Protein Deposition from Dry Powder Inhalers: Fine Particle Multiplets as Performance Modifiers

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Purpose. To evaluate the use of carrier-based dry powder aerosols for inhalation delivery of proteins and examine the effect of fine particle excipients as potential formulation performance modifiers.

Methods. Bovine serum albumin (BSA) was co-processed with maltodextrin by spray-drying to produce model protein particles. Aerosol formulations were prepared by tumble mixing protein powders with α-lactose monohydrate (63–90 μm) or modified lactoses containing between 2.5 and 10% w/w fine particle lactose (FPL) or micronised polyethylene glycol 6000. Powder blends were characterised in terms of particle size distribution, morphology and powder flow. Formulation performance in Diskhaler® and Rotahaler® devices was investigated using a twin stage impinger operating at 60 l min^{-1}.

Results. Inhalation performance of binary ordered mixes prepared using BSA-maltodextrin and lactose (63–90 μm) was improved by addition of FPL and micronised PEG 6000. For the addition of 5% w/w FPL the protein fine particle fraction (0.5–6.4 μm) using the Diskhaler® was increased from 31.7 ± 2.4% to 47.4 ± 2.2%. Inclusion of FPL and micronised PEG 6000 changed the bulk properties of inhalation powders and reduced powder flow but did not affect device emptying. Unexpectedly, improvements in performance were found to be independent of the order of addition of FPL to the ternary powder formulations. SEM studies revealed that this was probably the result of a redistribution of protein particles between the coarse carrier lactose component and added FPL during mixing.

Conclusions. Fine particle excipients can be used to improve the performance of carrier-based protein dry powder aerosols. Mechanistically, enhancement of performance is proposed to result from a redistribution of protein particles from coarse carrier particles to the fine particle component in the ternary mix.

KEY WORDS: dry powder aerosol; inhalation; protein; mixing; ternary component; polyethylene glycol.

INTRODUCTION

Inhalation delivery systems for the pulmonary administration of peptides and proteins include nebulisers (1,2), dry powder inhalers (3,4) and pressurised metered dose inhalers (5); of these, there is increasing interest in presenting recombinant proteins as dry powders. This technology avoids solution stability problems and addresses the concerns associated with protein denaturation during nebulisation (6).

For delivery using dry powder inhaler devices, proteins have frequently been processed by spray-drying (7,8) to produce particles with diameters in the range of 1–5 μm. Particles in this size range are generally considered to deposit in the tracheobronchial and pulmonary regions of the lung (9). However, such fine particles are characteristically cohesive and adhesive with poor flow and entrainment properties (10). Consequently, they are difficult to process and removal from the device is made inefficient. This problem has been addressed in commercial DPI formulations by producing loose agglomerates of the drug particles (e.g. Turbohaler™) or by blending the drug with coarse inert carrier particles. In the latter strategy, an ordered mix is formed where micronised drug particles are bound by physical forces at active sites on the carrier particles (11). This serves to improve the flow characteristics of the drug enabling efficient processing and promoting effective powder entrainment and device emptying.

However, the deep lung delivery performance of these carrier-based DPI formulations can be poor; principally, this is considered to be related to the inefficient detachment of fine drug particles from adherent sites on the carrier particle surface. At present this inefficiency is partially mitigated by the therapeutic index of the delivered drugs which allows generous margins for dosing without adverse effects. However, the efficient and effective use of the pulmonary route for delivery of proteins to the systemic circulation requires significant improvements in delivery efficiency and reproducibility of dosing (12).

Formulation modifications designed to increase deposition have concentrated on reducing the force of adhesion between the fine drug particles and the coarse carrier. Kassem and Ganderton (13) showed that it was possible to increase the separation of drug particles from a coarse particle surface by reducing the surface roughness of the carrier substrate. The inclusion of a ternary additive such as magnesium stearate into a formulation has also been shown to improve performance (14). However, a clinically more acceptable strategy was that used by Lord and Staniforth (15) to improve the performance of a salbutamol-based system. In this case, coarse carrier lactose particles were blended with fine lactose.

In the present study, we have investigated the *in vitro* deposition of a model protein (Bovine serum albumin) from a carrier-based dry powder aerosol formulation. Furthermore, recognising the poor general performance of these powder systems we have attempted to modify the micromeritic properties of the carrier lactose with a view to increasing redispersion of protein particles into the respirable aerosol; to achieve this, coarse lactose was pre-mixed with fine particle lactose (FPL) and micronised polyethylene glycol (PEG 6000). These excipients were chosen as they are generally recognised as safe. The mechanism of action of these fine particle performance modifiers was also examined.

MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA), molecular mass 66 kDa, was obtained as a lyophilised powder (>99% pure) from Sigma

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ABBREVIATIONS: BSA, bovine serum albumin; DPI, dry powder inhaler; FPL, fine particle lactose; FPF, fine particle fraction; FPM, fine particle multiplet; GSD, geometric standard deviation; MMAD, mass median aerodynamic diameter; P, protein; VMD, volume median diameter.

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Chemical Co. (Poole, U.K.). For this study, the protein was co-processed with maltodextrin (C★PUR 01908, Cerestar, Manchester, U.K.) by spray-drying to produce model particles having a diameter and size distribution suitable for inhalation.

Coarse carrier lactose (63–90 μm fraction) was prepared from α-lactose monohydrate (D30; Meggle, Wasserburg, Germany) by 2-stage sieve classification and air-jet sieved (Alpine, Augsburg, Germany) to remove adherent fine lactose particles.

The effect of fine particulate material on aerosol performance was investigated using both lactose and polyethylene glycol 6000. Fine particle lactose was obtained as Sorbolac 400 from Meggle. Fine particles of polyethylene glycol 6000 were produced from bulk material (Polyglykol 6000, Hoechst AG, Frankfurt, Germany) by fluid energy milling (Gem-T Air Pulveriser, Glen Creston, Stanmore, U.K.). During processing the mill was cooled using solid carbon dioxide in order to prevent excess heat softening the low melting point (55–63°C) PEG 6000.

Water used was MilliQ grade (Millipore, Watford, U.K.). Empty disk blisters for the Diskhaler® device were a gift from Glaxo Wellcome.

Spray-drying

Spray-dried powders in a size range suitable for inhalation were prepared using a laboratory scale co-current spray-dryer (Model 191, Büchi, Switzerland). Solutions containing 0.5% w/w BSA and 0.5% w/w maltodextrin in water were atomised at a rate of 4.5 ml min^{-1} using compressed air (600 l hr^{-1}, 0.7 mm nozzle). Inlet and outlet air temperatures were 90 and 55°C respectively. After spray-drying the product powder was collected by cyclone separation, transferred to glass vials and stored in a desiccator at room temperature over silica gel until used. The moisture content of spray-dried powders was determined by drying the sample at 100°C to constant weight (LP 16 Moisture Balance, Mettler, Greifensee, Switzerland).

Powder Characterisation

Particle Size Distribution and Morphology

The particle size distributions of spray-dried BSA-maltodextrin (50:50) and excipients were determined by laser diffraction (Mastersizer X, Malvern Instruments, Malvern U.K.). Size analysis was carried out in liquid suspension using cyclohexane + 0.1% w/w lecithin (BDH Ltd, Poole, U.K.) as the dispersing medium. Size distributions were expressed in terms of volume median diameter (VMD). The percentage of fine particles (<10 μm) contained in each powder was also determined (Table I). The aerodynamic diameter of spray-dried BSA-maltodextrin (50:50) was also measured using laser time-of-flight analysis (Aerosizer® with Aerodisperser®, API, Hadley, MA). The true density of BSA-maltodextrin (50:50) was determined as 1.43 g cm^{-3} by helium pycnometry (Accupyc 1330, Micromeritics, Norcross, GA). Morphology of protein and excipient particles was imaged by scanning electron microscopy (Model 6310, JEOL, Tokyo, Japan). Specimens were gold coated and examined at an accelerating voltage of 10 kV.

Table I. Particle Size Distribution of Powders

	Volume median diameter (μm)	% < 5 μm	% < 10 μm
BSA-maltodextrin (50:50)	2.1	90.4	99.6
Lactose (63–90 μm)	95.8	8.2	10.5
PEG 6000 (micronised)	4.0	61.7	97.5
Fine Particle Lactose	5.4	48.4	76.0

Protein Analysis

The amount of albumin in spray-dried protein powders and powder formulations was determined spectrophotometrically at 595 nm using a modified Bradford assay (Micro protein assay, Bio-Rad Laboratories, Hemel Hempstead, U.K.). For all assays, BSA from the same batch was used as a protein standard and samples were analysed in duplicate. Calibration plots for protein were linear over the range 1–7 μg ml^{-1}. For assay validation, replicate analysis (n = 5) of albumin standard solutions (2.5, 5.0 and 6.8 μg ml^{-1}) was performed. Experimentally, the mean albumin concentration of these solutions was determined as 2.5 ± 0.1, 5.1 ± 0.1 and 6.8 ± 0.1 μg ml^{-1} respectively.

