Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 38 (2005) 186–190
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Measurement of lactose crystallinity using Raman spectroscopy

Bridget M. Murphy[a], Stuart W. Prescott[b], Ian Larson[a,*]

[a] *Department of Pharmaceutics, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Vic. 3052, Australia*
[b] *Particulate Fluids Processing Centre, School of Chemistry, University of Melbourne, Vic. 3010, Australia*

Received 20 August 2004; received in revised form 9 December 2004; accepted 9 December 2004
Available online 22 January 2005

Abstract

Raman spectroscopy (RS) was used to determine the crystallinity of lactose (a commonly used carrier in dry powder inhaler (DPI) formulations). Samples of α-lactose monohydrate and amorphous lactose were prepared using ethanol precipitation and lyophilisation respectively. The anomeric forms were confirmed using DSC at a rate of 10 °C/min and heated to 250 °C. The Raman spectra of both α-lactose monohydrate and amorphous lactose were obtained. Distinguishable differences were seen between the two spectra including peak areas and intensities. Depolarisation ratios (ρ) of each form were then determined to identify the crystallinity of the lactose carrier samples. At the prominent Raman bands 865 and 1082 cm^{-1}, significant differences in ρ values were observed for crystalline (0.80 ± 0.07, 0.89 ± 0.06 respectively) and amorphous samples (0.44 ± 0.07, 0.51 ± 0.10).

Keywords: Raman spectroscopy; Depolarisation ratio; Lactose; Crystallinity; Anomeric form; Dry powder inhalation

1. Introduction

Knowledge of the crystallographic form of actives and excipients in solid dose forms is a necessity for the pharmaceutical industry. The ability of a compound to crystallise into two or more chemically identical, yet functionally or behaviourally different, polymorphic arrangements or into an amorphous form can greatly affect formulation, stability, and efficacy [1].

Dry powder inhalers (DPIs) for pulmonary delivery of therapeutic drugs have advantages over pressurised metered dose inhalation aerosols (pMDIs) in that they avoid the use of propellants and coordinated administration with inspiration [2]. DPI formulations generally consist of micronised drug (1–5 μm) and an inert coarse carrier particle (50–200 μm) which aids the flow and dispersion of the highly cohesive drug particles [3]. The carrier commonly used is α-lactose monohydrate.

As a solid, lactose is known to occur in either one of three crystalline forms (α-lactose monohydrate, α-lactose anhydrous, and β-lactose anhydrous) or in an amorphous state. The amorphous state, however, is a thermodynamically unstable state with a higher energy level than the crystalline forms, leading to problems regarding stability, hygroscopicity, and the transformation to the more stable crystalline form [4].

Deaggregation of lactose and drug during administration is affected by the crystalline form of lactose [5]. This determines the surface energy of the lactose particles and therefore the forces of interaction between lactose and drug particles [6]. Micronisation of crystalline lactose often leads to disruption or activation of the crystalline structure and varying degrees of disorder through the formation of residual amorphous regions [7]. Any amorphous content of micronised α-lactose monohydrate will affect these interactions and lead to issues in the efficiency of DPI formulations [5]. Therefore, a fast and inexpensive method for determining crystallinity is desirable.

X-ray powder diffraction (XRPD) is widely used to determine the crystallinity of lactose powders [8]; however, it requires advanced hardware and software and can be time

* Corresponding author. Tel.: +61 3 9903 9570; fax: +61 3 9903 9583.
E-mail addresses: bridget.murphy@vcp.monash.edu.au (B.M. Murphy), ian.larson@vcp.monash.edu.au (I. Larson).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.12.013

consuming [9]. Thermal analytical techniques such as differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) have also been used for the semi-quantitative evaluation of the crystallinity of lactose samples containing amorphous regions [10,11]. These techniques, however, are generally only used as complementary analytical tools.

As an alternative technique, Raman spectroscopy (RS) has many advantages over XRPD and other spectroscopic techniques such as infra-red spectroscopy. XRPD, DSC, and TGA all measure crystallinity as an average of an ensemble of particles within a solid sample whereas RS, when coupled to an optical microscope, has the potential to specifically determine the crystallinity of a single particle within that ensemble. The Raman method requires no sample preparation, is non-invasive, and non-destructive [12]. It is also relatively inexpensive compared to XRPD and provides a rapid technique for the measurement of sample crystallinity.

