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The use of different grades of lactose as a carrier for aerosolised salbutamol sulphate

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Abstract

Five different grades of lactose namely, anhydrous lactose, medium lactose, regular lactose, lactose crystals and foremost lactose were fractionated under similar conditions to obtain a size range of 63–90 μm and were characterised using laser diffraction and time-of-flight particle sizing techniques, scanning electron microscopy, optical microscopy image analysis, thermal gravimetric analysis and differential scanning calorimetry. Each of these lactose fractions were then blended separately with micronised salbutamol sulphate in a ratio of 67.5:1 (w/w). The mixing uniformity and percentage recovery of salbutamol sulphate in the powder blends were analysed using a validated HPLC method. The deposition profiles of the drug were determined using a 5-stage liquid impinger after aerosolisation at 60 l min^{-1} via a Rotahaler. Despite the identical processing conditions, the lactose fractions were shown to differ in particle size, size distribution and concentrations of fine particles. The particles from each fraction also exhibited different surface textures and dissimilar DSC thermograms. However, all the blends of the lactose with salbutamol sulphate were found to have a relatively high uniformity of salbutamol sulphate content, as suggested by a coefficient of variation of less than 3.2%. Anhydrous and medium lactose produced a more efficient delivery of salbutamol sulphate when aerosolised from the Rotahaler in comparison to other grades of lactose. For example, the fine particle fraction (FPF) and fine particle dose (FPD) of drug from formulations containing anhydrous lactose were $13.4 \pm 4.2\%$ and 57.3 ± 17.6 μg, respectively, which were approximately two times higher than the respective values of the formulation containing regular lactose. Medium lactose resulted in drug FPF ($7.9 \pm 2.7\%$) and FPD (32.4 ± 11.8 μg), which were significantly (ANOVA $P < 0.05$) higher than the same parameters obtained using lactose crystals, foremost lactose and regular lactose. More efficient drug delivery from anhydrous lactose may be partly attributed to the relatively higher concentration of fine lactose in this grade of carrier, although it showed a rougher surface than the other grades of lactose. However, the relatively high FPF of the drug from medium lactose may have been due to the relatively small mean particle size and smooth surface of the particles. Therefore, the source and grade of lactose may have a substantial effect on drug delivery from dry powder inhaler formulations and care should be

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taken in establishing appropriate quality control parameters when selecting an appropriate grade of carrier.

Keywords: Dry powder inhalers; Lactose; Salbutamol sulphate; Batch-to-batch variation; Dispersion; Deposition

1. Introduction

Dry powder formulations for inhalation are often composed of fine drug particles and inert coarse carrier particles. The carrier is used to aid the flow and dispersion properties of the drug, which is highly cohesive due to its small particle size (i.e. 1–5 μm). The materials that can be used as the carrier should be readily available in an acceptable pharmaceutical grade, chemically and physically stable and inert to the drug substance. Most importantly, the material should be readily cleared from the airways and should not exhibit harmful effects on especially the respiratory tract. Lactose is most commonly employed (Byron et al., 1990) since if appropriately processed it can fulfil many of the ideal requirements, although other sugars such as trehalose and mannitol, obtained as crystalline sieve fractions, also appear to be suitable as carriers for dry powder aerosols (Byron et al., 1996). It is possible to obtain lactose in a wide spectrum of particle size distribution but a typical particle size of lactose used as carrier for inhalation aerosols is between 63–90 μm (Bell et al., 1971).

Lactose can be obtained in either two basic isomeric forms namely, α and β-lactose or as an amorphous form. α-lactose exists both as monohydrate and as anhydrous forms, the former being the thermodynamically stable form which is usually employed as the carrier in dry powder aerosol formulations. The polymorphic forms of lactose have different physicochemical properties such as solubility, melting point, density and hardness. For example, α-lactose monohydrate is harder and more elastic than both β-lactose (Rowe and Roberts, 1995) and anhydrous α-lactose (Lerk et al., 1983). Depending on the crystallisation conditions, α-lactose monohydrate can be prepared in a variety of shapes, which may also differ in physical properties (Wong et al., 1988). Another important characteristic of lactose is the particle size and distribution which is crucial in determining nearly all aspects of powder performances such as flowability, compressibility and aerodynamic properties. Commercial grade lactose is available in a wide variety of particle sizes with diverse morphology and, in addition, different grades of lactose are available from suppliers (Wade and Weller, 1994). The physical properties of lactose such as particle size, shape and surface texture have been shown to govern the dispersion and deaggregation of any adhered drugs (Ganderton and Kassem, 1992; French et al., 1996; Steckel and Müller, 1997; Podczeck, 1998). It is probable that some previous studies have been carried out within the industrial environment to investigate the delivery of drugs from various commercial grades of lactose. However, the results from such studies, if they exist, will have been retained in-house. A recent study demonstrated that different grades of lactose produced different deposition profiles of a model drug, salmeterol xinafoate, from the Diskus® (Podczeck, 1998). This previous study employed lactose batches as supplied by the manufacturers which does not reflect the usual manufacturing process carried out in the Pharmaceutical industry where lactose is often sieved to obtain a particular size range (e.g. 63–90 μm) before it is blended with drugs. Furthermore, lactose batches from various suppliers can differ markedly in particle size distribution, and the latter is one of the dominant factors affecting deposition of drugs (Ganderton and Kassem, 1992). One objective of the present study was to obtain lactose batches which were sieved under similar conditions before blending the carrier, so produced, with a model drug salbutamol sulphate. The aim was then to establishing the relationship between in vitro deposition of the drug and physical properties of different grades of lactose; such as particle size distribution, morphological features and polymorphic forms were considered.

