Research Article

Characterisation and Deposition Studies of Recrystallised Lactose from Binary Mixtures of Ethanol/Butanol for Improved Drug Delivery from Dry Powder Inhalers

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Abstract. Dry powder inhaler formulations comprising commercial lactose–drug blends can show restricted detachment of drug from lactose during aerosolisation, which can lead to poor fine particle fractions (FPFs) which are suboptimal. The aim of the present study was to investigate whether the crystallisation of lactose from different ethanol/butanol co-solvent mixtures could be employed as a method of altering the FPF of salbutamol sulphate from powder blends. Lactose particles were prepared by an anti-solvent recrystallisation process using various ratios of the two solvents. Crystallised lactose or commercial lactose was mixed with salbutamol sulphate and *in vitro* deposition studies were performed using a multistage liquid impinger. Solid-state characterisation results showed that commercial lactose was primarily composed of the α -anomer whilst the crystallised lactose samples comprised a α/β mixture containing a lower number of moles of water per mole of lactose compared to the commercial lactose. The crystallised lactose particles were also less elongated and more irregular in shape with rougher surfaces. Formulation blends containing crystallised lactose showed better aerosolisation performance and dose uniformity when compared to commercial lactose. The highest FPF of salbutamol sulphate ($38.0 \pm 2.5\%$) was obtained for the lactose samples that were crystallised from a mixture of ethanol/butanol (20:60) compared to a FPF of $19.7 \pm 1.9\%$ obtained for commercial lactose. Engineered lactose carriers with modified anomer content and physicochemical properties, when compared to the commercial grade, produced formulations which generated a high FPF.

KEY WORDS: deposition study; dry powder inhaler; lactose; particle engineering; salbutamol sulphate.

INTRODUCTION

In order to maximise the probability of drug particles from a dry powder inhaler (DPI) reaching the lower respiratory tract system, their aerodynamic diameter should range between 1 and 5 μm (1–4), but fine drug particles with that size are known to have poor flowability, aerosolisation properties and high dose variability due to high forces of

cohesiveness (5,6). In order to overcome such a drug aggregation problem and to achieve effective delivery of the drug to the lungs from DPIs, several formulation approaches have been introduced, but the most extensively employed commercially involves the incorporation of an excipient carrier. Drug–carrier formulations are therefore considered the typical formulation for DPIs (7,8) and consist of interactive mixtures comprising drug particles with a diameter of 0.5–5 μm and carrier particles with a diameter of about 30–90 μm (9,10). In drug–carrier formulations, carrier particles reduce agglomeration of the drug particles and enhance their aerosolisation by reducing their cohesiveness, as a consequence of adhesion of the drug to the carrier surface (7). However, one of the major drawbacks of drug/carrier-based formulations is the inadequate detachment of drug from carrier due to high drug–carrier adhesive forces which can lead to poor drug deposition efficiency in drug–carrier DPI formulations (10). This can result in deposition of the large carrier particles in the mouth and oropharyngeal regions after aerosolisation, leading to subsequent clearance by swallowing. However, a fraction of drug particles can be deposited in the lower airways, which is the site of action for most respirable drugs (11,12). Several approaches have been

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described in the literature for enhancing drug-carrier detachment after the aerosolisation. For example, the modification of the size, shape and surface texture has been attempted (13–16). It was shown that by increasing the surface smoothness of the carrier particles, the flowability and dispersibility of salbutamol sulphate from a Rotahaler® device could be improved (17). Adding fine particles of carrier to the larger carrier particles resulted in improvement in the DPI performance, which was attributed to a reduction in the adhesive forces as a result of increasing the separation distance between drug-carrier particles (5,18,19). Introducing small cavities or asperities to smooth lactose surfaces resulted in a decrease in fine particle fraction (FPF) and dispersibility regardless of particle size (5), whereas the crystallisation of lactose particles from Carbopol gels led to an increase in the surface smoothness of the lactose carrier particles, and this promoted better deposition (20). Ethanol was also used as an anti-solvent to produce engineered lactose particles with higher elongation ratio, and this was shown to improve significantly the deposition profiles of salbutamol sulphate (21). Although such an approach proved interesting, little information has been provided since that time regarding the effect of using different solvents and in particular combinations of binary anti-solvent, on the physicochemical properties and aerosolisation efficiency of carrier particles produced by employing a multi-solvent crystallisation technique. Thus, the aim of the present study was to investigate whether the crystallisation of lactose from different ethanol/butanol co-solvent mixtures could be employed as a method of altering the FPF of aerosolised drug from powder blends. Salbutamol sulphate was the model drug used throughout. The physicochemical properties of the resulting crystallised or engineered lactose particles were recorded. To achieve the aim, aerosolisation performance of DPI formulations containing salbutamol sulphate and the engineered lactose was compared with that obtained from commercial lactose formulations.

MATERIALS AND METHODS

α -Lactose monohydrate (DMV International, the Netherlands) and salbutamol sulphate (LB Bohle, Germany) were used. Analytical reagent grade of absolute ethanol and 1-butanol were obtained from Fisher Scientific, UK.

Crystallisation Procedure

In order to investigate the effect of non-solvent polarity index on the properties of crystallised lactose, two non-solvents—ethanol and butanol with polarity indices of 5.2 and 4, respectively—were selected. Both of these non-solvents are miscible with each other and also miscible with lactose solvent (which is water). These were the principal reasons for selecting ethanol and butanol for use as non-solvents in the present study.

Lactose monohydrate was crystallised under different conditions to produce different batches of lactose particles. Lactose was dissolved in 100 mL of water at 45°C to produce

a 20% (w/v) lactose solution. A sample of this solution (5 mL) was added using a syringe to different combinations of anti-solvents, ethanol/butanol (80:0, 60:20, 40:40, 20:60 and 0:80, v/v) at a rate of 5 mL/min under constant stirring of 200 rpm (the length and width of blade were 3 and 0.5 cm, respectively). The total volume of non-solvent was kept constant (80 mL) for all experiments. Lactose crystals were formed as the lactose solution was added. Stirring was maintained under the same constant speed for 10 min at room temperature (22°C) after the lactose solution was added. The precipitated particles were collected by filtration under vacuum through a cellulose filter paper (<0.45 μ m) fitted into the filtration unit. The harvested crystals were dried for 24 h in an oven at 70°C, after which they were transferred to a glass vial and sealed until required for further investigation.

