 0.9	48.1 ± 0.2
5	110.8 ± 1.6	–	–	–	–
10	99.7 ± 0.2	–	–	–	–
20	89.9 ± 3.2	–	–	–	–
40	62.9 ± 0.4	–	–	–	–
50	47.9 ± 2	–	–	–	–
70	18.9 ± 0.7	–	–	–	–
80	6.7 ± 0.3	–	–	–	–
90	−7.6 ± 0.5	–	–	–	–
100	−17.2 ± 0.2	–[a]	38.3 ± 0.3	22.7 ± 0.5	46.7 ± 1.0

Mean ± S.D. are shown ($n = 4$).
[a] The value could not be determined because the thermogram did not show any clear end point of reaction.

solution calorimetry studies have typically been based on total dissolution of the sample in water [e.g. 12,20]. Recently, Harjunen et al. demonstrated an excellent correlation between the enthalpy accompanied by addition of a sample in an oversaturated aqueous solution and the amorphous content of the sample, suggesting that solution calorimetry may also represent a useful method in samples that are not completely dissolved in a solvent [21]. In the present study, the correlation between the heat of solvation of lactose and the amorphous content of the sample was determined. The heat of solvation was determined in nonaqueous solvents where lactose was not dissolved during the measurements. It is known, that the heat of solvation values may involve several processes, such as wetting, crystallisation, breakage of bonds, liquid penetration, hydration and possibly rearrangement.

Reaction enthalpies of lactose samples were measured in MeOH, EtOH, ACN, THF and acetone (Table 2). Lactose is virtually insoluble in ACN, THF and acetone and only slightly soluble in alcohols (MeOH, EtOH) [22]. Table 2 shows that compared to 100% crystalline lactose, the value of reaction enthalpy for 100% amorphous lactose in MeOH was substantially lower. In contrast, the amorphous content of the sample did not greatly affect the reaction enthalpy in ACN, THF and acetone. In EtOH, the reaction enthalpy of 100% amorphous lactose differed from that of 100% crystalline lactose, but the reaction enthalpy value for the 100% amorphous lactose could not be determined because no clear endpoint of the reaction was noted in the thermogram.

An excellent linear correlation was observed between the reaction enthalpy and the amorphous content of the lactose sample (physical mixtures) in MeOH ($r = 0.999$). The theoretical limits of detection (LOD) and quantification (LOQ) were calculated from the data (IUPAC). LOD and LOQ values were determined from the residual standard deviation (σ_0) in the y-intercepts from the linearity data and slopes (b) using the equations $\mathrm{LOD} = 3\sigma_0/b$ and $\mathrm{LOQ} = 10\sigma_0/b$. LOD and LOQ of 1.3 and 4.4%, respectively (amorphous content), were obtained for the physical mixtures in MeOH.

The unknown amorphous content of the spray-dried lactose samples was determined both by solution calorimetry using MeOH as a solvent and by Raman spectroscopy using the standard curve shown in Fig. 1, as described by Niemelä et al [17]. The amorphous content of spray-dried lactose increased in parallel with the increase in the amount of water in the feed solution (Table 3), as observed in an earlier study where the amorphous content of the corresponding samples was measured by isothermal microcalorimetry [18]. The results obtained by solution calorimeter correlated well with results calculated by Raman spectrometer (Table 3).

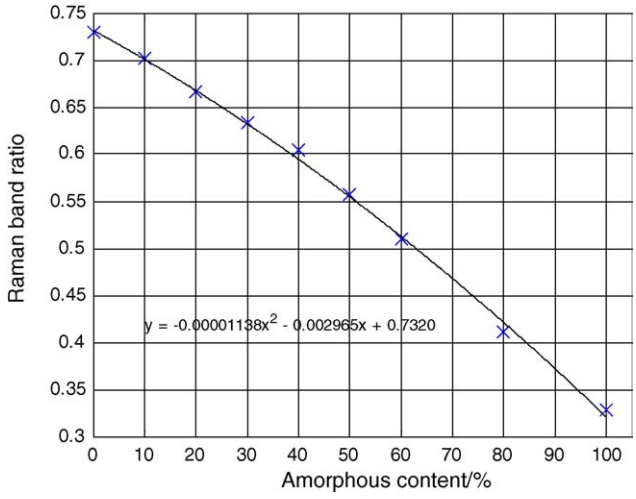

Fig. 1. Ratio of the Raman band areas as a function of the amorphous content in the physical mixtures. The correlation was determined for the mean values of amorphous content.

Table 3
Amorphous content of spray-dried lactose samples determined by solution calorimeter and Raman spectrometer

Ethanol:water ratio in feed solution	Amorphous content (%)	
	MeOH	Raman
10:90	98 ± 2	101 ± 2
20:80	60 ± 1	61 ± 3
25:75	44 ± 2	45 ± 3
30:70	36 ± 2	37 ± 3
35:65	30 ± 2	32 ± 1.4
75:25	7 ± 2	6.0 ± 0.7

Mean ± S.D. are shown ($n = 4$).

