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The influence of carrier and drug morphology on drug delivery from dry powder formulations

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Abstract

Lactose was crystallised either from neutralised Carbopol 934 gel or from water–ethanol solution without stirring, with a view to obtaining lactose α-monohydrate of favourable shape and smooth surface, suitable for use as carriers in formulations for dry powder inhalers (DPIs).

Crystallisation of salbutamol sulphate was carried out in the presence of water, lecithin and ethanol to form salbutamol crystals with defined shape and smooth surface. The crystals formed were needle-shaped, with a length of less than 6 μm and a width between 0.5 and 1 μm.

DSC and TGA showed that lactose crystals produced from Carbopol gel or from water–ethanol solution existed as α-lactose monohydrate. The DSC thermograms of micronised and crystallised salbutamol sulphate showed two similar endothermic transitions at 200 and 290 °C, respectively. The first transition was initially thought to correspond to the melting of salbutamol sulphate. However, the shape of the particles as observed by optical microscopy was not altered after heating the sample to 250 °C, suggesting that no transition from solid to liquid state occurred at 200 °C. This was confirmed by observations made using hot stage microscopy. The two endothermic transitions are suggested to correspond to the decomposition of the salbutamol sulphate molecule.

The elongation ratio of the commercial lactose crystals, lactose crystallised from Carbopol and from water–ethanol were 1.69 ± 0.05, 2.01 ± 0.13 and 6.25 ± 0.17, respectively. As the elongation ratio increased the flow properties of the carrier were affected detrimentally and this consequently reduced the content uniformity of salbutamol sulphate and drug emission from the inhaler device. Whereas, increasing the elongation ratio of the carrier or drug improved the deposition profiles of salbutamol sulphate, suggesting that the more elongated particles would be more aerodynamic and favour deep lung penetration.

Keywords: Dry powder inhalers; Lactose; Salbutamol sulphate; Decomposition; Crystallisation; Elongation ratio; Surface smoothness; Flow properties; Fine particle fraction

1. Introduction

In order for a drug to gain access to the lower airways, one of the primary requirements is that the drug particle has an aerodynamic diameter between 1 and 5 μm (Davies et al., 1976; Newman and Clarke, 1983; Gonda, 1990). The 1–5 μm-sized drug particles are

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rarely prepared directly by crystallisation. Generally, the crystals are left to grow to maturity in a crystallisation medium and the resulting crystals are harvested by filtration, followed by drying and subsequent processing by high-energy milling to produce micron-sized drug particles. This processing however can cause disruption to the crystal structure, resulting in products, which are highly charged, cohesive and difficult to process. Spray drying provides an alternative method of forming micron-sized drug particles, which are usually spherical with or without dimples. However, some concern remains with respect to a change induced in the solid-state characteristics of spray-dried products, particularly crystallinity (Elamin et al., 1995). Also, the yield is often low, due to loss of material in different parts of the spray dryer and furthermore, many drugs are subject to deterioration on heating which restricts the use of this technique.

Crystal and particle engineering of drugs and carriers can play a significant role in the formulation of materials for dry powder inhalation aerosols. Increasing surface smoothness of lactose carrier by means of crystal engineering has been reported to increase the percentage of fine particle fraction (FPF) of a salbutamol sulphate aerosol (Ganderton, 1992; Zeng et al., 2000a). It was also found that increasing the elongation ratio of the lactose carrier increased the FPF and dispersibility of salbutamol sulphate after aerosolisation of the formulations from both the Rotahaler® and the Cyclohaler® (Zeng et al., 2000a).

It is hypothesised that it may be possible to restrict nucleation and crystal growth by judicious additions to the crystallisation mediums. These may produce appropriately sized particles directly and materials such as surface active agents (surfactants) have been added in crystallisation procedures to achieve this end (Davey, 1982). The degree of interaction (adsorption) between the additive and the crystal surface depends on their respective chemical and physical properties such as the presence of anionic or cationic groups or the possibility of the formation of hydrogen bonds. One of the initial investigations into the effect of impurities on the crystal habit of pharmaceuticals was performed by Michaels and Colville (1960) using the pharmaceutical excipient, adipic acid. The facial growth rates of the adipic acid crystals were reduced by the presence of both anionic and cationic surfactants. An anionic agent (sodium tetrapropyl benzene sulphonate) caused the production of needle-like particles whereas a cationic surfactant (trimethyl dodecyl ammonium chloride) led to the formation of thin plates or flakes. In order for a surfactant to be employed to prepare drug crystals for lung delivery it should be non-toxic, especially to the respiratory tract and must be capable of being completely removed from the surface of the crystals so as not to affect the content uniformity of the drug. Lecithin (phosphatidyl choline), might provide a surfactant that would meet these requirements and it is soluble in absolute ethanol, a solvent in which salbutamol sulphate is insoluble. Therefore, it should be possible to remove any adsorbed surfactant with ethanol without substantially changing the morphology of the crystals. The first aim of the current study was to attempt to engineer the crystallisation of salbutamol sulphate from aqueous solution in the presence of lecithin, with a view to preparing drug particles with a smooth surface and regular shape combined with an acceptable size range for lung delivery. A second aim was to engineer smooth crystals of lactose and with different elongation ratios. In the field of aerosol science the use of elongated particles has attracted interest (Zeng et al., 2001). Fibres and needle-like crystals have aerodynamic diameters almost independent of their length and the diameter is approximately equal to the shortest dimension of the particle in question (Hinds, 1982). Elongated lactose crystals prepared previously (Zeng et al., 2000a) were either tomahawk or plate-like crystals. Such particles might be less aerodynamically favourable than needle-shaped particles since the latter might travel further in the air stream allowing a greater probability of drug detachment from the surface of the carrier, thereby promoting better deposition in the deep lung. The third aim was to investigate the deposition profiles of engineered and micronised salbutamol sulphate, from formulations containing, commercial lactose crystals, smooth lactose crystallised from Carbopol gel and needle-shaped lactose (LN) produced by crystallisation from a binary solvent water–ethanol.

2. Materials and methods

Lactose crystals (Batch no. S648090) were obtained as Lactochem™ from Borculo Whey Ltd., Chester,

UK and Carbopol 934 with an average molecular weight of approximately 3,000,000 from B.F. Goodrich Chemical Co., Cleveland, OH, USA. Micronised salbutamol sulphate (Batch no. 540330, BP 1993—USP XXII) was purchased from Allchem International, Maidenhead, Berkshire, UK and was further micronised using an air-jet microniser (JM-80, M&M Fryma Ltd., Herts, UK) with the nozzle pressure set to 6 bar. Ventolin® Rotahaler® and gelatin capsules (size 3) were supplied by GlaxoSmithKline Ltd., Ware, UK. Other materials were obtained as follows: lecithin-epicuron 200 (Batch no. 199047, Lucas Meyer, UK), hexane (Analytical grade, Rathburn, Walkerburn, UK), methanol (HPLC grade, Rathburn, Walkerburn, UK), ethyl parabens (Analytical grade, Aldrich, Poole, UK).

