Research Article

Engineered Mannitol Ternary Additives Improve Dispersion of Lactose–Salbutamol Sulphate Dry Powder Inhalations

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Abstract. The aim of this study was to evaluate the influence of novel engineered fine mannitol particles (4.7%, w/w) on the performance of lactose–salbutamol sulphate dry powder inhaler (DPI) formulations to obtain promising aerosolisation properties. The results showed that the more elongated the fine mannitol particles, the weaker the drug–carrier adhesion, the better the drug content homogeneity, the higher the amount of drug expected to be delivered to the lower airways and the higher the total DPI formulation desirability. Linear relationships were established showing that mannitol particles with a more elongated shape generated powders with broader size distributions and that were less uniform in shape. The weaker the drug–carrier adhesion, the higher the fine particle fraction of the drug is upon aerosolisation. It is believed that more elongated fine mannitol particles reduce the number of drug–carrier and drug–drug physical contact points and increase the ability of the drug particles to travel into the lower airways. Additionally, a lower drug–carrier contact area, lower drug–carrier press-on forces and easier drug–carrier detachment are suggested in the case of formulations containing more elongated fine mannitol particles. Ternary ‘drug-coarse carrier-elongated fine ternary component’ DPI formulations were more favourable than both ‘drug-coarse carrier’ and ‘drug-elongated coarse carrier’ binary formulations. This study provides a comprehensive approach for formulators to overcome the undesirable properties of dry powder inhalers, as both improved aerosolisation performance and reasonable flow characteristics were obtained using only a small amount of elongated engineered fine mannitol particles.

KEY WORDS: added fines; adhesive mixtures; engineered fine mannitol; formulation performance; ternary component.

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ABBREVIATIONS: ACM, Acetone crystallised mannitol; AR, Aspect ratio; CCM, Cooling crystallised mannitol; CI, Carr’s index; CM, Commercial mannitol; COD, Cutoff diameter; CV, Coefficient of variation; $d_{10\%}$, Particle diameter at 10% volume distribution; $d_{50\%}$, Particle diameter at 50% volume distribution (median diameter); $d_{90\%}$, Particle diameter at 90% volume distribution; d_{ae} , Theoretical aerodynamic diameter; d_e , Geometric diameter (volume mean diameter); d_i , Desirability function; ECM, Ethanol crystallised mannitol; ED, Emitted dose; DF, Overall desirability function; DPI, Dry powder inhaler; DSC, Differential scanning calorimetry; FMPs, Fine mannitol particles; FPF, Fine particle fraction; FPA, Fine particle aggregates; FR, Flatness ratio; GSD, Geometric standard deviation; HR, Hausner ratio; I + M, Inhaler with mouthpiece adaptor; IL, Impaction loss; IP, Induction port (throat); MD, Mean diameter; MMAD, Mass median aerodynamic diameter; MSLI, Multistage liquid impinger; RH, Relative humidity; PSD, Particle size distribution; RD, Recovered dose; SDM, Spray dried mannitol; SE, Standard error; SEM, Scanning electron microscope; SF, Shape factor; SM, Supplementary material; SS, Salbutamol sulphate; PXRD, Powder X-ray diffraction; ρ , True density.

INTRODUCTION

Dry powder inhalers (DPIs) have been the subject of widespread research interest over the past few years (1). However, obtaining a high drug aerosolisation efficiency is a major challenge for DPI formulations because most DPIs still have relatively poor drug aerosolisation efficiencies (2). The presence of a fine additive material could have a positive effect on DPI performance as assessed *in vitro* (3–6) and *in vivo* (7). In addition to lactose, several commercial fine additive materials have been investigated in ternary DPI formulations, including erythritol, trehalose (8), glucose (9), mannitol, sorbitol (7) and polyethylene glycol 6000 (10), and all were shown to enhance DPI performance.

Although several studies have highlighted the importance of the size and concentration of commercial fine additive components on drug inhalation behaviour, the influence of using engineered fine particle components on DPI performance has not been addressed. For example, the optimal size of fine carrier particles was suggested to be approximately 5–8 μm (8,11). Nevertheless, some reports showed that drug dispersion from DPIs increased when the size of the commercial fine carrier was increased (12) or decreased (13). In addition, the optimal concentration of commercial fine carrier particles varied between 5% (11), 9–10% (14), 15% (3), 20% (13) and 37.5% (w/w) (15), whereas after a specific concentration limit, increasing the amount of commercial fine carrier did not result in a higher fine particle fraction (FPF). This behaviour was explained by the ‘active site saturation’ theory (16), but there are still doubts about the specific

reason (17). At a high drug-to-fine carrier ratio, greater drug–drug agglomeration was obtained due to strong cohesive forces (18), leading to a decrease in DPI aerosolisation performance. This decrease was likely caused by an agglomerated–segregated system transformation (16). Linear relationships were reported between the FPF and the percentages of commercial fine carriers of $<5\text{ }\mu\text{m}$ (16) or $<10\text{ }\mu\text{m}$ (11) in size. However, the effect of fine carrier particles on DPI performance in these studies was dependent on the type of inhaler device used (19).

There is still disagreement in the literature as to the mechanism by which fine additive particles improve the performance of DPI formulations. The first mechanism proposed was the saturation of active sites (hot spots or high adhesion sites) on coarse carrier particles by fine particles, which leave the passive sites (low adhesion sites) available for drug (12), resulting in a decreased drug–carrier adhesion and an increased drug–carrier detachment upon aerosolisation. The second explanation proposed was the ‘agglomeration theory’ (9,10). In this theory, mixed aggregates were suggested to form between ternary components (commercial fine additive particles) and drug particles.

No consistent correlation was observed between the FPF and powder flowability because poor formulation flow properties were associated with both increased (20,21) and decreased (22) FPF. However, good flow is necessary to achieve a satisfactory DPI formulation in terms of handling, metering (e.g. capsule filling), fluidisation, deagglomeration and dispersion in an air stream (23). Additionally, formulated powders with better flowability were demonstrated to be advantageous in terms of higher drug emission (drug release from the inhaler device) upon aerosolisation (24). Moreover, the efficiency of blending during the preparation of DPI formulations is profoundly influenced by the flow properties of the powder components (12).

