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Effects of particle size and adding sequence of fine lactose on the deposition of salbutamol sulphate from a dry powder formulation

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Abstract

Ternary mixtures composed of coarse lactose (CL) (90.8 μm), salbutamol sulphate (SS) (5.8 μm) and either micronised lactose (ML) (5 μm) or intermediate sized lactose (IML) (15.9 μm) in a ratio of 66.5:1:1 w/w were prepared using different mixing sequences of the various components. In addition, a binary mixture composed of CL and SS (67.5:1 w/w) was also prepared as the control. The in vitro deposition of SS was measured using a twin stage impinger after aerosolisation at 60 and 90 l min^{-1} via a Rotahaler®. The aerodynamic particle size distribution of both the aerosolised SS and lactose was further analysed using an Andersen cascade impactor at 60 l min^{-1}. All ternary mixtures produced a significantly higher (analysis of variance, $P < 0.01$) fine particle fraction (FPF) and fine particle dose (FPD) of SS than the control after aerosolisation at either 60 or 90 l min^{-1}. Formulations containing the ML produced significantly ($P < 0.05$) higher FPF and FPD of SS than those containing the IML at both aerosolisation flow rates. Different mixing sequences were also shown to result in different deposition profiles of both SS and lactose after aerosolisation of the ternary mixtures containing ML at 60 l min^{-1}. The formulation prepared by first blending ML with CL before mixing with SS produced a higher FPF and FPD of SS but a lower FPF of lactose than the same formulation in terms of composition but prepared using different mixing orders of the three components. In contrast, the formulations containing IML produced a similar deposition profile to SS, regardless of the mixing sequences, and so did the formulations containing ML aerosolised at 90 l min^{-1}. These results suggest that the effect of mixing sequences on drug deposition may become more prominent at lower aerosolisation flow rates and by reducing the size of any added fine lactose.

Keywords: Dry powder inhalers; Lactose; Salbutamol sulphate; Ternary mixes; Dispersion; Deposition

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1. Introduction

All dry powder inhalers (DPIs) are composed of an inhaler device and a powder formulation. It is generally accepted that for deep lung penetration the drug should have an aerodynamic diameter between 1 and 5 μm (Newman and Clarke, 1983; Gonda, 1990). The powder formulation must also flow sufficiently well either to be dispensed from a bulk reservoir to give a reproducible dose, or be capable of being handled well on automatic filling machines to produce a unit dose for use in the device. Since the drug particles are highly cohesive and have poor flow properties, they are usually blended with coarser carrier particles (Ganderton, 1992), where the drug is thought to adhere to the carrier to form an ordered mix (Hersey, 1975). The drug is often present in low concentrations in the powder formulation, with a drug to carrier ratio of 1:67.5 w/w being typical. After inhalation, the carrier should ideally be retained in the inhaler device or deposit in the oropharyngeal region due to its large particle size (e.g. 63–90 μm). Therefore, drug detachment from the carrier is thought to be crucial in determining the overall delivery efficiency of drugs from dry powder aerosols (Ganderton and Kassem, 1992).

A number of previous studies have reported improvements which can be made to the amount of respirable drug delivered from a DPI by means of manipulating the powder formulation and these include smoothing the carrier surface (Ganderton, 1992), reducing the particle size of the carrier (Ganderton and Kassem, 1992; French et al., 1996; Steckel and Müller, 1997), and the use of ternary materials in the powder formulation (Staniforth, 1996). Addition of micronised lactose to powders employing coarse lactose as carrier was also found to improve the dispersion and deaggregation of both salbutamol sulphate (Zeng et al., 1996) and spray dried bovine serum albumin (Lucas et al., 1998). The use of fine carrier particles to improve drug delivery is likely to be preferential to the use of ternary materials, since the latter will require toxicological testing. Thus, ternary mixtures composed of coarse lactose, fine lactose and the drug may be more efficient at delivering drug to the lower airway than the binary mixtures containing only coarse lactose and drug in drug delivery. However, at least two potential problems are associated with the addition of fine lactose to powder formulations. First, fine lactose may result in poorer flow properties of the powder, which is one of the primary reasons for incorporating coarse carrier particles within the formulation. Second, on inhalation, some of the added fine lactose may be capable of penetrating into the peripheral airways, which is undesirable, especially for patients who are intolerant to lactose. Therefore, the ternary mixture should be carefully formulated in order to improve the efficiency of drug delivery to the lower airways without substantially affecting the flow properties of the formulation or introducing excessive fine lactose to the lung. We have previously reported that although the fine particle fraction (FPF) of salbutamol sulphate increased with increasing concentration up to 9% w/w added fine lactose (15.9 μm), the greatest stepped increase in the drug FPF occurred when the concentrations of the added fine lactose was increased from 0 to 1.5% w/w (Zeng et al., 1998). The aim of the present study was to investigate the effects of particle size of added fine lactose and mixing sequence of the three components (coarse lactose, fine lactose and salbutamol sulphate) of the ternary mixture on the in vitro deposition of the drug after aerosolisation at 60 and 90 l min^{-1}. The deposition profiles of lactose from the ternary mixtures were also studied.