Preparation of Powder Formulations for Inhalation

Powders for inhalation were prepared containing 2% w/w BSA-maltodextrin (50:50) and carrier lactose. BSA-maltodextrin (0.5 g) was accurately weighed together with lactose or fine particle treated lactose (24.5 g) into a glass container (125 ml) and mixed for 10 min in a turbulent tumbling mixer (Turbula T2C, Bachofen, Basel, Switzerland). Any remaining powder agglomerates were then removed by passing the mixture through a 180 μm aperture diameter sieve. This pre-mix was then returned to the glass vessel and mixed for a further 20 min. Powder blends (25 mg) were filled into disk blisters or size 3 hard gelatin capsules. Disk blisters were heat sealed using aluminum foil before use. Modified carrier lactose powders containing FPL at final concentrations of 2.5, 5.0, 7.5 and 10.0% w/w or micronised PEG 6000 (5.0% w/w), were prepared using the mixing method described above except that the pre-mixing and final mixing times were both increased to 30 min.

Homogeneity of the mixtures was evaluated by removing ten samples each weighing approximately 25 mg for assay of albumin content (the excipients did not interfere with the assay). Sampling was performed using a randomised grid method. The degree of homogeneity was expressed in terms of coefficient of variation (CV) of sample protein content; blended powders with CV's less than 5% were considered to be satisfactorily mixed (16,17). Protein content of formulations was approximately 230 μg in 25 mg (exact values were used in calculations).

Bulk Characterisation of Powder Formulations

Poured and tapped densities of powders were measured using a jolting volumeter (Engelsmann, Germany) and Carr's flowability index calculated (18). Powder flow was also measured directly using a Flodex™ test instrument (Hanson Research, Chatsworth, CA). In this technique the ability of powder to fall freely through an orifice of known diameter is used as an index of powder flow. For each sample, 75 g of powder was introduced into a flat-based cylindrical hopper fitted with one of a series of plates having orifices in the

diameter range 4–34 mm. After loading, the powder was allowed to stand for 2 min before the orifice shutter at the base of the hopper was opened. Powder flow was considered to have been established if a complete core was formed connecting the upper free surface of the powder bed with the orifice. Testing was carried out starting with the largest diameter orifice and sequentially reducing the orifice until the powder failed to discharge from the hopper. The flowability index was defined as the diameter of the smallest orifice through which the powder flowed.

In Vitro Aerosol Deposition

The aerosol performance of powder formulations was determined using Apparatus A (BP 1993, Appendix XVIIC), a glass twin-stage impinger (TSI) calibrated at a flow rate of 60 ± 5 l min^{-1}; prior to use, stage 1 and stage 2 chambers were charged with 7 and 30 ml of water respectively. The inhalation device was mounted in the TSI using a plastic adapter and primed by piercing the disk blister to release powder (Diskhaler®, Allen & Hanburys) or twisting the device body to break the capsule (Rotahaler®, Allen & Hanburys). Devices were then fired by switching on the pump for 4 s. Assay sensitivity did not permit single shot determinations of protein deposition. Therefore, sequences of four (Diskhaler®) or 5 (Rotahaler®) shots were fired into the TSI. The contents of the device, stage 1 and stage 2 were then rinsed into separate 50 ml volumetric flasks and assayed for protein content. Particles with mass median aerodynamic diameter (MMAD) 0.5-6.4 μm are trapped in stage 2. The aerosol performance parameters measured were the percent of the loaded dose retained in the device, fine particle dose, dispersing efficiency and fine particle fraction (FPF). The last three measures are defined as the cumulative mass of protein deposited in stage 2 of the TSI, the cumulative mass of protein in stage 2 expressed as a percentage of the doses loaded in the device, and the percent of protein emitted from the device reaching stage 2 of the TSI.

Statistical analysis of data was carried out using Minitab statistical software (Addison-Wesley Inc., MA). Post-ANOVA analysis was carried out by Fishers method.

RESULTS AND DISCUSSION

Physico-chemical Characteristics of Spray-dried Protein Particles

The co-processing of bovine serum albumin with maltodextrin by spray-drying yielded a white powder product with a residual moisture content of 5.4 wt. %. In this preparation of the model protein, maltodextrin acted as an amorphous diluent; although not currently used as an excipient for pulmonary administration it is included in USPNF XVII (Suppl. 8) for oral use and is a polymer of the monosaccharide dextrose which is in current use in dry powder inhaler formulations. Particles prepared using this controlled process were generally spherical in shape (Figure 1a). The powder was sized at 2.3 μm MMAD (± 1.5 μm GSD) and 2.1 μm VMD by aerodynamic time of flight analysis and laser light scattering respectively. From these analyses the narrow size distribution of the powder was evident with 99% of its mass <6.3 μm in aerodynamic diameter; particles of this size diameter are theoretically capable of penetrating to stage 2 of the impinger (19). Moreover, the protein powder had 95% of its mass <5 μm in aerodynamic diameter, a particle size usually considered as within the respirable range.