It has been shown that typical drug-commercial carrier DPI formulations have relatively satisfactory flow properties but poor aerosolisation performance (4,5). The aerosolisation performance was enhanced when elongated carrier particles were used (25) (or elongated drug particles (26)), but such carrier particles generated powders with very poor flow properties. Accordingly, the purpose of this study was to determine if formulations with both satisfactory aerosolisation and flowability properties could be achieved by employing only small amounts of engineered fine ternary component particles ($<20\text{ }\mu\text{m}$) with distinctly different morphologies. Unlike drug and carrier particles, the influence of using engineered fine ternary component particles on DPI performance has not yet been studied. Fine mannitol particles (FMPs) were used in this study because mannitol has been demonstrated to be an attractive carrier for aerosol delivery, as it does not have a reducing effect and is less hygroscopic.

MATERIALS AND METHODS

Materials

Micronised salbutamol sulphate (SS; LB Bohle, Germany, geometric diameter (d_e)= $1.9\pm0.2\text{ }\mu\text{m}$, true density (ρ)=

$1.39\pm0.01\text{ g/cm}^3$ (24,27)) and α -lactose monohydrate (Pharmatose, 100 M, DMV International, the Netherlands, $d_{10\%}$ = $58.5\pm0.3\text{ }\mu\text{m}$, $d_{50\%}$ = $87.9\pm0.4\text{ }\mu\text{m}$, $d_{90\%}$ = $128.5\pm0.7\text{ }\mu\text{m}$ (5)) were employed as a model drug and a model carrier, respectively. Spray-dried mannitol (SDM) and commercial mannitol (CM) were purchased from SPI Pharma, UK and Fisher Scientific, UK, respectively.

Preparation of Different Fine Mannitol Particles

To obtain FMPs with different morphologies, different engineered mannitol products were prepared, i.e. cooling-crystallised mannitol (CCM (28)), acetone-crystallised mannitol (ACM (4)) and ethanol-crystallised mannitol (ECM (25)). Different engineered mannitol bulk powders were poured separately through a $20\text{-}\mu\text{m}$ sieve (Retsch® GmbH Test Sieve, Germany) and then carefully sieved (Endecotts Ltd, England mechanical shaker) for 30 min. The obtained fine mannitol powders were collected and transferred into separate sealed glass vials until use.

Image Analysis Optical Microscopy

Quantitative optical image analysis was conducted using a computerised morphometric analysing system (Leica Q Win Standard Analyzing Software and Leica DMLA Microscope; Leica Microsystems Wetzlar GmbH, Wetzlar, Germany) adapted from Kaialy *et al.* (28). Briefly, for each mannitol sample, one drop of light paraffin oil was applied, and then a small amount of powder (approximately 20 mg) was homogeneously and gently scattered onto a glass slide. A minimum of 2,000 particles were detected and measured. Several particle shape descriptors were employed, including *aspect ratio* (AR), *flatness ratio* (FR) and *shape factor* (SF) (28).

Scanning Electron Microscopy

Electron micrographs of the FMPs, SS, lactose–SS formulation and lactose–SS–FMP formulations were obtained using a scanning electron microscope (SEM; HITACHI SU8030, Japan) operating at 0.5–1 kV. Prior to observation, the specimens were mounted on an aluminium metal stub under vacuum in an argon atmosphere. Several magnifications were used to evaluate the particle size, shape and surface texture properties of the different samples.

Laser Diffraction Measurements

Particle size distribution (PSD) analysis was conducted using a Sympatec (Clausthal-Zellerfeld, Germany) laser diffraction particle size analyser operated according to the experimental procedure described previously (25). A 50-mm lens was used, and all calculations were performed on the basis of Fraunhofer theory. MD (mean diameter based on the surface) was calculated using Sympatec software. The span ($d[v,90]-d[v,10]/d[v,50]$) was used as a measure of the width of the distribution relative to the median diameter. Because primary particle sizes were required, wet dispersion was used (29). Sonication and mechanical stirring (1,200 rpm) were applied to ensure that most agglomerates were entirely

dispersed into the primary particle size of the material. Each FMP sample was sonicated for 10 s before measurement.

Lactose sample was dispersed for 5 min by a magnetic stirrer (1,200 rpm). The dispersion medium was absolute ethanol saturated with lactose over 24 h and then filtered through a cellulose filter paper ($<0.45\ \mu\text{m}$). To ensure complete dispersion of lactose particles, each sample was sonicated for 10 s before measurement. All particle size data were collected following 5 min of dispersion, after which relatively 'stable' PSD descriptors were obtained (no particle dissolution or re-agglomeration being detected).

Differential Scanning Calorimetry

A differential scanning calorimetry (DSC)822° (Mettler Toledo, Switzerland) was employed with nitrogen as the purge gas ($50\ \text{mL min}^{-1}$) through the DSC cell. Aluminium non-hermetic DSC pans were used throughout the study. The instrument was calibrated using indium and zinc standards at the same heating rates used in the experiments. Approximately 4–5 mg of each FMP sample was used for each run. Before each measurement, the sample was allowed to equilibrate for 5 min at 25°C and then heated to 250°C at a heating rate of 30°C/min (28). Each sample was analysed in triplicate.

Powder X-ray Diffraction

Powder X-ray diffraction (PXRD) patterns of all FMP samples were collected on a Bruker D8 Advance Siemens powder diffractometer with Cu $K\alpha$ radiation ($1.54056\ \text{\AA}$) using the DIFFRACplus software, as explained in detail elsewhere (28). The diffraction peaks in all PXRD patterns were compared to the reference PXRD patterns of pure α -, β - and δ -D mannitol reported in the literature (30). The reproducibility of the PXRD patterns was verified by collecting multiple patterns of three different specimens of each sample.

Preparation of Formulation Powders

Each lactose–SS–FMP formulation was prepared in two consecutive blending steps. Commercial lactose was first blended (separately) with different 5% (w/w) FMP samples, after which each resultant lactose–FMP carrier powder mixture was blended separately with the SS drug in a 67.5:1 w/w ratio (in accordance with the ratio used in commercial Ventolin Rotacaps®, GSK). The per cent ratio (w/w) of lactose/SS/FMP in the final formulation was thus 93.8:1.4:4.7%. FMPs were pre-blended with lactose particles before mixing with SS based on the recommendation reported previously (12). Each blending process was carried out under standard mixing conditions in a cylindrical aluminium container ($6.5\times 8\ \text{cm}$) using a Turbula® mixer (Willy A. Bachofen AG, Basel, Switzerland) (100 rpm mixing speed and 15 min mixing time). The lactose–SS formulation was prepared using a similar protocol, except it was not blended with any FMP product. After the blending process was complete, all formulations were stored in tightly sealed glass vials for at least 24 h prior to any investigation to allow relaxation of any electrical charge.

