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Comparison of solid-state NMR and isothermal microcalorimetry in the assessment of the amorphous component of lactose

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Abstract

The aim of this study was to evaluate the use of solid-state nuclear magnetic resonance (NMR) in the assessment of the degree of disorder of processed lactose. The results obtained from ^{13}C cross-polarisation/magic angle spinning NMR were compared with those obtained from isothermal heat-conductive microcalorimetry, in which the heat flow during crystallisation of the amorphous lactose in the samples was recorded. A series of lactose mixtures was prepared and studied. In agreement with earlier studies, the microcalorimetric analyses resulted in a linear relationship between heat flow and the nominal crystallinity of the lactose mixtures. Mathematical models established from NMR spectra accurately predicted results even when the lactose mixture contained an additional component, acetylsalicylic acid. The results obtained by microcalorimetry and solid-state NMR were in agreement. Both methods were very sensitive and could detect as low as 0.5% amorphous lactose in a mixture. In addition, solid-state NMR has the advantage of being able to provide structural information about a sample containing several different components.

Keywords: Isothermal microcalorimetry; Solid-state NMR; Crystallinity; Disorder; Lactose; PLS analysis

1. Introduction

Over the years, numerous published articles have focused on the importance of being able to detect and measure the amorphous content of pharmaceutical solids; various techniques for

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achieving this end have been proposed. This is an important field, since disruption or activation of the crystal structure of a solid material, for example during drying and milling, will result in various degrees of disorder in the form of crystal defects and a subsequent increase in the molecular mobility of the activated substance (Ahlneck and Zografi, 1990). The result may be advantageous, for example dissolution rate may be enhanced, or it may be less desirable, for example chemical stability may be decreased (Otsuka and Kaneniwa, 1990). Additionally, physical properties such as powder flow and compactability may be influenced by the presence of an amorphous component in the solid powder (Vromans et al., 1987; Sebhatu et al., 1994; Buckton and Darcy, 1996).

Crystallinity changes or polymorphism have been demonstrated using a variety of techniques. The most widely used technique to measure the degree of crystallinity is X-ray powder diffraction (Klug and Alexander, 1974). Other techniques that have been applied are infra-red spectroscopy (Susi and Ard, 1973; Ek et al., 1994), density measurements (Duncan-Hewitt and Grant, 1986), solid-state nuclear magnetic resonance (NMR; Earl and Parrish, 1983) and water vapour absorption (Saleki-Gerhardt et al., 1994).

Isothermal heat-conduction microcalorimetry and differential scanning calorimetry (DSC) measure the heat evolved during the transformation of amorphous material to its crystalline state (Angberg et al., 1991a,b, 1992). With isothermal heat-conduction microcalorimetry, the heat flow from ongoing processes is measured at a specified relative humidity and temperature. The sensitivity of the microcalorimeter (it can detect less than 2% of disorder in a sample) offers an advantage over most other techniques used for crystallinity determinations (Sebhatu et al., 1994; Buckton et al., 1995; Giron et al., 1997).

High resolution ^{13}C solid-state NMR spectra are obtained using proton decoupling and magic angle spinning (MAS) and sensitivity enhancement is achieved by cross-polarisation (CP) (Schaefer and Stejskal, 1978). ^{13}C solid-state NMR has the advantage of being a non-destructive test method that provides information about the structure of the material; it is, for example, possible to gain information on polymorphism (Atalla et al., 1980; Sugiyama et al., 1991), specific interactions in powder samples or the presence of contaminants or other components in the sample. To date, the use of solid-state NMR has been somewhat limited in the characterisation of pharmaceutical materials. However, some studies (e.g. Ek et al., 1994) have indicated that it may enable increased appreciation of the physical and chemical properties of a powder sample.

The aim of this study was to evaluate the use of solid-state NMR in the assessment of the amorphous component in processed lactose. The results obtained using solid-state NMR were compared with measurements from isothermal heat-conductive microcalorimetry, since this technique is known to provide a very sensitive measure of the degree of disorder (Sebhatu et al., 1994; Buckton et al., 1995).