RS has already been applied to the identification and characterisation of the polymorphic forms of pharmaceutical actives [13–17]. Through the measurement of the wavelength and intensity of inelastically scattered light from molecules, RS probes the molecular and crystal lattice vibrations and is therefore sensitive to the composition, bonding, chemical environment, phase, and crystalline structure of the material [18]. Thus it has been identified as a possible method for determining the crystallographic form of common drug excipients such as lactose, where this is necessary.

The incident laser for Raman scattering is linearly polarised, while the positioning of a polarisation filter and half-wave plate between the sample and detector permits the collection of additional information in terms of the symmetry of the scattering tensor.

The Raman depolarisation ratio, ρ, is given by the intensity of the perpendicularly polarised response, $I_{\perp}$, divided by that of the parallel polarisation intensity, $I_{\parallel}$:

$$\rho = \frac{I_{\perp}}{I_{\parallel}}$$

The depolarisation ratio is typically in the range 0 to 3/4, with values approaching 3/4 indicating that the Raman band is depolarised [19] and lower values indicating that the band is polarised (typical of many crystalline substances [20]). Depolarisation ratios are used to determine the symmetry of a vibration of a molecule in the measured sample form. Thus, with ρ dependent on the crystallinity of the sample, numerical analysis of crystallinity is possible.

While the potential value of RS has been greatly enhanced over the years, previous studies on lactose have only distinguished between solid α- and β-lactose [21]; there appear to be no published Raman spectra of amorphous lactose.

The principle aims of this study were to prepare α-lactose monohydrate and amorphous lactose and to produce a valid method of characterisation using Raman spectroscopy.

2. Experimental

2.1. Materials

Hydrous lactose 100 mesh was supplied by The Lactose Company, Whyndale brand (New Zealand). Water was obtained from a MilliQ Academic A10 filtration system using Quantum™ EX Ultrapure Organex cartridge and 0.22 μm Millipak®40 (Millipore, MA, USA). Absolute ethanol was provided by CSR Ltd. (Yarraville, Australia).

2.2. Preparation of crystalline and amorphous lactose

Lactose (10 g) was dissolved in distilled water (100 mL) at 55 °C. Ten millilitres of this lactose solution was added to 90 mL of absolute ethanol at 55 °C without stirring. The solution was covered tightly with parafilm to prevent evaporation and left standing for 12 h at room temperature. The supernatant was decanted, and the remaining crystals dried for 12 h at 70 °C in a laboratory oven.

A 5% (w/v) lactose solution was lyophilised. The amorphous lactose was stored immediately in a refrigerated desiccator.

2.3. Differential scanning calorimetry

Differential Scanning Calorimetry (Perkin Elmer, DSC 7) was used to characterise the lactose samples. The DSC sample weights were between 3.5 and 5.0 mg. Sealed aluminium pans were used and measurements were made in an atmosphere of nitrogen, with a heating rate of 10 °C/min over a temperature range of 50–250 °C. Three measurements were taken for each sample.

2.4. Raman spectroscopy

A Raman microscope (Ramascope 2000 Renishaw PLC (UK) installed with a 782 nm diode laser together with a Leica DMLM optical microscope) was used to characterise the lactose samples and determine their depolarisation ratios. The instrument was calibrated by measuring the spectra of both silica and sulfur prior to each sample set. All measurements were conducted in a darkened room. A small amount of each sample was placed onto a microscope slide and the microscope objective focused using white light. Measurements were taken using a 50× objective with a grating scan over the region 50–1500 cm^{-1}. The scan time was 10 s and three scans were accumulated per measurement. After each initial measurement, a linear polariser was placed into the beam path and its transmission axis aligned to be parallel to that of the excitation beam and an accumulated scan taken to give a parallel polarisation measurement. This constituted the first of the two measurements. A half-wave plate was then inserted before the linear polariser to obtain the perpendicular polarisation measurement. This constituted the final of the measurements. To assess the reproducibility of the method,

three accumulated scans were taken on each of two different samples and assessed for inter- and intra-sample variance.

3. Results and discussion

3.1. Characterisation of lactose particles by DSC

During the crystallisation process of α-monohydrate lactose, water is incorporated into the crystal lattice. The DSC curve of α-monohydrate lactose shows an endothermic transition starting at approximately 140 °C (see Fig. 1a) which corresponds to the dehydration of the sample and is referred to as the peak of water of crystallisation. This thermal dehydration converts α-lactose monohydrate into its unstable anhydrous form, which is indicated by the melting endotherm of α-anhydrous lactose at approximately 220 °C and its subsequent degradation.

