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The effect of particle size on the dehydration/rehydration behaviour of lactose

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ABSTRACT

Ethanolic suspensions of spray dried and micronized alpha lactose monohydrate (Lα·H_2O) with average particle size between 3 and 200 μm, have been prepared and their dehydration behaviour was investigated by ^{13}C CP-MASNMR spectroscopy. Sub-micron lactose suspension prepared by a novel high pressure homogenisation method has been compared with the standard ethanolic suspensions of Lα·H_2O prepared by reflux or static room temperature methods. In all cases, suspensions were shown to contain the stable anhydrous form of lactose (Lα_S). Several approaches were employed to remove ethanol from these suspensions and the resulting dry lactose powders were then analysed by FT-IR, PXRD and SEM to evaluate the effect of drying procedure on type and distribution of lactose polymorphs and particle size. For samples with mean particle size greater than 1 μm, the stable anhydrous polymorphic form of lactose was retained on removal of the ethanol, although differences in the morphology and particle size of the crystals were apparent depending on method of suspension formation. Sub-micron Lα_S, while stable in dry conditions, has been shown to be less stable to atmospheric water vapour than Lα_S with particle size between 3 and 200 μm.

1. Introduction

The pharmaceutical industry has a critical interest in the polymorphism of active pharmaceuticals and excipients because it is widely understood that different polymorphic forms of drugs can have different manufacturing, solubility and bioavailability properties. While these revised properties can render the polymorph beneficial, it can have a detrimental affect on the formulation and even small concentrations of a second polymorph can have serious implications to a drug product.

Lactose is widely accepted to exist in four crystalline forms, a single hydrated form; alpha lactose monohydrate (Lα·H_2O) and three dehydrated forms; beta lactose (Lβ), stable anhydrous (Lα_S) and unstable hygroscopic (Lα_H). All three anhydrous forms are generated from the monohydrate which occurs naturally as the major constituent in the milk of most mammals. All forms of lactose, except the unstable hygroscopic form, are used in the food and pharmaceutical industry. Lactose monohydrate is used in dry powder inhalation (DPI) formulation, and the stable anhydrous polymorph in metered dose inhalers (MDI), while beta lactose is often used in tablets owing to its preferential compaction properties (Hein et al., 2008). As well as the crystalline forms, non-crystalline or amorphous lactose may be generated in the formulation process.

While the generic definition of polymorphism is a material that exhibits different crystal structures within the solid-state but has identical characteristics in solution, this does not strictly apply to the alpha and beta anomers of lactose generated by mutarotation, though it has been applied to lactose for more than 50 years. The rapid interconversion between the two forms in aqueous solution agrees with McCrone's definition of polymorphism and is akin to the examples of conformational polymorphism for mannose and melibiose described by Dunitz and Bernstein (1995). Therefore, the accepted polymorphism terminology will be maintained in this article.

Lβ can be readily obtained using aqueous Lα·H_2O solutions and a co-solvent such as acetone (Larhrib et al., 2003). The stable anhydrous form, Lα_S, is generated by two different methods, either by using hard thermal dehydration methods i.e. heating to high temperature for a prolonged period, or by using a dehydrating anhydrous solvent such as methanol (Lim and Nickerson, 1973) or ethanol (Kirk et al., 2007). The latter 'soft dehydration' conditions are often preferred as the products formed are more crystalline as the thermal treatment of sugars often results in discolouration of the product commonly associated with the Maillard reaction (Maillard, 1912).

The ever increasing number of uses of nanotechnology continues to generate interest due to the potential for increased solubility and hence bioavailability. In terms of applications for pharmaceu-

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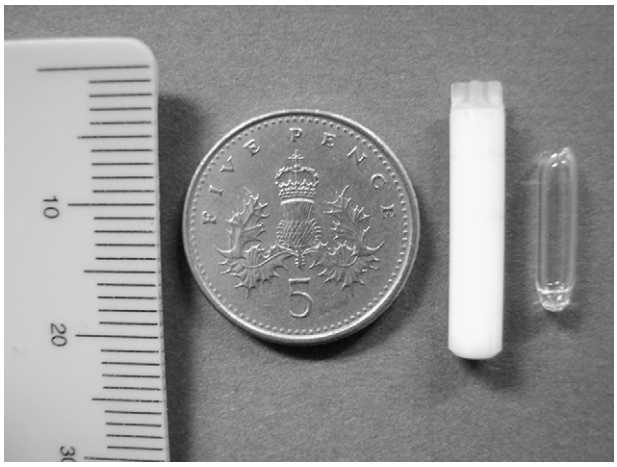

Fig. 1. Photographic size comparison of a glass insert, a standard 4 mm rotor and a 5p coin.

tical use, nanomaterials have been recognised as revolutionary technology for cell and tissue engineering and medical devices. Nanomedicine also generates improved tissue targeting, and as a consequence, a more accurate dose control. This essentially means that less active can be used per dose, which therefore reduces drug toxicity. Considering inhalation therapy in particular, nanoparticles provide the opportunity to further optimise the efficiency of drug delivery by deep lung penetration. The use of lactose as an excipient for inhalation therapy has been well documented in recent years (Larhrib et al., 1999; Malcolmson and Embleton, 1998; Iida et al., 2004; Zeng et al., 1999, 2001), as has the ever increasing need for the improvement of drug delivery. While modifications to inhalers themselves have generated much improvement in the success obtained by inhalation therapy (Tee et al., 2000), attention has more recently turned to the formulation and in particular the properties of the excipient. As a result of this, technology is becoming ever smaller in the hope that the improvement of these systems ensues (Patel et al., 2008; Morgan, 2005).

Dry powder inhalation formulations typically consist of an active pharmaceutical ingredient (API) and an excipient. Excipients are usually in the region of 63–90 μm particle size (Zeng et al., 1999; Elajnaf et al., 2006). This particle size is suitable since excipients are used as a carrier to ensure the active drug emerges to supply an accurate dosing from the drug delivery device. The carrier, typically $L\alpha{\cdot}H_2O$, is impacted in the oropharyngeal region and swallowed. The drug itself needs to be in the respirable range which is normally considered to be sub-5 μm. One of the skills of the formulators is to create a stable formulation with sufficient adhesion forces to keep the active attached to the carrier particles during storage, but to allow the particles to become separated in the event of turbulence created on actuation and dose delivery to the patient. The use of an excipient has proven to improve flow properties of the active into the lungs (Zeng et al., 1999; Crowder et al., 2002; Giry et al., 2006) as it prevents API agglomeration. However, should the dissociation mechanism fail, the active is also caught in the oropharynx and swallowed, effectively lowering drug activity. The potential for use of nanoparticles within this field means that, by lowering the particle size to within the respirable range (less than 5 μm) the carrier effect can be exploited without the need for the unreliable dissociation step, hence improving absorption of the active in the lower lung. This excipient effect becomes particularly prominent with the use of highly potent bronchodilators such as salmeterol, whereby a typical dose of 6 μg is administered per actuation, in comparison to more common dosages of 100–200 μg (Jinks, 2003).