2. Materials and methods

2.1. Materials

A sample of α-lactose monohydrate (Pharmatose, DMV, The Netherlands) was used as a reference material (corresponding to 100% crystalline lactose) and was also used to prepare lactose solutions which, after spray drying, resulted in totally amorphous lactose. In the evaluation of solid-state NMR, anhydrous α-lactose and β-lactose (AB Svenskt Mjölksocker, Sweden) were also used as reference substances. Physical mixtures of lactose of different degrees of order were obtained by mixing amorphous and crystalline lactose in different proportions ranging in amorphous content from 0.5 to 100%.

2.2. Methods

2.2.1. Preparation of powders

Crystalline α-lactose monohydrate was dissolved in water in a ratio of 1:6 to obtain a solution for spray drying. The solution was then spray dried in an A/S Niro atomizer (Anhydro, Denmark). The inlet temperature was 170–180°C

and the outlet temperature was 70–80°C. The solution feed flow rate was 0.6 l/h at a pressure of 4.5–5 kg/cm^2. After spray drying, the samples were dried at 60°C for 48 h to remove residual water and then stored at 0% RH (P_2O_5) before characterisation. This material was used as totally amorphous lactose (Sebhatu et al., 1994), as confirmed by measurement of a glass transition temperature (T_g) of at least 100°C using a differential scanning calorimeter, DSC (Mettler, TC10A/TC15, Switzerland).

To avoid segregation during mixing, a particle size fraction of 0–30 μm was obtained for both crystalline and amorphous lactose by elutriation using an air classifier (Alpine 100 MZR, Alpine, Germany). Density was measured using a helium pycnometer (Accu Pyc 1330, Micrometrics, USA). The powders were then stored at a constant relative humidity (0%; P_2O_5) at room temperature (22 ± 2°C) until mixed.

Physical mixtures of amorphous and crystalline lactose were prepared to give 0–100% crystalline content by weight. The components were weighted to a total amount of 25.00 g and were mixed in a turbula mixer (Turbula WAB, System Schatz, Switzerland) with 10 g of 2.0-mm glass beads for 100 min. The beads were added to enhance deagglomeration. The mixtures were sieved to remove the glass beads and then stored at 0% RH (P_2O_5) at room temperature (22 ± 2°C) until analysed.

2.2.2. Microcalorimetry

The microcalorimeter system used, the 2277 Thermal Active Monitor (TAM) (Thermometric, Sweden) has been described elsewhere (Suurkuusk and Wadsö, 1982; Angberg et al., 1992). This equipment consists of four independent isothermal heat-conduction microcalorimeters; the heat flow is monitored as a function of time. The heat evolved or absorbed can be obtained by integrating the heat flow curve over a specific time interval. The experimental temperature was 25°C and sample weights of 25–500 mg were used. The measurements were performed according to the miniature humidity chamber technique (Angberg et al., 1992). The powder sample was placed in the sample vessel together with a small tube filled with a saturated salt solution. Potassium bromide (81% RH) was used for samples with an amorphous content of 20% or more and sodium bromide (57% RH) was used for samples with lower concentrations of amorphous material (Nyqvist, 1983). Deionised water was used in the reference vessel. The sample and the reference were temperature equilibrated for 20 min within the TAM before the vessels were lowered into position for monitoring of the heat flow signal and the computer program was started. To minimise sample collection errors caused by the small sample size used and possible inhomogeneity of the mixtures, at least three samples from each lactose mixture were analysed and the mean value was calculated. Details of measurement, calibration and temperature equilibration have been described by Angberg (1992).