2. Materials and methods

Anhydrous lactose (batch no. E648036), regular lactose (batch no. 812701), medium lactose (batch no. 806703) and lactose crystals (batch no. S648090) were obtained from Lactochem™ lactose from Borculo Whey, Chester, UK. Foremost lactose (batch no. 85980/0710) was obtained from Foremost Farms, WI, 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, Herts, UK) with a nozzle pressure set to 6 bar. Ventolin Rotahaler® and gelatin capsules (size 3) were supplied by GlaxoWellcome Research and Development, Ware, UK.

2.1. Preparation of coarse lactose

The 63–90 μm particle size fraction of lactose was sieved 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, 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 subjected to the same procedure in order to ensure that the majority of particles fell within a size range of 63–90 μm. A portion of the sieved powder was stored in a sealed jar for further investigation. The remainder was allowed to dry in a vacuum oven at 50°C for 48 h and was then transferred to a sealed jar before placing in a desiccator over silica gel until required for further investigation.

2.2. Particle size measurement by laser diffraction and time-of-flight techniques

The particle size of both lactose and salbutamol sulphate was determined in a liquid medium by laser diffraction, according to an independent model, using a Malvern 2600 laser diffraction sizer (Malvern Instruments, Malvern, Worcs., UK). Salbutamol sulphate was measured, using a 63 mm lens, after dispersion in a solution of 1% (w/v) span 85 in hexane, saturated with the drug. The particle size of the lactose grades was determined using a 100 mm lens with chloroform as the liquid medium. Particle sizes were obtained using an independent particle size model and an obscuration between 0.16 and 0.27. Each sample was measured in triplicate.

An Aerosizer with an Aero-Disperser (API Aerosizer Mach-2, Amherst Process Instruments, MA, USA) was used as supplied. A small amount of powder (about 5 mg) was placed in the sample cup of the Aero-Disperser. Particle size measurement was then carried out at a medium feed rate, high shear force and a sample run time of 300 s. Time-of-flight data were processed with the operating software package Version 10.09 for API Aerosizer Mach-2 and the powder density was taken as that of lactose, 1.54 g cm^{-3} (Wade and Weller, 1994) and anhydrous lactose 1.53 g cm^{-3} (Whiteman and Yarwood, 1988). Both number and volume distributions were recorded. Each sample was analysed at least four times.

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

A small amount of powder was suspended in mineral oil (Sigma, St. Louis, USA), and the suspension was spread onto a microscopy slide. A cover slide was added allowing the suspension to settle homogeneously between the two glass surfaces. Particle size and shape were assessed parallel using an 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. 400 particles were measured for each sample and the surface volume mean diameter, roundness and elongation ratio were recorded, the latter two factors being defined as follows:

$$\text{Roundnes} = \frac{\text{perimeter}^2}{4 \times \pi \times \text{area}} \quad (1)$$

$$\text{Elongation ratio} = \frac{\text{maximum Feret diameter}}{\text{minimum Feret diameter}} \quad (2)$$

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. For the 10× objective, employed in these studies, the pixel resolution of the digitized image used for measurement was 1.13 μm per pixel in the *x* axis and 2.26 μm per pixel in the *y* axis.

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

2.4. Characterisation of crystal forms of lactose by differential scanning calorimetry and thermal gravimetrical analysis

Differential scanning calorimetry (DSC) was employed to determine the crystal form of each grade of lactose. The calorimeter used was a Mettler TA 4000 (Mettler Instrumente, CH-8608 Greifensee, Switzerland) thermal analysis system, with a DSC20 furnace. Thermograms were analyzed using Mettler GraphWare TA72PS.1 software. An empty aluminium pan (40 μl) was used as the reference for all measurements. The instrument was calibrated using tin, indium and gallium as standard materials. Weighed samples (5–10 mg) were measured in hermetically sealed aluminium pans. The pans after sealing were placed in the pre-equilibrated DSC furnace (25°C). Before each measurement was commenced, the sample was allowed to equilibrate for 5 min at 25°C and then was heated to 250°C at a heating rate of 10°C min^{-1}. Each sample was analysed in triplicate.

Thermal gravimetric analysis (TGA) was carried out using a Mettler TA 4000 thermal analysis system, with a TG 50 thermobalance. The sample (10 mg) was weighed into an aluminium crucible (70 μl) and placed into a pre-equilibrated furnace at 25°C. After equilibration at this temperature for at least 5 min, the sample was heated to 170°C at a heating rate of 10°C min^{-1}. The weight change with temperature was recorded and processed with Mettler GraphWare TA 72 to obtain the change in weight with temperature.

2.5. 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 grade of lactose, were placed in a Turbula mixer (Glen Greston, Middx., UK) and mixing was carried out for 30 min at 42 rev min^{-1}. Each blend was prepared in 5–10 g quantities.

All blends were then filled into hard gelatin capsules (size 3) manually such that each capsule contained 481.75 ± 0.59 μg salbutamol sulphate.