The DSC curve of amorphous lactose showed an initial exothermic transition at 110 °C (see Fig. 1b) indicating the crystallisation of amorphous to crystalline lactose through thermal treatment. This is subsequently followed by the endothermic transitions of crystalline lactose and its degradation. This behaviour is typical for amorphous lactose [22–24].

3.2. Characterisation of lactose particles using Raman spectroscopy

Raman spectra of α-lactose monohydrate and amorphous lactose (Fig. 2) were visually distinguishable permitting the crystallinity of the samples to be quantified using fitting [25] or peak height ratios [26]. Defined peaks occurred over the entire fingerprint region of the α-lactose monohydrate spectra, consistent with previously published results using RS [21]. Amorphous lactose showed an absence of peak splitting with wider peak areas occurring at similar Raman shift

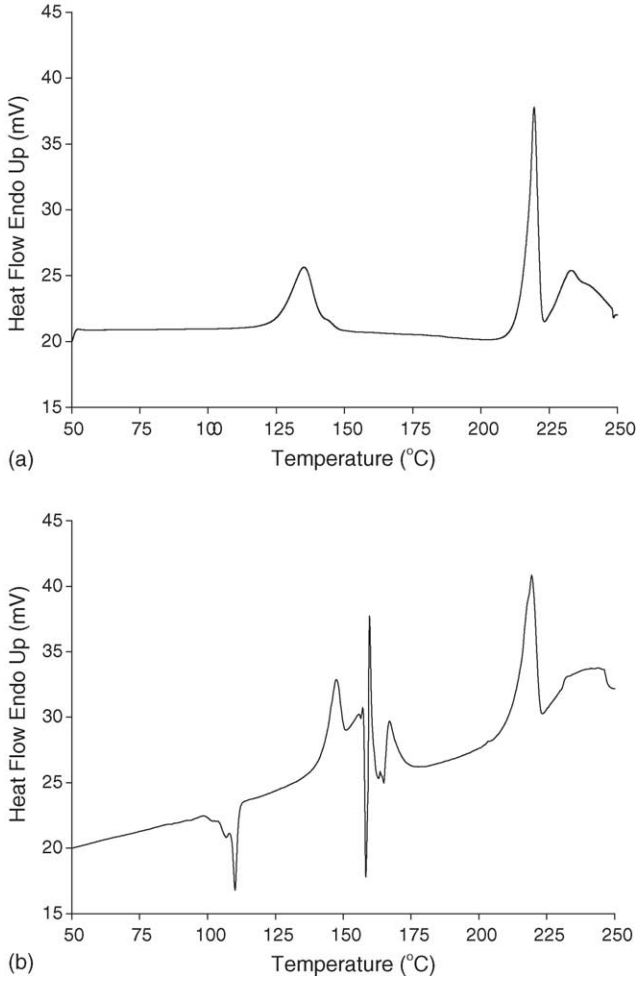

Fig. 1. DSC thermograms of (a) α-lactose monohydrate and (b) amorphous lactose. Endothermic transitions are seen at approximately 140 and 220 °C for α-lactose monohydrate. Conversion of amorphous lactose to crystalline α- and β-lactose is indicated by an exothermic transition at 110 °C with further endothermic transitions and subsequent decomposition of the sample.

