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Lactose: A definitive guide to polymorph determination

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Abstract

Lactose is a well-known molecule capable of forming a number of different polymorphs with varied chemical and physical properties. To date, no definitive guide for distinguishing between polymorphs using simple analytical techniques has been available. The information presented in this article aims to provide a conclusive guide for identifying the polymorphs of lactose and to successfully unravel years of contradictory research. Data have been collected on single phase polymorphs, prepared from an identical source, adopting the use of *in situ* and *ex situ* powder X-ray diffraction, CCD-Raman, FT-IR and ^{13}C–^{1}H cross-polarisation magic angle spinning NMR (CP-MASNMR) spectroscopy, in order to provide simple methods to discriminate between the polymorphs.

Keywords: Lactose; Polymorphism; X-ray diffraction; Raman; FT-IR; CP-MASNMR

1. Introduction

The breadth of research carried out on lactose, with the empirical formula, $C_{12}H_{22}O_{11}$, has been varied and immense. Much emphasis has been given to factors such as the determination of crystallinity and amorphous content and the analysis of the different lactose polymorphs themselves. The diverse chemical and physical properties known to be generated from the different polymorphs (Giron, 1995; Morris et al., 1998; Bernstein et al., 1999) affect the use of lactose in food stuffs, flavours and additives and its applications in pharmaceutical products such as dry powder inhalers (DPIs). Physical chemists, pharmaceutical chemists and food scientists have all, therefore, contributed to the lactose literature in terms of both the preparative techniques and characterisation methodology for each polymorph. Unfortunately, different methods of preparation and quality of starting materials have led to a wealth of literature containing contradictory information.

Before any discussion on lactose can be initiated, it is first important to clarify the use of the term polymorphism in relation to the different forms of lactose. The generic definition of polymorphism; a material that exhibits different crystal structures within the solid state but has identical characteristics in solution,(McCrone, 1965) has been applied to the different forms of lactose for more than 50 years. At first sight it is a fair assumption that, as they are anomers related via mutarotation, α- and β are not strictly polymorphs. However, the spontaneous equilibrium that exists of 40α:60β between anhydrous α- and β-lactose within solution agrees with McCrone's definition of polymorphism, where polymorphs are said to exist if there is a rapid interconversion between forms within solution. This use of the polymorphism terminology is akin to the examples of mannose and melibiose given by Dunitz and Bernstein (Dunitz and Bernstein, 1995) where the α- and β-forms of the aforementioned materials were interpreted to exhibit 'conformational polymorphism'. While in the case of lactose the application, by strict definition of polymorphism is debatable, since the different forms of lactose have been widely described as polymorphs for a number of years the terminology will be maintained.

In spite of this contradictory information pertaining to the analysis of lactose, there is notable agreement that there are four well-accepted lactose forms. These consist of a single hydrated form, α-lactose monohydrate ($L\alpha{\cdot}H_2O$) and three dehydrated forms, β-lactose (Lβ), stable anhydrous α-lactose ($L\alpha_S$) and unstable hygroscopic anhydrous α-lactose ($L\alpha_H$). To date, full characterisation of these polymorphs with a variety of techniques using the same initial starting material has not been published.

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Lα·H_2O is the major constituent of mammalian milk and all other polymorphs to date originate from this material. Currently this is the only single phase polymorph available commercially and hence the bulk of the research into lactose characterisation has been carried out on this polymorph. Single crystal X-ray diffraction data of Lα·H_2O have been collected and refined by a number of research groups (Beevers and Hansen, 1971; Fries et al., 1971; Noordik et al., 1984) the most recent of which (Smith et al., 2005) identified the hydrogen-bonding interaction in the structure.

Single crystals of Lα·H_2O are readily obtained at temperatures lower than 93.5 °C, using aqueous Lα·H_2O solutions in an antisolvent such as acetone (Larhrib et al., 2003). Above this temperature, it has been shown that single crystals of the more stereochemically favourable Lβ anomer are formed by mutarotation (Buma and Weigers, 1967). There has been much debate on the effects that the α- and β-anomers have on each other with respect to crystal growth (Van Krevald, 1969; Nickerson and Moore, 1974) and work has been carried out on both the promotion and inhibition of these anomers (Dincer et al., 1999). It has been known for many years that the crystallisation of lactose is slow compared to that of other sugars (Michaels and van Krevald, 1966) and this factor allows mutarotation to occur in solution.

Much research has also been devoted to the other polymorph, which can be prepared as a single crystal, Lβ. Although Lβ is commercially obtainable, it is currently unavailable as a single phase. Commercially available Lβ is usually a mixture of Lβ and $L\alpha_S$, normally as a 60β:40α mixture. This mimics the equilibrium of lactose in aqueous solution at room temperature. Although $L\alpha_S$ is stable in the solid form, in solution, the Lβ anomer has the more energetically favoured geometry. Mutarotation of lactose between the anomers occurs readily in solution, via ring opening and closing mechanisms, eventually reaching the equilibrium of 60β:40α (Raghavan et al., 2000).

Purification of Lβ to produce a single phase polycrystalline sample can be carried out using a number of methods including those described by Buma and Weigers (1967), Parrish et al. (1979) and Parrish et al. (1980). This procedure uses alcoholic potassium methoxide solutions, although the level of purification appears to be dependent on both the starting materials and experimental conditions. The crystal structure of Lβ was determined originally by single crystal methods by Hirotsu and Shimada (1974) and has since then been improved by Garnier et al. (2002).

In contrast, the literature surrounding the remaining two anhydrous forms of α-lactose is less reliable and often conflicting. Some of the first results to be published with regards to the anhydrous forms of lactose were those of Hockett and Hudson in the 1930s (Hockett and Hudson, 1931) whereby acidic methanol was used as a dehydrating solvent. Later research by Lim and Nickerson (Lim and Nickerson, 1973) and Buma (1978) termed this polymorph 'α_M', a terminology that has been maintained in some later literature. These authors also found that lactose could be soluble in ethanol, but only when the moisture content of the solution was above 10%. The solubility of lactose in methanol was initially thought to contribute to the dehydration process and hence formation of the anhydrous polymorph (Lim and Nickerson, 1973). Lim and Nickerson therefore concluded from their findings that an anhydrous anomer, similar to that produced from methanol, would not be produced from dissolution in ethanol due to the need for a high water content. It has since been shown by our research using various analytical techniques, including elemental analysis, that this is not the case; *vide infra*.

The solvent extraction discussed above is often described as a soft dehydration technique. Garnier et al. (2002) however, studied the mechanism of hard, or thermal dehydration by heating Lα·H_2O in air, termed as 'hard' due to the associated danger of sugar damage during heating. Methods adopted by Garnier et al. produced either $L\alpha_S$ or $L\alpha_H$, depending on the experimental temperature. It has been documented (Figura and Epple, 1995) using techniques such as differential scanning calorimetry (DSC), that $L\alpha_H$ is a precursor to the more stable $L\alpha_S$ when derived from thermal methods. At 120 °C, $L\alpha_H$ is formed due to the evacuation of water from within the crystal lattice of the Lα·H_2O unit cell. As the temperature rises, more thermal energy is available and a rearrangement occurs to form $L\alpha_S$. Whilst the transformation from Lα·H_2O to $L\alpha_H$ is rapidly reversible in conditions of ambient humidity, once $L\alpha_S$ is formed, Lα·H_2O will only be reformed in exceptional humidity (>50%). The formation of $L\alpha_S$ from Lα·H_2O has been shown, by DSC and temperature resolved X-ray diffraction (TXRD), to occur at 160–170 °C. In research carried out by Figura and Epple, 'α_S', their terminology indicating the stable anhydrous α-lactose polymorph, was prepared by quasi-equilibrium dehydration according to the methods developed by Nickerson (1974). $L\alpha_H$ was prepared by rapid dehydration, either by heating at 120 °C or vacuum drying for 3 h. Both procedures used Lα·H_2O as the starting material.