2.6. 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, FL, USA), a multiple wavelength UV detector (SpectroMonitor 3100, LDC Analytical) 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.

2.7. Measurement of dose uniformity

The homogeneity of the blends was examined by analysing the quantity of salbutamol sulphate in aliquots (33 ± 0.04 mg) of sampled powder, the amount of powder in each capsule. Each aliquot of blend was placed in a 100 ml volumetric flask and made up to the volume with the HPLC mobile phase containing the internal standard. Six aliquots 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.

2.8. Deposition test of salbutamol sulphate

Deposition of salbutamol sulphate from each blend was determined using a five-stage liquid impinger (Astra Draco, Lund, Sweden) after aerosolisation of three capsules at 60 l min^{-1} via a Rotahaler. The mobile phase (20 ml) containing the internal standard was introduced into each of the first four stages of the impinger. A Whatman filter paper (0.8 μm, Whatman International, Maldstone, UK) was placed in stage five of the impinger. A throat piece was connected to the neck of the first stage and sealed with Sellotape® to ensure that the connection was airtight. After the vacuum pump was calibrated to draw air through the apparatus at a flow rate of 60 ± 2 l min^{-1}, a Rotahaler® was fitted into the moulded rubber mouthpiece attached to the throat of the impinger. A capsule was inserted into the inhaler device and after the dose was released, the pump was switched on and allowed to run for 5 s at 60 l min^{-1}. The capsule shell was then removed from the inhaler device and the deposition test was repeated so that two more capsules were actuated in the same manner.

Once the three doses had been released, stage five was dismantled and the filter paper carefully washed with the HPLC mobile phase. The washing solution was then made up to a fixed volume (50 ml) with the same solvent for assay. The capsule shells and inhaler device were washed with the same solvent and made up to the required volume (50 ml). Each stage of the impinger was washed individually in a similar manner. The concentration of salbutamol sulphate in each of the samples was analysed using the HPLC method outlined above.

Deposited 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 drug recovered from the inhaler device, throat piece and all five stages of the impinger, whilst the emitted dose (ED) was the dose emitted from the inhaler device. Fine particle dose (FPD) was the sum of the amount of drug recovered from stages three, four and five and the fine particle fraction (FPF) was calculated as the ratio of the FPD to RD. The percent recovery was calculated as the ratio of RD to the theoretical dose and the percent emission was defined as the ratio of ED to RD.

3. Results and discussion

3.1. Particle size and morphology of lactose and salbutamol sulphate

The salbutamol sulphate supplied by the manufacturer was shown to have a volume median diameter (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 subsequently remicronised. The VMD of the remicronised salbutamol sulphate was found to be 2.75 μm when measured by laser diffraction, and 1.24 μm when measured by time-of-flight. No particles above 10 μm were observed in either technique. It is interesting to note that the VMD of the same sample was smaller when determined by time-of-flight than by laser diffraction and this discrepancy is probably due to the different principles employed in these methods. The Malvern instrument calculates particle size from the scattering angle of a laser beam induced by the particles dispersed in a liquid (as employed in the present study, although the particles may also be dispersed in a gaseous medium) whereas the Aerosizer measures the size of a particle based upon the time required to travel in an air stream across

a fixed distance. Both methods, however, confirmed that the remicronised salbutamol sulphate was of a suitable size to be used in dry powder inhaler formulations.

Tables 1 and 2 summarise the data obtained for the VMD, geometric standard deviation (GSD) and concentration of fine particles (< 5 and 10 μm) for each sieved fraction of lactose determined by laser diffraction and time-of-flight sizing techniques, respectively. It can be seen that different grades of lactose had different particle sizes and size distributions although they had been subjected to a similar sieving process. For example, the VMD of anhydrous lactose determined by laser diffraction (Table 1) was smaller than that of medium lactose (ANOVA $P < 0.01$), which in turn was smaller than either regular lactose or lactose crystals ($P < 0.01$). Of all these grades, foremost lactose showed the highest VMD. Anhydrous lactose also exhibited a higher GSD than the all the other grades of lactose, suggesting that the former had a broader particle size distribution than the latter. Anhydrous lactose was shown to have more than twice the concentration of fine particles (< 5 or 10 μm) than the other grades of lactose, which had a similar content of fine particles. Compared to the remaining grades of lactose, medium lactose had a smaller VMD and GSD.

The time-of-flight results (Table 2) also showed that anhydrous lactose possessed the lowest VMD and the highest GSD values of all the grades of lactose investigated. The concentration of fine particles (< 10 μm) of anhydrous lactose was over five times higher than the other grades of lactose, which exhibited similar VMD, GSD and fine lactose concentrations. These results showed similar trends to those obtained with the Malvern. However, in comparison to the results obtained with the Aerosizer, the concentration of fine lactose was consistently higher when measured using the Malvern. This can be explained by the fact that for the laser diffraction analysis, the sample was suspended in a liquid, which is likely to disperse particle aggregates more completely than the air stream generated by the Aerosizer.

