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RESEARCH PAPER

The Measurement of the β/α Anomer Composition Within Amorphous Lactose Prepared by Spray and Freeze Drying Using a Simple ^{1}H-NMR Method

Rim Jawad • Carole Elleman • Louic Vermeer • Alex F. Drake • Brendon Woodhead • Gary P. Martin • Paul G. Royall

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ABSTRACT

Purpose Reports of the anomeric composition of amorphous lactose are rare and state a highly variable range of composition (between 0% and 60% w/w β content). We aimed to develop a quantitative measurement by ^{1}H-NMR of α and β anomer content in amorphous lactose produced by different production methods.

Methods Amorphous lactose was prepared by spray and freeze drying 10% w/v aqueous solutions of lactose. NMR analysis was performed in DMSO; peak areas of partially resolved doublets at 6.3 and 6.6 ppm were used to calculate % of α and β lactose present. Polarimetery was used to determine optical rotation of lactose solutions.

Results Observed specific rotation for supplied crystalline alpha lactose monohydrate of 88° recorded in DMSO was constant for the length of a typical NMR experiment (max. 10 min). β/α anomer contents of amorphous lactose measured by ^{1}H-NMR had standard deviations as low as 0.1% w/w ($n=6$). Drying a lactose solution 4 h after its preparation led to almost 35% w/w difference in anomer composition within solid amorphous material compared to samples dried after only 30 min, *e.g.* for freeze dried samples, β content was 60 ± 0.1% w/w (4 h) and 25 ± 1.0% w/w (30 min). Mutarotation leads to this increase in β anomer concentration in aqueous solution and within the solid amorphous lactose stored at 25°C e.g. after 56 d storage the β content of freeze dried lactose (30 min solution) increased from 25 ± 1.0% to 50 ± 0.5% w/w.

Conclusion A simple solution-based ^{1}H-NMR method for measurement of anomeric composition of lactose has been established. The solution β/α ratio at the time of drying is mirrored in the composition of the resulting solid amorphous material. In order to produce a consistent anomer composition within spray and freeze dried amorphous lactose, the standing time for the feed solution should be greater than 4 h, such that the most dynamic region of the mutarotation profile has been exceeded. If the amorphous material has been formed from a solution that has not been allowed to equilibrate for 4 h, the resulting solid will continue to undergo mutarotation if trace amounts of moisture are present, until the anomeric β/α ratio slowly approaches 1.7.

KEY WORDS amorphous · freeze drying · lactose · NMR · spray drying · β/α anomer composition

INTRODUCTION

Lactose, as a consequence of its low cost and biological acceptability, is one of the most commonly used diluents in many pharmaceutical formulations (including tablets, capsules and inhalers) (1–4). In food and confectionary, lactose is employed as a colour enhancing and flavour generating sugar (4–8). Other favourable properties of lactose include high physical and chemical stability, low hygroscopicity, good compaction properties, high water solubility and high compatibility with numerous active ingredients (1–4). The amorphous form of lactose is used in direct compression tablets. Micronized and conditioned lactose are on occasion

R. Jawad · L. Vermeer · A. F. Drake · B. Woodhead · G. P. Martin · P. G. Royall (✉)
Institute of Pharmaceutical Science, King's College London
Franklin-Wilkins Building, 150 Stamford Street
London SE1 9NH, UK
e-mail: paul.royall@kcl.ac.uk

C. Elleman
The Reading Science Centre, Reading Scientific Services Ltd.
Whiteknights Campus, Pepper Lane
Reading RG6 6LA, UK

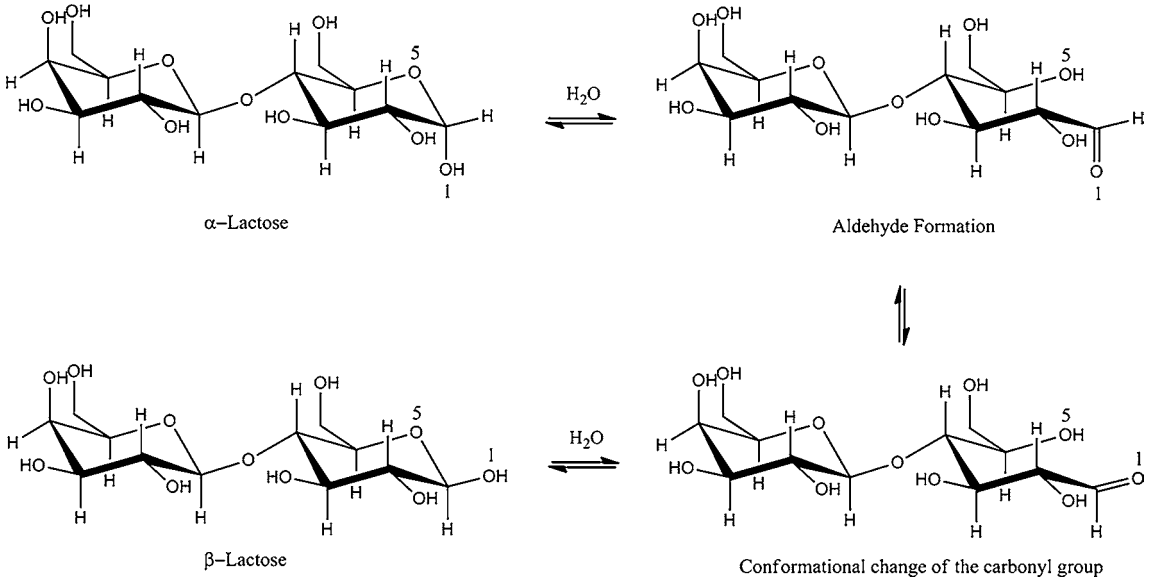

Fig. 1 A representation of the mutarotation mechanism of lactose based on the mechanism published by Silva *et al* (11) and drawn using Chemdraw®.

added as a fine particle fraction to crystalline lactose carrier particles in dry powder inhaler formulations. Amorphous lactose has also been employed as a model amorphous material used to validate methods for the detection of small amounts of amorphous content (9, 10).

Lactose is a reducing disaccharide that consists of β-D-galactose and α/β-D-glucose fragments bonded through a β1-4 glycosidic linkage; it constitutes approximately 2–8% w/w of milk (8). It is a complex material and can exist in two anomeric forms, α-lactose and β-lactose, which differ in the orientation of the hydrogen and the hydroxyl group on carbon atom number 1 (Fig. 1). The α and β anomers of lactose exhibit different physicochemical properties for example there is a 7 fold difference between the observed solubilities for the crystalline forms of the isomers in water (Table 1).

In aqueous solutions, both α-lactose and β-lactose may undergo mutarotation (8) and interconvert following the scheme given Fig. 1. This mechanism is based on the literature reports of the mutarotation for related sugars principally glucose (11, 12). It is currently believed that the mutarotation of lactose involves the formation of the free aldehyde form of the glucose unit which is initiated by the protonation of the O5, followed by breakage of the O1-H bond (11, 12). The starting state in the reaction scheme is the α form, (Fig. 1), its standard optical rotation has been measured as 84°. The final state in the interconversion process, as described in the reaction scheme, is the β form and its standard optical rotation is 34° (8, 13). Over time, a steady state is reached between the two forms of lactose present in solution. At neutral pH and at room temperature, the equilibrium amounts in water are approximately 63% β-lactose and 37% α-lactose (8). The exact composition and the time taken to reach equilibrium between the two forms are dependent on concentration, pH and temperature. Typical equilibration times are reported to fall within the 3.5 to 6.5 h range and it is generally accepted that the mutarotation of lactose in aqueous solution follows first order kinetics for reversible reactions approaching equilibrium (13–15).