2.2.3. Solid-state NMR

The ^{13}C CP/MAS NMR spectra were recorded on a Bruker AMX-300 instrument at ambient temperature. The spectrometer operated at 75.47 MHz using a double air-bearing probe and ZrO_2 rotors. Spinning rate was 5 kHz, contact time was 0.8 ms, acquisition time was 37 ms, sweep width was 368 ppm and delay between pulses was 2.5 s. For each spectrum, 2000–15000 transients were accumulated with 2048 data points and zero filled to 4096 data points. The spectra were referenced to carbonyl in external glycine ($\delta = 176.03$ ppm). The spectra were manually phased and intensities of 612 spectral points in the interval 110–55 ppm were used in the data analysis for each spectrum. The experimental parameters were the same for all samples measured, since the spectra of amorphous and crystalline lactose were non-discriminating, as confirmed by the calibration models where the slope is equal to unity (Figs. 4 and 5).

2.2.4. Data analysis

The composition data and NMR spectra of the lactose mixtures were correlated using partial least squares (PLS) analysis (Geladi and Kowalski, 1986; Martens and Naes, 1989). Two models were obtained. The first PLS model, model A, used the whole range of crystalline/amorphous lactose compositions (0–100% crystallinity) and the

mean-centred ^{13}C CP/MAS NMR data for those mixtures. The composition of the mixtures was used as the Y-matrix and the intensities of 612 evenly distributed datapoints in each of the NMR spectra formed the X-matrix. Four PLS components were obtained where 80.7% of the total variance in the X-matrix explained 98.9% of the total variance in the Y-matrix.

A second PLS model, model B, was established using the range of lactose mixtures containing 0–10% amorphous lactose, and the mean-centred ^{13}C CP/MAS NMR data for these mixtures. The composition of the lactose mixtures (Table 1) was used as the Y-matrix and the intensities of 612 evenly distributed datapoints in the NMR-spectra comprised the X-matrix. Three PLS components were obtained where 73.9% of the variation in the X-matrix explained 99.7% of the variation in the Y-matrix. All computations were carried out using SIMCA 5.0 software.

Table 1
Mixtures of crystalline α-lactose monohydrate and amorphous lactose for PLS model B (Fig. 5), and corresponding predictions of amorphous content from NMR data

Amount of amorphous lactose in mixture (% by weight)	Amount of predicted amorphous lactose in mixture (% by weight)
0.00	0.16
0.50	0.32
1.00	0.87
2.00	1.91
3.00	3.02
4.00	3.84
5.00	5.33
6.00	6.47
9.00	8.96
10.00	9.66
8.00[a]	7.28
5.00[a]	5.96
3.00[a]	2.94
1.50[a]	1.63
5.00+ASA[a]	5.27

ASA, acetylsalicylic acid.
[a] Lactose mixtures used for the validation of PLS-model B (Fig. 5).

3. Results and discussion

3.1. Microcalorimetry

The results obtained by microcalorimetry are in agreement with earlier studies of mixtures of amorphous and crystalline lactose (Sebhatu et al., 1994; Briggner et al., 1994; Buckton et al., 1995). Fig. 1a–c shows microcalorimetry heat flow curves typical of those that were obtained in this study. Due to lack of structural information on the complex polymorphism occurring during the crystallisation event, it is difficult to interpret the flow curve precisely and different explanations of the peak profiles have been suggested (Briggner et al., 1994; Buckton and Darcy, 1995, 1996). Generally, the crystallisation process of lactose can be divided into three distinct phases (Sebhatu et al., 1994): phase I—absorption of water vapour, phase II—crystallisation of amorphous lactose, and phase III—a probable recrystallisation of anhydrous β-lactose to the α-monohydrate-form (Fig. 1a). However, many different events occur simultaneously during the crystallisation of amorphous lactose, and only the sum of the responses at a particular moment is seen in the heat flow curve. No attempt is made in this study to explain the complex crystallisation event; the purpose is rather to compare the use of microcalorimetry with that of solid-state NMR in measuring the amorphous content of a solid mixture.