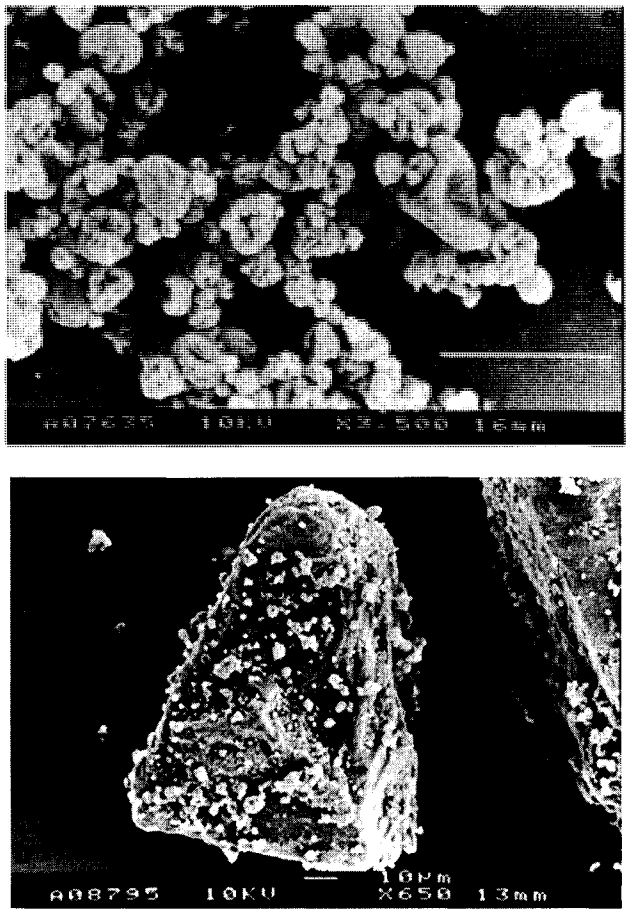

Fig. 1. Scanning electron micrographs of (a) spray-dried BSA-maltodextrin (50:50) particles (scale bar = 10 μm) and (b) coarse carrier lactose particle (63–90 μm) with adhering protein particles.

Influence of Fine Particle Modified Lactose on *In Vitro* Protein Deposition from the Diskhaler®

The simple binary dry powder aerosol formulation prepared by blending spray-dried BSA-maltodextrin (50:50) with air-jet sieved coarse carrier lactose (63–90 μm) had a high degree of homogeneity (CV = 2.3%). A microscopic examination of this powder blend indicating the formation of a true ordered system with spray-dried particles adhered to the surface of the larger lactose carrier crystals (Figure 1b).

Subsequent modified carrier systems were produced using the same coarse lactose fraction (63–90 μm diameter range) which was pre-mixed with a fine particle lactose (Sorbolac 400; VMD = 5.4 μm). This manipulation produced a significant effect on the particle size distribution of the coarse lactose. For example, addition of 7.5% w/w FPL to the coarse lactose decreased the VMD from 95.8 μm to 55.7 μm with 22.5% by volume of particles in this modified lactose <5 μm and 31.6 % <10 μm. Prior to mixing with FPL, the measured quantity of particles in the coarse lactose less than 5 μm was 8.2% and the proportion less than 10 μm was determined as being 10.5%.

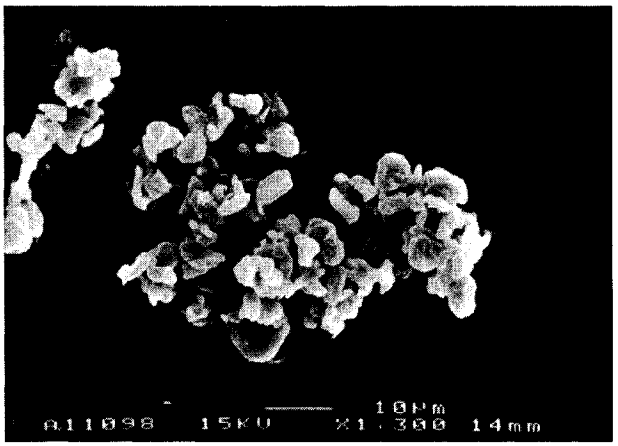

Fig. 2. Scanning electron micrograph of micronised PEG 6000 particles.

The subsequent addition of BSA-maltodextrin (50:50) to coarse carrier lactose pre-treated to contain 2.5, 5.0, 7.5 and 10.0% w/w FPL produced an homogeneous distribution of the protein throughout the ternary mixtures. Following mixing for 30 minutes all CV's were less than 5.0%.