Table I Physicochemical Properties of Crystalline α-Lactose Monohydrate and Crystalline β-Lactose (7, 29)

Property	α	β
Melting Pt °C [7]	202	252
$[\alpha]_D$ at 20°C [29]	+ 84°	+34°
Solubility (in H_2O) 20°C g/100 mL [7]	7	50
Specific heat [7]	0.299	0.285
Specific gravity 20°C [7]	1.54	1.59
Heat of combustion (kJ/mol) [7]	5687	5946

Spray or freeze drying are the methods most commonly reported for the production of amorphous lactose (8, 10, 16, 17). Both of these methods use aqueous solutions of lactose as their feed material; however, the anomeric composition of the resulting amorphous solids is rarely described. A survey of the pharmaceutical literature of the last 20 y conducted by the authors showed that of the 103 articles that contain "amorphous lactose" in their title only 4 articles declared the ratio of the β/α anomer composition within the amorphous lactose formed. Without measuring the β/α ratio, such studies are incomplete, not least due to the many differences in physicochemical properties (7).

Polarimetry, or a gas chromatographic method based on silylation are the methods typically used to determine the

anomer content in lactose samples (10, 18, 19). The complicated and time-consuming nature of these assays is the likely cause of the paucity of data describing the anomer composition of amorphous lactose. Powder X-ray diffraction (PXRD) can only be applied to crystalline materials and has been applied to determine anomer content in lactose; thus when it is applied to amorphous lactose such measurements are in reality made post re-crystallisation rather than on the true amorphous form (20). The assumption made is that the anomer content in the re-crystallised form reflects the anomer ratio in the amorphous material (20); this is unlikely unless water has been completely excluded from the amorphous material and from its storage environment during re-crystallisation. Lactose present in a disordered amorphous solid diffracts X-rays in a diffuse halo, rather than at discrete angles typical of ordered crystalline materials (21).

Solid state nuclear magnetic resonance, ^{13}C-NMR, has been used to characterise lactose samples (16, 22, 23). Gustafsson *et al.* used peak broadening to identify the presence of amorphous material and reported spray dried lactose to be 100% amorphous but the authors did not determine the β to α ratio within the amorphous lactose samples (16). Lefort *et al.* refined the NMR approach; their solid state ^{13}C-NMR work identified the fraction of the β anomer in milled lactose samples which also approached 100% amorphous (23). The time taken to conduct these experiments (4 to 6 h) has undoubtedly led to the lack of further published studies using solid state NMR to characterise the anomeric content in amorphous lactose. In order to allow NMR to be quickly and routinely applied in the characterisation of amorphous lactose, there is a need to develop a conventional solution based NMR approach using an accepted and widely used NMR solvent, for example dimethyl sulphoxide (DMSO). Since the mechanism for mutarotation requires the protonation of lactose (Fig. 1), then as DMSO is an aprotic solvent, it can be assumed that mutarotation in DMSO would be negligible. Thus the anomeric composition present in a DMSO solution should be equivalent to that found in an amorphous sample of lactose before the solution was prepared. The only report of the determination of the anomer ratio by solution based (DMSO) proton NMR makes this assumption (22) although this earlier work only describes the application of this technique to crystalline anhydrous α lactose, a milled and a re-crystallised sample of this material. The fractions of the β anomer were reported to be <1%, <4% and equalling 50% respectively (22). However no discussion of the validation of the technique is given, and no attempt has been made to apply this technique to spray or freeze dried lactose samples.

The results from the little work that has reported the anomeric composition of amorphous lactose have been equivocal. Lefort *et al.* produced predominantly amorphous α-lactose by controlled ball milling of anhydrous α-lactose for 30 h. Upon storage of these samples at elevated temperatures approaching the glass transition of lactose, a solid state mutarotation to the β anomer was observed (23). A final ratio of 50% β and 50% α indicated that the mechanism in the solid state is different from the mutarotation reaction pathway recorded in aqueous solution (23), where at equilibrium a typical ratio of 63% β and 37% α has been recorded (8, 11). A 1:1 ratio of anomers is almost never observed for spray or freeze dried amorphous lactose and furthermore there is a broad range of anomeric ratios reported (18, 19, 24, 25).

Roetman and van Schaik produced amorphous lactose employing a wide range of spray drying conditions (18). The amount of β lactose measured in these samples was always higher than the α anomer and it was observed that the ratio between the two was controlled by the temperature of the feed solution (before being passed into the spray drier), the outlet temperature of the spray drier and later, the storage temperature of the powder. This work indicated that mutarotation occurs in the feed solution, during spray-drying and subsequently in the solid amorphous state. Roetman and van Schaik claim that there is a universal β/α isomeric ratio of 1.25 (55% β and 45% α), which all amorphous forms of lactose approach and the rate of achieving this solid state equilibrium depends on storage temperature and water content (18). In their study, the feed solutions were held at different temperatures, but the length of the holding time was not described, so it is difficult to judge whether the mutorotation equilibrium had been attained before drying. Furthermore, both polarimetry and GC were used to measure the β/α ratio but there were significant differences between the β/α ratios measured by the two different methods (18). For example, a product produced using an outlet temperature of 85°C had, by polarimetry, a β/α ratio of 1.26 while by GC this ratio was determined to be 1.35.

Ramos *et al.* (10) produced samples of spray-dried lactose using the same feed concentration and similar spray drying conditions as the Roetman and van Schaik study (18). However, the former study reported the opposite in terms of the dominant anomer, with 34% β and 66% α present in the isolated amorphous lactose samples. It was reported that the amorphous material produced had the same composition irrespective of the isomeric composition of the starting material, but neither the feed temperature nor feed standing time were discussed (10).

Buckton *et al.* (24) investigated the influence of feed temperature on the anomer composition of spray dried lactose and observed that between room temperature and 40°C the amount of the β anomer was lower than the α anomer; however, the situation was reversed above 50°C. The conclusion drawn from this observation was that at low

feed temperature, mutarotation occurred during spray drying; however, at high feed temperatures, the composition of the amorphous material reflected the anomer ratio present in the feed aqueous solution at that storage temperature. The authors reported that the feed solutions were equilibrated at their assigned storage temperatures; however, the equilibration time for mutarotation to occur at room temperature is typically 3.5–6.5 h, and as no equilibration times are provided it is not clear whether anomer equilibrium had been achieved at the lower feed temperatures (24). To add further ambiguity in an earlier study by the same group of authors, it was reported that no β lactose was present in spray dried samples produced using similar parameters as the later study (19). For this study, the anomeric composition of zero β lactose content was determined by a combination of differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and microcalorimetric methods. Finally, a study by Listiohadi *et al.* characterised freeze dried amorphous lactose and observed an anomer of content of 59% β and 41% α, a β/α ratio of approximately 1.45 (25). Clearly the postulate of Roetman and van Schaik (18) of a universal anomer composition with a β/α ratio of 1.25 was not confirmed.

The wide range of reported anomer content of amorphous lactose is likely to be a result of the limited analytical methods available and a lack of control with respect to the equilibration time of the feed solution before drying. Spray and freeze drying are commonly employed in the pharmaceutical and food industries for the bulk manufacture of amorphous lactose (8). Therefore, monitoring the anomeric content of spray and freeze dried lactose does require a re-examination. In order to broaden the number of analytical approaches available and to address the contradictory reports concerning the anomeric content of amorphous lactose in the literature, the aim of this work was to establish a quantitative measurement by ^{1}H-NMR of the α and β content in amorphous lactose produced by different drying methods.

MATERIALS AND METHODS

The crystalline forms of lactose used for the preparation of the feed solutions for the drying experiments and as the controls in the NMR analysis were α-lactose monohydrate (SigmaUltra), and β-lactose (Fisher Scientific Ltd). The stated anomer purity from the suppliers for the α-lactose was 4% β and 96% α based on the supplied certificate of analysis using a GC analytical protocol, while the anomer purity for β-lactose was 80% β and 20% α respectively. These samples were used as received, but their anomer purity was confirmed by both polarimetry and proton NMR analysis. HPLC grade water was used throughout (Fisher Scientific). Phosphorous pentoxide P_2O_5 (≥98.5%) was supplied by Sigma-Aldrich.