Of the different microcalorimetric graphs obtained in this study, Fig. 1b is closest to the most common profile of the crystallisation of amorphous lactose seen in the literature (Briggner et al., 1994; Sebhatu et al., 1994). Phases I and II can clearly be seen but there is almost no sign of phase III. All of the samples in this study with an amorphous content of more than 20% had a profile like this. In Fig. 1c, phase III can be seen as a major peak, and an endothermic response (probably due to water desorption in phase II) is also seen (Darcy and Buckton, 1997). In almost all samples with an amorphous content of 10% or less, phase III was depicted as a relatively large peak, but this response did not appear to increase with the

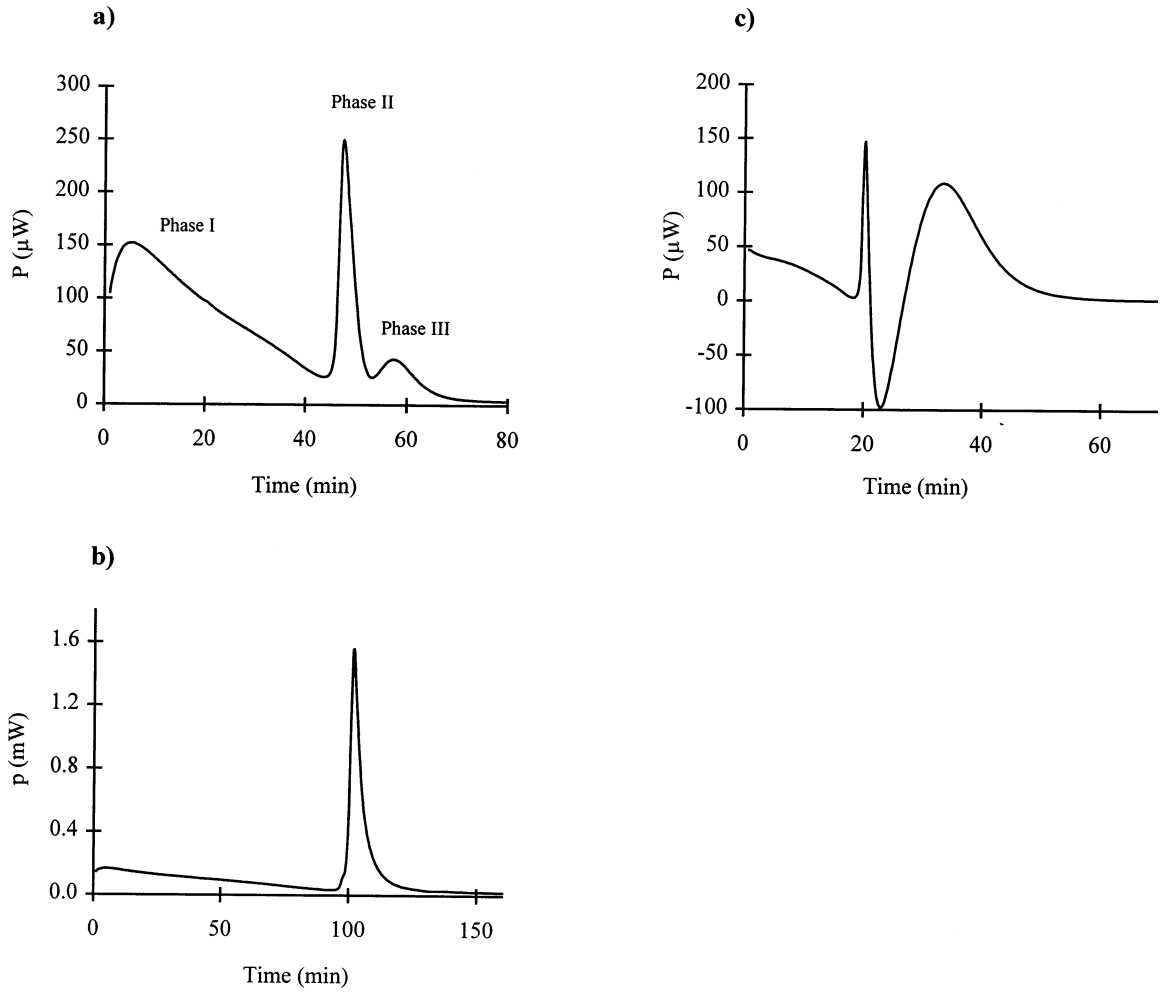

Fig. 1. Typical microcalorimetric profiles of lactose mixtures containing: (a) 1.5% amorphous lactose; (b) 40% amorphous lactose; (c) 4% amorphous lactose.

degree of order. For example, Fig. 1a shows the profile of a mixture containing 1.5% amorphous lactose and the phase III peak was smaller than that seen with 4, 6 or 8% amorphous lactose mixtures. Thus, it appears that the nature of phase III cannot simply be explained by an increase in the order of the mixture.