The functional effect of fine particle lactose concentration on the deposition of BSA from dry powder formulations containing a coarse carrier lactose is presented in Table II. Analysis of variance showed that addition of fine lactose at all the concentrations examined produced a statistically significant ($p < 0.05$) increase in the proportion of protein dose reaching stage 2 of the impinger. In the case of carrier lactose pre-treated with 5.0% w/w FPL, the protein fine particle fraction (0.5–6.4 µm) delivered from the Diskhaler® was increased from 31.7 ± 2.4% to 47.4 ± 2.2%. However, further increasing the proportion of FPL in the conditioned carrier to 7.5 and 10.0% w/w did not produce any additional increase in protein fine particle fraction ($p > 0.05$).

Influence of Polyethylene Glycol 6000 on *In Vitro* Albumin Deposition

Experimental evidence reported in the previous section indicates that addition of fine lactose can significantly increase deposition from dry powder inhalers. To investigate if this effect extended to other fine particle excipients, the ternary component was changed to polyethylene glycol (PEG 6000). This material has previously been used as a coarse carrier in an experimental dry powder inhaler system (20). To produce microfine particles of PEG 6000 (Figure 2) with a diameter comparable to the fine particle lactose used in the previous experiments, commercial grade PEG 6000 powder was fluid energy milled. In this process, the volume median diameter of the bulk powder was reduced from 52.7 µm to 4.0 µm. However, formulations containing PEG 6000 could not be tested in the Diskhaler® as the material was found to melt during heat sealing of the blister pockets causing the powder to agglomerate. For this reason, the comparative effect of pre-treating the coarse carrier lactose with FPL or micronised PEG 6000 was tested using the capsule-based Rotahaler® device.

Statistical analysis of the data presented in Table III showed that both PEG and FPL treatments increased deposition of protein in stage 2 of the impinger (ANOVA; $p < 0.05$). The mean fine particle fraction was increased from 24.4 ± 4.1% for the untreated carrier lactose system to 33.5 ± 4.5% and 36.8 ± 6.9% for powders containing coarse lactose pre-treated to contain 5% w/w PEG 6000 or FPL respectively. Further statistical analysis showed no significant difference in deposition between the treated samples ($p > 0.05$). That the levels of deposition were significantly lower than observed when the same FPL-containing formulation was tested in the Diskhaler® can be attributed to the lower efficiency of the Rotahaler® in producing particle deaggregation. These results indicate the possibility of using fine particles other than lactose as performance modifiers in dry powder inhalers. However, the low melting point and potential hygroscopicity of PEG 6000 make processing of this material difficult.

Influence of the Ternary Components on the Bulk Properties of Inhalation Powder Formulations

In using fine particle material to improve inhaler performance a potential problem is the effect produced on the powder flow of the formulation. Since this parameter can critically influence powder entrainment and emptying of drug from a device (21) then it is possible that a reduction in the flowability of a formulation could reduce the fine particle protein dose. As expected, the inclusion of micronised material into mixes, as either fine particle lactose or PEG 6000, was found to cause a reduction powder flow (Table IV). This was evidenced by the increase in both Carr's index and flowability index. Unlike Carr's index, the latter parameter does not infer flowability from packing densities but is a direct measure of flow.

Table II. Effect of Fine Particle Lactose (FPL) on *In Vitro* Albumin Deposition from the Diskhaler®

Formulation	Device retention (%)[a]	Fine particle dose (µg)	Dispersion efficiency (%)[a]	Fine particle fraction (%)[b]
BSA-maltodextrin (50:50) → coarse lactose	16.0 (3.2)	226 (14)	23.7 (1.5)	31.7 (2.4)
BSA-maltodextrin (50:50) → (coarse lactose + 2.5% w/w FPL)	16.5 (2.3)	279 (24)	29.2 (2.5)	40.3 (3.7)
BSA-maltodextrin (50:50) → (coarse lactose + 5.0% w/w FPL)	13.6 (1.2)	336 (16)	35.5 (1.7)	47.4 (2.2)
BSA-maltodextrin (50:50) → (coarse lactose + 7.5% w/w FPL)	12.7 (2.0)	343 (27)	36.0 (3.0)	48.8 (3.8)
BSA-maltodextrin (50:50) → (coarse lactose + 10.0% w/w FPL)	14.3 (3.0)	361 (45)	36.6 (4.6)	49.8 (5.1)

Note: Air flow rate = 60 l min^{-1}. Values are mean ± SD for five disks (four shots per disk).
[a] Results are percent of loaded dose.
[b] Results are percent of emitted dose.
→ Indicates the final mixing stage in the preparation of the formulation.