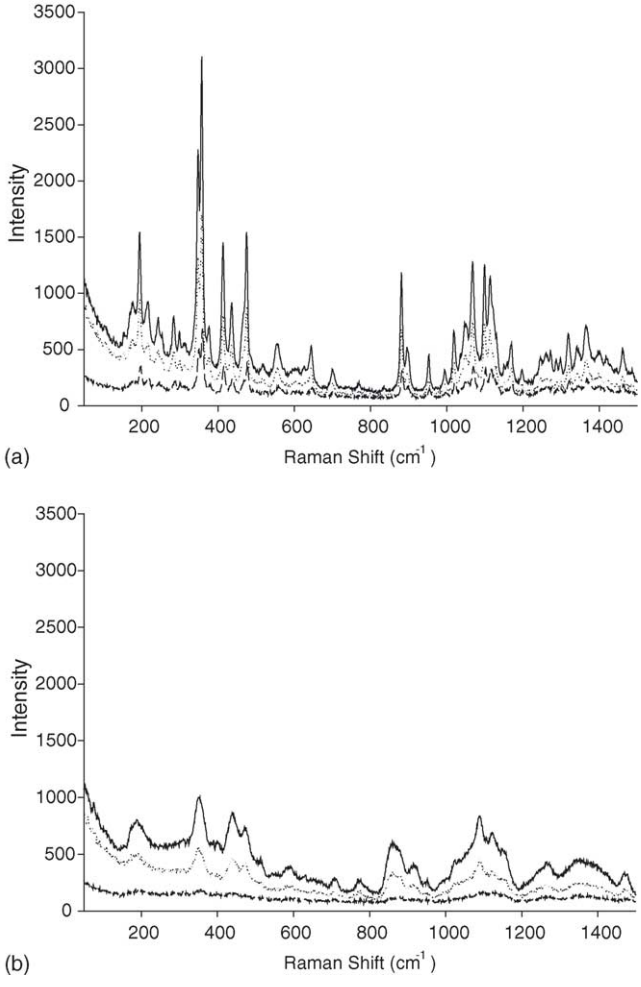

Fig. 2. Raman spectra of lactose samples showing the differences in intensity observed between normal (bold line) parallel polarised (dotted line) and perpendicular polarised (dashed line) scans for (a) α-lactose monohydrate and (b) amorphous lactose.

Table 1
Comparison of the polarisation ratios obtained for lactose samples at various Raman shift values

Sample	Raman shift	
	865 cm^{-1}	1082 cm^{-1}
α-Lactose monohydrate	0.80 ± 0.07	0.89 ± 0.06
Amorphous lactose	0.48 ± 0.04	0.55 ± 0.05

Standard deviation was calculated where $N = 6$.

values to the prominent peaks of α-lactose monohydrate. These broad peaks are indicative of a lack of crystallinity [20,26]. A large decrease in overall spectral intensity was also observed.

Table 1 shows the depolarisation ratios (ρ) of α-lactose monohydrate and amorphous lactose at two prominent Raman bands. Previous studies of Raman spectra of α-lactose assigned the band reported here as being at 1082 cm^{-1} to stretching vibration of the bridge COC group [21]. The tentative assignment of the 865 cm^{-1} band to an OCO bending mode could be made [21]; however, this mode is likely to be strongly coupled to other vibrations and full assignment of the vibrational spectrum would require considerable computational effort.

The obtained depolarisation ratios for crystalline and amorphous lactose are not as one would expect; the Raman bands 865 and 1082 cm^{-1} in the crystalline sample are significantly depolarised, while in the amorphous sample they are polarised. Further to this, the depolarisation ratios are outside the theoretical range of 0 to 3/4. At other prominent bands (185, 352 and 443 cm^{-1}) there is no marked difference between the depolarisation ratios of the amorphous or crystalline sample. Possible explanations for these data are as follows.

The lactose molecule is low symmetry (C_1), meaning that the Raman spectra of α-lactose monohydrate is quite complex, with 129 Raman-active vibrational modes [21]. The local symmetry of the nuclei in the vibrator are such that in solution and in the amorphous solid some of the Raman bands are polarised, as previously described for various gases [27] and solid state samples such as glasses, solidified melts and some types of polymer [28].

Secondly, the monoclinic and multiply hydrogen bonded crystal structure of α-lactose monohydrate [29] is known to rotate the plane of polarised light (optical activity or rotary polarisation). This could have the effect of depolarising the bands in the crystalline sample, with the path length for the incident light being different for each scattering event, thus rotated to a different degree.

Despite the unpredicted, yet not unprecedented depolarisation results, RS is capable of distinguishing crystalline and amorphous lactose. The depolarisation ratios at 865 and 1082 cm^{-1} are significantly different, as is the overall Raman spectra of crystalline and amorphous lactose visually, allowing for the simple determination of lactose anomeric form.

3.3. Reproducibility of Raman spectroscopic method

Inter- and intra-sample variance was assessed and found to be within acceptable limits. The inter-sample coefficients of variation (CV) for depolarisation ratios of Raman bands 865 and 1082 cm^{-1} were determined as 3.0% and 5.6% respectively for α-lactose monohydrate, and 7.8% and 4.1% for amorphous. Intra-sample CV values were also within acceptable limits with the highest CV values for α-lactose monohydrate 5.3% for 865 cm^{-1} and 6.7% for 1082 cm^{-1} and values of 13.78% and 5.70% respectively for amorphous lactose.

