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Adhesion and redistribution of salmeterol xinafoate particles in sugar-based mixtures for inhalation

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Abstract

The aim of this study was to evaluate coarse and fine sugars as potential alternative excipients in dry powder inhalation formulations and to develop a greater understanding of the key interactions between the particulate species in these mixtures. Interactive mixtures composed of salmeterol xinafoate (SX) and different type of sugars (lactose, glucose, mannitol and sorbitol) were prepared using validated laboratory scale mixing. The sugars and SX were characterised by laser diffraction, scanning electron microscopy, atomic force microscopy and loss on drying method. Deposition of SX was measured using a twin-stage impinger and analysed using validated HPLC method ($r^2 = 1.0$, CV = 0.4–1.0%). Good correlation existed between the fine particle fraction (FPF) of SX and both the adhesion force and the moisture content. The addition of 10% fine sugars to produce ternary mixtures (i.e. SX, coarse and fine sugars) generally increased dispersion, with the addition of fine glucose > fine mannitol > fine lactose > fine sorbitol. The dispersion of SX showed a reciprocal relationship with the moisture content of the sugars with glucose showing the greatest and sorbitol showing the lowest extent of SX dispersion. The study clearly demonstrated that strong SX adhesion to coarse sugars reduced the extent of dispersion and that surface detachment of the SX and fine sugar from the coarse sugar carrier was important in the dispersion process.

Keywords: Dry powder inhalation; Sugars; Salmeterol xinafoate; Interactive mixtures; Agglomerate

1. Introduction

Aerosol inhalation has become well established as a method of drug delivery to the respiratory tract in the treatment of lung disease. Most commercially available carrier-based dry powder inhalers (DPIs) have a relatively low efficiency with fine particle fractions (FPF) below 20% (Steckel and Muller, 1997). The performance of DPIs depends on the properties of the powder formulation, the design of the device and inspiratory manoeuvres by the patient. The rational development of powder formulations requires understanding of the basic material properties related to the aerodynamic particle behaviour and adhesion, combined with reliable methods for solid-state and particle size analysis (Shekunov et al., 2003). Drug particle sizes of less than 5 μm are required to penetrate into the lung airways upon inhalation and these particle are usually highly interactive. Coarse carriers such as lactose are combined with drug to improve flowability and ex-device delivery of the powder (French et al., 1996).

Dispersion of micronised drug in respiratory delivery will be dependent on its cohesive and adhesive properties in the powder formulations. Particulate interactions occur in mixtures of micronised drugs since particle detachment forces are relatively low and the balance between interactive and detachment forces favors cohesion to give agglomerates or adhesion to give interactive mixtures. Effective respiratory delivery requires the dispersion of drug from agglomerates and interactive units using the energy generated in the inhalation device.

The mechanism by which drugs are dispersed is not fully understood. The magnitude of particle interactions between drugs and between drugs and excipients will be important in the dispersion process. One theory that has been advanced to explain dispersion involves the interaction of fine lactose present in the lactose excipient with the high energy sites on the carrier's surface causing saturation, leaving low energy, passive sites available for drug adhesion (Zeng et al., 1996, 1998). The reduced adhesion between drug and carrier particles increased

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drug detachment. Recently, a hypothesis based on competitive adhesion and redistribution of the drug between the carrier surface and fine excipient to produce mixed agglomerates of drug and fine excipient was proposed to explain improved drug dispersion (Louey and Stewart, 2002). Greater efficiencies were likely to result through facilitated drug detachment from the carrier surface as agglomerates and better dispersion of mixed agglomerates by the dry powder inhaler.

Almost all DPI formulations use lactose monohydrate as carrier (Larhrib et al., 1999). Lactose monohydrate is commonly employed since it fulfils the many ideal requirements of excipients, although other sugars such as mannitol and trehalose obtained as crystalline sieve fractions are suitable as carriers for dry powder aerosols (Byron et al., 1996). Several authors have examined sugars as potential carriers (Louey and Stewart, 2002; Steckel and Bolzen, 2004; Tee et al., 2000). Formulations of salbutamol sulphate (SS) containing different sugars produced FPF in decreasing order of mannitol > sorbitol > lactose; however, the chemical nature of the fine sugar particles was found to play a less important role in determining respirable fraction of the drug than the coarse carriers (Tee et al., 2000). In contrast, higher FPF were obtained in ternary mixtures of SS and coarse lactose with micronised glucose (VMD of 4.4 μm) than with micronised lactose (VMD of 4.0 μm) (Louey and Stewart, 2002). The roles of both the coarse sugar as the carrier and the fine sugar associated with the coarse sugar are important in the drug dispersion because of their potentially differing propensities to interact with the drug. Therefore, the aim of this study was to gain a better understanding of adhesion and particulate redistribution, and their influence on drug dispersion from carried-based mixtures for inhalation using different coarse and fine sugars. Specifically, the study used salmeterol xinafoate as a model drug and sugars included lactose, glucose, mannitol and sorbitol. Scanning electron and atomic force microscopy were used to observe the multi-particulate species of the powder mixtures and to quantify adhesion, respectively.

2. Materials and methods

2.1. Materials

Salmeterol xinafoate, micronised (SX; volume mean diameter of 2.4 μm) (Glaxo SmithKline, Australia), inhalation grade of α-lactose monohydrate (Inhalac 120) (Meggle GmbH, Germany), D-glucose anhydrous (Ajax Chemicals, Australia), D-mannitol (BDH Laboratory Supplies, UK) and D-sorbitol (VWR International Ltd., UK). Chemical and solvents used were ammonium acetate (Analar, Australia), methanol (HPLC grade, Biolab, Australia) and propan-2-ol (Merck Pty. Ltd., Australia), were used as supplied.