Table III. Effect of Fine Particle Lactose (FPL) and Micronised PEG 6000 on *In Vitro* Albumin Deposition from the Rotahaler®

Formulation	Fine particle dose (μg)	Dispersion efficiency (%)[a]	Fine particle fraction (%)[b]
BSA-maltodextrin (50:50) → coarse lactose	138 (14)	10.9 (1.1)	24.4 (4.1)
BSA-maltodextrin (50:50) → (coarse lactose + 5.0% w/w PEG 6000)	204 (36)	16.4 (2.9)	33.5 (4.5)
BSA-maltodextrin (50:50) → (coarse lactose + 5.0% w/w FPL)	196 (35)	15.5 (2.7)	36.8 (6.9)

Note: Air flow rate = 60 l min^{-1}. Values are mean ± SD of five shots (five capsules per shot).
[a] Results are percent of loaded dose.
[b] Results are percent of emitted dose.
→ Indicates the final mixing step in the preparation of the formulation.

In relation to the effect on inhalation performance, device emptying was first assessed by analysing protein residues remaining in the device and blisters after firing. However, this was not possible for the Rotahaler® where contaminating protein from the gelatin capsules interfered with the protein assay.

For the Diskhaler®, increasing the concentration of FPL in the conditioned carrier formulation did not increase the proportion of the protein dose retained in the device on firing (Table II). Statistical analysis showed no significant difference in the device and blister residues for each formulation (ANOVA; $p > 0.05$). That approximately 15% of the nominal protein dose was found in the device would suggest poor powder emptying. However, for all the formulations tested the output from the device (calculated by mass) was greater than 95% of the disk blister fill weight (Figure 3a). A visual examination of device and blisters also confirmed little powder accumulation after firing. From these data, it would appear that although the addition of FPL to formulations reduced powder flow, emptying from the Diskhaler® was not affected. In this case, it was considered that the retention of protein in the device resulted from adhesion of primary particles to device surfaces immediately following separation from the coarse lactose carrier. Such losses are similar to those described by de Boer et al. (22) for a commercial beclomethasone dipropionate formulation in the Diskhaler®. The cause of this preferential surface attraction of protein particles was considered to result from electrostatic and density effects.

In the case of the Rotahaler® (Figure 3b), the emptying efficiency was found to be lower and more variable than for the Diskhaler®. However, the addition of either 5% w/w PEG 6000 or FPL as a performance modifier did not significantly affect the output of the formulation from the device (ANOVA; $p > 0.05$).

Mechanistic Interpretation for the Action of Fine Particle Excipients

Given that normalisation of protein deposition data by calculating the fine particle fraction ignores any dose metering or device deposition effects (23) then the use of coarse carrier lactose pre-treated with fine particle lactose would appear to improve the separation of protein particles from carrier particles, thereby increasing the fraction of the emitted protein dose reaching stage 2 of the TSI. Mechanistically, the reason for this improvement cannot be unequivocally explained. However, according to the ordered mixing theories developed by Hersey (11) it might be expected that during blending the microfine lactose particles would occupy the most active binding sites on the lactose crystal surface. In this case only more passive sites would remain available for adhesion of protein particles during the second stage of blending. According to these currently accepted theoretical considerations (14), protein particles should be more easily removed from formulations where the coarse carrier is pre-mixed with FPL rather than in cases where the protein particles are first mixed with the carrier and then FPL added into the system. In the former conditions, theory predicts that protein particles will only be weakly adhered to the least active sites. Whereas, in the latter case protein particles would be predicted to be very strongly bound to the highly active sites on the carrier surface.

To test this theory, ternary mixtures containing 2% w/w BSA-maltodextrin (50:50) and 5% w/w fine particle lactose

Table IV. Influence of Fine Particle Lactose (FPL) and Micronised PEG 6000 on the Bulk Properties of Inhalation Powder Formulations

Formulation	Poured density (g/cm^3)	Tapped density (g/cm^3)	Carr's index (%)	Flow index[a] (mm)
Coarse lactose (63–90 μm)	0.75	0.82	8.6	<4
BSA-maltodextrin (50:50) → coarse lactose	0.76	0.84	9.5	<4
BSA-maltodextrin (50:50) → (coarse lactose + 2.5% w/w FPL)	0.75	0.85	11.8	6
BSA-maltodextrin (50:50) → (coarse lactose + 5.0% w/w FPL)	0.74	0.88	15.9	12
BSA-maltodextrin (50:50) → (coarse lactose + 7.5% w/w FPL)	0.73	0.89	18.0	16
BSA-maltodextrin (50:50) → (coarse lactose + 10.0% w/w FPL)	0.72	0.90	20.0	22
FPL	0.32	0.56	42.9	>34
BSA-maltodextrin (50:50) → (coarse lactose + 5.0% w/w PEG 6000)	0.71	0.87	18.4	N.D.[b]

[a] Flowability index is defined as the diameter of the smallest orifice through which powder flows on the Flodex™ apparatus.
[b] N.D. Not determined due to a limited amount of material.