2. Materials and methods

Salbutamol sulphate (MMD) (5.8 μm of GSD 1.7), Ventolin Rotahaler® and gelatin capsules (size 3) were supplied by Glaxo-Wellcome Research and Development, Ware, UK. α-Lactose monohydrate (Lactochem®) was obtained from Borculo Whey, Chester, UK. D-Glucose monohydrate was obtained from Fisons Scientific Equipment, Loughborough, UK. *p*-Hydroxybenzoic acid ethyl ester was purchased from Sigma Chemical, Poole, UK, while ammonium acetate, methanol of high-performance liquid chromatog-

raphy grade and butan-1-ol of reagent grade were obtained from BDH Laboratory Supplies, Poole, UK.

2.1. Preparation of coarse lactose (63–90 μm)

Lactochem® lactose (100 g) was sieved for 15 min using a sieve shaker (Endecotts, London, UK) through a test sieve with an aperture width of 90 μm (Endecotts) which was placed over a test sieve with an aperture width of 63 μm (Endecotts). The particles collected on the 63 μm sieve were treated with an air stream with a flow rate of 160 l min^{-1} via a nozzle (i.d. ~ 1 cm) from a distance of approximately 15 cm above the powder. The compressed air treatment was continued such that the concentration of fine lactose (i.e. particles < 20 μm) reached a constant level, as monitored by laser diffraction particle size measurement. The lactose thus prepared was then placed in a desiccator over silica gel until further required.

2.2. Preparation of micronised lactose and intermediate sized lactose

Lactochem® lactose (< 63 μm) was subjected to up to nine passes through a jet mill (JM-80; M&M Fryma, UK) operated at an air pressure of 15 bar. The particles collected after one passage through the jet mill were termed 'intermediate-sized lactose' (IML) and those collected after nine passages were termed 'micronised lactose' (ML). All the milled particles were placed in a desiccator over silica gel until further required.

2.3. Preparation of powder formulations

All powder mixing was carried out using a Turbula® mixer (Glen Creston, Stanmore, UK) for 30 min.

Six formulations were prepared, each containing coarse lactose, ML (or IML) and salbutamol sulphate in a ratio of 66.1:1:1 w/w, using different sequences of powder component addition (Table 1). The first two components (Table 1) were mixed for 30 min using a Turbular® mixer before addition of the third component and blending for a further 30 min. A formulation composed of only coarse lactose and salbutamol sulphate in a ratio of 67.1:1 w/w was also prepared as the control.

All blends containing salbutamol sulphate were filled in hard gelatin capsules (size 3) manually such that each capsule contained 32 ± 2 mg of the powder.

2.4. Particle size measurement by laser diffraction

A small amount of lactose powder (about 5 mg) was dispersed in 5 ml butan-1-ol with the aid of sonication (Sonic water bath-Model F5100b; Decon Laboratories, Hove, UK) for 1 min. The particle size was measured by laser diffraction (Series 2600c; Malvern Instruments, Malvern, UK) using an independent particle size model and obscuration between 0.16 and 0.18. Each sample was measured at least six times.

Table 1
Mixing sequences employed to prepare the ternary ordered mixtures composed of salbutamol sulphate, coarse and fine lactose

Formulations	Mixing sequences	
	Initial blend[a]	Final component[b]
Control	Salbutamol sulphate + coarse lactose	
A	Coarse lactose + ML[c]	+ Salbutamol sulphate
B	Salbutamol sulphate + coarse lactose	+ ML[c]
C	Salbutamol sulphate + ML[c]	+ Coarse lactose
A′	Coarse lactose + IML[d]	+ Salbutamol sulphate
B′	Salbutamol sulphate + coarse lactose	+ IML[d]
C′	Salbutamol sulphate + IML[d]	+ Coarse lactose

[a] Components mixed for 30 min.
[b] Final component mixed with initial blend for further 30 min.
[c] Micronised lactose.
[d] Intermediate sized lactose.