Formulation Powder Density and Flowability

The bulk density (or apparent density), tap density (drop density), Carr's index (CI) and the Hausner ratio of all formulation powders were characterised as described in detail elsewhere (31).

Homogeneity Test

After preparation of the formulations, ten randomly selected samples ($27.4\pm 1\ \text{mg}$ for each sample, unit SS dose $400\pm 15\ \mu\text{g}$, in accordance with the dose used in commercially available Ventolin Rotacaps®, GSK) from different positions within each formulation (top left, top centre, top right, middle left, middle centre (two), middle right, bottom left, bottom centre and bottom right) were taken to analyse the SS content using HPLC (4). Each sample was dissolved in deionised water up to 100 mL in a volumetric flask. The average SS content for each formulation was expressed as the percentage of nominal SS unit dose (% potency, $400\ \mu\text{g}$). The degree of SS content homogeneity within all formulations was expressed using the coefficient of variation (%CV).

Drug–Carrier Adhesion Assessments

Air depression sieving (32) was employed to assess (indirectly) drug–carrier adhesion forces within all formulations. The air jet sieving machine (Copley Scientific, Nottingham, UK) was operated with a volume flow that generated a negative pressure of 4 kPa. Each formulation (1 g) was placed on top of the $20\ \mu\text{m}$ sieve, and a minimum of three samples ($27.4\pm 1\ \text{mg}$) was removed from different areas of each formulation after different functional sieving times (5, 30, 60 and 180 s). The SS content in each sample was quantified using HPLC (4). Upon air jet sieving, the particles were subjected to both aspiration (produced by the negative pressure) and airflow (produced by the blow nozzle). Aspiration generated the airflow, eventually resulting in (mostly) drag and lift forces to be exerted on the drug particles. Assuming that equal detachment forces were generated (for equal sieving times), less SS remained on the top of the sieve verifies weaker drug–carrier adhesive forces. Following sieving, each sample was stored at ambient conditions (20°C ; 50% RH) for at least 24 h prior to drug quantification to permit any charge relaxation to occur. The ratio of adhesion to detachment force was changed in favour of the detachment force when lower drug concentrations were measured in the material on the sieve. Drug–carrier adhesion assessments were carried out in an air-conditioned laboratory (22°C and 50% RH).

In Vitro Aerosolisation Deposition Study

After blending, each formulation was manually filled into hard gelatine capsules (size 3) such that each capsule contained $27.4\pm 1\ \text{mg}$ of the formulation powder. After filling, the capsules were equilibrated in sealed glass vials under ambient conditions (20°C , 50% RH) for at least 24 h prior to investigation. Different formulations were assessed *in vitro* using a Multi Stage Liquid Impinger (MSLI) equipped

with a USP induction port (Copley Scientific, Nottingham, UK) at a flow rate of 92 L/min, which corresponded to a pressure drop of 4 kPa across the Aerolizer® inhaler device (4,25). The MSLI was operated according to pharmacopoeial recommendations (33).

Each deposition experiment involved the actuation of ten capsules and was repeated three times. Several parameters were employed to characterise the deposition profiles of the different formulations under investigation, including the recovered dose (RD), emitted dose (ED), recovery, emission, mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), theoretical aerodynamic diameter (d_{ae}), impaction loss (IL) and fine particle fraction ($FPF_{\leq 5 \mu m}$) (4,6). The RD is the sum of the total mass of drug (in micrograms) recovered from the inhaler device with its fitted mouthpiece adaptor (I+M), induction port (throat, IP) and all stages of the impactor per capsule following each deposition. The ED is the sum of the amounts of drug recovered from the IP and all MSLI stages per capsule. Recovery is the per cent ratio of the RD to the theoretical dose (400 μg). Emission is the per cent ratio of the RD to the ED. To calculate the MMAD, the cumulative percentages of drug mass less than the stated aerodynamic diameter of MSLI stages from 1 to 4 were plotted against the effective cutoff diameter on log probability paper taking cumulative amounts less than MSLI stage 1 as 100%. MMAD is the particle size at which the line crossed the 50% mark (as the 50th percentile of the aerodynamic particle size distribution by mass). The same plot was used to calculate the GSD as the square root of the ratio of particle size at the 84.13th percentile to the 15.87th percentile. The d_{ae} of SS ($d_{ae} = d_e \times \sqrt{\frac{F}{X}}$) was calculated from the geometric mean diameter (d_e) and true density (ρ) (x is the dynamic shape factor for non-spherical particles, which was assumed to be 1). IL is the per cent amount of drug deposited on the IP plus stage 1 of the MSLI to the RD. The FPF was calculated from that plot as the fraction of powder emitted from the inhaler with an aerodynamic diameter of $\leq 5 \mu m$.

RESULTS AND DISCUSSION

Characterisation of Fine Mannitol Particles

Fundamentally, particle form is described by the first-order shape descriptors AR and FR (34). SF (0–1) is a second-order shape property reflecting variations in particle corners (shape irregularity). The AR/FR for different FMPs was in the following rank order: SDM < CM < CCM < ACM < ECM, whereas the reverse order was obtained for SF (Fig. 1a). Such results proved that, among the FMPs, ECM particles had the most elongated and irregular shape, whereas SDM particles had the most spherical and regular shape, observations which were supported by SEM micrographs (Fig. 1b–f). FMPs showed different PSDs (Fig. 2a), with a MD ranging from 3.3 ± 0.0 to $6.6 \pm 0.3 \mu m$ and span values ranging from 1.78 ± 0.4 to 2.67 ± 0.01 (Fig. 2b). Differences in size among FMPs could be attributed partly to their different morphologies (Fig. 1)

because particle size determinations are influenced by particle shape and vice versa (35). Indeed, laser diffraction does not take into account dynamic shape factors of particles (36).