The area under the heat flow curve for a specific time interval can be integrated to obtain the extent of crystallinity of a lactose sample. Buckton and co-workers integrated the area under phase II–III or the whole curve (phases I–III) while Sebhatu et al. (1994) integrated only the area under the phase II part of the curve. Both methods have been shown to result in a linear relationship between the amount of amorphous material and the heat flow i.e. the integrated area. In the present study, integration of the area under the phase II–III part of the curve (Fig. 2a,b) resulted in a linear relationship, while integration of only the area under the phase II part deviated slightly from linearity. Integration of the whole spectrum (phases I–III) was not undertaken. The reason for the better linearity when integrating both phase II and phase III is not clear, but the sometimes very pronounced peak of phase III does seem to be an important part of the total crystallisation process. Although the ratio be-

tween the area under peak II and that under peak III differed among the samples, the total area under peaks II and III resulted in a linear relationship. It therefore seems likely that, to obtain a representative value of the extent of crystallisation, the integration should be performed on the area under both peaks.

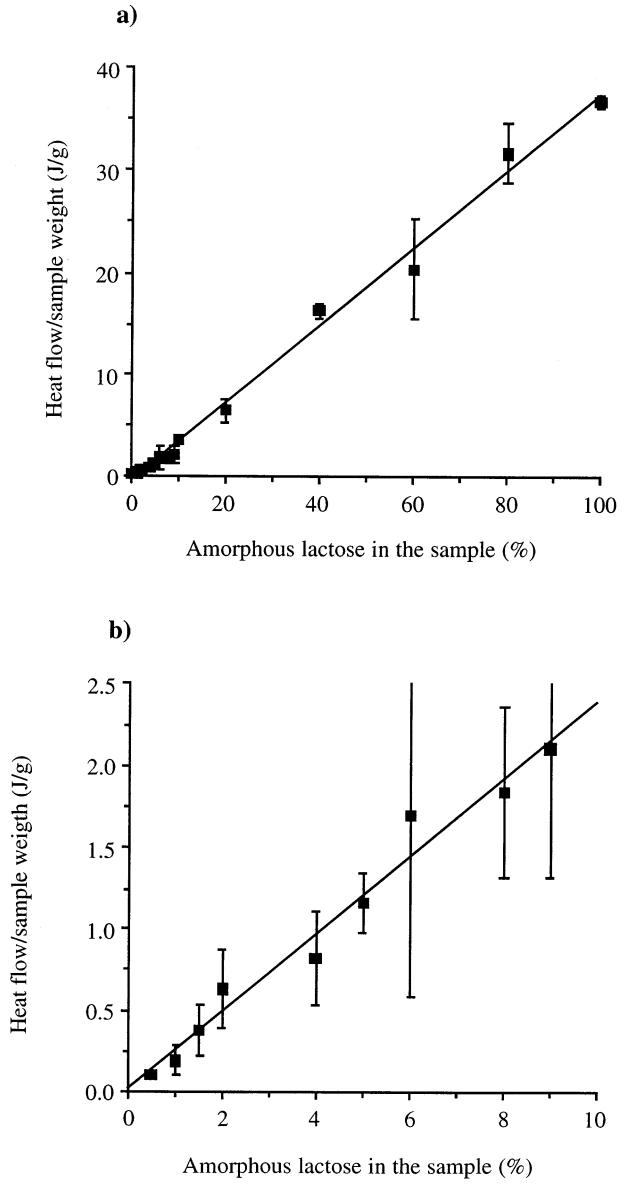

Fig. 2. Heat flow as a function of crystallinity for lactose mixtures containing: (a) 0–100% amorphous lactose, correlation coefficient 0.994; (b) 0–9% amorphous lactose, correlation coefficient 0.973 (bars representing standard deviations).