2.5. High-performance liquid chromatography analysis of salbutamol sulphate

Salbutamol sulphate was analysed by high-performance liquid chromatography (HPLC) employing a mixture of methanol and 0.1% w/w aqueous ammonium acetate (45:55, pH 4.5) as a mobile phase running at a flow rate of 0.8 ml min^{-1}, *p*-hydroxybenzoic acid ethyl ester (2 μg ml^{-1}) as an internal standard and UV detection at 276 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 15 cm (4.6 mm i.d.) column packed with 5 μm C-18 (Hypersil, Phenomenex, UK). The retention times for salbutamol sulphate and the internal standard were 2.71 and 5.49 min, respectively.

2.6. HPLC analysis of lactose

Lactose was analysed by HPLC employing a mixture of acetonitrile and water (75:25) as the mobile phase and d-glucose monohydrate as internal standard. The HPLC system consisted of a pump (CM 4000 Multiple Solvent Delivery System; LDC Analytical), an amino column (Apex; Jones Chromatography, UK) controlled at a temperature of 29°C and a refractive index detector (ERC-7512; Erma, Japan). The mobile phase was allowed to run at a flow rate of 0.8 ml min^{-1}. The retention times for lactose and the internal standard were 3.80 and 2.85 min, respectively.

2.7. Deposition tests of salbutamol sulphate

Deposition was determined using a twin stage liquid impinger (TI, Apparatus A, British Pharmacopoeia, 1993) after aerosolisation of five capsules, each containing a nominal dose of 32 ± 2 mg powder, equivalent to 480 ± 29 μg salbutamol sulphate, at 60 or 90 l min^{-1}, via a Rotahaler®. The aerodynamic particle size distribution of salbutamol sulphate from different formulations was also measured using an Andersen cascade impactor (ACI) after aerosolisation at 60 l min^{-1} via a Rotahaler®.

In the TI test, 7 ml of the mobile phase containing 2 μg ml^{-1} *p*-hydroxybenzoic acid ethyl ester was introduced in stage 1 and 30 ml of the same solvent in stage 2 of the TI. The capsule to be tested was placed in a Rotahaler®, which had been fitted into a moulded rubber mouthpiece attached to the throat piece of the impinger. Once the assembly had been checked and found to be airtight and vertical, the vacuum pump was switched on. After the pump had run for 5 s, the dose was released. The pump was allowed to run for another 7 s at 60 ± 2 l min^{-1} (or 90 ± 2 l min^{-1}) following the release of the dose and it was then switched off. The capsule shells were removed from the inhaler device and the deposition test was repeated until five capsules had been actuated in the same manner. The inhaler body, capsule shells and mouthpiece were washed five times with the mobile phase containing internal standard and the washing solution was made up to 100 ml with the same solvent. The sample thus obtained was used to measure the amount of drug retained in the inhaler device. The same process was carried out for both the upper and the lower stages of the twin impinger. All samples were analysed for the concentration of salbutamol sulphate using the HPLC method as described.

The ACI consists of eight stages, 0–7, and a preseparator. The impactor plates were coated with silicone oil by immersion in a 1% v/v solution of 200/1000 cS silicone oil (Hopkin and Williams, Essex, UK) in hexane and allowed to dry prior to each experiment. A filter paper (Whatman, cut-off < 0.45 μm) was placed in stage 8 of the impactor and 10 ml of the mobile phase with the internal standard was introduced into the preseparator. The impactor was then assembled and a Rotahaler® was then fitted into a moulded rubber mouthpiece attached to the throat of the impinger. A capsule was inserted into the inhaler device and, after the impactor was found to be airtight and the inhaler device lined up along the horizontal axis of the throat of the impactor, the ACI deposition test was carried out at 60 l min^{-1} under similar conditions to the TI test. Then, the inhaler body, capsule shells and mouth piece were washed five times with the mobile phase and the washing solution was made