Correlations were established when plotting AR/FR against the span ($r^2=0.9352$) and standard error (SE) of AR/FR ($r^2=0.7565$) for different FMPs (SM1, supplementary material), indicating that, generally, FMPs with more elongated shapes generated powders with broader (more heterogeneous) size distributions and that were less uniformly shaped. Particle size measurements using laser diffraction in the case of elongated particles are less accurate because laser diffraction presumes that all particles are spheres (37). Indeed, both laser diffraction and image analysis measurements are reliant on particle-direction and particle-particle contact area (38), which could negatively affect the accuracy of the measurements. Therefore, the size, shape and surface morphology of all FMPs were qualitatively validated by SEM.

The approximate size of only several particles as determined from SEM was consistent with the PSD data obtained by laser diffraction (Fig. 1c–f). In addition, the difference in morphologies could be clearly visualised from SEM images (Fig. 1b–f, SM2). Specifically, SDM particles exhibited a rounded-subrounded morphology with smoothly curved sides with no edges, consisting of small regular spherical-shaped nanoparticles that were 150–300 nm in size (Fig. 1b). Such primary nanoparticles could not be detected by laser diffraction because of the sensitivity of the technique and/or possible high particle agglomeration. CM showed angular-subangular particles (but not elongated) with less uniform grains (relatively less homogeneous particles) (Fig. 1c). CCM crystals presented an elongated columnar morphology with sharp edges (Fig. 1d). ACM particles revealed rod-shaped particles with relatively plain sides (Fig. 1e), whereas ECM particles showed a needle shape (Fig. 1f). FMPs displayed relatively smooth surface textures without significant irregularities. The degree of shape elongation of different FMPs was in the following rank order: SDM < CM < CCM < ACM < ECM (Fig. 1b–f), which matched the rank order assembled from the AR/FR data (Fig. 1a, SM3).

Similar DSC traces were produced by all FMPs, with one endothermic transition at $172.0 \pm 2.6^\circ C$ ($P > 0.05$) corresponding to the melting point of mannitol (SM4). No detectable events (e.g. dehydration endotherm) were observed below $100^\circ C$ for all samples, indicating that all FMPs had negligible amounts of surface water (free water). No significant difference in melting enthalpies (288.4 ± 6.8 J/g, $P > 0.05$) was found among the FMPs, which could suggest their similar relative degree of crystallinity. D-Mannitol has at least three common polymorphic forms: α -, β - and δ - (33). Indeed, α -, β - and δ -mannitol have comparable melting enthalpies, and their melting points differ only slightly (166.5, 166 and $158^\circ C$, respectively). In the DSC traces, α - and β -mannitol were indistinguishable, whereas δ -mannitol was difficult to detect, especially when present at low quantities within the sample (39). Therefore, all FMPs were further analysed using PXRD (Fig. 3), which is known to be an accurate method for polymorphic content analysis.

Fig. 1. Aspect ratio/flatness ratio, shape factor (mean $\pm$ SE) (a) and scanning electron microscope images for different fine mannitol particles: spray dried mannitol (SDM) (b), commercial mannitol (CM) (c), cooling crystallized mannitol (CCM) (d), acetone crystallized mannitol (ACM) (e) and ethanol crystallized mannitol (ECM) (f) (MD mean diameter)

The presence of characteristic diffraction peaks with a good peak resolution and no halo or peak broadening within all PXRD patterns confirmed the crystalline nature of all the tested FMPs (Fig. 3). For CM, only β -mannitol was detected (Fig. 3b). Analysis of the 2θ values showed the specific angles of β -mannitol (10.56° , 14.71° , 23.4° , 29.5° and 38.8°) within the patterns of all FMPs, whereas the specific angles of α -mannitol (9.57° , 13.79° and 17.2°) were detected in only SDM, CCM, ACM and ECM (Fig. 3). The specific angles of δ -mannitol (9.7° and 22.2°) were detected only in CCM and ACM (Fig. 4c, d). These results indicated that CM was pure β -mannitol, while SDM and ECM consisted of both α - and β -mannitol. By comparison, CCM and ACM were mixtures of α -, β - and δ -mannitol (Fig. 3). Acetone and ethanol are known to induce polymorphic transitions of mannitol due to their ability to interact with the mannitol lattice structure through solvent hydroxyl groups, which break the intra- and inter-hydrogen-bonding networks of solid-state mannitol and induce crystal solid-state form conversion. It is well-known that α -,

β - and δ -mannitol are kinetically stable for a minimum of 5 years at ambient conditions (25°C) (40,41).

Characterisation of Lactose-SS-FMP Formulations

Formulation Powder Bulk Properties

No significant difference was obtained among all formulations under investigation in terms of bulk density ($0.51 \pm 0.01 \text{ g/cm}^3$), tap density ($0.65 \pm 0.01 \text{ g/cm}^3$) (SM5) and flowability ($\text{CI} = 20.9 \pm 1.6\%$, fair 'reasonable' flow character, $P > 0.05$, $F = 2.69$) (SM6). Similar bulk properties are important in terms of direct comparison between different DPI formulations (20). Comparing the formulation flowability data of both lactose-SS-ACM $_{<20 \mu\text{m}}$ and lactose-SS-ECM $_{<20 \mu\text{m}}$ ternary formulations (investigated in this study) with the flowability data of both ACM $_{63-90 \mu\text{m}}$ -SS (4) and ECM $_{63-90 \mu\text{m}}$ -SS (31) binary formulations (investigated previously) suggested that the use of ternary formulations over binary formulations is advantageous for maintaining

Fig. 2. Cumulative (in per cent, undersize) size distribution (a), mean diameter (MD) and span (mean $\pm$ SD, $n \geq 12$) (b) for different fine mannitol particles: circle spray dried mannitol (SDM), square commercial mannitol (CM), plus sign cooling crystallized mannitol (CCM), triangle acetone crystallized mannitol (ACM) and diamond ethanol crystallized mannitol (ECM) (SS salbutamol sulphate)

formulation flowability (because both $ACM_{63-90 \mu m}$ (4) and $ECM_{63-90 \mu m}$ (31) products demonstrated very poor flow properties).