The two materials, formed by soft and hard dehydration methods, $L\alpha_M$ and $L\alpha_S$, are often treated as separate polymorphs, but recent research presented in the experimental section of this article has confirmed the work of Garnier et al. (2002) that in actual fact, these two materials are the same polymorph. Therefore, the term '$L\alpha_S$', defining α-lactose 'stable', will be used for the majority of this work to represent the stable anhydrous α-polymorph.

Figura and Epple carried out research on the stable and hygroscopic forms of anhydrous α-lactose using DSC and TXRD, an area also covered by many other research groups (Berlin et al., 1971; Itoh et al., 1977; Lerk et al., 1984; Reynhardt, 1990; Roos and Karel, 1990). Their aim was to investigate the physicochemical reasons for the different behaviour of excipients, which are identical in terms of chemical formulae. Their work focussed on the possibility that modification of lactose may occur during the drying processes of pharmaceutical products suggested by Fell and Newton (1970) and Vromans et al. (1997). The research highlighted a number of possible modifications that may occur such as partial crystallisation of Lβ, partial solidification of amorphous lactose or only partial dehydration of Lα·H_2O. These modifications would all potentially alter the physical properties of the lactose and hence have a deleterious effect on drug delivery.

Figura and Epple showed the differences between $L\alpha_S$ and $L\alpha_H$ by storing samples in different relative humidity (RH) and

measuring water uptake. An RH of 50% led to an uptake of approximately 1% for $L\alpha_S$ whereas $L\alpha_H$ showed a much larger weight gain of 4% in a much lower RH of 10%. This work demonstrated the contrasting hygroscopic behaviour of these polymorphs and the differences in their water sensitivity.

The importance of the comprehension of crystallisation processes and quantification of crystal forms, observed during the production of pharmaceutical products, is increasingly recognised. One of the main analytical tools used, when identifying and quantifying crystal forms in tablets, is powder X-ray diffraction (PXD) (Michaels and van Krevald, 1966; Suryanarayanan and Herman (1991) but this effective technique is not without its flaws. As with all techniques, there are advantages and disadvantages, the main disadvantages being the influence of preferred orientation of the crystallites on the diffraction pattern and the inability to quantify low level crystallinity. Uncertainties in both position and intensity of reflections leaves XRD periodically open to misinterpretation, therefore, a range of techniques is required to ensure that the characterisation is comprehensive.

The use of spectroscopic techniques such as Raman spectroscopy, for example, within the pharmaceutical industry, is becoming more and more common place and is the subject of much research (Bell et al., 2004; Pinzaru et al., 2004; Strachan et al., 2004). Raman is a non-destructive technique that is time efficient, giving almost instantaneous results. It is able to distinguish both stable and metastable crystal forms, polymorphs and solvates (Prasad et al., 1982; Bugay and Williams, 1995; Brittain, 1997) with a relatively high level of accuracy and reproducibility.

Although there is already a relatively large amount of research carried out on lactose using Raman spectroscopy, there is little literature on the use of this technique with regards to polymorphism. The vast majority focuses on the characterisation and quantification of amorphous content of lactose monohydrate (Campbell Roberts et al., 2002; Auer et al., 2003; Murphy et al., 2005).

Raman spectroscopy is sensitive to composition, bonding and chemical environment of a material, as well as the phase (Smith and Clark, 2004). Murphy et al. (2005) showed, both qualitatively and quantitatively, that Raman spectroscopy was effective at comparing crystalline and amorphous $L\alpha{\cdot}H_2O$. It was suggested that Raman spectroscopy had further advantages over PXD, other than those previously discussed, as optical methods meant that analysis could take place of a single particle rather than an average over polycrystalline material.

Qualitative results obtained by the above authors agreed with previous work carried out in 1974 by Susi and Ard (1974). Bands in the crystalline material were well resolved with a good intensity, compared to that of the amorphous material which had poorly resolved bands of comparatively low intensity, indicative of lack of crystallinity (Taylor and Zografi, 1998; Seyer et al., 2000). This work aims to summarise the essential differences between the Raman spectra of the polymorphs to provide a qualitative methods for distinguishing between the lactose polymorphs.

Until recently, Fourier transform infra-red (FT-IR) has been the spectroscopic method of choice, with little use of the complimentary technique of Raman, however, it has been shown that Raman spectroscopy gives much better resolved spectra compared to that of its counterpart (Ingle and Crouch, 1998). A number of research groups have demonstrated the importance of using this technique in the characterisation of both polymorphic and pseudopolymorphic forms of both drug and excipient (Bugay and Williams, 1995; Szelagiewicz et al., 1999; Taylor and Langkilde, 2000; Campbell Roberts et al., 2002; Auer et al., 2003) by comparing size and shape of high intensity bands to those of reference samples.

Factors such as mutarotation in solution make techniques such as solid state NMR invaluable when characterising lactose. The technique is non destructive, an advantage when investigating small quantities, and provides structural information such as that of polymorphism (Atalla et al., 1980; Sugiyama et al., 1991). Characterisation of pharmaceutical solids by solid state NMR has, until recently, been relatively limited, although an appreciation for the increased understanding of physical and chemical properties of polycrystalline samples, gained by the technique has been developed in the last decade or so (Ek et al., 1995). Earl and Parrish (Earl and Parrish, 1983) successfully carried out the first NMR study of lactose in 1983. The spectra of five different samples of lactose ($L\alpha{\cdot}H_2O$, $L\beta$, $L\alpha_S$ and two mixed crystals of α- and β-) were collected using ^{13}C–^{1}H cross-polarization-magic angle spinning (CP-MAS). From several data collections they were able to ascertain, without any knowledge of crystal structure, that $L\alpha_S$ had a much more complex structure than that of $L\alpha{\cdot}H_2O$. In 1983, solid state NMR was in its relative infancy, and since then advantages in the technique (higher field magnets, sample environment and advances in pulse programmes) have allowed more highly resolved data to be collected and state of the art spectra will be presented on all the lactose polymorphs.

Solid state NMR has also been used to investigate the quantification of amorphous content of lactose, another important factor with consequences for industry, in particular that of the pharmaceutical industry. Gustafsson et al. (1998) compared solid state NMR to isothermal microcalorimetry in the assessment of the degree of disorder of processed lactose. Their solid state NMR results were in agreement with both those of Earl and Parrish for both $L\alpha{\cdot}H_2O$ and $L\beta$ and the comparable microcalorimetry results within their investigation.

This paper aims to outline the key data from a number of different analytical techniques that allows the unequivocal recognition of the different lactose polymorphs. During the course of this work, a number of issues arising from conflicting past data are addressed and state of the art instrumentation has been utilised to collect *in situ* temperature resolved X-ray diffraction data for Rietveld refinement and solid state NMR data on all polymorphs for the first time.