Table 3 shows again that despite the same sieving procedure being employed, there were no-

Table 1
VMD, GSD and the percentage of fine particles for different grades of lactose measured by a laser diffraction technique after suspension in liquid[a]

Lactose (63–90 μm)	VMD (μm)	GSD	Percent < 10 μm (w/w)	Percent < 5 μm (w/w)
Anhydrous lactose	70.27 ± 1.76	1.57 ± 0.03	8.3 ± 1.1	4.8 ± 0.6
Regular lactose	90.81 ± 3.01	1.52 ± 0.03	3.4 ± 0.7	2.6 ± 0.4
Medium lactose	84.03 ± 1.75	1.48 ± 0.02	3.8 ± 1.1	2.3 ± 0.3
Lactose crystals	90.95 ± 0.96	1.52 ± 0.03	4.3 ± 0.5	2.7 ± 0.6
Foremost lactose	96.65 ± 1.12	1.52 ± 0.00	3.1 ± 0.2	2.3 ± 0.0

[a] Mean ± S.D., $n = 3$.

Table 2
VMD, GSD and the percentage of fine particles for different grades of lactose measured by a time-of-flight technique after aerosolisation in air[a]

Lactose (63–90 μm)	VMD (μm)	GSD	Percent < 10 μm (w/w)	Percent < 5 μm (w/w)
Anhydrous lactose	56.08 ± 0.43	1.30 ± 0.01	0.16 ± 0.05	0.05 ± 0.02
Regular lactose	60.88 ± 0.31	1.28 ± 0.03	0.03 ± 0.04	0.02 ± 0.02
Medium lactose	59.26 ± 0.27	1.29 ± 0.01	0.02 ± 0.00	0.01 ± 0.00
Lactose crystals	63.75 ± 0.21	1.26 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Foremost lactose	59.14 ± 0.86	1.27 ± 0.01	0.04 ± 0.01	0.01 ± 0.00

[a] Mean ± S.D., $n = 3$.

Table 3
The surface-volume mean diameter, roundness and elongation ratio of different grades of lactose measured by an optical microscopy image analysis ($n = 400$)

Carrier (63–90 μm)	Diameter (μm)	Roundness	Elongation ratio
Anhydrous lactose	66.84	1.34	1.43
Regular lactose	169.71	1.36	1.69
Medium lactose	96.74	1.34	1.59
Lactose Crystals	106.12	1.44	1.69
Foremost lactose	123.58	1.35	1.61

ticeable differences in the particle size of the various lactose fractions. Again, anhydrous lactose was found to have the smallest diameter followed by medium lactose. These confirm the results obtained using the laser diffraction technique. The diameter of foremost lactose particles determined by microscopy was found to be almost two times higher than anhydrous lactose. Regular lactose showed the highest diameter, determined by microscopy, which was approximately three times that of anhydrous lactose.

Different values for the roundness and elongation ratio were also obtained for the various grades of lactose (Table 3). Roundness, as defined in Eq. (1), is a factor which combines both geometric shape and surface smoothness. A sphere, represented by a two-dimensional circle, with surface asperities that are below the level of discrimination of the employed method will have a roundness value of 1. A sphere with rough surfaces and particles of any other shape will possess roundness values > 1. Thus, the higher roundness value of lactose crystals is indicative of either a more irregular shape or a rougher surface of the particular grade of lactose in comparison to other grades. The elongation ratio is defined as the length expressed as a function of the width and hence, spheres and perfect cubes have a ratio of 1.0. The higher the elongation ratio the more elongated and/or irregular the shape. Thus, regular lactose and lactose crystals were more elongated than medium and foremost lactose, which were in turn more elongated than anhydrous lactose. However, it has to be acknowledged that the two-dimensional shape descriptors obtained in the present study may not represent the actual three-dimensional morphological features of these crystals, which may be best shown by the SE micrographs (Fig. 1).

It can be seen from Fig. 1 that anhydrous lactose has a distinctively different shape from the other lactose grades employed, the former being rounder in shape in comparison to the latter. Further, the SE micrographs clearly show that Anhydrous lactose has more fine particles than the other lactose. Medium, foremost, regular and lactose crystals were shown to have a shape of a tomahawk, which is the shape of lactose crystal allowed to grow to maturity (Van Kreveld and Michaels, 1965). However, there are still slight differences in the morphological features of each of these latter lactose grades. For example, medium lactose appeared to have the smoothest surface whilst lactose crystals had the most pores or cavities on the particle surface.

The presence of a higher amount of fine particles in the sieved fraction of anhydrous lactose in comparison to that of the other grades of lactose could be due to different reasons. Anhydrous lactose powder as supplied was shown to have a smaller VMD and higher concentrations of fine particles (Table 4). The various grades of lactose before sieving differed greatly in particle size and size distribution: the VMD decreased in the order lactose crystals > regular lactose > foremost lactose > anhydrous lactose > medium lactose with the concentration of fine lactose particles (< 10 μm) following the reverse order. Therefore, compared to other grades but excepting medium lactose, anhydrous lactose might be expected to require a longer period of sieving in order to remove fine particles. Since the same sieving conditions were employed in the present study for each grade of lactose, the final fraction of anhydrous lactose powder might have been predicted to contain a higher concentration of fine lactose than other grades of lactose. The smaller VMD and the greater number of fine particles remained in the sieved fraction of anhydrous lactose in

comparison to medium lactose which was subjected to the same processing conditions may have been due to the difference in the physical properties of anhydrous lactose and α-lactose monohydrate. For example, anhydrous lactose is known to be more brittle than α-lactose monohydrate (Wong et al., 1988), due to the removal of water of crystallisation leading to the partial disruption