The microcalorimetry experiments in this study resulted in somewhat larger variation in the results than previously reported (Sebhatu et al., 1994; Briggner et al., 1994). There are several possible explanations for this. Firstly, the physical mixtures of amorphous and crystalline lactose were prepared by mixing the powders in a turbula mixer with glass beads in a manner comparable with the industrial production of pharmaceutical powder mixtures, and the level of variation would be expected to correspond with that seen in industrial production. In contrast, in microcalorimetric studies by Sebhatu et al. (1994) and Briggner et al. (1994), the amorphous and crystalline components were weighed directly into the sample vessel, thereby eliminating environmental stress and handling of the powders. Heterogeneity of the mixtures and the methods of sample collection and handling will probably be the largest source of variation. The glass beads may also have caused disturbances, although this was not confirmed when pure crystalline α-monohydrate was mixed with glass beads. Mixing and size fractionation of the powders was performed under ambient conditions rather than at 0% relative humidity and the possibility of the absorption of moisture during mixing and sieving cannot be excluded. In a recent study by Darcy and Buckton (1998), the relative humidity used during the crystallisation event is reported to affect the obtained size of the net calorimetric peaks. Since two different humidities were used in the present study, this may also have contributed to the size of the error. Further, commercial α-lactose monohydrate is known to contain a small amount of β-lactose anhydrate (Lerk, 1983). Variations in the proportion of the β-anhydrate form in the commercial α-lactose monohydrate used may have contributed to the variations in the phase III response if this phase is indeed caused mainly by the transition of the β-form of lactose to the α-monohydrate form.

3.2. ^{13}C CP/MAS NMR

The ^{13}C CP/MAS NMR spectra of the reference samples of α-anhydrate, β-anhydrate and

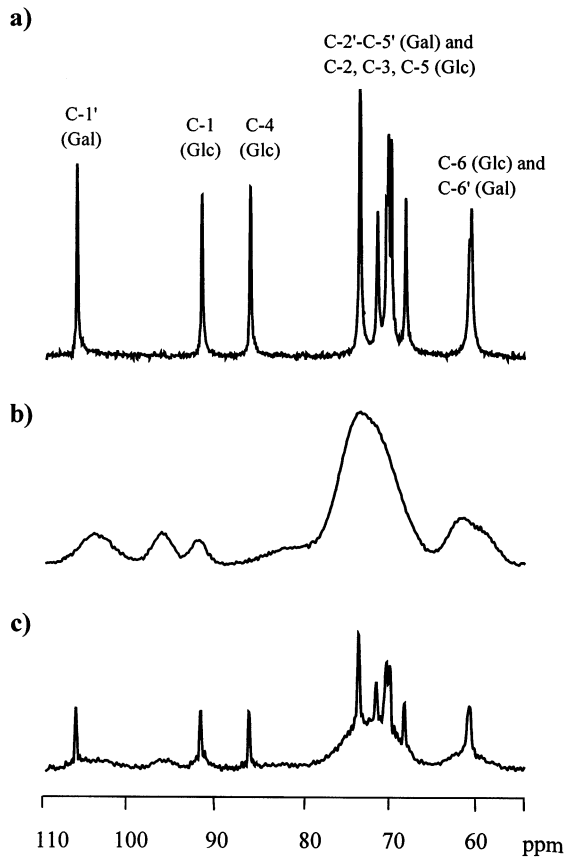

Fig. 3. ^{13}C CP/MAS NMR spectra of (a) 100% crystalline lactose sample; (b) 100% amorphous lactose sample; (c) 90% crystalline–10% amorphous lactose. Chemical shifts assigned to the corresponding monosaccharide and carbon atom in the α-monohydrate lactose molecule i.e. α-D-glucose and β-D-galactose denoted as Glc C-1–C-6 and Gal C-1′–C-6′, respectively (Earl and Parrish, 1983).