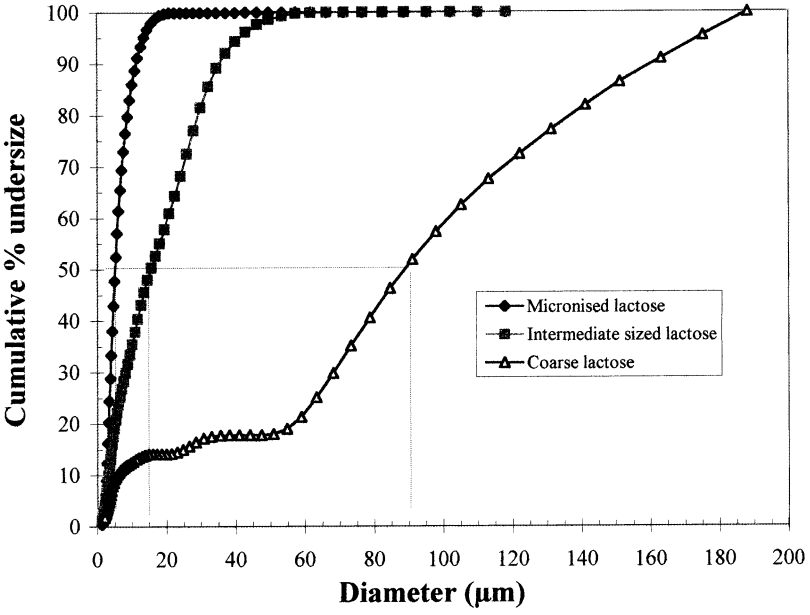

Fig. 1. Particle size distribution of different batches of lactose as measured by laser scattering.

up to 100 ml with the same solvent. Both the throat and preseparator were washed three times with the mobile phase and the separate washing solutions were made up to 100 ml with the same solvent. The impaction plates of the remaining stages of the impactor were also washed individually with the same solvent, but each washing solution was made up to 50 ml. The concentration of salbutamol sulphate in each of the samples was analysed using the HPLC method as already outlined.

Fine particle dose (FPD) was denoted as the quantity (μg) of the particles per capsule that deposited in the lower stage of the TI after aerosolisation at either 60 l min^{-1} (effective cut-off diameter (ECD) < 6.4 μm) or at 90 l min^{-1} (ECD < 7.2 μm) (Srichana et al., 1996). Each capsule contained a nominal dose of 480 ± 30 μg salbutamol sulphate. The recovered dose (RD) was taken as the total quantity of drug recovered per capsule after each actuation, while the emitted dose (ED) was that emitted from the inhaler device. Percent emission was calculated as the percentage of emitted dose to total dose. Fine particle fraction (FPF) was the ratio of FPD to RD, while dispersibility was the percentage of FPD to ED.

2.8. Particle size distribution of aerosolised lactose

Lactose deposition was determined using an ACI under similar operation conditions as those for the deposition of salbutamol sulphate except that the solvent used was the mobile phase for lactose analysis. The percentage of lactose deposited on each stage of the ACI was calculated as a fraction of the emitted dose. Each formulation was tested in triplicate.

3. Results

3.1. Particle size distribution of various batches of lactose

The particle size distribution of the various batches of lactose employed in the study is shown in Fig. 1. The coarse lactose had a volume median diameter (VMD) of 90.8 μm with a geometric

standard deviation (GSD) of 2.2 and, despite this batch of lactose having been subjected to air treatment, it still contained approximately 11% (v/v) particles < 20 μm and 7% (v/v) particles < 5 μm. The IML exhibited a VMD of 15.9 μm with GSD of 1.3 μm, and contained 60% (v/v) particles < 20 μm, 35% (v/v) particles < 10 μm and 20% (v/v) particles < 5 μm. The VMD of the ML was 5 μm (GSD of 1.8 μm) and all particles were less than 20 μm.

3.2. Deposition profiles of salbutamol sulphate at 60 l min^{-1}

The various formulations produced different FPF, FPD and dispersibility of salbutamol sulphate (SS) (analysis of variance, $P < 0.01$) as well as drug emission ($P < 0.05$) (Table 2). All formulations containing ML produced significantly higher ($P < 0.05$) FPF, FPD and dispersibility of SS than the control. These results are in agreement with those previously reported (Zeng et al., 1996), where addition of fine lactose to powder formulations was shown to improve the dispersion of SS. Of the three formulations containing the same amount of ML but prepared using different mixing sequences, formulation A produced the highest FPF, FPD and dispersibility of SS, while formulation B produced the lowest FPF, FPD and dispersibility of the drug. For example, formulation A resulted in a FPD of SS of 61.0 ± 4.6 μg, which was nearly twice that produced by formulation B. Formulation C resulted in an intermediate FPF, FPD and dispersibility of SS. The control produced a drug emission of 83.4 ± 2.4%, which was similar to those of the formulations containing IML but higher ($P < 0.05$) than those of the formulations containing ML.