2.2. Preparation of coarse and fine sugar

Coarse sugar carriers consisting of coarse lactose (CL), coarse glucose (CG), coarse mannitol (CM) and coarse sorbitol (CS) were prepared by dry sieve classification using a sieve shaker (Fritsch, Germany) and test sieves (106–180 μm (Endcotts, UK)) for 30 min. Fine lactose (FL), fine glucose (FG), fine mannitol (FM) and fine sorbitol (FS) were obtained by fluid energy milling (Chrispro Jetmill, USA) with injector pressure of 862 kPa and grinding pressure of 552 kPa. All sugar particles were stored in a desiccator over silica gel.

2.3. Particle sizing of sugar particles

The particle size distribution of powders was measured by laser diffraction (Malvern Mastersizer S, Malvern Instruments Ltd., UK) using the 300 RF lens and the small volume sample presentation unit (capacity 150 ml). Approximately 500 mg of sugar powder was dispersed in 5 ml of propan-2-ol with the aid of a sonication in a water bath for 3 min. Particle size analysis of each sample was performed using 2000 sweeps and analysed by suspending the sonicated sample in propan-2-ol (refractive index of 1.378) and using the estimated imaginary refractive index of the sugars as 0.1. The average particle size distribution was measured from five replicates of each sample. The particle size of the primary powders was described by the volume mean diameter (VMD). The size distributions were characterised by the 10 and 90 percentiles ($d_{10\%}$ and $d_{90\%}$, respectively). The residual values were always below 1%.

2.4. Formulation and preparation of interactive mixtures

The interactive powder mixtures were prepared by a validated laboratory mixing process (Liu and Stewart, 1998). Ternary interactive mixtures composed of SX (VMD of 2.4 μm), coarse sugar carrier (CL, CG, CM and CS) and fine sugar (FL, FG, FM and FS) were prepared (ratio of SX:coarse sugar:fine sugar of 87.5:2.5:10, w/w). In addition, binary mixtures consisting of different sugar carriers (ratio of coarse sugar:SX of 97.5:2.5, w/w) were also prepared in 5 g batches. All the mixtures were stored in a desiccator over silica gel until further required.

2.5. Drug dispersion by twin-stage impinger (TSI)

Using a Rotahaler® (Glaxo Wellcome), the in vitro aerosol dispersion of the powder formulations was determined using a twin-stage impinger (TSI, Apparatus, A; British Pharmacopoea, 2000) (Copley, Nottingham, UK). A solvent of 60% methanol (HPLC grade) was used as the collection liquid with 7 and 30 ml were placed in stage one and two of the TSI, respectively. The air flow was drawn through the TSI using a vacuum pump (Dynavac Engineering, Vic., Australia) and the air flow rate was adjusted to 60 l/min at the mouthpiece prior to each measurement (Fisher and Porter, UK). The temperature ($20.0 \pm 1.0\,^{\circ}C$) and relative humidity of the surrounding environment ($50 \pm 3\%$ RH) was measured by a thermo-hygrometer (Shinyei TRH-CZ, Japan). The aerodynamic cut-off diameter at 60 l/min was 6.4 μm. An air volume of 4 l (4 s at 60 l/min) was drawn for each measurement. Each TSI section (inhaler, stage one and stage two) was rinsed with 40% methanol–60% water, the liquid was collected and the volume adjusted to 100 ml. Five TSI replicates for each mixture were performed and were randomised for formulation. The SX concentration was determined by a validated high performance

liquid chromatography (HPLC) assay described below. The fine particle fraction (FPF) was defined as the amount of SX deposited in the lower stage (stage 2) of the TSI as a percentage of the total recovered dose (defined as drug content collected from all components of TSI and inhaler). The emitted dose (ED) was calculated as the amount of drug recovered from all stages of the TSI (excluding inhaler) as a percentage of recovered dose.

2.6. HPLC analysis of salmeterol xinafoate

SX was analysed by high performance liquid chromatography (HPLC) using a C_{18} column (μBondapak™, 3.9 mm × 300 mm, Waters) and an UV detector (Waters Tunable Absorbance Detector, USA) at a wavelength of 252 nm. A mixture of methanol and 0.2% (w/v) ammonium acetate solution (55:45, pH ∼ 6.9) was used as a mobile phase running at a flow rate of 1.0 ml/min by a HPLC pump (Waters 510, USA). The peak area was recorded by integration (Shimadzu CR6A Chromatopack, Japan). The retention time of SX was 4.2 min. The calibration plot of standard SX solutions was linear over the range of 0.4–10 μg/ml with $r^2 = 1.00$. Five replicates of standards samples of 0.4, 1.0, 4.0 and 10.0 μg/ml solutions were performed for assay validation and the mean accuracy was 91.4 ± 2.4%, 100.4 ± 5.3%, 101.3 ± 1.1% and 99.8 ± 0.6%, respectively (mean ± S.D.). The precision was tested before each experiment by analyzing 1.0 and 4.0 μg/ml standard solution of SX using five replicates and the CV was below 2%.

2.7. Scanning electron microscopy (SEM)

Powder samples were glued and mounted on metal sample plates. The samples were gold coated (thickness ≈ 15–20 nm) with a sputter coater (BAL-TEC SCD 005, Japan) using an electrical potential of 2.0 kV at 25 mA for 10 min. The surface morphology of the particles was examined using a Hitachi S-570 scanning electron microscope (Tokyo, Japan) operating at 15 kV.

2.8. Atomic force microscopy (AFM)

The force of adhesion between SX and the various lactose samples was determined using the colloid probe AFM technique. The methods of preparation and measurement of SX force measurements using this technique is described in more detail elsewhere (Islam et al., 2004b). Briefly, the AFM measurements were preformed using a commercial AFM (TMX 1010, Topometrix, USA). Prior to force measurement, particles of SX (2–3 μm diameter, $n = 3$) were fixed onto standard V-shaped cantilevers (Topometrix, CA, USA) using an epoxy resin glue (Araldite, Selley Pty. Ltd., Vic., Australia). A high resolution, reflective mode microscope was used throughout the procedure to evaluate tip cleanliness, quantity of glue and tip-particle integrity prior to and post-curing. The spring constant of the cantilevers ($k = 0.067$ N/m) was determined by using the inbuilt AFM software. The adhesion force distribution for each sample was obtained from adhesion measurements at greater than 50 individual sites on at least five different particles.