Preparation of the Feed Solutions for Spray and Freeze Drying

For both spray and freezing drying, a 10% w/v aqueous feed solution of α-lactose monohydrate with respect to the supplied α-lactose monohydrate with a purity of 96% α and 4% β, using crystalline material was prepared in HPLC grade water, this is a typical concentration used for these drying procedures (10, 18, 19).

All solutions were dissolved in glass beakers (200 mL for freeze drying and 1 L for spray drying) using a magnetic rotating stirring bar ~5 cm long. All solutions were prepared and stored at 25°C, but two different protocols were employed. The first set of amorphous samples was prepared by starting the drying processes soon after dissolution of the lactose (*i.e.* when no particulate matter was detected). For the 10% w/v solutions, the absence of particles was noted at 20 min after the water was initially added to the lactose powder and so the drying commenced 30 min after initial mixing. A second protocol involved leaving the solutions 4 h after initial mixing before drying was initiated.

Spray Drying

Aqueous 10% w/v solutions of α-lactose monohydrate, (either the 30 min or 4 h solutions), were spray dried using a Niro A/S micro-sprayer. The temperature of the feed solution for spray drying was 25°C ± 2°C. The inlet temperature applied was 195°C producing an outlet temperature of 95°C with a feeding rate of 20 mL.min^{-1} and an air flow of 67 kg h^{-1} (19). The outlet temperature (95°C) was measured by a temperature probe placed in the airstream of the tube joining the drying chamber to the cyclone collection vessel. This is a standard spray drying preparation procedure for amorphous lactose given in the literature and is claimed to produce 100% amorphous material (10, 18, 19). The spray drying process lasted a maximum of 50 min using 1 L of feed solution. The resulting spray-dried powders were collected from the cyclone sample chamber and placed immediately in a desiccator over P_2O_5, which was placed in a controlled-temperature room at 25°C for 48 h. This step allowed further drying of the amorphous material. After 48 h, the desiccator was placed in a disposable plastic glove bag (Aldrich Atmosbag, tape-seal, two-hand, non-sterile, size M, part number Z112818-1EA) filled with a continuous flow of nitrogen gas (oxygen free). Whilst in the bag and under nitrogen, the whole powder sample was mixed and then divided into approximately 50 mg aliquots within 7 mL

glass sample jars. The sample jars were sealed within the bag and placed back in a desiccator over P_2O_5. This procedure ensured that all of the samples were stored under nitrogen. Once loaded and sealed, the desiccator was placed back into the controlled-temperature room at 25°C. A sample jar was removed from the desiccator for each subsequent experiment and not replaced, *i.e.*, a fresh sample was used each time in order to minimise the exposure of the powder to atmospheric humidity.

Freeze Drying

Aqueous 10% w/v solutions of α-lactose monohydrate (either pre-stored for 30 min or 4 h) were freeze dried using a Varian freeze dryer Girovac model GVD4. Samples of lactose solution (2 mL) were placed in 7 mL glass sample jars, these samples jars were placed centrally on the sample shelf within the freeze-dryer. The freeze drying process involved a pre-freezing step at −80°C, a primary drying step for 72 h employing a temperature of −50°C and vacuum pressure of 0.06 mbar, then secondary drying over P_2O_5 for 48 h at 25°C. The sample manipulation *i.e.* aliquoting, sealing and storage was conducted in a similar way to the spray-dried samples.

Powder X-ray Diffraction, PXRD Analysis

PXRD analyses were performed on a PANalytical CubiX PRO Fast diffractometer PW3800/00 with a Cu LFF X-ray tube PW3373/00 (PANalytical, Almelo the Netherlands). The powders were spread on a zero background holder and placed on a spinner stage with a spinner revolution time of 1 s. Bragg-Brentano geometry was applied using monochromatic Cu K alpha radiation and soller slits (0.02 rad) for both the incident and diffracted beam paths. The instrument was operated at a voltage of 45 kV and a current of 40 mA over a scan range 2–70 °2Theta with a step size of 0.0201 °2Theta and a time per step of either 71.75 s (Slow scan) and 8.89 s (Fast scan).

Dynamic Mechanical Analysis, DMA

DMA (DMA 8000, PerkinElmer, UK) analyses were performed by first calibrating for the force component by using the method described in the manufacturer's manual (26). The temperature component was calibrated using Indium with a melting point of 156.6°C and comparing the obtained value to the literature value. DMA samples were prepared by loading 20–50 mg of lactose powder and loading them into a stainless steel metal pocket which was folded to form an angle of 60° between the inner face of the pocket. The powder was then crimped to ensure that the powder was fully compressed with the folded pocket and that a thin sandwich of 0.4 mm was formed (27). The sample pocket was then placed in the DMA where the temperature of the sample was recorded by a platinum resistor sensor that is located behind the middle of the pocket. The experimental parameters employed were a dynamic displacement of 0.05 mm, a multi-frequency mode of 1, 10 and 30 Hz and heating rate of 5°C.min^{-1} from 25°C to 250°C.

Thermogravimetric Analysis, TGA

A PerkinElmer Pyris 6 Thermogravimetric analyser was calibrated for temperature and weight according to the methods supplied in the manufacturer's manual. Samples of 5–10 mg of lactose were loaded into an open pan and heated at a heating rate of 10°C.min^{-1} over a temperature range from 25°C to 150°C, with the sample mass monitored as a function of temperature and time.

Proton NMR Analysis

NMR samples were prepared by dissolving 3–4 mg of lactose in 0.7 mL of DMSO—Dimethyl Sulfoxide- d6 99.9% at %D with 0.05% v/v Trimethylsilane TMS (Goss Scientific instruments Ltd). The lactose dissolved in DMSO within a few minutes (not longer than 10 min) before being placed on the NMR which took a maximum of another 10 min. 1-dimensional 1H spectra were recorded on a Bruker Avance 400-MHz spectrometer equipped with a QNP probe. The temperature was maintained constant at 298 K. 16 scans were recorded using a standard zg30 pulse sequence (a 30 ° proton pulse followed by acquisition); with a 1 s recycle delay. The 30° pulse length was calculated based on a 90° pulse with a frequency of 24.3 kHz. The sweep width was set to 20.69 ppm and the acquisition time was 3.96 s. Spectra were processed and analysed using the Bruker Topspin software. The free induction decay was multiplied with an exponential function corresponding to a line broadening of 0.3 Hz, and spectra were phase corrected manually prior to an automatic baseline correction. Peaks were integrated using the β-lactose peak as a reference by defining its peak area as 1. The ratios of the two areas were compared following the method described by Willart *et al.* (22). The TMS reference was used for comparing the chemical shifts.

Optical Rotation

PerkinElmer polarimeter 343 was utilised to determine the optical rotation of lactose using a sodium D line monochromatic radiation (λ=589 nm) and a 1 dm cell path length. The instrument was calibrated first against two standard blank solutions: water and DMSO. Lactose samples were prepared at 25°C±2°C by dissolving lactose

powder in HPLC grade water or DMSO. The following concentrations were prepared: a) in water, 10% w/v (corresponding to the concentration used to prepare freeze-dried and spray-dried lactose) and 4% w/v (corresponding to the concentration used in previous polarimetry studies on lactose (29)) and b) in DMSO, 0.7% w/v (corresponding to the concentration of lactose used in the NMR analysis). After the initial mixing of the solution, the optical rotation was measured immediately after a clear solution was noted. Thus for the 10% w/v solution optical rotation was measured after 20 min and for the 4% w/v after 10 min from the initial mixing. Measurements continued over 10-min time intervals for 400 min. The observed optical rotation versus time curve was fitted to an exponential decay and extrapolated to correct for the optical rotation at the initial time point.

RESULTS & DISCUSSION

The spray and freeze drying methods used in this study have all been reported at length within the literature and these are the standard preparations for producing highly amorphous forms of lactose (10, 18, 19). Many techniques that are highly sensitive towards measuring the amounts of disorder within materials have been used to confirm that the drying protocols reported here produce amorphous content approaching 100% (20, 21, 27, 28).