The formulations containing IML, A′ & C′, produced slightly but significantly ($P < 0.05$) higher FPF and dispersibility of SS than the control, while formulation B′ resulted in a similar deposition profile of SS to the control. Mixing sequences of the mixtures composed of IML generally had insignificant ($P > 0.05$) effects on the deposition of SS, although formulation B′ produced lower FPD and FPF of the drug than those of either formulation A′ or C′. Thus, the effect of mixing order on the delivery of SS appears to be less significant for the formulations containing IML in comparison with those composed of ML. For example, formulation A resulted in an approximately 62% higher FPF of SS than B as compared with the 26% difference in drug FPF between formulations B′ and A′. Furthermore, the formulations containing ML produced significantly ($P < 0.05$) higher FPF and dispersibility of SS than the corresponding formulation containing IML although only formulation A resulted in a significantly higher ($P < 0.01$) FPD of SS than all formulations containing IML. Therefore, smaller particles of added fine lactose produced higher dispersion and deaggregation of SS.

Table 2
Deposition profiles of salbutamol sulphate in a TI after aerosolisation of different formulations at 60 l min^{-1} via a Rotahaler® (mean ± SD, $n = 3$–6)

Formulations	FPD (μg)	FPF (%)	Dispersibility (%)	Emission (%)
Control	29.8 ± 2.7	6.7 ± 0.6	8.0 ± 0.7	83.4 ± 2.4
Containing ML				
A	61.0 ± 4.6	14.7 ± 1.1	19.8 ± 1.5	74.4 ± 0.8
B	35.8 ± 2.0	9.1 ± 0.5	11.6 ± 0.6	78.4 ± 2.5
C	42.2 ± 2.4	10.7 ± 0.6	14.1 ± 0.8	76.1 ± 2.3
Containing IML				
A′	39.5 ± 3.7	9.6 ± 0.9	12.1 ± 1.1	79.5 ± 1.1
B′	31.7 ± 2.1	7.6 ± 0.5	9.4 ± 0.6	80.8 ± 2.1
C′	42.9 ± 4.0	9.7 ± 0.9	12.0 ± 1.1	80.9 ± 3.3

Table 3
Deposition profiles of salbutamol sulphate in a TI after aerosolisation of different formulations at 90 l min^{-1} via a Rotahaler® (mean ± SD, $n = 3–6$)

Formulations	FPD (μg)	FPF (%)	Dispersibility (%)	Emission (%)
Control	47.5 ± 1.3	10.6 ± 0.3	13.2 ± 0.4	80.4 ± 2.1
Containing ML				
A	96.6 ± 7.2	22.9 ± 1.7	29.9 ± 2.2	76.7 ± 0.9
B	80.1 ± 3.9	18.6 ± 0.9	23.9 ± 1.2	77.9 ± 0.7
C	83.3 ± 7.2	18.6 ± 1.6	24.0 ± 2.1	77.5 ± 0.7
Containing IML				
A′	54.4 ± 3.7	13.3 ± 0.9	16.6 ± 1.1	80.0 ± 3.1

3.3. Deposition profiles of salbutamol sulphate at 90 l min^{-1}

Different deposition profiles of salbutamol sulphate were also obtained after aerosolisation of the various formulations at 90 l min^{-1} (Table 3). Similar to aerosolisation at 60 l min^{-1}, the formulations containing either ML or IML produced FPD, FPF and dispersibility of SS, which were significantly higher ($P < 0.01$) than those of the control. No significant difference ($P > 0.05$) was observed in the percent emission of SS from all these formulations. All three formulations containing ML produced similar FPD, FPF, dispersibility and emission of SS, suggesting that the mixing order did not have a significant effect ($P > 0.05$) on the delivery of salbutamol sulphate after aerosolisation at 90 l min^{-1}.

The formulation containing IML produced FPD, FPF and dispersibility of salbutamol sulphate, which were slightly higher than the control ($P < 0.05$) but much lower than those of the formulations containing ML. These results further confirm that the particle size of the added fine lactose plays an important role in determining the deposition of salbutamol sulphate, i.e. the smaller the fine lactose, the higher the drug dispersion.

Increasing the aerosolisation flow rate from 60 to 90 l min^{-1} was shown to increase the FPF of salbutamol sulphate from all formulations. Interestingly, the FPF of SS was shown to increase to different extents after aerosolisation from different formulations. For example, the highest increase in drug FPF and FPD (124% increase in FPD) was observed for formulation B, followed by formulation C (97% increase in drug FPF). Drug FPF and FPD were shown to be the least affected by the aerosolisation flow rate from formulation A′, containing IML (about 38% increase in the drug FPF and FPD), while formulation A and the control exhibited an approximately 60% increase in drug FPD after aerosolisation from 60 to 90 l min^{-1}.