2.1. Preparation of coarse lactose

A 63–90 μm particle size fraction of lactose was obtained by sieving, using an air-jet sieve (Alpine, Augsburg, Germany). Each grade of lactose (approximately 50 g) was first passed through a test sieve with an aperture width of 90 μm (Endecotts Ltd., London, UK) for 15 min and the sieved powder was then passed through a 63 μm sieve for a further 15 min. The powder retained on the 63 μm sieve was re-sieved using the same procedure in order to ensure that the majority of particles fell within a size range of 63–90 μm. The sieved powder was allowed to dry in an oven at 70 °C overnight and then transferred to a sealed jar before placing in a desiccator over silica gel until required for further investigation.

2.2. Crystallisation procedures

2.2.1. Crystallisation of salbutamol sulphate from aqueous solution

Salbutamol sulphate (20 g) was dissolved in 50 ml deionised water at 75 °C with the aid of stirring using a magnetic stirrer. When the salbutamol was completely dissolved, the solution was allowed to cool to 45 °C. The crystals were collected by filtration under vacuum through a Whatman filter paper (<0.2 μm) fitted into the filtration unit. The crystals were spread on a glass petri dish, and allowed to dry overnight in an oven at 70 °C. The recrystallised drug was then transferred to a vial, sealed and placed in a desiccator over silica gel until required for further investigation.

2.2.2. Crystallisation of salbutamol sulphate from a binary solvent: water–ethanol

Salbutamol sulphate (5 g) was dissolved in deionised water (100 ml) at room temperature with the aid of stirring using a magnetic stirrer. Once the salbutamol sulphate was completely dissolved, 10 ml of the resultant solution was added to absolute ethanol (90 ml). Crystals were formed within a minute, and were collected using a similar procedure to that described above (Section 2.2.1).

2.2.3. Crystallisation of salbutamol sulphate in the presence of water–lecithin–ethanol

Solutions of 0.4 g ml^{-1} salbutamol sulphate were prepared by dissolving 20 g salbutamol sulphate in 50 ml of deionised water at 75 °C with the aid of stirring using a magnetic stirrer and 14 mg lecithin was then added. When all the lecithin was dispersed in the solution, it was allowed to cool to 45 °C without disturbance. The salbutamol sulphate solution (20 ml) was introduced into a filtration unit (Sartorius Limited, Surrey, UK) and 50 ml of absolute ethanol was added rapidly. The precipitated crystals were collected immediately by filtration under vacuum, through a Whatman filter paper (<0.2 μm) fitted into the filtration unit. The resultant crystals were washed twice (20 ml total volume) with absolute ethanol to remove traces of lecithin. The crystals were allowed to dry overnight in an oven at 70 °C, then transferred to a vial, sealed and placed in a desiccator over silica gel until required for further investigation.

2.2.4. Crystallisation of lactose crystals from Carbopol gel

Carbopol (0.2 g) was dissolved in 100 ml deionised water at 60 °C in a 250 ml beaker with the aid of stirring (500 rpm), using a 4-bladed stirrer. Lactose (50 g) was then dissolved in the Carbopol solution at 60 °C with constant stirring at 500 rpm. A cloudy solution was produced with a pH of approximately 2.8. The resulting solution was allowed to cool to room temperature and sodium hydroxide solution (1 M) was then added drop-wise to the solution, whilst stirring at 800 rpm. The viscosity and clarity of the solution increased with pH. The addition of sodium hydrox-

ide was continued until the pH had risen to 7 and the resultant gel was covered and placed in the dark allowing crystals to grow. After 48 h, the pH of the gel was adjusted to 2.5–3.5 with 1 M hydrochloric acid, which caused it to liquefy and the crystals were allowed to settle for about 10 min. After decanting the supernatant, the crystals were washed with 60% ethanol, then with absolute ethanol three times, before allowing them to dry in an oven at 70 °C overnight. The resultant particles were poured into a 90 μm mesh size sieve, which had been placed upon a 63 μm mesh sieve. The particles were sieved by hand brushing slowly for 1 h so as to limit the rupture of any crystals. The resulting sieved fraction (63–90 μm) was transferred to a jar, sealed and placed in a desiccator over silica gel.

2.2.5. Crystallisation of lactose crystals from water–ethanol solution

Commercial lactose crystals (10 g) were dissolved in 100 ml distilled water at 55 °C. After cooling to room temperature, 10 ml of this solution was transferred to a 200 ml beaker containing 90 ml of absolute ethanol, which had been placed on a hot plate at 55 °C. The solution was stirred with a glass rod and then left to stand at 55 °C without disturbance. As soon as a precipitate started to form (usually within 10 min), the beaker was removed from the hot plate and placed at ambient temperature for 24 h. The resultant crystals were filtered through a glass filter under vacuum and allowed to dry in an oven at 70 °C overnight. The resulting crystal particles were fractionated by sieve to produce a 63–90 μm cut and stored in a sealed jar, as described in Section 2.2.4.

2.3. Particle size measurement of micronised salbutamol sulphate by laser diffraction

The particle size of micronised salbutamol sulphate was determined in a solution of 1% (w/v) span 85 in hexane by laser diffraction, using a Malvern 2600 instrument spectrophotometer (Malvern Instruments, Malvern, Worcs, UK) fitted with a 63 mm lens. The volume median diameter and associated geometric standard deviation (GSD) were obtained after the data had been fitted to an independent model of analysis. All measurements were carried out in triplicate.

2.4. Characterisation of particle shape by optical microscopy with image analysis and by scanning electron microscopy

A small amount of powder was suspended in mineral oil (Sigma Chemical Co., St. Louis, USA), and the suspension was spread onto a microscope slide and a cover slip was placed on the suspension which was allowed to settle homogeneously. 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 linked to an optical microscope (Nikon Labophot, Tokyo, Japan) via a miniature video camera. For the 10× objective the pixel resolution of the digitised image used for measurement was 1.13 μm per pixel in the *x*-axis and 2.26 μm per pixel in the *y*-axis. Four hundred particles were measured for each sample of lactose and the surface volume mean diameter (VMD) (Washington, 1992) and elongation ratio were recorded, the latter factor being defined as follows:

$$\text{elongation ratio} = \frac{\text{maximum Feret diameter}}{\text{minimum Feret diameter}} \quad (1)$$

where the minimum and maximum Feret diameters were calculated from 16 calliper measurements at 6° intervals around the particle. These two measurements were not necessarily at right angles to each other.

Double-sided adhesive tape was placed on an aluminium stub and after removing the protective covering, a few particles were scattered on the tape and dispersed by tapping lightly on the edge of the stub with a spatula. The particles were then coated with a 15–20 nm layer of gold using a sputter coater (Polaron E5100, Polaron Equipment Ltd., Watford, UK) under an electrical potential of 2.0 kV and a current of 20 mA. Several photomicrographs were produced by scanning fields, selected at random at different magnifications using a Philips SEM501B scanning electron microscope (Einhoven, Holland).

2.5. Characterisation of salbutamol sulphate and different samples of lactose by differential scanning calorimetry, thermogravimetric analysis and hot stage microscopy

DSC experiments were conducted using a 2920 Modulated DSC (TA Instruments), with a refrigerated

cooling system (RCS). Nitrogen was used as the purge gas, flowing at a rate of 33 ml min^{-1} through the DSC cell and at 150 ml min^{-1} through the RCS units. Aluminium non-hermetic DSC pans were used through the study. The mass of each empty sample pan was matched with the mass of the empty reference pan to ±0.1 mg. The instrument was calibrated using an indium standard and approximately 3 mg of sample was used for each run. After sealing, the pans were placed in the DSC furnace which had been pre-equilibrated at 25 °C. Before each measurement, the sample was allowed to equilibrate for 5 min at 25 °C and was then heated to 250 °C (lactose) and 350 °C (salbutamol sulphate) at a heating rate of 10 °C min^{-1}. Each batch was analysed in triplicate.