The PXRD diffractograms for crystalline lactose samples used in this study are typical for the results reported in the literature for highly crystalline material (20). The data for both α and β anomers (Fig. 2) exhibited strong regular diffraction signals (particularly at 10.5° and 12.6°, which are characteristic of crystalline β-lactose and α-lactose monohydrate, respectively) (24) while those corresponding to spray and freeze dried lactose exhibited a randomly dispersed diffraction pattern or "halo" which is similar to the pattern of amorphous lactose reported in literature (20). On this basis, the spray and freeze dried lactose fractions were assumed to be amorphous and identical as the ordered regular pattern of diffraction peaks produced by crystalline materials (Fig. 2) were obviated by processing. As there are no observed diffraction peaks for the spray and freeze dried samples the usefulness of PXRD to differentiate between the two anomeric forms of lactose in these samples is severely limited and therefore PXRD was not applied further in this study.

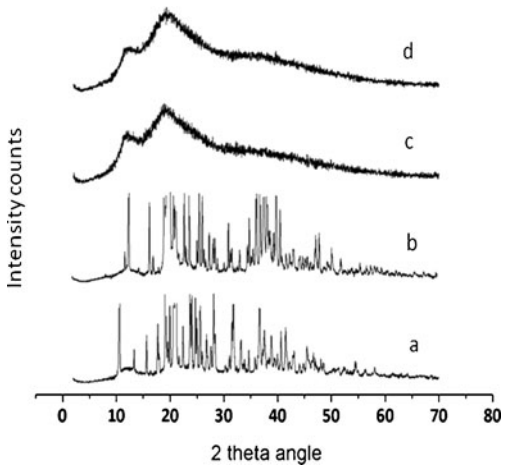

Fig. 2 PXRD of a) crystalline β-lactose (as received) b) crystalline α-lactose (as received) c) freeze dried lactose d) spray dried lactose (both freeze dried and spray dried samples were analysed at $t=0$ time point where the feed solution was dried after 30 min of its preparation time); $t=0$ refers to immediately after 48 h of secondary drying of the spray- and freeze-dried material over P_2O_5.

The DMA thermograms of crystalline α-lactose monohydrate and the spray or freeze dried lactose samples were markedly different (Fig. 3). The modulus signals for both the spray and freeze dried lactose samples (data not shown) were almost identical as a function of temperature. DMA is a technique that measures the modulus (strain/stress ratio) of a sample as an oscillating force is applied under the imposition of a controlled temperature programme. The apparent modulus or hardness of the lactose samples were measured as the temperature was increased at a rate of $5°C.min^{-1}$ (Fig. 3). The crystalline sample showed little alteration in the modulus signal until the melting point, when the modulus dramatically collapsed. For the spray and freeze dried samples, a loss in the modulus of a similar magnitude was observed just beyond 100°C. This sudden decrease in the viscosity or hardness was associated with the glass transition of the dried materials, whereby at the glass transition the molecular mobility within the amorphous lactose powders increases rapidly (27). The temperature at which the loss in modulus signal had reached its midpoint was determined to be approximately 120°C, and this was designated as the glass transition temperature for the two forms of amorphous lactose used. An observed T_g of 120°C falls within the glass transition range of amorphous lactose reported in literature (17, 28), which again implies that the spray and freeze dried samples were in a highly amorphous state.

On further heating, above the glass transition temperature, the amorphous lactose samples became liquefied allowing the molecules to diffuse more rapidly permitting crystallisation to begin (17, 28). Crystallisation was marked by the increase in modulus at approximately 150°C; however, as the onset of melting approaches for these newly crystallised forms of lactose, the modulus falls again, producing a peak in the modulus profile. The large change in the value of the modulus at the glass transition (Fig. 3) due to the high sensitivity of this method provides the key benefit of DMA when it is used to characterise amorphous powders (27).

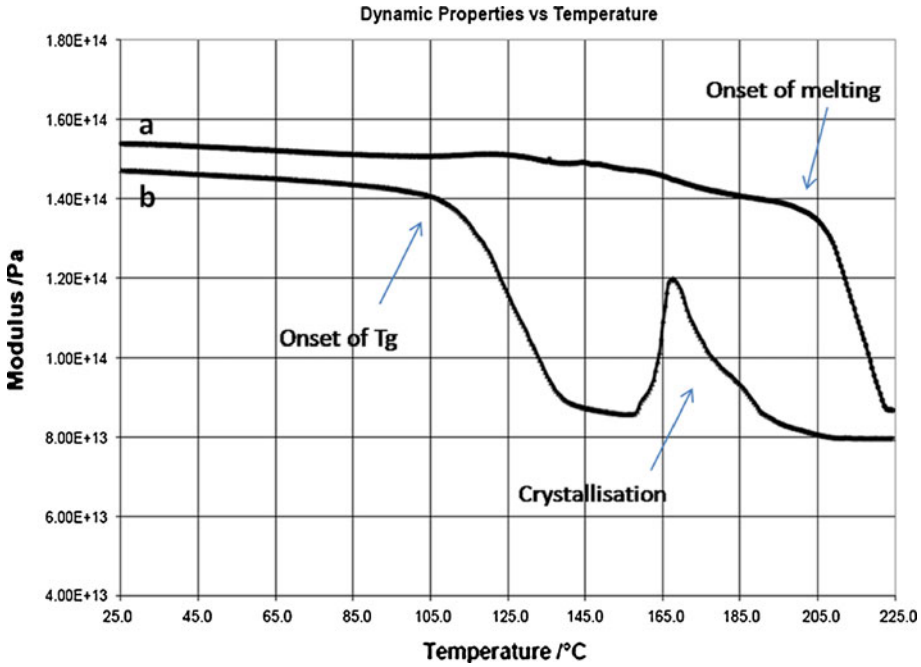

Fig. 3 An overlay of DMA thermograms of a) crystalline α-lactose monohydrate (as received) and b) spray-dried lactose (dried after 30 min of the preparation of the feed solution).

The water content of both spray- and freeze-dried lactose was determined by TGA and was found to be 1.3%±0.3 w/w and 1.4%±0.3 w/w respectively (n=3, ± SD). The % weight loss of water for both samples was determined by calculating the difference between the weight of the lactose sample at 25°C and that at 120°C where the baseline was stable, indicating no more water loss. No hydrate water was detected.

The ^{1}H-NMR spectra of crystalline α-lactose, β-lactose, freeze and spray dried lactose were all similar apart from the region corresponding to approximately 6 ppm (Figs. 4 and 5). ^{1}H-NMR analysis was performed to quantify the α and β anomers in lactose since the protons of the hydroxyl group (OH) group at carbon C1 in the anomers are apparent at different chemical shifts (δ) due to their existence in different chemical environments. The characteristic ^{1}H-NMR signals corresponding to the α and β lactose anomers (Figs. 4 and 5) appear as a partially resolved doublets at 6.3 and 6.6 ppm respectively (22).

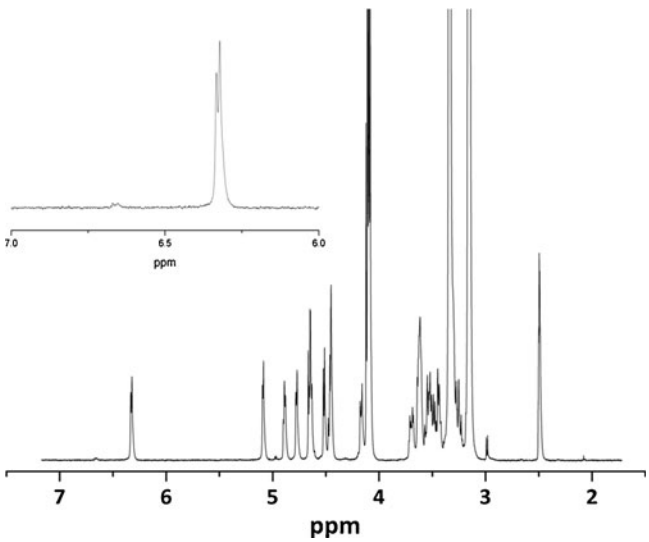

Fig. 4 A NMR spectrum (400 MHz) of crystalline α-lactose solution as received (0.7% w/v in DMSO); the inset shows the enlarged α and β anomer region (6–7 ppm).