2.9. Statistical analysis

Comparison between different groups of FPF was performed using one-way analysis of variance (ANOVA) (SPISS, USA), with probability values of less than 0.05 considered as statistically significant.

3. Results and discussions

3.1. Morphology of the sugar carriers and fine excipients

SEMs of the coarse sugars are presented in Fig. 1. The coarse lactose exhibited a tomahawk shape, typical of α-lactose monohydrate grown to maturity (Fig. 1A). Coarse glucose particles appeared to be more elongated with a smoother surface compared with lactose (Fig. 1B). Mannitol particles were observed as elongated, flaky particles with more surface asperities than lactose (Fig. 1C). The sorbitol particles were symmetrical, round containing many small pores (Fig. 1D). Pores on the coarse sorbitol were formed during the hydration and dehydration process as sorbitol is highly hygroscopic and easily loses its water of crystallisation (Tee et al., 2000). Interestingly, although sorbitol is a stereo-isomer of mannitol, it has different physical properties, including greater hygroscopicity (Tee et al., 2000).

3.2. Particle size distributions of coarse and fine sugars

The particle size parameters of coarse and fine sugars are summarised in Table 1. Not unexpectedly, the classified coarse sugars did not result in exactly similar particle size distributions. For example, coarse mannitol showed a VMD of 225.8 μm with 10% of particle below 109.3 μm, whereas coarse sorbitol possessed a smaller VMD of 187.5 μm with 10% of particle below 118.2 μm. The difference in particle size distributions may be attributed to differences in both the original particle distributions and the particle shape of these coarse sugars. As the particle shape deviates from spherical, the sieve and volume equivalent spherical diameters are more likely to differ giving rise to differences in the particle size distribution determined by laser diffraction. The particle size distribution of coarse sugars showed mono-modal distributions with the presence of fine sugar being relatively low (less than 1.4% <5 μm). Since previous findings concluded that drug dispersion was not intrinsically influenced by carrier VMD (Islam et al., 2004a), but associated

Table 1
Particle size distribution of coarse and fine sugars

Sample	d_{10} (μm)	VMD (μm)	d_{90} (μm)	Particle <5 μm
Coarse lactose	116.1 (0.4)	161.3 (0.3)	212.9 (0.6)	1.3 (0.2)
Coarse glucose	85.6 (0.5)	177.9 (0.6)	286.5 (1.7)	0.7 (0.1)
Coarse mannitol	109.3 (0.7)	225.8 (2.0)	208.7 (0.7)	1.4 (0.1)
Coarse sorbitol	118.2 (3.4)	187.5 (1.8)	269.2 (2.0)	0.0
Fine lactose	1.2 (0.1)	5.5 (0.1)	10.4 (0.1)	64.4 (0.6)
Fine glucose	0.7 (0.1)	3.9 (0.1)	7.5 (0.1)	69.4 (0.8)
Fine mannitol	0.9 (0.0)	3.3 (0.0)	6.5 (0.0)	78.4 (0.4)
Fine sorbitol	0.6 (0.1)	6.5 (0.4)	15.4 (1.8)	55.0 (1.5)