Thermogravimetric analysis (TGA) was used to determine the number of moles of water of crystallisation per mole of anhydrous lactose for different samples. The sample (10 mg) was weighed into an aluminium crucible (70 μl) and placed in the furnace which had been pre-equilibrated furnace at 25 °C. After equilibration at this temperature for at least 5 min, the sample was heated to 250 °C at a heating rate of 10 °C min^{-1} (TGA 2950, TA Instruments).

About 1 mg of salbutamol sulphate powder was suspended in silicone oil (Sigma Chemical Co., St. Louis, USA) and the suspension spread on a microscope slide. A cover slip was placed on the suspension, which was allowed to settle homogeneously between the two glass surfaces. The salbutamol sulphate was observed under an optical microscope whilst being heated from ambient to 250 °C at a heating rate of 10 °C min^{-1} using a Mettler FP82 hot stage and FP80 central processor.

2.6. Measurement of powder flowability

The angle of slide (θs) was used to characterise the flowability of different batches of lactose (Zeng et al., 2000b). A similar amount of lactose to that used to fill the capsule shells was placed on a stainless steel plate (6.55 cm × 7.00 cm), and this was then tilted by raising a supporting spindle vertically upwards from beneath. When the majority of the powder started to slide, the angle between the tilted plane and horizontal, θs, was measured directly. Each sample was measured in triplicate.

2.7. Preparation of powder formulations

Salbutamol sulphate and lactose were mixed in a ratio of 1:67.5 (w/w) in accordance with the ratio employed in commercial Ventolin® Rotacaps®. Stoppered vials, containing the separate blends of salbutamol sulphate with one batch of lactose, were placed in a Turbula mixer (Glen Greston Ltd., Middx, UK) and mixing was carried out for 30 min at 42 revolutions per min, 5–10 g of each blend was prepared.

All blends were then filled into hard gelatin capsules (size 3) manually such that each capsule contained 481.75 ± 14.60 μg salbutamol sulphate.

2.8. HPLC analysis of salbutamol sulphate

Salbutamol sulphate was analysed by HPLC employing a mixture of methanol and 0.25% (w/v) 1-heptane sulfonic acid sodium salt (40:60, v/v) as the mobile phase running at a flow rate of 0.9 ml min^{-1}, *p*-hydroxybenzoic acid ethyl ester (1 μg ml^{-1}) as an internal standard and UV detection at 238 nm. The HPLC system consisted of a pump (CM 4000 Multiple Solvent Delivery System, LDC Analytical Inc., FL, USA), a multiple wavelength UV detector (SpectroMonitor 3100, LDC Analytical Inc., FL, USA) and a 30 cm × 4.6 mm i.d. column packed with 5 μm Novapack C18 (Waters, Milford, MA, USA), which was maintained at 60 °C. The retention times for salbutamol sulphate and the internal standard were 6 and 10.6 min, respectively.

2.9. Dose uniformity and deposition study of salbutamol sulphate from different formulations

2.9.1. Measurement of dose uniformity

The homogeneity of the blends was examined by analysing the quantity of salbutamol sulphate in 33 ± 1 mg samples of powder, which was the amount of powder contained in each capsule. Each aliquot was placed in a 100 ml volumetric flask and made up to volume with the HPLC mobile phase containing the internal standard. Six samples were taken randomly from each blend and each solution was assayed in duplicate using the HPLC method described above. The coefficient of variation

(%CV) was used to assess the homogeneity of the blends.

2.9.2. Deposition test of micronised and recrystallised salbutamol sulphate

Deposition of salbutamol sulphate from each blend was determined using a twin-stage impinger after aerosolisation of three capsules at 60 l min^{-1} via a Rotahaler®; 7 and 30 ml, respectively, of the mobile phase containing the internal standard was introduced into the upper and lower stage of the impinger. The capsule to be tested was placed in the inhaler device (Rotahaler®, GlaxoSmithKline Ltd., Ware, UK), which had been fitted into a moulded rubber mouthpiece attached to the throat of the impinger. Once the assembly had been checked and found to be airtight and vertical, the dose was released; the pump was switched on, allowed to run for 7 s at 60 l min^{-1} and then switched off. The capsule shell was then removed from the inhaler device and the deposition test was repeated with two more capsules being actuated in the same manner. The capsule shells were washed five times and then made up to 50 ml with the mobile phase containing internal standard. The inhaler device, upper and lower stage were all washed with the same solvent and made up to separate 100 ml fractions. All the samples obtained were analysed for the concentration of salbutamol sulphate using the HPLC method outlined above.

Deposition of salbutamol sulphate from each formulation was determined four times and a variety of parameters were employed to characterise the deposition profiles of the drug. The recovered dose (RD) was the sum of the weights of drug (μg) recovered from the capsule shells, the inhaler device and the upper and lower stages of the twin impinger, whilst the emitted dose (ED) was the dose emitted from the inhaler device and depositing in the upper and lower stages of the twin impinger. Fine particle dose (FPD) was the amount of drug recovered from the lower stage (drug particles <6.4 μm). The FPF was calculated as the ratio of the FPD to RD and dispersibility as the ratio of FPD to ED (both expressed as a percentage). The percent recovery was calculated as the ratio of RD to the expected dose and the percent emission defined as the ratio of ED to RD (expressed as a percentage).

3. Results and discussion

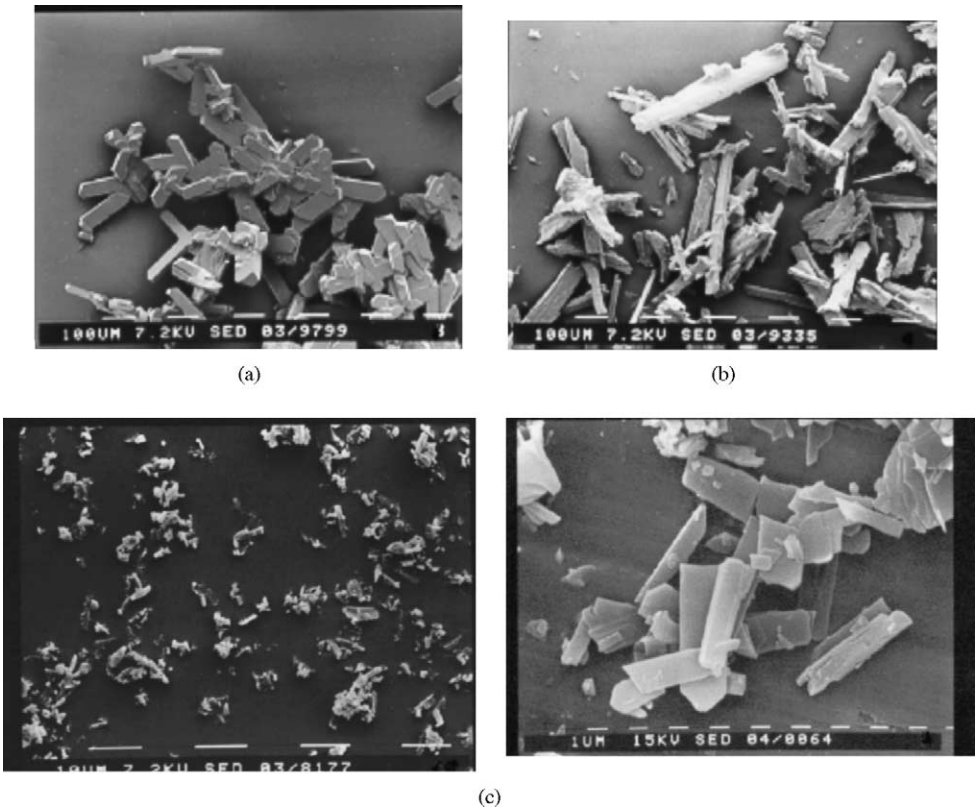
3.1. Particle size and morphology of salbutamol sulphate and lactose samples