There are a wide range of particle engineering approaches which can be used to produce particles of the inhalation particle size range, the most commonly used are micronization techniques such as ball milling or fluid jet milling. The particles produced by these techniques are irregular in shape and typically contain a high degree of amorphous material. There are approaches to reducing the amorphicity of these materials by conditioning (Traini et al., 2008) but typically they are used directly from the micronization process. Spray drying can provide a better particle size distribution and the particles often have a better shape, but they typically produce amorphous materials or partially amorphous materials which are notoriously difficult to stabilise in a product (Perkins et al., 2009). Other techniques that use supercritical fluids (e.g. SAS, Boutin et al., 2009) can provide an alternative but there is no clear indication that these techniques can produce powders with significantly improved performance over more traditional techniques.

A methodology to produce small particles of $L\alpha_S$ for aerosol formulations has been recently described (Jinks et al., 2006). A homogeneous mixture of $L\alpha{\cdot}H_2O$ with ethanol is treated at high pressure and produces cigar-shaped particles of $L\alpha_S$ less than 1 μm in size. This substance has been termed 'sub-micron' lactose and is an example of a bulking agent (or suspension aid) which can be used to improve the pharmaceutical performance of pressurised metered dose inhalers. The sub-micron lactose is added to the formulation and the active material (which is typically micronized) becomes "locked" into the formulation. This improves the suspension properties of the active and reduces problems such as deposition and shake dependence, which can be a problem with some formulations. As previously discussed, dose reproducibility is particularly important for highly potent drugs where it is neces-

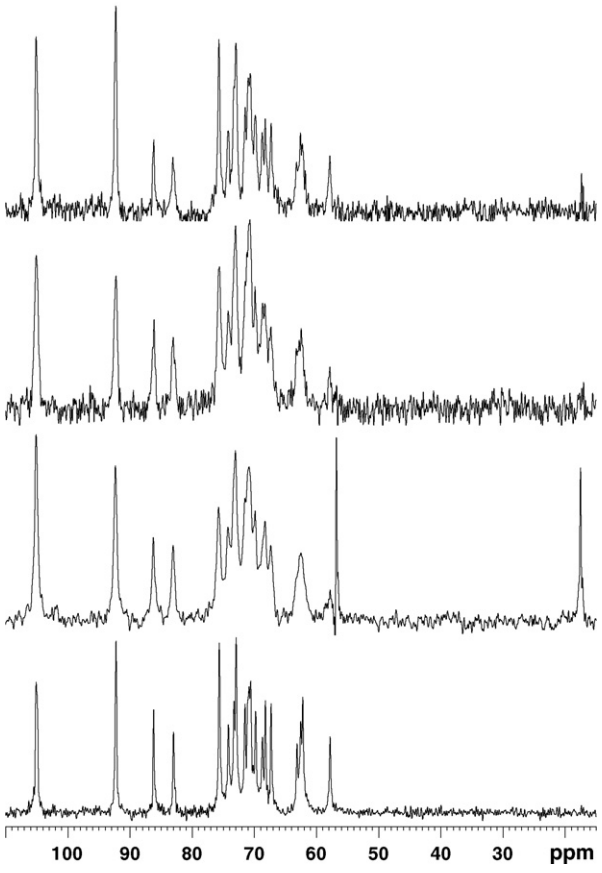

Fig. 2. ^{13}C CP-MASNMR spectral comparison of (top to bottom) sub-micron suspension, Lactochem extra fine crystals suspension (reflux), Lactochem extra fine crystals suspension (static dehydration), solid $L\alpha_S$.

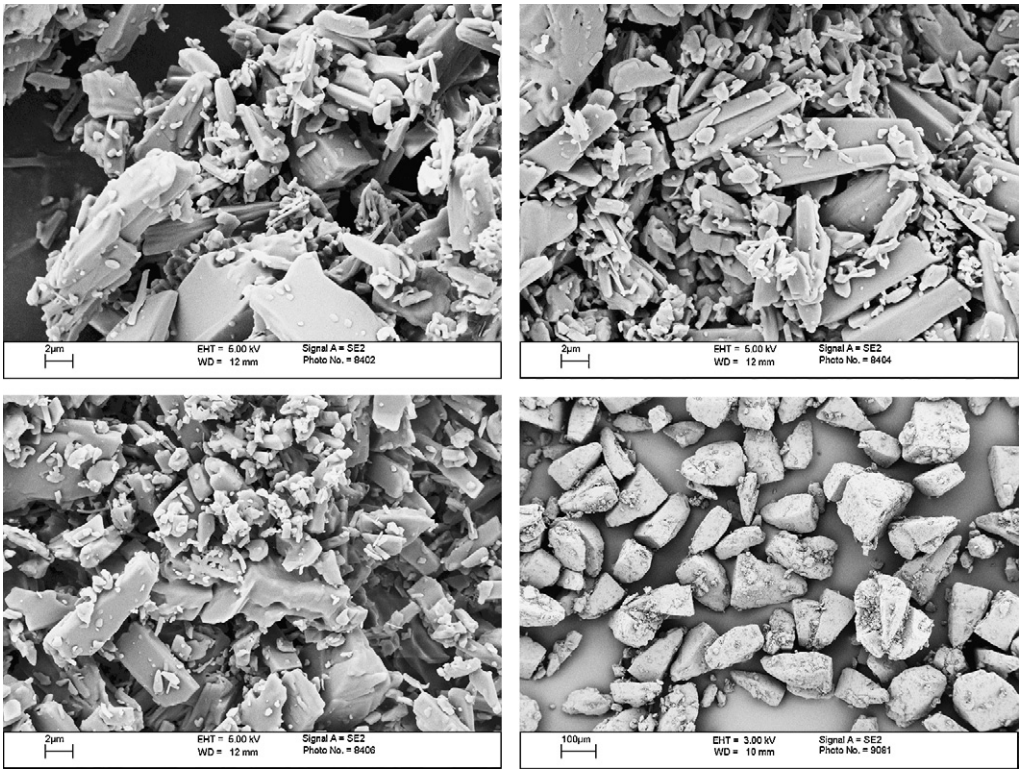

Fig. 3. SEM micrographs of Lactochem extra fine crystals refluxed suspensions after solvent removal (method 1, top left; method 2, top right; method 3, bottom left) in comparison to the starting material (bottom right).

sary to dose small quantities of an active pharmaceutical and the inclusion of a bulking agent can improve through-life dosing of the product.