The α and β protons (at C1) appear at the downfield of the ^{1}H-NMR spectrum as they are the most de-shielded atoms in the molecule. De-shielding takes place when the electron density, which shields a nucleus from the external field, on an atom is reduced by highly electronegative neighbouring groups. In the α anomer, the distance between the proton at C1 and the neighbouring oxygen atoms is greater than that of the β anomer (Fig. 6). Thus the influence of the neighbouring oxygen atoms on the protons at C1 of the beta anomer is greater causing a reduction of the electron density which leads to more de-shielding of the C1 proton of the β anomer. This results in the chemical shift observed for β-anomer occurring at a higher value (δ= 6.6 ppm) than the chemical shift of the C1 proton associated with the α-anomer (δ=6.3 ppm). The peaks appear as a doublet due to the spin-spin coupling resulting from the nearby hydrogen nuclei bound to C1 following the (n+1) rule. The peak areas of both signals were used to calculate the % of α and β lactose present. The ^{1}H-NMR spectra of both spray dried and freeze dried lactose showed that these different preparation methods produced lactose that exhibited quite different β/α ratios (Table II, Fig. 5). In

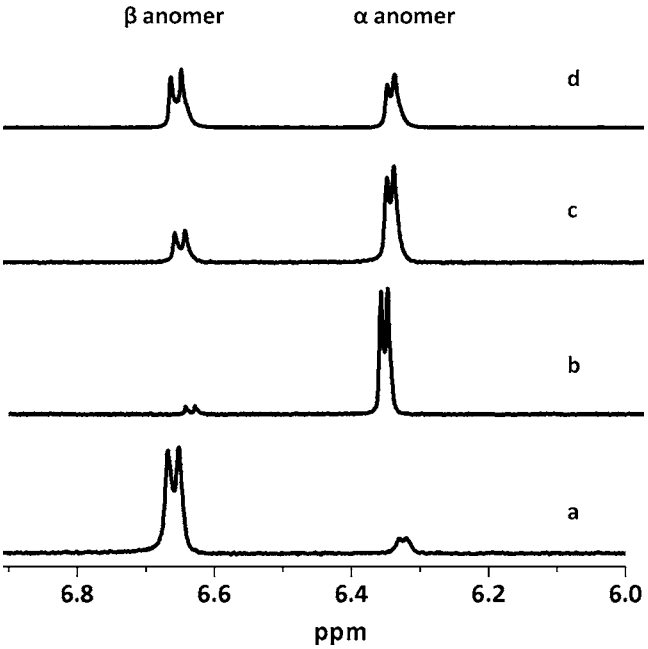

Fig. 5 An overlay of NMR spectra of (**a**) crystalline β-lactose (as received) (**b**) crystalline α-lactose monohydrate (as received) (**c**) freeze-dried lactose (analysed at $t = 0$ time point where the lactose solution was freeze-dried after 30 min of its preparation) (**d**) spray-dried lactose (analysed at 0 time point where the lactose solution was spray-dried within 30 min of its preparation); $t = 0$ refers to analysis immediately after 48 h of secondary drying of the spray- and freeze-dried material over P_2O_5.

addition, the ^{1}H-NMR analysis indicated that the crystalline α-lactose monohydrate was relatively pure with a 4% β and 96% α content while the β-lactose exhibited a composition of 87% β and 13% α.

The β/α ratio was recorded for different standing or storage times of the feed solutions showed large differences. For example, freeze drying within a 30-min standing time produced an anomer composition of 25% β and 75% α while a feed solution with a 4-h standing time produced a sample containing 60% β and 40% α. On the other hand, spray drying within a 30-min standing time produced 52% β and 48% α while a feed solution with a 4-h standing time produced 57% β and 43% α. The standard deviation in the measured anomeric ratios for the spray- and freeze-dried lactose samples all fall within the 0.1% to 1.4% range. The β/α ratio of freeze and spray dried lactose has also changed considerably upon storage, with the amount of the β anomer increasing significantly during a 56 d storage period.

To validate the NMR experiments and confirm that negligible mutarotation takes place whilst the samples were dissolved in DMSO and a number of polarimetry experiments conducted to determine the specific rotation of lactose solutions as a function of time. The observed specific rotation $[\alpha_{Obs}]_D$ normalises for solution concentration and path-length; in this study it was calculated by applying Eq. 1 to the measured or observed optical rotation,

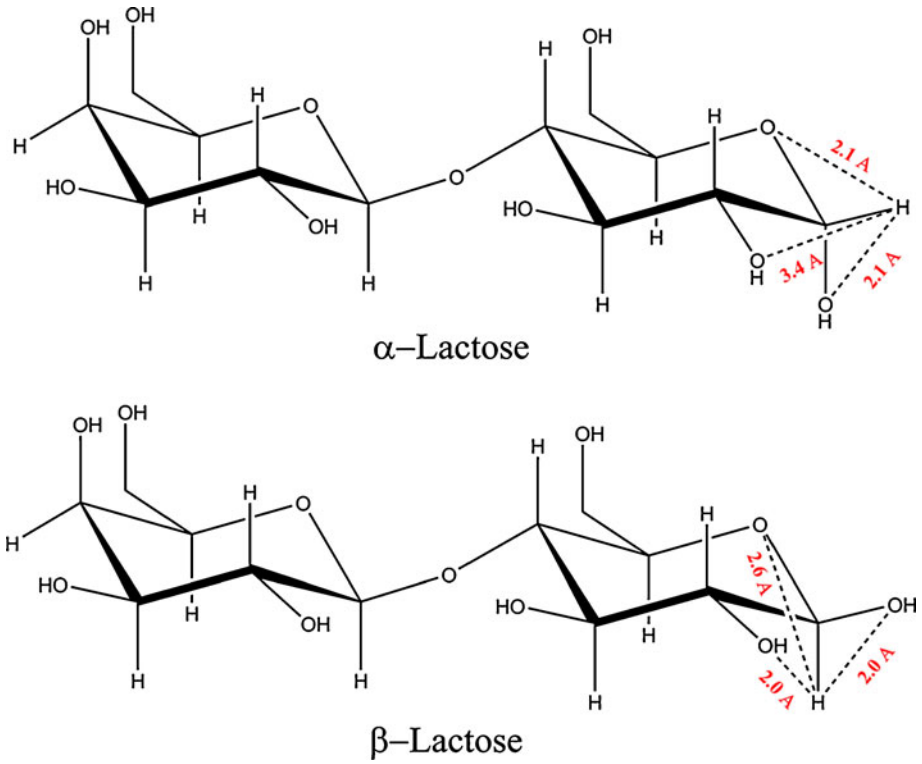

Fig. 6 A Chemdraw® representation of the two anomers of lactose showing the difference in distance between C1 and its surrounding environment. Dimensions are based on the published crystal structure of α-lactose monohydrate (35).

Table II β/α Ratio of Different Forms of Lactose Determined From Areas of the Peaks Attributed to the Carbon 1 β and α Protons. Thirty minutes or 4 h refer to the standing time of the feed solution prior to drying, after initial preparation. $t = 0$ Refers to Analysis Immediately After 48 h of Secondary Drying of the Spray- and Freeze-Dried Material Over P_2O_5, $t = 56$ d Refers to Analysis 56 d of Secondary Drying. (The SD of the β/α was Calculated Based on the Individual β/α Ratios Calculated)

Lactose	% β ± SD	% α ± SD	β/α ± SD	n
Crystalline α	4 ± 1.4	96 ± 1.4	0.04 ± 0.02	6
Crystalline β	87 ± 1.2	13 ± 1.2	6.7 ± 0.02	6
Freeze Dried ($t = 0$) 30 min	25 ± 1.0	75 ± 1.1	0.3 ± 0.02	6
Freeze Dried ($t = 0$) 4 h	60 ± 0.1	40 ± 0.1	1.5 ± 0.01	6
Freeze Dried ($t = 56$ d) 30 min	50 ± 0.5	50 ± 0.5	1.0 ± 0.02	3
Spray Dried ($t = 0$) 30 min	52 ± 0.3	48 ± 0.3	1.1 ± 0.01	6
Spray dried ($t = 0$) 4 h	57 ± 0.1	43 ± 0.1	1.3 ± 0.01	6
Spray Dried ($t = 56$ d) 30 min	60 ± 0.3	40 ± 0.3	1.5 ± 0.01	3

$$[\alpha_{Obs}]_D = \frac{100.\alpha}{1.C} \quad (1)$$

Where $[\alpha_{Obs}]_D$ = observed specific rotation, D = sodium D line monochromatic radiation (λ=589 nm), α = observed optical rotation, l= pathlength in dm, C = concentration in g/100 mL.