The salbutamol sulphate supplied by the manufacturer was shown to have a VMD of 4.02 ± 0.03 μm with some particles >10 μm. The drug was not thought to have an optimal particle size for inhalation and was therefore re-micronised. After this re-micronisation the VMD and GSD as measured by laser diffraction were 2.75 ± 0.02 and 1.68 ± 0.09 μm, respectively.

The crystal habit and size of salbutamol sulphate crystals were found to depend on the conditions used for crystallisation, such as the concentration of salbutamol sulphate in the solution and whether additives were introduced into the crystallisation medium. When the concentration of salbutamol sulphate was 0.4 g ml^{-1}, crystallisation occurred rapidly, while the solution was still hot (65 °C). The crystallisation of salbutamol sulphate from water favoured the formation of crystals with a prismatic shape and a particle size greater than 100 μm (Fig. 1).

Crystals were not formed when the concentration of salbutamol sulphate in water was reduced to 0.05 g ml^{-1}. However, the addition of 10 ml of this solution to 90 ml absolute ethanol, promoted spontaneous nucleation and elongated salbutamol crystals were formed within 1 min (Fig. 1). This occurred as a consequence of the reduced solubility of salbutamol sulphate in the presence of a water miscible solvent such as ethanol, which leads to an increase in the degree of supersaturation and hence an increased rate of drug crystallisation. The crystals formed in the presence of ethanol were thinner and more elongated than those produced from water alone at higher salbutamol sulphate concentrations (Fig. 1). The addition of lecithin to the crystallisation medium, reduced nucleation and crystal growth. When followed by the addition of a solvent which promoted rapid nucleation, such as ethanol, particles with the required size range for pulmonary drug delivery were produced (Fig. 1).

The scale bars in the SE micrographs (Fig. 1) indicate that the length of salbutamol sulphate crystals was less than 6 μm and the width of the crystals was found between 0.5 and 1 μm, thus the particles might be suitable for deposition in the lower airways and not



(a) (b) (c)

Fig. 1. SE micrographs of salbutamol sulphate crystallised from (a) water, (b) water–ethanol mixture and (c) water–lecithin–ethanol.

be exhaled. Due to the elongated nature of the particles, sizing data derived from techniques assuming broad particle sphericity become inadequate in generating reliable and meaningful results.

Table 1 shows the surface VMD for the different lactose samples. Lactose re-crystallised from a water–ethanol solution (LN) had the smallest surface VMD (68.68 μm), followed by the commercial lactose crystals (106.12 μm). The lactose re-crystallised from a Carbopol gel (L Carb) had the highest value of surface VMD, which was approximately 1.5 times that of LN.

Table 1
The surface VMD, elongation ratio measured by optical microscopy image analysis ($n = 400$) and the angle of slide (θs), $n = 3$, for lactose crystals (LC), lactose crystallised from Carbopol (L Carb) and lactose crystallised from water–ethanol solution (LN)

Carrier	Diameter (μm)	Elongation ratio	θs (°)
LC	106.12	1.69 ± 0.05	48.5 ± 2.12
L Carb	114.38	2.01 ± 0.13	43.0 ± 1.41
LN	68.68	6.25 ± 0.09	53.6 ± 3.51

The lactose samples also showed different values of the elongation ratio: the higher the elongation ratio, the more elongated and/or the more irregular the shape. Thus, LN was more elongated than L Carb, which was in turn slightly more elongated than the commercial crystals. The shapes of lactose particles are shown in the SE micrographs (Fig. 2) from which it can be seen that LN had a distinctively different shape from the other lactose samples; LN crystals were needle-shaped, whereas in the other two batches they were tomahawk-shaped. The angle of slide (θs) can be used to assess the flow properties powders, the lower the value of this angle, the better the flow properties of the powder. It can be seen from Table 1 that increasing the surface VMD, or decreasing the