This article investigates the stability of sub-micron lactose and the dehydration behaviour of Lα·H_2O samples of varying particle size in ethanol. Different methods of removing the solvent from the suspension were investigated to examine the effect of varying drying methods on both particle size and stability of the final product.

2. Experimental

2.1. Preparation of samples

2.1.1. Preparation of lactose polymorph standards

Single phase samples of the three major air stable polymorphs of lactose were prepared using standard methods for comparative purposes. Both Lβ and Lα_S were prepared from the Lα·H_2O sample purchased from Fluka (vide infra).

Alpha lactose monohydrate (Lα·H_2O) was analysed as provided (Fluka Biochemica, Cat. no. 61340). Analysis by powder X-ray diffraction (PXRD) was performed to check for the 18° 2θ reflection attributed to a crystalline impurity (Garnier et al., 2002), the absence of which implied sample purity.

Beta lactose (Lβ) and stable anhydrous alpha lactose (Lα_S) were prepared according to the method outlined by Kirk et al. (2007).

Beta lactose: Lα·H_2O was refluxed for 3 h in at least eight times the amount (w/v) of methanolic potassium methoxide solution (0.014 M). Methanol (Fisher Scientific Ltd.) was previously dried for 24 h over 4 A molecular sieves (dried at 400 °C prior to use).

Stable anhydrous alpha lactose: Lα·H_2O was refluxed for 24 h in at least 10 times the amount (w/v) of dry absolute ethanol (99.8%, Fisher Scientific Ltd., previously dried for 24 h over 4 A molecular sieves).

Lβ and Lα_S were analysed by PXRD to ensure sample purity, paying particular attention to any indication of the presence of the hygroscopic alpha lactose (Lα_H) polymorph. Lα_H readily rehydrates to Lα·H_2O, hence giving potential for a mixed (impure) sample. Lα_H has been well characterised by PXRD (Garnier et al., 2002; Platteau et al., 2004, 2005; Kirk et al., 2007) and neither of the samples displayed any of the reflections indicative of this polymorph. Due to the readily rehydrating nature of the polymorph, it was also necessary to check for Lα·H_2O in the patterns, again with a negative result.

2.1.2. Preparation of anhydrous alpha lactose ethanolic suspensions

The Lα·H_2O starting materials of particle size between 3 and 200 µm were provided by 3M Health Care (micronized lactose) and Friesland Foods DOMO (Lactochem extra fine crystals, Lactochem microfine, Lactochem fine powder, Lactopress spray dried, Lactopress spray dried 250) and were produced by micronization and spray drying techniques.

Absolute ethanol (99.8%, Fisher Scientific Ltd.) was dried by standing over 4 A molecular sieves (previously dried at 400 °C) for 24 h. The Lα·H_2O samples were refluxed in at least ten times the amount (w/v) of dry absolute ethanol and the suspension cooled and stored in a sealed container.

A second set of ethanolic suspensions were produced using the same materials and w/v quantities, however without the refluxing stage. PXRD of the dried products after four days showed incomplete dehydration (Lα·H_2O presence), implying that a longer dehydration period was necessary. Therefore, these suspensions were stored in sealed containers for four weeks to allow full dehydration to take place.

Sub-micron lactose was provided by 3M Health Care. It was produced by a standard in-house method (Jinks et al., 2006). Micronized Lα·H_2O was dispersed in anhydrous ethanol using a

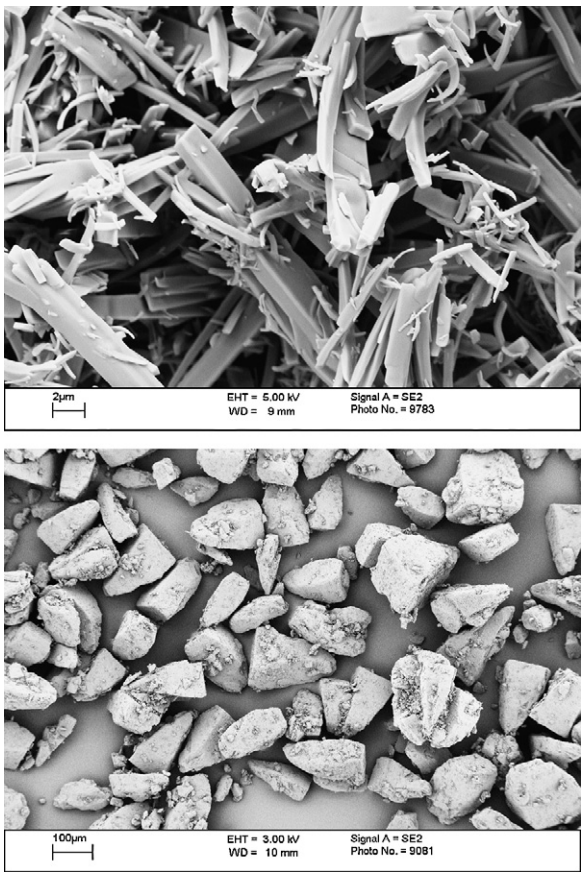

Fig. 4. SEM micrographs of Lactochem extra fine crystals non-refluxed suspension after solvent removal (method 3, top) in comparison to the starting material (bottom).

Silverson high shear mixer. The suspension was added to the product reservoir of an Avestin Emulsifier C160 homogeniser and re-circulated for 30 min at 10 kpsi. The dispersion was then passed out of the homogeniser at 20 kpsi and transferred to a sealed container. This process generated a suspension of final mass:volume ratio 1:8.4.

2.1.3. Methods for removing ethanol from the ethanolic suspensions

Ethanol was removed from each of the suspensions by three methods. Method 1, termed "open air evaporation" was implemented such that samples of each suspension (ca. 200 mg) were placed onto watch glasses and allowed to evaporate to dryness (typically 3 h). In method 2, "solvent removal by means of an absorbent medium", a thin layer (ca. 60 mg) of suspension was placed onto the surface of a piece of standard filter paper, such that the solvent was immediately absorbed (total drying time typically 5 min). Finally method 3, "solvent evaporation in a dry atmosphere", where suspensions (ca. 200 mg) were placed over a desiccant (self-indicating silica gel) and the solvent allowed to evaporate to dryness (approximately 3 h).

2.2. Instrumentation

2.2.1. Scanning electron microscopy (SEM)

The micrographs were produced on a Carl Zeiss 1530 VP field emission gun scanning electron microscope (FEG-SEM) with an EDAX pheonix EDX X-ray microanalysis system.

2.2.2. CCD-Raman

Data were collected on a Horiba Jobin Yvon LabRAM HR800 spectrometer using a 632.8 nm HeNe laser with 600 grating and 50× objective. The hole size used was 1000 μm and the slit aperture 150 μm. Multiscan collections were performed, with each 200 cm^{-1} scan comprising of 20 accumulations, each of 20 s exposure. The full range of each scan was 200–1500 cm^{-1}. Data were analysed using LabSpec software v5.25.