3.4. Aerodynamic particle size distribution of salbutamol sulphate from formulations prepared using different mixing orders

The particle size distribution of aerosolised salbutamol sulphate from different formulations, as measured by an ACI, is shown in Fig. 2. All formulations with added ML produced significantly higher ($P < 0.01$) fractions of fine particles (either < 3.20 or < 4.00 μm) than the control, composed of salbutamol sulphate and the coarse lactose only. Formulation A consistently produced the highest fractions of fine salbutamol sulphate. Formulation B produced significantly lower ($P < 0.05$) fractions of fine particles, between 0.76 and 2.30 μm, than formulations A and C. These results are in agreement with those obtained using a TI (Table 2). They further confirm that mixing order affects the dispersion of SS after aerosolisation at 60 l min^{-1} via a Rotahaler®.

3.5. Deposition profiles of lactose from formulations prepared using different mixing orders

Different deposition profiles of lactose were also observed for the various formulations containing ML and prepared using different mixing sequences (Fig. 3). A nominal dose of 33 ± 2 mg of the formulations containing 1.5% added ML produced less than 350 μg fine lactose that was < 6.18 μm, which amounted to approximately 1% w/w of the total lactose. The formulation containing ML, but prepared by first blending SS and lactose before mixing with ML (formulation B), produced a fraction of fine lactose < 6.18 μm almost twice as great as those of the same formulation but prepared using other mixing orders (formulations A and C). Fine lactose < 6.18 μm was not detected after aerosolisation of the control, despite approximately 8% of the composite course lactose having a particle size less than this diameter (Fig. 1).

4. Discussion

Addition of fine lactose to powder formulations was previously shown to improve the dispersion and deaggregation of salbutamol sulphate, resulting in higher FPF and FPD of the drug (Zeng et al., 1996, 1998; Lucas et al., 1998). It has been suggested by these previous workers that such an effect is partly due to the coverage of binding sites on the coarse lactose by the fine lactose. If this is so, then the mixing sequence of the various components would be expected to affect the drug dispersion by altering the particulate interactions between the drug and coarse carrier. Since formulation A was prepared by first blending the ML with the coarse lactose before mixing with SS, some of the binding sites on the coarse lactose would be either saturated or covered by ML and, hence, less of the subsequently introduced drug particles may be expected to adhere to these binding sites, leading to a greater number of drug particles being more readily detached from the carrier after inhalation when compared with formulations B and C, prepared using other orders of mixing the various components. Consequently, formulation A produced the highest potentially respirable fraction of the drug. In formulation B, SS was first blended with the coarse lactose before mixing with ML and, hence, more drug particles might be expected to adhere directly to the coarse

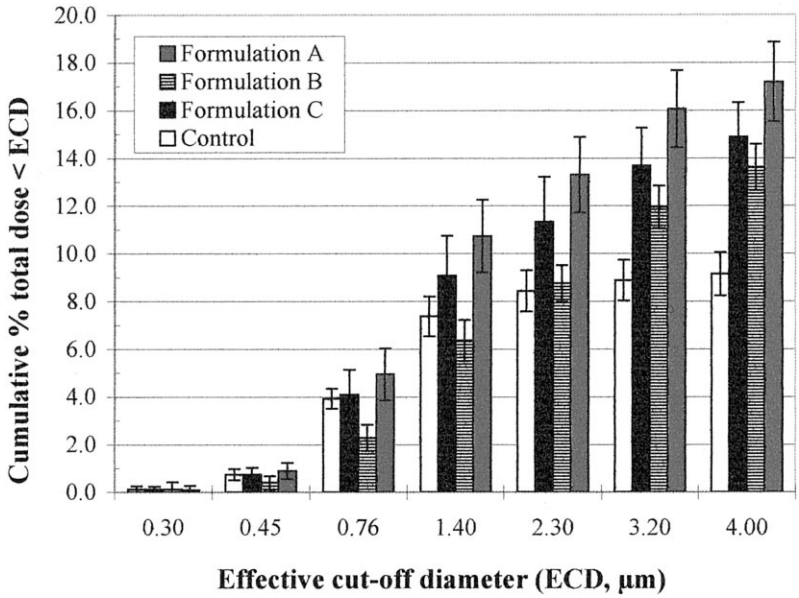

Fig. 2. Particle size distribution of salbutamol sulphate as measured by an ACI after aerosolisation at 60 l min^{-1} via a Rotahaler® (error bars denote standard deviation, $n = 5$).