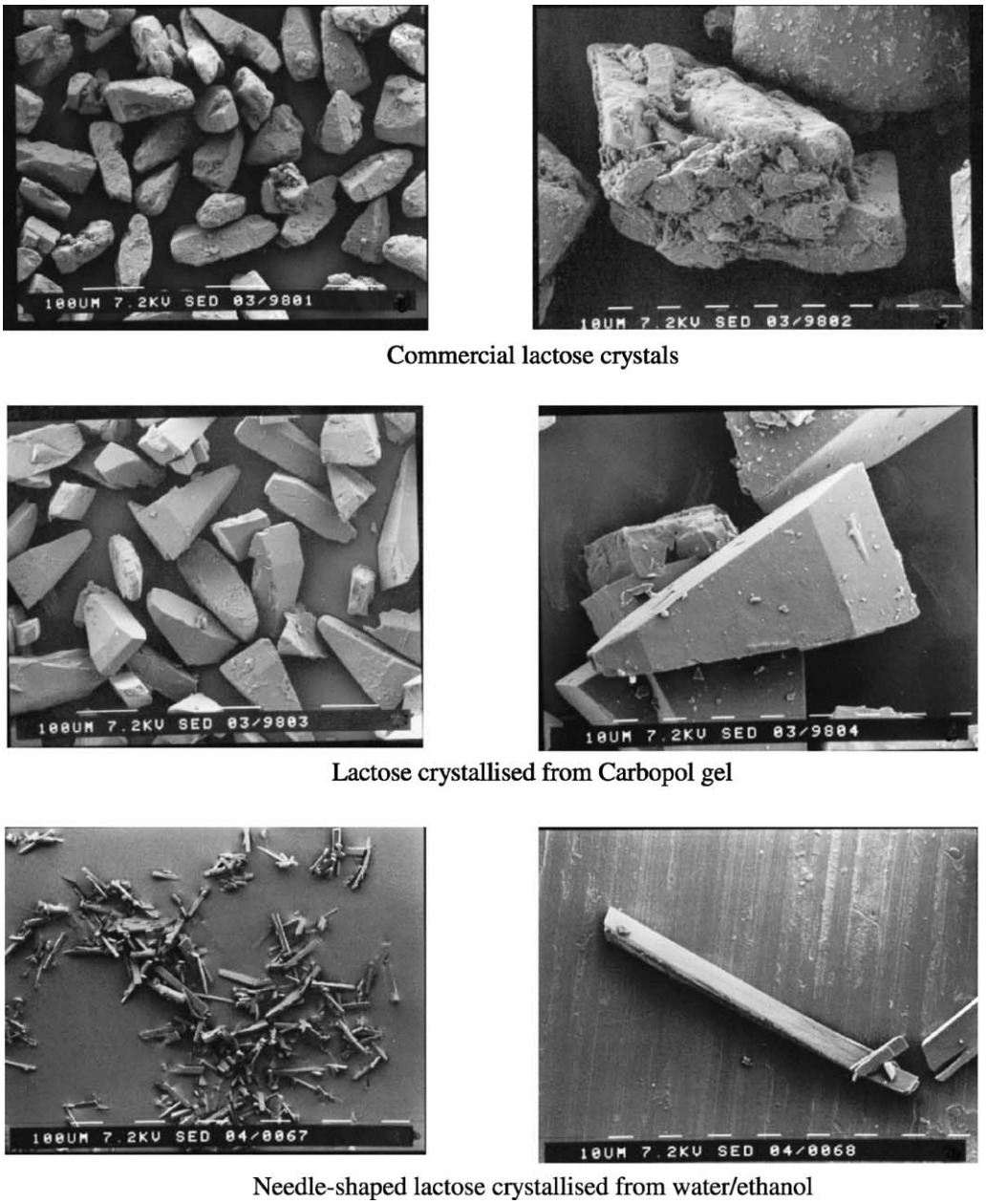


Fig. 2. SE micrographs of different batches of lactose.

elongation ratio of the particles, generally decreased the angle of the slide. LN crystals showed the lowest value of the surface VMD and the highest value of elongation ratio and exhibited the highest value of θs, suggesting that this batch possessed the poorest flow properties of the samples examined. There were comparably small differences between the surface VMD and elongation ratios of the particles obtained for the commercial lactose crystals and those obtained by crystallisation from Carbopol. However, a marked

difference was found in the values of θs obtained for the two batches of crystals. SE micrographs clearly showed that lactose crystals had many pores or cavities on the particle surface whereas such defects could not be detected using SEM in the case of lactose crystallised from the Carbopol gel. Therefore, the particles obtained from Carbopol gel had the smoothest surfaces, accounting, in part, for the reduction in the frictional forces between lactose particles.

Table 2
The moles of water of crystallisation per mole of anhydrous lactose calculated from the weight loss between 110 and 180 °C from TGA thermogram for different lactose samples

Carrier	Weight loss (%, w/w)	Moles of water/mole of anhydrous lactose (n)
LC	5.153 ± 0.027	1.033 ± 0.005
L Carb	5.056 ± 0.074	1.012 ± 0.015
LN	5.068 ± 0.064	1.015 ± 0.013

3.2. Characterisation of lactose and salbutamol sulphate by DSC and TGA

Jbilou et al. (1999) reported that the crystallisation of lactose from water–ethanol (50:50%, v/v) produced amorphous lactose and that the crystals obtained were a mixture of α- and β-lactose. The mechanical stirring used by these previous workers may have accelerated the mutarotation of lactose molecules from α- to β-lactose, and may have also introduced some disorder within the crystal lattice. In the current study, the crystals obtained from water–ethanol solution were allowed to grow without disturbance and as a result the DSC thermogram (Fig. 3) of the lactose showed two endothermic transitions which were similar to those of CL and L Carb, suggesting that all the lactose samples existed as α-lactose monohydrate. The first endothermic transition corresponds to the loss of water of crystallisation and the second transition corresponds to the melting of lactose followed by its decomposition. The

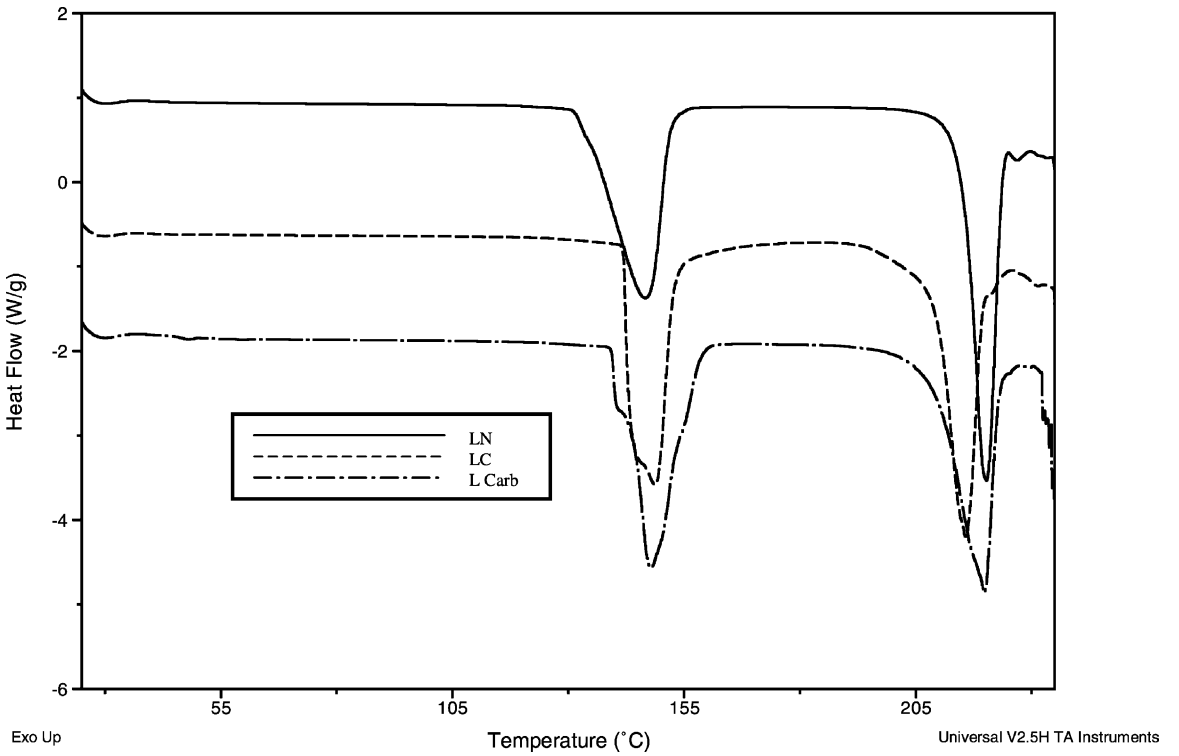


Fig. 3. DSC thermograms of commercial lactose crystals (LC), lactose crystallised from Carbopol (L Carb) and lactose crystallised from water–ethanol solution (LN).