2.2.3. FT-Infrared

Data were collected on a Shimadzu FTIR-8400 s Fourier transform infrared spectrophotometer. The spectrophotometer was set to a resolution value of 1.0 and 30 scans were performed per sample in the range of 400–4000 cm^{-1}. Sample preparation was as a CsI disc pressed to ~10 t using a 13 mm die. Spectra were analysed using Shimadzu IRSolution software v1.30.

2.2.4. Powder X-ray diffraction (PXRD)

Samples were analysed with a Bruker D8 Advance diffractometer, using monochromated CuKα_1 radiation at λ = 1.5406 Å and a position sensitive detector (PSD). Perspex flat plate sample holders

Table 1
Comparison of ^{13}C SSNMR peaks, FT-IR bands and XRD d-spacings unique to L$\alpha\cdot H_2O$ and Lα_S.

	L$\alpha\cdot H_2O$			Lα_S				
^{13}C SSNMR (ppm)[a]	106.6	92.2	86.6	105.9	93.1	83.9		
FT-IR (cm^{-1})	876	899	916	855	874	894	913	930
XRD (d-spacing, Å)	4.46	4.554	4.464	4.55	4.63	4.89		

[a] Referenced to adamantane solid.

Table 2
Polymorphic determination of the dried products from the refluxed suspensions.

Suspension	Approximate particle size of starting material (μm)	Polymorph produced after solvent removal		
		Method 1	Method 2	Method 3
Lactochem extra fine crystals	180–200	Lα_S	Lα_S	Lα_S
Lactopress spray dried	150–180	Lα_S	Lα_S	Lα_S
Lactopress spray dried 250	100–150	Lα_S	Lα_S	Lα_S
Lactochem fine powder	30–50	Lα_S	Lα_S	Lα_S
Lactochem microfine	5–10	Lα_S	Lα_S	Lα_S
Micronized lactose[a]	2–4	Lα_S	Lα_S	Lα_S
Sub-micron[a]	0.5–2	L$\alpha\cdot H_2O$	L$\alpha\cdot H_2O$[b]/Lα_S	Lα_S

[a] Provided by 3M Health Care Ltd. All other samples provided by Friesland Foods DOMO.
[b] Major component.

Table 3
Elemental analysis of the dried sub-micron suspension in comparison to the stable lactose polymorphs.

	Calc C (%)	Exp C (%)	Calc H (%)	Exp H (%)
$L\alpha_S$	42.11	41.97	6.43	6.34
$L\alpha{\cdot}H_2O$	40.00	40.12	6.67	6.79
Lβ	42.11	41.98	6.43	6.44
Sub-micron lactose (method 1)	42.11	40.07	6.43	6.71
Sub-micron lactose (method 2)	42.11	40.21	6.43	6.63
Sub-micron lactose (method 3)	42.11	42.00	6.43	6.44

were used to mount the samples and data were collected between the 2θ range of 5° and 35°, using 0.014767° steps over a period of 2 h.

2.2.5. Solid-state nuclear magnetic resonance spectroscopy (SSNMR)

Solid-state NMR spectra were recorded on Bruker Avance 500 MHz NMR spectrometer, equipped with a 2.5 mm MAS HX probe, 100 W proton amplifier and 500 W X amplifier. Cross-polarisation used a 1 H pulse of 2 μs (at −5.5 dB), a ramped proton CP pulse of 2 ms (at −4.0 dB) and a carbon CP pulse of 2 ms (at −3.1 dB). TPPM15 proton decoupling was applied during the 30 ms acquisition time. A relaxation delay of 60 s was used between each scan and 256 scans were acquired. FIDs contain 3k data points which were Fourier transformed into 16k data points, no function was applied to the FID. Line fitting and deconvolution was performed using Bruker Solid Line-Shape Analysis program. Spectra were referenced to external adamantane and the magic angle was set up using KBr. Poor quality spectra were recorded when a relaxation delay of 10 s was used, however, a delay of 60 s showed good quality spectra that were equivalent to test spectra recorded using 300 s delay.

2.2.6. Thermal analysis

Thermal analysis in the form of thermogravimetric analysis and differential thermal analysis were carried out using a TA Instruments 2960 SDT instrument. The samples were heated at 10 °C/min in air. Each sample was analysed once and data were plotted using microsoft excel.

2.2.7. Elemental analysis

Data were collected using an Exeter Analytical Inc. (EAI) CE-440 Elemental Analyzer.

3. Results and discussion

3.1. SSNMR of suspensions

SSNMR was performed on the suspensions to determine the polymorph present within the suspensions, and therefore assess the effect of ethanol removal on lactose polymorphism. ^{13}C SSNMR was performed to produce powder patterns of each sample while remaining in ethanol suspension. This unique method of data collection involved the preparation of a small glass insert (Fig. 1) made from a 3 mm solution state NMR tube (Norell S-3-HT-400-7), into which the suspension in question was placed. Centrifugation allowed effective packing of the insert (while the sample remained under ethanol) such that, following sealing, a sufficient quantity of material was present to produce a high quality SSNMR signal. Analysis of the insert was carried out by placing into the normal SSNMR rotor and spinning in the usual way. This type of analysis provided the opportunity for direct comparison of spectra of suspension and dried samples.

Results confirmed, by comparison to standard data, that the sub-micron suspension was in the stable anhydrous form. This was compared to the suspension produced from the largest particle size $L\alpha{\cdot}H_2O$, Lactochem extra fine crystals, which was also shown to be in the stable anhydrous form after complete dehydration via both refluxed and non-refluxed methods. The SSNMR results are shown in Fig. 2.

3.2. Analysis of powders after removal of ethanol

3.2.1. SEM analysis

Once each of the drying methods was employed, a primary point of analysis was to ensure that particle size was retained, and that agglomeration had not taken place during the drying. SEM analysis enabled the determination of particle size post ethanol removal, which showed that particle size reduction had occurred for each of the refluxed suspensions (Fig. 3), independent of method of ethanol removal. The particles were irregular in shape and typically between 3 and 20 μm. This irregularity in shape is not consistent with the expected characteristics of $L\alpha_S$ particles. Particle morphology was not affected by analysis processing (NMR and PXRD sample preparation, confirmed by SEM of non-processed material), hence it was concluded that production of these morphologies is most likely