2. Experimental

2.1. Preparation of polymorphs

2.1.1. α-Lactose monohydrate

$L\alpha{\cdot}H_2O$ powder (Fluka Biochemica) was analysed without purification. Although previous studies have reported the presence of an impurity in samples of $L\alpha{\cdot}H_2O$, this impurity is

Table 1
Elemental analysis of stable lactose polymorphs

	Exp C (%)	Calc C (%)	Exp % H (%)	Calc H (%)
Lα·H_2O	39.8	40.0	6.1	6.6
Lβ	41.9	42.1	6.1	6.4
$L\alpha_S$ (thermal)	41.8	42.1	5.9	6.4
$L\alpha_S$ (solvent)	42.1	42.1	6.4	6.4

readily identified by powder X-ray diffraction by the presence of a reflection at 18° 2θ (Walstra and Janness, 1984; Goodhart, 1994; Garnier et al., 2002). This reflection was not present in the X-ray diffraction of this starting material and hence no purification was undertaken.

2.1.2. β-Lactose

Lβ was prepared using Lα·H_2O (Fluka Biochemica) as the parent material. The method of preparation followed that which was initially outlined by Parrish et al. (1979, 1980) whereby Lα·H_2O was refluxed in eight times the amount (w/v) of 0.014 M methanolic potassium methoxide for 3 h. The purity of the Lβ compound was quantified using GC analysis and concluded to be >96% pure Lβ and not a 5α:3β (Hockett and Hudson, 1931) or 4α:1β (Olano et al., 1977) ratio as previously obtained by using acidic alcoholic methods of preparation and variable starting materials.

2.1.3. Stable anhydrous α-lactose

$L\alpha_S$ was produced by two different methods of dehydration of commercial crystalline Lα·H_2O. The first was by a thermal method: Samples of commercial Lα·H_2O were heated to 160 °C and held for 16 h (Garnier et al., 2002). The second method used solvent mediated dehydration: Commercial Lα·H_2O was refluxed in 10 times (w/v) of dry 99.8% ethanol (Fisher Scientific Ltd.), of which the solvent had previously been dried over 4A molecular sieves (Fisher Scientific Ltd.). A second solvent mediated dehydrated sample was obtained using the above method exchanging ethanol for methanol.

2.1.4. Hygroscopic anhydrous α-lactose

Three methods of preparation were used to form the $L\alpha_H$ polymorph; the first two methods used *ex situ* procedures and the third an *in situ* method. In the first method, $L\alpha_H$ was formed by heating commercial, crystalline Lα·H_2O in an oven at 120 °C and holding for 16 h (Garnier et al., 2002). Samples were then transferred Whilst hot to an argon filled environment for storage. The second method used an *in vacuo* technique similar to that of Figura and Epple (Figura and Epple, 1995). Samples of Lα·H_2O were heated to 120 °C under vacuum and held for 16 h. $L\alpha_H$ samples analysed by powder X-ray diffraction (PXD) were formed *in situ* using isothermal temperature resolved X-ray diffraction (TXRD) by holding a sample of LαH_2O at 120 °C for 3 h followed by a 10 h data collection maintaining the temperature at 120 °C.

Elemental analysis of the above polymorphs confirmed that all polymorphs, with the exception of LαH_2O, were anhydrous in character (Table 1).

2.2. Instrumentation

2.2.1. Powder X-ray diffraction

Data were collected on powdered samples using a Bruker D8 Advance diffractometer. The instrument used monochromated Cu Kα_1 radiation at λ = 1.5406Å and a position sensitive detector (PSD). Samples were mounted in Perspex flat plate sample holders and analysed through a 2θ range of 5–40°, using 0.014767° steps over a period of 20 min. The $L\alpha_H$ sample, analysed by TXRD, used the Bruker D8 Advance diffractometer, fitted with a Anton Parr HTK 1200 sample stage. Patterns were analysed using EVA software, v5.0 rev. 3.

2.2.2. CCD-Raman spectroscopy

The Raman data were collected on a Horiba Jobin Yvon LabRAM HR800 spectrometer using a 633 nm HeNe laser, a 1800 grating and 50× objective. Spectra were obtained using a multiscan collection of 200 cm^{-1} per scan, 10 collections per wavenumber range, with a scan time of 10 s, between 200 and 1500 cm^{-1} and analysed using LabSpec software v4.14.

2.2.3. FT-IR

Data were collected on a Perkin-Elmer FT-IR Paragon 100 PC instrument using pressed CsI discs pressed to 8 t using a 13 mm die. Spectra were analysed using Spectrum Graph Server software v1.60.

2.2.4. ^{13}C–^{1}H CP-MASNMR

Data were collected on a Bruker Biospin 500 MHz spectrometer using a 4 mm MAS BB ^{1}H probe spinning at 5 kHz and operating at 125.771 MHz for ^{13}C and 500.13 MHz for ^{1}H. A ^{13}C–^{1}H, cp.av pulse program, with ramped cross-polarisation was used in conjunction with 69 kHz spinal-64 decoupling. A recycle delay of 240 s and 256 scans were co-added to produce FIDs with 2 K data points, with an acquisition time of 34 ms. The FID were collected with a sweep width of 238.8 ppm and then Fourier transformed with no window function to produce 32 K data points for the spectrum. The spectra were referenced to tetramethylsilane at 0 ppm.

3. Results and discussion

3.1. PXD analysis

Characteristic PXD patterns of the four distinct single phase lactose polymorphs are shown in Fig. 1. Fig. 2 shows the crystallographically identical patterns of α-lactose prepared by thermal and solvent-mediated methods (Table 2).

3.1.1. α-Lactose monohydrate

The PXD pattern of Lα·H_2O has been known for a number of years. Data documented by the JCPDS (2002) database shows that patterns analysed by Folen (1975) concur with current data, as do previous single crystal data (Smith et al., 2005) and also data (Beevers and Hansen, 1971; Fries et al., 1971; Noordik et al., 1984) found on the Cambridge Structural Database (Fletcher et al., 1996; Allen, 2002) (version 5.26, August 2005 update)

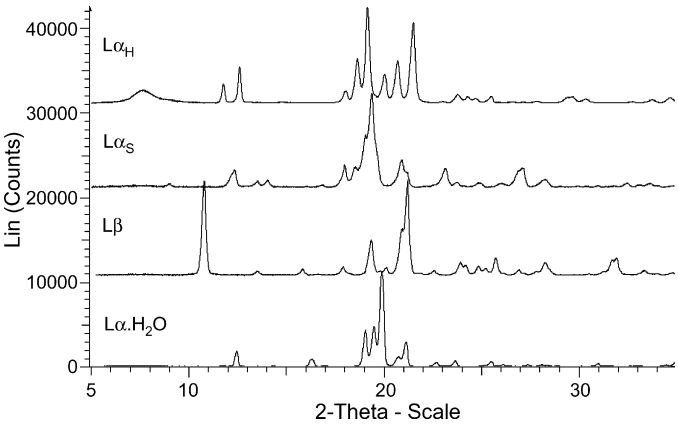

Fig. 1. PXD patterns of lactose polymorphs 5–40° 2θ using Cu Kα_1 radiation $\lambda = 1.54056$.