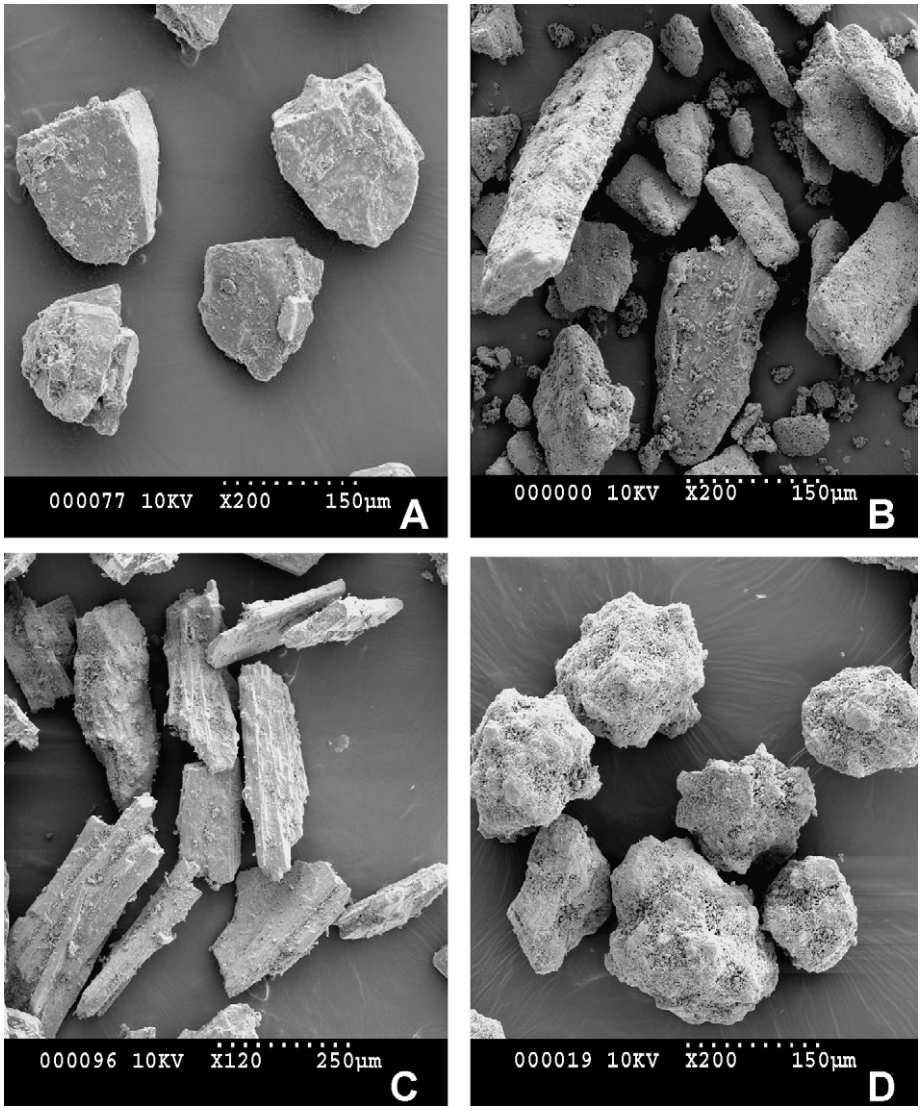

Fig. 1. Scanning electron micrographs of sugar carriers: (A) coarse lactose (magnification 500×), (B) coarse glucose (magnification 150×), (C) coarse mannitol (magnification 250×), and (D) coarse sorbitol (magnification 150×).

with the presence of fine lactose adhered on the surface of the lactose carrier, the effect of different sugar distributions was not further investigated.

The size distribution of fine sugars used as added ternary dispersion modifiers, showed VMD ranging from 3.3 to 6.5 μm. About 90–99% particles were below 10 μm, except for fine sorbitol (where 80% were less than 10 μm), and 55–78% particles were below 5 μm. FL and FS were slightly larger than FM and FG (Table 1). Milling conditions were similar for all sugars and the slightly different fine sugar distributions reflected the differing propensity of the sugar particles to undergo comminution.

3.3. Effect of the sugars as carriers in SX–sugar binary mixtures

3.3.1. In vitro deposition SX from SX–sugar binary mixtures

The FPF and ED of the binary mixtures were determined using SX at a concentration of 2.5% (Fig. 2A). This concentration was chosen since it provided a drug loading that was likely to result in agglomeration and to allow comparison with previous studies (Islam et al., 2004a,b). SX alone showed a FPF of 10.7% and the binary sugar mixtures produced FPF of SX ranging from 4.0% to 11.5%. The low fine sugar content of each of these coarse sugars would have little effect on the extent of dispersion of SX (Islam et al., 2004b). Therefore, the physicochemical nature of the coarse sugars, especially their adhesion capacity, was important in determining the FPF of SX from the binary sugar mixtures. The effect of the coarse lactose carrier in reducing the FPF from 10.7% to 4.0% was attributed to the strong adhesion of micronised drug particles to the coarse lactose particles and, therefore, a high detachment force was required to remove the drug particles from the carrier before they can be entrained in the air stream and efficiently delivered to the lungs (Visser, 1989).

The FPF of SX from sorbitol mixtures was not significantly different from that of lactose mixtures ($P > 0.05$); however,

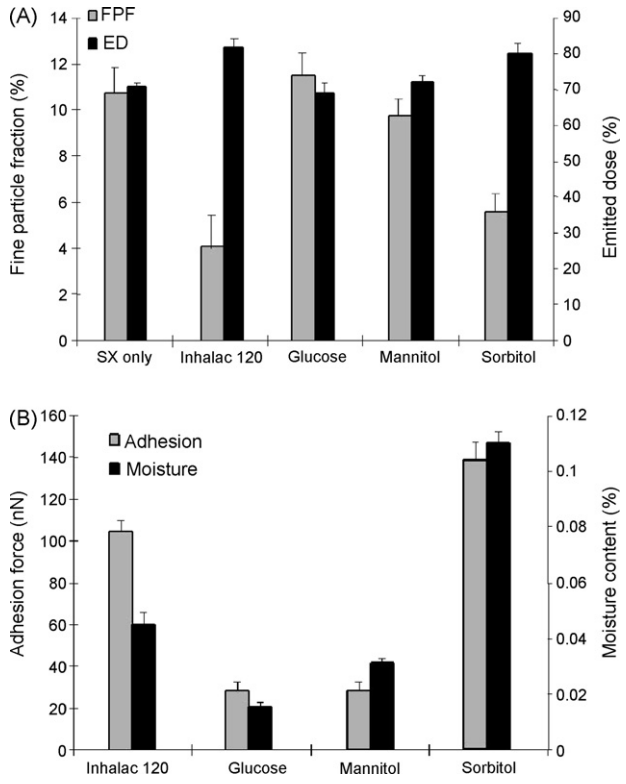

Fig. 2. Salmeterol xinafoate (SX) dispersion from mixtures of SX and coarse sugars. (A) Effect of different coarse sugars on the FPF and ED of SX, (B) Relationship between SX adhesion force and moisture content for the different coarse sugar.

the FPF of SX from glucose and mannitol mixtures showed significantly higher FPF of SX to those of lactose and sorbitol ($P < 0.05$). The FPF of SX from glucose mixtures was slightly higher than mannitol, and superior to all other coarse sugars.

SX emission from the Rotahaler®, measured by the ED, is a function of powder flow properties; the better the powder flow, the higher the drug emission will be from the binary interactive mixtures (Concessio et al., 1999). Inclusion of the coarse lactose increased the ED of SX from 71.3% for pure SX to 81.8% for the coarse lactose mixture (Fig. 2A). No significant difference in the ED occurred between coarse lactose and coarse sorbitol binary mixtures ($P > 0.05$); however, the ED reduced from about 80% to 70% when either glucose or mannitol was employed as the carrier (Fig. 2A).