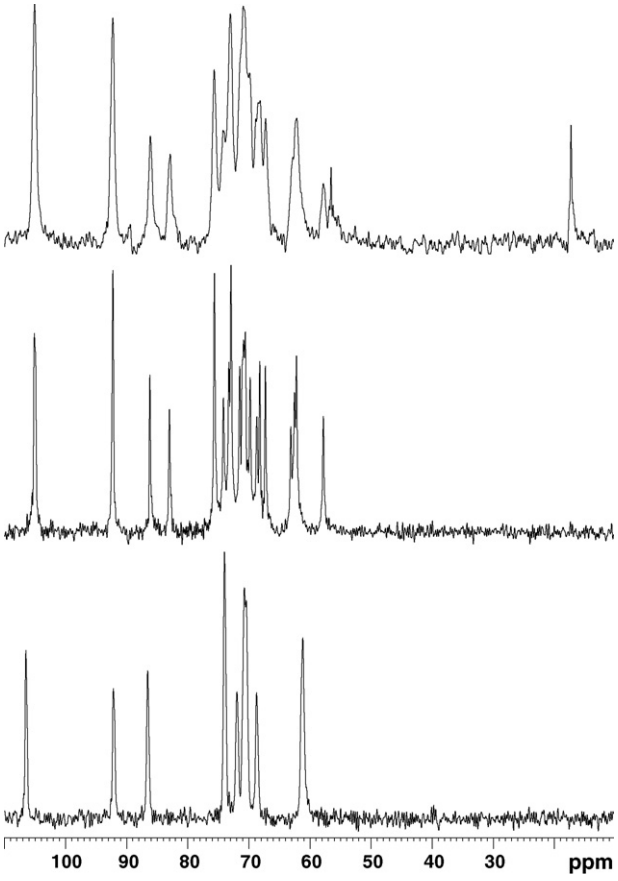

Fig. 5. ^{13}C CP-MASNMR spectral comparison of (top to bottom) 24 h treated sub-micron suspension, $L\alpha_S$ solid and $L\alpha{\cdot}H_2O$.

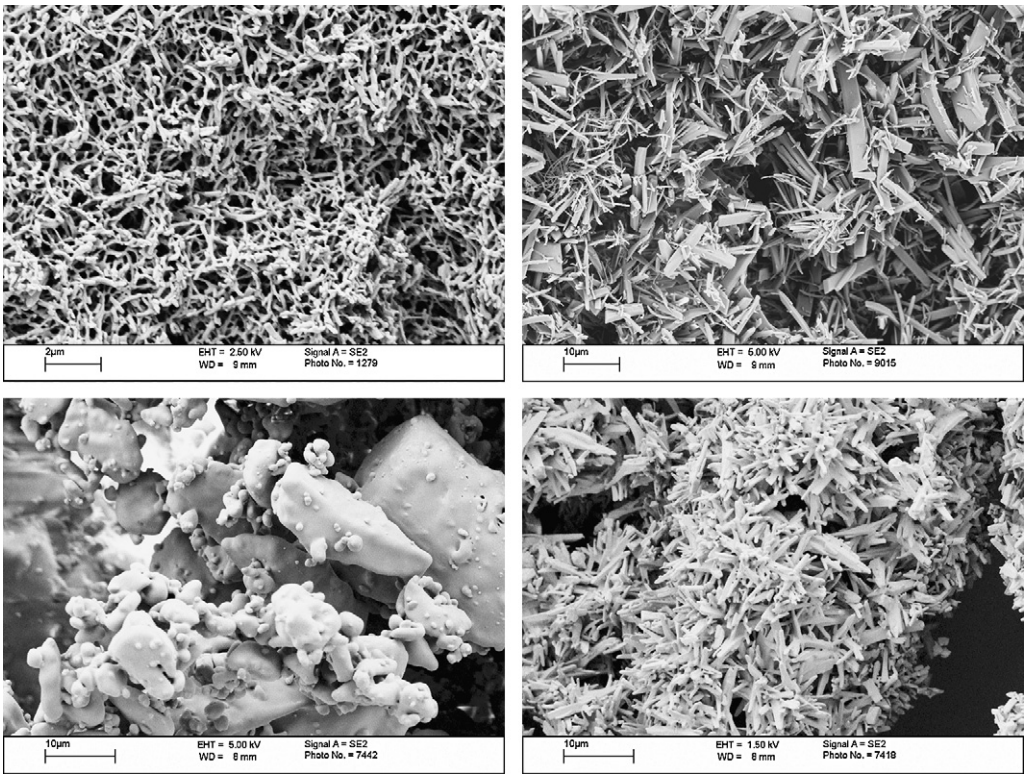

Fig. 6. SEM micrographs of sub-micron $L\alpha_S$ (clockwise starting top left) in comparison to standard $L\alpha_S$, $L\alpha{\cdot}H_2O$ and $L\beta$.

associated with the refluxing process. Vigorous stirring and the effect of boiling solvent during refluxing produces an impact and attrition environment (particle size reduction produced as a result of friction and abrasion similar to that observed by ball milling), which, in comparison to the starting material, reduces the particle size and generates a rounded particle with smooth surfaces.

To confirm the cause of irregular particle formation, SEM analysis was also carried out on the dried products of the non-refluxed suspensions (Fig. 4). Results showed each of the samples to have the expected elongated tape-like particles associated with $L\alpha_S$, with no evidence of the original starting material particle shapes remaining.

3.2.2. Solid-state analysis

Each suspension was subjected to the three solvent removal methods previously discussed (Section 2.1.3), and the resulting products initially characterised using FT-IR, PXRD and SSNMR analysis to identify the polymorph present. Characteristic peak positions of $L\alpha{\cdot}H_2O$ and $L\alpha_S$ for the techniques discussed are given in Table 1. The results, outlined in Table 2, imply that the drying methods were suitable for samples of a larger particle size, however, for the sub-micron sample, the slowest method of solvent removal (method 1) resulted in the rehydration of the anhydrous polymorph to form $L\alpha{\cdot}H_2O$. This was partially coincident with the result of solvent removal by means of an absorbent medium (method 2), which generated a mixture of $L\alpha{\cdot}H_2O$ and $L\alpha_S$.

This rehydration (or partial rehydration) of the sub-micron sample is an important observation, as the stable anhydrous polymorph should remain anhydrous until subjected to relative humidities in excess of 75% (Listiohadi et al., 2008).

Elemental analysis shows the extent of rehydration (Table 3). For example the sub-micron sample dried by method 1 is directly comparable, in terms of elemental composition, to that of the $L\alpha{\cdot}H_2O$ standard. Likewise, the mixed phase sub-micron sample dried by method 2 appears to be predominantly $L\alpha{\cdot}H_2O$, though the presence of $L\alpha_S$ is clearly indicated by carbon content.

It is thought that this rehydration of the sub-micron $L\alpha_S$ is as a result of water physisorption following solvent hydration. The ethanol solvent rehydrates by absorbing atmospheric moisture, at which point the sub-micron lactose is directly subjected to an aqueous environment. Due to its high chemical potential, it readily reabsorbs water into the lattice as the dry particles are produced, thus reforming $L\alpha{\cdot}H_2O$.