Particle Size Distribution Analysis

Particle size analysis was conducted using a Sympatec (Clausthal-Zellerfeld, Germany) laser diffraction particle size analyser. The volume mean diameter (VMD), particle size distribution ($D_{10\%}$, $D_{50\%}$, $D_{90\%}$) and span were calculated automatically using the software provided. Span, which is a measure of the width of the size distribution, was calculated using the following equation:

$$\text{Span} = (D_{90\%} - D_{10\%}) / D_{50\%} \quad (1)$$

where $D_{90\%}$, $D_{10\%}$ and $D_{50\%}$ are the equivalent volume diameters at 90%, 10% and 50% cumulative volume distributions, respectively.

Approximately 100–200 mg of each sample was dispersed into the cuvette containing ethanol saturated with lactose. The measurement was performed with the HELOS sensor using WINDOX software.

Selection of Lactose Particle Size Fraction

All crystallised lactose and commercial lactose samples were sieved to obtain particle size in the range 63–90 μ m. This was achieved by pouring the lactose monohydrate on top of a 90- μ m sieve which was placed on top of a 63- μ m sieve. The sieves were then shaken for 15 min, and the lactose particles were then collected and transferred to a glass vial and sealed until required for further investigation.

Characterisation of Particle Size and Shape of Lactose Crystals by Image Analysis Optical Microscopy

Powder (about 20 mg) was homogeneously dispersed on a microscope slide. Particle size and shape were assessed using image analysis software (designed in-house at King's College London) installed on an Archimedes computer, which was attached to an optical microscope (Nikon Labophot, Tokyo, Japan) via a miniature video camera. One hundred particles in each sample were selected and measured, and the surface volume mean diameter, Feret diameters, elongation ratio, and roundness were calculated.

Roundness and elongation ratio were calculated using Eqs. 2 and 3, respectively:

$$\text{Roundness} = (\text{perimeter})^2 / (4 \times \pi \times \text{Area}) \quad (2)$$

Elongation ratio

$$= \text{Maximum Feret Diameter} / \text{Minimum Feret Diameter}. \quad (3)$$

Powder Density and Flow Properties Assessment

True density, bulk density, tapped density, Carr's index and Hausner ratio of all the crystallised lactose samples were measured. The true density was measured using an ultracycrometer 1000 (Quantachrom, USA) under helium gas and was calculated from a mean of three determinations. The input gas pressure was 19 psi and the equilibrium time was 1 min.

Carr's index (CI), which is an indication of powder flowability, was calculated using the following equation (22,23):

$$\text{CI} = [(\text{Tapped density} - \text{Bulk density}) / \text{Tapped density}] \times 100. \quad (4)$$

In brief, the powder was filled in a 5-mL measuring cylinder and after recording the volume (bulk volume) the cylinder was tapped 100 times and the new volume was recorded (tapped volume). A preliminary experiment showed that 100 taps was sufficient to attain the maximum reduction in the volume of powder bed.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FT-IR) spectra were used to investigate any changes in crystallised lactose on the molecular level during the crystallisation or drying process. These spectra were determined with an FT-IR instrument (Perkin Elmer, USA) using a scanning range between 450 and 4,000 cm^{-1} . The sample (several milligrams) was placed in the middle of the sample stage and a force applied (120 bar) using the top of the arm of the sample stage. After obtaining sharp peaks of appropriate intensity, the spectra acquired were the results of averaging four scans at 1 cm^{-1} .

Scanning Electron Microscope

Electron micrographs of crystallised and commercial lactose samples were obtained using a scanning electron microscope (Philips XL 20, Eindhoven, the Netherlands) operated at 15 kV. The specimens were mounted on a metal stub with double-sided adhesive tape and coated under vacuum with gold in an argon atmosphere prior to observation.

Atomic Force Microscopy

Atomic force microscopy (AFM) was performed using a Veeco MultiMode AFM equipped with an E-type scanner

operating via a Veeco Nanoscope IIIa controller (Veeco Instruments, Cambridge, UK). Images were made in tapping mode using a Veeco RTESP phosphorous-doped silicon tip with a nominal force constant of 40 Nm at a scanning rate of 1 Hz and resolution of 256 lines per image. Sufficient particles were imaged at different scales of scrutiny to produce three representative images per sample at scales of 3 μm , 1 μm and 333 nm. Roughness analysis was performed using Veeco Nanoscope software (version 6.13) on a 333 $\times$ 333-nm region from each image determined to be flat and free of macroscale changes in height such as transitions between overlapping particles or between different faces of the same particle lying in different planes. Roughness values calculated were both the root mean square average (R_q) and arithmetic mean average (R_a) of the difference in height recorded at each data point and the mean plane and presented as the average of the three measurements for each sample.

Different Scanning Calorimetry

A differential scanning calorimeter (DSC7, Mettler Toledo, Switzerland) was used to investigate the anomer content and crystalline nature of the different lactose samples. Samples (4–5 mg) were heated from 25°C to 300°C at a scanning rate of 10°C/min in crimped aluminium pans with a pinhole. A purge gas of nitrogen was passed over the pans with a flow rate of 50 mL/min. The fusion points and their enthalpies were calculated using the supplied software (Mettler).

Different scanning calorimetry (DSC) was also used to measure the number of moles of water present as a crystalline hydrate within the samples. The only form of lactose which is known to exist as a hydrate is α -lactose monohydrate; thus, DSC may provide a simple method of estimating the number of moles of α -lactose monohydrate present within the sample. To this end, accurately weighed lactose powder was placed in crimped DSC pan containing a hole and heated from 40°C to 150°C at 10°C/min, and then the temperature of the sample was maintained at 150°C for 5 min to ensure complete water evaporation. Total water content was determined by weighing the pan before and after heating using DSC. Moles of water per mole of lactose (n) were calculated according to the following equation:

$$n = \frac{(W_1 - W_2) \times \text{MW}_{\text{lactose}, \text{H}_2\text{O}}}{W_1 \times \text{MW}_{\text{H}_2\text{O}}} \quad (5)$$

where W_1 is powder weight before heating, W_2 is powder weight after heating, $\text{MW}_{\text{lactose}, \text{H}_2\text{O}}$ is the molecular weight for lactose monohydrate (360.31 mg), and $\text{MW}_{\text{H}_2\text{O}}$ is molecular weight of water (18.02 mg).