3.3.2. *Effect of carrier moisture and SX–coarse sugar adhesion force on the dispersion of SX*

The relationship between moisture content, SX–coarse lactose adhesion force and FPF of SX is seen in Fig. 2A and B. Adhesion forces between SX and different sugar carrier surfaces were determined by AFM. Adhesion force distributions for the four sugars were broad ranging from about 28 to 138.7 nN. The mean adhesive forces were significantly different for the sugars with the extent of interaction being in the following decreasing order: sorbitol > lactose > mannitol > glucose (Fig. 2B). Good relationships existed between FPF, moisture content and mean adhesion force was observed. For example, the low FPF from the sorbitol was reflected in high adhesion force between SX and sorbitol and the moisture content of the coarse sorbitol, whereas glucose had the lowest moisture and highest FPF. The presence of the pores in the coarse sorbitol carrier may contribute to the higher presence of moisture, which consequently influences the adhesion of fine particles to the carrier surfaces probably by increasing capillary interaction. Previously adsorbed moisture layers on lactose surfaces were found to significantly affect the adhesion of salbutamol sulphate (Dey et al., 2000); capillary forces were found to play a dominant role in the adhesion between SS and lactose.

Capillary forces arose from a thin layer of water molecules adsorbed on the surfaces. As surfaces came in contact, condensed water vapour wicked into the capillary spaces between the contiguous surfaces of lactose. High moisture content in sorbitol might associate with the hydrophilicity of the surface. The greater the uptake of water vapour, the greater the moisture content

3.3.3. *TSI–SEM studies in the binary mixture*

SEM was used in conjunction with the TSI to study the distribution of the particulate components of the mixture during inhalation. Samples at several different stages of inhalation (capsule, inhaler, mouth, stages 1 and 2) were captured on adhesive tape, imaged using SEM and compared qualitatively for the different multi-particulate species present in the binary mixture (Fig. 3).

After inhalation, the coarse sugars were removed from the capsule for all mixtures; however, SX (and perhaps a small quantity of fine lactose) was seen adhered to the gelatin capsule wall. This occurred in all mixtures and is shown representatively by an SEM for the binary glucose mixture in Fig. 3A. SEMs of particles captured in the device before exiting to the TSI demonstrated some difference in dispersion behaviour between the mixtures. For the binary glucose mixture that gave the highest dispersion, the SEM showed a mixture of coarse sugars with some SX particles attached and detached dispersed and agglomerated SX particles (Fig. 3B). However, for the binary sorbitol mixture, where the dispersion was poor, the particulate species seen in the SEM were mainly coarse sugars with drug particles attached and a few detached SX particles (Fig. 3C). During passage of the particulate species through the TSI, the contrasting behaviour of the glucose and sorbitol binary mixtures was seen in the SEM images of particles captured on stage 1 of the TSI (Fig. 3D and E). For mixtures containing glucose (and also mannitol which is not shown), the captured coarse sugar particles contained few adhered particles, while the coarse sorbitol particles contain a higher load of adhered SX particles. This behaviour was consistent with the adhesion studies discussed in Section 3.3.3 and reinforced the fact that poor dispersion was associated with strong drug adhesion to the coarse sugar carriers, probably due to enhanced capillary interaction. Deposition of micronised SX from a binary glucose mixture was seen on stage 2 of the TSI (Fig. 3F). The particulate load on stage 2 was relatively low for the binary mixtures due to the generally poor dispersion of SX from these mixtures.

