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 1 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 stabil-ity 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 blinding 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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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 Sorbotalc 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 GiaXo 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). Suspensions 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 1 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.

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