Preparation of Powder Formulation

Crystallised sieved lactose particles (63–90 μm fraction) were blended with salbutamol sulphate (SS) in a ratio of 67.5:1 (w/w) in an aluminium container (batch size was 3 g). This blending was carried out using a Turbula® mixer (Willy A. Bachofen AG, Maschinenfabrik, Basel, Switzerland) at a constant speed of 100 rpm for 30 min. This speed was maintained constant for the blending of all formulations.

HPLC Analysis of Salbutamol Sulphate

Salbutamol sulphate was analysed by a HPLC (Waters, Milford, MA, USA) method employing a mobile phase containing a mixture of methanol and 0.25% (w/v) 1-heptane sulfonic acid sodium salt in water (45:55, v/v). The flow rate of mobile phase was 2 mL/min. The eluant was monitored using a UV detector set at a wavelength of 200 nm. The HPLC system consisted of a pump (CM4000 Multiple Solvent Delivery System, LDC Analytical Inc., FL, USA), a multiple wavelength UV detector (Spectro Monitor 3,100, LDC Analytical Inc., FL, USA) and a 30-cm×4.6-mm i.d. column packed with 5 µm Novapack C18 (Waters), which was maintained at 60°C. The retention time for salbutamol sulphate was 3.2 min.

Measurement of Dose Uniformity

After blending the drug with carrier, the homogeneity of the drug content in each of the powder formulations was examined by taking a minimum of five randomly selected samples from different positions within the blend. Each sample weighing 33 ± 1.5 mg (this was the amount of the powder mixture to be introduced into each capsule) was dissolved in 100 mL water contained in a volumetric flask. The amount of the active drug (salbutamol sulphate) in each powder formulation was determined using the HPLC method, as described in “Preparation of Powder Formulation”. The degree of uniformity (or homogeneity) was expressed in terms of the percentage coefficient of variation (CV%), and a percentage CV <6% was taken as an indication of acceptable uniformity.

Deposition Study

Powder pulmonary deposition profiles of all dry powders were assessed *in vitro* using a multistage liquid impinger (MSLI) equipped with a USP induction port (Copley Scientific, Nottingham, UK), as has been described in detail elsewhere (15). Each hard gelatin capsule was filled with 33 ± 1.5 mg of the powder formulation blend (ratio of drug to carrier was 1:67.5) and the capsule was pierced; ten actuations (ten capsules) of each powder were delivered consecutively to the multistage liquid impinger (15).

The procedure of drug collection from mouth piece adaptor (I+M), induction port (IP) and each stage of MSLI was described elsewhere (15). The deposition from each formulation was determined three times and several parameters were employed to characterise the powder deposition profile. The recovered dose (RD) was the sum of the weights of drug (µg) collected from inhaler device, induction port and all stages of the impinger. The emitted dose (ED) was the amount of drug emitted from the inhaler device and collected from the induction port and all stages of the impinger. The percentage total recovery was calculated as the ratio of the RD to the theoretical dose (481 ± 22 µg). The percentage emission was the ratio of the ED to RD. Impaction loss was the sum of drug amounts collected from the induction port and MSLI stage 1, expressed as a percentage of the RD. In order to calculate the FPF, the cumulative mass of powder less than the stated size of each stage of the impactor,

determined as a percentage of the total amount of drug collected from the impinger and induction port (*i.e.* ED), was plotted as a function of the log value of the effective cutoff diameter (15,24–27). The effective cutoff diameter was taken as the new cutoff diameter of each stage of the MSLI when it was operated at a different flow rate from 60 L/min. In order to calculate the effective cutoff diameter of each individual stage of the MSLI at a flow rate of 92 L/min, the following equation was employed (28):

$$D_{50,Q} = D_{50,Q_n} (Q_n/Q)^{1/2} \quad (6)$$

where $D_{50,Q}$ is the cutoff diameter at a flow rate of Q (L/min) and n refers to nominal values obtained. Q_n is equal to 60 L/min and Q is the operating flow rate during the test (which is 92 L/min). FPF was calculated from that plot as the cumulative amounts of drug with an aerodynamic diameter ≤ 5 µm taken as a percentage of the ED. A second cumulative plot was drawn between the cumulative mass of drug less than the stated size of stages 1, 2, 3 and 4, expressed as a percentage of the cumulative mass of drug less than stage 1, against the effective cutoff diameter of each stage. This plot was employed to calculate the experimental mass median aerodynamic diameter (MMAD), which was defined as the particle size at which the line crossed the 50% mark. In addition, the geometric standard deviation (GSD) was taken as the square root of particle size at 84.13rd percentile over particle size at 15.87th percentile (28).

Statistical Analysis

Where appropriate, results were evaluated using a one-way analysis of variance, where $p < 0.05$ was taken to represent a statistically significant difference.

RESULTS AND DISCUSSION

Crystallisation Procedure

Anti-solvent crystallisation techniques can produce low yields (29); however, this was not the case in this study, with yields generally being over 70% (Table I). In all cases, crystallisation of lactose was initiated immediately when the non-solvent medium (ethanol–butanol) was added.