Scanning Electron Microscopy of Lactose–SS and Lactose–SS–FMP Formulations

The lactose–SS formulation (Fig. 4a) exhibited relatively less fine particulates on the surface of lactose in comparison to the lactose–SS–FMP formulations (Fig. 4b–f). Except for the surface texture, no striking differences were observable in the blend structures of all formulations (Fig. 4). This concurred with the fact that the use of different FMPs did not profoundly affect the bulk properties of the formulation powders (SM5 and SM6). However, small differences in the physical properties of the formulation powders might have a remarkable effect on the powder dispersion behaviour (42). Unlike the lactose–SS formulation (Fig. 4a), the lactose–SS–FMP formulations revealed few free non-adhering particle assemblies ($<20 \mu m$) (Fig. 4b–f). In fact, adhesion and cohesion (autohesion) are inherent issues with fine particles

(such as SS and FMP), which could lead to the formation of different agglomerates and/or aggregates, including SS–SS, SS–FMP and FMP–FMP.

At higher magnifications, typical rectangular-shaped particles were observed for the SS alone powder, with sizes less than $5 \mu m$ and a high degree of agglomeration (Fig. 5a). Such agglomeration is well documented in micronised particles due to their small size, high surface to volume ratio and high surface energy (43). However, after blending with different lactose–FMP carriers, most of the SS particles were single (individual) particles adhered to the lactose surfaces (Fig. 5). However, a relatively high degree of randomisation was observed due to the higher concentration of fine particles. Indeed, based on the ‘total mixing concept’, when fine and coarse particles are mixed together, a dynamic equilibrium is suggested to take place between the adhesive and non-adhesive mix (44). The formation mechanisms of such mixtures have been described elsewhere (44–46).

Visual examination of the lactose–SS formulation showed that SS particles adhered particularly on cavities of the lactose surfaces, with an increased number of SS particles remaining in macroscopic depressions (indentations) (Fig. 5a, SM7). Drug particle detachment from crevices on a carrier surface is more difficult because it is believed that these drug particles can be protected from aerodynamic forces during aerosolisation and might even become relatively immobile in the deep cavities (12,47) (Fig. 6).

Two major mechanisms are believed to control drug particle detachment from carrier particles during inhalation: detachment by the flow stream (fluid forces, flow detachment) and detachment by impaction (mechanical forces, mechanical detachment) (48–50). The mechanism by which drug particles detach from carrier surfaces is dependent on the texture of the carrier surface. Detachment by flow is facilitated by flat, smooth surface–carrier particles (48,49), whereas drug detachment from carriers with rough surfaces is primarily dependent on impaction forces. In the case of lactose–SS–FMP formulations, FMPs adhering to the lactose surfaces formed ‘microscopic projections’ from which SS particles could be easily detached (Fig. 5b–f) because of reduced van der Waals SS–carrier interaction forces, which could lead to improved DPI performance (51) (Fig. 6).

Although the same batch of SS drug was used in the preparation of all formulations, the agglomeration/aggregation behaviour of SS varied considerably. In the case of lactose–SS–FMP formulations, some SS particles adhered to FMPs to form SS–FMP aggregates (fine particle aggregates, FPA) adhering to the large lactose surfaces (Fig. 5b–f). Generally, drug–fine ternary component aggregates are formed at the expense of drug–carrier interactive mixtures, and they are expected to behave differently during aerosolisation (18). According to ‘agglomeration theory’, the formation of such FPA (e.g. SS–FMP) is likely to improve DPI performance.

Homogeneity Test

Despite all formulations being blended following a similar protocol, the lactose–SS–FMP formulations exhibited

Fig. 3. XRPD patterns (cps counts per second) and polymorphic form for different fine mannitol particles: spray dried mannitol (SDM), commercial mannitol (CM), cooling crystallized mannitol (CCM), acetone crystallized mannitol (ACM) and ethanol crystallized mannitol (ECM)

lower percentage potency than the lactose–SS formulation ($83.0 \pm 7.9\%$ versus $91.0 \pm 8.8\%$, mean $\pm$ SD). This discrepancy could be attributed to decreased SS–carrier adhesive forces in the case of the lactose–SS–FMP formulations in comparison to the lactose–SS formulation (as proved later). This could then lead to higher amounts of SS being detached from the carrier surfaces during handling of the formulation powder (e.g. mixing, storage and metering).

The lactose–SS–FMP formulations exhibited improved SS content homogeneity in comparison to the lactose–SS formulation (%CV 2.0–7.9% versus 9.7%, SM8). CVs of 7.9 and 9.7% are an indication of inhomogeneous mixtures. This variation in homogeneity could be due to the relatively high degree of SS–SS agglomeration observed in the lactose–SS formulations (Fig. 5a, SM7). It was assumed that in the case of the lactose–SS–FMP formulations, the presence of FPA might not be sufficient to indicate a lower degree of drug content homogeneity

because such aggregates (FPA) themselves might be homogeneously distributed throughout the formulation powder, as suggested by Clark *et al.* (52) (Fig. 5b–f). FMP with a more elongated shape (higher AR/FR) generated formulations with improved SS content homogeneity (smaller % CV) (SM8). This improvement could be related to the reduced surface energy for mannitol particles with more elongated shapes (53) because particle surface energy could represent an ‘energetic barrier’ to drug–carrier blending (43,54).

Evaluation of SS–Carrier Adhesion Properties

For all formulations, the amounts of retained SS decreased with increasing sieving time (Fig. 7). The lactose–SS formulations produced the highest amounts of SS remaining after 30 s, 1 min and 3 min functional sieving times (Fig. 7), demonstrating the strongest SS–carrier adhesion

Fig. 4. SEM photographs of different dry powder inhaler formulations: lactose–SS (**a**), lactose–SS–SDM (**b**), lactose–SS–CM (**c**), lactose–SS–CCM (**d**), lactose–SS–ACM (**e**) and lactose–SS–ECM (**f**) (SS salbutamol sulphate, SDM spray dried mannitol, CM commercial mannitol, CCM cooling crystallized mannitol, ACM acetone crystallized mannitol, ECM ethanol crystallized mannitol, FPF fine particle fraction (mean±SD, $n=3$), CV coefficient of variation, FPA fine particle aggregates)

among the formulations. The lactose–SS–ECM formulation produced the lowest amount of SS remaining after all the functional sieving times (Fig. 7), indicating that this formulation generated the weakest SS–carrier adhesive forces among the formulations.