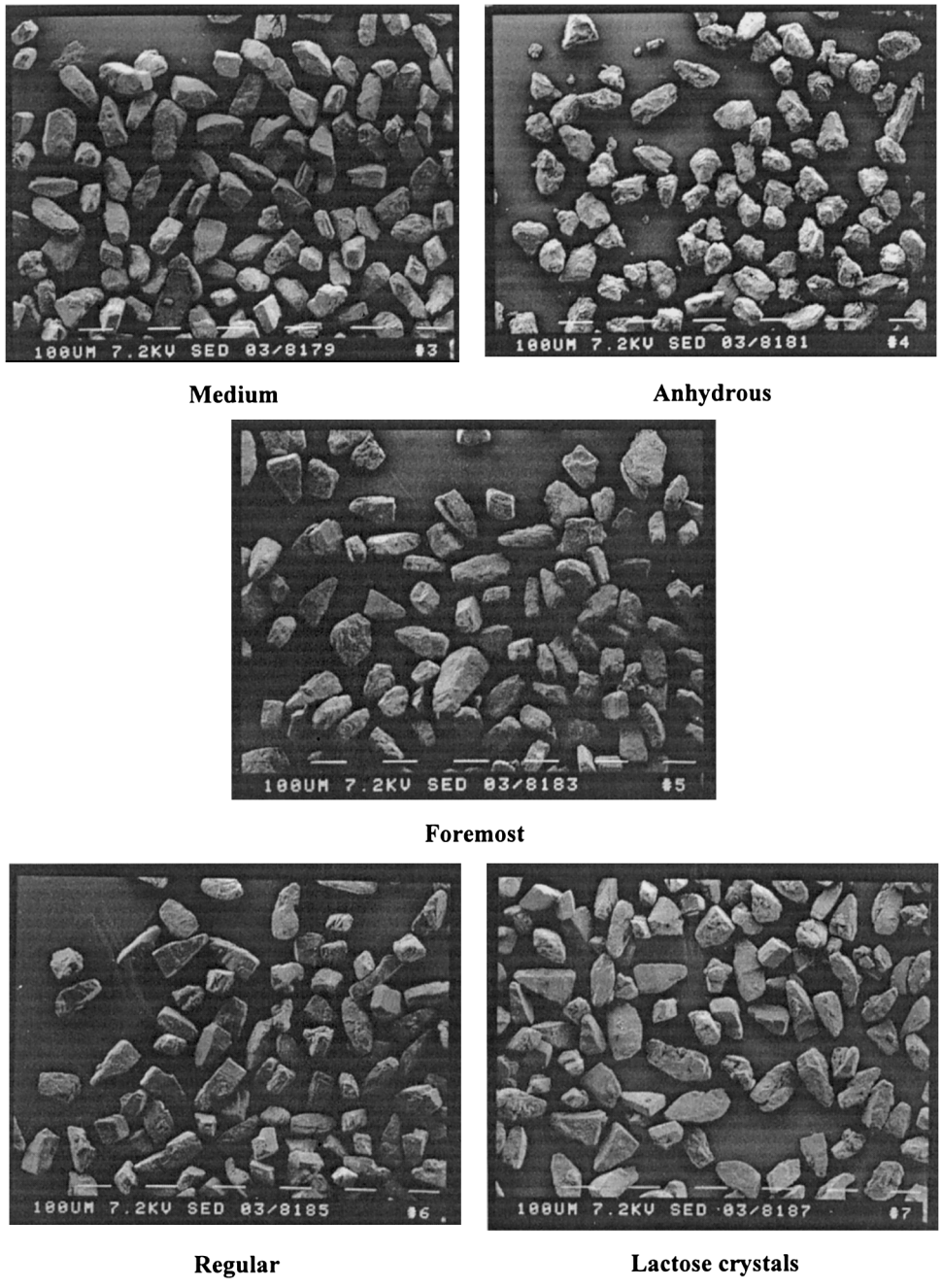

Fig. 1. The SE micrographs of different grades of lactose.

Table 4
VMD, GSD and the percentage of fine particles for the original grades of lactose measured by laser diffraction[a]

Lactose (63–90 μm)	VMD (μm)	GSD	Percent < 10 μm (w/w)	Percent < 5 μm (w/w)
Anhydrous lactose	38.38 ± 0.76	3.81 ± 0.04	23.6 ± 0.2	12.1 ± 0.5
Regular lactose	77.72 ± 1.50	2.97 ± 0.00	11.2 ± 0.2	4.7 ± 0.4
Medium lactose	34.59 ± 0.76	2.97 ± 0.08	18.1 ± 0.5	7.2 ± 0.4
Lactose Crystals	110.13 ± 1.73	2.29 ± 0.19	8.2 ± 1.0	3.9 ± 0.4
Foremost lactose	60.19 ± 1.35	3.38 ± 0.06	15.6 ± 0.6	7.4 ± 0.5

[a] Mean ± S.D., $n = 3$.

of crystalline order. During air-jet sieving, particle friction and collision may be more likely to induce fragmentation of anhydrous lactose than medium lactose and this phenomenon may also have contributed partly to the higher concentration of fine particles found in the sieved fraction of Anhydrous lactose. Medium lactose had a smaller VMD and a higher concentration of fine lactose than regular lactose, foremost lactose and lactose crystals. Yet, the sieved fraction of medium lactose had a similar VMD and concentrations of fine particles (Tables 1 and 2) to the other three grades of lactose.