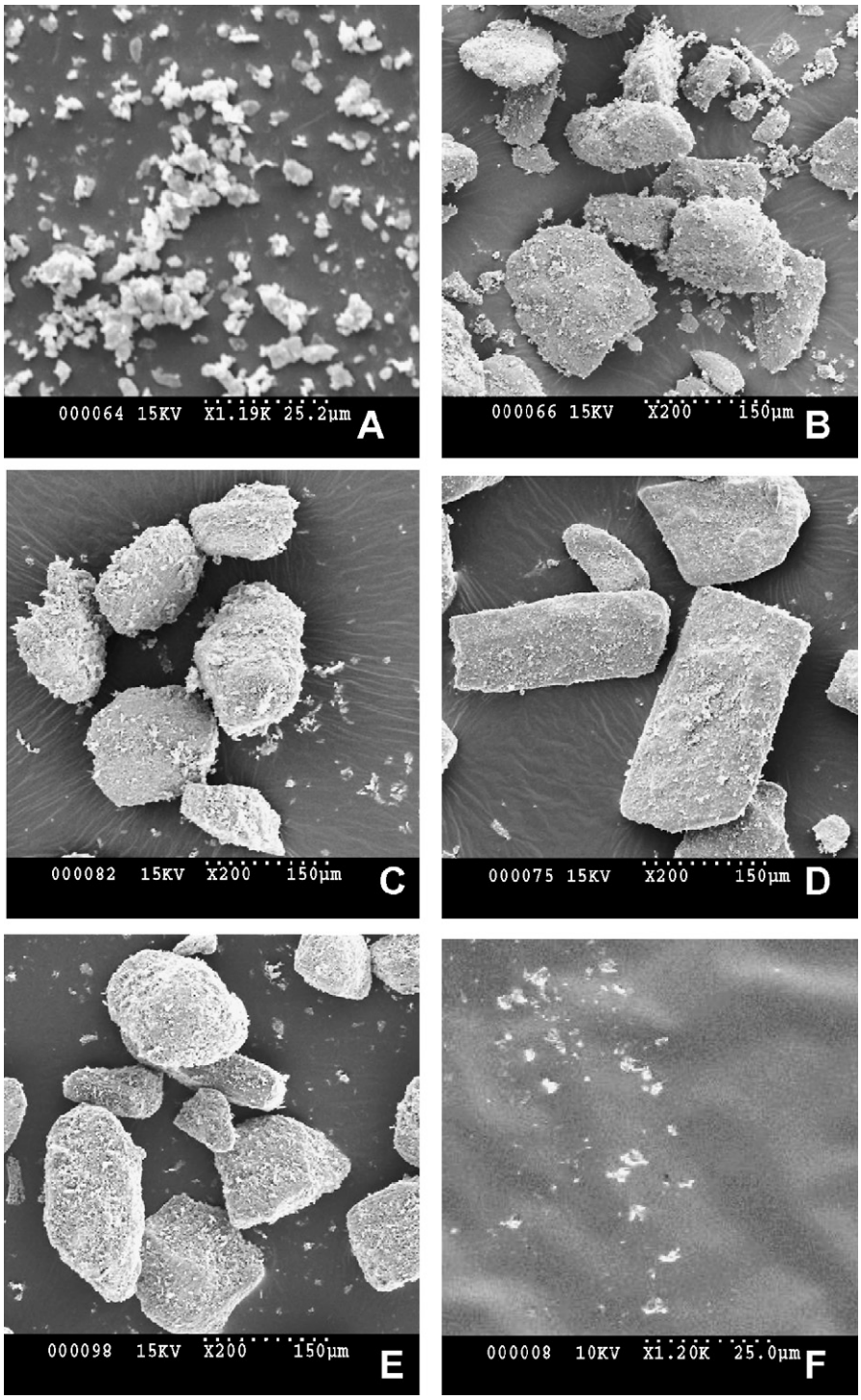

Fig. 3. Scanning electron micrographs of particulate species captured in the Rotahaler® and twin-stage inhaler after dispersion of SX from binary mixtures. (A) Capsule wall from binary glucose mixtures, (B) inhaler mouthpiece from binary glucose mixtures, (C) inhaler mouthpiece from binary sorbitol mixtures, (D) stage 1 from binary glucose mixtures, (E) stage 1 from binary sorbitol mixtures and (F) stage 2 from binary glucose mixtures (magnification 1.2k× for (A and E) and magnification of 200× for (B–E)).

3.4. Effect of fine sugars in SX–sugar ternary mixtures

3.4.1. In vitro deposition of SX in the ternary sugar mixtures

One of the key strategies to improve drug dispersion has been to incorporate fine sugars in the mixture as ternary components (Zeng et al., 1999). The presence of 20% fine lactose increased SX dispersion from 4.0% to 16.8% for a lactose-based mixture (Adi and Stewart, 2004). Therefore, the addition of fine sugars (FL, FG, FM and FS) and their interaction with the different coarse sugars to improve dispersion were investigated further. The effect of fine sugars (10%) on the dispersion of SX from the

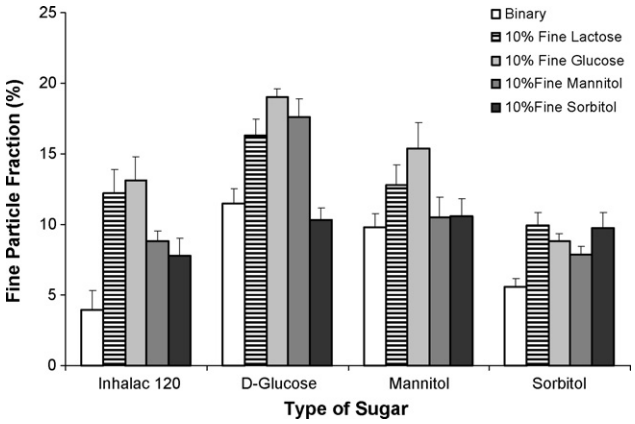

Fig. 4. Effect of fine sugars on the dispersion of SX (FPF) from the different coarse sugar mixtures.

coarse sugar mixtures is summarised in Fig. 4, clearly indicating that the dispersion of SX was dependent upon both the coarse and fine sugar.

The presence of fine sugars in the ternary SX–coarse sugar mixtures increased dispersion of SX with the addition of FG > FM > FL; however, the addition of FS showed a similar FPF to that of the SX–coarse glucose mixture. Ternary mixtures of SX consisting of coarse glucose and FG as ternary component resulted in the highest FPF of SX (19%) compared with other ternary coarse sugar mixtures. Glucose mixtures performed significantly better than currently used ternary mixtures consisting of coarse and fine lactose ($P < 0.05$) and could present as a potential alternative delivery platform for commercial formulations.

The efficiency of SX dispersion from ternary mixtures containing coarse and fine mannitol was less than ternary glucose mixtures possibly because the rougher surface of mannitol increased inter-particulate adhesion due to mechanical interlocking (Fig. 1B and C). Deformation of asperities may result in increased contact area and adhesion. Several studies have examined the effect of surface roughness on particle adhesion onto substrate surfaces during interactive mixing using various techniques (Podczeck, 1998). These studies suggest that higher surface roughness produced lower *in vitro* deposition due to increased adhesion of drug.

The FPF of SX for coarse lactose ternary mixtures followed a similar pattern to that of the glucose mixtures, where addition of fine glucose and fine sorbitol contributed to the highest SX dispersion (FPF of 13.1%) and lowest SX dispersion (FPF