This theory was confirmed by a simple SSNMR experiment on the sub-micron suspension. A large volume of the suspension was subjected to open air drying conditions (method 1) for a period of 24 h. The large volume employed slowed down ethanol evaporation, such that after the 24 h period, the sample was still in suspension form. Another glass insert was prepared using this sample, and was analysed with the SSNMR technique previously outlined (Fig. 5). At a first glance, the results can be interpreted to show $L\alpha_S$ resonances. However, there is a significant amount of line broadening present within the spectrum to imply a loss of crystallinity that may be associated to the rehydration transition.

3.3. Characterisation of sub-micron lactose

3.3.1. SEM

The SEM micrograph shown (Fig. 6) confirms that the average particle size of the sub-micron sample is below 1 µm. The shape of the particle is characteristic and is an oblate spheroid or cigar-shaped particle. This shape is also indicative of $L\alpha_S$ (Fig. 6, top right) as opposed to $L\alpha{\cdot}H_2O$ (Fig. 6, bottom left), which has a more rounded particle shape (when crystallised rapidly). $L\alpha_S$ is more comparable in morphology to that of $L\beta$ (Fig. 6, bottom right), as both have a smooth surfaced, needle shaped crystal. Sub-micron lactose has been shown to have excellent flocculation characteristics (Jinks, 2003) such that in an inhalable formulation, the high density of very small particles holds the active drug in suspension. This means the active drug is not able to sediment and the dosing characteristics of pMDI devices are significantly improved.

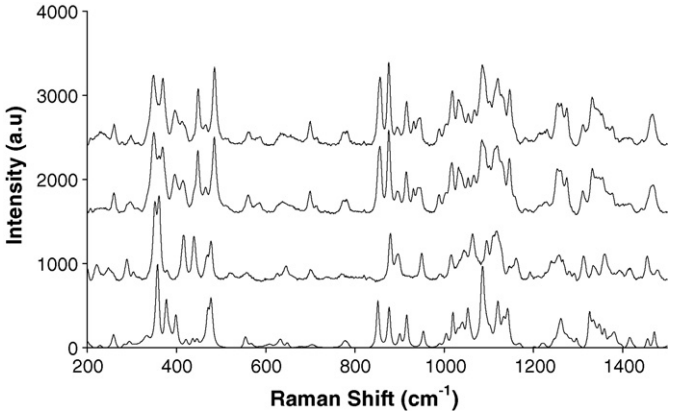

Fig. 7. CCD-Raman spectra of (top to bottom) sub-micron lactose, $L\alpha_S$, Lβ and $L\alpha{\cdot}H_2O$.

3.3.2. CCD-Raman

The spectra for the three forms of lactose discussed are shown in Fig. 7 along with a comparison to the sub-micron sample. Characterisation of lactose using Raman spectroscopy has been well documented since the 1970s (Susi and Ard, 1974). It is also well known that the spectra obtained for $L\alpha{\cdot}H_2O$ and its anomer, Lβ, are significantly different. A change in conformation leads to alteration of C–O–H vibrations, which has a marked effect (through coupling) on the vibrational behaviour of the ring structure. However, it should also be observed that the characteristic stretching and bending frequencies of the C–O–C glycosidic bond (the bond linking the two sugar moieties) are present in both spectra (~1100 and ~350 cm^{-1}).

With regards to the sub-micron sample, $L\alpha_S$, it is scientifically viable to expect similarities in the spectrum to that of its parent compound, $L\alpha{\cdot}H_2O$. The spectrum produced is a good match to the known $L\alpha_S$ sample, and also shows the characteristic C–O–C stretching and bending frequencies.

3.3.3. FT-IR

Previous study into the IR spectra of lactose polymorphs has shown that the main diagnostic region is 800–1000 cm^{-1} (Kirk et al., 2007). It is here where distinct differences between each of the polymorphs can be identified. The spectra collected indicate the characteristic vibrational bands for each polymorph (Fig. 8).

By comparing the sub-micron sample to the standards, it is clear that a match to the $L\alpha_S$ spectrum is seen.

Across the full range of the spectra, there are many similarities between the polymorphs. The spectra mainly comprise of a series of low wavenumber vibrations, though each have a strong vibrational band at ~3200 cm^{-1}, indicative of the intermolecular OH groups which show stretching frequencies in this range. One distinct difference between the $L\alpha{\cdot}H_2O$ spectrum and the anhydrous polymorphs are the vibrational bands at ~1650 and ~3500 cm^{-1}. These stretches are indicative of the free water molecules within the lattice.

3.3.4. PXRD

The PXRD patterns produced indicate distinct differences between the $L\alpha{\cdot}H_2O$, $L\alpha_S$ and Lβ samples. The patterns are compared to sub-micron lactose in Fig. 9.

The $L\alpha{\cdot}H_2O$ pattern shows the major reflections occurring at the following d-spacings; 4.46, 4.54 and 4.64 Å. The reflection with d-spacing 4.46 Å is particularly characteristic of $L\alpha{\cdot}H_2O$, hence its absence from the other patterns implies that there is no $L\alpha{\cdot}H_2O$ presence within the other samples.