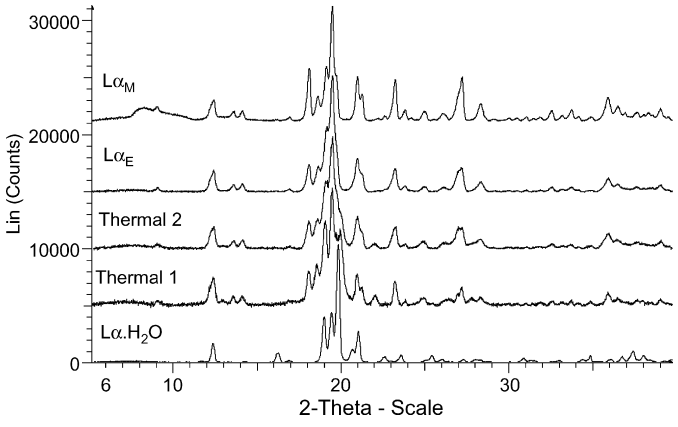

Fig. 2. PXD patterns of anhydrous α-lactose polymorphs: Lα·H_2O; thermal 1—surface layer of thermally treated lactose; thermal 2—protected layer of thermally treated lactose; $L\alpha_E$, $L\alpha_M$.

which showed that powder data constructed from parameters obtained by single crystal X-ray diffraction all produce the same characteristic pattern.

The *d*-spacings of the major reflections in the PXD pattern of Lα·H_2O occur at 4.46 Å (100% relative intensity (r.i.)), 4.55 Å (~42% r.i.), 4.66 Å (~39% r.i.) and 4.20 Å (~27% r.i.), taking into account instrumentation and sample factors such as zero point error and preferred orientation.

The major reflections referenced above are the key characteristics of the Lα·H_2O PXD pattern, in combination with the pattern shape itself, with particular reference to the lower angle reflections (10.0–23.0° 2θ). The pattern shows a highly crystalline material, with a high level of reflection overlap. The full width-half height maxima (FWHM) of Lα·H_2O is approximately 0.4° 2θ, compared to 0.1° 2θ for a quartz standard on this D8 diffractometer. The high degree of overlap is due to the large monoclinic unit cell (96 distinct atoms, $V = 768.85$ Å^3); space group $P2_1$, where $a = 4.7830(5)$ Å, $b = 21.540(2)$ Å, $c = 7.7599(8)$ Å and $\beta = 105.911(2)°$.[7] Crystallographic data obtained for lactose has shown that three out of four polymorphs of lactose discussed above, Lα·H_2O, Lβ and $L\alpha_H$, crystallise with monoclinic unit cells (Hirotsu and Shimada, 1974; Platteau et al., 2004; Smith et al., 2005). The crystal structure of the stable anhydrous α-lactose, $L\alpha_S$, is the exception, which has been shown to have a more complex triclinic unit cell (90 distinct atoms, $V = 720.18$ Å^3); space group $P1$ where $a = 7.6522(2)$ Å, $b = 19.864(1)$ Å, $c = 4.9877(1)$ Å, $\alpha = 92.028(1)°$, $\beta = 105.911(2)°$ and $\gamma = 97.153(1)°$.

3.1.2. β-Lactose

Lβ is the anhydrous anomeric equivalent to Lα·H_2O yet the PXD patterns are characteristically very different. Like that of the Lα·H_2O polymorph, much analysis of Lβ has been carried out; the first documented PXD data were collected in 1967 by

Table 2
Comparison of *d*-spacing and 2θ values of reflections (>5% r.i.) of lactose polymorphs

Lα·H_2O		Lβ		$L\alpha_S$		$L\alpha_H$	
2θ (°)	*d*-spacing (Å)	2θ (°)	*d*-spacing (Å)	2θ (°)	*d*-spacing (Å)	2θ (°)	*d*-spacing (Å)
12.42	7.121	10.55	8.377	8.96	9.849	7.60	11.62
16.28	5.440	13.29	6.658	12.29	7.199	11.75	7.528
16.97	5.221	15.63	5.667	13.49	6.558	12.59	7.027
19.04	4.657	17.69	5.009	14.01	6.314	14.79	5.984
19.48	4.554	19.14	4.633	16.82	5.267	18.03	4.916
19.88[a]	4.462	19.57	4.532	17.97	4.933	18.62	4.761
20.73	4.281	19.91	4.46	18.54	4.783	19.16[a]	4.629
21.12	4.204	20.73	4.282	19.05	4.655	20.02	4.433
22.69	3.916	20.99[a]	4.229	19.37[a]	4.580	20.69	4.289
23.67	3.756	22.37	3.971	20.89	4.249	21.51	4.128
25.16	3.537	23.73	3.747	21.16	4.198	23.02	3.860
25.50	3.490	24.98	3.709	22.55	3.939	23.78	3.738
26.11	3.410	24.66	3.607	23.14	3.841	24.29	3.661
27.39	3.254	25.00	3.558	23.75	3.744	24.71	3.601
28.13	3.170	25.55	3.483	24.87	3.577	25.49	3.492
28.39	3.141	26.74	3.332	26.03	3.421	27.15	3.200
		28.08	3.176	26.96	3.305	29.66	3.010
		28.35	3.145	27.11	3.286		

[a] Major reflections.

Buma and Weigers (1967). Data presented here confirms previous PXD analysis documented by the JCPDS, carried out by Folen (1975).

The major reflections of Lβ are found between 10.0° and 23.0° 2θ, comparative to that of Lα·H_2O. The five major reflections are notably sharp indicating high crystallinity, and have *d*-spacings of 4.229 Å (100% r.i), 8.377 Å (∼98%) 4.282 Å (∼49% r.i.), 4.633 Å (∼37% r.i.), subject to zero point error and preferred orientation. Single crystal X-ray diffraction data (Hirotsu and Shimada, 1974; Garnier et al., 2002) obtained from the Cambridge Structural Database concurs with the experimental results presented here and the JCPDS data card authenticating the procedure to prepare the single phase powder sample. When comparing the Lβ PXD pattern with other polymorphs, the most characteristic reflection is that *ca*. 10.5° 2θ (*d* = 8.377 Å), which is a strong reflection, unique to the Lβ material.

3.1.3. Stable anhydrous α-lactose

It has been discussed previously that an understanding of anhydrous α-lactose is often open to interpretation, largely due to differing terminology associated with it. There are currently two procedures for dehydrating lactose. The first is a 'soft' method, which uses dehydrating solvents such as anhydrous alcohols to produce α-lactose. The second method uses thermal or 'hard' dehydrating conditions. Separate terminology for the two documented techniques of hard and soft dehydration, suggests that each leads to materials with different crystal structures. The PXD characterisation of stable anhydrous lactose polymorph is highlighted below (Fig. 2).

3.1.3.1. Soft dehydration. The 'Lα_S' PXD pattern outlined in Fig. 1 is formed from solvent mediated methods using ethanol. In Fig. 2, the same PXD pattern is given the more specific terminology, Lα_E, to distinguish it as α-lactose formed from ethanol, rather than anhydrous lactose formed in methanol (Lα_M) or by thermal methods (Lα_S). It is important to note however that the generic term, Lα_S, may be given to any of the samples shown in Fig. 2 as the diffraction patterns show that the phases are identical. In each case the same characteristic PXD pattern is generated from samples created by the dehydration of Lα·H_2O using both thermal and solvent-mediated methods indicating the same crystalline product in each case. Similar to previous samples, and indeed, most PXD data, the lower angle reflections are the distinguishing features of the pattern. The major reflections are less well resolved than that of the parent Lα·H_2O pattern but occur within the same region, 10.0–23.0° 2θ. The major reflections are observed with *d*-spacings of 4.629 Å (100% r.i.), 4.655 Å (∼56% r.i.), 4.249 Å (∼28% r.i.), 4.933 Å (∼23% r.i.) and 4.783 (∼22% r.i.). The latter two reflections are *the* characteristic reflections of the Lα_S polymorph, although their intensity is relatively weak.