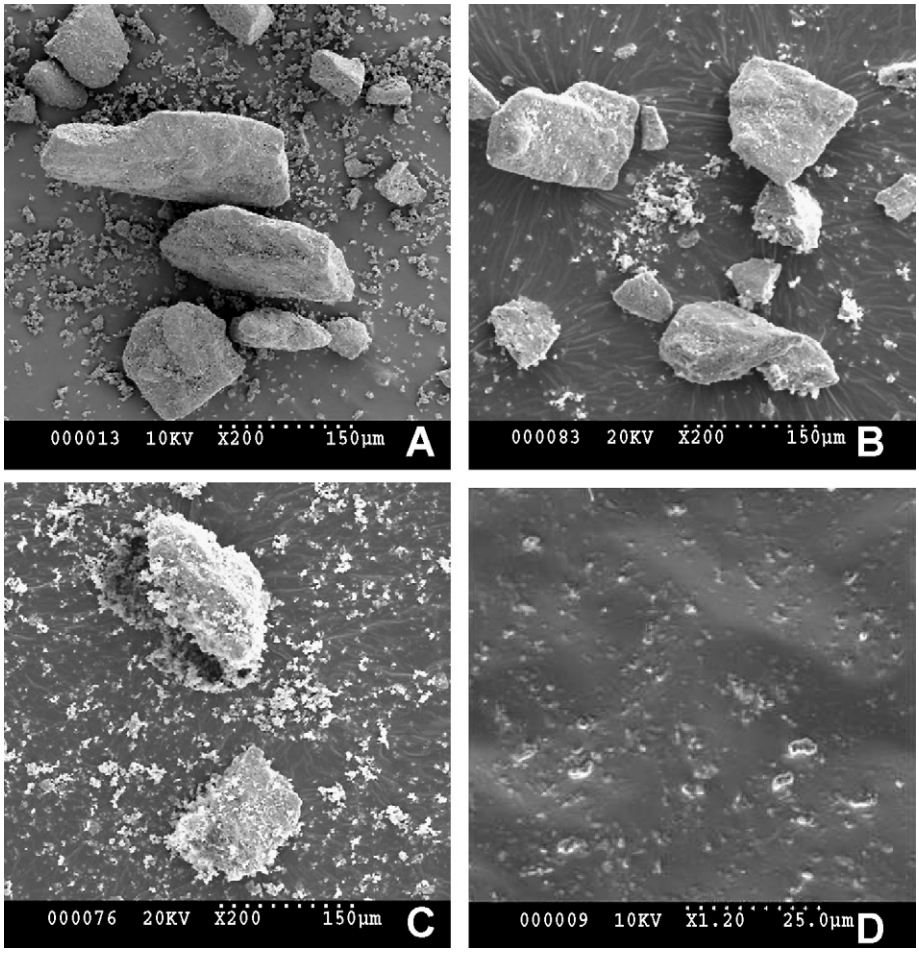

Fig. 5. Scanning electron micrographs of particulate species captured in the Rotahaler® and twin-stage inhaler after dispersion of SX from ternary mixtures containing glucose and fine glucose. (A) mixture before dispersion, (B) inhaler mouthpiece, (C) stage1 and (D) stage 2 (magnification 200× for (A–C) and magnification 1.2k× for (D)).

of 7.8%), respectively. Surprisingly, no difference in FPF of SX occurred between fine mannitol and fine sorbitol ($P > 0.05$). Similar results were observed for formulations containing coarse mannitol where the FPF of SX was found to increase significantly ($P < 0.01$) with added fine lactose whilst little change was observed when fine mannitol or sorbitol was added to the formulation (Tee et al., 2000).

Addition of fine sugars to the mixtures containing coarse sorbitol resulted in higher FPF of SX compared with that of the binary sorbitol mixture and dispersion was not significantly different for all the fine sugars added. Sorbitol showed very poor delivery of SX with FPF of SX ranging from 7.8% to 9.9% (Fig. 4), even when fine sugars were added. The addition of fine sorbitol produced the lowest dispersion in every coarse sugar mixture and the FPFs of SX were almost similar regardless the coarse sugar type (7.8–10.6%).

The study of *in vitro* SX dispersion using different coarse and fine sugars demonstrated better understanding in the formulation strategy using alternative sugars. Improvement of SX dispersion did not limit the choice of coarse carrier or fine ternary component. For example the improvement of SX dispersion from mixtures containing coarse lactose was not limited only with the addition of FL (FPF of 12.2%); fine glucose could be used as well as an alternative fine sugar (FPF of 13.1%). However, not all type of fine sugars improved the SX dispersion in comparison to the SX–sugar binary mixture; for example, mixtures containing coarse mannitol, SX and fine sorbitol.

3.4.2. TSI–SEM studies in the ternary sugar mixtures

As in Section 3.3.3, SEMs were used in conjunction with the TSI to study the distribution of the particulate components during inhalation of the ternary sugar mixtures. Samples of the CG/FG and CS/FS ternary mixtures of SX were captured on adhesive tape at several different stages during inhalation (capsule, inhaler, mouth, stages 1 and 2) and compared using the SEM. These mixtures represented the best and worst performing ternary sugar mixtures seen in Section 3.4.1.

As was seen for the binary mixtures, some SX and fine sugars were observed adhering on the capsule surface after dispersion, either as agglomerates or single particles, possibly due to a strong electrostatic interaction with the capsule wall resulting in the SX loss; however, coarse sugars were emitted from