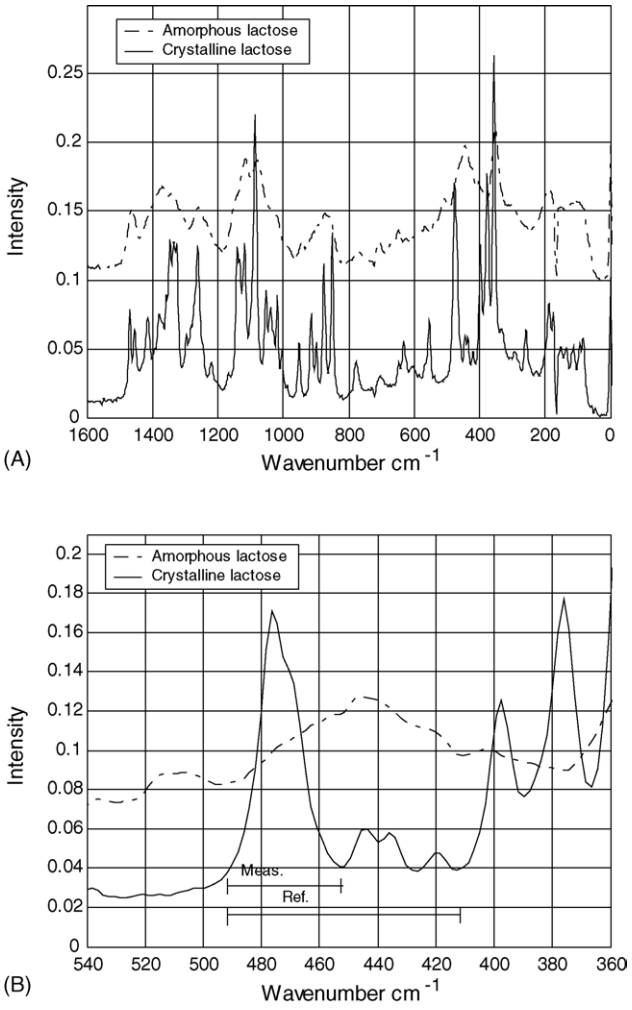

Fig. 2. Raman spectra of 100% crystalline α-lactose monohydrate (—) and 100% amorphous lactose (- - -) (A) and the area of the Raman spectra used in calculations (B).

The more heat evolved in the solution calorimetry process, the higher the amorphous content of the sample (Table 2). After the measurement in MeOH, the spray-dried lactose samples with the ethanol to water ratio 10:90 was taken off the reaction vessel, dried and weighed. The weight loss during measurement was only 0.5% (w/w), indicating that dissolution of the sample did not occur. Thus, what are the reasons for the observed energy change in the reaction? The process includes several steps, such as wetting, breakage of bonds, liquid penetration, and possibly rearrangement and crystallization. Crystallization of 100% amorphous lactose during measurement in MeOH was studied by Raman spectroscopy. The Raman spectra of 100% amorphous lactose and 100% crystalline α-lactose monohydrate clearly differ from each other over the whole spectral range (Fig. 2). Most bands of these two compounds are, however, overlapping and the largest shifts can be seen in the wave number range below 600 cm^{-1}. The bands centred at 470 and 440 cm^{-1} were chosen to represent the monohydrate and amorphous forms, respectively; and a simple band ratio method was used in the

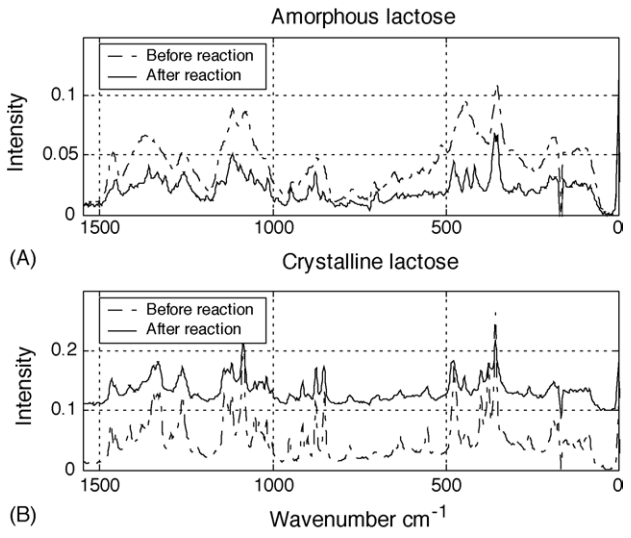

Fig. 3. Raman spectra of (A) 100% amorphous and (B) 100% crystalline α-lactose before and after reaction in MeOH.

calibrations. The spectrum of 100% amorphous sample after the reaction in MeOH reflects the crystallization of the sample (Fig. 3A). Thus, the observed results with solution calorimetry can be at least partly explained by the conversion of an amorphous sample to a crystalline sample. It is well-known that an amorphous material has a higher energy, entropy and free energy than the corresponding crystal form. As expected, a spectrum of 100% crystalline sample shows crystallinity also after the reaction (Fig. 3B).

4. Conclusions

The present results clearly indicate that total dissolution of a sample in a solvent is not necessary for the determination of its amorphicity by solution calorimetry. The amorphous content of a sample can be measured by solution calorimetry even when the sample is only slightly soluble in the solvent. However, the present results suggest that solvents where a sample is almost completely insoluble are not suitable for solution calorimetry determinations. The amorphous content values of the sample obtained with solution calorimetry, using a solvent in which the sample is slightly soluble, and Raman spectroscopy were in good agreement with each other.

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