These findings disagree with Lim and Nickerson (1973) who suggested that the same polymorph should not be obtained by treatment of samples with ethanol and methanol. The PXD analysis does show that treatment with methanol does result in a higher level of crystallinity as the reflections are sharper and more well-resolved. This effect may be due to the higher solubility of lactose in the more polar methanol allowing the formation of larger crystals generated by less rapid recrystallisation.

3.1.3.2. Hard dehydration. PXD patterns of samples formed through thermal dehydration initially appeared to be slightly different to those formed by solvent mediated methods (Lα_E, Lα_M). A single characteristic, reproducible reflection was observed at *ca*. 19.9° 2θ that was not observed in the patterns of the solvent treated samples. This reflection lies at the exact 2θ value of the major reflection of the parent Lα·H_2O material, implying that some of the lactose monohydrate starting material remained in the product and avoided the thermal dehydration process. When a sample of lactose, or indeed any sugar, is heated in air, a hard, yet brittle surface layer is formed, caused by the melting of the surface particles. This process occurs so rapidly that these particles do not undergo dehydration and a surface crust forms that has not passed through the anhydrous stage. The partially amorphous material forms a protective barrier from the harsh conditions and allows the remaining material beneath to dehydrate slowly. This hypothesis was confirmed by separate PXD analysis of the two layers, (Fig. 2) the surface layer (Thermal 1) and material which was below the protective molten sugar layer (Thermal 2). This experiment showed that the powder beneath the surface had an identical PXD pattern to that of solvent mediated samples, referred to as Lα_M/Lα_E in Fig. 2, thus confirming, as in previous work (Hockett and Hudson, 1931; Lim and Nickerson, 1973; Buma, 1978; Figura and Epple, 1995; Garnier et al., 2002) that the two dehydration methods form the same polymorph leading to the generic terminology of Lα_S for the single phase stable α-lactose polymorph irrespective of preparation method.

Crystallographic data are rare for the Lα_S polymorph. Growing large enough crystals for single crystal X-ray diffraction analysis is made difficult by the insolubility of lactose in non-aqueous solutions. Dissolution in aqueous conditions either; causes rehydration and produces Lα·H_2O crystals; or mutarotation leading to single crystals of Lβ. Investigations by Garnier et al. (2002) and Dincer et al. (1999) have indicated that there are some anhydrous solvents that Lα_S is soluble in, such as dimethylsulfoxide or *N*-methylpyrollidinone but at the same time have indicated that these may lead to different solvate polymorphs of Lα_S and not a the individual Lα_S polymorph itself (Garnier et al., 2002). Due to the difficulties in producing single crystals and the heavily overlapping PXD pattern, Rietveld refinement of a powder pattern of Lα_S structure has only recently been carried out successfully by Platteau et al. (2005). The diffraction pattern used in the refinement concurs with that presented here, although to date, no conclusive structural refinement has been carried out on these data.

With reference to existing PXD data, the JCPDS has a number of different patterns attributed to 'anhydrous lactose' which covers those of molecular ratios such as 5α:3β (Drapier-Beche et al., 1999) or 1α:4β (Olano et al., 1977), and that of single phase 'α-lactose' (Brittain et al., 1991) none of these patterns concur with either previous work carried out by research groups such as Garnier et al. (2002) or the data presented here.

3.1.4. Hygroscopic anhydrous α-lactose

$L\alpha_H$ is the least researched polymorph of lactose, largely due to its hygroscopic nature and therefore lack of industrial applications. Current work can be both compared and contrasted to that of the last decade. Comparisons may be made to that of TXRD investigation carried out by Garnier et al. in 2002 and contrasted to that of Figura and Epple (1995) and Platteau et al. (2004).

The PXD pattern for $L\alpha_H$, presented (Fig 1) indicates a different crystal structure to that of its counterparts. It has previously been inferred (Campbell Roberts et al., 2002) that hygroscopic lactose is amorphous and whilst large FWHM infer small particle size, the high reflection intensity and well resolved pattern describes a crystalline material.

The major reflections of $L\alpha_H$ occur in two regions of the diffraction pattern; between 11.5–13.0° 2θ and 18.0–22.0° 2θ. Individual reflections include those with *d*-spacings of 4.629 Å (100% r.i.), 4.128 Å (~83% r.i.), 4.761 Å (~46% r.i.), 4.289 Å (~44% r.i.) and 4.433 Å (~30% r.i.). These reflections were also observed by Garnier et al., but not however by Figura and Epple in 1995 or Platteau et al. in 2004. In fact, from their research, Figura and Epple concluded, by PXD analysis, that the transition between $L\alpha{\cdot}H_2O$ and $L\alpha_H$ occurred too quickly to allow structural rearrangement and hence, the structure of $L\alpha{\cdot}H_2O$ is preserved. The data presented here clearly shows that it is not the case and implies that these authors collected data on samples that had undergone incomplete reaction or partial rehydration.

The first crystallographic information pertaining to the $L\alpha_H$ polymorph was presented by Platteau et al. (2004). The previously discussed problems with growing single crystals of anhydrous α-lactose are even more enhanced for an unstable polymorph, therefore the Rietveld refinement of powder diffraction data was used to determine the crystal structure. This process involved the *ab initio* structure determination of $L\alpha_H$ using information from the $L\alpha{\cdot}H_2O$ single crystal determination. Unfortunately, the powder X-ray diffraction pattern obtained by Platteau et al. was mixed phase and contained significant amounts of $L\alpha{\cdot}H_2O$ making the refinement extremely challenging. The structure of $L\alpha_H$ was refined using Rietveld methods with $L\alpha{\cdot}H_2O$ as a second phase. As the authors always produced mixed phase samples, these findings led them to the conclusion that $L\alpha_H$ could not exist as a single phase.