4. Conclusion

Raman spectroscopy has been demonstrated to be an effective method to distinguish α-lactose monohydrate and amorphous lactose. Samples confirmed by DSC to be either of crystalline or amorphous form, displayed clearly different Raman spectra, mainly characterised by a decrease in peak definition and in peak intensity of the amorphous form. Depolarisation measurements of various Raman bands in the samples were shown to be affected by the crystallinity of the samples; the Raman bands in the crystalline samples were seen to be depolarised, while those of the amorphous sample were partially polarised.

Acknowledgements

Helpful discussions with Dr Stephen Best and A/Prof. Paul Mulvaney (University of Melbourne) are gratefully acknowledged. The Particulate Fluids Processing Centre is established and supported by the Australian Research Council's Research Centres Programme.

References

[1] K. Knapman, Mod. Drug. Disc. 3 (2000) 53–54.
[2] Y. Kawashima, T. Serigano, T. Hino, H. Yamamoto, H. Takeuchi, Int. J. Pharm. 172 (1998) 179–188.
[3] X.M. Zeng, G.P. Martin, C. Marriott, J. Pritchard, Int. J. Pharm. 200 (2000) 93–106.
[4] B.C. Hancock, G. Zografi, J. Pharm. Sci. 86 (1997) 1–12.
[5] P. Harjunen, V.-P. Lehto, K. Martimo, E. Suihko, T. Lankinen, P. Paronen, K. Jarvinen, Eur. J. Pharm. Sci. 16 (2002) 313–321.
[6] M.D. Louey, S. Razia, P.J. Stewart, Int. J. Pharm. 252 (2003) 87–98.
[7] A. Saleki-Gerhardt, C. Ahlneck, G. Zografi, Int. J. Pharm. 101 (1994) 237–247.
[8] H. Larhrib, G.P. Martin, D. Prime, C. Marriott, Eur. J. Pharm. Sci. 19 (2003) 211–221.
[9] X. Chen, S. Bates, K.R. Morris, J. Pharm. Biomed. Anal. 26 (2001) 63–72.
[10] M. Angberg, Thermochim. Acta 248 (1995) 161–176.
[11] P.S.-R.Á. GombÁs, M. Kata, G. Regdon Jr., I. Eros, J. Therm. Anal. Calorim. 68 (2002) 503–510.

[12] G. Fini, J. Raman Spectrosc. 35 (2004) 335–337.
[13] S.C. Pinzaru, I. Pavel, N. Leopold, W. Kiefer, J. Raman Spectrosc. 35 (2004) 338–346.
[14] C.J. Strachan, D. Pratiwi, K.C. Gordon, T. Rades, J. Raman Spectrosc. 35 (2004) 347–352.
[15] D. Pratiwi, J.P. Fawcett, K.C. Gordon, T. Rades, Eur. J. Pharm. Biopharm. 54 (2002) 337–341.
[16] S.E.J. Bell, J.R. Beattie, J.J. McGarvey, K.L. Peters, N.M.S. Sirimuthu, S.J. Speers, J. Raman Spectrosc. 35 (2004) 409–417.
[17] K.L.A. Chan, O.S. Fleming, S.G. Kazarian, D. Vassou, G.D. Chryssikos, V. Gionis, J. Raman Spectrosc. 35 (2004) 353–359.
[18] G.D. Smith, R.J.H. Clark, J. Archiv. Sci. 31 (2004) 1137–1160.
[19] E. Spinner, Spectrochim. Acta: Part A 59 (2003) 1441–1456.
[20] J.J. Seyer, P.E. Luner, M.S. Kemper, J. Pharm. Sci. 89 (2000) 1305–1316.
[21] H. Susi, J.S. Ard, Carbohydr. Res. 37 (1974) 351–354.
[22] A. Olano, N. Corzo, I.M. Castro, Food Chem. 14 (1984) 53–63.
[23] P. Darcy, G. Buckton, Thermochim. Acta 316 (1998) 29–36.
[24] C.F. Lerk, A.C. Andreae, A.H. De Boer, P. De Hoog, K. Kussendrager, J. Van Leverink, J. Pharm. Sci. 73 (1984) 856–857.
[25] C. Branca, S. Magazu, G. Maisano, P. Migliardo, J. Chem. Phys. 111 (1999) 281–287.
[26] L.S. Taylor, G. Zografi, Pharm. Res. 15 (1998) 755–761.
[27] A. Bramley, J. Franklin Inst. 204 (1927) 231–237.
[28] M.J. Gall, Spectrochim. Acta: Part A 28 (1972) 669–672.
[29] C.A. Beevers, H.N. Hansen, Acta Cryst. B 27 (1971) 1323.