3.2. Characterisation of the polymorphic forms of lactose

Medium lactose, regular lactose and lactose crystals exhibited a DSC thermogram (Fig. 2), which is typical of α-lactose monohydrate. The endothermic peak at around 150°C corresponds to the loss of water of crystallisation whilst the endotherm commencing at about 200°C is due to the melting of α-lactose monohydrate (Lerk et al., 1984a,b). Foremost lactose showed a slightly higher peak temperature of dehydration with a melting endotherm slightly different from that of

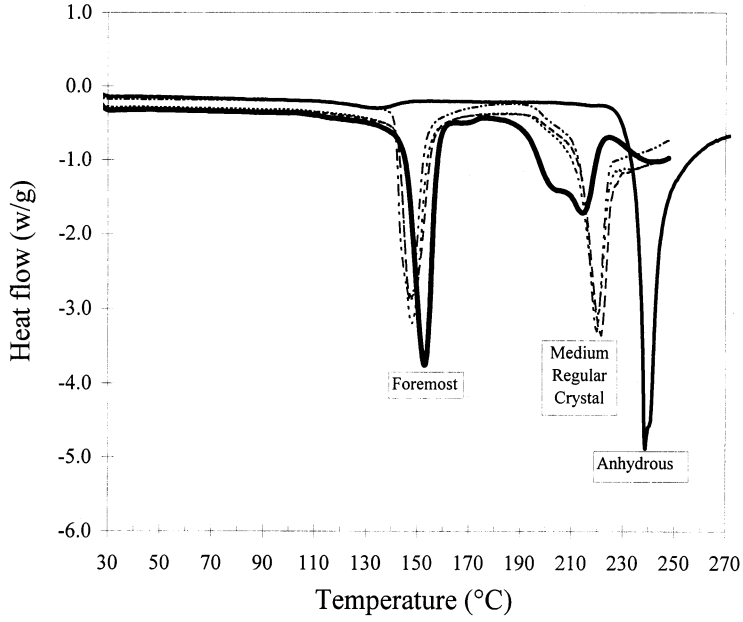

Fig. 2. The DSC thermograms of different grades of lactose.

α-lactose monohydrate (Fig. 2), the reason of which is not clear. The anhydrous lactose showed a small dehydration endotherm at around 120°C and a melting endothermic peak at 240°C, which is also typical of stable anhydrous lactose (Lerk et al., 1984b). All grades of lactose did not show any dehydration endotherms below 100°C, suggesting that they contain a negligible amount of free water which would have produced an endothermic peak at around 100°C on the DSC thermograms.

The TGA thermograms of these grades of lactose (Fig. 3) showed that no weight loss occurred below 100°C. This further confirmed the results obtained by DSC that which showed none of the lactose contained a significant quantity of free water. The weight loss between 105 and 125°C observed for lactose crystals, medium and regular lactose is due to dehydration of the water of crystallisation. It has to be noted that the dehydration temperature measured by TGA was lower than that obtained by DSC since an open pan was employed in the former measurement in contrast to a sealed pan in the latter. The partial pressure of water in the sealed pan of DSC measurement would be expected to be higher than in the open pan of the TGA measurement and hence, a higher temperature is required for the vaporisation of crystalline water in the DSC cell than in the TGA cell. Similar to the results obtained by DSC, foremost lactose was shown to dehydrate at higher temperatures when compared to lactose crystals, Regular and medium lactose. Anhydrous lactose showed only a small weight loss at around 100°C, confirming that this grade of lactose is mainly in the anhydrous form.

3.3. Content uniformity

Fig. 4 shows the percentage recoveries and coefficient of variations (%CV) of salbutamol sulphate obtained for each formulation. It can be seen that all formulations showed a recovery of salbutamol sulphate over 90% and a satisfactory mixing uniformity of the drug with %CV less than 3.5%. Slight differences were found in the drug recovery and mixing uniformity from the different formulations. For example, the formulation containing anhydrous lactose resulted in a lower percent recovery than other grades of lactose. This was initially thought to be due to water uptake by this lactose upon exposure to the ambient atmosphere during mixing and sampling. However, an analysis of water sorption of anhydrous lactose at ambient relative humidity failed to detect any significant water uptake over 3 h (data not shown). The lower drug recovery from samples taken from the formulation containing anhydrous lactose could be caused by detachment of the drug from adhering sites on the surface of carrier dur-

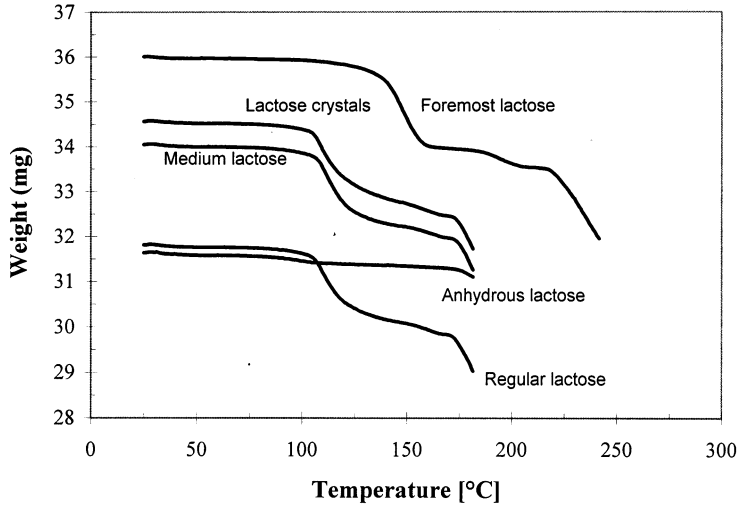

Fig. 3. The TGA thermograms of different grades of lactose.