Particle Size Distribution

The particle size distributions of all the sieved (63–90 µm) samples are presented in Table I. The VMD and span were used to express the size distribution of the samples. It can be seen that the crystallisation of lactose resulted in particles with a different size distribution when compared to commercial lactose sample. The VMD of commercial lactose was 91.1 ± 0.4 µm, whilst VMD for all the crystallised samples was lower ($p < 0.05$), in the range 70.3–81.1 µm (Table I). Despite all lactose samples being sieved in the same way, the crystallised lactose samples showed smaller $D_{10\%}$, $D_{50\%}$ and $D_{90\%}$ compared to commercial lactose. Differences in particle size distribution of sieved samples can be attributable to differences in particle shape, particularly when elongated particles are produced (5). The span values for all samples were small (0.79–1.16), indicative of carefully prepared

Table I. Particle Size Distribution (Expressed as $D_{10\%}$, $D_{50\%}$, $D_{90\%}$, VMD, Span, S_V and Percentage of Fine Particles $<10.50\ \mu\text{m}$) for Commercial Lactose and Lactose Crystallised from Different Ratios of Ethanol/Butanol ($n=5$)

Lactose samples	Yield (%)	$D_{10\%}$ (μm)	$D_{50\%}$ (μm)	$D_{90\%}$ (μm)	VMD (μm)	Span ^a	S_V^b (m^2/cm^3)	$<10.50\ \mu\text{m}$ (%)
Commercial lactose	–	58.5 ± 0.3	87.9 ± 0.4	128.5 ± 0.7	91.1 ± 0.4	0.80 ± 0.00	0.07 ± 0.00	0.0 ± 0.0
Crystallised using ethanol	74	52.1 ± 0.6	79.7 ± 0.6	115.4 ± 0.4	81.1 ± 0.9	0.79 ± 0.01	0.12 ± 0.00	0.9 ± 0.1
Crystallised using ethanol/butanol (60:20)	100	44.4 ± 0.9	70.9 ± 1.2	102.1 ± 1.5	71.7 ± 1.4	0.81 ± 0.00	0.15 ± 0.01	1.9 ± 0.4
Crystallised using ethanol/butanol (40:40)	100	51.7 ± 1.4	74.2 ± 0.5	99.4 ± 1.1	74.0 ± 0.8	0.64 ± 0.03	0.15 ± 0.04	2.3 ± 1.3
Crystallised using ethanol/butanol (20:60)	100	26.3 ± 10.9	77.2 ± 1.1	115.5 ± 1.3	75.7 ± 1.6	1.16 ± 0.14	0.26 ± 0.05	7.1 ± 2.0
Crystallised using butanol	50	42.1 ± 2.0	71.6 ± 0.8	101.1 ± 0.7	70.3 ± 1.0	0.82 ± 0.03	0.22 ± 0.01	4.8 ± 0.4

VMD volume mean diameter

^aSpan, a measure of the particle polydispersity, is calculated as the difference in particle diameters at 10% and 90% cumulative volume, divided by $D_{50\%}$ ^bVolume specific surface area

sieved fractions. Table I shows that unlike commercial lactose, fine lactose particles with a size $<10.50\ \mu\text{m}$ are present in the sieved crystallised lactose samples, and these might be expected to have an effect on DPI performance (15,16). It was found that

generally crystallised lactose samples prepared using higher volumes of butanol retained higher amounts of fines compared to lactose samples crystallised using low volume fractions of butanol (Table I). The higher amount of fines that resulted using

Fig. 1. SEM images for commercial lactose sample (a), recrystallised lactose samples using ethanol/1-butanol (80:0) (b), ethanol/1-butanol (60:20) (c), ethanol/1-butanol (40:40) (d), ethanol/1-butanol (20:60) (e) and ethanol/1-butanol (0:80) (f), salbutamol sulphate (g), commercial lactose with salbutamol sulphate formulation blend (h) and lactose (ethanol/butanol 80:0) with salbutamol sulphate formulation blend (i)

Table II. True, Bulk and Tapped Densities, Carr's Index, and Hausner Ratio for Commercial Lactose and Lactose Crystallised Under Different Ratios of Ethanol/Butanol ($n=3$)

Sample	True density (g/cm ³)	Bulk density (g/cm ³)	Tapped density (g/cm ³)	CI (%)	Hausner ratio
Commercial lactose	1.55±0.05	0.62±0.01	0.76±0.00	18.1±1.7	1.22±0.02
Crystallised using ethanol	1.57±0.01	0.18±0.01	0.23±0.01	21.5±1.7	1.27±0.03
Crystallised using ethanol/butanol (60:20)	1.57±0.01	0.20±0.00	0.25±0.00	20.0±1.1	1.25±0.02
Crystallised using ethanol/butanol (40:40)	1.54±0.00	0.18±0.00	0.22±0.00	23.2±1.4	1.30±0.02
Crystallised using ethanol/butanol (20:60)	1.64±0.00	0.28±0.05	0.33±0.04	19.4±7.8	1.25±0.12
Crystallised using butanol	1.70±0.01	0.24±0.01	0.32±0.00	27.2±2.0	1.37±0.04

higher volumes of butanol (Fig. 1 and Table I) compared to lactose crystallised from low volume of butanol could be due to the rougher surfaces of the resultant lactose particles recrystallised under these conditions. It might be anticipated that all fine carrier particles would be removed during the sieving process. However, carrier particles with a rougher surface texture are more likely to retain fine carrier particles within any micro-irregularities on the lactose surface. It was assumed that such entrapped fine carrier particles were lodged in the carrier surface depressions and thus could not be removed during the mechanical vibration process.

The volume specific surface area (S_v) values for crystallised lactose samples were significantly higher than the S_v values obtained for commercial lactose ($p<0.05$), which could be due to the presence of higher amounts of fines in these samples and lower VMD. Generally, as more butanol was used in the crystallisation of lactose, higher S_v values obtained (Table I).

The particle size distribution of SS indicated a monomodal distribution with a VMD of $1.86\pm0.16\ \mu\text{m}$, $D_{50\%}$ of $1.66\pm0.06\ \mu\text{m}$ and $D_{90\%}$ of the particles $<3.14\pm0.31\ \mu\text{m}$, which indicates the suitability of these particles for respiratory delivery (30).

Powder Density and Flow Assessment

The true density obtained for the commercial lactose was $1.546\ \text{g/cm}^3$, which is similar to the previously reported ($1.55\ \text{g/cm}^3$) value (31). Generally, a higher true density appeared to be obtained for lactose crystallised in the presence of high volumes of butanol (Table II), but statistical analysis of the data indicated that the true density of lactose was not significantly ($p>0.05$) affected when the amount of butanol used in the crystallisation medium was $<40\ \text{mL}$. When over $40\ \text{mL}$ butanol was used, the true density of crystallised lactose was increased compared to the commercial lactose ($p<0.05$). Generally, alcohols dehydrate the lactose samples during the crystallisation processes. However, the length of the alcohol chain plays a major role in lactose sample hydration (32). Butanol has a longer chain compared to ethanol; therefore, the more butanol used during crystallisation, the lower is the degree of sample dehydration. Thus, samples crystallised from anti-solvents containing a higher content of butanol are associated with more moles of water (Table III) and the generation of different polymorphs (Fig. 2 and Table III). On the other hand, the more ethanol used during crystallisation, the more readily does the dehydration of lactose monohydrate occurs. This could, in part, account for the variability of different recrystallised lactose particles in terms

of true density measurements since different polymorphs have different true densities.