The presence of FMPs on the lactose surface may decrease the SS–lactose contact area (Fig. 5b–f) (9), leading to a weaker SS–lactose adhesion force. Alternatively, in the case of the lactose–SS formulations, SS particles are adhered directly, and thus tightly, to the lactose surfaces or entrapped within a gap in the lactose surface (Fig. 5a, SM-7). Both of these situations could result in high lactose–SS interparticulate forces (9). Linear relationships were established when plotting the amounts of SS remaining after 5 s ($r^2=0.9619$) and 30 s ($r^2=0.9152$) functional sieving times against the AR/FR of the FMPs (Fig. 7). This linearity indicated that SS particles adhered to the surface of lactose–FMP carrier detached more easily (efficiently) in the case of FMPs with more elongated shapes (FMPs with more elongated morphology generated weaker SS–carrier adhesive forces).

Weaker adhesive forces indicated lower physical interactions. The dispersion behaviour of fine SS particles is believed

to be dependent on the agglomerate structure and mechanical strength (20). In comparison to isometric particles, the contact area in the case of elongated particles is unpredictable, leading to better deaggregation properties (12). It is believed that the detachment of SS from FMPs is easier in the case of more-elongated FMPs due to the lower SS–FMP interparticulate contact area (fewer points of physical contact). In contrast, when the flat region on the carrier surface increases (as in the case of more-flattened FMPs), higher degrees of SS–carrier adhesion and SS–SS cohesion are expected due to the higher probability of direct contact (12).

During blending, particle collisions could lead to greater lactose–SS adhesion due to the small SS particles becoming more tightly attached to the lactose surface or swept into the carrier surface crevices (55). Lower lactose–SS adhesive forces in the case of formulations containing FMPs could be attributed to lower lactose–SS press-on forces during mixing. FMPs could provide a ‘protection effect’ by coating lactose particles, thus cushioning SS particles from impact (generate weaker *in situ* collision and friction (push-on) forces during

Fig. 5. SEM photographs of different dry powder inhaler formulations: lactose-SS (a), lactose-SS-SDM (b), lactose-SS-CM (c), lactose-SS-CCM (d), lactose-SS-ACM (e) and lactose-SS-ECM (f) (↓ SS salbutamol sulphate, SDM spray dried mannitol, CM commercial mannitol, CCM cooling crystallized mannitol, ACM acetone crystallized mannitol, ECM ethanol crystallized mannitol, FPF fine particle fraction, FPA fine particle aggregates)

mixing). However, in theory, a high SS-carrier adhesion does not necessarily mean a small FPF of SS because it can be hypothesised that both SS particles and FPA might travel to lower airways depending on their inertial impaction. Therefore, all formulations were further assessed in terms of *in vitro* aerosolisation performance.

Deposition Tests of SS

Satisfactory drug recovery and a MMAD between 1 and 5 μm are key for successful pulmonary drug delivery *via* DPIs. In this study, no significant difference ($P>0.05$) was found among all formulations in terms of RD ($302.1 \pm 30.6 \mu\text{g}$), ED ($266.6 \pm 29.8 \mu\text{g}$) (SM9), recovery ($83.7 \pm 6.8\%$), emission ($87.9 \pm 2.2\%$) (SM10), MMAD ($2.8 \pm 0.1 \mu\text{m}$) and GSD (2.2 ± 0.0) (SM11). Similarity in terms of RD, ED, recovery and emission suggested similar amounts of SS available to the overall pulmonary region (or the impactor) when delivered from different formulations. Such similarity could be related to the similar weight of all filled doses within the capsules, the similar formulation powder bulk properties (SM5 and SM6) and the high number of actuations ($n=10$) used during each aerosolisation test of each formulation. The

experimental aerodynamic diameter of SS (MMAD) was slightly higher than its theoretical aerodynamic diameter ($d_{ae}=2.2 \pm 0.2 \mu\text{m}$), which could be related to both the rectangular shape of the SS particles and the SS-SS agglomeration of SS powder (Fig. 5a). In turn, the d_{ae} of SS was slightly higher than its geometric diameter ($d_e=1.9 \pm 0.2 \mu\text{m}$), which was expected because particle density for SS is >1 ($1.39 \pm 0.01 \text{ g/cm}^3$) (24). However, as a PSD descriptor, MMAD is more suitable than both d_e and d_{ae} because it reflects aerosol particles during aerosolisation.

A GSD >1.25 proved a heterogeneous (polydisperse or non-uniform) aerodynamic PSD of SS generated by all formulations upon aerosolisation. However, similarity between different formulations in terms of RD, ED, recovery, emission, MMAD and/or GSD does not necessarily indicate similar aerosolisation performance. Satisfactory RD (between 75% and 125%) and optimal MMAD (between 1 and 5 μm) confirmed the acceptable dispersible nature of all lactose-SS-FMP formulations. These RD, ED, recovery and emission values also indicated that drug recovery from the MSLI stages by washing was good and that the method used to assay the SS was reasonably accurate with good reproducibility.

Fig. 6. Schematic drawing representation of the role of fine mannitol particles with different morphologies (commercial mannitol: angular morphology, and ethanol crystallised mannitol: needle morphology) on the entrapment and aerosolisation capabilities of salbutamol sulphate drug. Less drug–carrier adhesion forces is suggested in the case of more shape-elongated fine particle additive

Because the same batch of drug (SS) and carrier (lactose) were used in the preparation of all formulations, variations in the aerosolisation performance could be attributed to different FMP products. All formulations produced a similar capsule retention (amounts of drug remained in capsules shells, $6.2 \pm 1.4\%$, $P > 0.05$) (Fig. 8). It can be assumed that SS particles detach from carrier particles even inside the capsules and consequently deposit on the capsule walls. In comparison to the lactose–SS formulations, the lactose–SS–FMP formulations generally deposited higher amounts of SS on I+M ($4.8 \pm 0.8\%$ versus 4.1 ± 0.4 – $7.8 \pm 0.5\%$) and throat (IP, $2.6 \pm 0.6\%$ versus 4.7 ± 0.7 – $6.5 \pm 0.5\%$) (Fig. 8). This deposition pattern agreed with previous studies and could be ascribed to the higher total fine content in the case of lactose–SS–FMP formulations compared to lactose–SS formulations (6). MSLI deposition data indicated that the lactose–SS–ECM formulation would deliver the highest amounts of SS to lower airways (Fig. 8). The results also showed that the amounts of SS deposited on MSLI stage 1 (representing the upper airways) decreased linearly ($r^2 = 0.9536$) with increasing AR/FR of FMPs (SM12). However, FMPs with more elongated shapes (higher AR/FR) produced higher amounts of SS on stage 2 ($r^2 = 0.9247$), stage 3 ($r^2 = 0.6595$), stage 4 ($r^2 = 0.8482$) and filter ($r^2 = 0.7477$) (SM12).