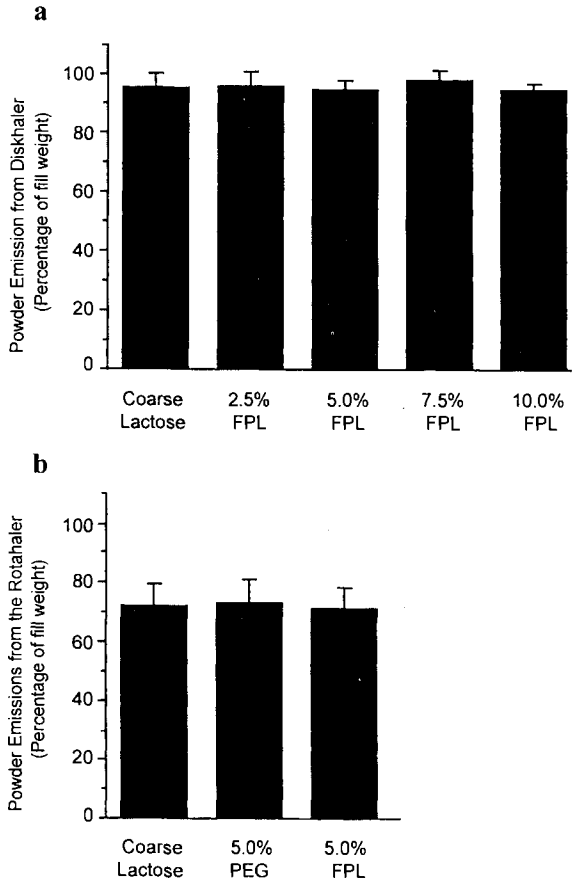

Fig. 3. Summary of the powder emissions of modified formulations from (a) Diskhaler® and (b) Rotahaler® devices. Data are calculated as a percentage of the blister fill weight. Air flow rate = 60 l min^{-1}. Values are mean (SD error bars) of four shots from five disks.

were prepared using different mixing orders and the deposition efficiency of each blend was determined (Table V). Unexpectedly, these data indicated that the effect of fine particle lactose on the deposition of protein from the Diskhaler® was independent of the order of addition to the ternary powder formulation. For example, addition of FPL to a preformed binary mixture of BSA-maltodextrin (50:50) and coarse carrier lactose produced an increase in the mean fine particle fraction from 29.3 ± 3.9% to 39.1 ± 3.9%. This was similar to the improvement produced by conditioning the carrier lactose with 5% w/w FPL prior to the addition of protein particles (FPF = 41.9 ± 1.7%). If FPL were acting only by occupation of binding sites on the surface of the carrier particles then addition to a binary ordered mix would, theoretically, have had no effect on the fine particle fraction. That the addition of fine particle lactose was improving protein deposition by a different mechanism was also suggested following examination of ternary systems by scanning electron microscopy.

Figure 4 shows the effect of adding BSA-maltodextrin (50:50) to a binary system containing coarse lactose pre-treated using 5% w/w FPL. In these conditions, the fine lactose particles

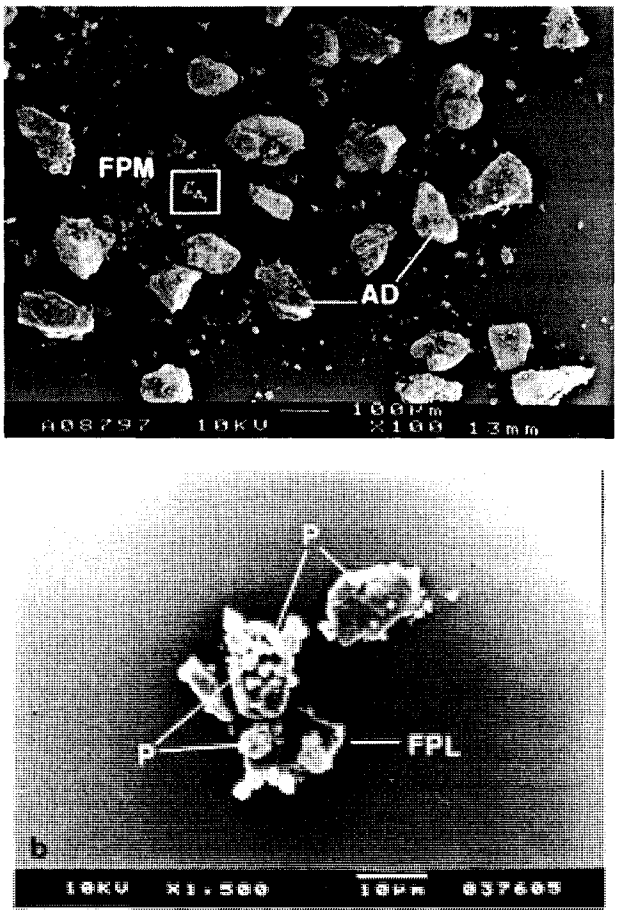

Fig. 4. Scanning electron micrographs of the BSA-maltodextrin (50:50)-lactose (63–90 μm)-FPL (5% w/w) ternary powder formulation. (a) hybrid adhesion system, showing the presence of conventional coarse carrier adhesion units (AD) and free fine particle multiplets (FPM) (b) composition of a free fine particle multiplet showing protein particles (P) adhered to fine particle lactose (FPL).