The polarimetry and ^{1}H-NMR data, (Table III) showed that for up to 30 min after the DMSO solution had been prepared there was no change in either the anomeric ratio determined by ^{1}H-NMR or the optical rotation of the DMSO solution prepared from crystalline α-lactose monohydrate. In further polarimetry experiments, the optical rotation began to decrease at a low rate only after 40 min (data not shown). All of the ^{1}H-NMR experiments were conducted within 20 min of the DMSO solution being prepared. Therefore, it was assumed that the anomeric ratios determined by the ^{1}H-NMR experiments reported here are true reflections of the composition within the solid phase. Thus the introduction that DMSO was proven to have little effect on the mutarotation of lactose.

The specific rotations of 4% and 10% w/v aqueous solutions prepared using crystalline α-lactose monohydrate were also determined by polarimetry to establish the time required for the lactose solutions to reach anomeric equilibrium. The optical rotation values of the lactose solutions were recorded over a period of 400 min (Fig. 7) and the results showed that the solution took approximately 4 h (240 min) to reach its equilibrium composition of 63% β and 37% α (8, 30). The equilibration time was taken to be the time required to reach a plateau or a constant value for the observed specific rotation.

The first step in the conversion of specific rotation data into the relative fractions of the two anomers was the extrapolation of the measured specific rotation to zero time after mixing (Fig. 7), *i.e.* to the theoretical point where the optical rotation is measured on a freshly prepared solution. This extrapolation was carried out by fitting the experimental data to a first order exponential decay, with the fits for all data sets having r^2 values >0.999. The observed specific rotation $[\alpha_{Obs}]_D$ of α-lactose monohydrate at this extrapolated intercept was determined for a 4% w/v solution to be 82.6°. This value is the specific optical rotation associated the sample of α-lactose monohydrate before any mutarotation could have occurred. From the independent NMR results reported above, this sample was found to comprise 4% β and 96% α, which correlates precisely with the stated purity provided by the suppliers. Thus, it could be concluded that a sample of lactose containing 96% of the α-anomer has an optical rotation of 82.6°. The other point where the anomer ratio is unambiguously known is at equilibrium in aqueous solution, the plateau region on the Fig. 7. The anomer ratio at this point has been reported as 63% β and 37% α (8, 30) and the specific rotation for this system was measured as

Table III Observed Specific Rotation and Anomer Concentration Determined by ^{1}H-NMR for 0.7% DMSO Solutions of α-Lactose Monohydrate, (Certificate of Analysis 96% w/w α and 4% w/w β Supplied by Sigma Aldrich). Values Determined as a Function of Time From Solution Preparation

Time (min) from solution preparation	Optical Rotation	Anomer concentration determined by ^{1}H-NMR	
	$[\alpha]_D$	% w/w β ± SD	% w/w α ± SD
0	88.3°	4.2 ± 1.4 ($n = 6$)	95.8 ± 1.4 ($n = 6$)
10	88.1°	–	–
20	87.8°	–	–
30	87.7°	3.9 ± 0.1 ($n = 2$)	96.1 ± 0.1 ($n = 2$)

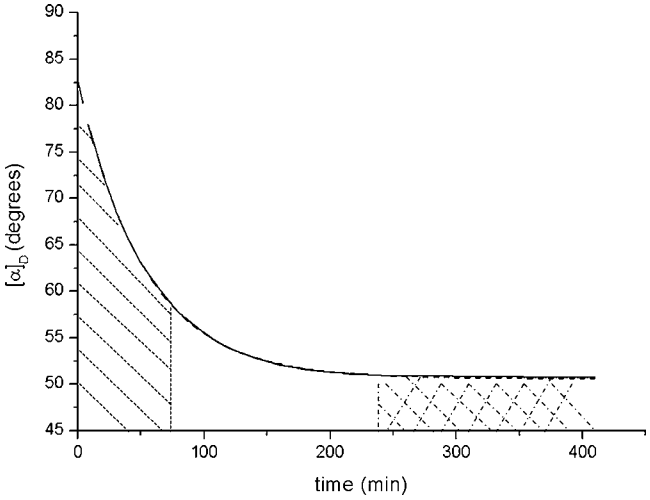

Fig. 7 Optical Rotation plot (fitted to exponential decay) of 4% w/v α-lactose monohydrate in water, experimental parameters are $\lambda = 589$ nm, 10 cm pathlength and 25°C Black—actual data; single hatch—time frame when spray-drying took place; cross hatch—time frame when equilibrium ratio is attained.

50.6°, the average specific rotation measured for the solution after 4 h.

To aid the determination of the anomer ratio from optical rotation measurements, the following constants and variables were defined: $[\alpha_{\alpha MH}]^\circ$ was defined as the specific optical rotation of 100% pure α-lactose monohydrate that has suffered no mutarotation; likewise, $[\alpha_\beta]^\circ$ was defined as the specific optical rotation of 100% pure β-lactose that has suffered no mutarotation; $[\alpha_{Obs}]_D$ was defined as the observed specific optical rotation for any sample of lactose under study (a measured experimental value, therefore it was a known dependent variable); f_α was defined as the fraction of α-lactose present in the aqueous solution, a variable which depends on time in solution, temperature, concentration and pH, the value of which value will lie between 0 and 1. Similarly, f_β was defined as the fraction of β-lactose present in the aqueous solution.

These parameters are related by the following equation.

$$[\alpha_{Obs}]_D = (f_\alpha \times [\alpha_{\alpha MH}]^\circ) + (f_\beta \times [\alpha_\beta]^\circ) \quad (2)$$

The aim of the optical rotation work was to determine the amount of β and α lactose present in aqueous solution as a function of time. Therefore, for the two special cases discussed above, for the pure α-lactose monohydrate at time 0 (extrapolated) and after 4 h equilibration, the known values for the composition and the measured optical rotation were substituted into Eq. 2 to produce the following simultaneous equations. It should be noted here that a sample containing 96% α-lactose monohydrate and 4% β-lactose, will have f_α and f_β values of 0.96 and 0.04 respectively.

At time 0 for a 4% w/v solution;

$$82.6 = (0.96 \times [\alpha_{\alpha MH}]^\circ) + (0.04 \times [\alpha_\beta]^\circ) \quad (3)$$

At equilibrium after 4 h for a 4% w/v solution;

$$50.6 = (0.37 \times [\alpha_{\alpha MH}]^\circ) + (0.63 \times [\alpha_\beta]^\circ) \quad (4)$$

For these experimental conditions $[\alpha_{\alpha MH}]^\circ$ and $[\alpha_\beta]^\circ$ are constant, so the two simultaneous Eqs. 3 and 4 may be solved to determine the values for $[\alpha_{\alpha MH}]^\circ$ and $[\alpha_\beta]^\circ$. This gave a value for $[\alpha_{\alpha MH}]^\circ$ of 84.8° and $[\alpha_\beta]^\circ$ of 30.5°. These values are close to the literature values reported for 4% w/v aqueous solutions of the pure forms of α-lactose monohydrate and β-lactose, which are 84 and 34 respectively (29). This calculation described above was also repeated for the 10% w/v solution, the results are listed in Table IV showing that as the concentration is decreased the optical rotation for the pure forms of lactose increases.