α-monohydrate lactose were in agreement with earlier studies (Earl and Parrish, 1983). In Fig. 3, the chemical shifts have been assigned to the corresponding monosaccharide and carbon atom in the α-monohydrate lactose molecule according to Earl and Parrish (1983). The two constituent monosaccharides of α-monohydrate lactose molecule are α-D-glucose and β-D-galactose which have been denoted as Glc and Gal, respectively. The figure contains examples of ^{13}C CP/MAS NMR spectra from samples containing 100% crystalline α-lactose monohydrate (Fig. 3a), 100% amorphous lactose (Fig. 3b) and a mixture of 90% crystalline α-lactose monohydrate and 10% amorphous lactose (Fig. 3c). A typical peak corresponding to amorphous lactose is shown at 93–96 ppm (Fig. 3b,c). The intensity of this peak is expected to increase with the amorphous content of the sample and in addition, a general broadening of all the peaks included in the spectra is seen.

The correlation plot for PLS model A (covering 0–100% lactose crystallinity) is shown in Fig. 4. The correlation coefficient is high (0.994) and the mixtures are spread evenly along the correlation line, indicating a good model. The spectra of amorphous and crystalline lactose are non-discriminating, as confirmed by the slope of the curve which is equal to unity.

PLS model B used only low amounts of amorphous lactose (0–10% amorphous content, Table 1). The correlation plot for this model is shown in Fig. 5 (denoted as filled boxes). Again, the correlation coefficient is high (0.998) and the mixtures are spread evenly along the correlation line.

PLS model B was validated by predicting the crystallinity composition from the NMR spectra of four freshly prepared lactose mixtures, containing 8, 5, 3 and 1.5% of amorphous lactose (de-

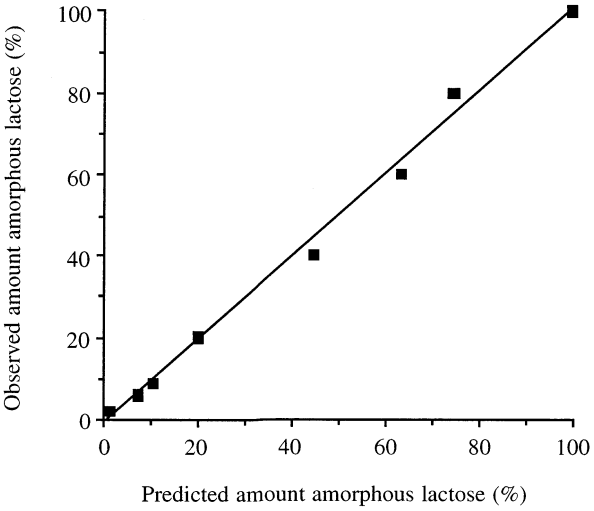

Fig. 4. The PLS correlation plot for model A. The actual values of the amorphous content of the mixtures were entered on the Y-axis, and the predicted values for amorphous content (obtained from NMR spectra) were entered on the X-axis. The correlation coefficient is 0.994.

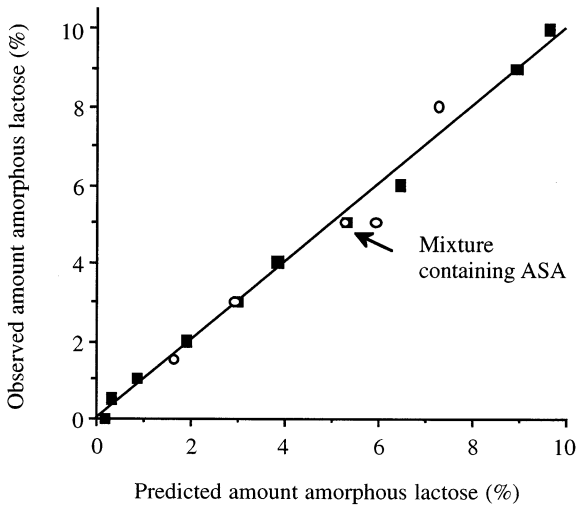

Fig. 5. The PLS correlation plot for model B (■). The actual values of the amorphous content of the mixtures were entered on the Y-axis, and the predicted values for amorphous content (obtained from NMR spectra) were entered on the X-axis. The correlation coefficient is 0.998. The predicted values from five new lactose mixtures (○).