Carr's index, the bulk and tap densities of the lactose batches were used as indirect measures of the interparticulate forces within each (Table II). There was no difference in the Carr's index of the different crystallised lactose samples (ANOVA, $p>0.05$), indicating similar flow properties of all tested samples, as confirmed by the similarity in Hausner ratios for all samples. All crystallised lactose samples showed markedly lower bulk and tapped densities ($p<0.05$) than commercial lactose powder (Table II), and generally the lactose crystallised from solvents with a high butanol content displayed higher bulk and tap densities compared to the samples obtained in the presence of lower concentrations of butanol. Similarly, changes in both the tap and bulk densities of recrystallised lactose particles could be related to the volume of butanol used during the crystallisation process as different lactose forms have different physicochemical properties. Higher bulk density and tap density for lactose particles crystallised using higher volumes of butanol could be due to the higher amounts of fine particles present in these samples (Table I). It can be assumed that powders with higher amounts of fine particle have a higher average number of interparticulate contact points and thus higher bulk density and higher tap density.

Solid-State Characterisation of Lactose Particles

DSC was employed to characterise the lactose before and after crystallisation (Fig. 2). The DSC thermogram of commercial lactose showed two distinctive endothermic peaks at about 149°C and 218°C , which correspond to the dehydration of crystalline hydrate water and the melting of anhydrous α -lactose, respectively (33,34). These two endothermic peaks appeared in the DSC thermographs of all crystallised samples with smaller intensity compared to commercial lactose. The DSC thermogram of commercial lactose also showed a small exothermic peak at about 173°C , which corresponds to the crystallisation of amorphous lactose to mostly α -lactose as reported by several researchers (33–35). A new endothermic peak at about 240°C appeared in the thermograms obtained for all of the crystallised lactose samples, which corresponds to the temperature of the melting of β -lactose (33,34). A broad endothermic peak after the melting of β -lactose is due to thermal degradation of lactose (36,37). The presence of two endothermic peaks at 218 and around 240°C indicates that the crystallised samples must comprise a mixture of α -monohydrate and β -lactose. The enthalpy values associated with each transition are listed in Table III.

Table III. Enthalpy of Dehydration (ΔH_v), Amorphous Content Recrystallisation (ΔH_c), α -Lactose Fusion (ΔH_{220}), β -Lactose Fusion (ΔH_{240}), Moles of Water Per Mole of Lactose and Amorphous Content Percentage for Commercial Lactose and Different Crystallised Lactose Samples for Commercial Lactose and Different Crystallised Lactose Samples ($n=4$)

Lactose sample	ΔH_v dehydration (J/g)	ΔH_c recrystallisation (J/g)	ΔH_{220} α -lactose (J/g)	ΔH_{240} β -lactose (J/g)	Moles of water per mole of anhydrous lactose ^a	Moles of water per mole of anhydrous lactose ^b	Amorphous (%)
Commercial lactose	119 $\pm$ 4.5	2.13 $\pm$ 0.69	143 $\pm$ 1.0	—	1.05 $\pm$ 0.04	1.07 $\pm$ 0.02	6.66 $\pm$ 2.16
Crystallised using ethanol (80 mL)	27.4 $\pm$ 4.7	2.66 $\pm$ 1.35	20.1 $\pm$ 2.0	118 $\pm$ 18	0.23 $\pm$ 0.04	0.17 $\pm$ 0.11	8.33 $\pm$ 4.22
Crystallised using ethanol/butanol (60:20 mL)	19.2 $\pm$ 3.9	2.20 $\pm$ 0.39	14.4 $\pm$ 2.0	114 $\pm$ 3	0.16 $\pm$ 0.03	0.22 $\pm$ 0.08	6.89 $\pm$ 1.23
Crystallised using ethanol/butanol (40:40 mL)	44.2 $\pm$ 2.4	3.31 $\pm$ 0.85	14.2 $\pm$ 2.6	99.2 $\pm$ 28	0.38 $\pm$ 0.02	0.45 $\pm$ 0.11	10.36 $\pm$ 2.65
Crystallised using ethanol/butanol (20:60 mL)	47.4 $\pm$ 19.1	2.95 $\pm$ 1.48	4.9 $\pm$ 0.9	97.7 $\pm$ 35.1	0.41 $\pm$ 0.69	0.52 $\pm$ 0.05	9.22 $\pm$ 4.63
Crystallised using butanol (80 mL)	80.1 $\pm$ 7.3	3.03 $\pm$ 1.52	66.6 $\pm$ 11.1	34.5 $\pm$ 0.48	0.69 $\pm$ 0.06	0.81 $\pm$ 0.05	9.48 $\pm$ 4.79

^a Calculated according to Eq. 7

^b Measured using gravimetric technique Eq. 5 described in DSC method

For comparison purpose, apart from Eq. 4, the following Eq. 7 was also used to calculate the number of moles of water per mole of anhydrous lactose (n) for all crystallised lactose samples:

$$n = \Delta H_d \times \text{RMM}_{\text{lactose}} / (\Delta H_v - \Delta H_d) \times \text{RMM}_{\text{water}} \quad (7)$$