Once $[\alpha_{\alpha MH}]^\circ$ and $[\alpha_\beta]^\circ$ were determined experimentally, Eq. 2 was re-arranged to solve for both f_α and f_β. This re-arrangement exploits the fact that $f_\alpha + f_\beta = 1$.

$$f_\alpha = ([\alpha_\beta]^\circ - [\alpha_{Obs}]_D)/([\alpha_\beta]^\circ - [\alpha_{\alpha MH}]^\circ) \quad (5)$$

$$f_\beta = ([\alpha_{\alpha MH}]^\circ - [\alpha_{Obs}]_D)/([\alpha_{\alpha MH}]^\circ - [\alpha_\beta]^\circ) \quad (6)$$

Equations 5 and 6 may be applied to lactose polarimetry data in order to determine the fractions of the anomers present in solution at any time point by simply measuring $[\alpha_{Obs}]_D$ at the time of interest. However, it should be noted that Eqs. 5 and 6 are only applicable if the experiments are conducted under the same conditions and concentrations as used for the determination of $[\alpha_{\alpha MH}]^\circ$ and $[\alpha_\beta]^\circ$ values. In the present study, Eqs. 5 and 6 were used to determine the composition of the 10% w/v feed solutions used for the spray and freeze drying at important time points. For example, for a 10% w/v aqueous solution of Sigma-Aldrich supplied α-lactose monohydrate, containing 4% β and 96% α, 30 min after preparation, the solution contains 30% β and 70% α, and after 4 h it contains 63% β and 37% α. The half life to reach the equilibrium composition for a

Table IV The Extrapolated Time Zero ($t = 0$) Observed Specific Rotation $[\alpha_{Obs}]_D$ and the Specific Optical Rotation for Pure α- and β-Lactose Determined for two Different Concentrations of α-Lactose Monohydrate Aqueous Solutions

Concentration % (g/100 mL)	$[\alpha_{Obs}]_D$ at $t = 0$	$[\alpha_{\alpha MH}]^\circ$	$[\alpha_\beta]^\circ$
4	82.6°	84.8°	30.5°
10	78.0°	80.0°	30.7°

10% w/v solution was 36 min and it took 4 h to reach the steady state equilibrium composition of anomers.

DISCUSSION

The ^{1}H-NMR using a DMSO solution based sample preparation method developed here is a simple and sensitive method for characterising the anomeric composition within lactose samples that are either amorphous or crystalline. Polarimetry has supported the hypothesis that DMSO as an aprotic solvent has little impact on mutarotation. When a lactose solution in DMSO is analysed within 20 min of preparation, the NMR spectra has been shown to reflect the anomeric composition present in the solid material (Table III). Therefore, a key aim of this study has been achieved by establishing a quantitative measurement by NMR of the α and β anomer content in amorphous lactose.

The ^{1}H-NMR method identified an identical anomer composition of the Sigma supplied crystalline α-lactose monohydrate as that stated on its certificate of analysis. A GC method was reported in the certificate of analysis, thus the NMR method described here has been validated employing a second method. In addition, the composition of the supplied crystalline α-lactose monohydrate determined by polarimetry for a freshly prepared solution also matched the certification of analysis and NMR determined compositions.

The standard deviation of the α content for the supplied crystalline α lactose monohydrate sample, determined by NMR was 1.4% w/w (n=6), which was larger than the value of 0.1% w/w (n=6), determined for both the spray and freeze dried samples produced from fully-equilibrated feed solutions (t=4 h). For the crystalline and amorphous samples, the NMR analysis was conducted on a number of different days, to investigate and accommodate the impact of any potential instrumental drift on the variability in response. The spray and freeze dried materials were both prepared in two batches and 3 samples from each batch were analysed, so as to comprise the 6 replicates for each type of dried material (Table II). These samples were handled with great care, ensuring thorough mixing and storage under a nitrogen atmosphere at 0% RH and 25°C. Thus, the narrow standard deviation was brought about by the tight control of the analyte preparative conditions. Since crystalline α-lactose monohydrate is relatively stable, it was considered unnecessary to store these samples under such tight control. Six samples were analysed from the Sigma supplied batch stored under ambient conditions. However, it appears that fluctuations in the homogeneity and storage conditions of the crystalline α-lactose monohydrate did influence the standard deviation of the determined anomer composition, while its overall composition also measured by NMR matched the supplied certificate of analysis. The standard deviations for the measured compositions reported here are much lower than the error range obtained from the solid state ^{13}C-NMR approach (of almost 10%) described by Lefort *et al.* (23). These data provide further justification for the development and use of the solution based ^{1}H-NMR analytical approach.

The amorphicity of the spray- and freeze-dried samples of lactose was confirmed by both DMA and PXRD. A glass transition at approximately 120°C was observed, with a large decrease in the storage modulus when samples of both spray and freeze dried lactose were heated. The lowering of the sample's resistance to deformation was attributable to the de-vitrification of the processed samples (31), but the storage modulus in the test samples was not completely lost because of the underlying contribution from the steal of the powder pocket (27). However, immediately after the glass transition, the modulus was reduced to a similar value to that observed after the melt associated with the crystalline material. The similar modulus values post melt and glass transition indicates that all of the spray and freeze dried material was amorphous. If any crystalline material had been present in the processed material, this would have made a contribution to the signal, giving an intermediate value in the modulus until the melting point of any residual crystalline material had been reached.

The PXRD data presented within this paper not only clearly shows the key benefits of this important technique but also highlights its central limitation. PXRD is reported to be the gold standard for amorphous detection (20, 28, 32); however, PXRD is unable to characterise mutarotation within amorphous materials and therefore supplementary techniques require development.

Combining the NMR data with the results from the polarimetry may be used to reveal the origins of the varying anomeric composition of amorphous lactose. The standing time for the feed solutions used for both freeze and spray dried lactose was found to have a large impact on the anomer composition of the final amorphous product. For both a 4% and a 10% w/v aqueous solution prepared from a typical batch of crystalline α-lactose monohydrate, there is a rapid change in the anomer content over the first 4 h. After this time, the mutarotation reaches equilibrium and a steady state or constant anomer composition was attained (Fig. 7). The data for the 10% w/v and 4% w/v lactose were very similar; both curves produced the same extrapolated onset of 4:96 β:α for the anomeric ratio and an equilibrium ratio of 63:37 β:α. Thus, spray- or freezing-drying a solution of lactose 4 h after preparation of the solution led to nearly a 35% w/w difference in anomer composition within the solid amorphous material compared to samples dried within 30 min of preparation. A

contributing factor to this difference in anomer composition is clearly the mutarotation taking place in the lactose feed solutions, an observation that has received little attention in the pharmaceutical literature.

The two concentrations of lactose in solution, 4% and 10% w/v, used in the polarimetry experiments were selected to mirror the concentrations used in previous polarimetry experiments (29) and to represent a typical values used in spray and freeze drying (20, 21, 27, 28). For the 4% w/v solution, the $[\alpha_{\alpha MH}]°$ and $[\alpha_{\beta}]°$ values were very close to those reported by Drapier-Beche (29) (Tables I and IV). Incorporating the $[\alpha_{\alpha MH}]°$ and $[\alpha_{\beta}]°$ values calculated for the 10% w/v solution into Eqs. 5 and 6 allowed the determination of the anomer composition for the freshly-prepared solution, 30 min later, and 4 h after the formation of the solution from the observed specific rotations measured in the polarimeter. The anomer content of the freeze dried lactose reflects most closely the extent of mutarotation observed in solution. A 10% w/v α-lactose monohydrate aqueous solution at 30 min after initial mixing contains, from the polarimetry reported here, 30% β and 70% α, and the equivalent freeze dried sample prepared at 30 min contained 25% β and 75% α as measured by NMR (Table II).