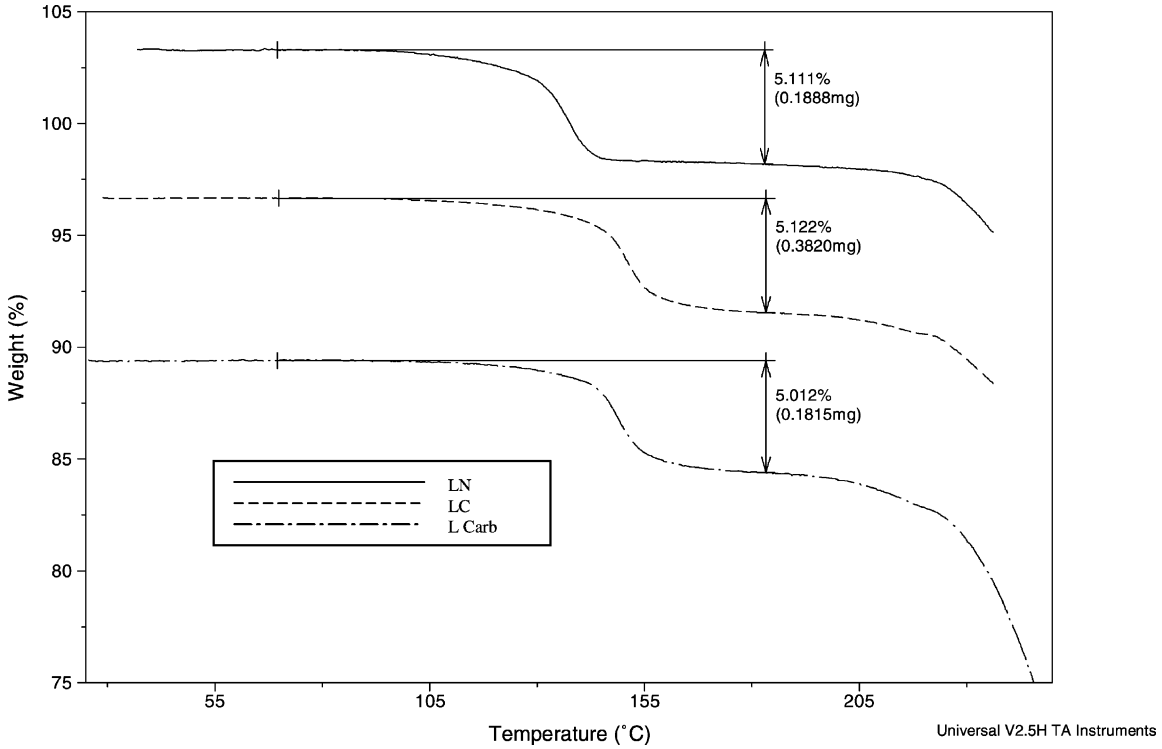


Fig. 4. TGA thermograms of lactose crystals (LC), lactose crystallised from Carbopol (L Carb) and lactose crystallised from water–ethanol solution (LN).

TGA thermograms showed that all three lactose samples exhibited similar weight loss (≈5%, w/w) profiles upon heating (Fig. 4). The loss of weight occurred between approximately 110 and 180 °C due to the loss of water of crystallisation and this was used to calculate the number of moles of water per mole of anhydrous lactose (Table 2) using the following equation:

$$n = \frac{W_{\text{loss}}/\text{RMM}_{\text{water}}}{(1 - W_{\text{loss}})/\text{RMM}_{\text{lactose}}} \quad (2)$$

where W_{loss} is the percentage weight loss between 110 and 180 °C, $\text{RMM}_{\text{lactose}}$ and $\text{RMM}_{\text{water}}$ are the relative molecular masses of anhydrous lactose (342.3) and water (18.0), respectively.

It can be seen from Table 2 that all the lactose samples were found to have one mole of water per mole of anhydrous lactose, suggesting that they all exist as the monohydrate.

The DSC thermograms of micronised and needle-shaped salbutamol sulphate crystallised from aqueous solution in the presence of lecithin were similar (Fig. 5). The first endothermic transition which started at about 180 °C and peaked at approximately 200 °C was initially attributed to melting. However, when the sample was heated to 250 °C and the contents of the DSC or TGA pan were observed under the optical microscope, surprisingly, the remaining particles were of similar shape to the starting material, although they had taken on a yellow colour. Further investigation was carried out by immersing a fresh sample of salbutamol sulphate in silicone oil and carrying out observations by hot stage microscopy. No transition from solid to liquid state was seen at 200 °C. This observation suggests that the first transition in the DSC thermogram was not due to melting of salbutamol sulphate, but to decomposition of the drug. From the structure of salbutamol sulphate, the most likely group to depart upon heating could be the *tert*-butylamine group, but further investigation is required. For example, the salbutamol sulphate could be heated to above 200 °C and the gas released at 200 °C collected, followed by analysis of the vapour by gas chromatography-mass

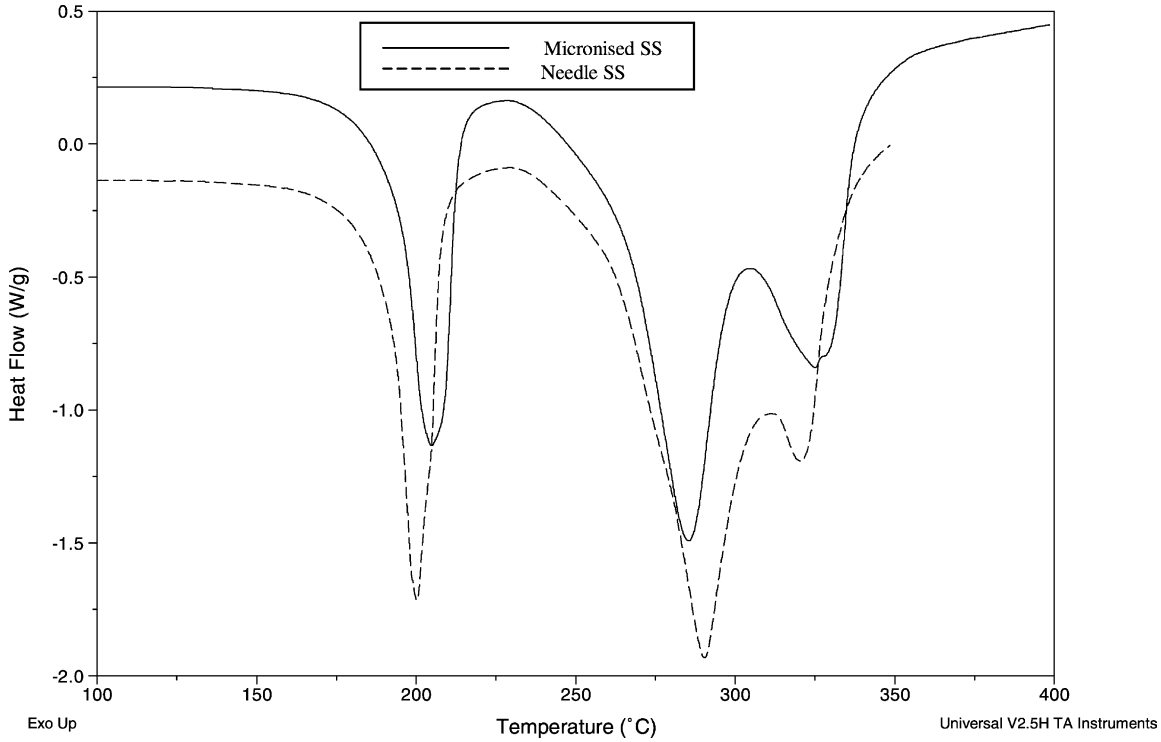


Fig. 5. The DSC thermograms of micronised and needle-shaped salbutamol sulphate (SS).

spectrometry. A further decomposition of salbutamol sulphate occurred at 290 °C, which was related to a second endothermic transition, above which the powder turned brown in colour.