where ΔH_d is the enthalpy of the dehydration obtained from the dehydration endotherm (J/g), ΔH_v is the enthalpy of vaporisation of water (which is 2,261 J/g) (38,39), $\text{RMM}_{\text{lactose}}$ is the relative molecular mass of anhydrous lactose (which is 340.3), and $\text{RMM}_{\text{water}}$ is the relative molecular mass of water (18.0). All crystallised lactose samples contained lower amounts of moles of water per mole of lactose compared to commercial lactose sample, which appeared to be ~ 1 mol of water of crystallisation per mole of anhydrous lactose (Table III). This value is very close to the value obtained using Eq. 5. When the number of water moles obtained using these two different techniques was plotted against each other (Fig. 3), it was found that a high correlation coefficient ($r^2=0.9968$) existed between the techniques used to calculate the number of water moles per number of lactose anhydrous moles. This indicates that both methods appear to be valid for these systems. The number of water moles calculated using the gravimetric method (Table III) was slightly higher than when the enthalpy technique using Eq. 7 was employed. This could be due to the presence of free water (non-crystallised water) in lactose samples which results in a higher number of water moles being determined as present. Table III also shows that the greater the butanol concentration used during the crystallisation process, the greater is the number of water moles per mole of lactose in the crystallised particles. This could be due to the presence of long chain with butanol which cannot facilitate the dehydration of lactose monohydrate compared to ethanol with low chain (32). It can be seen from Table III that Δh_c (the heat of crystallisation of amorphous lactose) was affected by the combination of non-solvents. Equation 8 was employed to estimate the amorphous content of the crystallised lactose samples using the heat of crystallisation of the amorphous lactose content (40), and all data are recorded in Table III.

$$\% \text{ amorphous content} = (\Delta h_c / \Delta H_c) \times 100 \quad (8)$$

where Δh_c is the heat of crystallisation of amorphous lactose (in J/g) and ΔH_c is the specific heat of crystallisation of amorphous lactose which can be taken as 32 J/g for amorphous lactose (41). Table III shows that all crystallised lactose samples contained more amorphous lactose (in the range from 6.89% to 10.36%) than the commercial lactose sample (6.66%). However, it should be noted that when the amorphous lactose percentage is $<20\%$ (as in the lactose samples crystallised in this study), conventional DSC gives only an approximate estimate of lactose amorphicity (33,42). The commercial lactose sample comprised primarily the α -anomer form with the fusion enthalpy of 143.86 J/g (Table III). The fusion enthalpy due to α -lactose in all the crystallised samples was lower (Table III), and the latter all showed a specific endothermic peak (Fig. 2) which related to the fusion of β -lactose. An increase in butanol concentration in the crystallisation medium decreased the enthalpy of β -anomer of the resultant lactose crystals. The lowest enthalpy for the fusion of β -lactose was observed when 100% butanol was

Fig. 2. DSC thermograms for different crystallised lactose samples prepared using different ratios of ethanol/butanol using a heating rate of 10°C/min

used to crystallise lactose. It appeared on the basis of these results that solvent crystallised samples comprise a combination of anhydrous α -lactose, β -lactose, and α -lactose monohydrate. It is difficult to calculate the percentage of α - and β -lactose in the crystals as it is not clear what percentage of amorphous lactose converted to α - or β -lactose during the DSC experiments. However, it is obvious that the contribution of each form in each crystallised sample depends on the ratio of ethanol/butanol used to crystallise the samples (see the enthalpy data in Table III).

Since β -lactose would be in the anhydrous form, it is expected that the low enthalpy of fusion for β -lactose should associate with low moles of water in the sample. Figure 4 shows that when the enthalpy for the fusion of β -lactose increases, the number of water moles per mole of lactose anhydrous decreases. The highest enthalpy for β -lactose was obtained for lactose samples crystallised in the absence of butanol or a low concentration of butanol relative to a higher concentration of ethanol.

The FT-IR spectrum of all samples (Fig. 5) showed a band at 1,650 cm^{-1} which is related to the vibration of the crystal water hydroxyl group (43), whilst the band at 1,200–1,070 cm^{-1} is attributable to the asymmetric vibrations of C–O–C in the glucose and galactose residues (43). It has been reported that the band at 920 cm^{-1} is a specific diagnostic band for α -lactose anomer (43,44), and this band is apparent in commercial lactose and all the crystallised lactose samples. Kirk *et al.* (44) reported that the band at 950 cm^{-1} is a specific diagnostic band for β -lactose anomer, which is absent from the spectra of the commercial lactose sample (Fig. 5a) but is apparent in all the crystallised lactose samples (Fig. 5b–f).

These results support those from the DSC experiments. Absorptions at 1,260, 900 and 875 cm^{-1} provide an indication of the presence of amorphous lactose (37), and these were apparent in the spectra of all samples (Fig. 5).

Particle Shape Analysis

Scanning electron microscopy (SEM) studies showed the presence of typical ‘tomahawk’-shaped particles covered with fines present in the commercial grade sample (Fig. 1a). SEM images of crystallised lactose samples contained particles with different sizes, shapes and surface textures as compared with

Fig. 3. Loss of water moles per mole of lactose calculated by enthalpy change as a function of the same parameter obtained gravimetrically from monitoring DSC pan weight after heating. Each data point represents the mean of three experiments

Fig. 4. Relationship between β -lactose fusion enthalpy and water dehydration enthalpy and amount of butanol used for the crystallisation of lactose (a) and the fusion enthalpy of β -lactose and dehydration enthalpy of crystallised water (b). Each data point represents the mean of three experiments

the commercial lactose. All crystallised lactose samples comprised aggregates of smaller particles, although the composition of the precipitating non-solvent used to crystallise lactose particles affects the shape and the size of these aggregates. The composite particles within the aggregate were finest when ethanol was employed alone as the precipitating solvent, and as the amount of butanol in the crystallisation medium increased, the generated particles became wider, thicker and larger in size. The lactose particles formed using ethanol alone appeared to be composed of flat rectangular plates (Fig. 1i). It is apparent from the micrographs that the recrystallised lactose samples have different surface roughness (Fig. 1). This was supported by the roughness data reported in Table IV.

All the crystallised lactose samples were found to have a higher roundness and lower elongation ratio compared to commercial lactose (Table IV). Higher roundness values indicate that the crystallised lactose particles are more irregular in shape (45). Lower elongation ratio measurements indicate that the crystallised lactose samples are less elongated and/or less irregular in shape (45). Thus, these findings tend to support the observations from the SEM micrographs. The crystallised lactose particles would appear to be composed of small, possibly partially formed particles aggregated together to form larger secondary particles.

Content Uniformity

After the blending of crystallised lactose with SS, five randomly selected samples of 33 ± 1.5 mg for each formulation blend indicated that the percentage uniformity was acceptable (94–99%). The %CV of SS content varied between 4% and 7% (refer to the data reported in [Electronic supplementary material \(ESM\)](#)) for the crystalline samples indicating adequate mixing, but this was higher for the commercial lactose-containing formulations (10.9%).