The presence of $L\alpha{\cdot}H_2O$ was not observed in the current PXD data. Maintaining a temperature of 120 °C during PXD data collection allowed the stability of the compound to be maintained during analysis and therefore gave the PXD pattern of what has been determined to be the $L\alpha_H$ single phase. Single phase $L\alpha_H$ was analysed *in situ* at 120 °C. A LeBail (LeBail et al., 1988) fit was carried out using GSAS (Larson and Von Dreele, 2000), Fig. 3, and unit cell parameters determined. $L\alpha_H$ was found to crystallise with a monoclinic unit cell, in the space group $P2_1$ with cell parameters of $a = 7.841$ Å, $b = 19.789$ Å, $c = 4.939$ Å and $\beta = 103.839$ Å. Goodness of fit parameters were $R_p = 4.9\%$, $R_{wp} = 6.5\%$ and $\chi^2 = 7.17$. When these parameters are compared to those of previous work carried out on a mixed phase sample (Platteau et al., 2004) an increase in each dimension is noted

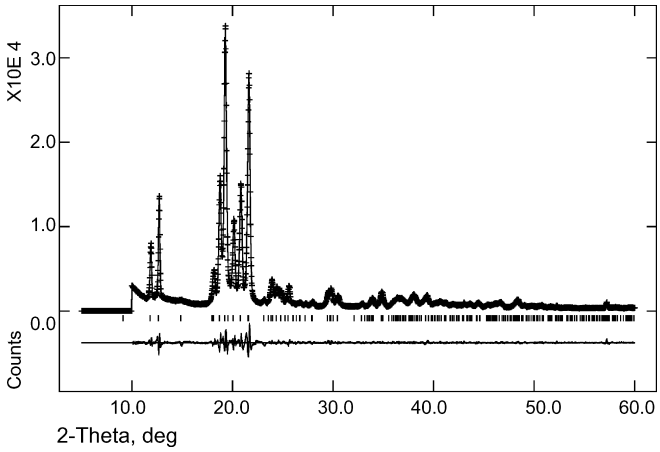

Fig. 3. LeBail fit of single phase $L\alpha_H$ at 120 °C.

($a = 0.79\%$, $b = 0.48\%$, $c = 0.67\%$), in the presented work compared to that of Platteau et al. coupled with an increase in β angle (0.14%). These increases in the cell parameters are due to thermal expansion of the unit cell as these X-ray data were collected *in situ* in the HTK 1200 furnace at 120 °C and previous work was carried out at room temperature.

3.2. CCD-Raman

A striking advantage that spectroscopic techniques have over PXD is the ability to analyse a solid phase comprising many components. Theoretically, all solid crystalline phases have a unique vibrational spectrum and make spectroscopy a useful tool for comparative purposes. However, the complexity of the spectra often make the use of the whole spectrum impractical and therefore, often, only certain unique bands are used to confirm the presence of these materials.

When comparing the Raman spectra of lactose polymorphs (Fig. 4) it is important that the effects of sample and instrument parameters be considered. Instrument parameters such as the size of the confocal hole affect band intensity in a similar way to changes in polymorphism. In order to minimise these effects, relative intensities are usually used in the same manner as XRD analysis. Table 3 compares the relative intensities of the three stable lactose polymorphs with bands of relative intensity ≥10%.

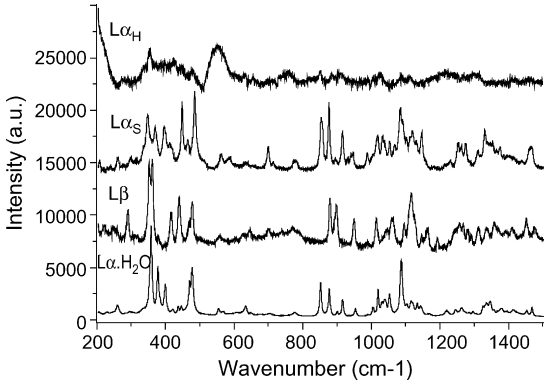

Fig. 4. CCD-Raman spectra of $L\alpha{\cdot}H_2O$; Lβ; $L\alpha_S$; $L\alpha_H$ 633 nm HeNe laser.

Table 3
Major Raman bands (>10% r.i.) of stable lactose polymorphs (200–1500 cm^{-1})

Band (cm^{-1})	Relative intensity (%)		
	Lα·H_2O	Lβ	$L\alpha_S$
287	–	39	–
345	–	–	80
357	100	100	–
375	55	–	–
397	35	–	65
437	–	57	–
445	–	–	91
475	55	50	–
485	–	–	100
851	40	–	73
876	30	50	82
915	20	–	56
952	10	29	–
1018	30	31	55
1051	24	–	–
1062	–	34	–
1086	64	–	76
1119	–	63	56
1375	–	–	62

Data collection for lactose, with particular reference to polymorphism, requires the use of a high resolution grating and a low signal:noise ratio. The low signal:noise ratio is advantageous in most analyses but is particularly important with respect to polymorphism, as small changes in spectra can be characteristic of different polymorphs and may be lost in noise in a more poorly resolved spectrum.

Assigning bands of a Raman spectrum of lactose is complex. Susi and Ard (1974) documented that there were 129 active Raman vibrational modes. Used as a comparative tool, however, it has proved to be an effective, efficient technique to distinguish between polymorphs.

3.2.1. α-Lactose monohydrate

The most intense band occurred at 355 cm^{-1}. The peak is single and clearly resolved. This peak shape disagrees with the results obtained by Murphy et al. (2005) which showed that this band was split into a doublet. The other major bands are present at 375, 475 and 1086 cm^{-1}. The latter of these two bands was used by Murphy et al. as a representative band of Lα·H_2O, due to the findings of Susi and Ard, who attributed this band to the C–O–C bridge of the glycosidic bond.

Comparing the results presented here, to those of Murphy et al. the spectra are reasonably similar. There are, however, discrepancies with peak intensity. For example, they noted the band at 443 cm^{-1} as a prominent band whereas the spectra presented here shows it to be minimal compared to the other bands. These factors may be attributed to the recrystallisation process carried out by Murphy et al. that was not carried out on the manufactured sample used in this investigation, or by instrumental factors described above.

3.2.2. β-Lactose

Raman spectroscopy is an effective tool for outlining structural differences between the two anomeric forms of lactose. The Raman spectrum of Lβ has previously been documented by Susi and Ard in 1974.

Notable Raman bands in the Lβ analysis are in the same region, common to lactose and carbohydrate is general. Clear, well resolved bands can be observed *ca*. 400–1500 cm^{-1}.

Major bands were present at 359, 437 and 1115 cm^{-1}.

3.2.3. Stable anhydrous α-lactose

A number of Raman bands can be observed in the spectrum of $L\alpha_S$, a number of which are comparable to that of the parent Lα·H_2O from which it was formed (Table 3). A review of previous work shows little research into the characterisation of the $L\alpha_S$ with respect to Raman spectroscopy.

The Raman spectrum of $L\alpha_S$, formed from solvent mediated methods, although lower in intensity, still implies a relatively crystalline material. High background levels suggests some level of amorphous content present within the sample, but the level of crystalline material was sufficient enough to be comparable.

When the background was removed from the above sample, the major bands were shown to occur at 485 and 875 cm^{-1}.

3.2.4. Hygroscopic anhydrous α-lactose

There have been several sets of Raman data collected for the analysis of $L\alpha_H$, with varying results.

In order to avoid rehydration, samples of $L\alpha_H$ were prepared by heating in a controlled environment at 120 °C for 16 h. The sample was then transferred to a quartz silica tube and sealed in the tube under vacuum.

The data collected were poor in both resolution and intensity and therefore no firm conclusions could be drawn.

3.2.5. Comparison by CCD-Raman

There are a number of bands present in the spectra of all three stable polymorphs which can therefore be assigned to common functional groups within the lactose molecules; functional groups that were not involved with either the anomeric carbon; differentiating between α- and β-polymorphs, or the presence of free O–H; indicating water molecules, unique to the Lα·H_2O polymorph.

The bands at ~875 and ~895 cm^{-1} show C–O stretching and C–H bending vibrations common to carbohydrates (Nørgaard et al., 2005).