3.3. Content uniformity of micronised and needle-shaped salbutamol sulphate in the formulations

Table 3 shows the percentage recovery and coefficient of variation (%CV) in salbutamol sulphate content obtained with all formulations. Although the sampling and mixing procedures were similar for all formulations, differences in %CV were observed, with those containing needle-shaped salbutamol sulphate producing the higher %CV. The highest variation in content was found when the needle-shaped salbutamol sulphate was mixed with LN, although the variation was still less than 7%. The needle-shaped carrier also showed the highest angle of slide and consequently exhibited the poorest flow properties of all the powders studied.

Table 3
Percent recovery and coefficient of variation (CV) in salbutamol sulphate (SS) content obtained from different formulations ($n = 6$)

Formulation	Percent recovery ± S.D.	%CV
Lactose crystals-micronised SS	98.20 ± 1.14	1.16
Lactose carb-micronised SS	96.19 ± 1.67	1.74
Lactose needle-micronised SS	101.78 ± 1.95	1.92
Lactose crystals-needle SS	98.59 ± 3.68	3.73
Lactose carb-needle SS	97.58 ± 3.13	3.21
Lactose needle-needle SS	96.74 ± 6.70	6.93

Crystallisation of salbutamol sulphate from water in the presence of lecithin and ethanol provided direct formation of micron-sized drug particles suitable for delivery to the lung without any additional operation such as milling. Also, the crystals obtained might be expected to have lower surface energy compared than the more cohesive, highly charged micronised salbutamol sulphate. The interaction of crystallised salbutamol sulphate with the carrier might therefore be expected to be less due to lower adhesion forces than that which exist between micronised salbutamol

Table 4
Recovered dose (RD), emitted dose (ED) and fine particle dose (FPD) of micronised and needle-shaped salbutamol sulphate (SS) from formulations containing different lactose carriers (mean ± S.D.)

Formulation	RD (μg)	ED (μg)	FPD (μg)
Lactose crystals-micronised SS	458.60 ± 15.30	366.20 ± 18.80	25.10 ± 5.90
Lactose carb-micronised SS	461.06 ± 12.23	373.79 ± 13.61	75.24 ± 8.18
Lactose needle-micronised SS	455.80 ± 22.30	333.50 ± 18.70	100.10 ± 16.10
Lactose crystals-needle SS	468.79 ± 18.25	363.12 ± 31.73	80.54 ± 7.99
Lactose carb-needle SS	446.75 ± 22.29	357.41 ± 23.67	103.86 ± 11.57
Lactose needle-needle SS	438.23 ± 45.52	304.49 ± 46.29	127.34 ± 14.14

sulphate and the carrier. If this were the case, mixing and demixing of the crystallised drug may have occurred continuously during the mixing period leading to the observed variation in content uniformity of needle-shaped salbutamol sulphate.

3.4. Deposition of drug from formulations containing different batches of lactose

Deposition data from different formulations (Tables 4 and 5) were calculated as a nominal dose of one capsule containing 481.75 ± 14.60 μg salbutamol sulphate. It can be seen that a similar RD was obtained for all formulations, ranging from 438 μg for the formulation containing lactose needle-needle salbutamol sulphate to 468 μg for blends composed of lactose crystals-needle salbutamol sulphate. These RDs correspond to percentage recoveries of between 93 and 99%. The formulations containing either needle-shaped salbutamol sulphate or LN, produced the highest variation in the RD as indicated by the magnitude of the S.D. values (Table 4). Such variations were not believed to be due to the procedure used to wash the TSI, but were mainly attributable to the poorer flowability of the formulation during mixing and the nature of adhesion of the drug to the carrier. The variation in RD only increased slightly when needle-shaped salbutamol sulphate replaced micronised drug using lactose crystals as the carrier (Table 4). There were many asperities and crevices on the surface of the lactose crystals and the latter may have trapped the drug leading to the formation of a more stable mix.

More powder was found to remain in the Rotahaler® after aerosolisation of the blends containing LN, whether combined with micronised or needle-shaped drug. This is demonstrated by the lower percent emission values for such formulations, compared to the formulations containing lactose crystallised from Carbopol gel (Table 5). Increasing the surface VMD or decreasing the elongation ratio of the carrier (Table 1), generally increased the percent emission of salbutamol sulphate (Table 5). These parameters have been shown to affect the angle of slide (θs) and thus, the flow properties of the carrier. The lower emission of salbutamol sulphate from the formulations containing the LN carrier was probably due to the poorer flow properties of this carrier leading to a lower amount of the drug being released from the inhaler device.

Despite the slightly lower emission of salbutamol sulphate from formulations containing LN, the latter produced the highest FPD, dispersibility and FPF

Table 5
Fine particle fraction (FPF), dispersibility, percent recovery and percent emission of micronised and needle-shaped salbutamol sulphate (SS) from formulations containing different lactose carriers (mean ± S.D.)

Formulation	FPF (%)	Dispersibility (%)	Percent recovery	Percent emission
Lactose crystals-micronised SS	5.50 ± 1.30	6.90 ± 1.60	93.50 ± 3.10	79.90 ± 5.10
Lactose carb-micronised SS	16.32 ± 1.72	20.15 ± 2.28	99.49 ± 2.64	81.10 ± 3.09
Lactose needle-micronised SS	22.10 ± 4.30	29.90 ± 3.60	93.00 ± 4.60	73.30 ± 5.70
Lactose crystals-needle SS	17.19 ± 1.67	22.29 ± 2.74	98.71 ± 3.84	77.35 ± 3.88
Lactose carb-needle SS	23.27 ± 2.61	29.09 ± 3.03	95.03 ± 4.74	79.98 ± 2.89
Lactose needle-needle SS	29.07 ± 1.35	42.07 ± 2.51	94.03 ± 9.77	69.22 ± 4.04

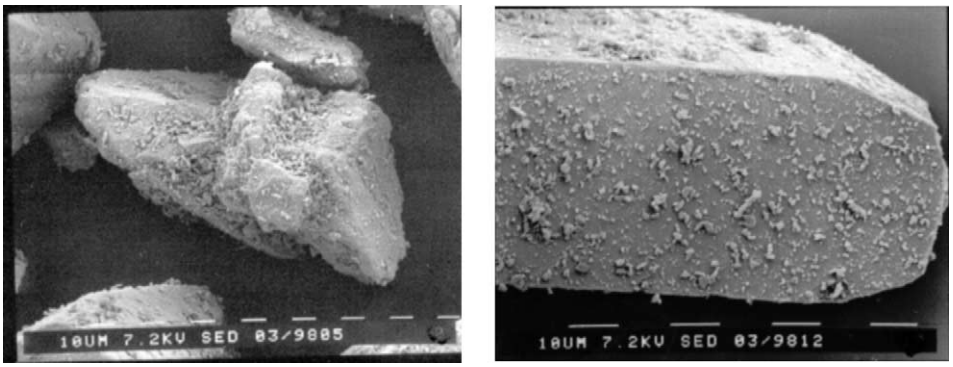


Fig. 6. Salbutamol sulphate adhered to the surface of commerciallised lactose crystals (left) and lactose crystallised from Carbopol gel (right).

whether micronised or needle-shaped drug was employed. FPD, dispersibility and FPF were reduced with the use of L Carb, but these values were still markedly better than the corresponding values using lactose crystals (Tables 4 and 5). The LN showed the lowest surface VMD and the highest elongation ratio (Table 1). Decreasing the particle size of the carrier has been shown to improve drug delivery from dry powder inhaler (DPI) formulations, possibly by reducing interparticulate forces between the drug and carrier (Staniforth, 1996; Lucas et al., 1998; Zeng et al., 1998; Larhrib et al., 1999). The higher FPD, dispersibility and FPF of salbutamol sulphate from formulations containing LN suggested that more drug was detached from this carrier than from L Carb or LC. Even for drug particles which are not detached, the LN may act as carrier to aid deep lung penetration of salbutamol sulphate due to its superior aerodynamic properties.