The aerodynamic PSD of SS generated by different formulations indicated that SS particles within the lactose–SS formulations were not sufficiently dispersed into individual particles (Fig. 8), likely due to a high SS–SS agglomeration (Fig. 5a, SM-7) (56) and/or high lactose–SS adhesion (Fig. 7). The rank order of FPF generated by different formulations

was lactose–SS ($14.9 \pm 1.2\%$) < lactose–SS–SDM ($18.9 \pm 0.7\%$) $\leq$ lactose–SS–CM ($20.1 \pm 5.3\%$) $\leq$ lactose–SS–CCM ($23.1 \pm 1.1\%$) < lactose–SS–ACM ($28.8 \pm 0.3\%$) < lactose–SS–ECM ($32.6 \pm 1.1\%$) (Fig. 8). The rank order of IL obtained from different formulations was lactose–SS–ECM ($46.8 \pm 4.1\%$) < lactose–SS–ACM ($53.9 \pm 1.3\%$) < lactose–SS–CCM ($61.1 \pm 2.1\%$) $\leq$ lactose–SS–CM ($64.9 \pm 6.8\%$) $\leq$ lactose–SS ($72.3 \pm 2.5\%$) < lactose–SS–SDM ($79.3 \pm 2.5\%$). The IL amounts could be related to the amounts of SS–SS agglomerates and/or the amounts of SS that were still adhered to the coarse carrier and had not been released during aerosolisation. Because the target site of SS is the lower airways, both smaller IL and higher FPF are indicative of higher pharmacologically active amounts of drug and hence a better DPI performance. The results showed that AR/FR of FMPs was inversely proportional to IL ($r^2 = 0.8892$) and proportionally related to FPF ($r^2 = 0.9345$) of SS (Fig. 9a), indicating better DPI performance from more elongated FMPs. It was assumed that the particle morphology of the FMPs affected the contact geometry between the SS and lactose particles and consequently could have a substantial impact on SS–lactose adhesion (Fig. 6).

FMPs with more elongated shapes could have a higher ability to reduce the number of lactose–SS physical contact points and thus are thought to lead to an easier SS detachment from the lactose carrier surfaces upon aerosolisation. Additionally, correlations were established when plotting the FPF of SS against the amounts of SS remaining after 5 s ($r^2 = 0.6892$), 30 s ($r^2 = 0.9197$), 1 min ($r^2 = 0.8919$) and 3 min ($r^2 = 0.7916$) functional sieving times (Fig. 9b). This indicated that the smaller the SS–carrier

Fig. 7. Percent amounts of remaining salbutamol sulphate (mean $\pm$ SD, $n\geq 3$) after different functional sieving times (5, 30, 60 and 180 s) obtained from lactose–SS formulation (black diamond) (no fines) and lactose–SS formulations containing different fine mannitol particles: red square spray dried mannitol (SDM), dark yellow triangle commercial mannitol (CM), white circle cooling crystallized mannitol (CCM), white square acetone crystallized mannitol (ACM) and blue circle ethanol crystallized mannitol (ECM) and % amounts of remaining SS (mean $\pm$ SD, $n\geq 3$) after (white diamond) 5 s and (blue triangle) 30 s functional sieving times in relation to aspect ratio/flatness ratio (AR/FR, mean $\pm$ SE) of fine mannitol particles. Less drug–carrier adhesion forces were obtained in the case of fine mannitol particles with more elongated shape

adhesion, the higher the amounts of SS expected to be delivered to the lower airways. These results confirmed that SS particles/agglomerates formulated with FMPs with more elongated shapes were easier to disperse/re-disperse during aerosolisation.

In the case of the lactose–SS formulation, SS particles are likely to occupy the ‘active sites’ on the lactose surfaces. However, in the case of lactose–SS–FMP formulations, FMP particles are expected to occupy the active sites, leaving SS particles to re-orient on less binding sites on the lactose surface. As a consequence, higher amounts of SS were dispersed upon aerosolisation.

SS particles were assumed to exist in different possible states: (1) individual SS particles, (2) SS–SS agglomerates, (3) SS–lactose aggregates and (4) SS–FMP aggregates. In the case of lactose–SS–FMP formulations, FPA (SS–FMP aggregates) (Fig. 5b–f) were expected to detach from the lactose particle surfaces more easily than single SS particles due to their relatively greater detachment mass (detachment by impaction, mechanical detachment) (9,48–50). In addition, it was assumed that the detachment of SS particles from FPA (Fig. 5b–f) was

easier than SS particle detachment from coarse lactose particles because fine carrier particles have smoother surfaces, leading to reduced drug–fine carrier adhesive forces (6,10) (Figs. 5 and 7).

The interactions between SS particles, SS–SS self-agglomerates, SS–FMP aggregates and lactose–SS units with single- or multi-SS particles were largely impacted by the morphology of the FMPs. The formation of SS–FMP aggregates is thus likely to reduce the number of SS–SS interactions and improve the aerosolisation performance of SS (8). During mixing, fine mannitol additives and drug compete for active sites. It is therefore possible that FMPs and SS particles are continually exchanged on the active sites of coarse lactose (dynamic situation) due to the competition between cohesion (SS–SS, FMP–FMP or SS–FMP) and adhesion (lactose–SS or lactose–FMP). The outcome of such competition in the resultant mixture is doubtful, but adhesion is believed to be favoured in the case of smaller drug or carrier particles and rougher carrier surfaces [46].