Fig. 5. FT-IR spectra of commercial lactose (a), crystallised lactose from ethanol/1-butanol (80:0) (b), ethanol/butanol (60:20) (c), ethanol/butanol (40:40) (d), ethanol/butanol (20:60) (e) and ethanol/butanol (0:80) (f)

Table IV. Roughness Data Measured by AFM and Also Roundness and Elongation Ratio for Untreated Lactose and Different Crystallised Lactose Samples

	Rq (Roughness mean square, nm)	Ra (arithmetic mean roughness, nm)	Roundness	Elongation ratio
Commercial lactose	3.3±0.9	2.6±0.7	1.36±0.13	1.64±0.35
Crystallised using ethanol	2.8±0.4	2.2±0.4	2.09±0.44	1.36±0.17
Crystallised using ethanol/butanol (60:20)	3.4±0.2	2.8±0.2	1.95±0.40	1.44±0.20
Crystallised using ethanol/butanol (40:40)	–	–	1.94±0.37	1.36±0.17
Crystallised using ethanol/butanol (20:60)	–	–	1.71±0.38	1.49±0.27
Crystallised using butanol	5.7±1.3	4.5±1.0	1.58±0.29	1.41±0.23

Aerosolisation Performance of Various Lactose Formulation Blends

When operated at a flow rate of 92 L/min, the MSLI separates particles according to their effective cutoff diameters, which are 10.49, 5.49, 2.50 and 1.37 μm for stages 1, 2, 3 and 4, respectively. The deposition profiles of SS from the formulations containing recrystallised lactose were markedly different compared to the profile obtained from the commercial lactose formulation blend. Formulation blends containing crystallised lactose carriers generated a higher RD, ED, percentage recovery and percentage emission (ANOVA, $p < 0.05$) in comparison to the formulation blend containing commercial lactose (Table V). An acceptable range of recovered dose is within 75–125% of the theoretical dose (46), which would correspond to a range between 360.75 and 601.25 μg SS. This was obtained for the experimental batches, but not for the formulation containing the commercial grade lactose.

The MMAD and GSD values obtained using the different lactose fractions were similar (Table V), probably as a consequence of the same batch of SS being employed. Such a particle size is thought to be ideal for delivery to the peripheral alveolar airway (47,48).

Crystallised lactose formulations produced lower impaction losses ($p < 0.05$) and higher FPFs ($p < 0.05$) compared to the commercial lactose formulation blend (Table V), indicating the better aerosolisation performance of the formulation blends containing engineered lactose particles. This could be due to an easier drug–carrier redispersion (detachment) from the more corrugated crystallised lactose carrier particles. The

formulation blend containing crystallised lactose obtained using ethanol/butanol (20:60) produced the highest FPF (37.96%) and the lowest impaction loss (40%). However, generally, it was found that formulations containing crystallised lactose generated using higher volumes of butanol produced higher FPF values. The blends containing lactose particles obtained from low amounts of butanol (<40 mL) generally deposited lower amounts of drug on the inhaler and mouthpiece adaptor (see ESM), whereas higher percentage amounts of drug were recovered from the IP for crystallised lactose formulations ($p < 0.05$). All the crystallised lactose formulations produced higher amounts on stages 2, 3, 4 and 5 ($p < 0.05$), the latter two fractions being highly respirable.

In order to investigate the effect of different polymorphs of lactose obtained from different ratios of ethanol/butanol on the deposition profiles of SS formulation blend in various regions of airway, the cumulative amounts of SS deposited on different MSLI stages were calculated as a percentage to the ED for all tested formulations (Fig. 6). It can be seen that all recrystallised lactose formulations resulted in higher amounts of SS <10.50, 5.49, 2.5 and 1.37 μm . Interestingly, Fig. 6 shows that of all the crystallised lactose formulations, the sample produced from ethanol/butanol (40:40) produced the lowest cumulative amounts of SS less than sizes of 10.50, 5.49, 2.50 and 1.37 μm and FPF (Table V).

The improvement in aerosolisation performance of formulations containing crystallised lactose could be explained by the differences in the physicochemical properties of these batches in comparison to the commercial lactose formulations. First, it was apparent from SEM images (Fig. 1)

Table V. RD, ED, Recovery (%), Emission (%), Impaction Loss, MMAD, GSD and FPF of Salbutamol Sulphate from Different Formulation Blends (Mean $\pm$ SD, $n=3$)

Lactose product formulations	RD (μg)	ED (μg)	Recovery (%)	Emission (%)	Impaction loss (%)	MMAD (μm)	GSD	FPF (%)
Commercial lactose	317±5	299±7	65.9±1.1	94.5±0.6	67.8±4.7	3.2±0.4	2.2±0.1	19.7±1.9
Crystallised using ethanol	400±5	380±5	83.2±1.0	95.0±0.1	55.6±6.4	3.2±0.1	2.2±0.0	28.9±5.6
Crystallised using ethanol/butanol (60:20)	419±13	403±13	87.1±2.6	96.1±0.3	51.8±4.0	3.2±0.1	2.1±0.0	32.1±2.6
Crystallised using ethanol/butanol (40:40)	398±16	383±14	82.7±3.3	96.3±0.4	56.1±5.1	3.6±0.0	2.1±0.0	27.3±3.6
Crystallised using ethanol/butanol (20:60)	405±39	381±42	84.1±8.1	94.1±1.2	40.0±3.6	3.4±0.0	2.1±0.1	38.0±2.5
Crystallised using butanol	393±40	372±40	81.6±8.2	94.8±0.6	49.2±3.1	2.9±0.0	2.2±0.0	35.4±2.5

RD recovered dose, ED emitted dose, MMAD mass median aerodynamic diameter, GSD geometric standard deviation, FPF fine particle fraction