The L Carb and the LC showed only slight differences in particle size and elongation ratio, but a large difference in surface smoothness. Increasing the surface smoothness of lactose was shown to improve the respirable fraction of salbutamol sulphate from the Rotahaler® (Ganderton, 1992; Zeng et al., 2000a). The higher FPF of the blends prepared from L Carb might be due to the smooth surface of the carrier and/or an increase in the elongation ratio. The lowest FPF, FPD and dispersibility of salbutamol sulphate, which was obtained using blends containing LC may be due to this lactose having the roughest surface with the lowest elongation ratio. SE micrographs (Fig. 6) clearly showed that drug particles could associate with the smooth surface of L Carb. Drug particles also adhered to the surface of commercial lactose crystals but were also entrapped in the crevices. The entrapped salbutamol sulphate may not be capable of detachment by the turbulence created by inhalation, leading to a lower proportion of the drug reaching the lower airways.

The blends containing crystallised salbutamol sulphate produced higher FPD, dispersibility and FPF than those containing micronised salbutamol sulphate (Tables 4 and 5). The salbutamol sulphate used in the current study has been re-micronised in order to achieve the optimum size range. The second micronisation may have increased the amorphous content, promoting adhesion of the drug to the carrier. This would result in a strong interaction of the micronised salbutamol with carrier particles, impeding drug detachment, dispersion in the air stream and consequently lead to a possible reduction of drug deposition in the respiratory tract. The crystallised salbutamol sulphate particles are also more elongated than the micronised drug. These needle-like crystals are therefore more likely to disperse in an air flow and remain airborne than the more isometric micronised particles of a similar geometric size (Hickey et al., 1992). As a result, more drug particles, may have been detached from the carrier particles, leading to an increased FPD.

4. Summary and conclusion

Lactose crystals of different elongation ratio were engineered either from aqueous solution in the presence of Carbopol gel or from a binary solvent,

water–ethanol. The resulting crystals were smoother and with very well-defined shape compared to the commercially available lactose. The engineering process did not cause any polymorphic transformation to the resulting crystals, which were found to exist in the α-monohydrate form.

Needle-shaped salbutamol sulphate of suitable size for delivery to the lungs, was produced by a simple crystallisation procedure from aqueous solution in the presence of lecithin and ethanol.

Engineered lactose carriers improved the deposition profiles of salbutamol sulphate compared to commercially available lactose crystals. Replacing micronised salbutamol sulphate with needle-shaped drug further enhanced deposition. The highest deposition of salbutamol sulphate was obtained by combining LN with needle-shaped drug. However, the latter combination produced some variations in the content uniformity of salbutamol sulphate and emptying of the capsules during inhalation flow rate. This was attributed to the poorer flow properties of the blends. Excellent results either in terms of both flow properties and deposition might be achievable by introducing a very small amount of the engineered lactose produced from Carbopol gel in the binary blend composed of LN with needle-shaped drug.

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References

Davey, J.R., 1982. In: Janic, S.J., De Jong, E.S. (Eds.), Industrial Crystallisation. North-Holland, Amsterdam, pp. 123–135.

Davies, P.J., Hanlon, G.W., Molyneux, A.J., 1976. An investigation into the deposition of inhalation aerosol particles as a function of air flow rate in a modified 'Kirk Lung'. J. Pharm. Pharmacol. 28, 908–911.

Elamin, A.A., Sebhatu, T., Ahlneck, C., 1995. The use of amorphous model substances to study mechanically activated materials in the solid state. Int. J. Pharm. 119, 25–36.

Ganderton, D., 1992. The generation of respirable cloud from coarse powder aggregates. J. Biopharm. Sci. 3, 101–105.

Gonda, I., 1990. Aerosols for delivery of therapeutic and diagnostic agents to the respiratory tract. Crit. Rev. Ther. Drug Carrier Syst. 6, 273–313.

Hickey, A.J., Falts, K.A., Pillai, R.S., 1992. Use of particle morphology to influence the delivery of drugs from powder aerosols. J. Biopharm. Sci. 3, 107–113.

Hinds, W.C., 1982. Aerosol Technology. Wiley, New York.

Jbilou, M., Piva, F., Barthelemy, P., Conflant, A.M., Guyot-Hermann, J.C., 1999. A new lactose for direct compression prepared by a phase separation process. In: The 18th Proceedings of Pharmaceutical Technology, vol. 1. Utrecht, The Netherlands, pp. 321–338.

Larhrib, H., Zeng, X.M., Martin, G.P., Marriott, C., Pritchard, J., 1999. The use of different grades of lactose as a carrier for aerosolised salbutamol sulphate. Int. J. Pharm. 191, 1–14.

Lucas, P., Anderson, K., Staniforth, J.N., 1998. Protein deposition from dry powder inhalers: fine particle multiplets as performance modifiers. Pharm. Res. 15, 562–569.

Michaels, A.S., Colville, A.R., 1960. The effect of surface active agents on crystal growth rate and crystal habit. J. Phys. Chem. 64, 13–19.

Newman, S.P., Clarke, S.W., 1983. Therapeutic aerosols. I. Physical and practical considerations. Thorax 38, 881–886.

Staniforth, J.N., 1996. Improvement in dry powder inhaler performance: surface passivation effects. Proc. Drug Delivery Lungs Lond. VII, 86–89.

Washington, C., 1992. Particle Size Analysis in Pharmaceutics and other Industries Theory and Practice. Ellis Horwood, London, pp. 23–25.

Zeng, X.M., Martin, G.P., Tee, S.K., Marriott, C., 1998. The role of fine particle lactose on the dispersion and deaggregation of salbutamol sulphate in an air stream in vitro. Int. J. Pharm. 176, 99–110.

Zeng, X.M., Martin, G.P., Marriott, C., Pritchard, J., 2000a. The influence of carrier morphology on drug delivery by dry powder inhalers. Int. J. Pharm. 200, 93–106.

Zeng, X.M., Martin, G.P., Marriott, C., Pritchard, J., 2000b. Crystallisation of lactose from Carbopol gels. Pharm. Res. 17, 879–886.

Zeng, X.M., Martin, G.P., Marriott, C., 2001. Particle Interactions in Dry Powder Formulations for Inhalation. Taylor & Francis, London, pp. 90–91.