noted as circles in Fig. 5). In addition, acetylsalicylic acid was added to a lactose mixture containing 5% amorphous lactose and estimated in the same manner (Fig. 5). The estimations from NMR data fitted accurately on the correlation line, demonstrating the high predictive ability of PLS model B. Thus, it is possible to use solid-state NMR measurements to estimate the crystallinity of lactose mixtures even if the amorphous content is very low or if the lactose mixture contains an additional component. The accuracy is within $\pm 1\%$.

3.3. Comparison of techniques

A summary of the two techniques evaluated in this study is shown in Table 2. Both techniques can detect lower than 0.5% amorphous content in a lactose sample. Accurate calibration models are necessary for both methods, and the preparation of these models takes both time and materials. A simplified approach to estimate the amorphous content based on calculation of the enthalpy of crystallisation and fusion has recently been suggested (Philips, 1997); this method would not require a calibration model. However, the exact determination of the amorphous content of drug substances requires an accurate calibration standard, and confirmation of the standard using definitive measures of crystallinity (Hancock, 1998).

In general, the range of time (30 min to about 4 h) required to obtain the crystalline content of the lactose samples used in this study was about the same with both isothermal microcalorimetry and solid-state NMR. However, while lower proportions of amorphous lactose in the sample required less time for analysis with microcalorimetry, the reverse was true for solid-state NMR. This is because of differences in the measured parameters: the response of a reaction or the actual structure of the substance.

The advantages of solid-state NMR are that it is a non-destructive analytical method, it is specific to the material under investigation and it can provide information about the structure of the material and thus also about additional components or contaminants in the sample. However, with ^{13}C CP/MAS NMR, only compounds containing carbon can be analysed. The microcalorimetry method has the advantage of

Table 2
Comparison of isothermal microcalorimetry and solid-state NMR for characterisation of the amorphous content of lactose

	Isothermal microcalorimetry	^{13}C CP/MAS NMR
Detection limit	$\leqslant 0.5\%$ amorphous lactose[a]	$\leqslant 0.5\%$ amorphous lactose
Time for analysis (h)	0.5–4	0.5–10
Destroys sample	Yes	No
Other components detected	No	Yes
Sample weight (mg)	20–300	500–700
Prediction limits	Within 1–2%[a]	Within 1%
Reproducibility[b]	Good	Very good
Provides structural information	No	Yes
Calibration model necessary	Yes	Yes

[a] Estimated using the results of this study and those from Buckton et al. (1995).
[b] Dependent upon the proportion of amorphous lactose in the sample and the homogeneity of the mixtures.

requiring a smaller amount of material for the analysis than NMR, although the amorphous portion of the sample crystallises during analysis, thus destroying the original sample. The small amount of material needed for analysis may also be a drawback, since large variations between the samples may arise due to inhomogeneity of the powder mixture. However, the lack of specificity is the main disadvantage of isothermal microcalorimetry.

3.4. Conclusions

Microcalorimetry and solid-state NMR provided comparable results in this study. It is important to emphasise that these good results were obtained for powder mixtures prepared in a manner comparable to industrial production of pharmaceutical mixtures. Isothermal microcalorimetry is already recognised as a very sensitive technique for measuring the degree of disorder of a substance and, in this paper, solid-state NMR has also been shown to be a sensitive method of assessing the amorphous content of lactose powder mixtures. In addition, solid-state NMR is a specific, non-destructive analytical method, which offers information about the structure of the material and any existing contaminants. However, additional studies are required to consolidate the detection limit of the technique and the reliability of the solid-state characteristics obtained for other pharmaceutical materials.

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