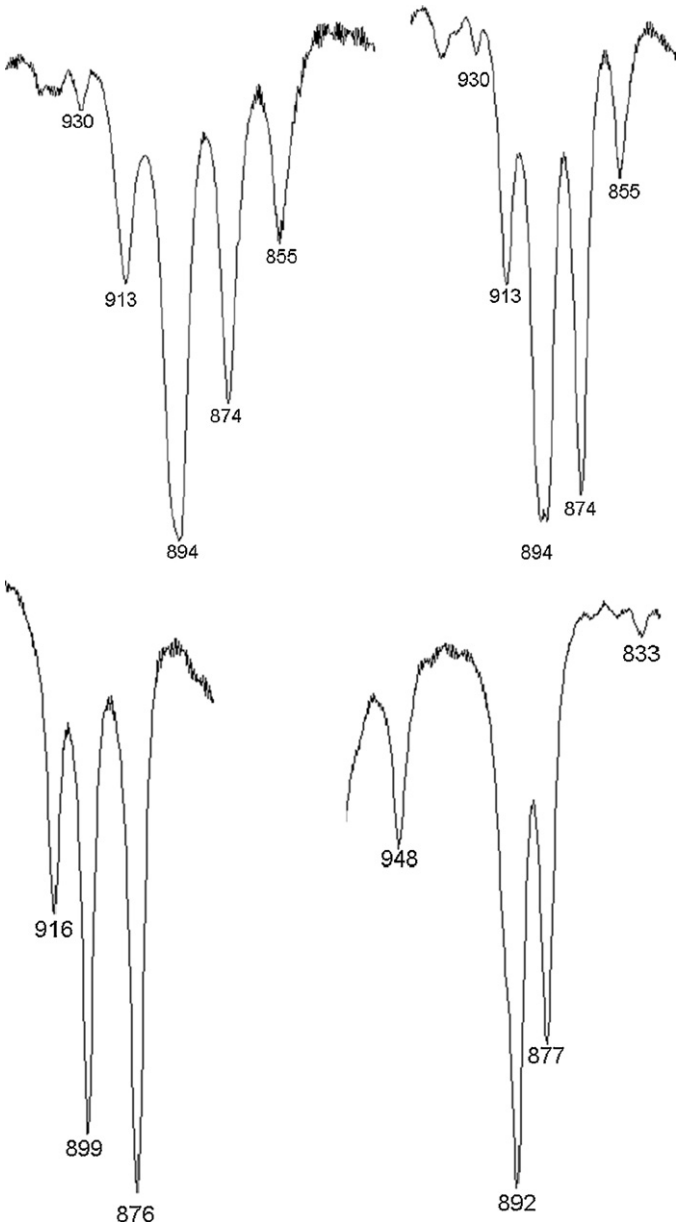

Fig. 8. Diagnostic regions of the FT-IR spectra of (clockwise starting top left) sub-micron lactose, $L\alpha_S$, Lβ and $L\alpha{\cdot}H_2O$.

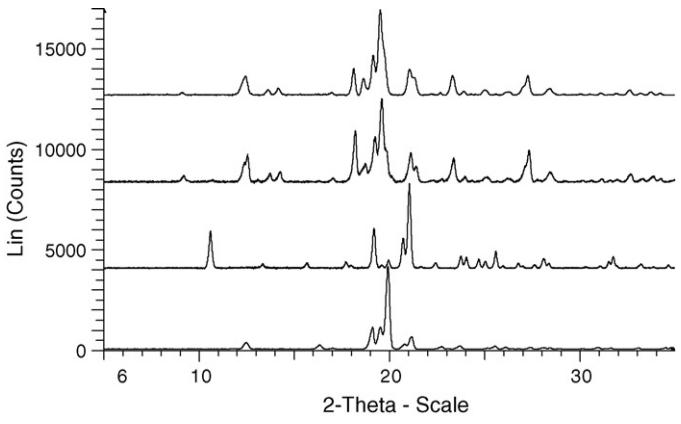

Fig. 9. Powder XRD patterns of (top to bottom) sub-micron lactose, $L\alpha_S$, Lβ and $L\alpha{\cdot}H_2O$.

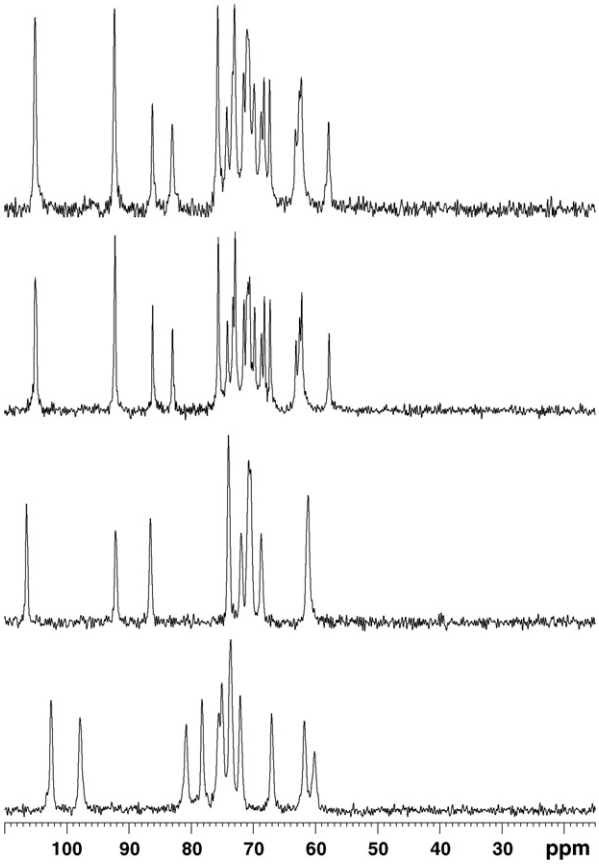

Fig. 10. ^{13}C CP-MASNMR spectra of (top to bottom) sub-micron lactose, Lα_S, Lβ and Lα·H_2O referenced to solid adamantane.

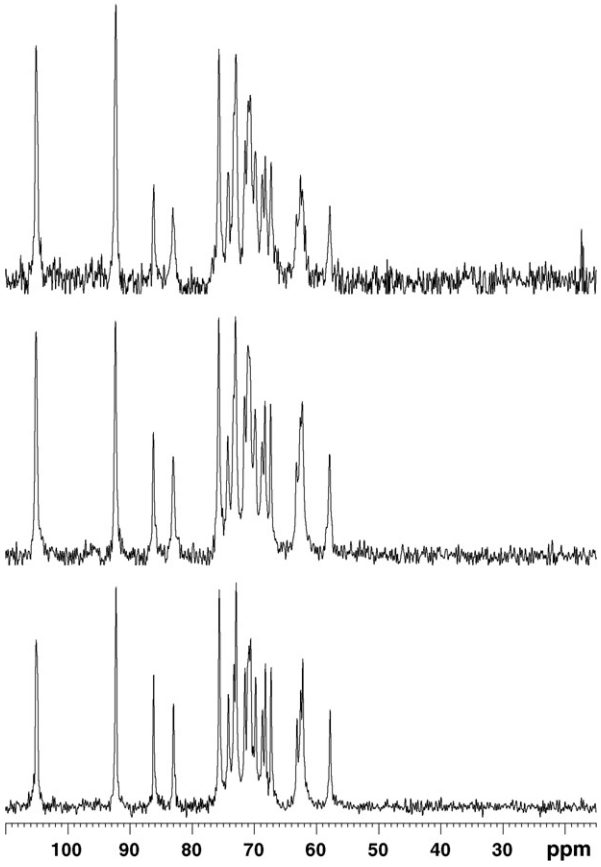

Fig. 11. ^{13}C CP-MASNMR spectral comparison of (top to bottom) sub-micron lactose suspension, sub-micron lactose (solvent removal by method 3) and Lα_S.

The d-spacings for the major reflections of the Lβ pattern occur at 4.28, 8.35 and 4.22 Å. The reflection with d-spacing 8.35 Å is indicative of the Lβ polymorph as none of the other polymorphs show reflections of this intensity in this range of the pattern. Again, the absence of this peak from the sub-micron sample implies that there is no beta lactose present.

The Lα_S polymorph also has a well researched PXRD pattern. The d-spacings of the three major reflections occur at 4.55, 4.63 and 4.89 Å. The pattern also has a characteristic reflection at approximately 9° 2θ. Other polymorphs do not exhibit reflections at such low angles, and hence this reflection can be used to detect even small quantities of Lα_S.