Freeze dried amorphous lactose dried within 30 min of solution preparation contains a high α-lactose content, whereas a solution held at 25°C for 4 h and then freeze dried, the resulting solid has a high β-lactose fraction (Table II). The actual amounts are within 3–5% of the anomer composition observed in solution at these time intervals by polarimetry. The initial stage of freeze drying, pre-freezing at −80°C is very rapid and therefore this process must capture and preserve the degree of mutarotation within the solution, with minimal mutarotation possible in the glassy matrix once the ice crystals have formed. The rate of mutarotation is known to slow dramatically as the temperature is lowered (13, 18). The following stage of the process, the low primary drying temperature of −50°C also negates mutarotation within the lactose-water glassy matrix. Therefore, the β/α anomeric composition of the initial feed solution was found to be very similar to the anomeric composition of the final amorphous cake for freeze dried lactose.

When the feed solution for the spray-dryer is held for 4 h at 25°C before processing, the resulting material broadly conforms to the findings obtained for the freeze dried samples, *i.e.* the solution β/α anomeric ratio reflects the composition of the resulting solid amorphous material. However, when the feed solution is spray dried within 30 min of preparation, the amount of the β anomer is much greater than predicted by considering the mutarotation occurring in solution as a function of time alone. Haase & Nickerson (13) recorded an increase in the rate constants for lactose mutarotation as a function of temperature. Thus, the 195°C inlet temperature of the spray drier, even though the lactose solution is only exposed to this temperature for a few seconds, would be expected to accelerate the mutarotation compared to the feed storage temperature of 25°C. This would result in the presence of a higher amount of the β anomer. The influence of the inlet temperature on solutions that have been equilibrated for 4 h at 25°C is less as these systems have reached their anomeric equilibrium, *i.e.* they already have a large proportion of β lactose present. A number of workers have developed equations based on first order reversible kinetics, which indicate that as the temperature increases, the rate to reach equilibrium in solution also increases (18). Rate constants range between 0.45 h^{-1} and 1.37 h^{-1} over the 25°C to 35°C temperature range (14) and to further this work, the authors are currently investigating mutarotation in solution over a wider temperature range.

The time taken for spray drying to be carried out would also be expected to have a major impact on the β/α anomeric composition of the spray-dried material prepared from the feed solutions dried within 30 min of solution preparation. The time taken to spray dry 1 L of feed solution was approximately 50 min. Since spray drying was begun within the 30 min window, the last portion of feed solution was not processed until 80 min after preparation of the solution. This 80 min zone (see Fig. 7), is the most dynamic part of the mutarotation equilibrium process such that at the end of this period the amount of β lactose present in the feed solution would be high and nearing its 63% concentration at equilibrium. Therefore, when all of the spray dried lactose from the 30 min feed solution is collected and mixed; the β lactose content is higher, 57%, compared to the 30% β lactose content of the aqueous solutions measured at 30 min by polarimetry.

The solubility of α-lactose monohydrate has been rarely addressed in the literature associated with spray and freeze drying (19, 25). The solubility of α-lactose monohydrate is 7 g/100 mL (8); in many optical rotation studies, which typically use a 4% w/v solution, the solubility is not exceeded (29). In contrast, the 10% w/v solutions typically used for spray and freeze drying do exceed the solubility of α-lactose monohydrate in water (8, 19, 25). However, mutarotation enhances the observed solubility because as the α-lactose monohydrate dissolves into water, then the β-anomer is rapidly formed, causing a reduction in the α anomer's concentration in water and therefore increasing the observed solubility until a final equilibrium is eventually reached in solution. The consequence of the initial rapid conversion to the β-anomer is that the half-lives taken to reach anomeric equilibrium are similar for solutions initially prepared from 10 g and 4 g lactose per 100 mL

of water (36 min). Two batches of each of the 4% w/v and 10% w/v lactose solutions were run in the polarimeter and in all four experiments a half-life of 36 min was measured. Therefore, using feed solutions which just exceed the solubility limit of 7% w/v is expected to have minimal impact on the observed β/α anomeric ratio of the resulting amorphous lactose produced by both spray and freeze drying.

Mutarotation was also found to occur in the solid amorphous lactose over a 56 d storage period (Table II), for example the 30 min freeze-dried samples when stored for this period suffered a 25% increase in their β-anomer content. Lefort *et al.* (23) also reported an increase in β-anomer via solid state mutarotation, which was initiated by heating amorphous lactose to temperatures close its glass transition, whereas no significant mutarotation was detected at 25°C. Their postulate was that the mechanism of solid state mutarotation is different from that observed in aqueous solution as water was excluded from their milled material; however, in the current work 1.3 and 1.4% w/w water was found to be present within the stored amorphous lactose. For both studies, no re-crystallisation was detected over the time frame of the experiments, which for the Lefort *et al.* study was up to 4 h and for the current study reported here was 56 d. It would have been interesting if in the earlier study (23) the experiments had been repeated after an extended period of isothermal storage.

In aqueous solution, mutarotation follows apparent first order kinetics for reversible reactions approaching equilibrium (13), observed here by the consistency of the half-lives measured at different concentrations. At 25°C, as a consequence of mutarotation, the anomeric composition reaches a constant β/α ratio of 1.7 after 4 h. In the amorphous state and in the presence of small amounts of water (1.3–1.4% w/w) the mutarotation of lactose continues towards this 1.7 ratio (Table II), however it takes much longer to achieve. For example after 56 d storage at 25°C the composition of the 30 min spray-dried lactose samples had moved close the 1.7 ratio with a content of 60% β and 40% α, (Table II). The presence of the small amount of water within the amorphous lactose samples prepared in this work indicates that the mutarotation mechanism shown in Fig. 1 may still be applicable when considering the spray and freeze dried materials. This finding does not contradict the earlier work (23) as it was not clear whether the anomeric equilibrium had been achieved in these studies.

The dynamic nature of the β/α composition for amorphous lactose produced by different methods and stored for different lengths of time has a significant impact on the applications of lactose in pharmaceutical science. The two anomers possess a range of physicochemical properties (7, 29) and so not recording and maintaining the anomeric composition may result in arrange of potential variability including: a) a lack of control of drug deposition profiles when lactose is used as a carrier in DPI formulations (33); b) misinterpretation of the data generated when amorphous lactose is used as a model system for studying relaxation and stability within pharmaceutical glassy materials (34); c) poor predictive capability when amorphous lactose is used to produce calibration plots for the measurement of small amounts of amorphous content in processed and milled lactose destined for use in tablets, DPIs and capsules (10, 27).

CONCLUSION

This study has shown that the β/α ratio for amorphous lactose is not constant. It depends on both the production method and storage conditions. These findings impact on the use of amorphous lactose in medicines and as a calibrant for amorphous content determination**.** The aim of the work was accomplished; a simple solution based ^{1}H-NMR method has been established which can measure the β/α anomer composition of spray and freeze dried amorphous lactose with a standard deviation as low as 0.1% w/w (n=6).

The ^{1}H-NMR method described here has given important insights into the properties and production of amorphous lactose. The control of the storage conditions of amorphous lactose is vital, as the α to β mutarotation continues even in the solid amorphous form. A contributing factor to the wide ranging anomeric composition of amorphous lactose reported in the literature, (10, 18, 19, 24, 25) is a poor appreciation of the mutarotation equilibrium within the feed solutions prior to initiation of the drying processes. Thus to control the anomer composition present in spray and freeze dried lactose it is prudent to monitor and control the standing time of the feed lactose solutions.

When lactose is in aqueous solution its β/α content approaches a ratio of 1.7. Therefore we recommend in order to produce a consistent anomer composition within spray and freeze dried amorphous lactose, the standing time for the feed solution should be greater than 4 h, so that the solution is well-removed from the most dynamic region of the mutarotation profile, (single hatched area on Fig. 7) and within the plateau region (double hatched area on Fig. 7) so that the equilibrium content of 63% β and 37% α is approached. If the amorphous material has been formed from a solution that has not been allowed to stand for 4 h, the resulting solid will continue to undergo slow mutarotation should trace amounts of moisture be present, with the anomeric β/α ratio also tending towards 1.7 over a number of weeks of storage.

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