By studying data of other disaccharides analysed by Raman spectroscopy, it is possible to gain more structural information relating to lactose, for example, the data presented by Taylor et al. (1998) on trehalose dihydrate. α-Lactose can be related to trehalose by the presence of the α-glucose moiety being bonded to the bridging O by the same C atom (1,4 bridging) as trehalose (1,1 bridging), giving structural similarity. From these data, the low wavenumber regions of Raman spectrum, as shown in this investigation, can be attributed to O–C–H, C–C–H and C–O–H deformations and to the C–C and C–O bands and are normally due to coupled vibrations rather than single functional groups.

One notable feature between the Lα·H_2O and the Lβ are the bands between 350 and 360 cm^{-1}. It would appear that the band at 356 cm^{-1} present in the Lα·H_2O is also present in the β-anomer but is split into the doublet of bands at 349 and 359 cm^{-1}.

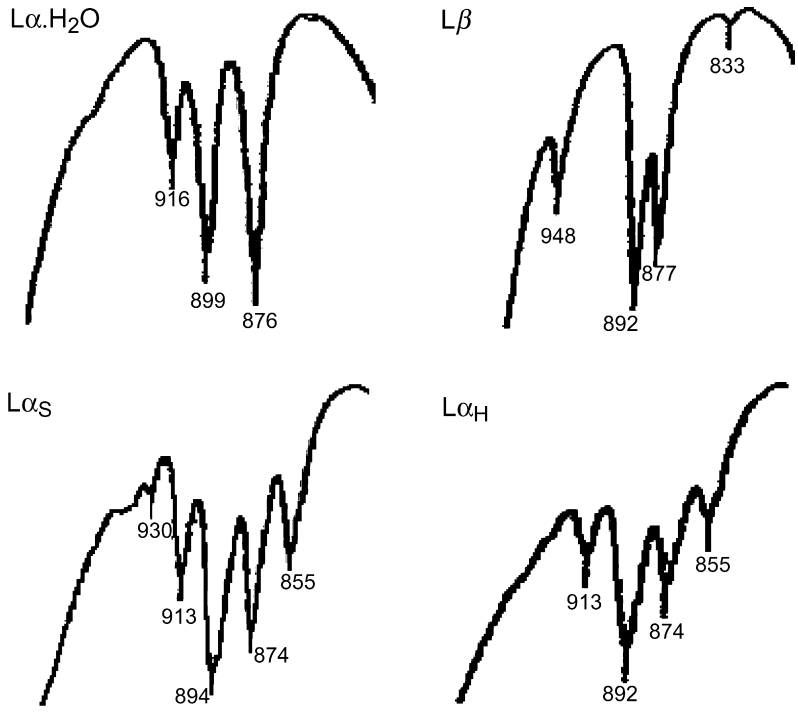

Fig. 5. FT-IR of designated diagnostic region 950–800 cm^{-1} Lα·H_2O; Lβ; $L\alpha_S$; $L\alpha_H$.

By comparing the spectrum collected by Murphy et al., this doublet is present in their sample of Lα·H_2O, as discussed previously, an example of the influence of instrument and sample parameters.

The two stable α-lactose forms, Lα·H_2O and $L\alpha_S$, can also be differentiated by Raman spectroscopy. Bands between 360 and 400 cm^{-1} show examples of this but major differences between the two α-lactose forms can be observed as bands at 345 and 483 cm^{-1}; which are present in $L\alpha_S$ polymorph but not the Lα·H_2O, and those at 375 and 397 cm^{-1}; present in the Lα·H_2O but not in $L\alpha_S$.

3.3. FT-IR

Analysis by FT-IR shows that the main diagnostic differences between lactose samples were found in the region between 1000 and 800 cm^{-1}. For example, when comparing spectra, the band that is present in Lβ *ca.* 950 cm^{-1}, is not present in any of the α-anomer samples, Lα·H_2O, $L\alpha_S$ or $L\alpha_H$ (Fig. 5).

Visual analysis of the complete spectra (4000–400 cm^{-1}) confirms that the expected broad vibrations bands at approximately 3200 cm^{-1}, corresponding to the intermolecular O–H stretching were present in all samples, as were the numerous vibrations found within the lower wavenumber region.

3.3.1. α-Lactose monohydrate

It could be noted from the spectrum that there were two vibrations present in the Lα·H_2O sample that were not observed within the remaining samples. These were attributed to free O–H vibrations from the water molecules (approximately 3500 cm^{-1}) and the distortion of the water molecules (approximately 1654 cm^{-1}).

Within the lower wavenumber region, there appeared to be a relatively well-defined diagnostic region, outlined in Fig. 5.

The vibrations at 3528 and 1654 cm^{-1} have already been explained. A broad vibration occurring at 3380 cm^{-1} corresponded to the intramolecular O–H stretching. The vibrations ranging from 916 to 976 cm^{-1} fit with what has been deemed the most diagnostic part of the spectrum, with respect to the contrasting polymorphs. From existing IR tables (Harwood et al., 1999) this region appears to consist mainly of out-of-phase ring stretching and twisting C–H bonds. The remaining vibrations lie within the shorter wavenumber region of the spectra. Although not much information can be obtained with respect to functionality, it was noted that this region of the spectra had an individual pattern for each polymorph.

3.3.2. β-Lactose

It is observed that the designated diagnostic region, as previously mentioned, is where the most obvious differences occur within the spectra. The vibration present in the Lβ sample *ca.* 950 cm^{-1} is not present in the Lα·H_2O sample. Similarly the vibration in the Lα·H_2O sample *ca.* 920 cm^{-1} is not present in the Lβ sample. Lβ is shown to have unique vibrations present within the fingerprint region, in particularly at 833 cm^{-1}.

Comparing spectra of Lα·H_2O, Lβ and a Lα·H_2O/Lβ mixture, the mixed sample can be determined as a mixture of these particular polymorphs, using FT-IR. There are two vibrations of interest; the first appears at approximately 950 cm^{-1}. The vibration present in the mixed sample corresponded to that present in the Lβ, diminished due to the opposing pattern of the Lα·H_2O; the second is at 918 cm^{-1}, which was applicable to the Lα·H_2O where the spectrum of the Lβ shows no vibration at this wavenumber; the third notable area of the spectrum is

between 900 and 890 cm^{-1}. The mixed phase sample shows that both the L$\alpha \cdot H_2O$ and Lβ vibrations occur in the mixed phase sample resulting in a double vibration.

3.3.3. Stable anhydrous α-lactose

Comparisons can be made between α-lactose polymorphs by using FT-IR spectroscopy. If the aforementioned diagnostic region is studied, observations may be made between the hydrated and anhydrous samples. Comparisons between L$\alpha \cdot H_2O$ and Lα_S show that, within this particular region of the IR spectrum, there is very little to distinguish between polymorphs. However, if the whole of the spectrum is observed, 4000–400 cm^{-1}, the vibration that occurs *ca.* 855 cm^{-1} is clearly observed within the spectrum of Lα_H. Therefore, in the case of L$\alpha \cdot H_2O$ *versus* Lα_S, it is also necessary to extend the data region to 4000 wavenumbers to confirm the presence of water. As stated previously, the two classic vibrations pertaining to the water molecules are not present in the anhydrous samples.