When comparing the Lα_S pattern to that of the sub-micron sample it is apparent that there are distinct crystallographic similarities. The absence of all characteristic reflections for the other lactose polymorphs discounts them as a crystallographic match. Consequently it can be concluded from the obtained data that the sub-micron sample consists of the Lα_S polymorph and is not contaminated with Lα·H_2O.

3.3.5. SSNMR

The resonances of Lα·H_2O and Lα_S have previously been documented and assigned to the corresponding part of the lactose molecule (Earl and Parrish, 1983). Comparison of the spectra produced indicates that the sub-micron lactose shows agreement with the other methods of analysis, and a clear match to Lα_S (Fig. 10).

Comparison of dried sub-micron lactose, Lα_S and the SSNMR spectrum of the sub-micron suspension (Fig. 11) shows that while in dried form (providing a suitable drying method was used) and in suspension the lactose present is in the stable anhydrous Lα form.

3.3.6. TGA and DTA

The TGA and DTA traces for each form of lactose are shown in Figs. 12–15.

The differences between Lα·H_2O and the anhydrous samples become apparent when paying particular attention to the number of endotherms observed in the results. Lα·H_2O undergoes two transitions, indicated by two mass decreases and corresponding endotherms in the plot. The first is due to loss of water. A natural assumption would be that this phase occurs at the boiling point of water, however due to interactions within the lattice, the boiling point effectively increases to overcome any forces of attraction. The transition is hence observed at approximately 120 °C. The second endotherm indicates the decomposition of the sample to oxides of carbon.

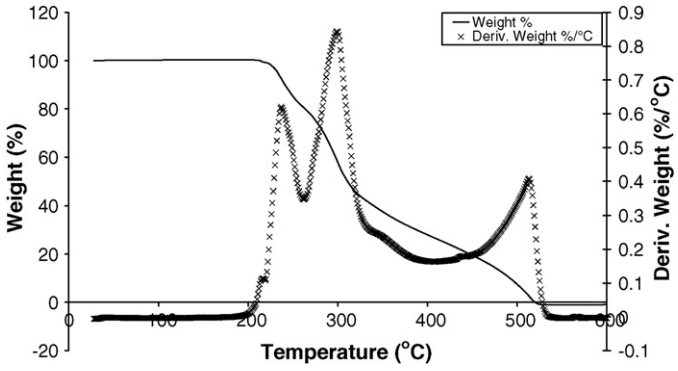

Fig. 12. TGA/DTA trace for sub-micron lactose.

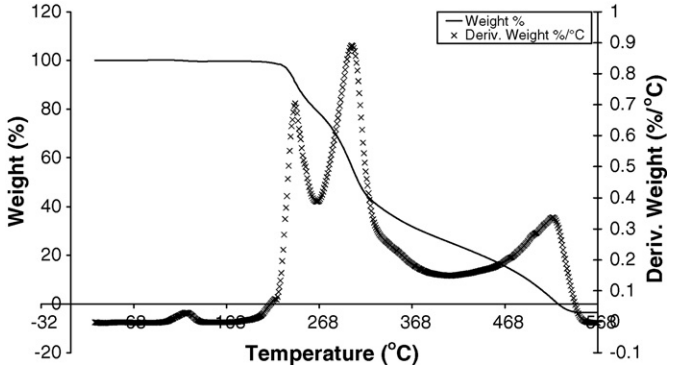

Fig. 13. TGA/DTA trace for Lα_S.

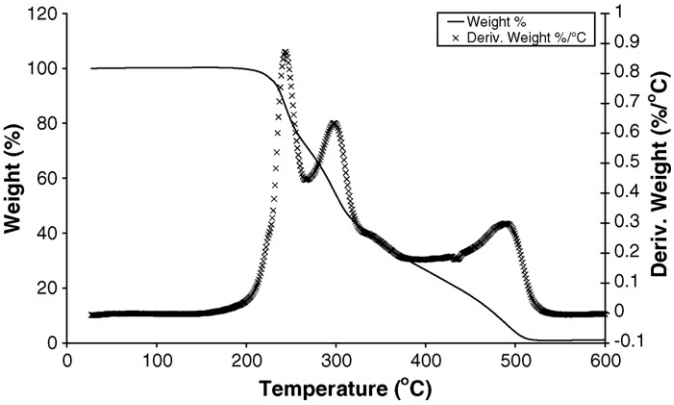

Fig. 14. TGA/DTA trace for Lβ.

For the anhydrous samples (Lα_S, sub-micron lactose and Lβ), there is no loss of water, hence a single mass loss is observed in the plot. This endotherm is consequently associated with the direct decomposition of the samples.

There are distinct similarities between the known Lα_S sample and the sub-micron lactose, such as the temperature of decomposition (onset at approximately 220 °C), hence strengthening the argument that the sample is in fact Lα_S and not Lα·H_2O.

3.3.7. Elemental analysis

The table shown compares the elemental composition of the sub-micron lactose in comparison to the three standards, and the expected values for this analysis. Results are shown previously in Table 3.

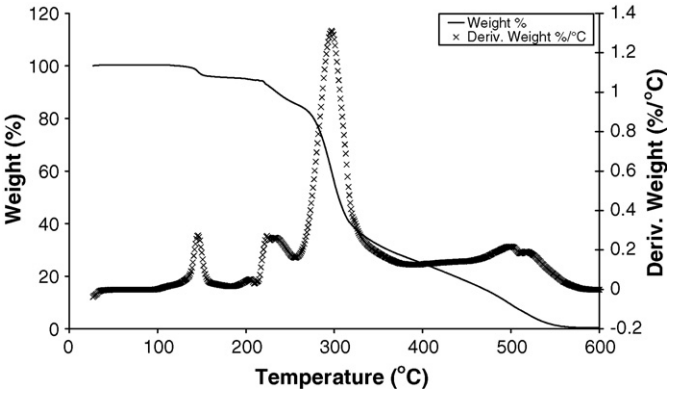

Fig. 15. TGA/DTA trace for Lα·H_2O.

4. Conclusions

Polymorph production from a suspension of lactose in anhydrous ethanol was analysed. Results indicate that particle size plays an important role in the rehydration behaviour of anhydrous lactose during the solvent removal stages. Where larger particles (up to 200 μm) commonly retain their anhydrous nature, smaller particles (1–2 μm) can be subjected to rehydration, even at low humidities.

It was proven that sub-micron lactose in ethanolic suspension is present in the stable anhydrous form. It has also been shown that polymorph retention is possible in the dried state, subject to the solvent removal conditions employed. Where a slow rate of solvent removal is employed (as was demonstrated by evaporation method 1) rehydration of the stable anhydrous product occurs to yield alpha monohydrate.

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