Fig. 6. Cumulative amounts of salbutamol sulphate less than the stated aerodynamic particle size diameter taken as a percentage to the total amount of drug recovered from the MSLI when operated at flow rate of 92 L/min for commercial lactose and different crystallised lactose formulations ($n=3$)

and roughness data (Table IV) that the crystallised lactose carriers have a greater rugosity (surface roughness) than commercial lactose, resulting in differences in the carrier–drug contact area and hence differences between the relative strengths of the van der Waals, capillary and electrostatic forces existing between particles in each formulation (49). The higher roughness of crystallised lactose particles could be responsible for better aerosolisation performance as a consequence of lower powder cohesiveness (5) and lower drug–carrier adhesion forces (50) which result from the lower drug–carrier contact area (or increased the separation distance between drug and carrier particles). By comparing recrystallised lactose formulation blends, it was observed that carriers with higher surface roughness provide higher FPF for SS upon aerosolisation (Fig. 7). It is suggested that although better aerosolisation performance could be obtained for carrier with rougher surfaces, the shape of carrier on the aerosolisation performance of drugs should not be ignored. Secondly, it is known that the crystalline form of the carrier and drug has a significant effect on drug–carrier interactions within a formulation (51). However, there is still little information regarding the effect of carrier (pseudo)polymorphism on aerosolisation efficiency of the resulted formulations.

Fig. 7. Relationship between roughness and FPF for recrystallised lactose samples

Fig. 8. FPFs for different formulations containing engineered lactose which have different physicochemical properties (bulk density, tap density and volume specific surface area). Each data point represents the mean of three experiments

Nevertheless, the exploitation of such a finding is likely to be limited since stability issues are likely to preclude the use of amorphous lactose in DPIs (40,52), with such instability affecting the powder dispersion and flowability (10). It has been shown that amorphous lactose also has a higher charge with higher internal and surface energy which leads to higher drug–carrier adhesion, hence resulting in lower drug–carrier redispersion (53). Meanwhile, it has been reported α -lactose-containing blends are preferred to β -lactose-containing blends due to the lower surface free energy of the former lactose particles, which results in a higher FPF (54). Traini *et al.* (54) investigated the influence of lactose pseudomorphic form on salbutamol sulphate–lactose interactions in DPI formulations. They showed that the aerosol performance of the drug from lactose followed the following rank order α -lactose monohydrate $>$ β -lactose anhydrous $>$ α -lactose anhydrous. In their study, the effect of mixtures of different polymorphs on the aerosolisation performance of SS was not studied as the pure form of polymorphs was used. Therefore, the present work would appear to be the first study of its kind to consider the functional relevance of different amounts of α - and β -anomers of lactose within the carrier particles on aerosol performance. Inverse gas chromatography has been used previously to detect differences in the surface energy of amorphous α - and β -lactose (55,56), and the logical conclusion is that these differences will affect the FPF obtained. It is known that surface energies affect the dispersibility of the particles in different media, the adhesion–

Fig. 9. Effect of the amount of fine carrier particles $<10.50\ \mu\text{m}$ on FPF of different DPI formulation blends of SS lactose. Each data point represents the mean of three experiments

cohesion interactions between particles and the adhesion of drugs to containers and carriers (57,58). The composition of the lactose carrier particles which in this study contained varying amounts of α - and β -anomers as well as different amorphous content affected the deposition of SS. It was found that lactose particles, which produced the highest FPF, contained lactose precipitated using ethanol/butanol (20:60).

However, it has to be appreciated that polymorphism may not be the only factor affecting respirable fraction. For example, the carrier volume specific surface area (S_v), carrier bulk density and carrier tap density all produced strong positive linear correlations with the FPF of SS (Fig. 8). In addition, the bulk and tap densities of the crystallised samples were markedly lower than those of the commercial lactose (Table II), whereas the FPF from the formulation containing commercial grade lactose was much lower (Table V). A similar observation has been reported by Bosquillon *et al.* (59) who showed better aerosolisation performance when the powder tapped density was lower. The correlation between volume specific surface area and FPF has been reported previously when recrystallised mannitol was employed as a carrier (15). Other factors that showed weaker positive correlations with the FPF of SS were elongation ratio and true density (refer to the figure reported in ESM). These findings concur with those reported by Kaialy *et al.* (15,16) for mannitol and Zeng *et al.* (20) for lactose particles.

In addition, a further factor that confounds the isolation of the effect of polymorphic form is the presence of different amounts of fine (<10.50 μm) lactose particles in the blends (Table I). It has been shown that the presence of such particles leads to a better DPI aerosolisation efficiency (5,18,19). The crystallisation process used in the present study appears to produce carrier particles with surface irregularities covered with fine lactose particles, which were not removed by mechanical sieving. Such fines may occupy the high energy sites on carrier crystals, leaving drug particles to occupy weaker binding sites (60). Similar to previous publications (61,62), it was found that the more fines in crystallised lactose carrier, the higher is the resultant FPF (Fig. 9).

As the same batch of drug was used in all DPI blends under investigation, any difference in drug deposition properties could be attributed to the batch of lactose used in DPI formulation. However, a different aerosolisation performance might be expected when different APIs are used as it is established that the respiratory deposition pattern of the inhaled drug-carrier mixtures is dependent on drug type/physicochemical properties (7,63–65).

Also, it should be noted that all the 63–90 μm engineered lactose particles were stored for 6 months in sealed glass vials, and the results showed that there were no marked changes in their properties in terms of the PSD, polymorphic form and *in vitro* deposition behaviour (data not shown). Stability issues may provide a focus for further research. The research is required to be undertaken systematically under different temperatures and relative humidities for the optimised formulation.

CONCLUSION

This study demonstrated that the use of different concentrations of anti-solvents in recrystallisation can be used as a potential particle engineering technique for preparing

crystals with different habits which might have application for modifying the aerosolised delivery of drugs to the lower airways as dry powders. Using this technique, particles could be prepared with predetermined physicochemical properties leading to enhanced aerosolisation performance from the resultant DPI formulation. The relative percentages of α - and β -lactose and amorphous content present in the carrier particles correlated with FPF of SS. An explicit interpretation of the significance of this finding was confounded by correlations between FPF of SS and (a) true, bulk and tapped densities, (b) elongation ratio, (c) volume specific surface area of the carrier particles and (d) amount of carrier fines present in the final formulation. However, overall engineered lactose carriers with modified anomer content and physicochemical properties, when compared with the commercial grade, produced formulations which generated a high FPF

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