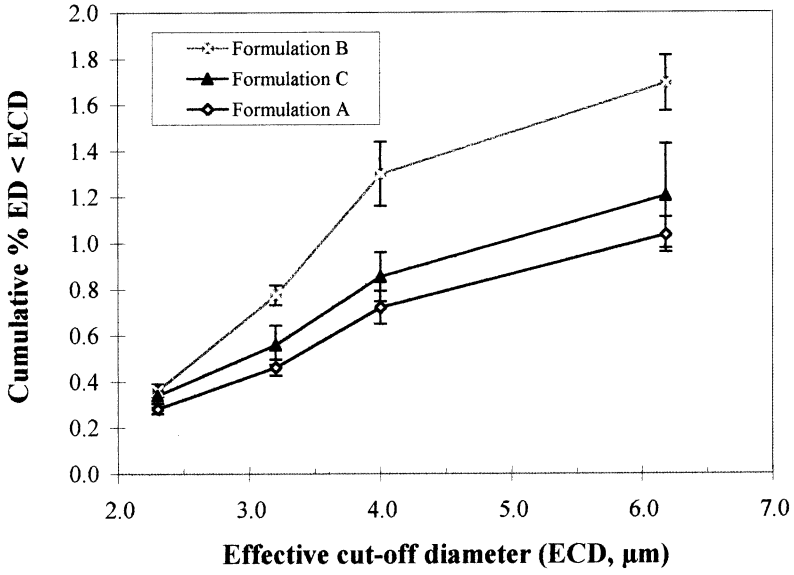

Fig. 3. Particle size distribution of lactose after aerosolisation at 60 l min^{-1} via a Rotahaler® as measured by ACI (error bar denotes standard deviation, $n = 3$–6).

lactose than in formulations A and C, and thus, the former exhibited the lowest dispersion of the drug in comparison with the latter two formulations. However, some redistribution of SS and ML at adhesion sites on the surface of the coarse lactose would appear to have occurred in formulation B during powder mixing since this formulation still produced significantly higher FPF and FPD of drug compared with the control.

The IML might be expected to adhere to the coarse lactose less tenaciously than the ML due to the larger particle size of the IML (VMD 15.9 μm) as compared with the ML (VMD 5 μm). According to Zimon (1982), the adhesion force due to van der Waals forces is directly proportional to the particle diameter (d) but Coulombic and electrical double layer forces are proportional to d^{-1} and d^{-2}, respectively. In contrast, the gravitational force acting on a particle is proportional to d^3, assuming sphericity in both cases. Thus, the ratio of the adhesion forces to the gravitational forces, the so-called apparent adhesion, increases rapidly with decreasing particle size regardless of the components of the adhesion forces, and such a ratio is thought to be the primary factor that govern the interacting states between two particles (Otsuka, 1998). Thus, the IML, with its larger particle size, may be less efficient in covering the binding sites on the coarse lactose than ML and, hence, formulations containing IML might be expected to produce a lower drug FPF and FPD than those containing ML, as seen in this study. However, IML contains approximately 35% (v/v) particles < 10 μm and it may be this portion of particles that was responsible for the higher drug dispersion observed for the formulations containing IML in comparison with the control.

The influence of aerosolisation flow rate on drug deposition is well documented (see, for example, Timsina et al., 1994). Increasing flow rate is known to improve the deaggregation and dispersion of drug particles and, therefore, all formulations were shown to produce a higher FPF and FPD after aerosolisation at 90 l min^{-1} than was achieved at 60 l min^{-1}. However, it has to be acknowledged that the comparison of drug FPF and FPD, as reported in the present study at different air flow rates, only provides a qualitative insight into the dependence of drug dispersion on air flow rate, since the effective cut-off diameter of the impinger changes as a function of flow rate. A

stricter measurement of particle size distribution of aerosolised drug at varying air flow rates might employ isokinetic sampling techniques (Kassem, 1990)