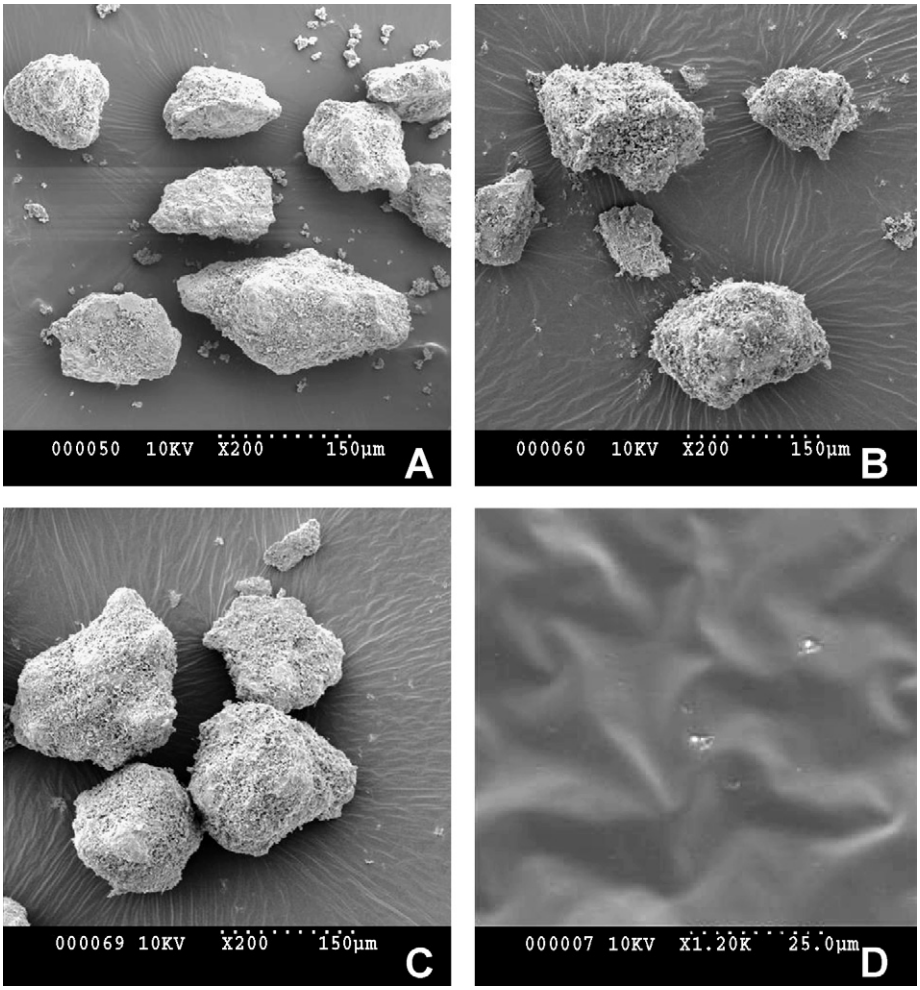

Fig. 6. Scanning electron micrographs of particulate species captured in the Rotahaler® and twin-stage inhaler after dispersion of SX from ternary mixtures containing sorbitol and fine sorbitol. (A) mixture before dispersion, (B) inhaler mouthpiece, (C) stage1 and (D) stage 2 (magnification 200× for (A–C) and magnification 1.2k× for (D)).

the capsule through the inhaler device. Addition of FG to the coarse glucose mixtures of SX disrupted the strong adhesion of SX particles to the coarse glucose surface resulted in particle detachment form the surface and the formation of dispersed and loosely agglomerated particles generally off the surface of the coarse (Fig. 5A), whilst addition of FS to coarse sorbitol mixtures of SX resulted in strong adhesion of SX and FS to the surface of the coarse sorbitol with minimal particulate detachment (Fig. 6A). The structure of the loose agglomerates seen in the ternary glucose mixtures is unknown, but some agglomerates are likely to contain SX and FG.

SEM images of the particulate species emitted from the coarse glucose ternary mixture and captured on the inhaler mouthpiece and stage 1 of TSI (Fig. 5B and C) showed the presence of coarse sugars with some adhered particles and agglomerates impacted on the tape surface. Individual particles are seen in stage 2 (Fig. 5D). This implies that any entrained and detached mixed agglomerates experiencing air turbulence during these stages would lead to the de-agglomeration of drug and fine sugar particles into single particles in stage two (Fig. 5D). In contrast, particles were strongly adhered to the coarse sorbitol; few detached particles were seen in the Rotahaler® mouthpiece and in stage 1 (Fig. 6B and C). The presence of FS failed to interrupt the strong adhesion of SX to the carrier due to the high capillary interaction, resulting in the low FPF value of SX (Fig. 6D).

The results in Sections 3.4.1 and 3.4.2 are important in the understanding of the mechanism of dispersion of drugs in carrier-based mixtures for inhalation. The dispersion of SX showed a reciprocal relationship with the moisture content of the sugars. For example, the SX mixture containing glucose showed the greatest extent of dispersion and contained the lowest moisture while the SX mixture containing sorbitol showed the lowest dispersion and possessed the greatest moisture content. In a close relationship with this result, the SEM–TSI study showed that where the moisture content was highest, the greatest SX adhesion was seen on the coarse sugar carrier and the least displaced fine particles of SX/fine sugar was observed. The increased moisture content is likely to increase the adhesion of the particulate species through increased capillary interaction. Thus, both coarse and fine sugars of highest moisture content will have an increased propensity to interact with lactose. SX is likely to adhere more strongly to the coarse lactose carrier reducing the tendency to be detached from the carrier during inhalation or will interact with the fine sugar to form an agglomerate in which the agglomerate strength is high due to the increased work of adhesion with less tendency to de-agglomerate. This research therefore through the novel SEM/TSI studies associates agglomerate formation with better dispersion.

4. Conclusion

This study clearly demonstrated that strong SX adhesion to coarse sugar carriers reduced the extent of dispersion. The coarse sugar carriers displayed differing adhesional propensities to SX; some of these differences may have been inherent differences in intermolecular interaction due to the differing chemistries, but there was a strong relationship between adhesion force and moisture content indicative of enhanced capillary interactions.

Surface detachment of the SX and fine sugars from the coarse sugar carrier was important in the dispersion process. In ternary mixtures of coarse and fine glucose, SX dispersion increased when the SX was removed from the carrier surface and existed in the mixture as agglomerates. The mechanism of SX surface detachment in the ternary powder mixtures is not known and interpretations remain speculative. It is likely to be related to the extent of interaction between fine sugars and SX during mixing. Fine sugars may preferentially interact with the coarse sugar surface causing SX displacement, but the appearance of the coarse carriers under SEM indicates that both SX and fine sugar are removed from the carrier surface. Interaction between the fine sugars and SX produces agglomerates and it will be the properties of the agglomerates including the extent of particle interaction and the packing fraction that dictate its tensile strength and its propensity to de-agglomerate during inhalation. It is of note that the influence of the micronised sugars was related to their moisture content where sugars like sorbitol with high moisture contents and presumably greater capillary interaction in the agglomerate caused little dispersion enhancement.

This study demonstrates the adverse influence of strong drug-carrier adhesion shows the essential multi-particulate species that must be present in powder mixtures to enhance dispersion efficiency. However, a greater knowledge of inter-particulate adhesion and probably particulate redistributions are necessary to fully understand drug dispersion in powders for inhalation.

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