3.3.4. Hygroscopic anhydrous α-lactose

Lα_H may be characterised using FT-IR but the hygroscopic nature of the material forces reproducibility issues. The designated diagnostic region for the sample, shows that the polymorph may be distinguished from the L$\alpha \cdot H_2O$ parent material, but only on immediate analysis after preparation within an anhydrous environment. The sample begins to rehydrate after a period of approximately 10 min and the FT-IR spectrum indicates L$\alpha \cdot H_2O$. However, this observation becomes advantageous with respect to determining between the stable and hygroscopic forms of α-lactose due to the similar FT-IR spectra obtained from these two samples. Repeat analysis of a Lα_H sample will show rehydration occurring, and hence give the spectrum of L$\alpha \cdot H_2O$. This will not occur with Lα_S.

3.4. ^{13}C–^{1}H CP-MASNMR

Fig. 6 outlines the ^{13}C–^{1}H CP-MASNMR spectra for the single phase lactose polymorphs.

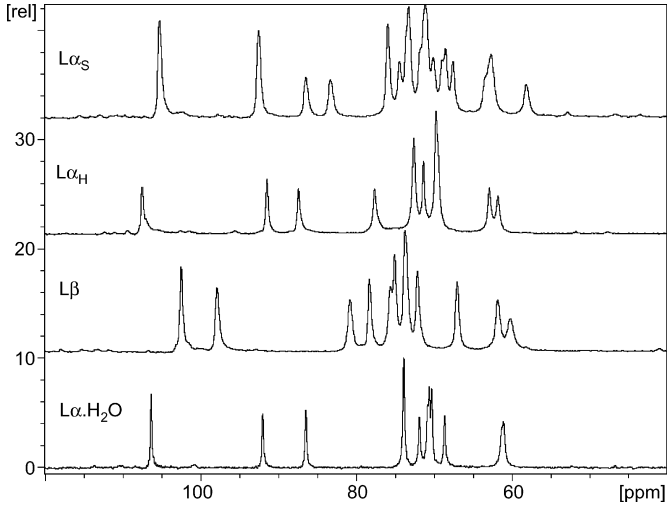

Fig. 6. ^{13}C–^{1}H CP-MASNMR spectra for lactose polymorphs.

3.4.1. α-Lactose monohydrate

The spectrum of L$\alpha \cdot H_2O$ has relatively well defined resonances. It shows that there are at least nine visible carbon environments within the unit cell. Closer inspection of peak shape suggests that a further two may be present at ~62 and ~72 ppm. From single crystals X-ray analysis it is known that $Z = 2$ and that the space groups $P2_1$ indicates the presence of a screw-axis. This concurs with the CP-MASNMR data. The lack of the remaining environments suggests a degree of symmetry between the two molecules within the unit cell. However, it is also possible that due to the relatively broad peak width (~30 Hz) overlapping resonances may be present if the frequency was not high enough to separate them. Analysis has shown that spinning sidebands do not affect peak intensity. All sidebands present lie out of the vicinity of any resonances.

3.4.2. β-Lactose

Previously published crystallographic data (Buma and Weigers, 1967; Hirotsu and Shimada, 1974; Garnier et al., 2002) state that Lβ also has the space group $P2_1$ and $Z = 2$. Fig. 6 clearly shows 11 different carbon environments, therefore indicating that the majority of the carbons within the molecule are in different environments and both of the lactose molecules within the unit cell have the same symmetry. Comparing this spectrum to that of the L$\alpha \cdot H_2O$, obvious differences are apparent. It is possible that the downwards shift occurs in the L$\alpha \cdot H_2O$ spectrum compared to that of the Lβ due to the present of the electronegative oxygen of the water molecule. There are a number of resonances unique to Lβ, including those at 79, 82, 99 and 104 ppm, making it readily distinguishable from the remaining polymorphs.

3.4.3. Stable anhydrous α-lactose

It can be assumed by the large number of peaks within the spectrum that there is a lack of symmetry within this structure. This is confirmed by the crystallographic findings of Platteau et al. (2005). Platteau et al. found that, similar to that of the L$\alpha \cdot H_2O$, Lα_H and Lβ, $Z = 2$ for the stable anhydrous polymorph but that the two molecules within the unit cell were different. The difference between the two molecules within the same unit cell would account for the large number of resonances within the ^{13}C MASNMR spectrum. 13 peaks maybe seen within the spectrum of Lα_S. Shoulders that appear *ca.* 74, 72, 68 and 64 ppm suggest that resonances occur at these ppm. Although resolution is questionable with respect to these shoulders, they indicate that at least 17 unique carbon environments are present within the unit cell. Further optimisation of the parameters is required to confirm these data.

With respect to the question of preparation methods, solvent mediated *versus* thermal methods, the results of CP-MASNMR experiments confirm previous findings by alternative techniques outlined in this paper, that the two methods do produce the same polymorph. Fig. 7 outlines the three different samples. Original naming convention, outlined in previous sections and work (Lim and Nickerson, 1973; Garnier et al., 2002) will be used for the purpose of clarity. Lα_S, is attributed to the thermally dehydrated α-lactose, Lα_M to that of α-lactose dehydrated using anhydrous

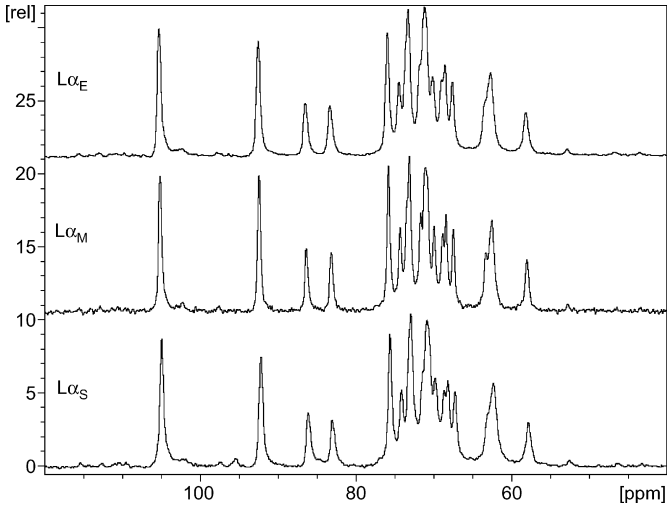

Fig. 7. ^{13}C CP-MASNMR spectra for anhydrous forms of α-lactose.

methanol and Lα$_E$ to that of α-lactose dehydrated using anhydrous ethanol. The CP-MASNMR data show that with respect to carbon environments, the three samples have the same NMR spectrum. This concurs with X-ray data.

3.4.4. Hygroscopic anhydrous α-lactose

Crystallographic data (Platteau et al., 2004) show that this polymorph has the space group $P2_1$ $Z = 2$. Ten different carbon environments can be observed in the spectrum suggesting that there is a degree of symmetry within the structure. There is a slight downwards shift compared to that of the Lα·H_2O possibly due to the relationship between the O–H groups of the molecules interacting with each other. The increase in bandwidth between Lα·H_2O and Lα$_H$ indicates a less crystalline, more disordered material.

4. Conclusions

The techniques outlined within this article show, in full agreement, that characterisation of lactose polymorphs is possible, without prior knowledge of the structure within a sample.

Although a large proportion of the work presented here concludes that much research already carried out on lactose is authentic, it has helped to highlight, clarify and condense those areas of analysis that were contradictory and, as all analysis were carried out using the same batch of Lα·H_2O parent material and secondary single phase polymorphs, validate procedures of both preparation and analysis.

Acknowledgements

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