The FPF and FPD of SS is dependent upon the mixing order of the formulations containing ML but would appear to be less dependent on the order in which components are blended for formulations containing IML. Thus, increasing the size of added fine lactose reduced the significance of mixing order of the various components in the ternary mixtures. Due to the lower tendency of IML to adhere to the coarse lactose, the tendency will be for the smaller sized drug particles to occupy the sites where adhesion can occur on the coarse lactose due to the greater availability of such sites in formulations containing IML when compared with those containing ML. This will render the mixing order less significant in determining the final interacting state between the coarse lactose and SS. Although mixing order was important in determining drug dispersion after aerosolisation of the ternary mixtures containing ML at 60 l min^{-1}, the FPF and FPD of salbutamol sulphate were shown to be independent of the mixing order at an air flow rate of 90 l min^{-1}, suggesting that the energy input generated at this flow rate may have diminished the importance of differences in the particulate interaction between the drug and carrier brought about by different mixing sequences. The effect of mixing order on drug deposition may also be dependent on the design of inhaler device. Drug deposition from devices with a higher air resistance may be less dependent on the mixing order as compared with that obtained from a device with lower air resistance, since the same volumetric flow rate from the former devices will generate higher linear velocity than that from the latter. For example, mixing order of the formulations containing spray dried maltodextrin–bovine serum albumin, and added fine and coarse lactose was reported to have no significant effect on the dispersion of the drug after aerosolisation at 60 l min^{-1} from a Diskhaler® (Lucas et al., 1998). The air resistance of the Diskhaler® and Rotahaler® have been reported to be 0.067 and 0.040 (cmH_2O) l^{-1} min, respectively (Olsson and Asking, 1994). It can thus be expected that at the same volumetric flow rate (e.g. 60 l min^{-1}), the linear velocity generated in the Diskhaler® will be higher than that in the Rotahaler®. It is the linear velocity rather than the volumetric flow rate that governs the drag force and turbulence of the air stream (Baron and Willeke, 1993). The average linear velocity of the airstream in the inhaler device may be roughly estimated by dividing the volumetric flow rate by the cross-sectional area of the inhaler and, thus, a volumetric flow rate of 60 l min^{-1} will generate a linear velocity of around 4 m s^{-1} in the Diskhaler® (cross-sectional area ~ 2.5 cm^2), which is similar to that in the Rotahaler® (cross-sectional area ~ 4.0 cm^2) resulting from a volumetric flow rate of 90 l min^{-1}. Since the effect of mixing order is more prominent at low inhalation flow rates via a device with relatively low air resistance, such a strategy to improve drug delivery from DPI may be particularly beneficial for severely compromised asthmatic patients, and for children and infants.

Different deposition profiles of lactose were also observed for the various formulations. The control did not show any detectable fine particles of lactose less than 6.18 μm. Much of the fine lactose had been removed by subjecting the 63–90 μm sieved fraction to compressed air treatment; any remaining fine particles would be expected to adhere to the coarse fraction. Formulation B produced fractions of fine lactose almost twice as great as those of formulations A and C. In formulation B, salbutamol sulphate was first blended with the coarse carrier particles, while in formulation A, ML was first blended with the coarse carrier particles. Therefore, ML is likely to adhere more strongly to vacant adhesion sites on the coarse carrier in formulation A rather to the sites on the coarse lactose contained in formulation B, which will be initially covered with drug and, hence, the former formulation produced lower fractions of aerosolised fine lactose as compared with the latter. These results further support the possible mechanisms by which fine lactose affect the dispersion of salbutamol sulphate from powder blends.

5. Summary

A ternary mixture composed of salbutamol sulphate, fine and coarse lactose may be superior to the binary mix containing the drug and coarse carrier only, for a more efficient delivery of drug from dry powder aerosols. The mixing sequence of the various components of the ternary mixtures and the particle size of the fine lactose were shown to affect the FPF and FPD of the drug. Formulations prepared by first blending the coarse and fine lactose before mixing with drug produced higher FPF and FPD of salbutamol sulphate but lower fractions of fine lactose than the same formulation prepared using other orders of mixing the three components. The formulations composed of smaller-sized fine lactose generally exhibited a more efficient drug dispersion than those containing larger-sized fine lactose. However, mixing order may be more important in determining drug delivery when the formulations are aerosolised at relatively low flow rates via inhalers of low air resistance. Thus, the control of mixing order may be particularly useful strategy in the preparation of powder formulations intended to be aerosolised at low flow rates.

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Appendix A. Abbreviations

ACI	Andersen cascade impactor
CL	Coarse lactose
ECD	Effective cut-off diameter
ED	Emitted dose
FPD	Fine particle dose (also referred to as respirable dose) is the mass of drug particles <6.4 μm per capsule after tests using a twin impinger
FPF	Fine particle fraction (also referred to as respirable fraction) is the percent of drug particles <6.4 μm after tests using a twin impinger
GSD	Geometric standard deviation
IML	Intermediate sized lactose
L	Lactose
ML	Micronised lactose
SS	Salbutamol sulphate
TI	Twin stage liquid impinger
TOF	Time of flight
VMD	Volume median diameter

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