Table V. Influence of Mixing Order on *In Vitro* Albumin Deposition from the Diskhaler®

Formulation	Device retention (%)[a]	Fine particle dose (μg)	Dispersion efficiency (%)[a]	Fine particle fraction (%)[b]
BSA-maltodextrin (50:50) → coarse lactose	17.5 (3.0)	209 (29)	22.6 (3.0)	29.3 (3.9)
BSA-maltodextrin (50:50) → (coarse lactose + FPL)	15.9 (2.4)	278 (17)	30.8 (1.9)	41.9 (1.7)
FPL → (BSA-maltodextrin (50:50) + coarse lactose)	15.I (1.9)	269 (24)	28.8 (2.6)	39.1 (3.9)

Note: Air flow rate 60 l min^{-1}. Values are mean ± SD for five disks (four shots per disk).
[a] Results are percent of loaded dose.
[b] Results are percent of emitted dose.
→ indicates the final mixing step in the preparation of the formulation.

were found to be associated with the carrier surface or to exist as small aggregates or individual particles free within the mix (Figure 4a). Further close examination revealed adhered protein particles, identifiable from their unusual spherical morphology, to be associated with these fine lactose particles as multiplets (Figure 4b). A similar distribution of these protein-lactose multiplets was also evident throughout other blend formulations prepared using different orders of mixing, and when lower concentrations of FPL (2.5% w/w) or PEG 6000 were used for conditioning of carrier particles.

From these data, a redistribution of protein particles between the original coarse carrier lactose component and the added fine particle lactose would appear to have occurred during the mixing process. This resulted in the formation of a hybrid ordered system containing protein-coarse lactose adhesion units and protein-fine lactose multiplets. Such a redistribution of microfine drug particles is similar to that previously described in ternary granulated systems by Soebagyo and Stewart (24). In producing these protein fine lactose multiplets the inhalation performance of the formulation could be increased by two distinct processes. Primarily, it is considered that the liberation of protein particles from the surface of fine lactose crystals within multiplets occurs more readily than from the coarser lactose particles as a result of a lowered adhesion force. This could be expected as smaller particles have a lower degree of surface roughness which will restrict opportunities for contacts at clefts and asperities on substrate surfaces. In this case, more protein particles would be detached from the multiplets than from coarse carrier adhesion units for a given inspiratory force. Secondly, it is also possible that a part of the aerosolised particle population contains protein-fine lactose multiplets with an aerodynamic diameter < 6.4 μm. Under such conditions, some protein particles may become deposited in stage 2 of the impinger as intact multiplets without being redispersed. That systems of this type could develop is likely given that the fine particle lactose used in this study contained a significant proportion of particles < 5 μm. Indeed, multiplets formed from these small lactose particles could be observed on the surface of coarse carrier particles. These smaller multiplets were similar in appearance to those described in Figure 4b. However, it is currently unclear as to the relative contributions of lactose particles of differing sizes to the formation of multiplets and the overall enhancement in formulation performance although the presence of the protein-coarse lactose adhesion units is still considered as an important requirement for the finished formulation so that entrainment and device emptying are not impeded.

CONCLUSIONS

Therapeutic and economic imperatives of using the pulmonary route for administration of peptides and proteins, demand a highly efficient and reproducible delivery system. The evidence presented in this study shows that by inclusion of fine particle lactose or PEG 6000 in carrier-based formulations the performance of a dry powder inhaler system can be significantly improved. The influence of the fine particle lactose was shown to be concentration dependent with optimum levels of deposition achieved from carrier lactose conditioned with 5% w/w FPL. It was considered that the improvements found in the present study resulted from redistribution of protein particles to form multiplets with fine lactose particles. In these circumstances the increase in fine particle fraction was postulated to result from either a reduction in the adhesion force between the protein particles and the fine lactose particles in a given multiplet or that the protein-fine lactose multiplets were deposited in stage 2 of the impinger as a result of their small aerodynamic diameter. Although the effect reported in the present study was for a protein-based system it is apparent that the phenomenon may also extend to formulations prepared using small molecule drugs.

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