Upon aerosolisation, fine ternary component particles might have similar deposition profiles to the drug

Fig. 8. Amounts of salbutamol sulphate (SS) (mean $\pm$ SD, $n=3$) deposited on capsules, inhaler with mouthpiece adaptor (I + M), indication port (IP) and different MSLI stages, and fine particle fraction (FPF) obtained from lactose-SS formulation and lactose-SS formulations containing 4.7% (w/w) of different fine mannitol particles: spray dried mannitol (SDM), commercial mannitol (CM), cooling crystallized mannitol, acetone crystallized mannitol and ethanol crystallized mannitol (ECM) (S statistically similar, RD recovered dose, ED emitted dose, $MMAD$ mass median aerodynamic diameter, GSD geometric standard deviation). Aerodynamic particle size distribution of SS obtained from lactose-SS formulation (no fines) (orange circle) and lactose-SS formulations containing different fine mannitol particles: SDM (red square), CM (dark yellow triangle), CCM (blue diamond), ACM (gray diamond) and ECM (white circle) (mean $\pm$ SD, $n=3$). DPI formulations containing fine mannitol particles with more elongated shape generated drug particles with smaller aerodynamic size upon aerosolisation

(provided that they have similar density) (57). It is thus acknowledged that morphology, surface area and projected area in the settling direction of particles have a decisive effect on the drag forces on the particles.

Needle-shaped crystals are easier to keep airborne within airflow in comparison to isometric particles with similar size; thus, needle-shaped crystals could deposit further on the lower airway regions (58). Under similar conditions,

Fig. 9. White circle fine particle fraction (FPF) and white square impaction loss (IL) of salbutamol sulphate (SS) (mean $\pm$ SD, $n=3$) in relation to aspect ratio/flatness ratio (AR/FR) of different fine mannitol particles (mean $\pm$ SE) (a) and FPF of SS (mean $\pm$ SD, $n=3$) relative to % remained amounts SS (mean $\pm$ SD, $n \geq 3$) after different functional sieving times (black circle 5, red square 30, dark yellow triangle 60 and blue diamond 180 s) obtained from lactose–SS formulations containing different fine mannitol particles (b)

non-isometric (more elongated) particles have more separation distance than isometric (more spherical) particles (12). For formulations with a fixed mass of SS, the mass of SS particles available for contact with the lactose particle surface per unit area of lactose carrier was lower in formulations containing FMPs with a more elongated shape due to their higher theoretical specific surface area (data not shown). As a result, FMPs with more elongated shape could have a higher potential to fill the active sites on the lactose surfaces. Finally, this could lead to a decrease in the mass of SS particles adhered to the adhesion active sites per unit area of lactose carrier in the case of FMPs with more elongated shape.

To evaluate the influence of several formulation parameters simultaneously, the overall desirability function parameter (DF) was employed (59). Different formulations were compared quantitatively using a desirability function (d_i) (27) ($d_{i\max} = \frac{Y_i - Y_{\min}}{Y_{\max} - Y_{\min}}$, where Y_i is the actual observed value of the response variable, $Y_{\max}$ is the maximum acceptable value of the variable and $Y_{\min}$ is the minimum acceptable value of the variable). Except for

the *in vivo* lung deposition pattern and drug safety, FPF was considered a good quality control indicator of DPI pharmaceutical performance. Homogeneous drug content with the DPI formulation is critical during DPI design to attain regular doses during treatment, whereas better flowability is desirable for satisfactory dosing, managing and industrial processing. The maximum response variables were defined as follows: FPF=100%, CV=40% and CI=40%, whereas the minimum response for the measured variables were set as follows: FPF=10%, CV=1% and CI=5%. The overall desirability function was calculated as follows: $DF = (d_{FPF} + 1/d_{CV} + 1/d_{CI})^{1/3}$. The DF obtained for different formulations was in the following rank order: lactose–SS < lactose–SS–SDM $\leq$ lactose–SS–CM $\leq$ lactose–SS–CCM < lactose–SS–ACM < lactose–SS–ECM (Fig. 10). These results suggested that the lactose–SS–ECM formulation produced the best overall performance in terms of the highest amounts of drug potentially deliverable to the lower airways with satisfactory flowability and minimum dose variability. Interestingly, the lactose–SS–ECM_{<20 μ m} formulation demonstrated both suitable flow properties (CI=23%) and improved aerosolisation performance (FPF=33%) (Fig. 10). The results showed that the DPI formulation desirability increased as the shape elongation (AR/FR) of FMP increased (Fig. 10).

Further studies should be carried out to evaluate the influence of FMP on DPI performance in the case of higher drug and FMP masses *in vitro* and *in vivo*. Stability studies should also be performed at higher temperatures/humidities. More efforts are warranted to evaluate the influence of fine mannitol particles on DPI performance in the case of a wider diversity of drugs, drug-to-fine additive ratios, carriers, inhaler devices and flow rates. Moreover, further mechanistic understanding of the influence of engineered fine additive materials on DPI performance is required, which will be the subject of further research.

CONCLUSIONS

A significant improvement in the performance of a dry powder inhaler was achieved by the careful selection of an engineered fine additive particle product. When employing fine mannitol particles with aspect ratio/flatness ratio values between 1.7 and 5.8 within the typical salbutamol sulphate–lactose formulation, fine mannitol particles with more elongated shapes produced better drug content homogeneity, weaker drug–carrier adhesion, higher fine particle fraction of drug and higher overall formulation desirability. The fine particle fraction of the drug tends to increase linearly as the drug–carrier adhesion decreases. The limitation of poor flowability associated with the elongated-shaped coarse carrier particles could be overcome by using only 5% (w/w) elongated fine particle excipient as an additive. The use of engineered fine additive particles with pre-determined ‘selected’ properties can be a new potential approach to improve the performance of a dry powder inhaler. It is believed that this is the first study to provide a systemic evaluation of the effect of engineered fine particle

Fig. 10. Over all desirability function (DF; mean $\pm$ SD, $n=3$) for formulations composed of lactose-salbutamol sulphate (SS) (a), lactose-SS formulations containing different fine mannitol particles: spray dried mannitol (SDM) (b), commercial mannitol (CM) (c), cooling crystallized mannitol (CCM) (d), acetone crystallized mannitol (ACM) (e) and ethanol crystallized mannitol (ECM $_{\leq 20 \mu\text{m}}$) (f) and DF for ECM $_{63-90 \mu\text{m}}$ -SS formulation (g) (CI Carr's index, FPF fine particle fraction)

additives on the performance of a dry powder inhaler. This study provides a new insight for researchers to

investigate the influence of engineered fine particle sugars (in addition to engineered mannitol) on the aerosolisation

performance of binary drug-carrier dry powder inhaler formulations. However, it should be kept in mind that different fine additive excipient materials (e.g. lactose) might have different interaction forces with the same model drug investigated in this study.

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