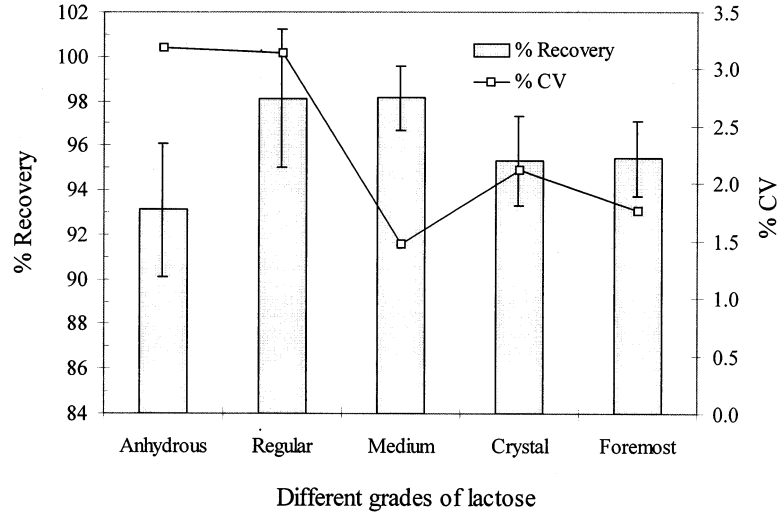

Fig. 4. Content uniformity (mean percent recovery ± S.D.) and mixing homogeneity (%CV) of salbutamol sulphate in different powder blends.

ing mixing and storage, leading to a substantial amount of drug becoming adhered to the surface of the glass containers. A fine layer of powder was observed to adhere on the inner walls of the container in which the salbutamol sulphate was blended with anhydrous lactose. This powder would be expected to be mainly composed of salbutamol sulphate since it was not seen in the container with the anhydrous lactose alone.

3.4. The deposition profiles of salbutamol sulphate from different powder formulations

Powder formulations containing different grades of lactose as the carrier were shown to produce dissimilar deposition profiles of salbutamol sulphate (Tables 5 and 6). The RD of salbutamol sulphate varied from 375 μg for formulations containing lactose crystals to 427 μg for those containing anhydrous lactose, corresponding to a percentage recovery of between 81.8 and 95.3%. The ED of the drug ranged from 258 μg for the formulations containing lactose crystals to 339 μg for those composed of anhydrous lactose, corresponding to a percent emission between 67 and 79%.

Blends containing anhydrous lactose produced an FPD and FPF, which were more than twice the respective values of the formulations containing lactose crystals, regular lactose and foremost lactose (Tables 5 and 6). The drug dispersibility is also higher ($P < 0.01$) from anhydrous lactose than from lactose crystals, regular and foremost lactose. The FPF and FPD of salbutamol sul-

Table 5
RD, ED and FPD of salbutamol sulphate using different grades of lactose[a]

Lactose (63–90 μm)	RD	ED	FPD
Anhydrous lactose	427.27 ± 3.57	339.12 ± 15.67	57.16 ± 17.64
Regular lactose	397.64 ± 37.72	293.31 ± 34.23	22.07 ± 5.52
Medium lactose	407.57 ± 16.20	303.91 ± 27.39	32.44 ± 11.79
Lactose Crystals	375.40 ± 77.27	258.42 ± 70.41	14.99 ± 5.30
Foremost lactose	426.25 ± 26.93	287.18 ± 38.39	22.44 ± 11.79

[a] Mean ± S.D., $n = 3$.

Table 6
Fine particle fraction, dispersibility, percentage recovery and percentage emission of salbutamol sulphate using different grades of lactose[a]

Lactose (63–90 μm)	FPF	Dispersibility	Recovery (%)	Emission (%)
Anhydrous lactose	13.4 ± 4.2	16.7 ± 4.5	95.3 ± 0.8	79.4 ± 4.1
Regular lactose	5.5 ± 0.9	7.5 ± 1.4	84.1 ± 7.9	73.7 ± 4.4
Medium lactose	7.9 ± 2.7	10.6 ± 3.5	86.2 ± 3.4	74.5 ± 5.3
Lactose Crystals	3.9 ± 0.8	5.7 ± 0.5	81.8 ± 16.8	78.3 ± 7.7
Foremost lactose	5.2 ± 1.7	7.8 ± 2.5	92.7 ± 5.8	67.1 ± 4.8

[a] Mean ± S.D., $n = 3$.

phate from medium lactose were significantly higher ($P < 0.05$) than the corresponding values for the formulations containing lactose crystals. Formulations containing either regular or foremost lactose produced similar deposition profiles of the drug in terms of both FPF and FPD as well as the drug dispersibility.

Fig. 5 shows the aerodynamic particle size distribution of aerosolised salbutamol sulphate from different powder formulations. Formulations containing anhydrous lactose produced significantly higher ($P < 0.01$) fractions of fine particles (either < 3.1 or < 6.8 μm) than the formulations containing regular lactose or foremost lactose or lactose crystals. However, there was no significant difference ($P > 0.05$) in the fractions of fine particles (< 6.8 μm) from the formulations containing anhydrous and medium lactose although the former produced significantly higher cumulative percent fine particles (< 12 μm) of the drug than the latter. Lactose crystals resulted in the lowest fractions of fine salbutamol sulphate whilst foremost and regular lactose appeared to produce a similar particle size distribution of the aerosolised drug.

The more efficient delivery of salbutamol sulphate from anhydrous lactose may be largely attributable to the higher concentration of fine carrier present in the powder. Addition of micro-

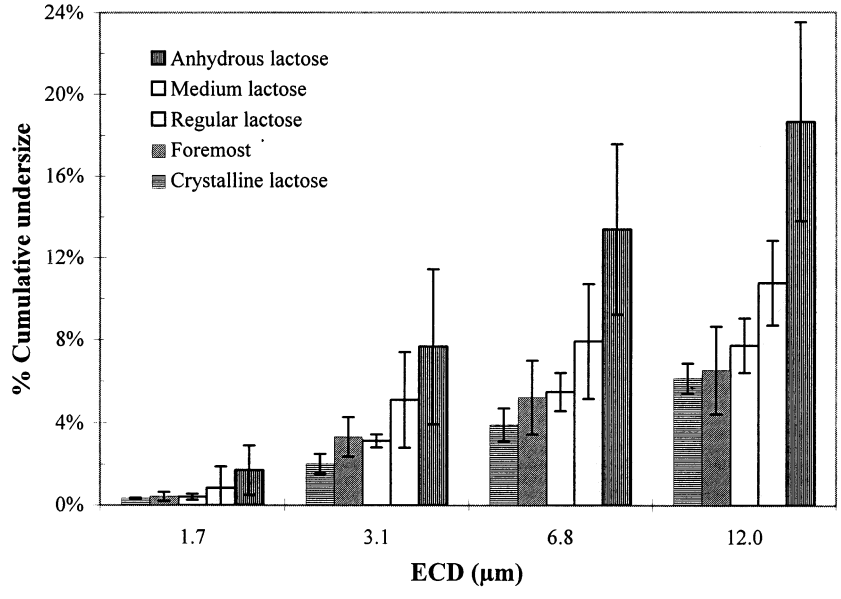

Fig. 5. Aerodynamic particle size distribution of salbutamol sulphate after aerosolisation at 60 l min^{-1} from a Rotahaler (Error bars denote S.D., $n = 4$).

nised lactose to powder formulations employing coarse lactose as the carrier has been previously shown to improve the dispersion and deaggregation of salbutamol sulphate (Zeng et al., 1998). The fine particles of lactose are thought to reduce the drug-carrier interaction by occupying possible drug binding sites on the larger lactose particles. The formation of multiplets between fine lactose and drug may also occur in the presence of excess fine particles, thereby hindering direct contact between the drug and the coarse carrier thus promoting drug particle detachment from the carrier surface during aerosolisation, leading to more drug particles being aerosolised.

The relatively high FPF of salbutamol sulphate from medium lactose may have been primarily due to the small VMD and a smooth surface of this grade of lactose as compared with lactose crystals, regular and foremost lactose. Either increasing surface smoothness or reducing the particle size of lactose has been reported to improve the dispersion and deaggregation of salbutamol sulphate (Ganderton, 1992; Ganderton and Kassem, 1992). The existence of surface pores or cavities on lactose crystals may have resulted in a relatively low FPF and FPD of salbutamol sulphate from this grade of lactose. After blending with lactose crystals, a portion of the drug particles may have been entrapped or incorporated into these surface asperities and the mechanically entrapped drug particles may not be available for dispersion and deaggregation induced by the inhaled air stream, resulting in a reduced fraction of aerosolised drug. Higher turbulence might detach these particles but it would only be produced at flow rates that patients could not be expected to achieve.

4. Conclusions

Lactose was characterised in terms of particle size, size distribution, morphological features and polymorphic forms. Different grades of lactose were shown to differ in these physical properties which in turn resulted in dissimilar dispersion properties of salbutamol sulphate incorporated as a powder blend. Anhydrous lactose contained the highest concentration of fine lactose and after blending with salbutamol sulphate, it produced a more efficient delivery of the drug than the other grades of the lactose. Medium lactose, due to its relatively small diameter and smooth surface, induced a better dispersion of salbutamol sulphate than lactose crystals, the latter producing the lowest fine particle fraction of the drug. Foremost lactose and regular lactose appeared to render a similar dispersibility of salbutamol sulphate. Since different grades of lactose are likely to produce varying delivery profiles of inhaled drug, the control of particle morphology and particle size distribution of the carrier may be crucial in determining the reproducibility and efficiency of drug delivery to the respiratory tract from dry powder inhaler formulations.

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